EQUINE INTERNAL MEDICINE

Stephen M. Reed Warwick M. Bayly Debra C. Sellon



ELSEVIER

FOURTH EDITION

130+ VIDEO CLIPS

EQUINE INTERNAL MEDICINE

This page intentionally left blank

EQUINE INTERNAL MEDICINE

FOURTH EDITION

Stephen M. Reed, DVM, Dipl ACVIM
Rood & Riddle Equine Hospital • Lexington, Kentucky
Professor Emeritus, Department of Veterinary Clinical Sciences • The Ohio State University • Columbus, Ohio

Warwick M. Bayly, BVSc, MS, PhD, Dipl ACVIM
Professor, Department of Veterinary Clinical Sciences
Washington State University • Pullman, Washington

Debra C. Sellon, DVM, PhD, Dipl ACVIM
Professor, Department of Veterinary Clinical Sciences • College of Veterinary Medicine,
Washington State University • Pullman, Washington

ELSEVIER

ELSEVIER

3251 Riverport Lane
St. Louis, Missouri
63043

EQUINE INTERNAL MEDICINE ISBN: 978-0-323-44329-6

Copyright © 2018, Elsevier Inc. All rights reserved.
Previous editions copyrighted 2010, 2005, 1999.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies, and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors assume any liability for any injury and/or damage to persons or property as a matter of product liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data

Names: Reed, Stephen M., editor. | Bayly, Warwick M., editor. | Sellon, Debra C., editor.
Title: Equine internal medicine / [edited by] Stephen M. Reed, Warwick M. Bayly, Debra C. Sellon.
Other titles: Equine internal medicine (Reed)
Description: Fourth edition. | St. Louis, Missouri : Elsevier, [2018] | Includes bibliographical references and index.
Identifiers: LCCN 2017028516| ISBN 9780323443296 (hardback : alk. paper) | ISBN 9780323443098 (Ebook)
Subjects: LCSH: Horses--Diseases. | Veterinary internal medicine. | MESH: Horse Diseases
Classification: LCC SF951 .E565 2018 | NLM SF 951 | DDC 636.1/089--dc23 LC record available at https://lccn.loc.gov/2017028516

Senior Content Strategist: Jennifer Flynn-Briggs
Content Development Manager: Luke Held
Associate Content Development Specialist: Anna Miller
Publishing Services Manager: Deepthi Unni
Project Manager: Radhika Sivalingam
Design Direction: Amy Buxton

Printed in United States of America

Last digit is the print number: 9 8 7 6 5 4 3 2 1

ELSEVIER Book Aid International Working together to grow libraries in developing countries
www.elsevier.com • www.bookaid.org

Contributors

Monica Aleman, MVZ Cert, PhD, DACVIM
Medicine and Epidemiology
University of California
Davis, California

Warwick M. Bayly, BVSc, MS, PhD, DACVIM
Professor
Department of Veterinary Clinical Sciences
Washington State University
Pullman, Washington

Michelle Henry Barton, DVM, PhD, DACVIM
Fuller E. Callaway Endowed Chair
Large Animal Medicine
University of Georgia
Athens, Georgia

Etta Agan Bradecamp, DVM, DACT, DABVP
Theriogenologist
Rood and Riddle Equine Hospital
Lexington, Kentucky

Teresa Ann Burns, DVM, MS, PhD, DACVIM
Veterinary Clinical Sciences
Clinical Assistant Professor, Equine Internal Medicine
The Ohio State University College of Veterinary Medicine
Columbus, Ohio

Jennifer L. Davis, DVM, MS, PhD
Associate Professor of Clinical Pharmacology
Department of Biomedical Sciences and Pathobiology
Virginia-Maryland College of Veterinary Medicine
Blacksburg, Virginia

Elizabeth Davis, DVM, PhD, DACVIM
Associate Professor
Clinical Sciences
Kansas State University
Manhattan, Kansas

Igor F. Canisso, DVM, MSc, PhD, DACT, DECAR
Assistant Professor of Theriogenology
Department of Veterinary Clinical Medicine
College of Veterinary Medicine
University of Illinois Urbana-Champaign
Urbana, IL

Katarzyna Dembek, DVM, PhD, DACVIM
Assistant Clinical Professor
Veterinary Clinical Sciences
Iowa State University
Ames, Iowa

Thomas J. Divers, DVM, DACVIM, DVECCS
Professor of Medicine
Clinical Sciences
Cornell University
Ithaca, New York

Bettina Dunkel, DVM, PhD, DACVIM, DECEIM, Dip ACVECC, FHEA, MRCVS
Senior Lecturer in Equine Medicine
Veterinary Clinical Sciences
The Royal Veterinary College
North Mymms
Hatfield, UK

Katherine S. Garrett, DVM, DACVS
Rood and Riddle Equine Hospital
Lexington, Kentucky

Ray Geor, BVSc, MVSc, PhD, DACVIM
Professor and Pro Vice-Chancellor College of Sciences
Massey University
Palmerston North, New Zealand

Tiffany L. Hall, DVM, DACVIM, DACVECC
Internal Medicine and Critical Care
Associate
Equine Medical Center of Ocala
Ocala, Florida

Rachel C. Hector, DVM
Resident, Anesthesia and Pain Management
Clinical Sciences
Colorado State University
Fort Collins, Colorado

Kenneth W. Hinchcliff, BVSc, MS, PhD, DACVIM
Journal of Veterinary Internal Medicine
Co-Editor-in-Chief
Faculty of Veterinary Science
Professor
University of Melbourne
Melbourne, Victoria
Australia
President and CEO
Trinity College
Parkville, Victoria
Australia

Melissa T. Hines, DVM, PhD, DACVIM
Professor
Large Animal Clinical Sciences
University of Tennessee
Knoxville, Tennessee

Samuel D. Hurcombe, MS, DACVIM, DACVECC
Associate Professor–Clinical
Cornell Ruffian Equine Specialists
Cornell University
Elmont, New York

Mary Lassaline, DVM, PhD, MA, DACVO
Associate Professor of Clinical Equine Ophthalmology
Surgical and Radiological Sciences
University of California – Davis
Davis, California

Maureen T. Long, DVM, MS, PhD, DACVIM
Associate Professor
Infectious Diseases and Pathology
University of Florida
Gainesville, Florida

Khursheed R. Mama, DVM, DACVA
Professor of Anesthesiology
Clinical Sciences
Colorado State University
Fort Collins, Colorado

Dianne McFarlane, DVM, PhD, MS, DACVIM, CVSH, OSU
Center of Veterinary Health Sciences
Professor of Physiological Sciences
Oklahoma State Univeristy
Stillwater, Oklahoma

Harold C. McKenzie III, DVM, MS, DACVIM
Associate Professor
Large Animal Clinical Sciences
Virginia Maryland College of Veterinary Medicine, Virginia Tech
Blacksburg, Virginia

Robert H. Mealey, DVM, PhD, DACVIM
Professor
Department of Veterinary Microbiology and Pathology
Washington State University
Pullman, Washington

Francisco J. Mendoza, DVM, PhD, MSc, DECEIM
Professor of Internal Medicine
Department of Animal Medicine and Surgery
College of Veterinary Medicine
University of Cordoba, Spain

Yvette S. Nout-Lomas, DVM, PhD, DACVIM, DACVECC
Assistant Professor
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, Colorado

Alejandro Perez-Ecija, DVM, MS, PhD, DECVP
Associate Professor of Internal Medicine
Department of Animal Medicine and Surgery
College of Veterinary Medicine
University of Cordoba, Spain

Ann M. Rashmir-Raven, DVM, MS, DACVS, PGCVE, FHEA
Associate Professor
Large Animal Clinical Sciences
Michigan State University
East Lansing, Michigan

Stephen M. Reed, DVM, DACVIM
Rood and Riddle Equine Hospital
Lexington, Kentucky
Professor Emeritus
The Ohio State University,
Columbus, Ohio

Chris Sanchez, DVM, PhD, DACVIM
Associate Professor
Department of Large Animal Clinical Sciences
College of Veterinary Medicine, University of Florida
Gainesville, Florida

Debra C. Sellon, DVM, PhD, DACVIM
Professor
Department of Veterinary Clinical Sciences
College of Veterinary Medicine
Washington State University
Pullman, Washington

Maria R. Schnobrich, VMD, Dip ACT
Leblanc Reproduction Center
Theriogenologist
Rood and Riddle Equine Hospital
Lexington, Kentucky

Harold C. Schott II, DVM, PhD, DACVIM
Professor of Internal Medicine
Veterinary Clinical Sciences
Michigan State University,
East Lansing, Michigan

Colin C. Schwarzwald, PhD, DACVIM, DECEIM
Professor of Equine Internal Medicine
Equine Department
Vetsuisse Faculty
University of Zurich
Zurich, Switzerland

Charlie Scoggin, DVM, MS, DACT
LeBlanc Reproduction Center
Associate Veterinarian
Rood and Riddle Equine Hospital
Lexington, Kentucky
Clinical Sciences
Affiliate Faculty
Colorado State University
Fort Collings, Colorado

Sharon J. Spier, DVM, PhD, DACVIM
Professor
Department of Medicine and Epidemiology
University of California
Davis, California

Patricia Talcott, DVM, MS, PhD, DABVT
Professor of Toxicology
Washington Animal Disease Diagnostic Laboratory
Washington State University College of Veterinary Medicine
Pullman, Washington
Veterinary Diagnostic Toxicologist
Washington Animal Disease Diagnostic Laboratory
Pullman, Washington

Ramiro E. Toribio, DVM, MS, PhD, DACVIM
Professor
Veterinary Clinical Sciences
The Ohio State University
Columbus, Ohio

Stephanie J. Valberg, DVM, PhD, DACVIM
Professor and Mary Anne McPhail Dressage Chair in Equine Sports Medicine
College of Veterinary Medicine
Michigan State University
East Lansing, Michigan

Bryan M. Waldridge, DVM, MS, DABVP, DACVIM
Veterinarian
Park Equine Hospital at Woodford
Versailles, Kentucky

This page intentionally left blank

Preface

This is the fourth edition of *Equine Internal Medicine.* Like its three predecessors, it has been written and edited with the aim of promoting a clearer comprehension of the principles of medical disease and/or problem development by focusing on the basic pathophysiologic mechanisms that underlie the development of various equine diseases. As with previous editions, basic information is presented and then related to the clinical characteristics of each disease and its therapy and management.

Most of the chapters that appeared in the previous three editions have been updated, and a number of them have been extensively revised or rewritten. Although the bulk of the chapters address specific diseases along systems-based lines, we realize that the practitioner is initially confronted with a specific problem that may have its origin in one or more of the body's systems. The first section of the book is therefore devoted to an in-depth discussion of the basic mechanisms by which problems might develop, and the principles underlying the treatment of many of them. The reader can build on this foundation by reading about specific disorders in the second section of the book, which is divided into chapters dealing with problems of a particular body system or of a specific nature.

Many true experts have contributed to this text. Their depth of knowledge about all aspects of equine internal medicine is encyclopedic and daunting. We are grateful for their efforts and diligence in helping us to produce what we hope will continue to be regarded as the definitive text on medical diseases of horses. We are indebted to them for their efforts. We trust that they derive a sense of pride from the part they have played in producing what we hope represents the gold standard in equine medical textbooks.

In these days of progressive globalization of the world's societies and associated growth in the international movement of horses for breeding, recreational, and competitive purposes, there also has been a worldwide increase in expectations relating to the standard of veterinary care and evaluation of sick horses. The sophistication of specialist training programs and the increased number of equine internists also taking advantage of postgraduate doctoral opportunities have resulted in a wealth of new information and the maturing of an increasingly complex and challenging discipline—equine internal medicine. The delivery of superior health care and increased client expectations that have been associated with the growth of this discipline have led to the appearance of extremely well-informed and astute equine general practitioners everywhere, and specialist equine internists on most continents. More than ever before, equine internal medicine now stands as an autonomous specialty in the veterinary profession. We trust that the fourth edition of *Equine Internal Medicine* will prove to have as much universal appeal and application as those editions that preceded it.

Finally, we would be remiss if we did not thank the many people at Elsevier for their persistence and efforts. Penny Rudolph, Lauren Harms, Radhika Sivalingam and Anna Miller in particular deserve our gratitude. They and many others have assisted in manuscript preparation, correspondence, and all the other tasks that must be completed to get a book like this into print. Without them and the generosity of our colleagues, this book would not have been published. We think that everyone's efforts have been worthwhile.

Stephen M. Reed, DVM, Dipl ACVIM
Warwick M. Bayly, BVSc, MS, PhD, Dipl ACVIM
Debra C. Sellon, DVM, PhD, Dipl ACVIM

This page intentionally left blank

Contents

PART 1
Mechanisms of Disease and Principles of Treatment

1 Mechanisms of Disease and Immunity, 3
2 Pharmacologic Principles, 79
3 Recognizing and Treating Pain in Horses, 138
4 Critical Care, 158
5 Internal Medicine and Clinical Nutrition, 191
6 Clinical Epidemiology and Evidence-Based Medicine, 218
7 Clinical Approach to Commonly Encountered Problems, 232

PART 2
Disorders of Specific Body Systems

8 Disorders of the Respiratory System, 313
9 Disorders of the Cardiovascular System, 387
10 Disorders of the Musculoskeletal System, 542
11 Disorders of the Neurologic System, 580
12 Disorders of the Gastrointestinal System, 709
13 Disorders of the Liver, 843
14 Disorders of the Urinary System, 888
15 Disorders of the Hematopoietic System, 991
16 Disorders of the Endocrine System, 1029
17 Disorders of the Eye and Vision, 1139
18 Disorders of the Skin, 1159
19 Disorders of the Reproductive Tract, 1217
20 Disorders of Foals, 1365
21 Toxicologic Problems, 1460
Appendix I Aspects of Clinical Relevance in Donkeys, 1513
Index, 1525

This page intentionally left blank



PART 1

Mechanisms of Disease and Principles of Treatment

This page intentionally left blank

CHAPTER 1

Mechanisms of Disease and Immunity

Robert H. Mealey and Maureen T. Long*

The Microbiome

Maureen T. Long

Dermal and mucosal surfaces provide a life-preserving protective barrier composed of physical, chemical, and microbial defenses.[1] In the past 5 years, the concept of normal flora has been broadened to include the whole of "microbiota" that colonizes skin, gut, and mucosal surfaces. The microbiome is the science of analyzing the total number of organisms in an ecosystem, which in our case are animals. The practical application of this science to the clinician is that the microbiome differs among many niches of the body, varies with individuals, and has an interactive role with actual disease states. Microbiota is the microbial population within the different sites of the body. *Commensal bacteria* are those living on or within a host in a way such that both derive mutual benefit and in which interruption of this association results in abnormal host development or overt disease. These represent that part of the microbiome that has intimately coevolved with the different niches of the body.[1]

Environment is local, such as within different areas of the gut, and also external, and these can have either positive or negative effects on the microbiome. For instance, poor ventilation in animal housing not only is a direct irritant, but changes in pH, metabolism of particulates, and activation of the immune system will dramatically alter the structure of the microbiome.

Age is also an important factor. Colonization of skin and mucosal surfaces occurs at birth and is highly variable in early life. Changes in management from birth to weaning are accompanied by changes in diet and husbandry for horses. They create a highly dynamic environment that eventually can be used to predict best practices once data on the microbiome in healthy horses are obtained in a methodical and scientific manner.

Skin Microbiome

The combination of normal flora and mucosal immunity provides an effective barrier against infectious colonization of nondisrupted skin surfaces. Even though horses inhabit an environment heavily contaminated with fecal flora, normal dermal flora in the horse is surprisingly devoid of members of the Enterobacteriaceae.[2] Normal inhabitants include mixed populations of bacteria of species of *Acinetobacter, Aerococcus, Aeromonas, Bacillus, Corynebacterium, Flavobacterium, Micrococcus, Nocardia,* coagulase-negative *Staphylococcus, Staphylococcus aureus, Streptomyces,* and nonhemolytic *Streptococcus* generae.[3] Certain *Staphylococcus* spp. have been associated with skin disease in the horse, and these include *S. aureus, S. intermedius,* and *S. hyicus,* whereas species such as *S. xylosus* and *S. sciuri* were more associated with normal skin. More than 30 species of fungi can inhabit the skin and *Alternaria, Aspergillus, Candida, Fusarium, Rhizopus,* and *Trichophyton* spp. are commonly present.[2] Until recently the presence of *Malassezia* yeast species has been considered pathogenic. Recent fungal culturing of the skin of normal, healthy horses has confirmed colonization by a species of *Malassezia* yeast that is novel to the horse (tentatively named *M. equi*). Colonized sites include the groin, axilla, and perineal regions.[4] To date there are no studies describing the microbiome of the skin of horses.

Oral Microbiome

The oral and pharyngeal mucosa is richly populated with many bacteria, including obligate aerobes, anaerobes, and facultative anaerobes.[5] By culture, gram-positive and gram-negative anaerobes are the predominant flora in the mouth and pharyngeal tonsils of the normal horse, with *B. fragilis* and *Bacteroides* spp. most commonly found. Genera consisting of *Fusobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Veillonella* spp., and *Megasphaera* spp. are also cultured. Aerobic and facultative anaerobic populations mostly include *S. zooepidemicus, Pasteurella* spp., *E. coli, Actinomyces* spp., and *Streptococcus* spp. In a 16S metagenomics analysis of the subgingival sites of the horse, although highly diverse, many similarities to human and other species were observed.[6] The most common bacterial phyla included Gammaproteobacteria, Firmicutes, Bacteroidetes, Betaproteobacteria, Fusobacteria, Actinobacteria, Epsilonproteobacteria, TM7, Deltaproteobacteria, Synergistetes, GN02, Tenericutes, Spirochaetes, Chloroflexi, and Alphaproteobacteria. The Gammaproteobacteria had the highest population of species, with *Moraxella* spp. and *Pasteurellaceae* spp. overrepresenting the population. In the former, *Actinobacillus* species and unclassified *Pasteurellaceae* were the most common bacteria.

* The editors and authors acknowledge and appreciate the contributions of J. Lindsay Oaks, Thomas R. Klei, D. Paul Lunn, and David W. Horohov as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

The finding of *Actinobacillus* species is not unexpected; however, by comparison these taxa are relatively absent from cat and dog oral metagenomes. Of the highly abundant Firmicutes, many classes of *Bacilli* were detected; several *Neisseria*-like bacteria were detected. Of particular interest is the population of oral spirochetes that reside in the equine mouth because of its relationship to periodontal disease in humans, and although there were *Treponema,* spirochetes, and leptospires present and abundant, "periodontopathogens" were present in low numbers. When healthy horses were compared with horses with oral disease, *Actinobacillus* (Gammaproteobacteria) and the *Neisseria*-like *Gemella* sp. (Firmicutes) predominated compared with *Prevotella* (Bacteroidetes) and *Veillonella* (Firmicutes).[7]

Pharyngeal and Respiratory Microbiome

Because these same genera are also consistently found in horses with lower respiratory infections, opportunistic colonization by pharyngeal flora is the likely mechanism of disease.[5] Contamination of the trachea of the horse is a frequent occurrence, as evidenced by the fact that transtracheal aspiration yields positive bacterial cultures in approximately 30% of both normal adult horses and foals.[8] As with skin flora, normal horses have multiple fungal species inhabiting conjunctival, nasal, and oral mucosa.[8] Stabling increases the frequency of ocular fungi in normal horses.[9]

Intestinal Microbiome

In animal models and chronic human conditions, the presence of normal flora is considered important for intestinal maturity and containment of disease. Changes in cecal weight, villus:crypt ratio, and volatile fatty acid (VFA) production and the development of gut immunoglobulin A (IgA) responses are all affected by suboptimal cecal colonization in germ-free animals.[10] A relationship between severity of mucosal disease and normal flora has also been demonstrated in models of inflammatory bowel disease of humans.[11]

Bacteria are present in all parts of the intestinal tract of the horse, and the microbial fauna increases in complexity and density aborally.[12] The stomach of the horse is not a sterile environment. A dense population of gram-positive bacterial rods, primarily composed of *Lactobacillus* spp., colonizes the nonsquamous portion of the equine stomach. Substantial colonization of the duodenum is present with a large population of proteolytic bacteria, and this colonization increases by tenfold in the ileum.[13]

Microbial degradation and fermentation of plant material in the large intestine are important components of nutrient acquisition in the equid. The consumption of cellulose and starch results in the production of VFAs.[14] The major cellulolytic bacterial strains in the horse produce arrays of fermentation products that differ from those of cattle.[15] Early genetic techniques also demonstrate that the predominant flora are the low guanine-cytosine (GC)-content bacteria, which include *Cytophaga-Flexibacter-Bacteroides* and *Clostridium* bacteria; the actual species are completely novel.[16] Standard microbiologic techniques specifically demonstrate Enterobacteriaceae, *Butyrivibrio* spp., *Streptococcus* spp., *Bacteroides* spp., *Lactobacillus* spp., *Selenomonas* spp., *Eubacterium* spp., *Propionibacterium* spp., and *Staphylococcus* spp. in residence.[17] In addition, there are completely different compositions of bacteria among the differing segments of the colon, especially between the ascending colon and cecum, indicating highly specialized digestive functions associated within the large intestine itself.[16] Yeasts and fungi of the order Mucorales have been identified in the cecum of normal horses and are capable of digesting cellulose and starch.[18] By deep sequencing, Firmicutes and Bacteroidetes (or in another study, Verruccomicrobia) are the two must abundant phyla.[19] The common bovine rumen bacteria *Ruminococcus flavefaciens* is one of the most predominant cellulolytic bacteria of the equine cecum based on standard microbiologic techniques.[15]

Since 2012 several studies have been published investigating the equine microbiome, and these have been recently reviewed.[17] The techniques employed in each investigation could highly bias study results. Fecal samples or samples from specific gastrointestinal locations have been used. Most studies had a stated goal of attempting to define the flora of the hindgut of the horse.

Routine surveillance demonstrates a relative lack of intestinal pathogens in the flora of normal horses. In the largest study to date, fecal shedding of *Salmonella enteriditis* as detected by fecal culture in normal horses from farms without evidence of salmonellosis was 0.8% in resident horses.[20] Molecular diagnostics, once hoped to provide a tool for understanding the incidence of *Salmonella* spp. in the clinically normal horse, has provided inconsistent information, and polymerase chain reaction (PCR) is most useful for identification of subclinical shedders and environmental contamination during an outbreak.[21,22] On the basis of limited investigations, the carriage rates of *C. difficile* in normal horses and foals appear to be low (less than 1.5%).[23]

Intestinal flora in the horse is an important source for extraintestinal pathogens. In studies examining the carriage rate of *Rhodococcus equi,* all horses cultured carried the bacteria regardless of age.[24,25] If a farm had endemic *R. equi* and respiratory isolates contain the 90-kDa plasmid that is associated with disease, fecal isolates also contained this plasmid. In a recent study, there was little difference in the composition of the phyla of fecal bacteria measured via deep sequencing between normal foals and those with either subclinical or clinical *R. equi.*[26] However, a striking difference in flora was observed over the first few weeks of life among all foals demonstrating the transition from a juvenile gut to that of the adult. Flora transitioned from equal abundance of Firmicutes to Bacteroidetes over this short interval.

The right dorsal colon contains the highest numbers and the most diversity of protozoal species.[17] Four classes of protozoa species, Rhizopoda, Mastigophora, Cliata, and Suctoria, have been described based on relative prevalence in ascending and descending parts of the large colon. The importance of protozoa for normal gastrointestinal function is debated, but these organisms are presumed to play an important role in degradation of plant fiber. Despite this presumption, one investigation demonstrated little effect of protozoa on the digestibility of dry matter with little effect on cellulose digestion.[27]

Urogenital Microbiome

By far, most of the work that characterizes equine normal flora has focused on urogenital flora to address infertility and fetal loss. Although vaginal and vestibular mucosa of mares should be colonized with normal mucosal flora, the uterus

is considered sterile. However, typical culturing techniques result in frequent isolation of what could be considered pathogens, and cytology and bacterial counts are essential supplemental tests for detecting true uterine infection. Counts less than 10 colony-forming units and lack of inflammatory cells indicate uterine or technical contamination.[28]

Many bacteria inhabit the external genitalia of stallions, including bacteria considered to be associated with metritis in mares. The predominant aerobe isolated is coagulase-negative *Staphylococcus* spp., followed by *Corynebacterium* spp., α-hemolytic *Streptococcus* spp., and *Lactobacillus* spp. Pathogens such as β-hemolytic *Streptococcus* spp., *Pseudomonas aeruginosa,* and *Klebsiella* spp. can be frequently found in servicing stallions.[29,30] Pregnancy rates appear to be the same in mares bred to stallions with *P. aeruginosa*–infected semen.[31]

Fungal Microbiome

Essentially the same principles apply regarding normal flora, host immunity, and specific virulence factors for the pathogenesis of fungal infection. Fungal infections can be divided into primary or opportunistic pathogens. True pathogens are less dependent on host status than opportunistic pathogens, although even a true pathogen may require some degree of alteration of normal flora or host immunity to become established. Long-term antibiotic use, immunosuppression, and compromised organ function (especially involving the pulmonary or endocrine system) are three primary host factors highly associated with the establishment of opportunistic fungal infection. Fungi in particular can adapt to the mammalian environment over a relatively short course in order to become established. Establishment usually requires a change in thermal range, oxygen requirements, and resistance to host defenses.

Nosocomial Infections

Development of colitis in the horse has been associated with feed change, antibiotics, surgery, nonsteroidal inflammatory drugs, and transport, all events that disrupt flora.[32,33] Rapid change from a roughage diet to concentrate results in increased anaerobes, decreased cellulolytic bacteria, decreased cecal protozoa diversity, and decreased pH in the equine cecum.[32] Isolation of *Clostridium difficile* is more likely from horses treated with antibiotics, and clinical disease has been associated with ampicillin, erythromycin, penicillin, and potentiated sulfonamide administration in adult horses.[34,35] In ponies infected with *Salmonella* spp., transport and surgery reactivated infection and diarrhea, and antibiotics (oxytetracycline) prolonged shedding but did not induce recrudescence.[33] In a case-control study, use of potentiated sulfonamides was not significantly associated with the development of diarrhea in hospitalized horses; however, overall antibiotic use was highly associated with the occurrence of diarrhea.[36]

Antibiotics disrupt normal gastrointestinal flora and function,[37] and changes in carbohydrate metabolism are a large intestinal event secondary to reduced microbial reduction of carbohydrates to short-chain fatty acids (SCFAs). Because SCFA metabolism and absorption result in fluid and electrolyte absorption, a sudden decrease in SCFA leads to osmotic diarrhea with an intraluminal accumulation of organic acids, cations, and carbohydrates. Erythromycin and amoxicillin directly affect colonic motility.[37] Erythromycin is a motilin receptor agonist resting in contraction of antral and duodenal smooth muscles.[38] In the horse erythromycin results in a dose-dependent increase in ileocecal emptying.[39] Motility-enhancing effects have also been observed in human patients treated with amoxicillin.[37]

Occurrence of infectious lower respiratory disease in adult horses is an example of how contamination of a normally sterile site with several commensal bacteria results in disease. The tonsillar mucosa of the oropharynx is heavily colonized with *S. equi* subsp. *zooepidemicus,* and necrosis of this tissue occurring during viral infection is associated with spread to the lower respiratory tract.[5] Transport of horses (especially for distances greater than 500 miles) is a primary risk factor for pleuropneumonia as demonstrated in a large retrospective study.[40] Elevation of the head for an extended period of time is likely a contributing factor. Horses normally feed from the ground for most of the day, and this posture promotes effective tracheal clearance of inhaled debris and particulate matter. *Pasteurella, Actinobacillus,* and *Streptococcu*s spp. are the most frequent and prolific colonizers of the trachea after prolonged head elevation.[41,42]

Nosocomial infection (health care–associated infections) is defined by the Centers for Disease Control and Prevention as a localized/systemic condition resulting from an adverse reaction to the presence of an infectious agent or its toxin. There must be no evidence that the infection was present or incubating at the time of hospital admission.[43] Nosocomial infections are becoming a major problem for large animal veterinary teaching and private referral hospitals. Infections with *Serratia marcescens, Acinetobacter baumannii, S. aureus,* methicillin-resistant *Staphylococcus* spp., *Enterococcus* spp., and various *Salmonella enteritidis* serovars have all been reported in association with nosocomial infection in equine patients.[44,45] Surgical incision infection, joint sepsis, catheter phlebitis, wounds, and diarrhea represent the common clinical syndromes reported in horses.[44-46] When nosocomial infection involves the acquisition of isolates from the hospital environment, these isolates are more difficult to treat because they frequently undergo high-level antibiotic pressure and attain multiple-drug resistance (MDR). Nosocomially transmitted salmonellosis in equine hospital wards is increasingly reported, with *Salmonella enteritidis* serotypes Krefeld, Saint Paul, DT104, and Anatum all demonstrating attainment of MDR over the course of the outbreak.[47,48] Only one study of a nosocomially transmitted *S. enteritidis* (serotype Heidelberg) did not demonstrate significant acquisition of MDR over time.[49]

Pathogenesis of Bacterial Infections

The ability of bacteria to gain entry and cause disease results from a combination of factors possessed by the agent itself, environmental conditions, and status of host defenses. General mechanisms that are specific to bacteria and enhance disease are virulence factors that enhance the entry, spread, and damage to host tissues (Table 1.1). Major virulence factors are listed for specific equine pathogens. Protein secretion systems (PSSs) are a structurally diverse complex of essential virulence factors for bacteria that allow specialized

interactions among cells.[50] These main systems function to translocate various sized molecules and are important in the formation of adhesins on attachment to host cells. Fibrillar adhesins (FAs) and nonfibrillar adhesins (NFAs) are the most important PSS subgroup not used for bacterial conjugation. They specifically target host cells and biofilms for enhancement of colonization and invasion. There are multiple types of FAs in both gram-positive and gram-negative bacteria, with gram-negative types the most well characterized (Table 1.2).[51,52] Pili or fimbriae are rod-shaped structures composed of an orderly array of a single protein usually arranged in a helical fashion to form a cylinder. The tip of the fimbria mediates attachment to carbohydrate moieties on cell surfaces and is integral to bacterial invasion and colonization. Bacteria can also contain multiple types of pili. Both the bacterial pili themselves and the cellular pathways they use for secretion and formation of pili are targets for pharmacologic intervention, and there are multiple subclasses depending on their configuration.[51]

Afimbrial adhesins are cell proteins that enhance the binding of bacteria to host cells. They are also called *microbial surface components recognizing adhesive matrix molecules.*[53,54] Gram-positive organisms possess afimbrial proteins on their surfaces that presumably aid in binding to host cells. The three most commonly studied afibrillar adhesins are those that bind salivary glycoprotein, bind fibronectin, or are composed of lipoteichoic acid.[53,55] Salivary binding proteins are commonly found in pathogens and commensals of the oral cavity. Both *Streptococcus* spp. and *Actinomyces* spp. possess these proteins. Fibronectin binding protein (FBP) is necessary for *S. aureus* invasion and binds both fibronectin and collagen to form a bridge between the FBP and the host cell integrin (integrin $\alpha_5\beta_1$).[56,57] Heterologs of FBP have been demonstrated in *S. pneumoniae* of humans, *S. equi* subsp. *equi,* and *S. equi* subsp. *zooepidemicus.* Other potential equine pathogens that have FBPs on their surface include *Actinomyces* spp., *E. fecalis,* and *L. monocytogenes.*[55,58] Lipoteichoic acid, a common binding factor found in *Streptococcus* group A bacteria, is important in adhesion of bacteria to cells.[59] This protein is also important in stimulation of cytokine secretion from the cells during infection and has been demonstrated in group B *Streptococcus,* including *S. equi* subsp. *equi.*[60] A less commonly described afibrillar adhesin is composed of surface polypeptide chains in *Corynebacterium* that binds to lectin.[61] Afibrillar adhesins are also present in gram-negative organisms; the most commonly studied are conserved high-molecular-weight adhesion proteins of *Haemophilus influenzae* and *Bordetella pertussis.*[59]

Bacterial ecology has emphasized the importance of biofilm for colonization of both biotic and abiotic surfaces of bacteria.[50] A requirement for biofilm formation is a tight interaction of bacterial adhesins with surface receptors that promote further bacterial aggregation. The most important family of biofilm adherence proteins is that of *Staphylococcus aureus.* These proteins have high molecular mass and repetitive structures whose size and number can be varied during the course of infection, possibly allowing for immune evasion.

The most common virulence proteins of both gram-positive and gram-negative organisms are bacteria lectins.[53-62] Although attachment is thought to be the primary role of these proteins, attachment itself results in an intracellular change, including actin rearrangements, cell signaling regulation, or actual secretion of bacterial substances into the

TABLE **1.1 General Mechanisms of Bacterial Pathogenesis**

Action	Mechanism	Examples
Entry of bacteria	Adhesion Entry	Fibrillar adhesins Nonfibrillar adhesins Curli fimbriae Lipoteichoic acid Biofilm adhesion proteins Membrane ruffling
Enhancement of spread	Immune resistance Host substrate utilization	Capsule Lipopolysaccharide Anticomplement Resistance to phagocytosis Phagolysosomal survival Iron acquisition
Damage to host membranes	Toxins	Exotoxin Endotoxin Apoptosis

TABLE **1.2 Major Types of Bacterial Adhesins**

Adhesin	Definition	Example
Type 1 fimbriae	Cell surface structure primarily on gram-negative bacteria that bind to the terminal mannose of glycoproteins on cells	*Escherichia coli*
Type 4 pili	Cell surface structure primarily on gram-negative bacteria that function in adhesion, twitching, and DNA uptake, which bind on CD46 and other glycolipids	*Pseudomonas aeruginosa*
Curli fimbriae	Coiled aggregative fimbrial structures that bind to fibronectin, laminin, and plasminogen and function in adhesion, aggregation, and biofilm formation	Enterohemorrhagic *Escherichia coli* (EHEC) *Salmonella*
Fibrils and flexible rods	Short, thin rodlike adhesins that bind to fibronectin for adhesion to host tissues	*Streptococcus* species
Lipoteichoic acid	Part of the peptidoglycan layer of cell walls	Gram-positive bacteria
Biofilm	Exopolysaccharide produced by bacteria that allows matrix formation of embedded material	Gram-positive and gram-negative bacteria

host cell. These proteins are highly conserved in bacteria and are important targets for immunoprophylaxis. Membrane transformation for uptake of intracellular bacteria such as *Yersinia* spp., *Listeria monocytogenes, Salmonella* spp., and *Shigella flexneri* can be either zipperlike or triggerlike. Alternatively, *Salmonella* and *Shigella* bacteria adhere and secrete proteins that are translocated into the host cell cytoplasm and trigger actin polymerization.[63] In addition to membrane ruffling, *Mycobacterium avium* and *Salmonella* spp. also rely on activation of intracellular GTPases, leading to phagocytosis.[64,65]

Once colonization occurs, multiplication and spread of bacteria are enhanced through virulence factors. These factors assist bacteria in survival in the hostile host environment and breakdown of tissue barriers. One of the most common and potent strategies for avoidance of phagocytosis is the presence of a capsule. Despite the remarkable diversity of bacteria, capsule assembly and structure are remarkably similar across species. Early studies with *S. equi* subsp. *equi* demonstrated that resistance to phagocytosis was associated with an increase in capsule and M-protein,[66] and in a model of *S. equi* subsp. *zooepidemicus* infection in mice, enhancement of virulence was associated with increased amount of capsule, which increased resistance to phagocytosis.[67] Although colonization of the guttural pouch occurs with nonencapsulated *S. equi* subsp. *equi* strains, induction of lymphadenopathy is associated with capsular strains.[68] In more recent studies of *S. equi* subsp. *equi* infection, rapid colonization in the lingual and pharyngeal tonsil is dependent on genetically associated virulence factors that control colony morphology, with the mucoid strain having enhanced virulence.[69]

Capsules of anaerobic bacteria are unique, and these structures may directly account for the formation of abscesses within the host. The capsule of *B. fragilis* has two distinct polysaccharides composed of repeating subunits with oppositely charged groups (Zwitter ion).[70] This polysaccharide complex injected alone promotes the induction of abscess. Infection of rodents with the encapsulated form of *Bacteroides* and *Fusobacterium* spp. results in the formation of intraperitoneal abscesses, whereas nonencapsulated bacteria do not cause abscessation.[71-73] Synergism of capsular anaerobes with other bacteria occurs; nonencapsulated bacteria have enhanced survival in abscesses and produce capsule when cultured or inoculated with encapsulated bacteria.[72]

Similar to and overlapping with capsule are structural proteins that block complement. The O side chain of lipopolysaccharide (LPS) on gram-negative bacteria is an anticomplement factor.[74] The longer the side chain, the greater the distance between phagocytes and bacteria. The capsular component, sialic acid, interacts with O antigen to prevent the formation of C3 convertase.[75] Bacterial enzymes are formed by *Streptococcus* spp. and other organisms that damage the polymorph chemoattractant, C5a.[76,77] Production of a protein in *Salmonella* spp., encoded by the *rck* gene, prevents insertion of the C9 fragment of complement into the bacterial membrane.[78] The M-protein of *S. equi* subsp. *equi* appears to decrease deposition of complement on the surface of bacteria.[79]

Recent studies of streptococci have shown that when M-protein content is kept constant, the amount of capsule is correlated with resistance to phagocytosis.[68] Resistance to in vitro phagocytosis can be abolished with treatment with hyaluronidase and induction of specific immunity against M-protein of *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus.*[80] The M-proteins of *Streptococcus* spp. are also essential for resistance to phagocytosis blocking complement.[66,81-83] This mechanism for complement resistance appears to be through enhancement of binding of fibrinogen to the bacteria in the presence of M-protein.[79,84,85]

Apoptosis is a distinctive morphologic process that results in cleavage of nuclear material and scavenging of unwarranted cells without immune activation. Apoptosis or programmed cell death is an important pathway for complex organisms to deal with damaged and diseased tissue. Apoptosis avoids the release of the tissue-damaging enzymes and nonspecific elimination of tissue that occurs in cellular necrosis. Several bacteria have modulated the host apoptotic pathways for enhancement of survival.[86] *Shigella flexneri, S. typhimurium,* and toxins of *S. aureus, Pseudomonas* spp., and *C. diphtheriae* have demonstrated programmed cell death as a consequence of cellular infection or exposure.[87,88] The protein of *S. flexneri,* IpaB, induces apoptosis by binding to and activating the cellular enzyme caspase 1, which induces apoptosis of macrophages.[89] *Staphylococcus aureus* α-toxin, which is similar to listeriolysin O, presumably escapes the macrophage after engulfment and induces host cell apoptosis.[90] The TSST toxin of *S. aureus* induces B-cell apoptosis and blocks immunoglobulin production.[91]

Pathogenesis of Fungal Infections

Maureen T. Long

Of the 250,000 species of fungi, fewer than 200 are true pathogens.[1] Superficial mycoses affect the hair shaft and the superficial epidermis. Cutaneous mycoses (dermatophytosis) infect the epidermis, dermis, hair, and nails of animals, and *Microsporum, Trichophyton,* and *Epidermophyton* spp. are the most commonly associated pathogenic genera. Subcutaneous tissues can become infected with *Sporothrix, Conidiobolus, Basidiobolus* spp., and members of the Dematiaceae fungi, including the *Chromoblastomycosis, Mycetoma,* and *Phaeohyphomycosis* spp. infections. Most of these infections are introduced by penetration through skin or opportunistic invasion of damaged skin surfaces. *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis,* and *Paracoccidioides brasiliensis* are the four most important fungal pathogens that can cause systemic infection. The most common opportunistic infections include *Candida albicans, Aspergillus* spp., *Cryptococcus neoformans, Mucor* spp., and *Pneumocystis carinii.*

Fungal virulence factors may be more complex than those of bacteria because of the higher degree of opportunism that occurs with a change in host status. There may be subtle factors that, combined with host status, result in a certain fungus attaining a virulent state. For instance, the typical fungal wall is composed of three major polysaccharides: mannose; β-1,3 and β-1,6 linked glucans; and chitin. Chitin mutants in *C. albicans* are less virulent when tested in rodent models than are wild-type fungi.[92] Further, a mutant *C. albicans* that cannot synthesize complex mannose oligosaccharides does not adhere to other yeast and epithelial cells and has lost virulence

in a guinea pig model.[93] Both of these mutants can proliferate in vitro normally, and whether or not this is an actual virulence factor is unclear.

As with bacteria, cellular adherence is an important prerequisite for infection and colonization of the host. Adhesins have been identified in *C. albicans* and *B. dermatitidis.* Two genes have been associated with adhesion in *C. albicans.* The first is a glycoprotein that has sequences consistent with agglutinating activity. Transformation of this gene into other nonadherent fungal species results in adhesion of the transformed yeast to cells.[94] *Candida albicans* also has integrin-like proteins, the disruption of which results in diminished hyphal growth, adhesion to cells, and loss of virulence in mice.[95,96] The *B. dermatitidis* adhesin mediates binding to human monocyte-macrophages through the CD14 receptor.[97]

Many fungi have polysaccharide capsules that, like bacteria, help resist phagocytosis and immune activation. The capsule of *C. neoformans* inhibits leukocyte accumulation, cytokine secretion, and macrophage phagocytosis.[98] Mutants without capsule are highly infective and avirulent. As indicated earlier, many fungi are engulfed by macrophages, and intracellular survival is mediated by virulence factors. Macrophages are decimating cells for *C. albicans,*[99] *H. capsulatum,*[100] and *B. dermatitidis. Histoplasma capsulatum* is primarily a yeast in vivo, and this form infects macrophages. Phagolysosomal fusion occurs at a normal rate,[101] but blockage of acidification of the phagolysosome occurs.[102]

Exposure to both pathogenic and saprophytic fungi is an everyday occurrence. Respiratory contamination and infection are important for many pulmonary species, but skin penetration and dissemination from necrotic gut are important portals for large animals also. Dissemination after the initial infection is dependent on previous damage to host tissues, deeper mechanical penetration, or actual invasion of new tissues. *C. albicans* can actually grow through and replace cell membranes.[99] True molds invade blood vessels and grow along the intima of the vessels. Fungi secrete many degradative enzymes, including proteinases, phosphatases, and DNAses, to surmount structural barriers.[103] A group of genes called *secreted aspartyl proteinase (SAP)* genes allow more persistent colonization of host surfaces and deeper penetration.[104]

When *C. immitis* invades the host, the fungi form endospores. These endospores secrete a proteinase and a urease that likely aid in the breakdown of pulmonary tissues.[105-107] The two proteinases of *A. fumigatus* break down elastin, a major component of lung tissues.[108,109] Phospholipase activity has been demonstrated in *C. albicans, C. neoformans,* and *A. fumigatus.*[110] Strains of *Candida* spp. with high amounts of this enzyme have higher virulence,[111] and abolishing this activity results in decreased adherence of the organism.[112] Host eicosanoids enhance fungal colonization. Recent evidence demonstrates production of eicosanoids by both dermatophytosis and systemic fungi.[113]

Fungi induce apoptosis, which may be due either to the direct effect of a fungal toxin or secondary to host cell cytoskeleton rearrangements.[114] The gliotoxin of *A. fumigatus* can induce DNA fragmentation and apoptosis in macrophages.[115] This toxin also has many other immunosuppressive qualities, which include inhibition of the neutrophil respiratory burst and T-cell activation.

Pathogenesis of Viral Infections

Viral infections such as equine influenza (Orthomyxoviridae), rhinopneumonitis and abortion (Herpesviridae), African horse sickness (Reoviridae), equine infectious anemia (Retroviridae), and various encephalitides (Alphaviridae and Flaviviridae) are responsible for some of the most medically and economically important diseases of horses. Specific therapy of viral infections remains a significant challenge because antiviral drugs are generally ineffective, impractical, or prohibitively expensive for the treatment of horses. Treatment of most viral infections focuses on supportive care of the affected organ systems and control of secondary complications such as bacterial infection. Currently control of most clinically significant viral diseases in horse populations relies on vaccination, quarantine, or even destruction of infected animals.

Despite the great significance of some viral infections, recognizing that many equine viruses are ubiquitous, weakly pathogenic, or not associated with any known disease under normal circumstances is also important. Some examples include equine adenovirus, respiratory and enteric reoviruses (the term *reo* is derived from the acronym for respiratory enteric orphan, indicating that these isolates have not been associated with disease), and equine herpesvirus (EHV) type 2. Some host-virus relationships may be mutualistic in that virally derived genetic elements are theorized to benefit the host by facilitating genetic variability and evolution.[116] Thus many viruses are of no practical clinical significance, and no control efforts are warranted. For this reason, the veterinarian should never assume that the recovery of a virus from a clinical specimen is significant without proof that the virus can cause the disease in question.

An in-depth discussion of viral structure, taxonomy, and replication is beyond the scope of this chapter, and the reader is referred to textbooks of veterinary or human virology for more detailed information.[117,118] A brief overview is presented to emphasize those features that have clinical relevance.

The fundamental structure of all viruses is a DNA or RNA genome enclosed by a coat of protein called the *capsid* (Fig. 1.1). For viruses that are enveloped, the capsid is enclosed further by a host cell–derived lipid membrane into which viral proteins have been incorporated. In addition to protecting the viral genome, the capsid and other associated structural proteins (e.g., matrix proteins) are important for virus assembly, packaging the viral genome, releasing the genome into a target cell, and for nonenveloped viruses providing receptors that bind to host cells. For enveloped viruses the receptors are incorporated into the lipid membrane. The primary clinical significance of these features is that enveloped viruses, because of their fragile lipid membrane, are highly susceptible to inactivation by heat, desiccation, or detergents, and transmission typically requires direct exchange of body fluids, short distance aerosols, or arthropod vectors. In contrast, nonenveloped viruses (e.g., equine rotavirus) are resistant to physical inactivation, and environmental contamination is more likely to be a significant factor in their transmission.

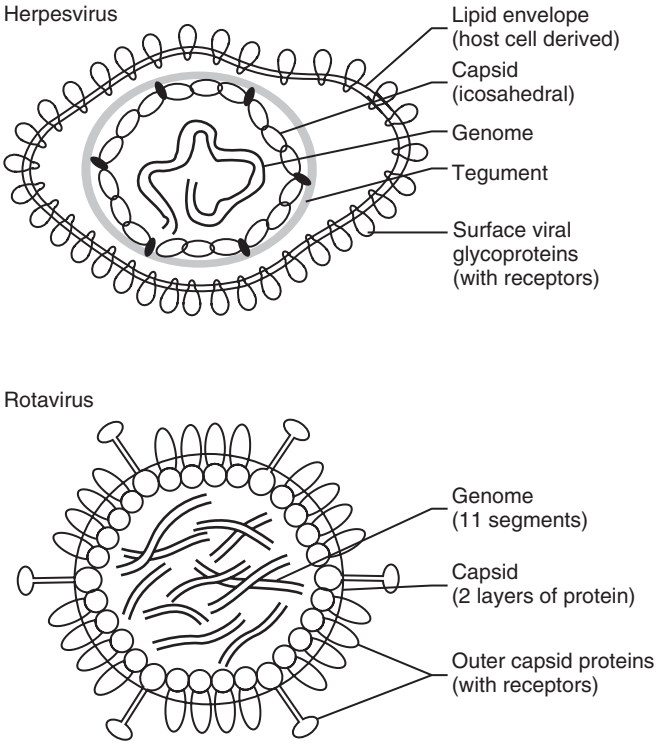


FIG. **1.1** Schematic representations of basic viral structure. The basic structure of an enveloped virus is shown by the drawing of a herpesvirus. The basic structure of a nonenveloped virus is shown by the drawing of a rotavirus.

The composition of the viral genome is an important basis for virus classification (Fig. 1.2). The type of viral genome also determines the strategies required to replicate the genome and transcribe messenger ribonucleic acid (mRNA; Fig. 1.3). Viral genomes may be single-stranded RNA, double-stranded RNA, single-stranded deoxyribonucleic acid (DNA), or double-stranded DNA. The genomes of single-stranded RNA viruses may have positive polarity, in which the genome also serves directly as mRNA for translation of viral proteins. To replicate genomes, these viruses must first synthesize a strand of complementary RNA that can be used as a template to replicate genomes and transcribe new mRNA. Retroviruses are a subset of single-stranded, positive-polarity RNA viruses that use their RNA genomes as templates to produce double-stranded DNA, which in turn is used for the transcription of mRNA and new viral genomes. Single-stranded RNA viruses may also have negative polarity, in which the genome is antisense to mRNA, and synthesis of a complementary RNA strand is required to serve as mRNA and as a template for new genomes. The clinical significance of these types of replication strategies is that they require polymerases and other enzymes not normally found in eukaryotic host cells. These unique viral enzymes are important targets for antiviral drugs because they can be used selectively to inhibit viral replication.

Viral RNA polymerases are low fidelity and lack proofreading functions and thus randomly introduce errors into new RNA at an average rate of about one nucleotide mismatch per 10,000 bases copied.[119] In a population of viruses, therefore, virtually every individual virus differs slightly, and this population is referred to as a *quasi-species.* Although many of these mutations are neutral or even deleterious, in the face of selective pressures such as the host immune response or antiviral drugs, this genetic plasticity allows rapid development of resistant virus populations.[120] Secondary structures or certain sequences in the viral genome may facilitate polymerase errors further at selected regions that are important for immune evasion, such as in sequences that code for neutralizing epitopes.[119] The genome of some viruses, such as influenza, comprise separate segments, which allows reassortment of entire gene segments and sudden and dramatic changes in antigenicity.[121]

All viruses are obligate intracellular parasites. Viral replication can occur only within living cells, and all viruses to some extent depend on the host cell synthetic machinery. The life cycle of all viruses includes the following steps: attachment to the target cell, entry into the cell, uncoating and release of the viral genome, transcription and translation of viral proteins, replication of the viral genome, assembly of new virions, and release of progeny virions[117,118] (Fig. 1.4). Although the biochemistry of these steps is beyond the scope of this discussion, recognizing that they are all specific, energy-requiring interactions between the virus and the host cell is important. The inability of the virus to interact appropriately with a cell at any of these steps prevents replication in that cell type and defines the tropism of the virus. All of these steps are also important potential targets for antiviral drugs and host immune responses.

Once the viral nucleocapsid gains entry to the cytosol of the cell, the viral genome is released through the process of uncoating. After uncoating, the viral genome localizes to the appropriate regions of the cell for replication and mRNA transcription. DNA viruses typically replicate genomes and transcribe mRNA in the nucleus and then transport the mRNA to the cytoplasm for translation. RNA viruses typically replicate, transcribe mRNA, and translate viral proteins in the cytoplasm. These sites of replication account, respectively, for the location of viral inclusion bodies that are diagnostically useful in histopathologic sections.

Cells that replicate virus often are killed as a direct consequence of infection. One mechanism by which viruses kill cells is lysis, often associated with the release of progeny virions. Insertion of viral proteins into cell membranes, budding, direct toxicity of viral proteins, and diversion of normal host cell homeostatic processes to viral replication may result in death of the cell.[117,118,122] Viruses also may activate the cellular self-destruct mechanism of programmed cell death (apoptosis). Although cells may induce apoptosis in an attempt to prevent completion of the virus life cycle, viruses also may use this mechanism to kill the cell and facilitate release of virions.[123]

Viral infection can cause neoplastic transformation of infected cells. The most common examples in horses include warts (equine papillomavirus) and sarcoids (bovine papillomavirus). Virally induced invasive neoplastic diseases such as leukemia or lymphosarcoma have not been recognized in the horse. Viral proteins that activate the cell cycle into the growth and division phases may lead to neoplastic transformation if expressed in a cell that is not killed by the infection. Papillomavirus infections induce epithelial neoplasms (fibropapillomata) using a virally encoded protein (E5 oncoprotein) that induces proliferation of normally quiescent cells and that presumably is needed for viral replication.[124] Oncogenic retroviruses, including leukemia and sarcoma viruses, induce neoplastic transformation by integration into the host cell genome and activation of cellular oncogenes.[125]

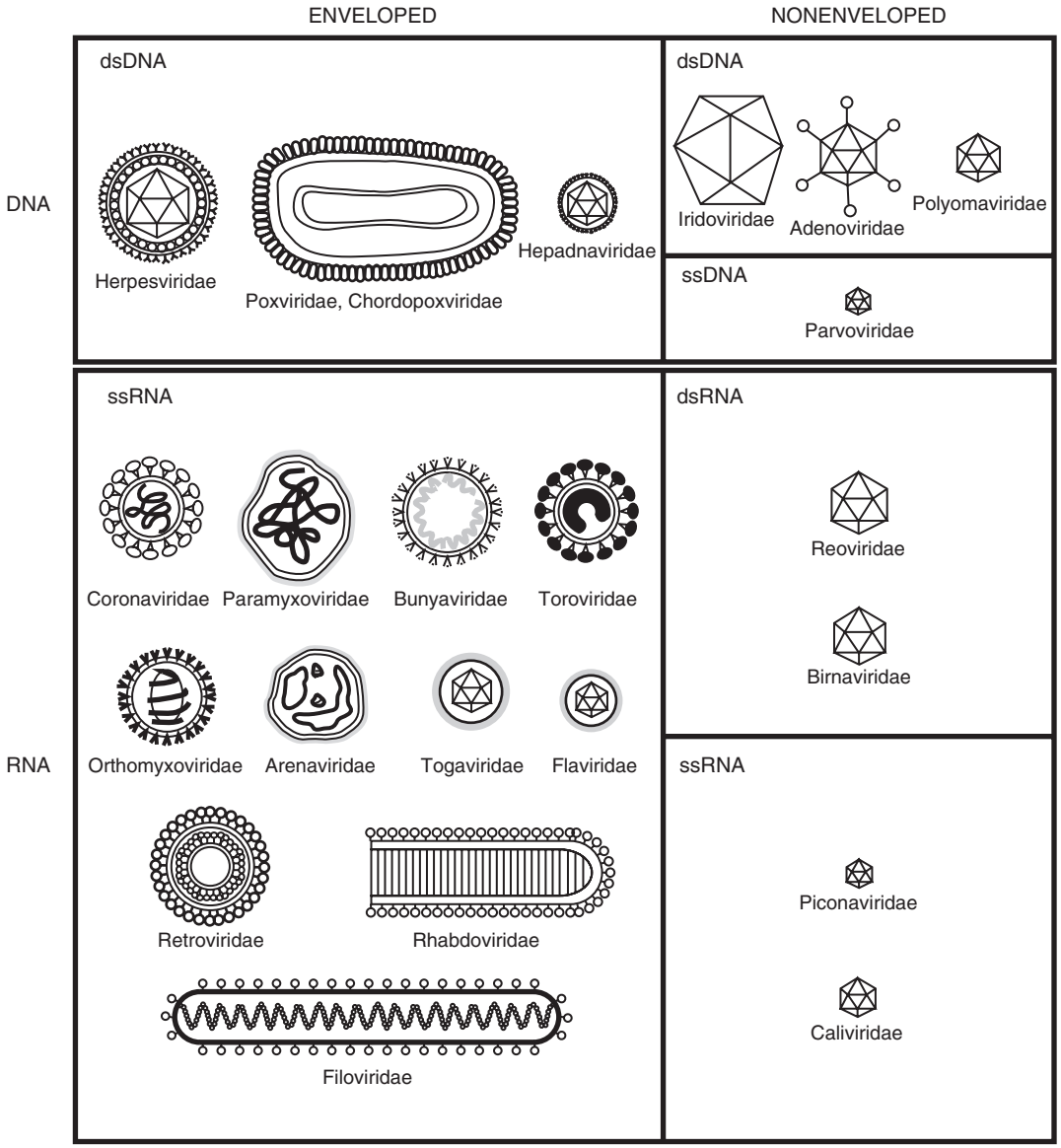


FIG. **1.2** Virus family classification based on genome composition and presence of envelope or absence of envelope. The relative size of the viruses to each other is also shown.

At the host level viruses may use several mechanisms to establish persistent infections and avoid immune clearance. *Virologic latency* is defined as the presence of a viral genome that is not producing infectious virus.[126] The genomes of latent viruses are transcriptionally suppressed and translationally silent so that no viral proteins are expressed that may identify the cell to the immune system as infected. The definition of *latency* also stipulates that on reactivation, viral gene expression and the production of infectious progeny virions can be resumed, differentiating latently infected cells from cells infected with defective viruses. In contrast, some persistent viral infections are characterized by continual replication despite the presence of antiviral immune responses. Even in the absence of recognizable clinical signs, such infections are not truly latent. The classic latent infection is that of the herpesviruses. For the α-herpesviruses, such as EHV1 and EHV4, latent infections are established in the nuclei of sensory neurons and can be maintained indefinitely, and infected animals serve as the reservoir of the virus.[127-129] On reactivation viral nucleic acids are translocated across synapses to epithelial cells of the nasopharynx, which produce infectious virus. The stimuli that induce reactivation are poorly defined, but reactivation can be induced by immunosuppression (e.g., corticosteroids) and presumably by other stressors, such as pregnancy, transport, and social stress.[128,130]

The severity of disease in a virus-infected horse, or whether infection even results in clinical disease at all, is the result of a complex interaction among the triad of virus, host, and environment. Critical factors include viral virulence, viral spread within the animal, the intensity of direct and immune-mediated pathologic response elicited by the virus, and the ability of the virus to avoid clearance by the host. Other than the virulence of the virus, which is strictly a property of the virus, the other virus-host interactions can be influenced by the age and genetics of the host and by environmental factors such as stress and nutrition. These factors account for

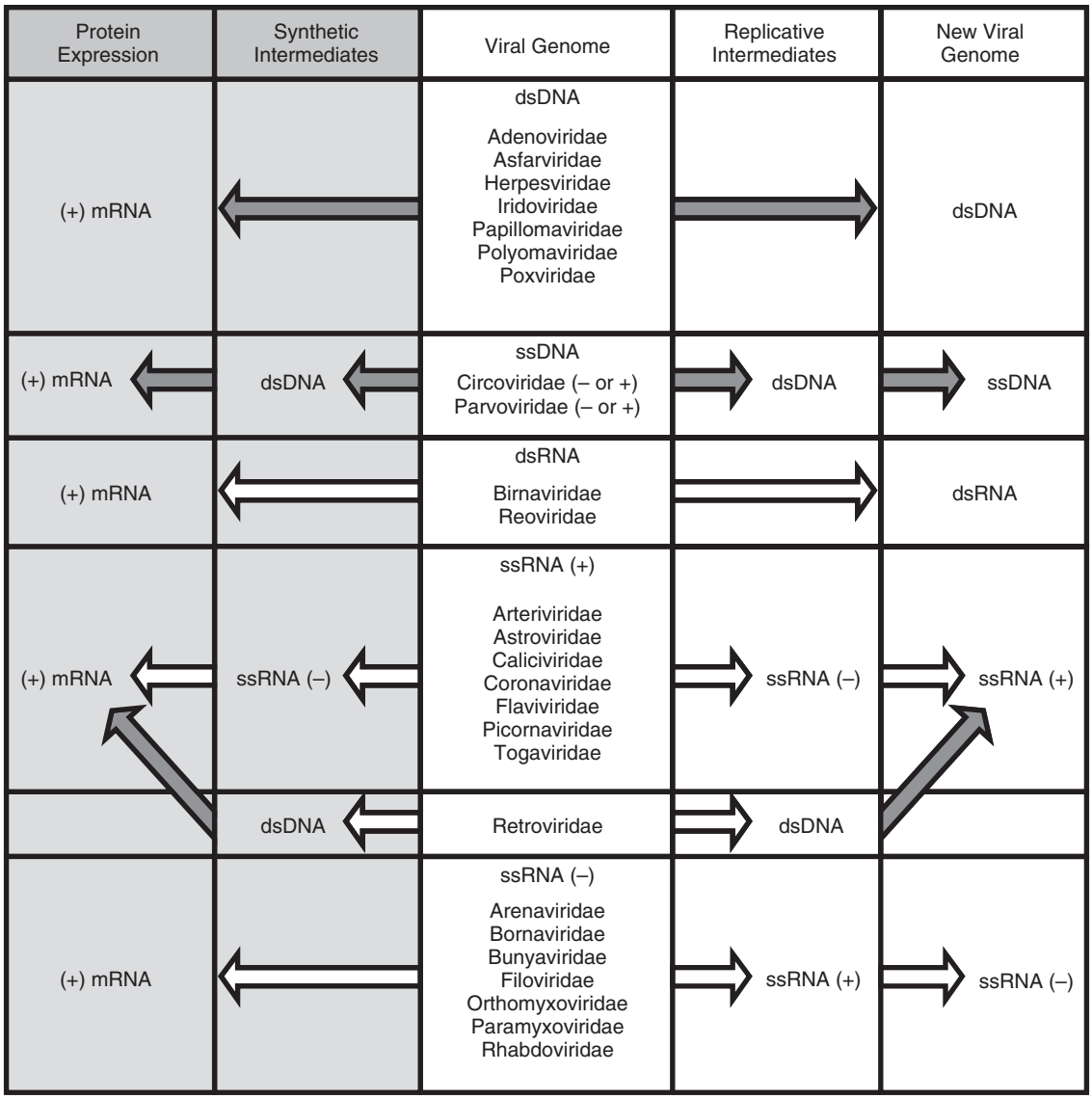


FIG. **1.3** Summary of the main virus families that infect vertebrates and general strategies employed by these viruses to produce mRNA for protein expression and to replicate genomes. Required intermediate molecules are indicated. *White arrows* indicate the need for unique viral polymerases, including RNA-dependent RNA polymerase and RNA-dependent DNA polymerase (reverse transcriptase). *Dark arrows* indicate the use of cellular polymerases or viral homologs of cellular polymerases. *ds,* Double-stranded; *ss,* single-stranded; (+) for RNA = positive polarity, polarity of RNA used for protein translation; (+) for DNA = coding strand, sequence same as for (+) RNA; (– or +) DNA = contains single strands of DNA of both polarities. (Modified from Baltimore D: Expression of animal virus genomes, *Bacteriol Rev*. 35:235-241, 1971.)

the observation that considerable variation in disease signs can occur among a group of animals infected with the same viral strain.

Certain strains of a virus may cause more severe disease than other strains. The main properties of a virus that may affect virulence include host cell tropism and replication rate. A tropism change that leads to involvement of additional tissues or facilitates virus spread generally results in more severe disease. Outbreaks of EHV1 abortion or neurologic disease strongly suggest that EHV1 strains exist that have a tropism for these tissues compared with EHV1 strains that cause respiratory disease. An increase in the viral replication rate is usually associated with an increase in virulence, presumably because of the greater number of infected cells and amount of tissue damage. The virulence of equine infectious anemia virus strains can be correlated directly to plasma virus titers and numbers of infected cells, without any changes in tropism.[131,132] The molecular basis for the increased replication rate is not clear but most likely is caused by variation in viral regulatory sequences and proteins.[133,134]

Viral infections generally are regarded as localized or systemic. Localized viral infections are those that are restricted to a single organ system, often at the site of entry. Because infection of the tissue is direct, the incubation period for localized viral infections is usually short, often only a few

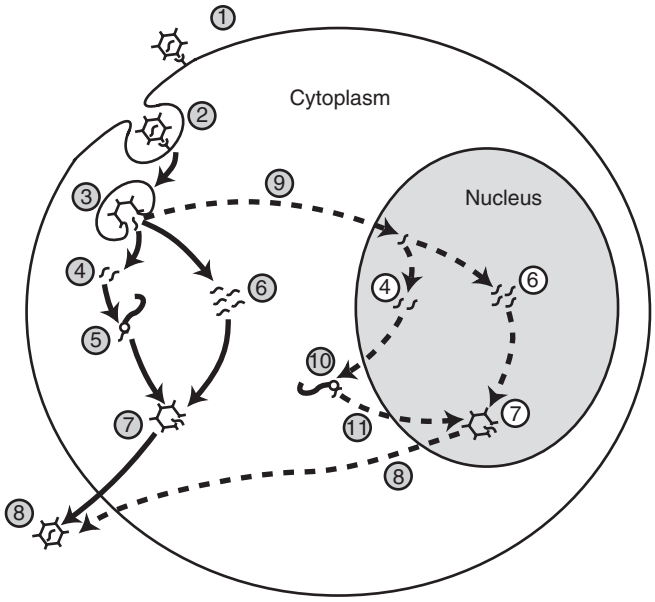


FIG. **1.4** Schematic representation of the general virus life cycle. Both RNA and DNA viruses share the initial steps, including attachment (1); entry/fusion (2); and uncoating and release of viral genome into the cell (3). *Solid arrows* indicate remaining steps for RNA viruses, which all occur in the cytoplasm, including transcription of mRNA (4); translation of viral proteins (5); replication of the viral genome (6); assembly of new virions (7); and release of progeny virions (8). *Dashed arrows* indicate steps for a DNA virus. The life cycle is similar except that the DNA genome is translocated to the nucleus (9) for transcription (4) and replication of viral genomes (6). Viral mRNA is then translocated back to the cytoplasm for translation (10), and newly synthesized viral proteins are translocated back to the nucleus (11) for assembly (7); new virions are then released from both nuclear and cytoplasmic membranes (8).

days. Many infections of the skin or mucosal surfaces are localized, and examples in the horse include infections with enteric rotavirus and influenza. For influenza, virus is inhaled into the nasopharynx and replicates in epithelial cells of the upper respiratory tract and trachea. Virus is not present in the blood or tissues outside of the respiratory tract. In general, viruses remain localized because they lack the receptors to infect cells of other tissues or circulating cells, such as monocytes or lymphocytes, that can disseminate the virus. Some viruses are temperature sensitive and remain localized because they are unable to replicate efficiently at core body temperatures. EHV3, the cause of coital exanthema, is restricted to the surface of the genitalia in horses because of its temperature sensitivity.[135] EHV1 and EHV4 are not temperature sensitive, however, and systemic infection may occur with these viruses. Temperature sensitivity is also a means by which some viruses, such as equine influenza and infectious bovine rhinotracheitis virus, may be attenuated for use as modified-live intranasal vaccines. Infection by the vaccine strain is limited to the cooler mucosal surfaces; the inability to spread systemically prevents sequelae such as abortion and pneumonia.[136,137]

Systemic infections are those in which virus is disseminated to multiple tissues by blood or lymph. This viremia may exist in the form of cell-free virions in the plasma or lymph or may be cell associated in circulating blood cells, usually monocytes or lymphocytes. The classic paradigm for a systemic infection is infection of mice with ectromelia virus[138] (Fig. 1.5). Localized viral replication first occurs at the site of entry and in regional lymph nodes. Depending on the level of replication, clinical disease may be present. The virus then enters the blood or lymphatics and spreads to other tissues, such as spleen and liver, in which clinical disease may occur. Virus is amplified and then released again for a second, usually higher-titered, viremia that further disseminates the virus to other organs. Each viremic episode is associated with a febrile response and is the basis for the biphasic fever response associated with some viral infections. Because systemic infections require multiple steps, the incubation periods are longer than for localized infections, typically 1 to several weeks. Infections in the horse by eastern, western, or Venezuelan equine encephalitis virus closely follow this paradigm. Localized viral replication occurs at the site of entry (mosquito bite) followed by viremia and dissemination to the central nervous system.[139] For most horses, even nonvaccinated horses, dissemination is controlled before infection of the brain, and neurologic disease is a rare outcome of infection. A variation on the theme is infection of horses with EHV1. The most common clinical disease associated with EHV1 infection is rhinopneumonitis caused by a localized infection of the nasopharyngeal mucosa.[140] In almost all cases, a cell-associated viremia also occurs in lymphocytes, but in most infected horses this does not result in disease. However, in some cases, viremia is associated with infection of endothelial cells, and in the pregnant mare vascular damage to the uterus and placenta may lead to abortion.[140,141] Similarly, infection of the vascular endothelium in the central nervous system results in neurologic disease.[142]

Some viruses also may spread in the host through nerves. In the horse rabies is the best-known infection that relies on neural spread. Following local replication in myocytes at the site of entry, usually a bite wound, rabies virus ascends peripheral nerves into the central nervous system, where it replicates in neurons and then egresses by way of cranial nerves to the salivary gland.[143] EHV1 and EHV4 establish latency in the nuclei of sensory neurons that innervate the nasopharynx and reach the nucleus by ascending nerve axons. Similarly, on reactivation these viruses egress back down the axon to infect epithelial cells.[127,144]

Once a virus reaches a target organ, virally mediated cell death is the fundamental source of pathologic response, disease, and clinical signs observed by the veterinarian. Despite the great complexity of virus-host interactions and the many factors that influence the expression of clinical disease, in actuality viruses have a limited number of ways by which to cause pathology. Cells and tissues may be destroyed directly by cytolytic viral infections or by infections that affect the differentiated function of target cells (e.g., neoplasms and immunodeficiencies). Viral infections of organ systems with bacterial flora (e.g., intestinal and respiratory tracts) can disrupt the normal barrier functions of these organs and result in secondary bacterial infections and toxemia that may contribute significantly to the pathologic response. Cell death and pathologic response also may be caused by host immune responses specifically directed against virally infected cells or by indiscriminate inflammatory responses. Virally induced autoimmune diseases have not been described in horses but are another potential source of pathologic response that may be identified in the future.

For most of the clinically important viral diseases of horses, disease manifestation results from some combination of

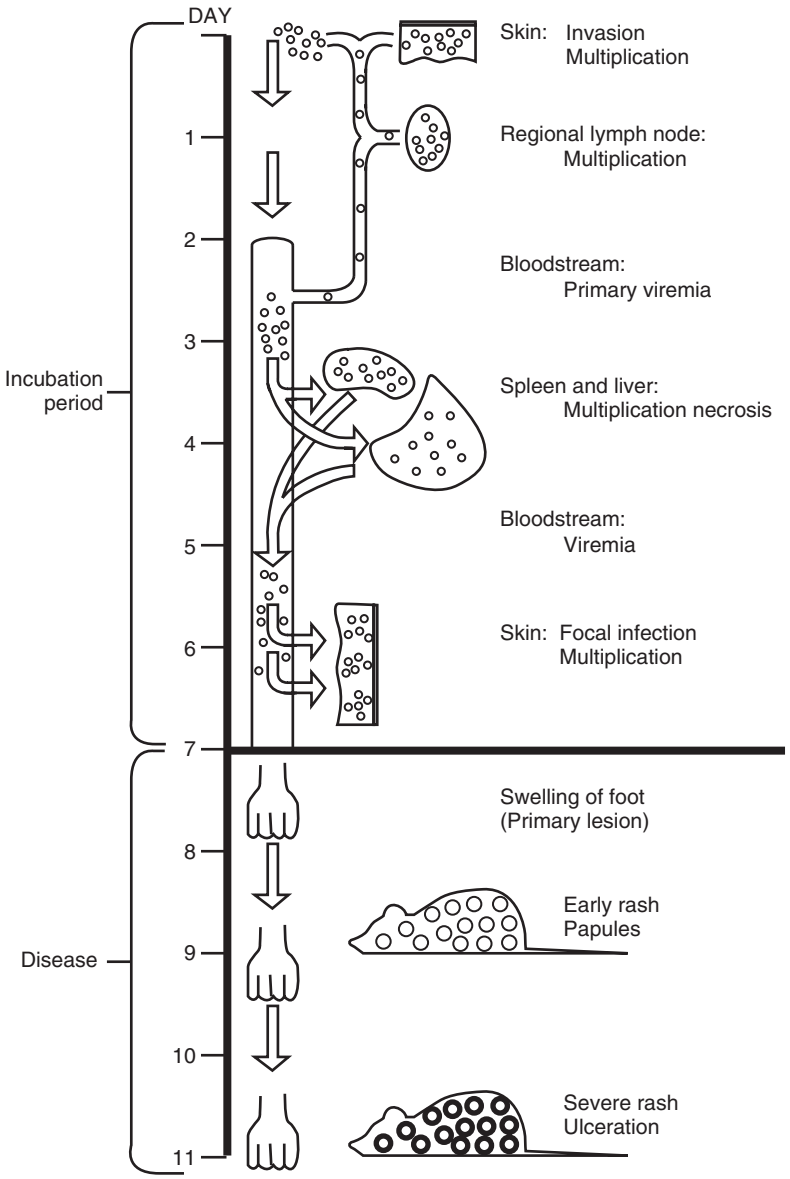


FIG. **1.5** Schematic representation of the pathogenesis of mousepox (ectromelia), illustrating the classic paradigm for the events in a systemic infection. (From Fenner F: The pathogenesis of the acute exanthems, *Lancet*. 252:915, 1948.)

cytolytic infection and immune-mediated tissue destruction. The relative contribution of these mechanisms is primarily a function of viral virulence and host factors that influence the type and intensity of immune responses. The predominant mechanism of pathologic response also can vary with different stages of the same disease, as seen in acute versus chronic equine infectious anemia. In acute disease most of the disease manifestation is caused by direct viral damage and cytokines, whereas in chronic disease immune complex–mediated anemia and glomerulonephritis become more significant.

The disease associated with a given viral infection is related to the affected organ system(s), the number of cells destroyed, and the sensitivity of the affected organ system to dysfunction. If the number of infected cells is not sufficient to lead to clinically significant organ dysfunction, the result is a subclinical infection. When enough cells are infected to lead to overt organ dysfunction, clinical disease becomes apparent. EHV1 infections of the respiratory epithelium, which has a large number of cells with a high turnover rate, produce mild clinical disease even with a high rate of infection. On the other hand, much more significant clinical manifestations of EHV1 infection such as abortion and neurologic disease are caused by infection of a few endothelial cells because minimal vascular damage can lead to thrombosis, ischemic necrosis, and damage to large amounts of tissue. If viral infection results in neoplastic transformation of a cell type, disease may progress according to the characteristics of the neoplasm, whether or not the virus remains associated with the tumor.

In most viral infections immune-mediated pathologic response contributes significantly to disease and in some cases may be the predominant cause of disease manifestation. Equine immune and inflammatory responses are covered elsewhere in this chapter.

Although not described in horses, viral infections in other species can induce immune-mediated responses to host cell antigens and autoimmune diseases. The best documented human autoimmune disease suspected to be initiated by viral infections is Guillain-Barré syndrome, in which infection with cytomegalovirus or Epstein-Barr herpesvirus elicits antinerve ganglioside immune responses and demyelinating disease.[145] Postinfluenza myocarditis is an occasional sequela in horses and human beings and is a potential autoimmune disease. Although no direct evidence supports this theory, influenza virus is not identified consistently in affected heart muscle, and the pathogenesis is not known.[146]

A key requirement for viruses to be maintained in nature is to persist successfully in a reservoir host (if the reservoir is an infected animal) and to be transmitted to another susceptible host. One of the most important obstacles to persistence and transmission is detection and elimination by the host immune system. Within the host rapidly replicating viruses such as influenza may shed and transmit virus before the host can mount specific antiviral immune responses. Herpesviruses avoid detection during latency by not expressing any viral proteins. Immunodeficiency viruses may cripple antiviral immune responses by directly infecting immunoregulatory $CD4^+$ T lymphocytes. One of the most important mechanisms of immunologic avoidance is antigenic variation in which neutralizing viral antigens are altered so that they are no longer recognized or accessible by host immune responses. Antigenic variation is generated by nucleotide errors during transcription or replication, which result in amino acid substitutions in the relevant epitopes. Other mechanisms by which some viruses may modify their antigenicity is through intramolecular recombination/duplication or reassortment of segmented genomes (e.g., influenza and African horse sickness).[120,121] For reassortment, coinfection of a single cell with genetically different virions may result in a progeny virion with segments derived from both virions and a major change in antigenicity. In influenza these are called *antigenic shifts,* and the radical change in the antigenicity of the virus may render preexisting immunity in the host population ineffective at preventing outbreaks of disease with high morbidity and mortality rates.[121]

Genetic differences in susceptibility to disease have been well documented. In an outbred population of animals, the considerable variation in the type or severity of clinical disease is well recognized, even when animals are infected with the same virus strain and have no recognizable differences in other factors such as age, challenge dose, nutrition, and general health status. Conversely, highly inbred populations may be more uniformly susceptible to a viral disease.[147] Thus inbreeding can pose problems for endangered species, such as Przewalski's horse, or other populations with limited genetic variability, which may incur high rates of morbidity or mortality if the animals are infected with a virulent virus. Host genetics may affect the tropism of the virus and influence the type and intensity of immune responses to a viral infection.

Pathogenesis of Parasitic Infections

Horses serve as hosts for numerous parasites, which induce a wide range of pathologic and immunologic responses.[148,149] Many of the latter are of a hypersensitive nature and also result in disease conditions. Immune responses leading to protective resistance against reinfection occur, but the level of this resistance is most often incomplete. Mechanisms associated with these responses have not been investigated extensively in the horse, but information is available from other host-parasite systems that may be relevant to equids. The purpose of this section is to acquaint the reader with contemporary thoughts on host-parasite interactions. Because of their prevalence, major importance to equids, and information available, coverage is limited to helminth parasites that occur in most developed and nontropical countries.

Infection with most metazoan parasites results in inflammation and structural and functional changes of the organs invaded. The outcome of these changes is an alteration of the host's physiologic state. The degree of alteration depends on the existing physiologic condition of the animal, which is dictated to a great degree by its age, nutritional status, and previous immunologic experience with the parasite. The numbers of parasites introduced and the specific parasite also affect the degree of physiologic change that occurs. When these factors favor major alterations, the results are readily identifiable clinical signs of infection. Subclinical infections, although less apparent, are potentially important to the general health of the animal and continued transmission of the agent. The pathophysiologic effects of infection by ectoparasites, helminths, and microorganisms are in many cases similar.[150,151] Abnormalities in weight gain, skeletal growth, reproduction, and lactation may result from infections with any of these agents. These changes are often directly related to parasite-induced anorexia, disruption of metabolic processes, and anemia. An understanding of the morphologic and biochemical lesions produced by specific parasites clarifies the role of these agents in clinical and subclinical conditions associated with the infections. Most detailed studies of the pathophysiology of parasitic infections have been conducted in laboratory animal models and domestic animal species other than the horse.[151] However, the classical pathology of parasitic infections of the horse has been reviewed.[149,152] The following discussion outlines some recent observations on host-parasite interactions that may be of significance to equine medicine. Examples of host-parasite interactions responsible for alterations in host homeostasis are presented as they relate to the gastrointestinal tract, lungs, and skin.

GASTROINTESTINAL TRACT

Internal parasites are most important to equine health as mediators of gastrointestinal problems, including colic and diarrhea. Although almost all internal parasites have been inferentially implicated as causative agents of colic at some time, significant evidence-based experimental or field observations have emerged to support this contention for some parasites. The helminth parasites include large strongyles, principally *Strongylus vulgaris, Parascaris* spp., *Anoplocephala perfoliata,* and as a group the cyathostomins.

The pathogenesis of colic associated with migration of *Strongylus vulgaris* through the mesenteric arteries and the resultant thrombosis, infarctions, and necrosis of the intestine have been described in detail elsewhere.[149,152,153] Large strongyles are easily controlled with currently available macrocyclic lactone anthelmintics and are rare in horses kept on well-managed farms in developed countries. Histologic studies of experimentally infected parasite-free pony foals during the initial stages of infection indicate that the severity of

lesions produced in the intestine cannot be attributed solely to mechanical disruption caused by larval migrations and that these larval stages induce some biologic amplification system within the mucosa, which results in the degree of inflammation observed.[154] Although the mechanisms involved in this response have not been investigated, the histologic nature of the lesion is characteristic of an Arthus reaction, suggesting an involvement of the immune response. Other experimental studies using the parasite-free pony–*S. vulgaris* system have implicated a role for the immune response in the mediation and regulation of the arterial lesions produced by this parasite. Passive transfer of immune serum but not normal serum reduced the severity of arteritis seen and clinical signs associated with experimental infections without reducing the numbers of parasites that develop in these ponies.[155] However, treatment with immune serum also induced an anamnestic eosinophilia and marked perivascular infiltration of eosinophils in the cecum. The reduction in intravascular lesions may have been associated with an inactivation of parasite-secreted inflammatory factors either by antibody or serum enzymes or circulating cytokines. This serum may also have contained nonspecific host-derived antiinflammatory substances. The exacerbation of the eosinophil response may have been associated with the formation of immune complexes. Although the mechanisms are unknown, the results suggest that the immune response may simultaneously modulate and potentiate inflammation. It has been postulated that larvicidal treatment of *S. vulgaris*–infected horses and killing of intravascular larvae may release a bolus of antigenic factors from these larvae within the mesenteric vasculature, resulting in an exacerbation of arterial and intestinal lesions and colic. Experimental testing of this hypothesis indicates that this phenomenon does not occur and further that viable larvae are necessary to maintain the arteritis and eosinophilia seen.[156] Experimental studies using parasite-free ponies that were immunized with crude adult worm antigens and subsequently challenged with *S. vulgaris* larvae showed an exacerbation of the pathologic responses seen in the mesenteric vasculature and including an anamnestic eosinophilia, further suggesting a role for the immune response in the development of these lesions.[157]

Colic associated with *Parascaris* spp. infection in foals has been related to intestinal impaction and rupture and is not considered to be of major significance in adult horses.[158] These conditions in foals may become more important with the widespread identification of *Parascaris* spp. resistance to ivermectin.[159] However, ascarid nematodes are particularly potent sources of allergens, and it is not inconceivable that the hypersensitized mature horse may respond to low-level infections with this parasite. Observations made in the author's laboratory are noteworthy in this regard. Two mature *Parascaris*-free adult horses were inoculated intradermally with less than 90 μg of saline-soluble somatic extract of adult *Parascaris* spp. to test for immediate hypersensitivity to this antigen. Both horses experienced an immediate systemic response and colic. One of the horses died within 3 hours of intradermal inoculation. Necropsy results were consistent with the diagnosis of acute severe colitis. Because of the allergic potential of ascarid nematodes and the sensitivity of the equine gut to immediate hypersensitivity reactions, this potential is worthy of further characterization and consideration.

Clinical observations have maintained an interest and concern over the pathogenic potential of *Anoplocephala perfoliata* infections. Earlier case reports have described cecal ruptures and intussusceptions of the cecum and colon associated with these infections.[160,161] Several case-control studies have documented an association between colics in the ileal region and *A. perfoliata* infection.[162] These parasites inhabit the region of the ileocecal junction and produce ulcerated lesions of the mucosa and submucosal inflammation. Detailed experimental investigations of these infections have not been conducted, and thus specific details on the pathogenesis and relevance of these lesions are lacking. An association between severity of observed lesions and tapeworm burden has been observed.[163,164]

Cyathostomins (cyathostomes or small strongyles) have not generally been considered to be of major importance, particularly as causative agents of colic. In this regard, Uhlinger's field studies are of particular importance.[165,166] In these controlled experiments different anthelmintic treatment regimens were used to test their efficacy in reducing the incidence of colic. The more efficacious treatment programs significantly reduced the incidence of colic by 2 to 13 times that seen in the same herds before implementation of the more efficacious treatment. Because of the management programs used before the initiation of this study and the results of fecal cultures, it can be assumed that the primary parasites present in these horses were cyathostomins. These data strongly implicate a role for cyathostomins in a substantial proportion of colics observed under field conditions. The parasite or host factors involved in these colic cases are unknown but may be the dynamic turnover of parasites during the life cycle in the mucosa and the related inflammatory responses seen that they induce.

Cyathostomins have been implicated in numerous case reports with seasonal diarrhea in adult horses, which is a condition called *larval cyathostominiasis.* These cases are characterized by a sudden onset of diarrhea during the late winter or spring. Horses younger than 5 years of age are particularly at risk, but mature horses can be affected as well, and a case-fatality rate of 50% has been reported.[167] These are difficult to diagnose, and the only consistent signs are weight loss, hypoproteinemia, and diarrhea.[166] Large numbers of larval cyathostomins can be found in the feces or in intestinal contents and within the mucosa of these horses. These symptoms are related to the synchronous emergence of fourth-stage larvae of these parasites from the mucosa. These larvae build to potentially large numbers within the mucosa owing to the arrested development of infective larvae. The seasonality of the occurrence of this condition at present does not appear to vary in different climatic regions as does the analogous bovine condition of type II ostertagiasis. Specific parasite or host factors associated with the regulation of the hypobiotic state of the larvae or the inflammatory response initiated at parasite emergence have not been described. Other clinical reports have suggested that cyathostomin-related diarrhea and weight loss are not related to the seasonal presentation described previously.[168] This is supported by experimental studies demonstrating that pathophysiologic effects occur as large numbers of parasites enter the intestine, as well as when they leave.[169]

In view of the paucity of specific mechanistic information on the pathophysiologic effects of equine gastrointestinal parasites, a synopsis of relevant information gathered from other model systems is warranted, particularly for nematodes.[151,170] Parasitic organisms may induce changes in gastrointestinal function directly by mechanical disruption of tissues and cells

or by the release of factors that directly alter cell function. Induction of the immune response serves as an anamnestic amplification system. The result of these changes is an alteration in function of the smooth muscle and epithelium of the bowel.

A number of helminth parasites, including *Parascaris* spp., stimulate intestinal smooth muscle hyperplasia. This response may be induced by intestinal inflammation or stenosis associated with parasitism. Contractility of these muscles can be induced in a Schultz-Dale reaction by stimulation with parasite antigens. This response is mediated in rats by mast-cell–derived 5-hydroxytryptamine (5-HT) and in guinea pigs by histamine. A regulatory relationship of myenteric neurons to these antigen-induced changes has also been demonstrated in this model system. These latter experiments suggest that antigen-induced stimulation of smooth muscle contractility may be correspondingly blocked by γ-aminobutyric acid similarly stimulated by mast cell products. This complex system may be an adaptation by the host to maintain homeostasis in the face of continued antigenic stimulus. It is noteworthy that strongyle-induced alterations in myoelectric activity of the equine small intestine and colon have been demonstrated in vivo.[171,172] In some of these experiments, dead *S. vulgaris* larvae evoked an alteration of the smooth muscle response in previously exposed ponies, suggesting a role for the immune response in the stimulation of the hyperactivity.[173]

There is a rapidly growing literature on the host-parasite interactions of a number of mouse models of gastrointestinal nematode infections.[174-177] The mouse models include infections of *Trichinella spiralis, Nippostrongylus brasiliensis, Heligmosomoides polygyrus,* and *Trichuris muris.* Because of the use of the mouse the most contemporary of immunologic, genetic, molecular, and cell biologic techniques are available and may provide useful insights into potential explanations of immunologic and pathophysiologic responses of the horse to nematode parasites, particularly the cyathostomins. Although differences in specifics exist among these murine models, it is clear that these worms induce a type 2 T-helper cell cytokine response consisting of interleukins (ILs) IL-4, IL-5, IL-9, and IL-13. IL-4 and IL-13 induction of the Stat6 pathway is central to most of the resulting responses. There is a consistent mastocytosis and eosinophil infiltration of the intestine. The resulting responses also involve goblet cells, the enteric nervous system, epithelial cells, and alternately activated macrophages as effector cells. Cell junctions are disrupted, smooth muscle hypercontractility is stimulated, epithelial cell secretion increased, and goblet cell production of mucus is stimulated. Although these mechanisms are used most often to explain expulsion of the nematodes in question, they could be equally important in the disruption of the homeostasis seen in the parasitized equine intestine.

RESPIRATORY SYSTEM

Several nematode parasites infect the equine lung. These include migrating stages of *Strongyloides westeri* and *Parascaris* spp. en route to the small intestine. Migrating stages of aberrant parasites, such as *Habronema* sp., *Draschia megastoma*, and *Strongylus* spp., which induce granulomatous foci in the lung parenchyma, and adults and larvae of the lungworm *Dictyocalus arnfieldi,* which inhabit the bronchi, also occur. Host responses to two of these are noted.

Parascaris spp. larval migrations in the lungs of yearling horses produce more severe clinical signs and inflammatory responses than in foals that are reared parasite free. These infections in yearlings are accompanied by focal accumulations of lymphoid tissue, indicating an induction of an active local immune response. It is suggested that this is an age-related phenomenon.[178] However, it is likely that more severe reactions could be the result of previous sensitization to *Parascaris* spp. antigens. Increased responses of this nature have been described in the livers of pigs immunized with *Ascaris suum* antigens following challenge infections.

Dictyocalus arnfieldi infections of donkeys rarely produce clinical signs, and it has been suggested that these equids are the natural host for this parasite. Infections of horses produce a more severe and prolonged bronchial inflammatory response similar to *Dictyocalus* sp. infection in other hosts. The mechanisms associated with this differential response have not been defined but are not uncommon in unadapted host-parasite associations. It is possible that the more marked inflammatory reaction of the horse to these parasites is due to the absence of downregulatory mechanisms that are established in the more adapted natural host, the donkey.

SKIN

Whereas *Onchocerca cervicalis* infections in horses are rare where macrocyclic lactone anthelmintics are regularly used, reactions to the filarial nematode illustrate variations seen in responses to chronic parasite infection. Focal, alopecic, depigmented, pruritic lesions are often seen in infected horses. Not all infected horses react to this infection, and the appearance of clinical signs is often seasonal. Detailed studies have not been conducted on the pathogenesis of these lesions in horses. However, similar conditions occur in human onchocerciasis,[179] and it is likely that the host-parasite responses active in humans are also present in the horse. Lesion development is associated with immune-mediated killing of microfilariae in the skin. Parasites appear to be killed in an antibody-dependent cell-mediated reaction. In this response antimicrofilarial surface IgG and IgE antibodies mediated adherence and degranulation of granulocytes, which are predominantly eosinophils. Major basic protein of eosinophils has been demonstrated in the tissues of patients with dermal lesions, and it has been suggested that eosinophil toxic enzymes and proteins are responsible for many of the changes seen. The reason for the absence of these lesions in most horses is unclear. Human onchocerciasis and filariasis are spectral diseases. In these diseases regulation of immune responses has been associated with the lack of pathologic responses to the parasites.[180,181] Immune regulatory mechanisms associated with these infections include immune tolerance, anergy, induction of immune regulation involving Treg cells or macrophages, and the production of IL-10 and transforming growth factor beta (TGF-β)–altering Th-cell subsets and the production of specific cytokines during different phases of the infections. Recent studies have also focused on the role of an intracellular commensal microorganism, *Wolbachia,* which is a parasite of *Onchocerca* and other filarial nematodes. These bacteria have been shown to mediate type 1 inflammatory lesions in humans and mouse models.[180]

The presence of these types of parasite-associated immune regulatory events has yet to be critically studied in the horse. However, the seasonal variability in skin responses to the

Onchocerca microfilariae by horses in some regions has been investigated. In this instance the onset of ventral-midline dermatitis during the summer may be related to a seasonal fluctuation in microfilarial burdens of the skin, which have been demonstrated to correspondingly peak at this time.[182] Not only do total numbers increase, but microfilariae are also found more commonly in the surface layers of the skin. Interestingly, this period of abundant microfilariae corresponds with the seasonal peak in numbers of the vector, *Culicoides varripennis.* Although speculative, correlations in the peak availability of microfilariae and vectors may be an evolutionary adaptation by these parasites to maximize transmission and survival of this parasite species.

PROTECTIVE RESISTANCE

Resistance to infection may be innate or acquired. In some instances innate resistance to equine parasites has been attributed to age, with older individuals being resistant. Most equine helminth parasites develop only in the horse, and conversely the horse exhibits an innate resistance to most nonequine parasites. Exceptions to this rule are parasites with a broader host range that occasionally infect horses, such as larvae of the tapeworm *Echinococcus granulosus* and the liver fluke *Fasciola hepatica. Trichostrongylus axei,* a parasite of ruminants, establishes readily in the equine stomach and produces significant lesions only when present in large numbers. In some cases parasites that develop in the horse induce more severe lesions and clinical signs than in their apparent normal host, as has been described for *D. arnfieldi.*

Age resistance to *Parascaris* spp. and *S. vulgaris* has been described in horses by comparing susceptibility of young and old ponies reared under parasite-free conditions. It is apparent that the reaction of the lung to migrating *Parascaris* larvae is more marked in mature horses and suggests that an immune response occurs in this site.[160,161] Initial reports on age-acquired resistance to *S. vulgaris* infection[183] have not been substantiated, and experimental observations in our laboratory indicate that this does not occur.

The occurrence of acquired resistance to equine parasites can be inferred from the observation that older chronically exposed horses generally have lower burdens of parasites than do similarly exposed young horses. On the basis of these criteria, acquired resistance is apparent to infections of *S. westeri, Parascaris* spp., *Strongylus* spp., and cyathostomin species. Extensive experiments are limited, however, to those on *S. vulgaris* and to some degree cyathostomins.

Although *S. vulgaris* has been largely eliminated from well-managed horse farms, examination of details of the immune response to it illustrates some equine immune mechanisms. Resistance that is acquired to *S. vulgaris* in most cases is partial and of a concomitant type; that is, some stages of the parasite, such as arterial larvae of *S. vulgaris,* may reside within the horse in the face of an active acquired resistance against newly acquired infective stages.

Resistance to infection with *S. westeri* adult parasites is inferred by the short duration of their life cycle within the small intestine and the failure of subsequent exposures to establish patent infections. Mares, however, remain infected with arrested third-stage larvae, which subsequent to foaling are transmitted to the foals in milk starting at 4 days postpartum. Similar phenomena occur in swine strongyloidosis. In these infections there is apparent protective resistance against the migrating L_3, which is effective in preventing reestablishment of the intestinal infection but is ineffective against L_3, which are sequestered in the abdominal fat of the sow.[184] Similar epidemiologic phenomena occur in *S. westeri* infection of horses, and it may be implied that similar immunologic mechanisms are also active.

Immunologic mechanisms associated with protective resistance are presented primarily as they relate to parasites that inhabit the lumen of the gastrointestinal tract and secondarily as those that undergo extraintestinal tissue migration.

Immune responses directed toward gastrointestinal nematodes vary significantly among hosts and against different parasite species within a given host. However, some generalities may serve as a background for understanding these responses in the horse. A phenomenon termed *self-cure* has been described in sheep, in which the ingestion of significant numbers of infective larvae induces the expulsion of existing adult parasites. This expulsion is initiated by a species-specific immediate-type hypersensitivity response that may cause the nonspecific expulsion of other nematode species. Although this phenomenon has not been examined in the horse, experimental infections of naturally parasitized ponies with large numbers of *S. vulgaris* L_3 induced a dramatic decrease in preexisting strongyle fecal egg counts, suggesting that a self-cure–like reaction may occur under some conditions.

More typically, establishment of primary infections results at some time in spontaneous expulsions of these worms as a result either of senility or, as demonstrated in laboratory animal model systems, of active acquired immune responses. This phenomenon occurs experimentally in the absence of reinfection and is thus separate from the self-cure phenomenon. A large number of immune effectors have been identified with this phenomenon in various model systems, and it is likely that some if not all are at some time active in the equine intestine. The mechanisms involved are T-cell dependent. Antibodies may be involved but are not sufficient in themselves to induce expulsion. T-cell–mediated mastocytosis, eosinophilia, and goblet cell hyperplasia have all been demonstrated to be related to expression of expulsion in some systems. These accessory cells are involved in the nonspecific efferent arm of this response. Mediators of inflammation, such as vasoactive amines, prostaglandins, and increased production of mucus, have been linked to immune elimination of primary infections in some but not all model systems. The increased secretion and hyperreactivity of intestinal smooth muscle associated with worm expulsion have been termed *weep and sweep* phenomena. It is likely that a number of specific immunologic events initiate several nonspecific effector mechanisms, resulting in this expulsion. These mechanisms vary with the species of parasite involved. The elimination of adult *S. westeri* and *Parascaris* spp. from maturing horses and the hypothetical seasonal turnover in *Strongylus* spp. and cyathostomin spp. may be mediated by such responses.

In addition to immune responses that occur during tissue migrations, protective resistance to reinfection by gastrointestinal nematodes occurs at the surface of the epithelium. This reaction, termed *rapid expulsion* or *immune exclusion,* is separate from self-cure or immune expulsion of primary infection. Infective larvae are expelled from the intestine in a matter of hours. Again, mechanisms of expulsion described vary between parasite and host species. However, anaphylactic reactions and mucus entrapment have been observed. Some experiments using the *T. spiralis*–rat system suggest

that alterations in the epithelial cells in immune animals are directly involved in the exclusion of these parasites. Although immune-mediated damage of intestinal helminths such as decreased fecundity, reduced size, and morphologic alterations have been noted, infective larvae expelled by rapid expulsion mechanisms remain viable and undamaged. It may be speculated that reactions of this nature are responsible, in part, for resistance to reinfection of equines with cyathostomins.

Some specific observations have been made on the equine acquisition of resistance to reinfection with cyathostomins.[185] This resistance is acquired over time with continued exposure to pasture L3; is incomplete; and, like other parasitic helminth infections, appears to be genetically regulated in that parasite numbers are overdispersed within a given herd of adult animals. The limited number of experimental and field studies completed to date suggests that immunity may be directed toward all stages of the parasite life cycle. Challenge infections of previously exposed and naive ponies with mixed strongyle infections indicate that a nonspecific self-cure–like phenomenon occurs, reducing numbers of adult and fourth-stage larvae (L4) in the lumen as well as within the mucosa.[157] Fecal egg counts are significantly reduced in older animals compared with yearlings even when adult parasite numbers are similar. These observations suggest that immune responses are directed at female fecundity or that the species of cyathostomins present in the older animals are inherently less fecund. Numbers of early L3 (EL3) are reduced following challenge infections of previously exposed adult ponies compared with previously exposed young animals or age-matched naive controls.[186] Other studies have suggested that acquired resistance is important in the induction of hypobiosis.[169,187] Resistance to acquisition of EL3 was not seen when numbers were compared in young previously exposed and age-matched ponies raised under parasite-free conditions. However, these previously exposed young animals demonstrated significantly fewer developing larvae (DL) in the mucosa, suggesting that once the EL3 began to develop, they were susceptible to immune attack.[186] This response is likely driven by cytokines produced by T cells of the Th2 lineage with demonstrated increases in IL-4 and IL-5. This response mimics in some ways the results described in studies of murine nematodes.[177]

A number of intestinal helminths, as well as others, migrate through extraintestinal tissues as part of their life cycle. These include parasites such as *Parascaris* spp., *S. westeri,* and *Strongylus* spp., all of which stimulate an acquired immune response in the horse. During this migration these larvae are vulnerable to attack by immune effectors that may either encapsulate them in an immune-mediated inflammatory response, disrupt their migrations by interfering with important metabolic or invasive processes, or inhibit molting from the L3 to L4 stages. The most studied phenomenon in this regard is antibody-mediated adherence of inflammatory cells, which may result in killing of the larvae. This phenomenon involves many cell types and immunoglobulin isotypes in different host-parasite systems. In vitro studies of this nature have been conducted using *S. vulgaris* third-stage larvae and equine immune effectors.[188] In these experiments an antibody-dependent adherence of cells was demonstrated and shown to be parasite species specific. In vitro killing was mediated by eosinophils and not by neutrophils or monocytes. Activated eosinophils were necessary to mediate this response, and *S. vulgaris* infections have been demonstrated to activate eosinophils and neutrophils in vivo.[189] Although it is not certain that eosinophils are essential in this protective immune response, an anamnestic eosinophilia is characteristic in immune ponies but not nonimmune ponies following experimental *S. vulgaris* challenge.[157] Because of its prominence and compelling in vitro and correlative in vivo data, the eosinophil is considered a major effector in immune-mediated helminth killing. However, recent studies in murine parasite model systems in which eosinophilia was blocked by anti–IL-5 treatment suggest that this type of cell is not essential for protective resistance in some systems.[190] Significant increases in equine IL-5 have been measured in ponies vaccinated against *S. vulgaris.* It is possible, in vivo, that a number of cells function as effectors and may overcome the absence of sufficient eosinophils under some circumstances. Antibody reactivity with parasite-secreted enzymes and molting fluids, factors important in parasite homeostasis, has been demonstrated in vitro; similar reactions may be important in vivo.

T-cell responses are essential for the induction of protective resistance to tissue-migrating helminths in most systems studied, including the experimental *S. vulgaris* pony model. This dependency is likely due to the T-cell dependency of the antibody response and to the mediation of secondary effector cell responses. It is likely that antigenic substances secreted or excreted (ES) by migrating nematodes are important in the induction of these responses. It is probable that a combination of immune responses elicited by a combination of specific parasite antigens, including surface antigens and ES products, is necessary to induce an immune response sufficient to provide protective resistance.

PARASITE-INDUCED REGULATORY RESPONSES

Helminth parasites have evolved elaborate mechanisms to evade the host's immune responses, live in the face of an active specific host response, and yet establish chronic infections. These immune evasion strategies include the production and secretion of molecules such as proteases, protease inhibitors, antioxidants, prostaglandins, and phosphorylcholine-antigens, which disrupt host effector responses.[191,192] Other molecules that mimic host immune regulatory factors, such as TGF-β and (macrophage migration inhibitory factor) MIF, have also been identified in parasitic helminth genomes.[191] These mimics likely down-regulate host-parasite–directed protective response and add to immune evasion.

Helminth parasites can induce a regulatory immune response, which promotes their survival but also has bystander effects on host responses to other infectious agents, vaccines, allergic responses, and autoimmune diseases. These observations have progressed to the point that helminth therapy is being developed for the treatment of human inflammatory bowel disease and ulcerative colitis.[193,194] In this situation infection with the swine whipworm *T. suis* impedes the regulation of type 1 inflammatory responses responsible for these conditions and reduces disease activity. The generally accepted and simple model that has emerged for this type of helminth-induced regulation is that parasite factors stimulate dendritic cells that activate regulatory T cells and alternately activated macrophages. These cells, through the production of TGF-β and IL-10, may suppress both Th1- and Th2-mediated inflammatory responses.[195-197]

Detailed studies of the effects of equine helminths on the regulation of parasite-induced responses or to heterologous immunogens have not been reported. The effects of different levels of gastrointestinal helminth infection on the response of ponies to keyhole limpet hemocyanin immunization was measured.[198] Antibody levels determined by enzyme-linked immunosorbent assay showed that animals with low levels of parasites had a trend toward increased KLH-specific total immunoglobulin, IgG(T), and IgA compared with heavily parasitized ponies. Moderately and heavily parasitized ponies demonstrated a trend toward reduced lymphoproliferative response to KLH that was not restored after the addition of IL-2. Cells from these ponies also produced significantly lower levels of IL-4 compared with lightly parasitized ponies. These data indicate that heavily parasitized animals have uniformly decreased cellular and humoral immune responses to soluble protein immunization. The mechanisms involved are unknown. A recent study evaluated the inflammatory and immunologic response to three different antigens given simultaneously to ponies concurrently dewormed with either ivermectin or pyrantel pamoate or kept as untreated control. This study found no difference in cytokine expression, acute-phase inflammatory markers, or antigen-specific antibody titers between the anthelmintic treatment groups.[199] Taken together, the possible interaction between resident intestinal nematodes and responses to vaccination in horses is in need of further investigation.

Infection and Immunity

Robert H. Mealey

BASIC PRINCIPLES OF INFECTIOUS DISEASE

Equine clinicians devote tremendous effort to the prevention, diagnosis, and treatment of infectious diseases. Detailed and current information on specific infectious diseases in the context of organ systems is provided elsewhere in this book, and in-depth discussions of infectious diseases of horses are provided in a recent text[200] and review.[201] The field of equine infectious disease research is growing rapidly, and modern genomics techniques such as massively parallel deep sequencing have enabled the recent discovery of previously unknown equine pathogens and potential pathogens.[202,203] In addition, continued application of proteomics approaches[204] will lead to a better understanding of infectious disease pathogenesis and the discovery of novel vaccine and therapeutic targets, as well as improved diagnostics.

On a more basic level, the following events occur in all infectious diseases: encounter, entry, spread, multiplication, damage, and outcome.[205] Encounter occurs when the host comes into contact with an infectious agent. The encounter is endogenous when infections are caused by normal microbial flora. An example is pneumonia caused by *Streptococcus zooepidemicus,* a member of the normal flora of the equine nasopharynx. Exogenously acquired infections result from encounter with an infectious agent in the environment or an agent transmitted from other animals or via insect or tick vectors. There are many examples of exogenous infectious diseases, and an important one is pneumonia caused by *Rhodococcus equi.* Entry into the host can occur by ingestion, inhalation, or penetration through epithelial barriers such as the skin or mucosal surfaces. Regarding the entry of organisms, inoculum size is a key factor in determining whether or not disease will develop. Following entry, microorganisms must spread from the entry site to local tissues or disseminate to distant sites in the body. Bacteria such as *Staphylococcus aureus* produce enzymes such coagulase, protease, and hyaluronidase that facilitate their ability to spread through tissues and cause disease. Substances produced by microorganisms that enhance their ability to cause disease, as well as microbial structures that do the same, are collectively referred to as virulence factors. The next step in the development of disease is replication of the infectious agent to levels that result in clinical signs. This period of replication before the development of clinically apparent disease comprises the incubation period. Microorganisms that cause disease by producing toxins, such as botulinum toxin produced by *Clostridium botulinum,* are an exception to this rule. Disease in the host ensues when the infection results in tissue damage. There are many ways in which damage can occur. Cell death caused by replication of the agent, extracellular toxins produced by the agent, and, importantly, the host's inflammatory and adaptive immune responses directed against the agent all result in tissue damage and disease. The systemic inflammatory response elicited by endotoxin during gram-negative bacterial infections is an excellent example of host-mediated damage. The final outcome depends on the interplay between infectious agent factors and host factors, which include the inoculum size and virulence of the agent, as well as the immune status of the host. The host may mount a successful immune response and clear the organism, as occurs with equine influenza virus infection, or the immune response may control replication of the agent such that disease resolves but the agent is not eliminated. This results in persistent inapparent infection as occurs with equine infectious anemia virus infection. Finally, highly virulent agents such as rabies virus and Hendra virus will kill the host, whereas less pathogenic agents may kill hosts that are immunocompromised in some way.

Successful pathogens have developed efficient mechanisms for entry, spread, and replication, all of which enhance their ability to cause disease. Importantly, each one of these steps requires breaching or avoiding host defense mechanisms, which include physical barriers, innate immune responses, and adaptive immune responses. These same immune responses ultimately control most infectious agents but also participate in the pathogenesis of disease. Thus understanding how infectious diseases develop and how they are controlled requires a fundamental knowledge of immunology, which is the primary focus of this chapter.

EQUINE IMMUNOLOGY

Although our modern understanding of the immune system is primarily based on humans and rodent models, the horse has contributed significantly to our understanding of many immunologic processes. These contributions include the earliest work on serotherapy and passive transfer of antibodies, antibody structure and function, immunity to infectious agents, immunodeficiencies, and reproductive

immunology.[206] Work in the horse continues in many of these areas from the standpoint of equine medicine and comparative immunology. With some exceptions, the overall organization and function of the equine immune system is similar to other mammalian species. A number of texts provide excellent in-depth reviews of basic and clinical immunology, and much of the general information provided in this chapter is drawn from these resources.[207-210] This chapter will focus on aspects of the immune system that may be of most interest to equine clinicians and researchers, with pertinent references to equine work provided when possible.

Innate Immunity and the Acute Inflammatory Response

Immune defenses include both innate responses and adaptive responses, each mediated by cellular and soluble components. Although fundamentally different, the innate and adaptive immune systems are intimately related and share many of the same processes and components. Innate immunity is rapid, is nonspecific, results in acute inflammation, and has no memory. In contrast, adaptive immunity takes time to develop, is antigen specific, and has recall capability. It is both the specificity of adaptive responses and the capacity for immunologic memory that allow complete protection against a particular pathogen. However, innate responses play key roles in both triggering the adaptive response and providing valuable time for specific adaptive responses to develop.

The horse, like every other species, is under constant assault from a variety of microbes that share its living space. Although most of these organisms are thought to be harmless, their disease-causing potential is evident when they cause opportunistic infections in individuals with compromised immune systems. In general, mammals have evolved a variety of defensive measures to prevent infections from occurring. The first line of defense includes the physical barriers provided by the skin and the mucosal surfaces of the digestive, respiratory, and urogenital tracts. In addition to providing a barrier to penetration, the surface of the skin contains various enzymes, fatty acids, and oils that inhibit the growth of bacteria, fungi, and viruses. Mucous membranes and mucosal secretions contain bacteriolytic enzymes, bactericidal basic polypeptides, mucopolysaccharides, and antibodies that prevent colonization and penetration of these surfaces. Mucus also provides a physical barrier that entraps invading organisms and leads to their eventual disposal.[211] Particles trapped in the mucus secretions of the respiratory tract, for example, are transported upward through the action of ciliary cells to the trachea where they are swallowed.[212] Once swallowed, the acidic secretions and digestive enzymes of the stomach destroy most organisms. Normal epithelial and tissue architecture is essential for successful exclusion of bacteria, and the disruption of this mechanism makes the host susceptible to infection by bacteria that normally colonize the upper airway.[213,214] The protective role of gastric acidity against gastrointestinal pathogens is supported by the observation that acid-suppressive drugs increase the risk of diarrhea in neonatal foals treated in intensive care units.[215] In humans, administration of proton pump inhibitors in critically ill patients increases the risk of *Clostridium difficile*–associated diarrhea.[216]

Proinflammatory Cytokines, Acute Phase Proteins, and Complement

After physical barriers have been breached, the innate immune system provides a variety of internal defenses to contain and eliminate the invading organisms. Inflammatory responses are initiated either via the activation of plasma protease systems directly, such as by bacterial cell wall components, or by the secretion of toxins or other proteins that can directly activate the inflammatory response.[217] The cell walls and membranes of bacteria contain various proteins and polysaccharides with characteristic, often repeating, molecular structures. These pathogen-associated molecular patterns (PAMPs) include lipopolysaccharides (LPSs), peptidoglycans, lipoteichoic acid, and flagellins.[218] Other PAMPs include viral nucleic acids and unmethylated bacterial cytosine-guanosine dinucleotides (CpG). PAMPs are recognized by pattern recognition receptors found on cells of the immune system, particularly those cells involved in the initial encounter with invading microbes. These "sentinel cells" include macrophages, dendritic cells, and mast cells, with macrophages being the most important for initiating the inflammatory response. Pattern recognition receptors are located on the cell surface, as well as intracellularly, and include toll-like receptors (TLRs), retinoic acid–inducible gene–1 (RIG-1)–like receptors, and nucleotide-binding oligomerization domain–like receptors. The TLRs are particularly important in the induction of inflammation. In addition, binding of PAMPs leads to intracellular signaling events culminating in the expression of costimulatory signals for the developing adaptive immune response. Injured cells release products collectively termed *damage-associated molecular patterns* (DAMPs) that bind TLRs on macrophages and other cells leading to the production of proinflammatory cytokines that augment the inflammatory process. Resident macrophages that encounter the invader initiate the inflammatory response through the production of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor–alpha (TNF-α).[217] Cytokines are hormone-like proteins that mediate a variety of cellular responses. A vast number of cytokines are involved in the regulation of innate and adaptive immune responses. IL-1, for example, is a pleiotropic mediator of the host response to infections and tissue injury (Table 1.3). Many of the effects of IL-1 are mediated through its capacity to increase the production of other cytokines, such as granulocyte colony-stimulating factor (G-CSF), TNF-α, IL-6, and platelet-derived growth factor (PDGF). IL-6 is responsible for the increased production of acute phase proteins by hepatocytes, including complement proteins, C-reactive protein, and serum amyloid A (SAA).

TABLE **1.3** **Biologic Activities of Interleukin-1**

Activates T cells	Induces fever
Activates B cells	Cytotoxic for some tumor cells
Enhances NK cell killing	Cytostatic for other tumor cells
Fibroblast growth factor	Stimulates collagen production
Stimulates PGE synthesis	Stimulates keratinocyte growth
Stimulates bone resorption	Stimulates mesangial cell growth
Chemotactic for neutrophils	Activates neutrophils
Activates osteoclasts	Induces IL-6 production

Many of these proteins and the cytokines that elicited them are responsible for the characteristic physical signs of inflammation, including increased blood flow and vascular permeability, migration of leukocytes from the peripheral blood into the tissues, accumulation of leukocytes at the inflammatory focus, and activation of leukocytes to destroy any invading organisms.[219]

During viral infections, intracellular pattern recognition receptors (such as RIG-1) bind to viral PAMPs (such as double-stranded viral ribonucleic acid [RNA]) and induce the production of type I interferons (IFNs), including IFN-α and IFN-β. These are produced in virus-infected cells within hours and inhibit viral replication through various mechanisms. In addition, natural killer (NK) cells are capable of lysing virus-infected cells via recognition of different types of receptors on the surface of infected cells, as well as recognizing cells expressing fewer major histocompatibility complex molecules (see later discussion), the expression of which can be down-regulated by viral infection. Finally, NK cells have surface Fc receptors (see later discussion) that can bind the Fc portion of antibodies bound to the surface of infected cells and subsequently kill the infected cell through the process of antibody-dependent cellular cytotoxicity. NK cells are of the lymphocyte lineage but do not possess antigen-specific receptors such as T and B lymphocytes (discussed later) and thus act as innate effector cells.

The complement system consists of over 30 different plasma proteins that are produced primarily in the liver. This complex interacting series of proteases and their substrates results in the production of physiologically active intermediaries that can damage membranes, attract neutrophils and other cells, increase blood flow and vascular permeability, and opsonize bacteria and other particles for phagocytosis.[220] The complement cascade can be activated in three ways. The *lectin pathway* is initiated when soluble carbohydrate-binding proteins (such as mannose-binding lectin) bind to carbohydrate structures on microbial surfaces. Proteases associated with these carbohydrate binding lectins then initiate the cleavage of complement components and activate the pathway. The *classical pathway* involves the binding of C1q to antibodies already bound to the surface of a microbe. Bound C1q is proteolytic and cleaves other complement components activating the pathway. The activation of complement via the *alternate pathway* does not involve antibodies. Instead, certain microbial products stimulate the association of factor D, a proteolytic enzyme, with the complex of factor B and C3b leading to the formation of C3 convertase and its deposition on the microbial surface. All three pathways converge with the generation of C3 convertase and the cleavage of C3. Soluble C3a, produced by the cleavage of C3 by the C3 convertases, can bind to mast cells causing them to degranulate and is thus referred to as an anaphylatoxin, as is C4a. C3b serves as an opsonin for C3b receptor-bearing phagocytic cells. C3b is also required for the formation of the membrane attack complex by the terminal complement components, C5 through C9. In this process C5 is cleaved and C5a is generated, which, along with C3a, is a chemoattractive factor for neutrophils and monocytes.[221] C5b forms a complex with C6, C7, and C8 on cell surfaces. This leads to the insertion and polymerization of C9 that forms a pore in the membrane leading to cell lysis. A summary of the three complement pathways and the resultant effector mechanisms of microbial destruction is provided in Fig. 1.6.

Lipid Mediators. Prostanoids are lipid mediators that regulate the inflammatory response.[222,223] The prostanoids include the prostaglandins (PGs), leukotrienes (LTs), and prostacyclin (PGI_2), and they are the product of cyclooxygenase cleavage of arachidonic acid followed by endoperoxidation. The major sources of prostanoids in acute inflammation are the phagocytes, endothelial cells, and platelets. Although prostanoids, in general, mediate the cardinal effects of pain, fever, and edema characteristic of the acute inflammatory response, their particular roles are somewhat confounding and can be either pro-inflammatory or antiinflammatory.[224] Prostanoid production depends on the activity of the two isoforms of the cyclooxygenase (COX) enzymes within cells: COX-1, which is present in most cells and its expression is generally constitutive, and COX-2, for which expression is low or undetectable in most cells but its expression increases dramatically on stimulation, particularly in cells of the immune system. Increased COX-

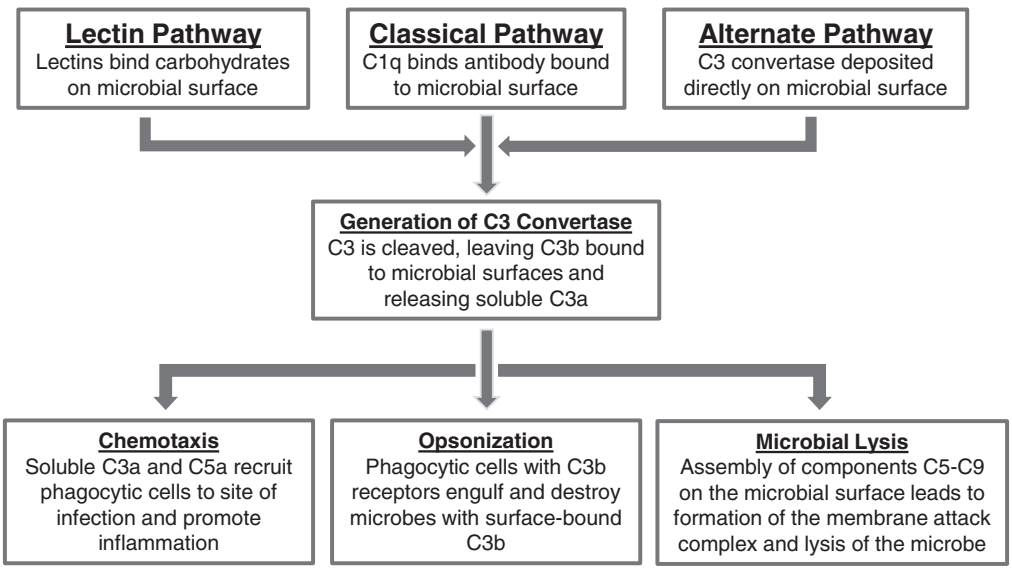


FIG. **1.6** Simplified pathways of complement activation and basic effector mechanisms of microbial destruction. See text for explanation.

2 expression by inflammatory stimuli likely accounts for the high levels of prostanoids found in inflammatory lesions and is the basis for the development of COX-2–selective nonsteroidal antiinflammatory drugs (NSAIDs).[225] Because there are species differences in the pharmacokinetics and COX selectivity of NSAIDs, extrapolation of studies performed in other species should be interpreted with caution with respect to the potential COX selectivity and efficacy in the horse.[226] Meloxicam has COX-2 selectivity and efficacy in the horse[227-229] but at the time of this writing is not approved in the United States for use in horses (but is approved in Europe). Firocoxib is a selective equine COX-2 inhibitor with documented safety and efficacy,[210-236] and it is currently the only COX-2–selective inhibitor approved by the Food and Drug Administration for use in horses in the United States.

Both COX isoforms produce PGH_2, which is the common substrate for a series of specific synthase enzymes that produce PGD_2, PGE_2, PGF_2, PGI_2, and TXA_2. It is the differential expression of these enzymes within cells present at sites of inflammation that determines the profile of prostanoid production. Likewise, the biologic effect of a prostanoid depends on binding to G protein–coupled cell surface receptors. Many cells of the immune system express multiple receptors that couple to apparently opposing pathways. The impact of prostanoids present during an inflammatory response is thus determined by the array of receptors the cells express and the intracellular pathways to which they are coupled. Activation of these receptors, even when coupled to similar pathways, might evoke different responses because of differences in the levels of expression (both constitutive and induced) or in the patterns of desensitization. The role of prostanoids in a given inflammatory response depends not only on the presence of the lipid mediators in the lesion but also the receptor profile on immune cells and the biochemical signaling pathways of these receptors.[237] Thus PGE_2 is considered proinflammatory because it promotes vasodilation by activating receptors on vascular smooth muscle and increases vascular permeability indirectly by enhancing the release of histamine and other mediators from tissue leukocytes such as mast cells. PGE_2 is also the prostanoid responsible for fever production. However, as inflammation progresses, PGE_2 synthesis by macrophages is enhanced as a result of increased expression of COX-2 and PGE-synthase, and the resulting increased levels of PGE_2 inhibit leukocyte activation, inhibit mast cell degranulation, and relax smooth muscle contractions. In the lung, PGE_2 promotes bronchodilation. Thus in these situations PGE_2 may be considered antiinflammatory.

Chemotaxis and Leukocyte Trafficking. One of the initial and most crucial aspects of the acute inflammatory response is the recruitment of leukocytes (primarily neutrophils) to the site of injury. Neutrophils constitute the first line of the cellular defense and are the initial cells involved in an inflammatory response. These phagocytic cells are derived from multipotent stem cells located chiefly in the bone marrow. Under the influence of a variety of signals provided from both within and outside of the bone marrow, these stem cells become committed to developing into cells of the granulocyte lineage. The critical signal is provided by a family of growth factors known as colony-stimulating factors (CSFs) that provide both proliferative and differentiating signals leading to the development of granulocytes and other leukocytes. Once released into the circulation these cells must find their way to the site of the inflammatory response. The production of various chemotactic factors by host cells, bacteria, and other invaders causes various leukocytes to enter the circulation and be carried to the site of the injury.[238] Chemokines are chemotactic cytokines. These are soluble proteins produced by host cells that induce the directional migration and activation of leukocytes, as well as other somatic cell types, and thus play major roles in the inflammatory response.[239] The chemokine CXCL8 (previously known as IL-8) plays a central role in the migration of neutrophils, including equine neutrophils.[240,241] Other chemokines promote humoral and cell-mediated immune reactions; regulate cell adhesion, angiogenesis, leukocyte trafficking, and homing; and contribute to lymphopoiesis and hematopoiesis.[242]

The specific trafficking of leukocytes from the blood to inflammatory sites is dependent on both the production of chemotactic factors and the interaction of specific receptors on the leukocytes with corresponding adhesion molecules on the endothelial surface of the blood vessels. Neutrophil adherence is a two-step process first involving endothelial cell surface molecules known as selectins. Small venular endothelium overlying a site of inflammation and exposed to leukotrienes, platelet activating factor (PAF), IL-1, complement protein C5a, histamine, LPSs, TNF-α, or other mediators released by clotting, platelet activation, or mast-cell activation express P-selectin. P-selectin mediates the process in which neutrophils initially interact with the endothelial surface in a process known as "rolling" in which the circulating neutrophil interacts with the endothelial cell before the actual adherence. P-selectin appears on the endothelial cell surface within minutes of an inflammatory stimulus, followed by E-selectin expression within a few hours. Endothelial selectins function by binding to carbohydrate ligands present on the leukocyte surface. In the case of neutrophils, the ligand is sialyl-Lewis^X, an oligosaccharide present on cell surface glycoproteins. Equine P-selectin binds the sialyl-Lewis^X moiety on equine leukocytes, mediating adhesion and stimulating the production of CXCL8.[243] The second part of the adherence process is the tight binding of integrins on the neutrophil surface to intracellular adhesion molecules on the endothelial cell surface. Leukocyte integrins are heterodimeric proteins and include leukocyte functional antigen–1 (LFA-1, also called CD11a:Cd18) and complement receptor type 3 (CR3, also called CD11b:Cd18). Neutrophils can be activated by a number of soluble proteins present in bacterial but not eukaryotic proteins. Host factors present at the site of inflammation can also activate neutrophils. These include complement proteins (C5a, C3a), the chemokine CXCL8, cytokines (especially TNF-α), and immune complexes.[244] Expression of integrins by the activated neutrophil allows them to become tethered to the endothelial surface. The migration of neutrophils through the vascular wall is less well understood than these initial events leading to firm adhesion. Integrins LFA-1 and CR3, as well as PECAM-1 (CD31), another cell adhesion molecule, appear to play a role in this process. Endothelial cell–produced CXCL8 also has a critical role in this process. Once through the endothelium, the phagocytes will follow chemotactic signals and migrate toward the point of injury. They may adhere to other cells during migration to the site of inflammation, and these interactions also are dependent on integrins.

Neutrophils recruited and activated in this manner will actively phagocytose microscopic invaders and attempt to destroy them using reactive oxygen products generated via a nicotinamide adenine dinucleotide phosphate

(NADPH)-oxidase–dependent respiratory burst. During this process, neutrophils release additional proinflammatory mediators, thus amplifying the response. Among those cells attracted to the area are NK cells, and the production of IFN-α or IFN-β by macrophages and other cells enhances NK cell cytolytic activity. The NK cells themselves can be a source of IFN-γ, another proinflammatory cytokine (discussed in detail later). Depending on the magnitude of the initial insult and the susceptibility of the invader to neutrophil-mediated destruction, the inflammatory response may be either acute or chronic.

Acute inflammation is thus a rapid response to an injury that is characterized by accumulations of fluid, plasma proteins, and neutrophils that rapidly resolves once the initial inflammatory stimulus is removed. Deactivation signals include PGE_2, cortisol, IL-10, and transforming growth factor–β (TGF-β). Some of those chemotactic agents responsible for initiating the response (CXCL8, FMLP, C5a, LTB4, and PAF) also serve to downregulate its intensity by inducing the shedding of IL-1 receptors from neutrophils.[245] The shedding of this decoy receptor may have antiinflammatory effects as it effectively binds and neutralizes this cytokine. Likewise many of the acute phase proteins are thought to have immunomodulatory activity downregulating neutrophil function.[246] Acute inflammatory responses may often be subclinical and resolve without complications. However, if the invader is resistant to neutrophil-mediated destruction or the degree of injury is large, the response may become more chronic with the added recruitment of macrophages, lymphocytes, and fibroblast growth.

The essential characteristic of the innate immune response is that it does not exhibit specificity for the invading organism. Thus the induction of an innate immune response does not require prior exposure to the invading organism nor is it augmented by repeated exposure to the same organism. In most instances these mechanisms are adequate for eliminating casual invaders. However, pathogenic organisms have evolved various methods for avoiding elimination. In response to these organisms, the specialized cells and products of the adaptive immune response are mobilized.

Adaptive Immunity

The adaptive immune response is initiated in response to the encounter with a foreign agent and depends on antigen-specific immune responses mediated by different divisions of the lymphocyte family (Fig. 1.7). In contrast to the nonspecific nature of the innate immune response, an important characteristic of the adaptive immune response is the specificity of this interaction. Thus exposure of the host to a particular microbe or parasite results in the induction of immune responses that are directed against specific components of the invading organism that do not affect unrelated organisms. The specificity of the adaptive immune response is the result of the interaction of specific molecular structures, or *antigens,* of the invader with antigen-specific receptors on lymphocytes. All types of chemical structures can serve as antigens, including proteins, nucleic acids, lipids, and polysaccharides. However, foreign molecules that are large, complex, and stable are the most antigenic. Large antigens, such as proteins, contain multiple *antigenic determinants* or *epitopes* that interact with lymphocytes via their antigen-specific receptors. *Haptens* consist of single antigenic determinants and can effectively combine with the binding site of antibody molecules. However, as they only consist of a single antigenic determinant, they cannot cross-link B-cell receptors (antibody molecules), and they are also unable to stimulate T-cell responses. Therefore haptens cannot stimulate an immune response unless multiple haptens are physically attached to a larger molecule, known as a *carrier.*

Like the innate response, the adaptive immune response to a specific antigen consists of both humoral and cellular effector mechanisms. The humoral component is mediated by immunoglobulins or *antibodies* found in plasma and tissue fluids. Antibodies are produced by B lymphocytes, small lymphoid cells characterized by the cell surface expression of immunoglobulin molecules. B cells represent less than 15% of the circulating peripheral blood mononuclear cells but are present in higher proportions in lymph nodes and the spleen. B cells are derived from the fetal liver and bone marrow of mammals and the bursa of Fabricius of birds. In the bone marrow, B

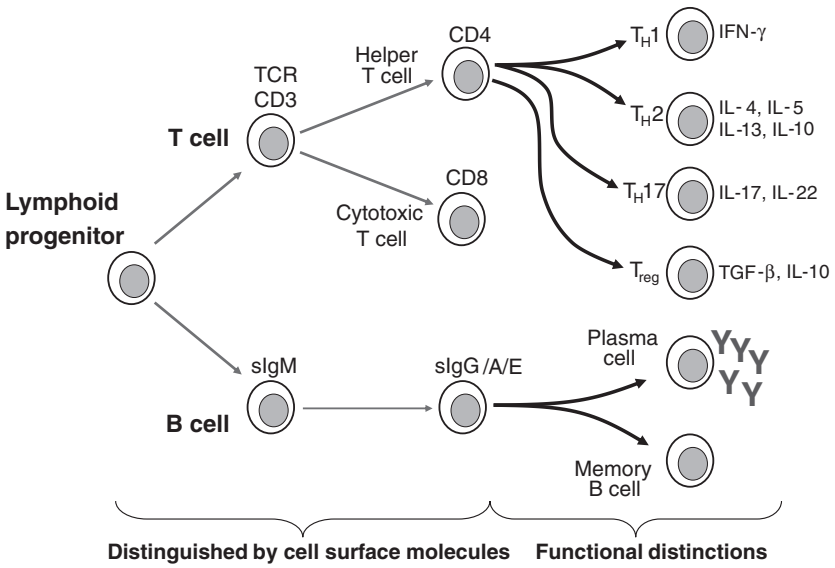


FIG. **1.7** Major divisions of the lymphocyte family. To the left of the diagram different populations of lymphocytes are distinguished by expression of different cell surface molecules. To the right of the diagram the distinctions are functional.

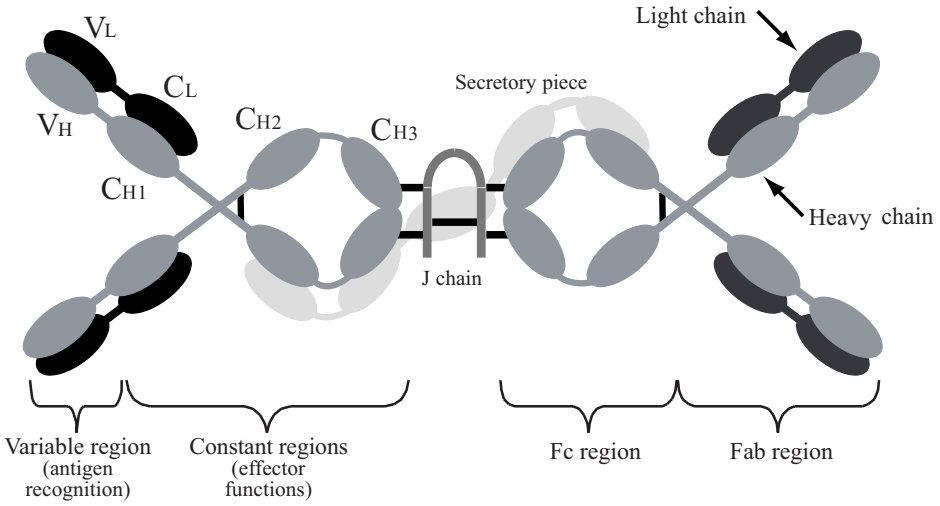


FIG. **1.8** Molecular structure of secretory IgA. This schematic illustrates the major features of immunoglobulin molecules. Whereas the illustrated IgA molecule is dimeric, with the two Ig units joined by a "J chain" and a series of disulphide bonds, IgG molecules are monomeric. Each Ig unit consists of two heavy chains and two light chains. The heavy chains have four subunits and the light chains two. One end of the Ig unit has a highly variable protein structure and is involved in antigen recognition, and the remainder of the Ig unit has a constant structure in each Ig class and subclass and determines the functional characteristics of the molecule, such as binding complement, or recognition by macrophages or neutrophil Fc receptors. This specialized dimeric IgA molecule also has a secretory piece that increases its stability in the harsh mucosal environment. In this diagram, the Fc portion is depicted as a circular structure, consistent with actual x-ray crystal structure determinations.

cells are the products of a putative lymphoid stem cell derived from the pluripotent stem cell. Under the influence of various cytokines produced by bone marrow *stromal* cells, the B-cell precursor undergoes its 3-day development into a mature B cell. On stimulation with a specific antigen, B cells differentiate into *plasma cells* that produce enormous quantities of a specific antibody. The activation, proliferation, and differentiation of B lymphocytes into plasma cells is dependent on other cells, including T lymphocytes, which represent the cellular component of the adaptive immune response. The T lymphocyte is also derived from the multipotent stem cell and lymphoid precursor in the bone marrow, though its subsequent development into the mature T cell occurs in the *thymus*. Within the thymic environment the prothymocyte undergoes a developmental and selective process while emigrating through the cortex into the medullary region of the thymus. Less than 3% of all the immature thymocytes found in the cortex survive to become peripheral T cells.

Though the induction of an antibody response requires the interaction of B and T lymphocytes, these cells recognize different epitopes on the same antigen. Indeed, antigen recognition by B cells and T cells is fundamentally quite different. B cells, and antibodies, recognize antigens in solution or on cell surfaces in their native conformation, whereas T cells only recognize antigen in association with self molecules known as major histocompatibility complex molecules found on most cells surfaces (see later discussion). The adaptive immune response thus differs from innate immunity in that it is antigen driven, and those cells that mediate the adaptive immune responses, T and B lymphocytes, express specific receptors for the antigen. Because the immune system will respond to the antigens of both live and killed pathogens, it is possible to stimulate immunity without causing infection and/or disease (the basis of vaccination). Although this principle appears to be straightforward, vaccination does not always yield the expected result. Why some vaccines work and others fail is a complex issue, a major component of which is the nature of the antigen-specific receptors of lymphocytes.

Immunoglobulin: Antigen-Specific Receptor of B Lymphocytes

The antigen-specific receptor of the B cell is cell surface bound immunoglobulin, also known as antibody. An immunoglobulin molecule is composed of two identical light chains and two identical heavy chains that form a disulfide-linked Y-shaped molecule (Fig. 1.8). The light chain can be divided into two domains, a conserved carboxy-terminal domain and a highly variable amino-terminal domain. Analysis of heavy chains reveals a similar domain structure with the amino-terminal domain being highly variable and the presence of three constant domains. The antigen-binding region of an immunoglobulin molecule is formed by the association of the amino ends of a light and a heavy chain, and the carboxyl end of the heavy chain determines the isotype of the molecule. Similar to most species, five different immunoglobulin *isotypes* (classes) have been identified in the horse: IgM, IgD, IgG, IgE, and IgA (Table 1.4).[247-250] Before the availability of genetic characterization of the equine immunoglobulin heavy chain gene loci, at least four IgG subclasses were identified by physicochemical means and defined serologically by monoclonal antibodies as IgGa, IgGb, IgGc, and IgG(T).[251] More recently, seven IgG subclasses have been defined (IgG1–IgG7) and named based on the designation of the corresponding Ig heavy chain constant region genes.[248,250] The original IgGa corresponds to IgG1, IgGb to both IgG4 and IgG7, IgGc to IgG6, and IgG(T) to both

TABLE **1.4 Immunoglobulin Isotypes**

Isotype	Immunologic Function
IgM	Surface IgM is found on naive, activated, and memory B cells. Secreted IgM is a pentamer and represents the major antibody produced during a primary response. IgM efficiently mediates agglutination, neutralization, opsonization, and complement activation.
IgD	Antigen receptor of naive B lymphocytes. An IgD heavy chain gene has been identified in the horse, and it is expressed.
IgG	The principal immunoglobulin found in plasma representing up to 80% of the total immunoglobulin concentration. Seven subclasses of IgG have been identified (see text). The major functions of IgG include opsonization and neutralization reactions. IgG1, IgG3, and IgG4/7 are effective in fixing complement, and IgG1, IgG4/7, and IgG3/5 bind Fc receptors so they can function as opsonins and mediate antibody-dependent cellular cytotoxicity. IgG6 and IgG5 may play an important role in exotoxin neutralization and immunity to parasites.
IgE	Most IgE is found associated with the surface of mast cells and basophils and only very small amounts are present in the plasma. The cross-linking of two IgE molecules with specific antigen results in the degranulation of the mast cells and basophils. Thus IgE is the primary antibody responsible for type I hypersensitivity reactions and appears to play a central role in immunity to parasites.
IgA	The most abundant antibody in secretions (tears, mucus, saliva, colostrum, etc.) is a dimer composed of two IgA molecules joined by a J chain. IgA in the plasma is predominantly monomeric. IgA antibodies can be neutralizing but do not fix complement efficiently nor do they have efficient opsonization activity.

IgG3 and IgG5.[247,250] All seven IgG subclasses are expressed and functional, with IgG1, IgG3, IgG4, IgG5, and IgG7 able to elicit a respiratory burst in equine peripheral blood leukocytes (predictive of binding to Fc receptors), whereas IgG1, IgG3, IgG4, and IgG7 bind complement C1q and activate the classical complement pathway.[247] Some functional discrepancies exist between the new and original designations. For example, IgG(T) does not fix complement and inhibits complement fixation by IgGa (IgG1) and IgGb (IgG4/7).[252] This is not consistent with the complement fixing properties of IgG3, which, along with IgG5, corresponds to IgG(T).[247] Perhaps new reagents will provide clarification.

Membrane-bound immunoglobulin serves as the antigen-specific receptor for B lymphocytes (B-cell receptor [BCR]). Thus antibodies in serum are secreted soluble forms of BCRs. Each BCR contains a membrane-spanning region near its carboxy end that is inserted into the mRNA during differential splicing of the heavy chain exons. Membrane IgM and IgD comprise the BCRs on the surface of naive B lymphocytes, although the function of IgD is unclear. The IgD isotype is secreted in very small quantities, if at all, and is rarely detectable in the circulation. Once activated, B cells cease to express IgD but continue to express the membrane form of IgM. Early on in an immune response the B cell secretes large amounts of the pentameric form of IgM. As the immune response proceeds, the B cell will switch the isotype of its heavy chain. Isotype switching involves the substitution of one heavy chain constant region in place of another. In the horse, the genes encoding the 11 different constant (C) regions (five primary isotypes including the seven IgG subclasses) of the heavy chain are sequentially arranged on chromosome 24 in the following order: Cμ(M), Cδ(D), Cγ1(G1), Cγ2(G2), Cγ3(G3), Cγ7(G7), Cγ4(G4), Cγ6(G6), Cγ5(G5), Cε(E), Cα(A).[250] Initially, the first two constant region genes encoding the M and D constant regions are used to form the heavy chain. When switching occurs a new constant region segment is selected, and the intervening genes are removed either by splicing or looping out. Isotype switching only affects the heavy chain constant domains and has no effect on the antigen specificity of the immunoglobulin molecule. Isotype switching occurs in lymphoid follicles within the lymph nodes and spleen (and other secondary lymphoid organs) during a primary immune response and requires interaction with T lymphocytes. Cytokines secreted by these interacting helper T cells provide the signals driving class switching. For example, IL-4 and IL-13 induce isotype switching to IgE, and interferon-gamma (IFN-γ) inhibits this induction and augments IgG production. IgA is produced in response to the combination of the cytokines IL-4, IL-5, and transforming growth factor-β (TGF-β).

The antigen specificity of a particular antibody molecule (and the B cell that produces it) is determined by the combination of the variable domains of the light and heavy chains. The association of these two domains results in the formation of an antigen-binding cleft or pocket that contains regions of hypervariability that define the specificity of a particular antibody molecule. It has been estimated that over 10^8 different antibody specificities are possible. The generation of this tremendous amount of diversity in antibody specificity occurs during B-cell ontogeny in the bone marrow. Within a given B cell, the genes encoding the heavy and light chains of an antibody molecule are organized into specific gene segments. Thus the light chain is formed from variable (V_L), joining (J_L), and constant (C_L) gene segments, which together form the variable and constant domains of the light chain. In the germ line of an undifferentiated human cell one finds several hundred different V_L and several dozen J_L gene segments. In the horse, there are 204 V_L genes, 12 J_L genes, and 8 C_L genes.[253] Likewise the heavy chain of a B lymphocyte is composed of V_H, diversity (D), and J_H segments, which form the variable domain, and these join to the constant region genes (discussed earlier) to form the complete heavy chain molecule. Similarly, in the germ line of humans one finds a large number of V_H gene segments and a smaller number of D_H and J_H segments. There are 54 V_H, 40 D_H, and 8 J_H genes in the horse.[253] During the differentiation of a B cell (Fig. 1.9), there is the sequential selection and rearrangement of a V_L segment with a J_L segment and the accompanying deletion of intervening V_L and J_L segments (Fig. 1.10). The VJ segment is joined with a C gene, and the rearranged VJC sequence is then transcribed into mRNA and translated into the light chain. A somewhat similar sequence follows for heavy chains except that two

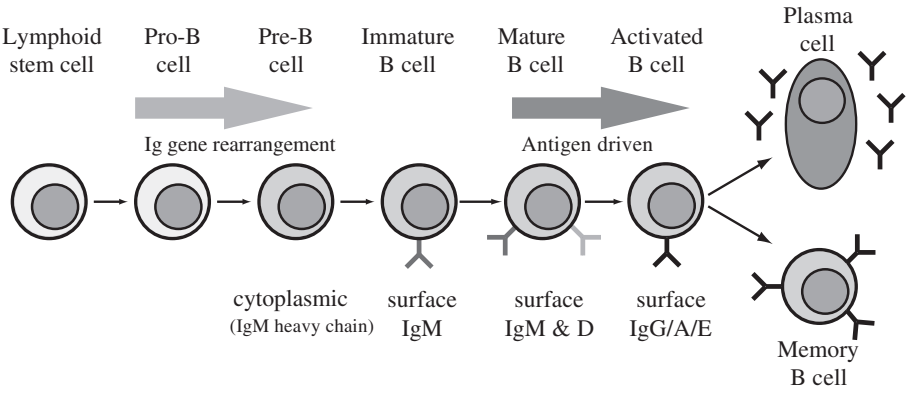


FIG. **1.9** B-cell differentiation. Different stages of B-lymphocyte development can be recognized by expression of immunoglobulin molecules. This maturation requires a series of gene rearrangements to select the genes that will encode the antigen binding part of the immunoglobulin molecule (variable region), and subsequently to select the genes that determine the class or subclass of the antibody molecule. Initially immature B cells express IgM (the majority of peripheral blood B cells), but after antigen exposure the B cell becomes activated and may express any of the immunoglobulin classes or subclasses. This decision depends in large part on cytokine signals from helper T cells. Activated B cells either mature into short-lived antibody-secreting plasma cells or become long-lived memory B cells.

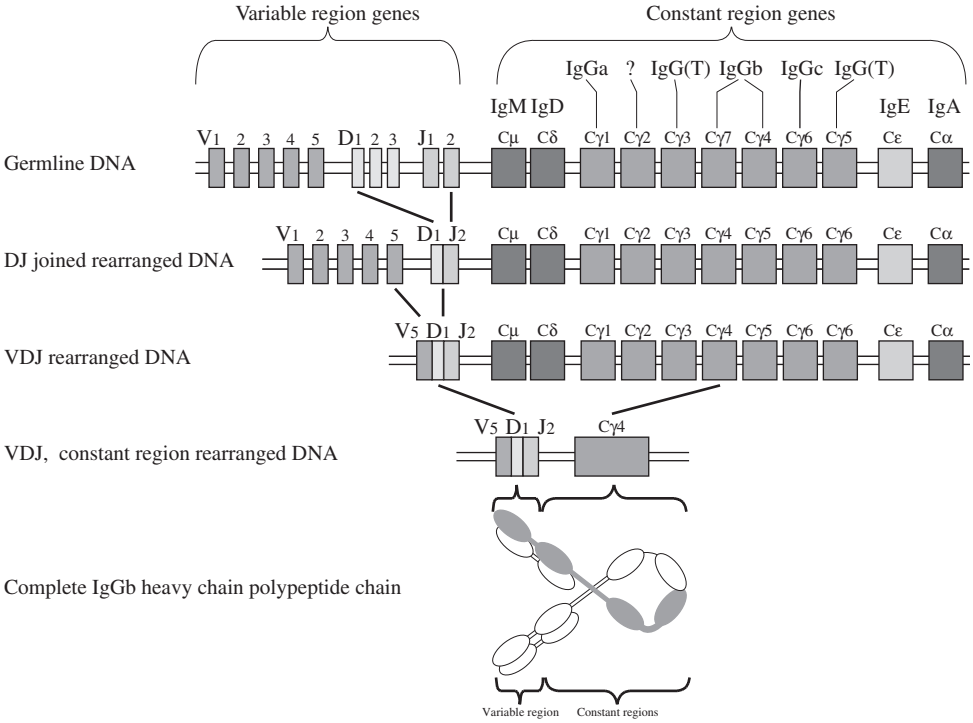


FIG. **1.10** Immunoglobulin gene rearrangement: somatic recombination process for production of an immunoglobulin heavy chain. The figure shows a hypothetical series of V, D, and J variable heavy chain genes, positioned 5′ to the known equine heavy chain constant region gene loci. In the first step in somatic recombination a D and a J gene segment are joined, and in the second step a V gene segment is joined to complete the VDJ recombination and form a gene capable of encoding the variable region. Subsequently one of the seven equine γ heavy chain constant regions, labeled with their corresponding IgG subclass, was selected to complete the gene rearrangement. Because the Cγ4 heavy chain constant region gene was selected, this leads to production of an IgG4 heavy chain.

rearrangements are necessary, a D to J rearrangement followed by a V to DJ rearrangement. Once completed, the VDJ segment is brought into proximity of the appropriate C_H segment and transcribed. During gene rearrangement, intervening deoxyribonucleic acid (DNA) is looped out and excised, which requires V(D)J recombinase. This enzyme is made up of RAG-1 and RAG-2 components, encoded by recombination-activating gene–1 and recombination-activating gene–2. To reconstitute the rearranged gene the cut ends of the DNA must be rejoined, which requires the enzyme DNA-dependent protein kinase (DNA-PK). Not all of the gene segment rearrangements produce functional genes. Because a B cell

has two sets of heavy chain genes, one on each chromosome, and most species have two different sets of light chain genes, including the horse,[253-255] there are several chances to form appropriate heavy and light chains. Once the heavy and light chain gene segments are successfully recombined, the genes on the sister chromosome neither recombine nor are they expressed. This process of allelic exclusion ensures that the B cell produces antibodies of a single specificity. Although this random assortment of gene segments accounts for much of the diversity in antibody specificity, additional mechanisms are also involved, including gene conversion (insertion of pseudogenes), junctional diversity (resulting from the imprecise joining of gene segments), and somatic mutations. Somatic mutations are point mutations in the hypervariable region of either the heavy or light chain that occur during the proliferation of antigen-activated B lymphocytes within germinal centers of lymph nodes and other secondary lymphoid organs and tissues. Such mutations play an important role in increasing antibody affinity for its antigen. Thus fewer than 500 genes can give rise to over 10^8 molecules of the various specificities needed to recognize the vast number of antigens the host may encounter.

T-Cell Receptor and CD3 Complex: Antigen-Specific Receptor of T Cells

T lymphocytes can be differentiated from B lymphocytes in that they do not express surface immunoglobulins but instead express the T-cell receptor (TCR). T cells also express another surface molecule called CD3. (The designation CD stands for cluster of differentiation and is the result of an international workshop to standardize the terminology used to describe leukocyte surface antigens recognized by monoclonal antibodies.) The TCR and CD3 form a multimeric complex on the T-cell surface, and this complex is involved in antigen-specific recognition. The TCR was originally described as a heterodimer comprised of an α chain and a β chain. Peptide mapping studies of the α and β chains from many different T-cell lines demonstrated that they contained variable and constant domains reminiscent of immunoglobulin structure. Further analysis indicated that, like immunoglobulin genes, TCR genes undergo gene rearrangements during T-cell development to generate tremendous diversity in antigen specificity. Subsequently, two additional TCR genes were identified, the γ chain and δ genes corresponding to a second heterodimer. Thus two types of TCRs exist, an αβ heterodimer and a γδ heterodimer. Although the percentages of T cells expressing the γδ receptor vary among mammalian species (ruminants and pigs have higher percentages of γδ T cells than other species), most T cells are αβ T cells with fewer than 5% of all T cells expressing the γδ receptor. The function of γδ T cells is poorly understood. In general, γδ T cells generate less diversity in their TCRs, can recognize nonpeptide antigens such as lipids, do not require major histocompatibility complex presentation for antigen recognition (see later discussion), and participate in inflammatory responses. They secrete cytokines and can have cytotoxic activity and may be important for recognizing antigens frequently encountered at mucosal surfaces and epithelial boundaries, at the interface between the host and the external environment.[207] As such they are thought to play an important role in immunologic surveillance. In the horse, $CD8^+$ T cells expressing the γδ TCR have been identified in peripheral blood.[256]

Analysis of the predicted amino acid sequences for the TCR proteins confirmed a structural similarity with antibody molecules. One peculiarity in the structure of the TCR was observed from the amino acid sequence analysis. Although both the α and β chains of the TCR contained a transmembrane region, both proteins had very short cytoplasmic tails. However, the TCR heterodimer is noncovalently associated with the CD3 complex of proteins. The five proteins of the CD3 complex are involved in signal transduction following TCR binding to antigen. Unlike the TCR α and β proteins, the CD3 proteins have large intracellular domains, some of which are phosphorylated in response to stimulation of the TCR (see later discussion). In addition to providing a signaling mechanism for the TCR, the CD3 complex is also required for the expression of the TCR heterodimer on the cell surface.[257]

The generation of diversity in the TCR during T-cell ontogeny employs a mechanism similar to that used to generate immunoglobulin diversity. The TCR α chains resemble immunoglobulin light chains in that they are composed of V, J, and C gene segments. The particular V, J, and C segments used are selected from a germ line configuration containing a few (C region) to several hundred (V region) gene segments. The selection and rearrangement of the gene segments are similar to those employed by the immunoglobulin light chain and appear to involve the same recombinases and DNA-PK. Likewise the β chains resemble heavy chains, each being composed of V, D, J, and C gene segments, and their selection and rearrangement from germ line genes also parallels immunoglobulin heavy chain rearrangement. Thus the generation of diversity is the result of the combination of multiple gene segments and junctional diversity. However, unlike immunoglobulins, the TCR genes do not undergo gene conversion or somatic mutations.

T-Lymphocyte Subsets

Mature T lymphocytes can be further divided into two distinct populations on the basis of their expression of either the CD4 or CD8 molecule.[258] The expression of these surface molecules is directly correlated with the specificity of the T cell (see later discussion). The expression of either CD4 or CD8 also correlates with T-cell function. Thus those cells that express CD8 are typically cytotoxic effector cells (cytotoxic T lymphocytes [CTLs]), whereas those that express CD4 are typically helper cells that produce cytokines that enhance antibody and cell-mediated immune responses. Whereas the T lymphocytes in the periphery express either CD4 or CD8, cortical thymocytes (immature T cells in the thymus) express both antigens. During maturation in the thymus, these cells convert to either $CD4^+$ or $CD8^+$ cells or they are eliminated (Fig. 1.11). Within the cortex of the thymus, thymocytes are exposed to thymic epithelial cells that present self peptides in the context of major histocompatibility complex (MHC) molecules (see later discussion). The thymic selection process involves both positive and negative selection. During positive selection, thymocytes with TCRs that bind self peptide–MHC complexes with low avidity (weakly) are stimulated to survive. This ensures that the developing thymocyte has a functional TCR. During negative selection, thymocytes that bind self peptide–MHC complexes with high avidity (strongly) are deleted. The end result is that mature T lymphocytes leave the thymus with functional TCRs capable of binding self MHC molecules without autoreactivity.

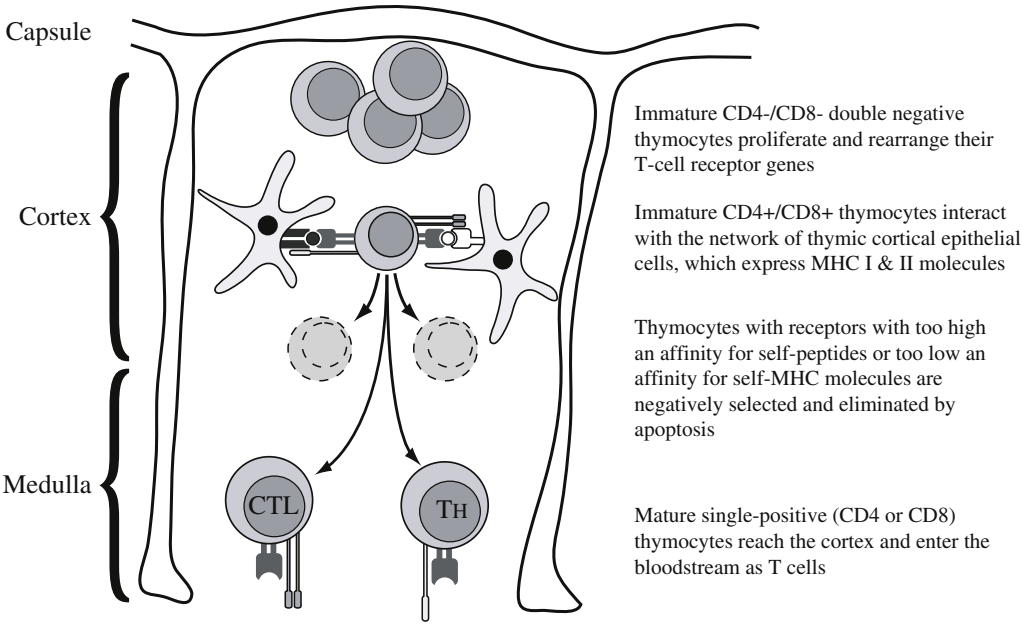


FIG. **1.11** T-cell maturation and selection in the thymus.

Major Histocompatibility Complex Molecules and Antigen Presentation

Unlike B cells and antibodies, which recognize antigens in solution or on cell surfaces in their native conformation, T cells only recognize processed antigen bound by antigen-presenting molecules on the surface of antigen-presenting cells (APCs). These antigen-presenting molecules are glycoproteins encoded by genes within a large gene cluster called the *major histocompatibility complex* (MHC), which also happens to be the most polymorphic gene region known in vertebrates. The MHC was originally defined in terms of its role in allograft rejection. Following the rejection of a primary allograft, antibodies that reacted with the allograft could be found in the recipient's sera. These antibodies could be used to identify or type tissues to determine the suitability of a donor for transplantation. Genetic analysis of the MHC region demonstrated that there were a number of closely linked genes encoding several different, though related, molecules that were involved in allograft rejection. These closely related genes are collectively referred to as MHC class I (MHC I) genes and their products as MHC I molecules. Similar to the anti–donor MHC antibodies found in allograft recipients, it was also demonstrated that multiparous mares had antibodies in their sera as a result of the exposure to paternal MHC antigens on the fetus.[259,260] These sera provided the earliest means to serologically define MHC I haplotypes (the complement of MHC alleles in a given individual) in horses.[259] Equine MHC I haplotypes are designated using the equine leukocyte antigen (ELA) prefix, similar to the nomenclature used in humans and other species (i.e., "HLA" in humans). In addition to the serologically defined MHC I molecules, another group of molecules were identified within the MHC that were involved in the stimulation of mixed lymphocyte responses and the control of immune responsiveness. These MHC class II (MHC II) molecules are structurally and functionally distinct from the MHC I molecules, but both are involved in T-cell recognition of antigen.

MHC I molecules are cell surface glycoproteins consisting of two noncovalently associated proteins, an MHC-encoded α chain (consisting of α1, α2, α3, transmembrane, and cytoplasmic domains) and β_2-microglobulin, a protein encoded outside of the MHC that stabilizes the molecule on the cell surface (Fig. 1.12). The α1 and α2 domains are variable regions encoded by polymorphic genes and form the antigen binding cleft of the molecule. MHC I molecules are expressed on the surface of most nucleated cells, with the highest level of expression on lymphoid cells and lower expression on fibroblasts, muscle cells, and neural cells. MHC I molecules are not detectable on early embryonal cells, placental cells, and some carcinomas. The level of expression of MHC I molecules can be modified by treatment with cytokines or infection with viruses, including equine herpesvirus–1 (EHV-1), which downregulates expression.[261] Interferons and TNF-α augment MHC I expression. This augmented expression is the result of increased production of MHC I mRNA, and the regulatory region of the MHC I genes has been shown to contain interferon and TNF-α–responsive elements that control the transcriptional activity of these genes.

The MHC I region of most species contains a large number of polymorphic genes within several different loci (locations on the chromosome). In humans there are three MHC I loci (A, B, and C) with 2000 to 3000 expressed alleles identified for each locus (Immuno Polymorphism Database; http://www.ebi.ac.uk/ipd). Comparably very few MHC I gene sequences are known in the horse. A recent BLAST query (February 2016) returned approximately 100 equine MHC I sequences, and fewer have been published (approximately 60).[262-264] Although seven loci were identified in MHC I homozygous horses (ELA-A3 haplotype),[264] a more recent study in horses representing 10 different haplotypes revealed that the number of loci differs by haplotype, with 4 confirmed and 3 provisional loci identified.[263] MHC I typing methods in horses include serologic typing,[259] reverse transcriptase polymerase chain reaction (RT-PCR) cloning and sequencing to identify

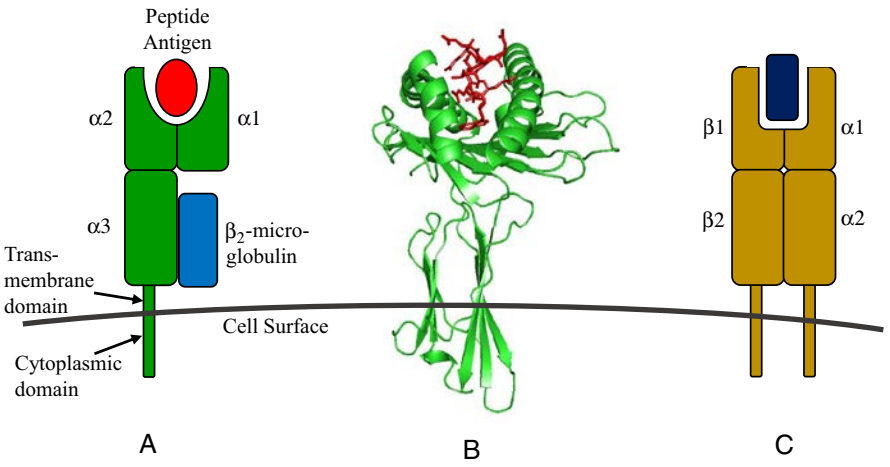


FIG. **1.12** MHC class I and II molecules . (A) Schematic depiction of an MHC molecule expressed on the cell surface, with peptide bound in the cleft formed by the α1 and α2 domains. (B) Molecular model of equine MHC class I molecule 7 to 6 (Eqca-N*00602) presenting the Rev-QW11 peptide, a known CTL epitope within the Rev protein of equine infectious anemia virus. β2 microglobulin not shown. (C) Schematic diagram of an MHC class II molecule expressed on the cell surface, with peptide bound in the cleft formed by the α1 and β1 domains.

specific expressed alleles,[263,265] and DNA microarray.[63] Most recently, MHC haplotypes have been identified using microsatellite markers.[266,267]

MHC I polymorphism is primarily localized in the α_1 and α_2 domains, with the α_3 domain being more conserved. The polymorphism of these two domains is related to their role in presenting antigen to T cells. The physiologic role of MHC I molecules was defined when it was discovered that CTL lysis of virus-infected cells was restricted to target cells expressing the same MHC I molecules as the CTL.[268] This observation led to the realization that T cells recognized the combination of self-MHC and foreign antigen. Furthermore, those T cells that recognized MHC I antigens invariably expressed the CD8 coreceptor. The nature of the association between MHC I and the foreign antigen remained unclear until x-ray crystallographic studies of human MHC I antigen were performed.[269] In addition to revealing the structural organization of the domains of the MHC I antigen, the image also revealed a cleft that lay between the α_1 and α_2 domains. It was proposed that this cleft binds the processed peptide epitopes for presentation to the T-cell receptor. Indeed, the cleft of the crystalized protein used for the x-ray diffraction studies was found to contain a contaminating peptide.[269] Other experiments showed that the incubation of cells with purified viral peptides resulted in the lysis of the cells by virus-specific MHC I–restricted CTL.[270] It is now known that the endogenous processing of viral antigens leads to the association of the viral peptides with MHC I antigens on the surface of the infected cell, and this is recognized by the TCR in association with CD8. How these viral antigens get to the cell surface is the result of a peptide transport system whose function is to transport processed peptides from the cytosol to the endoplasmic reticulum (ER). Foreign and other cytosolic proteins are marked for degradation by covalent linkage to a small polypeptide called ubiquitin. These ubiquinated proteins are targeted for entry into a proteasome, a cylindrical complex that degrades the protein into small peptides. These are then transported into the ER by the transporter associated with antigen processing (TAP). Once in the ER, peptides are handed off to newly formed MHC I molecules and stabilize a trimolecular complex with β2 microglobulin. This complex is then transported to the cell surface, where antigen presentation occurs. Because this is a normal cellular process for eliminating degraded proteins from the cell it is not surprising that MHC I molecules are normally loaded with these self-peptides. Indeed it is this encounter with MHC I loaded with self-peptides in the thymus that is responsible for the deletion of autoreactive clones during T-cell ontogeny. This unique peptide-binding characteristic of MHC I molecules has led to their use as immunologic reagents (tetramers) for the identification and enumeration of antigen-specific $CD8^+$ T cells.[271] In the horse, tetramers based on the equine MHC I molecule 7-6 (Eqca-N*00602), associated with the ELA-A1 haplotype, have been used to identify and quantify equine infectious anemia virus (EIAV)-specific cytotoxic T cells.[272,273] Similar approaches are in development to analyze CTL responses against other viruses and provide important information on the role these cells play in protection from these infections.

Peptides that bind in the MHC I binding cleft are typically 8 to 9 amino acids (aa) in length. Interestingly, three EIAV-specific CTL epitope peptides that bind to two closely related equine MHC I molecules associated with ELA-A1 haplotype are 11 to 12 aa long.[274] The two MHC I molecules, 7-6 (Eqca-N*00602) and 141 (Eqca-N*00601), differ in only one aa in the α2 domain, yet present peptides to CTL differently, resulting in differential CTL recognition. Molecular modeling suggests that these peptides bind in a bulged conformation and provides an explanation for the experimentally observed differential recognition by CTL.[274] Recently an independent group solved the crystal structures of these same MHC I molecules with the bound peptides.[275] This work confirmed the previous molecular modeling conclusions[274] and has provided the first crystal structures of equine MHC I–peptide complexes. In addition, a shorter peptide (9 aa) with similar anchor residues bound the clefts of these molecules in a more typical conformation.[275] Despite MHC I binding, this 9 mer peptide may not

be a CTL epitope. The original study confirmed experimentally that although 1 of the 12 mer peptides bound the Eqca-N*00601 molecule, there was no CTL recognition.[274] Recent work with the Eqca-1*00101 MHC I molecule (associated with the ELA-A3 haplotype) has further defined equine MHC I peptide binding motifs and has demonstrated a narrow peptide binding repertoire.[276] Moreover, $CD8^+$ T-cell recognition of a large panel of EHV-1 peptides that bound this molecule was restricted to a single 9 mer peptide. It will be of interest to determine whether any other equine MHC I molecules share the limited peptide binding and TCR epitope recognition properties of Eqca-1*00101, as this would have implications for the control of intracellular infections in horses with some MHC I haplotypes.

MHC II molecules are heterodimeric, transmembrane glycoproteins composed of an α chain and a β chain (see Fig. 1.12). A third chain, the invariant chain, has been shown to be associated with the MHC II molecule during assembly in the endoplasmic reticulum but is not expressed on the cell surface. Both the α and β polypeptides are encoded within the MHC region. Both polypeptides possess cytoplasmic, transmembrane, and two extracellular domains (α1, α2 and β1, β2). The α1 and β1 domains comprise the peptide binding cleft. The α chain has a single disulfide bond located in its membrane proximal (α2) domain, and the β chain has a disulfide bond in both of its extracellular domains. Structurally, the MHC II molecules resemble MHC I antigens and are also members of the immunoglobulin superfamily, a group of proteins that have structural similarities to immunoglobulin molecules, including conserved domains and variable antigen binding domains.

The MHC II genes are functionally and structurally distinct from the MHC I genes. Unlike MHC I molecules, MHC II molecules are restricted in their expression to certain cells of the immune system: B lymphocytes, dendritic cells, macrophages, and activated T lymphocytes of some species. Other cells may also express MHC II molecules after treatment with various cytokines.[277-279] Interferon-γ, TNF-α, 1,25-dihydroxyvitamin-D3, and granulocyte-macrophage colony-stimulating factor can induce MHC II molecule expression on monocytes and macrophages and other cells. IL-4 enhances MHC II expression on B cells. A number of agents have been shown to downregulate MHC II expression, including glucocorticoids, prostaglandins, and α-fetoprotein. Similar to MHC I, MHC II expression is regulated at the transcriptional level. The regulatory regions are quite different, however, and this probably accounts for the differences in tissue distribution for these two MHC molecules.

Like the MHC I genes, the MHC II region contains polymorphic genes encoding multiple MHC II molecules. There are loci for genes encoding the α chain (including DRA and DQA loci) and β chain (including DRB and DQB loci), with multiple alleles at most loci. In most species there is limited diversity in the α chain genes, with the β chain genes being the most polymorphic. For example, only two expressed alleles occur at a single DRA locus in humans, whereas there are multiple DRB loci containing over 1400 expressed alleles. Although the horse also has a single DRA locus, horses have greater DRA allelic diversity than any other species.[280-282] In addition, there are multiple DQA, DRB, and DQB loci, each with multiple alleles contributing to the level of MHC II diversity in equids.[282-285] Because of the critical role MHC II molecules play in antigen presentation to helper T cells (see later discussion), susceptibility to some diseases is associated with particular MHC II haplotypes. For example, analysis of microsatellite markers and direct sequencing has demonstrated that the MHC II haplotype is a risk factor for insect bite hypersensitivity in defined populations of horses.[286]

Whereas intracellular antigens are processed via the endogenous pathway and are associated with MHC I molecules that present them to $CD8^+$ cytotoxic T cells, extracellular antigens are processed via the exogenous pathway and are associated with MHC II molecules that present them to $CD4^+$ helper T cells (Fig. 1.13). Here endocytosed antigens, such as that phagocytosed by a macrophage, are degraded into peptide fragments within a phagolysosome compartment. Processed peptides then bind within the peptide binding cleft at the junction of the α_1 and β_1 domains of the MHC II molecule. This association of the epitope with the MHC II molecule protects it from further degradation. The MHC II molecule is then transported and expressed on the cell surface for subsequent presentation to the helper T cell. The immune system contains a distinct group of professional antigen-presenting cells called dendritic cells that are specialized to capture antigens and initiate T-cell immunity and move freely from epithelial surfaces to adjoining lymph nodes. Dendritic cells can be found in a variety of locations in the body and are often named based on their microscopic appearance. Thus interdigitating cells found in lymph nodes, veiled cells in lymphatics, and Langerhans cells in skin are all dendritic cells. Immature dendritic cells take up antigens by micropinocytosis using their extensive cellular processes or by receptor-mediated phagocytosis. This results in activation and migration to a regional lymph node where antigen presentation to T lymphocytes occurs. Dendritic cells in the spleen capture blood-borne antigens and similarly become activated, presenting antigens to T cells in the periarteriolar lymphatic sheaths within white pulp. Mature dendritic cells have high levels of MHC II expression on their surface. Although no longer phagocytic, they are extremely efficient stimulators of both MHC I– and MHC II–restricted T-cell responses in the draining lymph node or other secondary lymphatic tissue (Fig. 1.14).

In a complex antigen, certain epitopes are particularly effective at stimulating an antibody response. These *immunodominant* epitopes are often located at exposed areas of the antigen such as in polypeptide loops. These types of structures are often quite mobile and may allow for easier access to the antibody binding site. T-cell epitopes have been shown to possess a particular structural characteristic in that they involve the formation of amphipathic helices. However, structure alone does not determine the immunogenicity of a particular antigen, and T-cell recognition of foreign antigen requires more than just the expression of the processed antigen on the surface of the antigen-presenting cell. Additional signals provided by the antigen-presenting cell are also required for the activation of the T lymphocytes. Among these are signals provided by other accessory molecules found on the antigen presenting cell and various cytokines present in the extracellular environment.

Signaling through Antigen-Specific Receptors

The binding of a specific antigen by either the TCR of a T cell or the BCR of a B cell results in an intracellular signaling

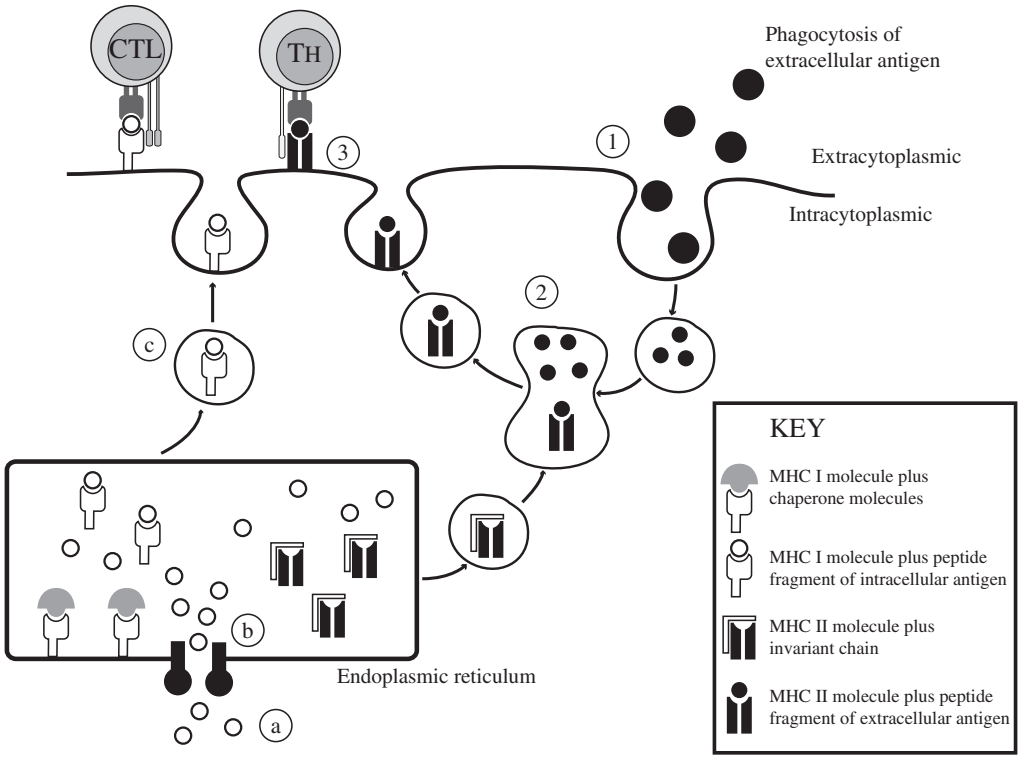


FIG. **1.13** Antigen processing pathways. This figure depicts MHC I antigen presentation to the left of the diagram, and MHC II antigen presentation to the right. In MHC I antigen presentation (a) peptides generated by degradation of proteins in the cytoplasm are transported into the endoplasmic reticulum by TAP (b). In this location, MHC I molecules anchored by calnexin bind the antigenic peptides, which allows release of the MHC I–peptide complex and transport through the Golgi to the cell surface (c). In MHC II antigen presentation, antigen is taken up by phagocytosis (1) into the endosome compartment that fuses with lysosomes for degradation. Vesicles containing MHC II molecules produced in the endoplasmic reticulum fuse with the endosomes (2), and the MHC II molecules bind with the degraded peptides for transport to the cell surface (3). The MHC II molecules are prevented from binding the endogenous peptides in the endoplasmic reticulum by the presence of an invariant chain, which is lost in the acidic endosomal environment.

cascade that eventually leads to the production of various cytokines and the proliferation of the stimulated cell. T-cell recognition of antigen involves the engagement of a TCR-CD4 or TCR-CD8 complex with processed peptide bound by an MHC II or MHC I molecule (Fig. 1.15). The engagement of the TCR with the appropriate MHC-peptide complex also results in the binding of CD4 (or CD8) to a dedicated region of the MHC II (or MHC I). In doing so the lck protein tyrosine kinase associated with the cytoplasmic tail of CD4 (or CD8) phosphorylates the cytoplasmic regions of the CD3 proteins (associated with the TCR) in regions known as immunoreceptor tyrosine–based activation motifs (ITAM). A series of subsequent phosphorylation events ultimately leads to the release of stored Ca from the endoplasmic reticulum. The increase in intracellular Ca levels and the activation of protein kinase C leads to the phosphorylation of various transcriptional factors that regulate the expression of the genes for various cytokines and/or their receptors. The process is subsequently downregulated by various phosphatases that are recruited to and subsequently dephosphorylate the CD3 ITAMs. A similar process occurs in B cells when their surface immunoglobulin receptor is cross-linked on binding to specific antigen. The reader is referred elsewhere for additional information regarding intracellular signaling in lymphocytes.[207,209]

Costimulatory Signals

In addition to the interaction of TCR-CD3, CD4/CD8, and MHC-peptide antigen complexes, other cell surface molecules are involved in signaling pathways. Of greatest importance is the interaction of CD28 on the T cell with B7 (CD80/86) on the antigen-presenting cell (APC). In the absence of CD28-CD80/86 costimulation, T cells are rendered functionally inactive or anergic. On restimulation, anergic T cells fail to proliferate or to produce cytokines such as IL-2 (a cytokine required for T-cell proliferation). The induction of anergy can be prevented by either the addition of exogenous IL-2 or, more important, by interaction of the CD28 cell surface antigen with its ligands (CD80/86). Stimulation of CD28 appears to be necessary for subsequent intracellular signaling events following TCR stimulation as CD28 cross-linking enhances various biochemical events triggered by TCR-mediated signaling. Other molecules, including the TNF-receptor family member CD40, regulate T-cell growth and cell death. The engagement of CD40 on the T cell with its ligand, CD40L (on APC), promotes cell survival and cell cycle progression. The binding of other members of this family, notably TNF-α, to their receptors on activated T cells typically results in the activation of a biochemical cascade of caspases that leads to apoptosis (programmed cell death). The cytocidal activity of these receptors is the result of

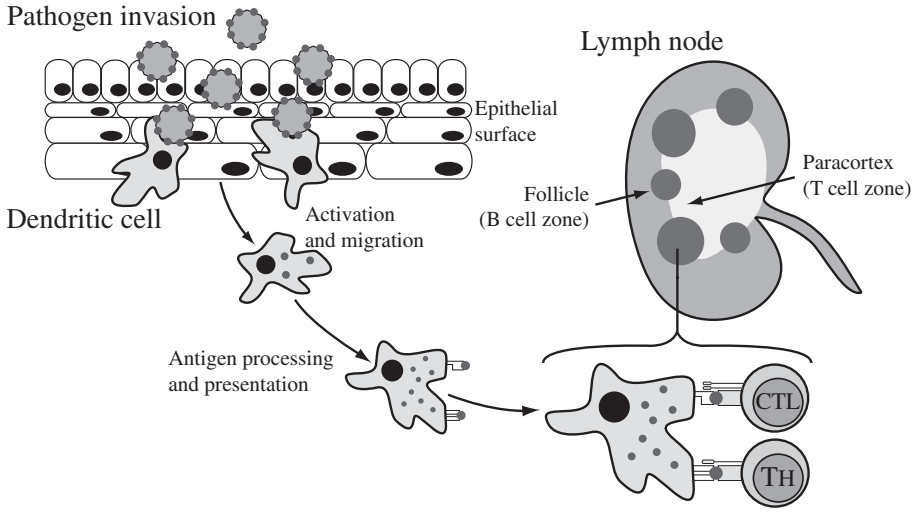


FIG. **1.14** The role of professional antigen-presenting cells (APCs). In this figure pathogen invasion is followed by antigen uptake by a dendritic cell, the most potent of the APC family. The dendritic cells become activated and migrate to a local lymph node where they are extremely effective at stimulating naive T cells in the paracortex, including both T-helper cells and CTLs.

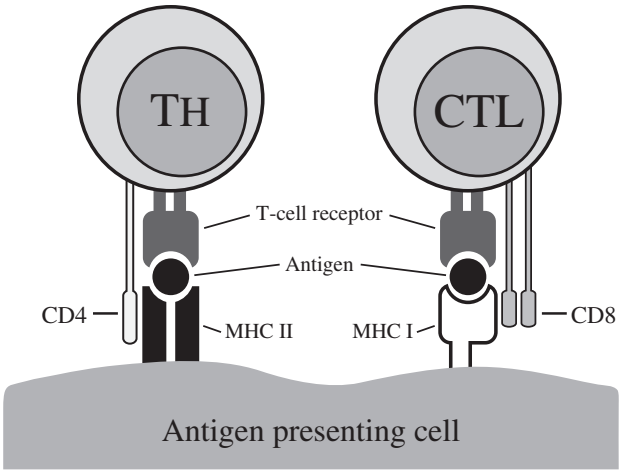


FIG. **1.15** MHC class I and class II restricted T cell recognition: the role of T-cell CD4 and CD8 molecules. T cells use their TCRs to recognize processed antigen presented in combination with either MHC I or MHC II molecules. T cells exclusively express either CD4 (T-helper cells) or CD8 (cytotoxic T lymphocytes [CTLs]). The CD4 molecule is required for interaction with MHC II molecules, and CD8 is required for MHC I interaction. As a result, helper T cells only recognize antigen presented by MHC II molecules, and CTLs only recognize antigen presented by MHC I molecules.

their intracytoplasmic portion of the receptor containing death effector domains. By contrast, CD40 lacks the intracellular death domains and instead has amino acid motifs that promote activation. In addition to their role in promoting T-cell activation and growth, both the CD28-CD80/86 and TNF-receptor pathways may also play a dominant role in the induction of specific T-helper cell subsets (see later discussion).

Cytokines and Cell-Mediated Immunity

As indicated previously, cytokines are hormone-like proteins that mediate a variety of cellular responses. Over 100 different cytokines and chemokines are known, and the reader is referred elsewhere for comprehensive lists of their designations and functions.[207,209] Table 1.5 provides an abbreviated list of cytokines with critical roles in innate and adaptive

TABLE **1.5 Selected Cytokines**

Cytokine	Biologic Activities and Source
IL-1	Multiple proinflammatory effects. Produced by macrophages. See Table 1.3.
TNF-α	Multiple proinflammatory effects. Produced by macrophages.
IL-2	Stimulates growth and proliferation of T cells. Produced by activated $CD4^+$ T cells, T_H1 cells, and some $CD8^+$ cells.
IL-4	Activates B cells and isotype switching to IgE and IgA. Produced by T_H2 cells. Promotes differentiation of T_H2 cells.
IL-5	Activates B cells and isotype switching to IgA. Recruits and stimulates eosinophil growth. Produced by T_H2 cells.
IL-6	Proinflammatory. Induces fever and the production of acute phase proteins by hepatocytes. Produced by macrophages. Also activates B cells and stimulates growth in stromal cells, fibroblasts, and a variety of other cell types.
IL-8	Now called CXCL8. A chemokine that is chemotactic for neutrophils. Produced by macrophages, endothelial cells, and various other cells.
IL-10	Inhibits the production of IL-12 by dendritic cells and IFN-γ by T_H1 cells. Suppresses macrophages and T-cell function. Produced by T_H2 cells and T_{regs}.
TGF-β	Produced by T_{regs} and other cells. Promotes differentiation of T_{regs}. Inhibits cell growth. Antiinflammatory. Inhibits T-cell proliferation and function. Promotes tissue repair. Induces isotype switching in B cells to IgA.
IL-12	Stimulates T_H1 differentiation. Augments NK cell and CTL function. Produced by dendritic cells and macrophages.
IFN-γ	Enhances phagocytosis and killing by macrophages. Produced by T_H1 cells. Enhances MHC expression and antigen presentation by APCs. Suppresses T_H2 responses. Induces isotype switching in B cells to IgG.
IL-13	Produced by T_H2 cells. Activates B cells and isotype switching to IgE. Inhibits T_H1 cells.
IL-17	Proinflammatory. Produced by T_H17 cells. Recruits neutrophils via induction of cytokine and chemokine production by other leukocytes, epithelia, endothelia, and fibroblasts.
IL-21	Produced by T_H17 cells and amplifies T_H17 responses. Promotes B-cell and T-cell responses.
IL-22	Proinflammatory. Produced by T_H17 cells. Induces chemokine production by epithelia and promotes epithelial barrier function. Induces production of defensins.
IL-23	Promotes T_H17 differentiation. Produced by dendritic cells and macrophages.

immune responses. A significant number of equine cytokines have been cloned and sequenced, and specific protocols are available to measure their expression. Numerous studies confirming the role of cytokines in equine sepsis, joint disease, airway diseases, and a variety of viral and bacterial diseases emphasize the similarities between equine and human immune systems.[206] As such, the potential for manipulating these responses using recombinant cytokines or anticytokine reagents is as applicable to equine medicine as it is for human medicine. The reader is referred to the U.S. Veterinary Immune Reagent Network (http://www.umass.edu/vetimm/equine) for information and links regarding cloned equine cytokines and other equine immune reagents currently available or in development.

Cell-mediated immunity is the component of adaptive immune defense mediated by T lymphocytes. Helper T cells are critical in these responses because depending on the cytokines they produce, they can activate macrophages to kill phagocytosed microbes, promote $CD8^+$ CTL responses, provide help for B cells in the production of antibodies, and, conversely, inhibit any of these responses. Helper T cells can be divided into distinct effector subsets based on the cytokines they produce. These subsets include T_H1, T_H2, and T_H17 cells, as well as a regulatory T-cell subset (T_{reg}), which has suppressive functions (see Fig. 1.7).

T_H1 $CD4^+$ Effector T Cells

The predominant cytokine produced by T_H1 cells is IFN-γ, but they also secrete IL-2 and TNF-α.[287] Although classically considered a T_H1 cytokine, IL-2 is produced transiently by $CD4^+$ T cells in general, within hours after their initial interaction with the APC during recognition of antigen and costimulators. IL-2 supports the stimulation, proliferation, and differentiation of all antigen-activated T cells very early in the immune response. Thus it is IFN-γ production that best characterizes T_H1 cells. The primary function of T_H1 cells is to activate macrophages to phagocytose and kill invading pathogens and to support the activation of CTL responses. Thus T_H1 cells are critical for controlling intracellular infections. In addition to its primary effect on activating macrophages to destroy microbes, IFN-γ produced by T_H1 cells enhances MHC antigen presentation and costimulation by APC, promotes activation of $CD8^+$ CTL, promotes further differentiation of T_H1 cells, inhibits the differentiation of T_H2 and T_H17 cells, and acts on B cells to promote isotype switching to IgG subclasses while it inhibits switching to T_H2-type isotypes such as IgE (Fig. 1.16). During antigen activation, $CD4^+$ T cells differentiate into T_H1 cells primarily during intracellular infections under the influence of IL-12 produced by dendritic cells and macrophages, as well as IFN-γ produced by other T_H1 cells and by NK cells.

T_H2 $CD4^+$ Effector T Cells

In contrast to T_H1 cells, T_H2 cells promote phagocyte-independent immune responses, including those mediated by eosinophils and mast cells, and they provide help to B cells for the induction of antibody responses. These responses are important for the elimination of extracellular infections, including parasitic infections and microbes infecting mucosal tissues. Detrimentally, T_H2 responses are involved in the development of allergic diseases. Antigen-activated $CD4^+$ T cells differentiate into T_H2 cells in the absence of IL-12 and IFN-γ, under the influence of IL-4 produced by other T_H2 cells, and perhaps by mast cells or even the $CD4^+$ T cell itself during initial activation. Regardless, T_H2 cells are best known

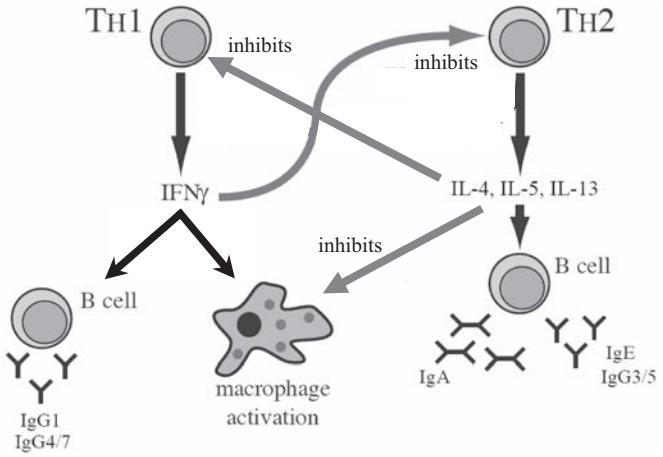


FIG. **1.16** T_H1 and T_H2 regulation. T_H1 lymphocytes provide help for macrophage activation, cytolytic activity, and production of a subset of IgG subclasses. T_H2 lymphocytes promote antibody responses, including IgA, IgE, and the remainder of the IgG subclasses. This is mediated by production of cytokines, which have a regulatory effect on each other.

for their production of IL-4, IL-5, and IL-13.[287] The combined effects of these cytokines include stimulation of isotype switching in B cells to produce IgE (IL-4 and IL-13), IgA (IL-4 and IL-5), and some IgG subclasses; further differentiation of T_H2 cells and suppression of T_H1 development (IL-4); recruitment of eosinophils and increased production of mucus in the gastrointestinal tract and airways (IL-4 and IL-13); and suppression of IFN-γ activation of macrophages and stimulation of alternative activation of macrophages with an antiinflammatory phenotype that promotes collagen synthesis and fibrosis (IL-4 and IL-13).

T-Helper Paradigms

The role of helper T-cell subsets and the cytokines they produce in promoting cell-mediated immune responses can best be illustrated in two scenarios, the first involving the induction of a T_H1 immune response in response to viral infection, and the second an allergic response to biting insect salivary antigens. In the first scenario, viral antigen present at the site of an ongoing infection in the lungs is processed by resident dendritic cells via the endogenous pathway (cytosolic viral proteins associated with intracellular infection), as well as the exogenous pathway (endocytosed virus and/or viral particles released by dead and dying infected cells). The exogenously processed epitope is presented on the surface of the dendritic cell in the context of an MHC II molecule to a naive $CD4^+$ T cell in a regional lymph node. The APC produces IL-12, which induces NK cells attracted to the site of the infection to produce IFN-γ that, along with antigen presentation and production of IL-12 by the APC, activates the T cell and drives it toward a T_H1 phenotype. Meanwhile, naive $CD8^+$ T cells encounter endogenously processed viral antigen on the surface of the APC in the context of MHC I molecules and become activated. Newly activated $CD4^+$ helper T cells (including T_H1 cells) produce IL-2, and differentiated T_H1 cells produce IFN-γ. The IL-2 drives the clonal proliferation of the activated $CD8^+$ T cells, and the IFN-γ stimulates $CD8^+$ T cells to become activated CTLs that produce additional IFN-γ. These activated CTLs lyse virus-infected target cells through the production of perforin and granzyme, which lead to apoptosis of the infected target cell via the activation of cytoplasmic caspases. In addition, IFN-γ secreted by T_H1 cells enhances phagocytosis and destruction of microbial invaders by macrophages. Meanwhile, virus-specific B cells have also encountered antigen and, within the paracortical areas of the regional lymph node, interact with the T_H1 cells by presenting antigen via MHC II molecules. The B cell and T_H1 cell also interact via costimulatory signals through CD28-CD80/86, as well as CD40-CD40L. This further stimulates the T_H1 cell to secrete IFN-γ, inducing the B cell to undergo isotype switching to produce IgG. Within germinal centers of lymphoid follicles in the lymph node cortex, activated B cells in the presence of antigen undergo somatic hypermutation of their BCR variable region genes as part of the affinity maturation process. This results in selection and expansion of B-cell clones that produce antibodies with the highest affinity for the viral antigen. Finally, these B cells differentiate into high-affinity IgG-secreting plasma cells. This combination of high-affinity IgG antibodies and $CD8^+$ CTLs serves to eliminate the virus.

In the second scenario, the introduction of salivary antigens into the skin by the bites of blood-feeding *Culicoides* midges results in the exogenous processing of antigen by dendritic cells. However, in the absence of IL-12 and interferon-γ, and likely in the presence of IL-4, there is the induction of T_H2 cells that produce additional IL-4, IL-5, and IL-13. These cytokines inhibit T_H1 responses and cause antigen-specific B cells to isotype switch to IgE antibodies that bind to mast cells. Subsequent exposure to antigen causes degranulation of these mast cells as the result of antigen cross-linking surface-bound IgE. Mast cell release of vasoactive mediators such as histamine, lipid mediators such as PGE_2, and additional production of IL-4, which exacerbates the response and leads to the accumulation of eosinophils, further contributes to the inflammatory process. The end result in some horses is a seasonally recurrent allergic dermatitis known as insect bite hypersensitivity (IBH; see Hypersensitivity and Autoimmunity later in this chapter). Strong evidence for this paradigm as it relates to susceptibility to IBH is provided by a recent study in IBH-affected and unaffected ponies.[288] Following intradermal injection of *Culicoides* antigen, IL-4 expression was increased in skin biopsies from IBH-affected ponies, whereas IFN-γ expression increased in those from unaffected ponies. Moreover, antigen-specific

stimulation of peripheral blood CD4+ T cells demonstrated a higher percentage of IFN-γ producing CD4+ T cells in the unaffected ponies. Finally, the serum of all ponies contained similar levels of antigen-specific antibodies, but the affected ponies had higher titers of antigen-specific IgE. These results indicate that IBH affected ponies are T_H2 biased with respect to *Culicoides* antigen and that a T_H1-biased response in unaffected ponies is protective against IBH.

In general, T_H1 and T_H2 responses along with their respective roles in immune protection and susceptibility to disease have been well described in horses.[206,208,249,289,290]

T_H17 CD4+ Effector T Cells

The function of T_H17 cells is to recruit neutrophils and induce inflammation, primarily to destroy extracellular bacteria and fungi. Therefore T_H17 cells represent an interaction between the adaptive and innate immune systems. Although T_H17 responses are important in resolving extracellular infections, they also significantly contribute to the pathogenesis of inflammatory diseases. As discussed earlier in the section on innate immunity, PAMPs associated with invading microbes bind pattern recognition receptors on macrophages and dendritic cells, leading to the production of proinflammatory cytokines. During such an infection, the combination of cytokines produced by dendritic cells, including IL-1, IL-6, and IL-23, along with IL-21, promotes the differentiation of activated CD4+ helper T cells to the T_H17 phenotype. Strong T_H1 and T_H2 responses tend to inhibit T_H17 differentiation. Once differentiated, T_H17 cells produce IL-17, an important contributor to the strong acute inflammatory reaction that sometimes occurs during T-cell responses (immune inflammation). IL-17 induces neutrophilic inflammation by stimulating the production of other cytokines and chemokines (such as TNF-α and CXCL8) that attract neutrophils to the site and also stimulates the production of antimicrobial substances (defensins) by numerous other tissue cell types. T_H17 cells also produce IL-22 and IL-21, which further promote inflammation, stimulate production of defensins, and improve barrier function in epithelial tissues. In humans, T_H17 cells have been implicated in a variety of autoimmune and inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease.[291,292] In horses, T_H17 cells may be involved in the pathogenesis of inflammatory bowel disease,[293] recurrent uveitis,[294] and recurrent airway obstruction.[295,296] Finally, peripheral blood mononuclear cells (PBMCs) and bronchoaveolar lavage cells from foals within the first 3 weeks of life increase expression of IL-17 mRNA in response to *Rhodococcus equi* stimulation in vitro.[297] Whether T_H17 cells play a role in the pathogenesis of *R. equi* pneumonia is currently speculative.

Regulatory T Cells

Regulatory T cells (T_{reg}) are a specialized subset of CD4+ T cells that play important roles in suppressing immune responses and maintaining self tolerance. T_{regs} express high levels of the IL-2 receptor α (CD25) and the transcription factor FoxP3, which is critical for their development and function. Thymic T_{regs} (also known as natural T_{regs}) are generated by self recognition in the thymus. Although these cells are directed against self antigens, they do not induce autoimmune responses. Rather, they inhibit other T cells recognizing self antigens in the same tissue. Peripheral T_{regs} (inducible T_{regs}) are generated from naive CD4+ T cells in peripheral secondary lymphoid tissues in response to particular antigens. These could be self or foreign antigens, and the antigen-specific peripheral T_{regs} inhibit other T cells recognizing the same antigen. Development of some T_{regs} requires TGF-β, and T_{regs} are dependent on IL-2 for survival and maintenance of function. T_{regs} suppress immune responses by producing inhibitory cytokines (TGF-β and IL-10), by reducing the ability of APCs to stimulate T cells (through CD80/86 interactions), by triggering apoptosis of effector T cells, and by disrupting metabolic pathways.[298] Although TGF-β has multiple effects on the immune system, its suppressive effects are due to its ability to inhibit the proliferation and function of T cells and inhibit the activation of macrophages. IL-10 has several suppressive properties, including inhibiting IL-12 production by dendritic cells and macrophages and reducing expression of MHC and costimulatory molecules on APCs.

A variety of human autoimmune diseases are associated with loss of T_{reg} function, including systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, rheumatoid arthritis, psoriasis, and inflammatory bowel disease.[298] In the horse, reagents and methods are available for the identification of T_{regs}.[299-301] Equine T_{regs} produce IL-10 and TGF-β and demonstrate suppressive function[302] and may be involved in trophoblast T-cell tolerance during pregnancy in the mare.[300] In foals T_{regs} appear to have higher suppressive activity than those derived from adult horses.[303] Finally, failure of T_{reg} suppression could be involved in several inflammatory/allergic diseases in horses, including IBH,[304-306] inflammatory bowel disease,[293] and possibly recurrent airway obstruction.[307] During persistent infections, such as HIV-1 infection in humans, T_{regs} may promote persistence by suppressing virus-specific T cells.[308] Whether this occurs during persistent infections in horses is under investigation.

Lymphocyte Trafficking Pathways

Leukocyte trafficking has been reviewed previously, with a particular emphasis on the innate immune response and the migration of neutrophils into inflamed tissues. Lymphocytes involved in adaptive immune responses differ in their migration from most other cells, in that they recirculate instead of making one-way trips. Memory and naive T lymphocytes, with their different capacities for response to antigens, differ also in their migration pathways through the body. Two general pathways of lymphocyte recirculation have been demonstrated. Naive T lymphocytes take the most common route, which involves entry into the lymph node by extravasation from the high endothelial venule (HEV) and return to the peripheral circulation via the efferent lymphatic. The endothelial cells of the HEV have a distinctive appearance, have specialized receptors, and can support a lot of lymphocyte migration. This allows rapid repeated circulation of naive lymphocytes through lymph nodes where there is the greatest chance of exposure to their specific antigens. Memory lymphocytes, on the other hand, leave the bloodstream in peripheral vascular beds, particularly in inflamed tissues, and return to lymph nodes via afferent lymphatics. This leads to the exposure of primed memory lymphocytes to the most likely early sites of antigenic encounter and allows for an early response to recall antigens. Thus memory lymphocytes are most common in inflammatory lesions and in the epithelial surfaces of the lung and gut wall. Differing expression of the adhesion and homing molecules may play an important role in mediating these different migration pathways.

For lymphocytes to follow the maturation and migration pathway outlined previously, the first step is for the naive lymphocyte to get into a lymph node so that it can meet its antigen on a professional APC. To achieve this the T lymphocyte needs to exit in the HEV. The naive lymphocyte expresses L-selectin, and this can bind to the vascular addressins GlyCAM-1, CD34, and MAdCAM-1, which are expressed on HEVs and promote rolling similar to that mediated by P- and E-selectin when they bind to phagocytes. These molecules are expressed on a variety of tissues, but in HEVs they have specific patterns of glycosylation that make them bind L-selectin. These differences represent the key to the specificity of the migration of lymphocytes to HEVs. This weak interaction initiates the process of extravasation, promoted by locally bound chemokines (i.e., CXCL8), which increase the affinity of the lymphocyte integrins for their ligands.

Approximately 25% of lymphocytes passing through an HEV leave, and this could mean 1.4×10^4 cells in a single lymph node every second, and in the body 5×10^6 lymphocytes may extravasate through HEVs every second (human). The "sticking" process (rolling, activation, arrest) takes a few seconds, with transendothelial migration and passage through the HEV basement membrane occurring in about 10 minutes. Most T cells after leaving the blood travel through the lymph node uneventfully and leave via efferent lymphatics; however, in rare events a naive T cell recognizes its specific peptide/MHC complex and becomes activated, eventually leading to formation of effector and memory T cells. That process takes 4 to 5 days, and once activated the migration pathway of memory T cells differs considerably from naive cells. All activated T cells lose the L-selectin molecules that mediated homing to lymph nodes and increase the expression of other adhesion molecules. The homing of individual lymphocytes to specific sites is regulated by expression of specific adhesion molecules. Memory cells are specifically attracted to areas of inflammation because of the increased levels of adhesion receptor ligands expressed on vascular endothelium in these regions. This is typically a result of TNF-α production by regional macrophages encountering infections. Sometimes infections do not result in TNF-α production, but memory cells also migrate randomly throughout the body. When they encounter their antigen, they can produce cytokines such as TNF-α themselves, which in turn causes local endothelial cells to increase expression of E-selectins. This will subsequently recruit more effector and memory cells to the region.

Mucosal Immunity

The mucosal immune system comprises a series of distinct compartments within the immune system, which are adapted to immunologic responses in unique environments such as the gut, respiratory tract, or urogenital tract. The mucosal immune system is perhaps the most important component of the adaptive immune system, and the reader is referred elsewhere for an appropriately detailed description of its general features[209] and of its role in equine immunity in the context of respiratory disease.[309] The mucosal immune system may represent the original vertebrate immune system, and it certainly protects the largest vulnerable area of the mammalian body and comprises a very large proportion of the total lymphocyte populations and immunoglobulin pool.[310,311]

The mucosal immune system consists of organized and dispersed lymphoid tissues that are closely associated with mucosal epithelial surfaces, and mucosal immune responses generated in one location are transferred throughout the mucosal immune system by lymphocytes programmed to home to regional effector sites. The principal immunoglobulin produced by the mucosal immune system is secretory IgA, which, in humans, is the most abundant immunoglobulin class in the body. Secretory IgA has unique adaptations that promote transport out onto mucosal surfaces, where it protects the body from bacteria and viruses principally by immune exclusion, that is, by physically preventing attachment to mucosal surfaces. The importance of mucosal IgA has already been demonstrated in immunity to numerous equine diseases.[309] Secretory IgA (sIgA) is formed by dimerization of two IgA monomers, which are attached by means of disulfide bonds to a J chain also produced by the same plasma cell that secretes the IgA. This confers the advantage of increased valency to sIgA, which can bind up to four of its targets, increasing its agglutinating ability. Immunoglobulin A is relatively "noninflammatory" (i.e., does not fix complement as effectively as IgG1 or IgG4/7), consistent with a role in defense by immune exclusion. Similarly, although myeloid cells possess Fc receptors for IgA it is not clear that it functions as an efficient opsonin or promotes phagocytosis.

Coordination of the mucosal immune response depends on organized mucosal-associated lymphoid tissue (MALT), principal examples of which are the pharyngeal tonsils and the intestinal Peyer's patches. In the gastrointestinal tract MALT is distributed throughout the gut, but in the respiratory tract these tissues are only found in the nasopharynx and oropharynx. MALT consists of lymphoid follicles containing IgA-committed B cells, surrounded by interfollicular T-cell areas with APCs and HEVs, with an overlying follicle-associated epithelium (FAE). Naive lymphocytes enter the MALT by extravasation from the HEVs (there are no afferent lymphatics in MALT), and after antigen encounter in the MALT they leave through efferent lymphatics. The FAE is specialized for antigen sampling, by having reduced secretion of mucus, and through the presence of specialized antigen uptake cells termed *microfold* or *M cells*. These M cells are typically closely associated with underlying aggregates of lymphocytes, often within large basolateral membrane pockets, and play a critical role in mucosal immune surveillance. Adherent macromolecules or particles bound to the apical M-cell membrane undergo endocytosis or phagocytosis and are released at the pocket membrane, where antigen presentation is initiated by dendritic cells, resulting in activation of antigen-specific B cells (Fig. 1.17). Subsequent trafficking and recirculation of memory IgA–positive B cells to the other components of the mucosal immune system (respiratory tract, intestinal tract, etc.) are responsible for the dissemination of local mucosal IgA responses throughout what is termed the *common mucosal immune system*. After homing of these B cells to effector sites such as the lamina propria of the gut and respiratory tract, and extravasation into the lamina propria from HEVs, further antigen encounter and second signals from APCs and from helper T cells result in further differentiation into IgA-producing plasma cells. The short half-life of IgA-secreting plasma cells requires a constant generation of precursors in induction sites and flow to effector sites. Antigen sampling and presentation are not restricted to organized MALT, as throughout the mucosal surfaces dendritic cells play a key role in antigen uptake and presentation, subsequently migrating to local lymph nodes or MALT to initiate immune responses.

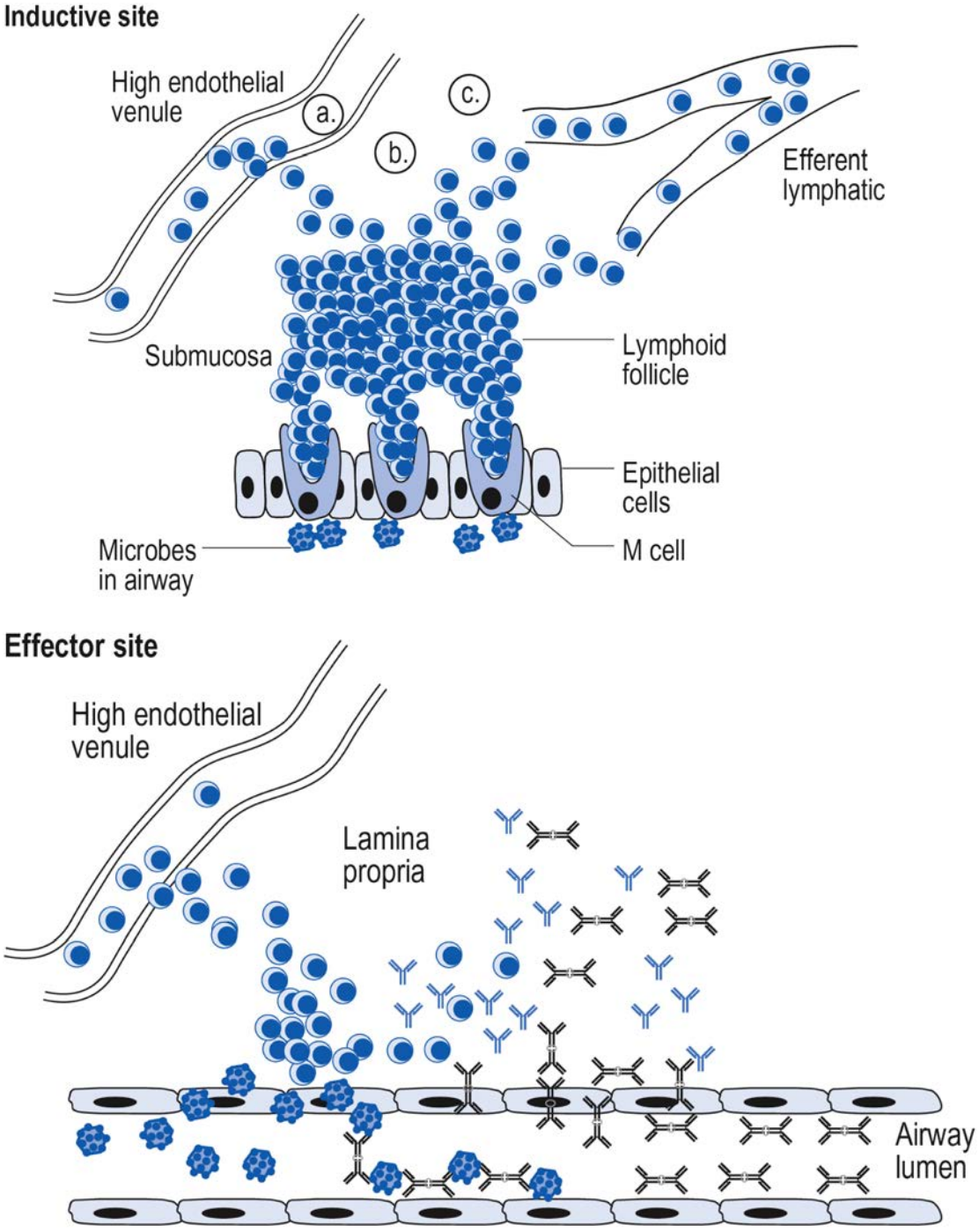


FIG. **1.17** Initiation of mucosal immune responses. Respiratory mucosal immune responses typically originate after antigenic encounter at inductive sites, which are the tonsils of the nasopharynx and oropharynx in the horse. Naive lymphocytes enter the inductive sites from high endothelial venules via the specialized cuboidal endothelium of those vessels in response to specific molecular signals. Antigens, such as microbes, are taken up by microfold or M cells, which are part of the highly specialized follicle-associated epithelium present at these sites. Antigenic material is transported across the M cell, and antigen presentation to B and T lymphocytes is accomplished by dendritic cells in the underlying tissues. The underlying lymphoid follicle is composed primarily of B lymphocytes, surrounded by areas of T lymphocytes. Antigen-specific B lymphocytes become committed primarily to IgA production at these sites, although some IgG-producing B lymphocytes are also generated. Subsequently, the primed lymphocyte populations exit the inductive site via efferent lymphatics, eventually reaching the blood circulation through the thoracic duct. These cells traffic to HEVs of effector sites throughout the respiratory epithelium and extravasate to make up the intraepithelial lymphocyte and lamina propria lymphocyte population and to give rise to lymphoid aggregates. Subsequent antigen encounter results in terminal differentiation to plasma cells, primarily IgA producing, although some IgG plasma cells are also formed. IgG is largely restricted to tissues, but secretory IgA is transported to the respiratory epithelial surface where it can agglutinate and neutralize infectious organisms. (From Lunn et al.,[309] with permission.)

After release of secretory IgA by plasma cells into the interstitium, it is bound by the polymeric Ig receptor on the abluminal surface of epithelial cells. Subsequently the sIgA is transported across the epithelial cell and released at the luminal surface together with the secretory component formed by cleavage of part of the polymeric Ig receptor. The secretory component can also be found in a free form in mucosal secretions. The secretory component confers resistance to proteolytic enzymes found in the respiratory and gastroenteric environment, some of which are secreted by pathogens, and prolongs the longevity of sIgA. During its transit through the epithelial cell sIgA can neutralize intracellular infections encountered in the endosomal compartments of cells. In addition, sIgA can bind antigens in the submucosa and transport or excrete them to the mucosa by this mechanism. The majority of the IgA in the mucosa is dimeric sIgA, whereas the bone-marrow–derived IgA in circulation is predominantly monomeric.

In the horse our understanding of the architecture and functions of the mucosal lymphoid system is best developed for respiratory lymphoid tissues.[309] Although lymphoid tissues are distributed throughout the respiratory tract, the greatest masses comprise the nodular lymphoid tissue of the nasopharynx and oropharynx, which can have an overlying lymphoepithelium specialized for antigen uptake and processing as in the case of tonsillar tissues. Additional nodular lymphoid tissues are typically present at sites where antigen-laden mucus and air currents converge throughout the trachea and bronchi and is called bronchus-associated lymphoid tissue. Tonsils represent the most complex mucosal nodular lymphoid tissues. Horses possess all of the various tonsillar tissues that are recognized in other species, and they are anatomically complex.[309] The nasopharyngeal tonsil is the largest mass of lymphoid tissue in the respiratory tract of horses of all ages, and its epithelium has been extensively characterized.[312] This epithelium has a classical FAE and is heavily folded, forming crypts, and also contains M cells. The nasopharyngeal tonsil exists in the dorsal recess of the nasopharynx and extends ventrally toward the opercula on either side of the nasopharynx. Therefore it is ideally placed for the sampling of antigens before entry to the airways or alimentary tract and may serve as an important target for intranasal vaccines. This tissue appears to be most abundant in young foals and atrophies with age, although many lymphoid follicles remain throughout the nasopharynx.

The nasopharyngeal epithelium also contains numerous lymphocytes. Immunohistochemical studies indicate that the majority of these lymphocytes are $CD8^+$ T lymphocytes, although B lymphocytes are also present.[312] The contribution of these lymphocytes to mucosal cellular immune defenses of the upper respiratory tract is poorly studied. However, following intranasal challenge of yearling and 2-year-old horses with EHV-1, virus-specific cytotoxic activity is detectable in several mucosal lymphoid tissues of the upper respiratory tract, as well as the local draining lymph nodes, and is particularly evident in the nasopharyngeal lining.[313] This cellular immune response is presumably mediated by $CD8^+$ T lymphocytes found in the nasopharyngeal epithelium and underlying lamina propria and may provide an important contribution to the clearance of infectious virus from the upper respiratory tract.

Ontogeny of the Equine Immune System

There have been few studies of the prenatal development of the equine immune system. As in other species, the thymus is the first lymphoid organ to develop, and mitogen-responsive cells can be identified there from day 80 of the 340-day gestational period of the horse.[314] Subsequently these cells appear in peripheral blood at 120 days, lymph nodes at 160 days, and the spleen at 200 days. Cells responsive in mixed lymphocyte reactions are detectable in the thymus from 100 days and the spleen at 200 days. Immunoglobulin production is detectable before 200 days of gestation, and newborn foals have IgM and IgG in their serum.[314-317] Presuckle serum concentrations of these immunoglobulins in the newborn foal are low. The mean concentration of IgM is 31.7 mg/dL, and that of IgG (comprised of IgG1 and IgG4/7) is 7.5 mg/dL.[316] Overall it appears that functional T lymphocytes are present by day 100 and B lymphocytes by day 200 of gestation. Immunologic competence of the equine fetus has been assessed in terms of specific antibody responses. In utero immunization of foals in late gestation with keyhole limpet hemocyanin in an alum adjuvant results in detectable specific antibody production and T-cell responsiveness at the time of birth.[318] In addition, the equine fetus can respond to coliphage T2 at 200 days and Venezuelan equine encephalitis virus at 230 days.[319,320]

Although detailed studies are few, some data regarding the maturation of thymocytes in young horses are available. As discussed, stem cells migrate into the thymus and mature into T cells under the influence of the epithelial microenvironment. In this process different patterns of cell surface molecule expression distinguish successive stages of thymocyte maturation. In humans, the earliest thymic precursor cells express low levels of CD4. This CD4 expression is lost as early thymocytes become double-negative $CD4^-CD8^-$ cells and then demonstrate their T-cell commitment by TCR-β gene rearrangement, which is an essential trigger for subsequent events and leads to low levels of expression of a cell surface TCR-β–CD3 complex. Intermediate thymocytes are $CD4^{lo}CD8^{lo}$, but after TCR-α gene rearrangement and expression of cell surface αβ TCR they rapidly become $CD4^{hi}CD8^{hi}TCR–CD3^{hi}$. Subsequently, thymocytes selected on the basis of productive TCR gene rearrangement and lack of self reactivity become mature T cells expressing either CD4 or CD8 (single positive) in combination with high levels of TCR-CD3. Maturation of T cells and similar patterns of T cell CD3, CD4, and CD8 expression can be demonstrated in the equine thymus.[321-324]

Mature B and T cells are present in the equine fetal spleen and lymph nodes by 90 to 120 days of gestation.[316] B cells are IgM^+, consistent with their naive status, and do not occur in germinal centers. Both $CD8^+$ and $CD4^+$ T cells are present in these tissues. By the time foals are born, $CD8^+$ and $CD4^+$ T cells are abundant in these secondary lymphoid tissues, as are IgM^+ B cells within well-developed germinal centers.[316]

Immunocompetence in Foals

Although normal foals are immunocompetent at birth they are immunologically naive, and infectious disease in neonatal foals is associated with high morbidity and mortality. Although failure of passive transfer is a major cause of this problem, as discussed later, immaturity of the immune system has also been considered a potential contributing factor. As a result numerous studies of neonatal immunocompetence have been completed and reviewed.[249,325,326]

Innate Immune Responses in Foals

Multiple studies indicate that expression of TLRs, proinflammatory cytokines, and acute phase proteins in foals is

comparable to that of adults.[326] A number of studies have reported neutrophils to be fully functional from birth,[327-329] but their function is significantly impaired before absorption of colostral antibodies, which are required for opsonization.[329,330] Killing by foal neutrophils is reduced in the first 2 weeks of life, as is phagocytic ability when assays are performed using autologous serum.[331] However, when serum from adult horses is used, neutrophil phagocytosis and oxidative burst capacity are normal and similar to those of adults from birth through 4 months of age.[331,332] These studies indicate that neutrophil function in foals depends on immunoglobulins and/or complement with adequate opsonizing activity. Another study of foals less than 7 days of age indicates that phagocytosis and oxidative burst activity of neutrophils is reduced compared with adult levels, even when adult serum is used.[333] Although the assays were similar, these apparently disparate results may have been due to the procedures used. Regardless, the importance of complement in foals is illustrated by the finding that the opsonic capacity of foal serum for bacteria is halved when complement is inactivated by heat.[220] Interestingly, complement activity in the first week of life is considerably elevated in colostrum-deprived foals, possibly as an alternative defense mechanism.[334] In bovine colostrum-fed foals, serum complement levels reach adult levels by 1 to 3 weeks of age.[335] Finally, alveolar macrophages recovered from bronchoalveolar lavages may be low in number up to 2 weeks of age and have impaired chemotactic function.[336]

Taken together, available studies indicate that innate immunity is intact in foals and leads to functional neutrophil responses similar to those in adults but that adequate levels of immunoglobulins and complement with opsonizing activity are critical.

Adaptive Cell-Mediated Immunity in Foals

Several studies have measured lymphocyte numbers and subpopulations in foals.[332,337,338] Foals are born with B and T lymphocytes, and with $CD4^+$ and $CD8^+$ T-lymphocyte subsets. Lymphocyte counts rise in the first 4 months of life, and the proportion of B lymphocytes increases. Although proliferation of peripheral blood T and B lymphocytes is lower during the first few weeks of life, it rises to adult levels by 4 weeks of age.[332] During the first 3 months of life, follicular development and the density of B- and T-cell zones increases in secondary lymphoid tissues.[336] In contrast, organized lymphoid tissue is not present in the lungs at birth, and very few lymphocytes are present in bronchoalveolar lavage (BAL) samples during the first week of life.[326,332] The appearance of organized lymphoid tissue in the lungs, along with T and B cells in BAL samples, gradually increases during the first 3 months. These factors likely increase the susceptibility of foals to respiratory infections during the neonatal period.

Compared with adult horses, foals exhibit reduced levels of IFN-γ production in stimulated BAL cells and PBMCs throughout the first 6 months of life, and this reduced T_H1 response might contribute to their susceptibility to *Rhodococcus equi* and other intracellular pathogens.[339] Adult horses immunized with an experimental EIAV plasmid DNA vaccine developed antigen-specific antibodies, lymphoproliferative, and CTL responses, whereas foals immunized within the first week of life with the same construct (and boosted at the same intervals as the adults) developed antigen-specific antibody and lymphoproliferative responses but failed to develop CTL responses.[273] These foals were born to EIAV-negative dams and thus had no passive transfer of EIAV-specific antibodies. Seroconversion occurred later than in the adults, and antibody levels waned sooner than in the adults. Adult horse dendritic cells stimulated with CpG-ODN (a PAMP that binds TLR-9) increase IL-12 expression, but foal dendritic cells do not.[340] This study further demonstrates that although foal dendritic cells express the costimulatory molecule CD86 similar to adult levels, MHC II expression is reduced at birth and does not reach adult levels until 3 months of age. A follow-up study found that foal dendritic cells infected with *R. equi* produce IL-12 and express costimulatory molecules (CD40 and CD86) similar to adult levels, but as previously observed, MHC II molecule expression is limited.[341] Thus foal APCs can produce the costimulatory and IL-12 signals (albeit variably depending on stimulus) required for T_H1 differentiation, but MHC II presentation may be compromised.

Foals less than 1 week of age have significantly lower whole blood basal expression of IFN-γ and IL-4 than adult horses and, unlike adults, fail to develop antigen-specific lymphoproliferative responses following immunization with a killed adjuvanted bovine vaccine.[342,343] Similarly, foals less than 1 week of age and foals between 3 and 6 months of age have a lower frequency of IFN-γ and IL-4 secreting PBMCs than adult horses.[344] Bronchial lymph node lymphocytes from *R. equi*–infected foals have significantly reduced *R. equi*–specific proliferative responses than those from adult horses but, interestingly, have lower IL-4 expression, higher IFN-γ expression, and higher IFN-γ/IL-4 ratios on *R. equi* stimulation than those of adults.[345] Although quantitatively lower than adults, IFN-γ production by foal T_H1 cells (and by $CD8^+$ T cells) following nonspecific stimulation is detectable a few days after birth and increases with age, whereas IL-4 production by T_H2 cells is virtually absent during the first 3 months.[346] In addition, *R. equi* infection of BAL cells from 10-day-old foals results in increased expression of IFN-γ and IL-4 and in PBMCs results in consistent IFN-γ expression during the first 3 weeks of age but decreased IL-4 expression at birth.[297] Finally, foals orally inoculated with *R. equi* within the first 2 weeks of life develop *R. equi*–specific CTL responses and IFN-γ expression by *R. equi*–stimulated PBMCs at similar levels as adult horses, with lower levels of IL-4 expression.[347]

Taken together these observations indicate that foals are capable of mounting T-cell responses, including T_H1 and CTL responses, and under some circumstances these responses can be similar to those of adult horses. Based on the latter studies documenting lower IL-4 expression/production relative to that of IFN-γ, foals appear to have a T_H1 bias. These observations challenge the view that foals are inherently T_H2 biased similar to mouse and human neonates.[348] It should be noted that the equine studies evaluated different cell populations, different stimulation conditions, different methods to measure cytokine expression/production, and, in the immunization studies, different immunogens and routes of immunization, likely contributing to some of the disparate results. Nonetheless, these studies agree for the most part that overall, helper T-cytokine expression/production and T-cell responses are quantitatively lower than those of adult horses. Although the precise mechanisms underlying these differences between foals and adults are unclear, contributing factors likely involve the overall naivete of T cells in young

foals and the relative immaturity of APCs in terms of PAMP responses and MHC II presentation, all leading to inefficient priming of naive T cells.

Antibody-Mediated Immunity in Foals

Passive Transfer of Maternal Antibodies. As a result of epitheliochorial placentation in horses, transfer of maternal antibodies does not occur in utero, and foals are born essentially agammaglobulinemic. Foals therefore depend on the ingestion of colostrum and intestinal absorption of maternal antibodies within the first 24 hours of life for protection against infectious pathogens. See discussion of failure of transfer of passive immunity under Secondary Immunodeficiencies later in this chapter.

De Novo Antibody Production in Foals. Few studies of de novo antibody production have been conducted in foals without the effect of transferred maternal antibodies. In a study of 10 pony foals fed only bovine colostrum, endogenous equine antibody production measured by radial immunodiffusion resulted in serum concentrations of IgG of 200 mg/dL by 2 weeks of age, 400 mg/dL by 1 month, and 1000 mg/dL by 3 months.[349] In a smaller study of two colostrum-deprived pony foals, comparing them to 18 colostrum-fed foals, and measuring serum γ-globulin levels by immunoelectrophoresis, very similar results were obtained, although it was apparent that the colostrum-deprived foals achieved higher serum γ-globulin levels between 6 weeks and 3 months of age than colostrum-fed foals.[350] In a third study, antibody concentrations in six colostrum-deprived foals were substantively higher than in five control foals between 3 and 5 months of age.[334] These three studies provide evidence for substantial endogenous production of IgG in the first month of life in foals deprived of equine colostrum and suggest that the onset of production is earlier and the rate is higher in foals deprived of colostrum. This observation is consistent either with nonspecific immunosuppression in colostrum-fed foals or stimulation of immunoglobulin production in colostrum-deprived foals. In another study of foals from mixed-breed horses fed only bovine colostrum, endogenous IgG production started later and was first detected at 1 month of age in the majority of foals, reaching similar levels to foals fed equine colostrum by 2 months of age.[351] Foals immunized with an experimental EIAV plasmid DNA vaccine within the first week of life and boosted on days 15, 36, and 50 seroconverted between days 47 and 53.[273] By comparison, three adult horses similarly immunized seroconverted on day 29. The foals were born to EIAV-negative dams and thus had no passive transfer of EIAV-specific antibodies. Vaccination of 3-day-old foals with a killed adjuvanted bovine vaccine, for which there was no maternal antibody interference, resulted in considerably decreased antibody responses compared with adults.[343] Although 3-month-old foals had improved antibody responses, the magnitude was less than in adults. In contrast, a similarly designed study demonstrated that vaccination with bacillus Calmette-Guérin (BCG; a modified live *Mycobacterium bovis* vaccine) elicited robust antibody responses similar to those of 4-month-old foals and slightly less than adults, although neither group of foals produced vaccine-specific IgG4/7.[342] The latter result was consistent with the findings of other studies (see later discussion). Taken together, neonatal foals are clearly capable of endogenous antibody production in the absence of maternal antibody, although responses tend to be less robust than in older foals and adults. However, the magnitude of the antibody response in neonates depends on the nature of the antigenic stimulation. Overall, when foals are vaccinated with antigens against which they have no passively transferred antibody, normal antibody responses have been documented from at least 3 months of age.[352]

In colostrum-fed foals, serum IgG concentration falls to its lowest level at 1 to 2 months of age as a result of catabolism of maternally transferred immunoglobulin, subsequently rising toward adult levels as a result of endogenous immunoglobulin production.[331,332,338,353] A study of five Quarter Horse foals in the first 9 weeks of life showed that IgG (the equivalent of IgG1 plus IgG4/7) concentrations were lowest at 1 month of age. However, the subsequent increase in IgG concentration was due to de novo IgG1 production, not IgG4/7. At the end of this study (at 9 weeks of age) there was still no clear evidence of IgG4/7 production, although IgG1 and IgG3/5 concentrations had reached or exceeded adult levels. In adult horse serum, IgG4/7 comprises greater than 60% of total serum IgG and is by far the dominant subclass in foal serum after passive transfer of immunity.[249,317,326] IgG4/7 has also been shown to have a critical role in immunity to a variety of pathogens,[354,355] and it is possible that the naturally late onset of endogenous production may be a factor in the increased susceptibility of foals to bacterial respiratory infections at this age.[249,356,357] A study investigating this possibility during the first 42 days of life measured the opsonic capacity of foal serum against *Escherichia coli* and *Actinobacillus equuli*.[220] No differences were detected over time, and foal serum was as effective as adult horse serum. Because IgG1, IgG4/7, and IgG3 all activate complement, bind Fc receptors, and bind bacterial cell walls,[247] IgG1 and IgG3 may functionally compensate for the slower onset of IgG4/7 production in young foals. Finally, in a study of immunoglobulin concentrations in foals throughout the first year of life, serum levels of all measured isotypes decreased at 4 weeks of age, after which point all but IgG4/7 began to rise. This pattern was the result of catabolism of maternal antibody and the different times of onset and rates of endogenous antibody production as follows: (1) IgA and IgG3/5 levels stabilized at 8 to 12 weeks of age; (2) IgG1 levels peaked at 8 weeks of age and then slowly declined throughout the duration of the study; and (3) IgG4/7 levels reached their nadir at 2 to 5 months of age and did not begin to rise until after 16 weeks of age. These observations could provide a basis for reduced endogenous antibody-mediated immunity during the first year of life. This study also showed that at 1 year of age serum immunoglobulin concentrations had still not reached adult levels.

A factor that significantly affects de novo immune responses in foals is the suppressive effect of passively transferred antigen-specific maternal antibodies. The rate of decline of these antibodies varies for both individuals and different infectious agents. The half-life for maternal IgG in foals is estimated at 20 to 30 days.[350] Studies of antigen-specific antibodies demonstrate similar half-lives for anti-influenzavirus and anti–tetanus antibodies of 27 to 29 days for IgG1, 35 to 39 days for IgG4/7, and 35 days for IgG3/5.[358] For many important pathogens, the concentration of maternal antibodies in foals falls to nonprotective levels by 2 to 3 months of age.[359,360] However, the remaining antibody can still render the foal unresponsive to vaccination for weeks or even months to come. In the case of equine influenzavirus[361,362] and tetanus toxoid, maternal antibodies can persist until 6 months of age and inhibit antibody responses in foals vaccinated before that

age.[358] In contrast, a recent study indicates that 3-month-old foals immunized with a three-dose-series multivalent vaccine had similar antigen-specific antibody and T-cell responses as foals initially vaccinated at 6 months of age.[363] Based on this study it is reasonable to begin vaccinating foals earlier than 6 months of age in circumstances where the risk of infectious disease is high.

Implications for Immunocompetence in Foals

The evidence presented previously indicates that the immune system of the foal is competent in many ways, with the innate immune system completely functional at least by the second week of life, and with the full complement of lymphocytes present from birth. Antibody is entirely provided by passive transfer at first, although endogenously produced immunoglobulin is detectable within a few weeks of birth and predominates from 1 to 2 months of age. Nevertheless, there are some key features of the foal immune system that can limit its ability to defend against infection. A critical factor is maternal antibody interference, which inhibits the binding of antigens to BCRs of naive B cells in the foal, reducing endogenous antibody responses. As foals age, a continuing immunomodulatory effect of maternal antibody may limit foal immunoresponsiveness, while no longer providing comprehensive protection itself. Of similar importance is that although the adaptive immune system is complete from the time of birth, it is naive. In addition, APCs are immature.[364] Neonates can mount normal immune responses but require appropriate presentation of antigen and costimulatory signals. Reduced antigen presentation by APCs, or antigen presentation in the absence of costimulatory signals, can result in a failure to mount the appropriate immune response. This is particularly an issue in neonates. The absence of memory responses and a well-developed repertoire of immune effectors is a serious handicap that only appropriate antigenic encounters can overcome. Despite the fact that humoral and cell-mediated immune responses are less robust during the first year of life, the majority of foals survive to adulthood. However, a better understanding of immune regulation in foals and the precise mechanisms underlying the differences between foal and adult immune responses is needed to develop improved immune-based strategies to prevent critical infectious diseases in foals such as *R. equi* pneumonia.

HYPERSENSITIVITY AND AUTOIMMUNITY

Hypersensitivity refers to an altered state of immunoreactivity resulting in self-injury. Four different types of hypersensitivities can be defined by the type of immunologic process underlying the tissue injury (Table 1.6). The most common and important type of hypersensitivity disease in most species is type I hypersensitivity, mediated by IgE. In these diseases some individuals produce IgE antibodies against a normally innocuous antigen, which is called an *allergen*. Exposure to the allergen triggers mast cell degranulation as described later, and a series of responses result that are characteristic of allergy. Allergic diseases are so important that more is known about the function of IgE in hypersensitivity than about its normal role in host defense. In this definition, and throughout this chapter, the term *allergy* refers only to type I hypersensitivity diseases mediated by IgE, whereas in other definitions allergy can refer to the entire spectrum of hypersensitivity diseases.[365] Other forms of hypersensitivity disease depend primarily on IgG antibodies (type II and III hypersensitivities) or T cells (type IV hypersensitivity). Each of these disease processes can play a role in the immunopathogenesis of autoimmune disease, in which the body mounts an adaptive immune response to self tissue antigens.

Clinical hypersensitivity diseases, such as recurrent airway obstruction (RAO) or equine recurrent uveitis (ERU), can involve more than one type of hypersensitivity reaction simultaneously, which limits the utility of this classification for clinical diagnosis. Alternative strategies for classifying these diseases may have greater clinical utility. For example, antibody-mediated hypersensitivity diseases (types I, II, and III) are immediate in onset if preformed antibody exists in circulation or tissues, with some variation in time course dependent on the antibody isotype involved. Cell-mediated hypersensitivity diseases (type IV) are delayed, even in sensitized individuals, for 1 to 3 days, while effector cells are recruited to

TABLE **1.6 Hypersensitivity**

	Type I	Type II	Type III	Type IV		
Immune Mediator	**IgE**	**IgG**	**IgG, IgA**	**T_H1**	**T_H2**	**CTL**
Antigen	Soluble antigen	Cell or matrix associated antigen	Immune complex formation	Soluble antigen	Soluble antigen	Cell-associated antigen
Effector mechanism	Mast-cell degranuation	Fc-receptor–positive cells (phagocytes of reticuloendothelial system)	Fc-receptor–positive cells, complement	Macrophage activation	Eosinophil activation	Cytotoxicity
Examples of hypersensitivity reaction	Systemic anaphylaxis, *Culicoides* hypersensitivity	Immune-mediated hemolytic anemia, neonatal isoerythrolysis	Purpura hemorrhagica, serum sickness	Equine recurrent uveitis	Chronic *Culicoides* hypersensitivity	Contact dermatitis

Note: The four types of hypersensitivities can be differentiated by the immune mediator involved, the form of antigen recognized, and the effector mechanism elicited in producing pathology. Equine examples of each condition are given when available.

the site of antigen exposure.[365] The goals of this section are as follows:

- Review the classical hypersensitivity types to explain the immunopathogenesis of hypersensitivity diseases
- Describe antibody and cell-mediated hypersensitivities of horses and their immunologic basis
- Identify autoimmune conditions of horses with a known immunologic basis

Detailed descriptions of clinical aspects of hypersensitivity and autoimmune diseases, their diagnosis, and their management are presented elsewhere in this book. Detailed explanations of many immunologic mechanisms involved in these disease processes are provided previously in this chapter.

Classical Types of Hypersensitivity Reactions

Type I Hypersensitivity

As described, type I hypersensitivity, also known as immediate hypersensitivity or allergy, is mediated by IgE antibody specific for allergens, which are extrinsic antigens normally not recognized by the healthy immune system. IgE is predominantly found in tissues, where it is bound to mast cells through the high-affinity IgE receptor, which is called FcεRI and has been identified in the horse.[366] When antigen binds to IgE on the surface of mast cells, cross-linking two or more IgE molecules and their FcεRI receptors, this triggers the release of chemical mediators from the mast cells, which cause type I hypersensitivity reactions. Basophils and eosinophils (when activated) also possess FcεRI receptors and therefore can participate in the same process. In addition to FcεRI receptors, there is an unrelated low-affinity IgE receptor called CD23 that is present on many lymphocytes, monocytes, eosinophils, platelets, and follicular dendritic cells. The role of CD23 appears to be to enhance IgE responses to specific antigens when those antigens are complexed with IgE. Thus CD23 on APCs can capture IgE-bound antigens. In the horse CD23 has been identified and its expression is upregulated by equine IL-4.[367] Monoclonal antibodies against equine CD23 are now available and have been used to identify CD23 primarily on a subpopulation of B cells.[368] These reagents should be useful for further evaluating the role of this low-affinity receptor in IgE-mediated responses in the horse.

The selective stimulation of IgE responses depends on characteristics of the antigen (allergen), the individual affected (genetic factors such as MHC haplotype), and the mechanism of antigen presentation. The antigen must be capable of eliciting a T_H2 immune response to stimulate IgE production. Small, soluble proteins, frequently enzymes, containing peptides suitable for MHC II antigen presentation, and presented to mucosal surfaces at low doses, are particularly efficient at generating IgE responses. Low doses of antigen specifically favor T_H2 over T_H1 responses, and exploiting this relationship is the basis of some therapeutic hyposensitization strategies (see under Immunomodulators). These processes are regulated by T_{regs} (discussed previously), which suppress T_H2 cytokine production in healthy individuals. When T_{regs} are deficient, atopy can result. The process of T_H2 differentiation is critical to promoting IgE responses and may be favored at enteric and respiratory mucosal surfaces, or skin, where parasite invasion typically occurs. This makes teleologic sense, as IgE responses are important for antiparasitic immunity.[369] Dendritic cells at such locations are frequently programmed to stimulate T_H2 responses. Cross-linking of FcεRI receptors on granulocytes also results in CD40L expression and IL-4 secretion, which further promotes IgE production by B lymphocytes and sustains allergic reactions.

Some individuals are predisposed to IgE-mediated hyperactivity to a wide variety of environmental allergens, and this condition is termed *atopy.*[370] Affected individuals have high levels of IgE in the blood and increased eosinophil populations. In humans this condition depends partly on genetic factors, including genetic variations in the IL-4 promoter sequence or association with particular MHC II genes. Nevertheless, environmental factors are also important, as atopy is increasingly common in humans in economically developed parts of the world. Four potential explanations for this observation are decreased exposure to infectious disease during childhood, environmental pollution, allergen levels, and dietary change. The first explanation, known as the "hygiene hypothesis," is supported by epidemiologic and experimental evidence. The premise is that living in areas with poor sanitation, microbial exposure, and infectious diseases early in life biases the immune system toward T_H1 responses and away from T_H2 responses, thus reducing the later development of allergic diseases. This is thought to be due in large part to the composition of the intestinal microbiota, including the presence of helminths, and the resulting induction of T_{regs} that modulate T_H1/T_H2 balance.[371] In developed societies, decreased microbial exposure during early childhood and decreased prevalence of infectious diseases leads to a depleted microbiota and an increased tendency to mount T_H2 responses, which may be the natural bias of the neonatal immune system.[348] It is unclear if atopy occurs in horses and whether or not the hygiene hypothesis applies. Interestingly, a recent study in 593 Warmbloods in Switzerland found that some horses had multiple hypersensitivities.[372] For example, horses with RAO had a 13.1 times higher odds ratio of being affected with IBH, and horses with IBH had a 7.1 times higher odds ratio of being affected with urticaria. In addition, the multiple hypersensitivity phenotype was associated with the absence of nematode eggs in the feces. Icelandic horses living in Iceland have high intestinal helminth burdens and low numbers of IL-4–producing T cells along with significantly higher IL-10 and TGF-β production by PBMCs than Icelandic horses living in Switzerland.[373] These horses do not develop IBH in Iceland because *Culicoides* midges do not occur in Iceland, but they are prone to IBH when imported into countries where *Culicoides* spp. are endemic. This susceptibility is associated with deworming before import, along with increased IL-4 production and decreased regulatory cytokine production.[373] Taken together, the results of these studies suggest that some horses are atopic and that this might be associated with reduced intestinal nematode exposure, consistent with the hygiene hypothesis.

Effector Mechanisms in Type I Hypersensitivity Allergic Reactions. When triggered by antigen cross-linking of IgE bound to FcεRI cell surface receptors, activated mast cells release chemical mediators stored in preformed granules and synthesize leukotrienes and cytokines. In type I hypersensitivity reactions the outcome of this reaction can vary from anaphylactic shock to minor localized inflammation. Mast cell degranulation causes an immediate allergic reaction within seconds, but there is also a sustained late-phase response that develops over up to 8 to 12 hours due to recruitment of T_H2 lymphocytes, eosinophils, and basophils.

Mast cells are highly specialized cells of the myeloid lineage that are common in mucosal and epithelial tissues near small

blood vessels. The range of inflammatory mediators released by degranulating mast cells is wide and includes enzymes that can remodel connective tissues; toxic mediators such as histamine and heparin; cytokines, including IL-4, IL-5, IL-13, and TNF-α; and lipid mediators, including leukotrienes and PAF. Histamine causes an increase in local blood flow and capillary permeability. Enzymes activate matrix metalloproteinases, which cause tissue destruction, and TNF-α increases expression of adhesion molecules and attracts inflammatory leukocytes. These reactions are all appropriate when the mast cell is reacting to an invasive pathogen, but in allergy this is the basis of the immediate inflammatory response and also the initiating step in the late-phase response.

The role of eosinophils in inflammation is tightly controlled at several levels. Synthesis in the bone marrow depends on IL-5 produced by T_H2 cells in the face of infection or other immune stimulation. Transit of the eosinophils to tissues depends on two chemokines, eotaxin 1 and eotaxin 2. Activation of eosinophils by cytokines and chemokines induces them to express FcεRI and complement receptors and primes the eosinophil to degranulate if it encounters antigen that can cross-link IgE on its surface. Mast cell degranulation and T_H2 activation recruit and activate large numbers of eosinophils at the site of antigen encounter. Basophils are similarly recruited, and together their presence is characteristic of chronic allergic inflammation. Eosinophils can trigger mast cells and basophil degranulation by release of major basic protein. This late-phase response is an important cause of long-term illnesses such as chronic asthma in humans.

Clinical Manifestations of Type I Hypersensitivity Reactions Depend on Their Site. The clinical outcome of type I hypersensitivity reactions depends on the amount of IgE present, the dose of allergen, and the site of allergen introduction. Direct introduction of allergen into the bloodstream or rapid enteric absorption can lead to widespread activation of connective tissue mast cells associated with blood vessels. This potentially disastrous event, termed *systemic anaphylaxis,* can cause catastrophic loss of blood pressure and airway obstruction resulting from bronchoconstriction and laryngeal swelling. This leads to anaphylactic shock and can follow administration of drugs against which an individual has an established IgE response. Treatment with epinephrine can control these potentially fatal events.

Penicillin is one example of a drug that can cause type I hypersensitivity reactions in humans, and it may induce this type of hypersensitivity reaction in the horse.[374] Penicillin can act as a hapten (see under Equine Immunology). Penicillin alone can elicit antibody formation by B cells but cannot elicit T-helper cell responses as it is not a protein. However, the β-lactam ring of penicillin can react with amino groups on host proteins to form covalent conjugates, and the modified self peptides can generate T_H2 responses in some individuals. The T_H2 cells can in turn release cytokines, which activate penicillin-binding B cells to produce IgE. In this scenario penicillin is a B-cell antigen and becomes a T-cell antigen by modifying self peptides. Intravenous penicillin results in protein modification and recognition and cross-linking of mast cell IgE leading to anaphylaxis.

Allergen inhalation, in contrast, induces local inflammation of the respiratory tract—for example, in the upper airways as in allergic rhinitis or in the lower airways in human asthma. Similarly allergen introduction into the skin causes local histamine release initially and a wheal-and-flare reaction, followed by a late-phase response several hours later. When allergens are ingested and reach the skin from the bloodstream, a disseminated form of the wheal-and-flare reaction occurs that is termed *urticaria* or *hives.* Prolonged inflammation of the skin results in eczema or atopic dermatitis in some individuals. Ingestion of allergens causes activation of gastrointestinal mast cells resulting in fluid loss across the bowel and smooth muscle contraction. The clinical presentation is diarrhea and vomiting. Sometimes ingestion of allergens can lead to systemic anaphylaxis if they are absorbed rapidly, or urticaria, as is sometimes seen in humans after oral penicillin administration.

Type II Hypersensitivity

This form of hypersensitivity disease occurs when the causal antigen is associated with cells or tissue components of the body, and there is an IgG antibody response to this antigen. Phagocytes, or other cells expressing Fcγ receptors, mediate destruction of the affected tissue, or removal from the circulation by the reticuloendothelial system in the case of antibody-positive erythrocytes or platelets. Additionally, antibody bound to erythrocytes may activate complement resulting in hemolysis. Blood transfusion reactions and neonatal isoerythrolysis are examples of type II hypersensitivities. Immune-mediated hemolytic anemia and thrombocytopenia are examples of drug-associated type II hypersensitivities. In the case of the horse, penicillin is an established cause of hemolytic anemia.[375] Diagnosis can be accomplished using a Coombs test (Fig. 1.18). Penicillin binds to the erythrocyte surface and is targeted by antipenicillin antibodies of the IgG isotype. Interestingly, quite large numbers of horses have antipenicillin antibodies of the IgM isotype, but this does not lead to disease.

Type III Hypersensitivity

In type III hypersensitivity the antigen is soluble and present in the circulation. Disease results from formation of antibody-antigen aggregates or immune complexes under certain specific conditions. Although immune complexes are generated in all antibody responses, they are generally harmless. Large complexes fix complement and are removed from circulation by the reticuloendothelial system. However, small complexes can form at antigen excess (Fig. 1.19), and these can deposit in blood vessel walls and tissues where they ligate Fc receptors on leukocytes, causing an inflammatory response, increased vascular permeability, and tissue injury. Complement activation also contributes to this process. Local injection of antigen can sometimes lead to a necrotizing skin lesion due to type III hypersensitivity, and this is termed an *Arthus reaction.*

The classical example of a type III hypersensitivity reaction is serum sickness, which can be seen after administration of horse antiserum in humans, such as in treating snakebites. After an IgG response to the horse serum is generated (7–10 days), signs of fever, urticaria, arthritis, and sometimes glomerulonephritis result. The foreign antigen is cleared as part of this process, which makes this condition ultimately self-limiting. Alternative scenarios for induction of type III hypersensitivity reactions include persistent infectious diseases where pathogens (or their antigens) are not completely cleared from tissues or autoimmune diseases in which antigen persists. Glomerulonephritis in horses with chronic equine infectious anemia is one example of the former.[376] Purpura hemorrhagica following *Streptococcus equi* infection is another. Inhaled antigens associated with moldy hay that induce IgG responses

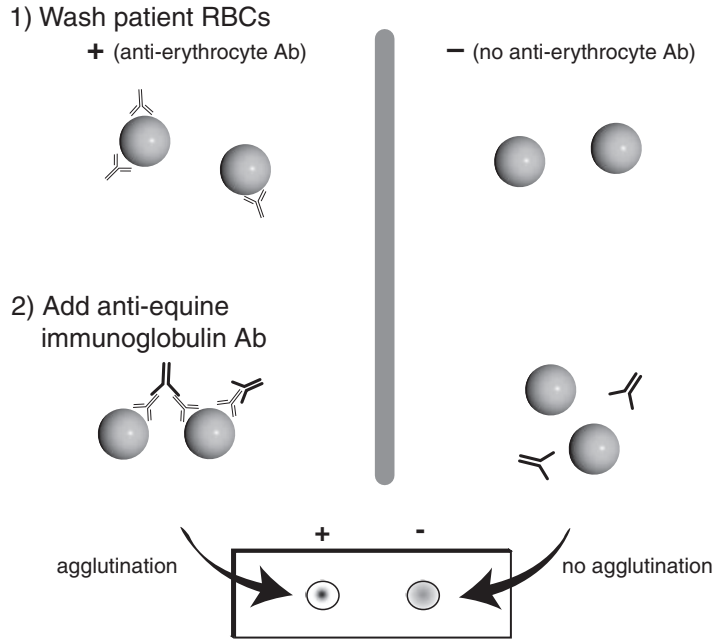


FIG. **1.18** Direct Coombs test. *Ab,* Antibody; *RBCs,* red blood cells.

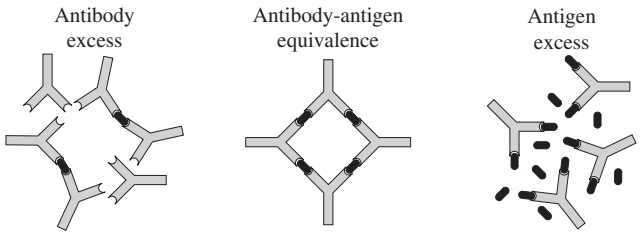


FIG. **1.19** Antibody-antigen precipitation. Antibody can precipitate soluble antigen in the form of immune complexes. This is most efficient when concentrations of antibody and antigen reach equivalence, and large immune complexes are formed. However, when antigen is in excess some immune complexes are too small to precipitate and can produce pathologic changes such as are seen in type III hypersensitivities.

can lead to immune complex formation in the alveolar wall, as occurs in cattle with hypersensitivity pneumonitis. This can also occur in humans and is known as farmer's lung. Any such circumstance in which immune complexes are deposited in tissues can lead to this type of pathology.

Type IV Hypersensitivity

Cell-mediated type IV hypersensitivities cause delayed hypersensitivity reactions. A variety of cutaneous hypersensitivity reactions are seen, such as the contact hypersensitivity seen after absorption of haptens such as pentadecacatechol in poison ivy or the local T_H1 response seen in the diagnostic tuberculin reaction. When type IV hypersensitivity results in a T_H2 response the principal outcomes are eosinophil activation and recruitment such as in chronic asthma in humans.

Antibody-Mediated Hypersensitivity Diseases in the Horse

The study of hypersensitivity disease in the horse was hampered in the past by the lack of reagents capable of detecting equine IgE. Although equine IgE has long been known to exist,[377,378] and the genetic sequence has been known since 1995,[379-381] the only reagents for studying it have historically been conventional polyclonal antisera produced by vaccination with physicochemically purified IgE[382,383] or made in chickens after vaccination with recombinant fragments of the IgE heavy chain.[384] Although many valuable studies have been performed using these reagents,[385-388] the availability of well-characterized monoclonal antibodies recognizing equine IgE has moved the field forward.[389,390] Passive transfer of maternal IgE via colostrum occurs in equine neonates, and cross-linking of this maternal IgE on the surface of neonatal basophils results in intracellular IL-4 production.[391,392] This might help promote adaptive T-cell responses in newborn foals. However, the onset of endogenous IgE production in horses does not occur before 9 to 11 months of age and does not reach adult levels before 18 months.[391] This may help explain why hypersensitivity disease is uncommon in horses before puberty.

The following section describes a series of equine diseases with characteristics of immediate hypersensitivity disease. This is not an exhaustive list of equine hypersensitivity diseases and additional examples can be found throughout this book.

Systemic Anaphylaxis

Systemic anaphylaxis is a severe immediate type I hypersensitivity reaction mediated by IgE. The incidence of true systemic anaphylaxis in horses is unknown, although the condition has been reported in association with administration of a wide range of compounds, including serum, vaccines, vitamin E–selenium preparations, thiamine, iron dextrans, and antibiotics, including penicillin.[393,394] Target organs in experimental equine anaphylaxis are the lung and the intestine.[393] Sudden dyspnea; hypotension, as evidenced by poor peripheral pulse character; rapid onset of urticaria; and collapse are cardinal signs of the onset of systemic anaphylaxis.

The therapeutic goals in treating systemic anaphylaxis are to (1) prevent or reverse the complications caused by mediator release, (2) maintain respiratory integrity, and (3)

maintain cardiovascular stability. Not all anaphylactic reactions require therapy. However, rapid recognition of those that do is critical to patient survival. Intravenous access via an indwelling intravenous catheter and airway patency should be established immediately because cardiovascular collapse and upper airway obstruction caused by angioedema can occur rapidly. The conscious horse does not tolerate tracheal intubation so emergency tracheotomy may be required. Oxygen should be administered if available because bronchoconstriction and cardiovascular collapse result in hypoxemia. The fluid requirement of horses in anaphylactic shock is not known, but large volumes of balanced polyionic fluid should be administered rapidly.

The principal therapeutic agent is epinephrine, which is a potent sympathetic stimulant. Epinephrine administration may cause excitement in the horse. Epinephrine should be administered intramuscularly (0.01–0.02 mg/kg, equivalent to 4.5–9 mL of 1:1000 dilution of epinephrine for a 450-kg horse) if dyspnea and hypotension are not severe.[395] Epinephrine should not be administered subcutaneously because its potent vasoconstriction can lead to poor absorption and tissue necrosis. If dyspnea or hypotension is severe, epinephrine should be administered slowly intravenously at the same dose as above. If there is no venous access, 2 times the above dose intramuscularly or 5 times the above dose intratracheally can be administered.[395] Epinephrine doses can be repeated every 15 to 20 minutes until hypotension improves. The side effects of epinephrine therapy are tachyarrhythmias and myocardial ischemia, which can be life-threatening. Alternatively, an epinephrine or norepinephrine drip can be instituted for cases of refractory hypotension. Other therapeutic agents such as antihistamines, β-agonists, or other pressors may be indicated, although their value is less certain. Though its effects may be delayed, glucocorticoid therapy is indicated to help reverse persistent bronchospasm, reverse angioedema, and break the cycle of mediator-induced inflammation triggered during hypersensitivity reactions. Ideally a rapid-acting glucocorticoid should be used such as prednisolone sodium succinate (0.25–10.0 mg/kg intravenously), but dexamethasone may also be used (0.2–0.5 mg/kg intravenously). Glucocorticoid therapy during the acute phase will aid in preventing the late-phase reaction.

Insect Bite Hypersensitivity

Insect bite hypersensitivity (IBH) occurs in response to salivary allergens of *Culicoides* midges. It is the most common allergic skin disease of horses and is characterized by intense seasonal pruritus, alopecia, and excoriation.[306,396] The clinical signs combined with increased numbers of IgE-positive cells in the skin along with high levels of *Culicoides*-specific IgE in serum provided evidence for immediate (type I) hypersensitivity in the immunopathogenesis of IBH.[385,386] This pathogenesis was further supported by studies using monoclonal antibodies against equine IgE[390] and confirmed by transferring the intradermal allergic reaction to normal horses using IgE from IBH-affected horses.[397] In some breeds a genetic predisposition based on an MHC II linkage has been demonstrated.[286,398,399] Interestingly, Icelandic horses, which are particularly affected, do not develop IBH in Iceland because *Culicoides* spp. do not exist in Iceland. However, when these horses born in Iceland are exported later in life to *Culicoides*-endemic countries, a high percentage develop IBH.[306,400] In terms of lack of exposure to *Culicoides* antigens early in life along with high intestinal nematode burdens in Iceland,[373] these observations are consistent with the hygiene hypothesis. Moreover, it has been shown that horses with IBH have reduced T_{reg} responses compared with unaffected horses.[304-306,400]

Recurrent Airway Obstruction

Recurrent airway obstruction (RAO) is a severe inflammatory disease of middle-aged and older horses induced by exposure of susceptible horses to inhaled organic dust, generally from hay, although a summer pasture–associated form is also observed in the southern United States and in Europe.[296,401,402] Hay dust contains a mixture of mold spores, forage mites, particulates, and endotoxins, which can induce and exacerbate airway inflammation. Removal of the hay dust by returning the horse to pasture leads to decreased inflammation within a few days. In RAO-susceptible horses, exposure to hay dust leads to invasion of the lungs and airways by neutrophils within 4 to 6 hours and concurrent airway obstruction resulting from bronchospasm, inflammation, and increased mucus viscosity, which principally affect the bronchioles. RAO-affected horses develop nonspecific airway hyperresponsiveness, which is a bronchospasm in response to a wide variety of stimuli including inflammatory mediators and neurotransmitters. Horses affected by RAO demonstrate increased histologic lesions and worsening airway function with increasing age. In addition, significant histopathologic changes are present before abnormal airway function can be detected.

The immunologic basis of RAO remains incompletely understood. Two pieces of evidence suggest a role for type I hypersensitivity in this disease. First, IgE levels are increased in bronchoalveolar (BAL) fluid of RAO-affected horses,[382] and second, allergen-specific IgE is increased in affected horses.[387,403,404] However, the immediate onset of airway obstruction typical of type I hypersensitivity to exposure to allergens is rarely observed, as clinical signs of RAO only develop several hours after antigenic exposure.[405] In addition, influx of eosinophils is not a feature of RAO. A study of immunoregulatory cytokines in RAO demonstrated evidence for a T_H2 bias with increased levels of IL-4 and IL-5 and decreased IFN-γ mRNA in BAL cells.[406] However, other investigators have documented increased IFN-γ levels in RAO and a lack of T_H2 polarized responses.[407,408] It seems likely that a number of complex immunologic processes are involved in the pathogenesis of RAO,[296,401] including a predominant T_H2 response or at least a dysregulation of cytokine responses associated with a disruption in T_H2/T_H1 balance,[206,409] IgE-mediated pathology, and T_H17 responses[295] accompanied by increased expression of TLR-4 and CXCL8,[410] leading to neutrophilic inflammation. Neutrophil-mediated inflammation predominates in RAO, and several studies suggest that IL-17 responses, and by extension T_H17 responses, are involved in its pathogenesis.[206,295,296] Finally, the percentage of T_{regs} in the airways of horses with RAO increases, and this may be a mechanism to limit immunopathology.[307]

Neonatal Isoerythrolysis and Alloimmune Thrombocytopenia

Neonatal isoerythrolysis (NI) is a common condition of foals and is extensively reviewed elsewhere in this text. The condition results from the passive transfer of maternal antibodies in colostrum, which recognize allogeneic foal erythrocyte antigens principally of the Aa and Qa blood group inherited from the sire. A similar condition occurs in mules due

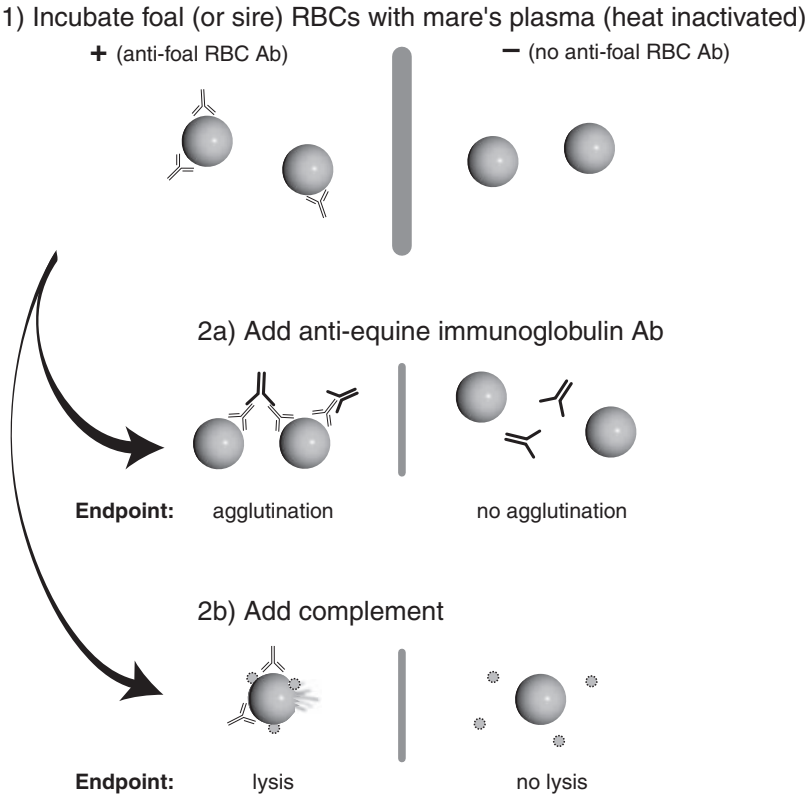


FIG. **1.20** Neonatal isoerythrolysis test. *Ab,* Antibody; *RBCs,* red blood cells.

to inheritance of a donkey-specific erythrocyte antigen.[411,412] A severe, potentially life-threatening, anemia results as the antibody-positive erythrocytes are removed by the reticuloendothelial system or lysed by complement. A similar condition causing severe neonatal thrombocytopenia in horses and mules less commonly affects platelets.[413-415] These conditions are typical of type II hypersensitivities and are mediated by circulating IgG recognizing cell surface antigens on erythrocytes. For newborn foals at risk of NI, a jaundice foal agglutination test can be performed before allowing the foal to nurse by making 1-mL serial dilutions of the mare's colostrum in saline (1:2–1:128), adding 1 drop of the foal's anticoagulated (EDTA) whole blood to each tube and mixing, centrifuging at medium speed (300–500 × g) for 2 to 3 minutes, then observing for agglutination of the red blood cell pellet by inverting the tubes. If strong agglutination occurs at or above the 1:16 dilution (≥1:64 for mule foals), the foal should not be allowed to nurse the mare. To prevent NI, the foal is muzzled and not allowed to nurse during the first 36 hours. An alternative source of colostrum (or commercial equine immunoglobulin product) is administered to the foal, and the mare is milked out during this period and her colostrum discarded. Diagnosis of NI can be confirmed using a variation of the Coombs test (Fig. 1.20).

Purpura Hemorrhagica

Purpura hemorrhagica is an uncommon acute type III hypersensitivity (immune complex) disease in horses characterized by edema of the head and limbs; leukocytoclastic vasculitis; petechial hemorrhages in mucosae, musculature, and viscera; and sometimes glomerulonephritis.[416,417] It is most commonly associated with *Streptococcus equi* exposure or infection but can also occur after *Corynebacterium pseudotuberculosis* infection or after vaccination with *S. equi* M protein (SeM).[418] Serum of affected horses contains immune complexes of *S. equi*–specific antigens (SeM) with IgA.[416] These immune complexes become deposited on the walls of small blood vessels, resulting in vasculitis, as discussed previously. The glomerulonephritis sometimes seen in association with purpura has been attributed to deposition of similar immune complexes containing streptococcal antigens and IgG.[419]

Cell-Mediated Hypersensitivity Diseases in the Horse

There are relatively few documented type IV (cell-mediated or delayed) hypersensitivity conditions in the horse in which the immunologic mechanism has been confirmed, although contact hypersensitivities have been reported.[365] One exception is recurrent uveitis, which is a very well-characterized example of type IV hypersensitivity in the horse.

Equine Recurrent Uveitis

Equine recurrent uveitis (ERU), also known as moon blindness or periodic ophthalmia, is the most important cause of blindness in horses.[420] The disease results in both acute and chronic ocular inflammatory disease, and chronic sequelae include development of posterior and anterior synechiae, cataracts, lens opacities, secondary glaucoma, and blindness. Eyes of affected horses contain IgG antibodies and autoreactive T cells specific for retinal antigens.[421] Although a specific cause has not been identified, sensitization to a variety of pathogens, and in particular to *Leptospira* spp.,[422,423] is

thought to induce the immune-mediated pathology that is central to the disease.[424] Early and aggressive treatment with corticosteroids and other antiinflammatory agents is essential to avoid visual debility or blindness.[425] Unfortunately, treatment failures are common, and the disease frequently recurs with further ocular damage months after the initial event, commonly leading to euthanasia.[420] Of horses with ERU, 56% become blind in one or both eyes, and 20% become completely blind.[426]

The understanding of the immunologic basis of ERU has been extended by studies of the immunoregulatory events in the eyes of affected horses. It has been shown that the T lymphocytes that invade the iris-ciliary body during this disease produce a pattern of IFN-γ cytokine production typical of a T_H1 response.[427] More recently, mononuclear cells in eyes from horses with ERU were found to be positive for IL-17 and IL-23, strongly suggesting that T_H17 cells are involved in the pathogenesis of ERU.[294] Taken together, these studies indicate that type IV hypersensitivity mediated by T_H1 and likely T_H17 cells contributes to the immunopathogenesis of ERU.

Autoimmunity

Autoimmune diseases occur when T and/or B cells respond to self antigens resulting in tissue damage. The hypersensitivity reactions discussed earlier comprise the mechanisms of this damage mediated by self reactive antibodies and/or T cells. Autoimmune diseases are rare and only arise when there is a break in self tolerance. A break in central tolerance occurs when self reactive B or T cells are not negatively selected during the maturation process in the bone marrow and thymus, as discussed previously under Equine Immunology. When self reactive lymphocytes escape deletion in the primary lymphoid organs and are released into the circulation, peripheral tolerance mechanisms exist to control autoreactivity. One of these peripheral tolerance mechanisms is the downregulation of self reactive T cells by T_{regs}. Because B cells have additional mechanisms to generate receptor diversity (i.e., somatic hypermutation), B cells have a higher probability of developing self reactivity than T cells. Thus many autoimmune diseases with known mechanisms involve autoantibodies. Autoreactive lymphocytes may respond to self antigens due to molecular mimicry, in which foreign antigens are similar enough to self antigens that the activated lymphocytes cross react with self antigen. Molecular mimicry is involved in the thrombocytopenia associated with HIV-1 infection in people where antibodies against HIV-1 envelope proteins also react with surface glycoproteins on platelets.[428] It is possible that a similar mechanism contributes to thrombocytopenia in horses infected with EIAV. Autoreactive lymphocytes can also cause damage when they gain access to immunologically privileged sites where they are normally excluded and in which self antigens are normally sequestered. Examples of these sites include the central nervous system and the eye. The autoimmune basis for ERU likely involves molecular mimicry between the *Leptospira* LruA and LruB antigens and equine intraocular proteins,[429] as well as self reactive T cells gaining access to the immunologically privileged intraocular location where they then cause damage.

A number of equine diseases are considered to be autoimmune in etiology, but few have been extensively studied.[430] Much of the explanation given earlier for the immunopathologies involved in hypersensitivity disease can be applied to autoimmune disease. Well-described equine diseases with autoimmune components include those with characteristics of type II hypersensitivities such as immune-mediated hemolytic anemia in adults.[431,432] Autoimmune diseases with skin manifestations, including pemphigus foliaceus, pemphigus vulgaris, and systemic lupus erythematosus (SLE), have been well characterized in humans and dogs. The pemphigus complex involves autoantibodies against skin adhesive proteins (desmoglein 1 and desmoglein 3 for pemphigus foliaceus and pemphigus vulgaris, respectively) and thus is characteristic of type II hypersensitivity disease. SLE is multifactorial, involving autoantibodies and immune complexes, and thus has characteristics of type II and type III hypersensitivity diseases. Although rare, pemphigus foliaceus and vulgaris[433-436] and SLE[435,437] have been described in horses. As discussed, equine recurrent uveitis has features of type II and type IV hypersensitivity, and there is some morphologic and immunologic evidence for classifying polyneuritis equi (cauda equine syndrome) as an autoimmune disease with immunopathology consistent with type IV hypersensitivity.[438-442]

With the exception of a few conditions, including some of those discussed previously, there are few autoimmune diseases where the cause is well understood. One of the exceptions is the anemia that can develop subsequent to administration of human recombinant erythropoietin to horses.[443,444] There is substantial evidence that horses mount an antibody response to the exogenous human erythropoietin that cross reacts with the endogenous equine hormone (molecular mimicry) and results in erythroid hypoplasia. The lesson of this example may be that in the modern world, with increasing availability of recombinant drugs that mimic natural biologic compounds, we would do well to remember that the immune system has an exquisite ability to distinguish what is foreign and to reject it vigorously.

IMMUNODEFICIENCY

Immunodeficiencies occur in both primary and secondary forms and have been extensively reviewed.[325,445-448] Primary immunodeficiencies are congenital and have a genetic basis, whereas secondary immunodeficiencies result from failure of transfer of passive immunity (FTPI) in foals, immunosuppressive infections or drug treatments, neoplasia, stress, or malnutrition. Immunodeficiencies can affect specific components of the immune system, such as the lymphoid or phagocytic system. Typically immunodeficiency is suspected in any of the following circumstances:

- Onset of infections in the first 6 weeks of life
- Repeated infections that are poorly responsive to therapy
- Infections caused by commensal organisms or organisms of low pathogenicity
- Failure to thrive
- Familial history of immunodeficiency
- Disease resulting from the use of attenuated live vaccines
- Failure to respond to vaccination
- Marked neutropenia or lymphopenia that persists for several days

Equine immunodeficiency is most commonly suspected on the basis of the first three reasons: increased susceptibility to infections. The most common immunodeficiency recognized in clinical practice is FTPI in foals.[325,449-451] Other causes of immunodeficiency vary from well-defined clinical entities, such as severe combined immunodeficiency of Arabian

TABLE **1.7 Components of the Immune System That Can Be Evaluated in Horses and Appropriate Tests for Quantitative or Functional Analyses of Each Component**

Component	Quantitative Tests	Functional Tests
Immunoglobulin	**Radial immunodiffusion, membrane-ELISA, electrophoresis, precipitation/coagulation tests**[a]	**Response to vaccination**
Lymphocytes	**Complete blood cell count, DNA-PK$_{cs}$ and SLA5A3 genetic evaluation**,[b] flow cytometric evaluation of lymphocyte subsets	**Response to vaccination, intradermal PHA test**, in vitro lymphoproliferation assays
Neutrophils and macrophages	**Complete blood cell count**	Chemiluminesence and bactericidal assays, flow cytometric evaluation of phagocytosis and oxidative burst
Eosinophils and basophils	**Complete blood cell count**	No commonly available tests
Complement	No commonly available tests	No commonly available tests
Acute phase proteins	SAA,[c] electrophoresis	No commonly available tests

Note: The list of tests is not exhaustive but is restricted to tests of likely practical value for which normal data are available. Tests listed in **bold** are routinely available to clinicians.
[a]Zinc sulfate turbidity, glutaraldehyde coagulation.
[b]See SCID and FIS for description of genetic testing.
[c]Serum amyloid A handheld test.

foals,[452,453] to cases where immunodeficiency is suspected on clinical grounds but the specific cause or nature of the problem is difficult or impossible to define. Regardless of their cause, immunodeficiencies result in increased susceptibility to infections, which are in turn poorly responsive to appropriate therapy. Defects in antibody production tend to predispose to pyogenic bacterial infection, whereas deficiencies in cell-mediated responses lead to infections with organisms normally nonpathogenic in horses such as *Candida albicans, Cryptosporidium* spp., or adenovirus. When any immunodeficiency is suspected, specific diagnostic tests are indicated to define the deficiency. The aim of the next section is to identify tests clinicians can apply practically in such cases and to explain their merits and limitations.

Tests of Equine Immune Function

Tests of components of the immune system (e.g., lymphocytes, immunoglobulins) generally can either quantitate that component or measure its functional capacity. In Table 1.7 the components of the immune system that currently can be analyzed in this manner are identified, and corresponding quantitative and functional tests are listed. The table also identifies those tests that are likely to be commercially available, and it should be noted that few of the functional tests are available unless the clinician is able to identify a capable equine immunologic research laboratory. Despite these limitations, the available tests do permit the identification of many of the well-defined causes of immunodeficiency in horses.

Tests of Antibody-Mediated Immunity

Although some assays of B lymphocyte function and number are described later, the principal tests of humoral immunity are quantitative assays of immunoglobulin concentration and measurements of specific antibody responses to vaccination. The variety of classes of immunoglobulins in the horse is complex, as reviewed earlier in this chapter. For practical purposes attention is generally focused on IgG, IgA, and IgM.

The current "gold standard" for measurement of concentrations of immunoglobulin classes is the radial immunodiffusion (RID) assay. The disadvantages of this test are its cost and the time required to perform the assay (24 hours or longer), which make it generally unsuitable for screening for FTPI in foals. Nevertheless, this form of test remains the single most valuable assay available to the clinician trying to measure total antibody concentrations in the horse. Currently, test kits are available for equine IgG and IgM (Triple J Farms, Bellingham, WA). The RID test is based on the ability of antigen and antibody to precipitate at equivalence when combined in proportion in agar gel plates. The serum being tested is added to punched-out wells in agar, impregnated with antibody to the specific immunoglobulin class being measured, and allowed to diffuse outward and bind with the anti–class-specific antisera. A precipitate forms when equivalence is reached and the area within the precipitate ring is directly proportional to the concentration of the patient's immunoglobulin class. Enzyme-linked immunosorbent assay (ELISA) test kits are also available for quantitation of equine IgA, IgG3/5 (IgG[T]), and IgM (Bethyl Laboratories, Montgomery, TX). Although not commercially available, serum concentrations of the IgG subclasses (IgG1, IgG4/7, and IgG3/5) can be determined by ELISA in equine immunologic research laboratories. Normal ranges of serum immunoglobulin concentrations are typically provided with commercial kits, and normal serum, milk, and colostrum concentrations of equine immunoglobulins have been described in numerous published studies. These results have been recently summarized and are available in tabular form.[249,326] One of these representative studies reported IgM, IgG, and IgA concentrations in normal adult horse serum to be 103 ± 5 mg/dL, 1913 ± 754 mg/mL, and 225 ± 140 mg/dL, respectively.[316]

By far the most common clinical question regarding immune status is whether a foal has achieved adequate transfer of passive immunity, which is defined as a serum IgG concentration greater than 800 mg/dL. Serum IgG concentrations less than 400 mg/dL and between 400 and 800 mg/dL have defined complete and partial FTPI, respectively. Despite these traditional cutoff values, a recent study in a large number of hospitalized foals demonstrated that foals with serum IgG

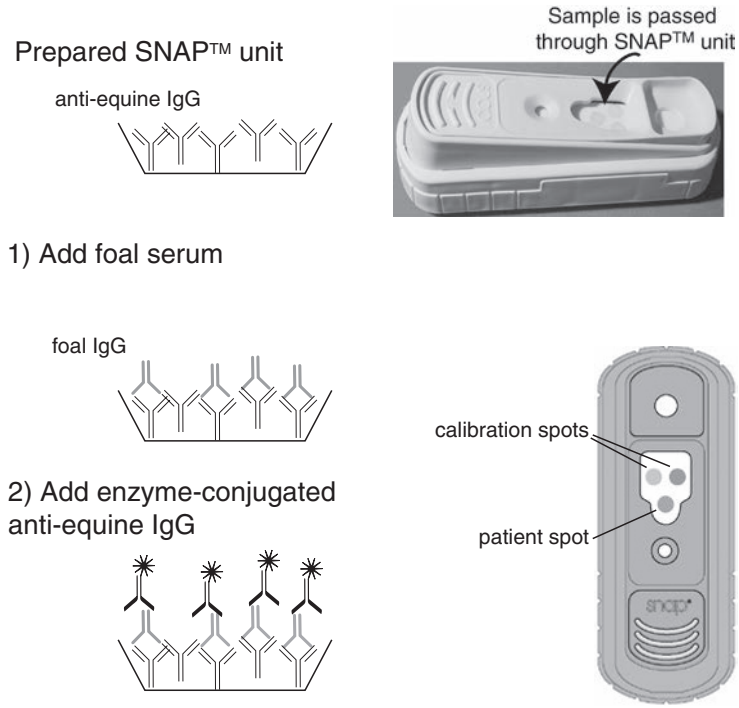


FIG. **1.21** Membrane-based ELISA system (SNAP, Idexx) for measuring serum IgG concentration. The diluted test equine serum sample is applied to a "patient spot" on a membrane impregnated with a capture antibody recognizing equine IgG. Calibration spots corresponding to specific concentrations of equine IgG (400 and 800 mg/dL) are adjacent to the patient spot. An enzyme-conjugated second antibody against equine IgG is applied to the entire membrane, and finally the device is triggered to release an enzyme substrate that produces a colored reaction corresponding to the amount of enzyme-conjugated antibody on the membrane. By comparison with the calibration spots the test sample IgG concentration can be estimated.

concentrations below 800 mg/dL are more likely to die than foals with serum IgG concentrations above 800 mg/dL.[454] The distinction between complete and partial FTPI is therefore not particularly relevant in making therapeutic decisions, at least in the hospitalized foal population. Tests used to determine transfer of passive immunity status should be rapid to allow early initiation of therapy, which decreases the utility of the RID test. A wide variety of other tests have been used for this purpose, including zinc sulfate turbidity, latex bead agglutination, ELISA, turbidometric analysis, glutaraldehyde coagulation, and infrared spectroscopy.[455-460] In the glutaraldehyde coagulation test (Gamma-Check E, Plasvacc USA, Templeton, CA), glutaraldehyde forms insoluble complexes with basic proteins in the serum. Clot formation in 5 minutes or less is equated with a serum IgG concentration of 800 mg/dL or greater. This test is good for initial screening because it is simple, rapid, inexpensive, and accurate for confirming an IgG concentration ≥800 mg/dL (high sensitivity for ruling out FTPI). However, the lack of clot formation within 5 minutes does not always indicate FTPI (can have low specificity), so confirmatory testing is warranted.[461] Although more expensive, the higher specificity and the ease of use and rapid results make the membrane filter–based ELISA tests (e.g., SNAP, Idexx Laboratories, Westbrook, ME) the preferred choice for many (see Fig. 1.21). Like the glutaraldehyde coagulation test, this test can be performed foal-side with whole blood. Although the membrane-filter ELISA is more specific in confirming FTPI, it is slightly less sensitive in detecting FTPI than the glutaraldehyde coagulation test.[461]

An additional test that provides information about serum immunoglobulin content is serum protein electrophoresis. This test gives quantitative information about albumin, α- and β-globulins (acute inflammatory proteins), and γ-globulin (immunoglobulin) concentrations (see Fig. 1.22). The utility of protein electrophoresis is demonstrated when detecting the monoclonal gammopathies that accompany plasma cell myelomas.[462] Hyperglobulinemia can occur during inflammation with increased production of α- and β-globulins and during infections in normal horses with polyclonal production of γ-globulins (immunoglobulins). Nevertheless in the diagnosis of immunodeficiencies, electrophoresis should be seen as an adjunct to RID assays, which are superior in terms of specificity and sensitivity.

Tests of Cellular Immunity

The simplest test of the cellular arm of the immune response is a complete blood count (CBC) to determine total and differential leukocyte numbers, and this should be the starting point for any evaluation. Identification of an absolute lymphopenia, for example, is a critical finding in a suspected case of severe combined immunodeficiency (SCID) in an Arabian foal, although the result must be repeatable in a series of tests given the variability of blood lymphocyte counts. Such a finding would logically lead to genetic testing to confirm the diagnosis.[463] The evaluation of a lymph node biopsy for normal architecture, including the presence or absence of cells in either the B- or T-lymphocyte–dependent areas, is another powerful test of the immune system.

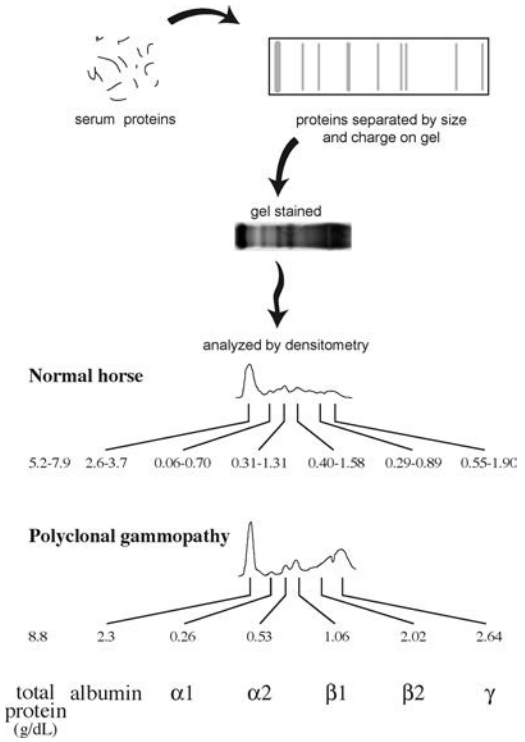


FIG. **1.22** Serum protein electrophoresis. The complex mixture of serum proteins are separated by migration through an agarose gel slab in response to an electric field. Proteins are stained and the intensity of staining of different bands is measured by densitometric scanning. These measurements are used to identify different types of globulins and albumin corresponding to stained bands.

However, in profound immunodeficiencies such as SCID, lymphoid organs may be impossible to locate antemortem. Beyond these readily available conventional techniques, three other more complex types of analyses can be of value: flow cytometric analysis (primarily of lymphocytes, although other cell types can be analyzed), lymphocyte function testing, and functional analysis of phagocytic cells.

Flow Cytometry. Flow cytometry provides additional cellular information by using monoclonal antibodies conjugated to fluorochromes to differentiate morphologically identical lymphocytes into distinct subsets. Flow cytometers use lasers to measure multiple parameters including light scatter and fluorescence characteristics of cells. The fluidics system of the flow cytometer delivers cells one by one to a point in space intersected by a laser beam. The laser beam emits light of a defined wavelength to illuminate the cell, which results in both scattered light of the same wavelength and fluorescent light of a different wavelength that is collected by photodetectors and converted into electronic signals. Optical filtration separates scattered light and fluorescent light to permit their independent measurement. Side-scatter light is dependent on the granularity of cells, whereas forward-scatter light is dependent on the size of the cell. Fluorescent light can be detected independently for a number of fluorochromes of different wavelengths, and typical examples are fluorescein and phycoerythrin. High-end flow cytometers have multiple lasers with different wavelengths and can detect a large number of colors (e.g., 5 lasers with detection of 18 colors), as well as side and forward scatter.

Signals are typically displayed as either histograms or dual-parameter correlated plots (dot plots), and statistical analysis is completed by computer. Histograms are analyzed by setting markers in particular channels and dot plots by drawing rectangular or polygonal boxes around data points. The software also allows the setting of "gates" for determining which events are collected in the first instance or which events are to be included in later analyses. Typically these gating techniques are employed using forward and side scatter to differentiate cell types, such as lymphocytes, monocytes, and granulocytes. The final key characteristic of flow cytometers is their capacity to analyze large numbers of cells in a short time, making it possible to analyze many thousands of cells in a matter of seconds.

An example of such an analysis is shown in Fig. 1.23. The upper panels (A) show side- versus forward-scatter plots of PBMCs isolated from a normal foal (left panel) and SCID foal (right panel). A typical distribution of lymphocytes (red gate), monocytes/blasting cells (blue gate), and granulocytes/neutrophils (green gate) is observed in the normal foal. It should be noted that the Ficoll centrifugation procedure for PBMC isolation removes the vast majority of neutrophils. In the SCID foal, there are very few cells in the normal lymphocyte gate, with the majority of cells being monocytes and larger granular cells, which are most likely NK cells. Standard two-color dot plots analyzing all cells within the lymphocyte and monocyte/blasting cell gates are shown in the next panels (B). In the normal foal, 43% of the PBMCs are labeled by the fluorochrome-conjugated anti–equine CD3 monoclonal antibody and are therefore T cells (upper and lower right quadrants combined). Of these T cells, 26% (or 11.2% of all PBMCs analyzed) are $CD8^+$ T cells (upper right quadrant). These cells are double labeled with the anti–equine CD3 monoclonal antibody and an anti–equine CD8 monoclonal antibody conjugated to a fluorochrome with a different color. The cells in the upper left quadrant are $CD8^+$ but are not T cells and likely represent $CD8^+$ NK cells. Compared with the normal foal, the SCID foal has virtually no T cells but does have a similar percentage of $CD8^+$ non–T cells (NK cells). Additional analysis using fluorochrome-conjugated monoclonal antibodies against equine B-cell and $CD4^+$ T-cell markers would reveal a similar absence of these lymphocyte populations in the SCID foal. Finally, the lower panels (C) show a standard single-color dot plot and corresponding histogram analysis of $CD8^+$ cells in the normal foal.

Lymphocyte Function Testing. Unfortunately tests of lymphocyte function are generally limited in their availability in the field. In vitro tests of lymphocyte function include lymphocyte proliferation responses to mitogens such as pokeweed mitogen, phorbol 12-myristate 13-acetate (PMA) with ionomycin, phytohemagglutinin (PHA), or concanavalin A (ConA). These assays are generally not commercially available, although they are commonly performed by immunologic researchers. It is essential to perform parallel studies on suitable age-matched control horses. The endpoint of these tests is usually read by determining the incorporation of radioactive ^3H-thymidine into the total population of proliferating cells.[464] Nonradioactive alternatives exist, and one strategy utilizes the thymidine analog 5-ethynyl-2′-deoxyuridine (EdU), which readily incorporates into the DNA of replicating cells.[465] The alkyne group of EdU reacts with fluorescent azides in a Cu(I) catalyzed "Click" reaction, which can then be detected using flow cytometry. An EdU incorporation–Click assay kit is available to researchers (Click-iT EdU Imaging Kit, ThermoFisher Scientific),

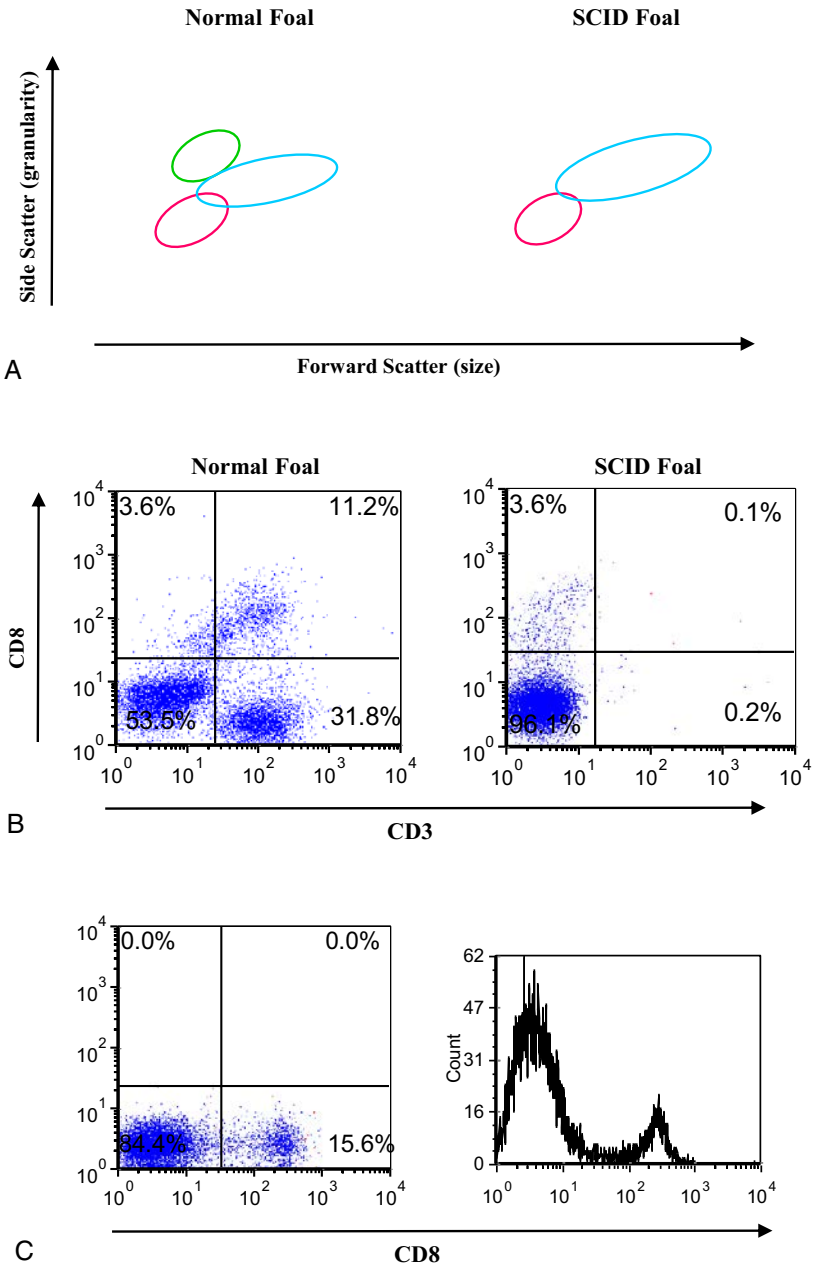


FIG. **1.23** Flow cytometric analysis of PBMCs from a normal foal and an SCID foal. See text for description.

and has shown utility for detecting equine lymphoproliferative responses (see Fig. 1.24). Finally, flow cytometry can be used to determine functional characteristics of various equine lymphocyte subsets by detecting intracellular production of cytokines following nonspecific stimulation with mitogens or antigen-specific stimulation (Fig. 1.24).

Two tests that can be of value and are readily available in practice are response to vaccination, as measured by rising serum titers, and response to intradermal PHA, which is dependent on a delayed-type hypersensitivity T lymphocyte response and develops in normal animals without prior sensitization.[466] A 50-µg dose of PHA in 0.5 mL of phosphate-buffered saline is injected intradermally, and 0.5 mL of phosphate-buffered saline is administered intradermally at a distant site. At the PHA site, an increase in wheal size of 0.6 mm or less indicates a defect in cell-mediated immunity. Response to vaccination has proven to be a potent means of identifying immunodeficiency in such conditions as juvenile llama immunodeficiency syndrome.[467] Similarly the equine immune response to a polyvalent inactivated bovine vaccine has been used to document the immunosuppressive effects of corticosteroid administration.[468] For practical purposes, response to rabies or tetanus vaccination may be the most suitable available tests provided that no routine vaccination had been administered in the immediate past. Equine rabies or tetanus antibody titer determination is typically commercially available, and the majority of available vaccines are sufficiently potent to provoke a fourfold increase in titer in normal horses.

Phagocyte Function Testing. Testing of equine neutrophil migration, phagocytic function, and bactericidal activity has been reported by several investigators,[328,330,469,470] and more quantitative information has been obtained using flow

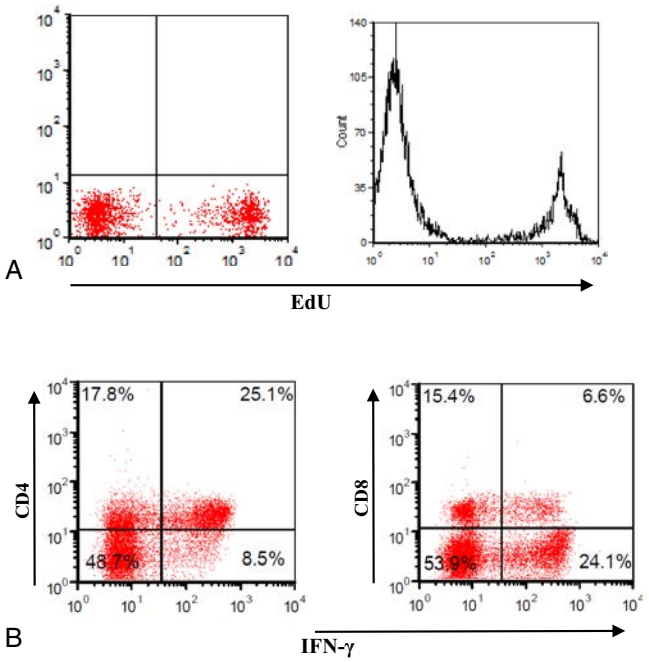


FIG. **1.24** Flow cytometric evaluation of lymphoproliferation and intracellular cytokine production. ***(A)*** Dot plot *(left panel)* and histogram *(right panel)* showing 5-ethynyl-2′-deoxyuridine (EdU) incorporation by proliferating equine PBMCs following in vitro stimulation with antigen. In this case the PBMCs were isolated from a horse infected with *Theileria equi* and stimulated with a *T. equi* sporozoite lysate for 4 days. ***(B)*** Two-color analysis of $CD4^+$ T cells *(left panel)* and $CD8^+$ T cells *(right panel)* for IFN-γ production. Isolated PBMCs were stimulated 24 hours with PMA + ionomycin, then surface labeled with fluorochrome-conjugated monoclonal antibodies against equine CD4 or CD8, followed by intracellular staining using a different fluorochrome-labeled monoclonal antibody against equine IFN-γ. $CD4^+$ cells (or $CD8^+$ cells) producing IFN-γ appear in the upper right quadrants.

cytometric approaches.[220,331,471] The techniques employed are typically only available in research laboratories.

Tests of Innate Immunity

Components of the innate immune response that have been measured in the horse include the numbers of granulocytes and monocytes in peripheral blood (CBC) and their phagocytic function (see earlier discussion), natural cytotoxicity in terms of lymphokine-activated killer (LAK) cell activity,[469,472-474] and measurement of soluble factors including several acute phase proteins.[475-480] Serum amyloid A (SAA) is one acute phase protein that can serve as an early indicator of inflammation, and a rapid stall-side test is now available (StableLab, Sligo, Ireland). Equine complement activity can be measured using hemolytic or flow cytometric assays.[481,482] With the exception of the CBC and SAA tests, tests of innate immune function have very limited availability.

Primary Immunodeficiencies

Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is a lethal primary immunodeficiency affecting Arabian foals, characterized by failure to produce functional B and T lymphocytes and resulting in the complete lack of adaptive immune responses.[445,453,483,484] The vast majority of affected foals are Arabians, in which the condition is inherited as an autosomal recessive trait[485] and results from a lack of DNA protein kinase (DNA-PK) activity that prevents V(D)J recombination.[452,486] In studies conducted in the United States and reported in 1977, the incidence of SCID among Arabian foals was at least 2% to 3%,[487] suggesting a carrier prevalence rate between 25% and 26%. However, in more recent studies conducted in the United States using a precise molecular diagnosis of the carrier state, carrier prevalence was consistently 8%.[488,489]

Clinical Signs and Laboratory Findings. Affected foals are clinically normal at birth but develop signs of infection during the first 1 to 3 months of life. The age of onset of infection depends on the adequacy of transfer of passive immunity and degree of environmental challenge. As maternal antibodies are catabolically eliminated SCID foals are increasingly susceptible to infections with bacterial, viral, fungal, and protozoal agents. Bronchopneumonia is a prominent disease, often caused by adenovirus (the most significant pathogen of SCID foals, affecting two thirds of them),[490] *Pneumocystis carinii,* or *Rhodococcus equi.* Enteritis, frequently caused by *Cryptosporidium parvum,*[491] arthritis, and omphalophlebitis are common. Adenoviral infection frequently extends to the gastrointestinal and urogenital systems and causes pancreatic disease leading to loss of endocrine and exocrine tissue and possibly contributing to the impaired growth and weight loss observed in SCID foals.[445]

Clinical signs include nasal discharge, coughing, dyspnea, diarrhea, fever, and weight loss. Although antibiotics, plasma, and supportive care prolong the course of disease, death invariably occurs before 5 months of age. The only exception was a single foal experimentally treated with a bone marrow transplant from a histocompatible donor that lived until 5 years of age before dying of an unrelated cause.[492,493]

A consistent hematologic finding is absolute lymphopenia (less than 1000/µl; usually less than 500/µl), with neutrophilia a variable finding resulting from bacterial infection. Total serum globulins and serum IgG can be normal in the first weeks of life if transfer of passive immunity is adequate but decline as maternal immunoglobulins are catabolized. Normal foals synthesize IgM from 180 days of gestation and have detectable IgM at birth in presuckle samples, whereas SCID foals have no IgM.[314] After colostral ingestion SCID foals also have IgM, although with its relatively short half-life it is undetectable by 2 to 4 weeks of age.[445]

Etiology and Pathogenesis. Normal maturation of T and B lymphocytes requires rearrangement of germ line V, D, and J genes to encode the required wide diversity of TCRs and BCRs, through the process of V(D)J recombination (see under Equine Immunology). This process depends on two groups of enzymes, the recombinase activating gene (RAG) products, which cut DNA, and DNA-PK, which is critical for DNA double-stranded break repair. In equine SCID, the genetic defect is a 5-base pair deletion in the gene encoding the catalytic subunit of DNA-PK (DNA-PKcs).[452] This frameshift mutation truncates the protein, resulting in a complete lack of DNA-PK activity.[452,486] Because DNA-PK is required for DNA strand break repair, V(D)J recombination does not occur.[486] As a consequence, developing T cells and B cells do not produce functional TCRs and BCRs and thus fail the positive selection process in the primary lymphoid organs.

Diagnosis. Previously antemortem diagnosis of SCID was suggested by appropriate clinical signs in a foal of Arabian breeding with persistent marked lymphopenia (usually less than 500/µL) and the absence of serum IgM by RID. If presuckle serum is unavailable for testing, serum IgM cannot be used as a diagnostic aid until the foal is older than 3 weeks. All suspected cases required confirmation by the necropsy finding of hypoplasia of the spleen, thymus, and lymph nodes with the absence of any normal lymphoid architecture. With the identification of the genetic defect causing SCID, the current standard for definitive diagnosis is demonstrating that the foal is homozygous for the defective SCID gene. This test depends on PCR amplification of a specific region of the DNA-PKcs gene, and evaluation of the amplicon using probes specific for normal and mutant sequences.[463] This test is commercially available (VetGen, Ann Arbor, MI); can be performed on whole blood, pulled hair, or cheek swabs; and identifies homozygous-affected, heterozygous carrier, and normal animals.

Treatment. Supportive care may prolong the course of disease, but affected foals die by 5 months. Immunologic reconstitution is currently impractical and an ethically questionable procedure.

Client Education. Arabian mares and stallions intended for breeding should be tested for SCID carrier status. When two heterozygous carriers are bred the progeny will include 25% SCID foals, 50% carriers, and 25% homozygous normal foals. Therefore prevention of SCID requires identification of carriers and either their removal from the breeding population or breeding exclusively with homozygous normal animals and subsequent testing of progeny before their own breeding future is planned.

Foal Immunodeficiency Syndrome

In the late 1990s a syndrome of anemia, immunodeficiency, and peripheral ganglionopathy was described in Fell pony foals and termed *Fell pony syndrome*.[494,495] This syndrome has also been identified in Dales pony foals and is now known as foal immunodeficiency syndrome (FIS).[496-498] Affected foals become ill within 2 to 3 weeks of birth and die by 3 months of age. Affected animals have been described outside the United Kingdom[499] and in the United States.[500]

Clinical Signs and Laboratory Findings. Clinical signs include ill thrift, anemia, respiratory infection, glossal hyperkeratosis, and diarrhea. Anemia can be severe and is normochromic and normocytic to macrocytic, with small numbers of late erythroid precursors in bone marrow.[495,501] Although bone marrow erythroid precursors are present at birth, erythroid hypoplasia develops rapidly and progresses to aplasia, resulting in severe progressive nonregenerative anemia.[502] Some foals are affected by cryptosporidial enteritis and adenoviral bronchopneumonia and pancreatitis. Plasma proteins and blood lymphocyte counts can be normal or low. Immunodeficiency is due to profound B-cell lymphopenia,[503,504] with a lack of B cells in the bone marrow and lymph nodes and very few within germinal centers in the spleen.[500,502] Concentrations of IgG subclasses are significantly lower in FIS foals compared with age-matched controls, coinciding with reduction of maternally derived antibodies and correlating with B-cell lymphopenia.[503] Some studies have described normal IgG concentrations in FIS foals, although decreases would likely have been observed had the foals survived longer.[500,502] IgM concentrations consistently decline in FIS foals compared with controls.[502,503] Taken together these observations indicate an absence of endogenous immunoglobulin production in FIS foals caused by the lack of functional B cells. In contrast, T-cell subsets measured by flow cytometry are normal, and lymphoproliferative responses are normal.[500,505] However, MHC II expression is low,[500,505] thymic hypoplasia is observed,[495,500] and T cells can be absent in the bone marrow.[502] Thus it is possible that an unidentified defect in T-cell function exists. This is further suggested by the occurrence of opportunistic infections in the face of adequate levels of maternally derived antibodies. On necropsy, lymphoid organs can be small, and secondary lymphoid follicles and plasma cells are absent.[495,500,502] Neuronal changes are characterized by neuronal chromatolysis in the cranial mesenteric, dorsal root, and trigeminal ganglia.[495]

Etiology and Pathogenesis. FIS is inherited as an autosomal recessive trait,[496] and a mutation in the sodium/myo-inositol cotransporter gene (SLA5A3) on chromosome 26 is strongly associated with the syndrome.[498] This is an osmotic stress response gene that encodes a cell membrane transporter protein that cotransports sodium ions and myo-inositol. Despite the strong association, the specific role of this gene in erythrocyte and B-cell development is unknown, and formal proof that the SLA5A3 mutation is the direct cause of FIS requires additional functional studies.

Diagnosis. The presence of anemia, diarrhea, ill thrift, and/or respiratory and other infections in young Fell and Dales pony foals along with low IgM levels and B-cell lymphopenia (as measured by flow cytometry) allows a presumptive diagnosis. Confirmation of FIS can now be made by demonstrating that the foal is homozygous for the SLA5A3 mutation, using the available DNA test (Animal DNA Diagnostics, Cambridge, UK). This PCR-based test is performed on pulled hair and identifies homozygous-affected, heterozygous carrier, and normal animals.

Treatment. Treatment of specific secondary infections is of limited efficacy in affected foals, particularly those affected by severe anemia and diarrhea, and all die by 3 months of age.

Client Education. The Fell and Dales pony breeds lost significant genetic diversity during World War II, and the overuse of a few popular sires likely resulted in the emergence of FIS, which until very recently affected up to 10% and 1% of Fell and Dales foals, respectively.[498] Genetic testing results reported in 2011 indicated that 38% of adult Fell ponies and 18% of adult Dales ponies were carriers of the FIS defect.[497] Similar to preventing SCID in Arabian horses, all Fell and Dales ponies used for breeding should be tested for FIS carrier status so that carrier-carrier matings are avoided. This will eliminate the production of FIS-affected foals and will eventually reduce the prevalence of the gene defect in the population. Implementation of this testing strategy has significantly decreased the number of FIS-affected foals, but it will take time to reduce the mutant gene frequency in the population given the large number of carriers.[506]

Common Variable Immunodeficiency

In humans, common variable immunodeficiencies are a heterogeneous group of disorders that do not become evident until late childhood or adulthood and comprise the most common forms of primary immunodeficiency.[209] In horses, common variable immunodeficiency (CVID) is a rare adult-onset fatal disorder characterized by B-cell depletion and inadequate antibody production caused by failure of B lymphocyte maturation in the bone marrow.[448]

Clinical Signs and Laboratory Findings. Over 30 cases of CVID have been reported in recent years.[507-511] Age of onset ranges from 2 to 23 years, there is no sex or breed predisposition, and cases are sporadic. Hypogammaglobulinemia leads to recurrent infections, typically caused by *Streptococcus, Staphylococcus, Actinobacillus,* and *Klebsiella* spp. Affected horses can present with pneumonia, sinusitis, meningitis, ataxia, diarrhea, peritonitis, gastrointestinal parasites, uveitis, conjunctivitis, skin infections, weight loss, and muscle atrophy.[448] Neutrophilia and hyperfibrinogenemia occur during infections, and transient lymphopenia (less than 1200 cells/μL) is common. Serum IgG is uniformly low in affected horses (less than 800 g/dL) as is IgM (less than 25 mg/dL), and IgA concentrations tend to decrease as the disease progresses (less than 60 mg/dL). Flow cytometry reveals B-cell lymphopenia (less than 2% of PBMCs) in all cases. Tetanus toxoid vaccination does not result in a rise in antibody titer in affected horses. Necropsy reveals lesions associated with infection in the affected organ systems. Lymph nodes are small and lack germinal centers, plasma cells are absent in lymphoid and mucosal tissues, and B cells are typically absent in the bone marrow and spleen.[448,511]

Etiology and Pathogenesis. To maintain populations of B cells in secondary lymphoid tissues, hematopoietic stem cells in the bone marrow must continuously generate B cells throughout an individual's lifetime. Although the pathogenesis of CVID is still unclear, the progressive B-cell depletion indicates a failure of B-cell development in the bone marrow. Early B-cell commitment genes, including E2A and PAX5, are decreased in the bone marrow of CVID-affected horses.[511] The E2A gene encodes a transcription factor that initiates B-cell differentiation and PAX5 expression, which encodes the B-cell-lineage–specific activator protein (BSAP) that is absent in the bone marrow of affected horses.[511] Thus disruption of pro–B-cell development during the transition from pre–pro–B cells could be the cause of CVID, and epigenetic gene silencing mechanisms may be involved.[448,510,511]

Diagnosis, Treatment, and Client Education. CVID is rare but should be suspected in adult horses with recurrent infections as described previously, hypogammaglobulinemia, B-cell lymphopenia, and failure to respond appropriately to vaccination. Affected horses with mild infections can be managed for variable lengths of time with continuous or intermittent oral antimicrobial therapy, but severe infections require aggressive intravenous antimicrobials and appropriate supportive therapy. Although regular immunoglobulin replacement is used in human patients, this is not practical in affected horses. Affected horses are usually euthanized within 6 months of diagnosis because of financial considerations and poor quality of life.[448]

Selective IgM Deficiency

Selective IgM deficiency is characterized by substantially reduced or absent serum IgM with normal or increased concentrations of other immunoglobulins and no other evidence of immunodeficiency.[445,512] Serum IgM concentrations are more than 2 standard deviations below the mean of age-matched control animals. All other immunologic parameters are normal, although a lack of response to the B-cell mitogen lipopolysaccharide was reported in an affected Standardbred foal.[513] The syndrome has been most frequently described in Arabians and Quarter Horses, although the diagnosis has been made in other breeds.

Clinical Signs and Laboratory Findings. Two clinical syndromes have been described. The first condition affects foals 2 to 8 months of age, which develop severe pneumonia, arthritis, and enteritis with or without septicemia, and many die before 10 months of age. Gram-negative bacterial infections are common (especially with *Klebsiella* species), and age at onset of signs is generally older than in foals with combined immunodeficiency. Some affected foals survive but have a history of repeated bacterial infections that respond temporarily to therapy but recur once antimicrobials are discontinued. These foals grow poorly and generally die within 2 years. Rarely foals can recover from IgM deficiency, suggesting that such cases may actually be secondary rather than primary immunodeficiencies.[514]

The second presentation involves horses between 2 and 5 years of age, many of which have or ultimately develop lymphoma. These individuals may have external or internal lymphadenopathy or both. Chronic weight loss, depression, and other nonspecific signs usually accompany lymphoma. In cases associated with lymphoma, the IgM deficiency is presumed to be a secondary rather than a primary immunodeficiency.

Routine laboratory findings are not diagnostically specific. Hematologic abnormalities consistent with chronic inflammatory disease, such as anemia, neutrophilia, and hyperfibrinogenemia, are commonly present. The total plasma protein and serum globulins are usually normal.

Etiology and Pathogenesis. Although a genetic basis is suspected, the pathogenesis of selective IgM deficiency is unknown.[445] It seems likely that there are primary and secondary forms of this syndrome. The pathogenesis of IgM deficiency is also unclear in humans but might involve decreased T-helper activity, increased isotype-specific suppressor T-cell activity, and intrinsic B-cell defects.[515]

Diagnosis. Definitive diagnosis of selective IgM deficiency is made by measuring the major serum immunoglobulins by RID and determining the absolute lymphocyte count. Horses

with selective IgM deficiency have serum IgM concentrations persistently less than 2 standard deviations below that of age-matched controls (less than 15 mg/dL at 4–8 months; less than 25 mg/dL at greater than 8 months) coupled with normal concentrations of IgG (≥1000 mg/dL) and a normal lymphocyte count. The normal IgM concentration in healthy fit horses is 103 ± 40 mg/dL, and a cut-point for IgM deficiency has been defined as ≤23 mg/dL.[516] Because seriously ill foals may have transiently depressed serum IgM concentration, suspected cases should be tested at least twice to document that IgM concentrations remain low. All other immunoglobulin concentrations are normal.

Treatment and Client Education. Other than supportive care and antimicrobial therapy, there is no effective treatment for selective IgM deficiency. Transfused plasma concentrations of IgM are low and the half-life is quite short; thus any benefit would be only temporary. The prognosis must be guarded; however, recovery has been reported.[514] Because primary immunodeficiency is likely in foals affected during the first year of life, it may be inadvisable to remate the sire and dam.

Other Primary Immunodeficiencies

Transient hypogammaglobulinemia and agammaglobulinemia are reported as established primary immunodeficiency syndromes in horses. However, these conditions have been infrequently reported and consequently remain poorly defined. Available information is presented here. A further form of immunodeficiency affecting humoral immunity was described by Boy and colleagues[517] in a 10-month-old Arabian colt. The animal exhibited an absence of serum of IgM, IgA, and IgG(T) and a normal concentration of IgG. In vitro testing of PBMCs with T-cell mitogens elicited normal responses, whereas responses to B-cell mitogens were weak. On postmortem examination there was generalized lymphocyte depletion of lymphoid organs.

To increase our understanding of these and other currently unidentified immunodeficiency syndromes of horses, it is critical that every effort be made to identify such cases and thoroughly investigate them. New immunologic resources now available may make it possible to further define these diseases and increase our diagnostic and prognostic resources, provided the case material can be identified.

Transient hypogammaglobulinemia has been reported in only two foals, an Arabian and a Thoroughbred, and is characterized by delayed onset of immunoglobulin synthesis.[514,518] Affected foals manifested signs consistent with bacterial and viral infections when passively acquired immunoglobulins are catabolized to nonprotective concentrations. For unknown reasons, the onset of autologous immunoglobulin production, which generally occurs at birth, is delayed until these foals are approximately 3 months of age. Hematologic studies may be suggestive of chronic infection, although total plasma protein is normal or reduced slightly. Diagnosis is based on the presence of low serum IgG (less than 200 mg/dL) and IgG3/5 (less than 20 mg/dL) at 2 to 4 months of age, with low-normal serum IgM (greater than 15 mg/dL) and IgA (greater than 20 mg/dL). Lymphocyte counts are normal. Antimicrobial therapy and plasma transfusions are necessary to minimize infections. Affected foals usually survive if they have not concomitantly suffered FTPI and they receive appropriate support between 2 and 4 months of age. Because foals spontaneously recover from this condition, it may be a secondary immunodeficiency.

Agammaglobulinemia is characterized by absence of B lymphocytes and failure to produce immunoglobulins in the presence of normal cell-mediated immunity.[445] The disease has been described in five colts of Thoroughbred, Standardbred, or Quarter Horse breeds.[514,519,520] Clinical signs commence between 2 and 6 months of age and result from bacterial infections such as pneumonia, enteritis, and arthritis. Multisystemic infections that respond poorly to therapy are common, and laboratory changes reflect chronic inflammatory disease. The fact that this syndrome has only been described in colts suggests an X-linked mode of inheritance, as occurs in X-linked agammaglobulinemia in humans, which is caused by a mutation in a protein tyrosine kinase gene called Bruton's tyrosine kinase *(btk)*.[521] The lack of this kinase activity results in a failure of pre–B cells in the bone marrow to develop into mature B cells. A maturation defect from stem cells to B cells has been suggested in affected horses.[445] Affected foals have persistently subnormal serum concentrations of all immunoglobulin classes and normal lymphocyte counts. Serum IgM and IgA are generally absent at the time of evaluation, and maternally derived IgG and IgG3/5 decline with time. At 2 months of age, IgG is less than 300 mg/dL, which declines to less than 100 mg/dL by 6 months. There is no serologic response to immunization, and B lymphocytes, as determined by immunofluorescence, are absent. Tests of cell-mediated immune function such as intradermal PHA and in vitro blastogenesis are normal. Plasma and antimicrobial therapy only result in transient improvement. Affected horses die of disseminated infection between 1 and 2 years of age.

Secondary Immunodeficiencies

Failure of Transfer of Passive Immunity

FTPI is the most common immunodeficiency disorder of horses and has been extensively reviewed.[325,446] It occurs in all breeds secondary to inadequate absorption of colostral antibodies. FTPI is significantly correlated with increased susceptibility to infectious disease and death in neonatal foals.[522,523] The newborn foal is capable of mounting a normal immune response, as described earlier in this chapter. However, neonatal foals are immunologically naive and thus have not developed memory responses or produced antigen-specific antibody or T-cell responses.

During the first 1 to 2 months of life, foals are dependent on transfer of passive immunity for protection from infectious disease. The diffuse epitheliochorial nature of the equine placenta does not allow for in utero immunoglobulin transfer to foals. Although minor concentrations of IgM and IgG can be detected at birth, the foal is born essentially agammaglobulinemic and acquires passive immunity by the ingestion and absorption of colostrum from the dam.[350,353,522] Colostrum is a specialized form of milk containing concentrated immunoglobulins that is produced during the last 2 weeks of gestation under hormonal influences. Colostrum contains primarily IgG4/7, along with IgG1 and IgG3/5, and lesser amounts of IgA and IgM, all of which have been concentrated into mammary secretions from the mare's blood.[249,315,317,326,524] Although yet to be identified in foals, the neonatal Fc receptor (FcRn) transports maternal IgG from the intestinal lumen through enterocytes and into the systemic circulation in mice.[525] This receptor has also been identified in the mammary gland of cows and other species, but its functional role in transporting maternal IgG into colostrum has not been

confirmed.[526-528] Whether FcRn is involved in this process in the mare is unknown. Colostrum is only produced one time each pregnancy and is replaced by milk that contains negligible immunoglobulins within 24 hours of the initiation of lactation.[350,524] This extremely rapid decline in immunoglobulin concentrations in mammary secretions is consistent with equine colostrum production ending at or even before parturition.[317] Intestinal absorption of immunoglobulins occurs in the small intestine, is greatest during the first 6 hours after birth, and then steadily declines until immunoglobulins can no longer be absorbed when the foal is 24 hours old.[529,530] Absorption is not selective for immunoglobulin as other macromolecules are absorbed,[529,530] potentially along with maternal T cells present in colostrum.[531] Thus the nonspecific intestinal absorption of large molecules within the first 24 hours in foals and other livestock species is the result of pinocytosis by specialized enterocytes and is FcRn independent.[526] This gut "closure" most likely results from the replacement of these specialized enterocytes by more mature cells within the first day of life.

The incidence of FTPI is highly variable among groups of horses and seems to depend primarily on management factors that ensure early colostral ingestion.[532] The reported prevalences of at least partial FTPI have ranged from 3% to 37%.[514,522,532,533]

Clinical Signs and Laboratory Findings. FTPI does not directly cause any clinical signs of disease. FTPI is suspected when signs of generalized or localized bacterial infections such as septicemia, pneumonia, enteritis, and arthritis develop during the first 3 weeks of life. Routine laboratory findings may be suggestive of sepsis, but the presence of infection in the neonatal period is not pathognomonic for FTPI. Common abnormalities include neutropenia or neutrophilia, hypoglycemia, and hyperfibrinogenemia. The total plasma protein may be low, normal, or elevated in foals with FTPI because of the wide variation in normal presuckle total plasma protein and the confounding effects of dehydration secondary to sepsis.

Etiology and Pathogenesis. Causes for FTPI in foals include (1) failure of the foal to ingest an adequate volume of colostrum in the early postpartum period; (2) loss of colostrum via premature lactation; (3) inadequate immunoglobulin content of the colostrum; and (4) insufficient immunoglobulin absorption via the intestine.[325,446,534] There is a highly negative correlation between foal serum IgG concentration and the incidence of severe infections[522]; however, the minimum amount of IgG necessary for protection of a foal from infection varies with the amount and virulence of environmental pathogens, concomitant stress factors, and colostral antibody titer against specific pathogens. Although a serum IgG concentration of at least 400 mg/dL has been considered evidence of adequate transfer of passive immunity, most normal foals attain values more than twice this high,[522,532] and serum IgG greater than 800 mg/dL is likely required for adequate immunity.[523] A recent study in hospitalized foals supports this latter conclusion.[454] Numerous other colostral factors may be important for the immune protection of foals. Colostrum has been variously shown to regulate cell-mediated immunity, activate granulocytes, promote intestinal absorption of macromolecules, decrease intestinal colonization by pathogens, and contain constituents of innate immunity (e.g., lactoferrin, complement) and leukocytes that have a local protective role in the neonatal digestive tract and may be systemically absorbed.[249,531,535,536] At this time the significance of these various phenomena for the health of neonates and their immunologic development is largely unknown. The one exception is the finding that colostral ingestion suppresses de novo antibody responses in foals in both a nonspecific and an antigen-specific manner.[350,358]

Neonatal weakness, musculoskeletal problems, or lack of maternal cooperation (maiden mares) are common reasons for failure to ingest an inadequate volume of colostrum. If colostral ingestion is delayed beyond 6 hours, the absorption of immunoglobulins is significantly reduced. Lactation before parturition is another common reason for FTPI, because colostrum is only produced one time each gestation. The causative factors for premature lactation are unknown at this time, but foals from mares that "leak" milk hours to days before parturition are likely to suffer FTPI.[534]

Subnormal colostral immunoglobulin content (less than 3000 mg/dL) is rare in mares that do not prelactate,[532] but wide individual variation in colostral concentration of immunoglobulins does occur.[532,537-539] Poor-quality colostrum will undoubtedly cause FTPI. Colostral immunoglobulin content can be estimated by specific gravity or quantitated by RID.[534] A Brix refractometer provides a quick and easy stall-side method to accurately estimate colostral immunoglobulin content, with a reading of 20% to 30% equating to an IgG concentration between 5000 and 8000 mg/dL.[540] A glutaraldehyde coagulation test is also available for measuring colostral IgG (Gamma-Check C, Plasvacc USA, Templeton, CA), with clot formation within 10 minutes indicating an IgG concentration of greater than 3800 mg/dL. Malabsorption is implicated as a cause of FTPI when foals are known to have ingested an adequate volume of good-quality colostrum within 12 hours of birth. Because glucocorticoids hasten the maturation of specialized enterocytes, stress-causing endogenous corticosteroid release may be a cause of reduced immunoglobulin absorption. However, obvious stress factors are often not found in foals with apparent impaired ability to absorb IgG.[205]

Diagnosis. Subnormal serum IgG concentration 24 hours after birth is the basis for diagnosis of FTPI. Serum IgG of less than 400 mg/dL is indicative of complete FTPI, and 400 to 800 mg/dL is considered partial FTPI. With the exception of hospitalized foals,[454] many foals under good management conditions may remain healthy if the serum IgG concentration is at least 400 mg/dL, and consequently this cut-point is measured by several rapid diagnostic tests. The most quantitatively accurate method to determine serum IgG is by RID as discussed earlier. However, this assay is time-consuming and expensive and thus inappropriate for the diagnosis of FTPI when timely therapeutic intervention is paramount. Numerous field screening procedures for IgG have been evaluated.[446,455-457,459-461,541] Criteria for selecting a screening test for equine FTPI must include accuracy, the time necessary to perform the test, ease of performance, and cost. The ease and reported accuracy of membrane-based ELISA tests often make them the test of choice in many practice situations. Because failure to diagnose and treat the condition could result in the death of many foals, a sensitive test is required. However, specificity is also important, particularly given the cost of treatment and the fact that treatment is not without its own inherent complications. Generally, all the available immunoglobulin screening tests are relatively accurate in identifying foals with complete FTPI; however,

there is variation in their ability to detect marginally deficient foals.[325,446,461] Individual clinician judgment is therefore important in test selection. As discussed, the glutaraldehyde coagulation test (Gamma-Check E) is good for initial screening because it is simple, rapid, inexpensive, and sensitive for ruling out FTPI (IgG concentration ≥800 mg/dL). However, this test lacks specificity, so a diagnosis of FTPI based on this test should be confirmed with a more specific test, such as the membrane filter ELISA (SNAP).[325,461] Overall, the relatively good performance and the convenience of the semiquantitative SNAP test will continue to make it a popular first choice.

Treatment. If FTPI is anticipated because of premature lactation, neonatal weakness, dam death, or low-specific-gravity colostrum, an alternative source of colostrum or a commercial concentrated equine IgG product (Seramune, Sera, Inc., Shawnee Mission, KS) should be given orally. A minimum of 2 L of equine colostrum given in 500-mL increments during the first 8 hours after birth is optimal. Bovine colostrum may be safely substituted if equine colostrum or commercial equine IgG product is not available[349,351]; however, foals given bovine colostrum may also require plasma transfusion because bovine immunoglobulins have a very short half-life in foals and are not specifically directed against equine pathogens.

If the foal is more than 12 to 18 hours old when FTPI is suspected or diagnosed, an intravenous plasma transfusion is indicated. The U.S. Department of Agriculture (USDA) has approved numerous commercial sources of equine plasma, and use of these products is convenient, saves time, and is safe because donors are free of alloantibodies and negative for infectious diseases. The only potential drawback to the use of commercial plasma is that antibodies specific for pathogens in the foal's environment may be lacking. Optimal plasma would be obtained from a local blood-typed donor known to lack serum alloantibodies and alloantigens Aa and Qa. The volume of plasma necessary to bring serum IgG into an acceptable range cannot be accurately predicted because it is dependent on the severity of FTPI, the immunoglobulin content of the plasma, and concomitant diseases, which may hasten immunoglobulin catabolism. Generally, 1 L of plasma will increase the serum IgG concentration of a 50-kg foal by 200 to 300 mg/dL[237]; thus 2 to 4 L may be necessary to achieve serum IgG greater than 800 mg/dL. A therapeutic dose of plasma should be administered and then serum IgG concentration reassessed. If the desired concentration has not been attained, more plasma is necessary. Some foals with partial FTPI (IgG greater than 400 and less than 800 mg/dL) may do well without plasma therapy if there are no preexisting infections and exposure to pathogens is minimized. These foals should be monitored closely for the development of infections. However, hospitalized foals with IgG concentrations in this range should receive plasma.[454]

Client Education. The prognosis for foals with FTPI depends on the degree of failure, the environment to which the foal is exposed, the foal's age at the time of diagnosis, and the presence and severity of secondary infections. Management factors that ensure the ingestion of at least 2 L of high-quality colostrum within 6 hours of birth are paramount in FTPI prevention. Foaling should be witnessed so that any malpresentations can be corrected, and foals that do not readily nurse within 3 hours can be given colostrum or suitable substitute via nasogastric tube. Colostral IgG concentration can be used as a predictor of FTPI. As measured by specific gravity, a colostral IgG concentration of 3000 mg/dL is the minimum acceptable value, and when colostrum contains less than this amount, some degree of FTPI should be suspected in the foal and corrected.[534] Brix refractometry[542] or the Gamma-Check C test could also be used for this purpose. Routine screening of foal serum IgG at 24 to 48 hours after birth allows necessary plasma therapy before the onset of infections.

Foals that are born prematurely, are weak, or are from prelactating mares should be provided with an alternative colostral source or commercial equine IgG product within 6 hours of birth. A colostrum bank can be established by collecting and freezing (−20°C) 250 mL of colostrum from mares that have not prelactated within 6 hours of foaling, once their own foals have suckled. Ideally, banked colostrum should be screened for alloantibodies, although they are unlikely if the mare's own foal remains healthy. The immunoglobulins in banked frozen colostrum are stable for at least 1 year.

Exercise

Exercise, when conducted at a stressful level, can significantly affect equine immune function.[543] Strenuous exercise significantly suppresses lymphoproliferative responses, whereas lymphokine activated killer (LAK) cell activity increases.[544,545] In racehorses, decreased lymphoproliferative responses can be demonstrated 12 to 16 hours after racing.[546] Protracted high-intensity training results in decreased phagocytosis and oxidative burst activity in neutrophils and lymphocytes, although pulmonary alveolar macrophage function is unaffected.[547] Other studies have demonstrated decreased neutrophil and pulmonary alveolar macrophage function in response to single bouts of intense exercise[548,549] and prolonged suppression of innate immunity after exercise of long duration.[550] Incremental exercise in unfit Standardbred mares results in an increase in inflammatory cytokine expression in blood (IFN-γ, TNF-α, and IL-1) and in muscle tissue (IFN-γ, TNF-α, and IL-6).[551] Strenuous exercise results in neutrophilia, increased apoptotic activity in leukocytes, and decreased leukocyte reactive oxygen species production in response to PMA stimulation, with some of these effects persisting for 72 hours after exercise.[552] In unconditioned ponies strenuous exercise increases susceptibility to experimental infection with equine influenza, associated with alterations in the virus-specific cell-mediated immune response.[553] In an influenza infection study in trained horses, moderate exercise led to increased signs of clinical disease, although duration of disease was unaffected.[554]

These various studies have demonstrated an immunomodulatory effect of exercise, with some specific evidence for increased susceptibility to infectious disease. Some of these effects are likely due to increases in plasma cortisol, which is correlated with the intensity and duration of exercise and with alteration in immune function in horses.[555] In general, moderate exercise appears to be beneficial to the immune system, whereas prolonged and intense exertion likely impairs immune function. The potentially immunosuppressive effect of high-intensity exercise, particularly of protracted duration or in unconditioned animals, should be recognized.

Age

There is a high incidence of respiratory infections in foals and weanlings, which frequently relapse on cessation of antibiotic

treatment.[556] It is also possible that the normal level of immunocompetence in this age group leads to increased susceptibility to respiratory infections, particularly under group housing conditions. Features of the foal's immune system that predispose to such infections have been discussed earlier in this chapter.

Geriatric horses experience a general reduction of immune function similar to humans, a phenomenon called immunosenescence. Also like humans, old horses tend to produce exaggerated inflammatory responses, known as "inflamm-aging."[557,558] Immunosenescence in humans is associated with age-related defects in the thymic and bone marrow microenvironment and a predominance of memory T and B cells with limited repertoire diversity.[559] In addition, increased cell death in the elderly, along with hormonal dysregulation, increased oxidative stress, and the presence of chronic diseases, contribute to a low-grade activation state in innate cells such as macrophages, which characterizes inflamm-aging.[559] Many of these effects are likely due to immune dysregulation, and the underlying cause could involve age-related alterations in the membrane composition and in critical signaling and regulatory molecules in immune cells such as neutrophils and T cells.[560]

Horses greater than 20 years old have decreased total lymphocyte counts and reduced numbers of B cells and $CD4^+$ and $CD8^+$ T cells, as well as decreased lymphoproliferative responses to mitogens.[561,562] In addition, old horses demonstrate reduced immunologic responses during exercise.[544] Although lymphoproliferative responses are decreased in old horses, T cells still produce IFN-γ and other inflammatory cytokines, and elevated expression of inflammatory cytokines occurs in peripheral blood along with elevated levels of TNF-α in serum, consistent with inflamm-aging.[563] Obesity likely contributes to the age-related proinflammatory state as obese old horses have higher frequencies of lymphocytes and monocytes producing IFN-γ and TNF-α than old thin horses.[564] Interestingly, a proinflammatory bias may not occur in the lungs of old horses. As evidence, the frequency of IFN-γ producing lymphocytes in both PBMCs and BAL cells is elevated in old horses, whereas age-related production in TNF-α is only present in PBMCs.[565] Moreover, an age-related decrease in BAL cell proinflammatory cytokine expression has recently been documented in horses.[566] Thus the process of inflamm-aging may be organ specific in the horse.

Telomeres protect the ends of chromosomes and contribute to the stability of the genome; maintenance of telomere length and telomerase activity inversely correlate with age, and shorter telomeres are associated with age-related chronic diseases.[567] Telomere length in PBMCs declines with age in horses and is inversely correlated with inflammatory cytokine expression and positively correlated with lymphoproliferative responses and total immunoglobulin levels.[568] Although these observations suggest a role for telomere length in equine immunosenescence and inflamm-aging, the associations between telomere length and immune function do not apply in the oldest horses, indicating that other factors are also involved.

Finally, old horses can mount a primary humoral immune response against a killed rabies vaccine similar to young horses, but their memory response against a killed influenza virus vaccine results in lower antibody titers than in young horses.[569] Lower antibody titers following influenzavirus vaccination in old horses has been observed by others.[544] A recent study measured antibody and cell-mediated responses in old horses following vaccination with a canarypox recombinant vector influenza vaccine.[570] In young naive horses the vaccine induced antibody and cell-mediated responses as measured by IFN-γ production and reduced viral shedding and protected against clinical disease following influenzavirus challenge. The old horses in this study were not naive, and vaccination slightly enhanced antibody titers whereas vaccine-elicited cell-mediated memory responses were not detectable. Nonetheless, virus shedding was reduced, and clinical protection occurred in the old horses.

The geriatric horse population is on the rise,[557,558] requiring an increased appreciation for age-related diseases and other problems unique to the older horse. Immune responses are diminished in old horses, and old horses are prone to produce inflammatory responses. Some vaccine strategies that are effective in younger horses may result in less robust immune responses in old horses, leading to lower levels of protection and an increased need for boosting. Further studies in this area will hopefully improve the understanding of immune regulation and function in old horses to better inform immunoprophylactic and therapeutic approaches.

Leukoproliferative Disease–Associated Immunodeficiency

Lymphoma is often associated with IgM deficiency[446,516,571] and can also be associated with decreased lymphocyte blastogenesis.[572,573] Affected horses have been diagnosed with bacterial pneumonia in some instances, as well as pulmonary aspergillosis in a horse with myelomonocytic leukemia.[574] These cases demonstrate the importance of considering leukoproliferative disease, and particularly lymphoma, in cases of persistent infections that are refractory to treatment.

Drug-Induced Immunodeficiency

The most common iatrogenic cause of immunosuppression is corticosteroid treatment, often administered with the aim of treating a hypersensitivity disorder. The mode of action and the immunosuppressive effects of corticosteroids are reviewed in the next section, but there is evidence for the capacity of corticosteroids to induce recrudescence of viral diseases such as equine infectious anemia[575-577] or EHV-1 infection[578] and to lead to development of life-threatening bacterial infection.[579] Based on the EIAV studies, dexamethasone administration results in profound reduction of peripheral blood B- and T-cell numbers,[575] and the recrudescence of viremia is most likely due to the suppression of T-cell responses because the virus-specific neutralizing antibody titers do not change.[576] There is also evidence that corticosteroid treatment can bias adaptive immune responses to vaccination in horses, specifically suppressing IgG1 and IgG4/7 responses without affecting IgG3/5 responses.[468] This phenomenon may be consistent with the capacity of corticosteroids to specifically suppress T_H1 immune responses with minimal effect on T_H2 responses.[580,581]

Infectious Disease

Several infectious diseases have been associated with immunodeficiency in horses. The best-characterized example may be perinatal EHV-1 infection.[582] Foals that are infected late in gestation with EHV-1 are often born weak with interstitial

pneumonia and develop a variety of bacterial diseases.[583] Affected foals have profound lymphopenia and generally die, despite therapy. The immunodeficiency is thought to be due to viral-induced lymphoid damage, because necropsy reveals marked necrosis of lymphoid tissue in the thymus, spleen, and lymph nodes. Another example is EIAV infection. During acute viremic episodes, reduction of peripheral blood $CD4^+$ and $CD8^+$ T-cell numbers occurs as does suppression of lymphoproliferative responses.[584,585]

Undifferentiated Immunodeficiencies

A group of foals with oral candidiasis and bacterial septicemia between 2 weeks and 4 months of age were found to have laboratory or histologic evidence of immunodeficiency that did not fulfill diagnostic criteria for any of the recognized primary immunodeficiencies.[586] Oral lesions ranged from focal white plaques on tongue margins to a generalized thick, white pseudomembrane covering the tongue and gingiva. Affected foals showed bruxism, ptyalism, fever, and depression in addition to pneumonia, arthritis, or diarrhea, singly or severally. The lymphocyte counts of the foals were usually normal. Several foals had IgM deficiency coupled with depressed blastogenesis, suggesting cellular immune dysfunction. Many of the foals had low or marginally reduced serum IgG in addition to IgM deficiency or reduced blastogenesis. It was not determined whether the immunologic defects were primary or secondary. All foals died despite extensive therapy with parenteral antimicrobials, topical antimycotics, and intravenous plasma.

Acquired immunodeficiency was identified in a 7-year-old Appaloosa gelding that had no history of previous illness.[587] Clinical signs included lethargy, anorexia, and dyspnea. Pneumonia and septicemia due to *Rhodococcus equi* were confirmed by tracheal wash and blood culture, respectively. Immunologic evaluation revealed marked lymphopenia, subnormal serum IgG and IgA with marginally low IgG concentrations, failure to respond serologically to immunization, and reduced in vitro lymphocyte blastogenesis. Histologic examination of lymph nodes and spleen revealed lymphoid atrophy.

IMMUNOMODULATORS

Clinicians frequently seek to increase normal, restore deficient, and temper overexuberant host immune responses. For these reasons, modulation of the immune system remains an area of intense interest in clinical medicine. Both immunostimulants and immunosuppressants are considered immunomodulators. There are a variety of ways to classify immunomodulators beyond this distinction, but for practical purposes they may be best classified based on their origin: that is, physiologic products (actual normal components of the immune response), microbial products, and chemically defined agents. Immunomodulation can also result from modes of therapy not considered here, including bone marrow transplantation and irradiation.

Although a scientific rationale for the use of immunomodulators exists, a major limitation is the complexity of the immune response to be modulated. There are several major problems in the rational clinical application of immunomodulators. Commonly used diagnostic methods do not allow precise identification of the in vivo defects, deficiencies, or excesses of substances or regulators present within the immunoregulatory network. Consequently, attempts to intervene with immunomodulators are often nonspecific. Some of the information on which the use of immunomodulators is based has come from controlled experimental studies, some performed in vitro and others in vivo. In clinical patients, expectations based on these types of studies are frequently not realized. Reasons for this include the timing of the administration of the immunomodulator during the course of disease and the fact that observation of a single immunologic phenomenon resulting from use of an immunomodulator—for example, an increase in lymphocyte count or lymphoproliferative responses—does not necessarily translate into improved clinical performance in the face of an infectious disease. Because of the complexity of the immune network, the rational use of immunomodulators can be considerably more difficult than the use of antimicrobial agents. An example of the importance of timing in immunomodulatory intervention is the relative success of immunosuppressive therapy in the context of organ allografts, compared with the frequent treatment failures experienced in treating autoimmune disease. In the former case therapy is planned in advance of the introduction of the allograft, whereas in the case of autoimmunity the immune response and resulting disease are well established before they are detected and therapy is initiated.

Immunomodulators have been embraced by clinicians, and the concept of their use remains appealing. The clinical value of immunosuppressive drugs such as corticosteroids and the immunostimulant adjuvants used in vaccines is clearly established. Although the body of work evaluating immunostimulants has grown in recent years, the evidence for the clinical value of many immunostimulants is still sparse and sometimes conflicting. It is important to look critically at the immunomodulatory drugs on the market and make attempts to objectively evaluate the effects of therapy.

This discussion will be limited to drugs currently marketed for use as immunomodulators or for which there is published evidence of activity. Advances in molecular biology have made available recombinant forms of many cytokines with immunomodulatory potential, but only those with some established value will be discussed.

Immunosuppressors

Corticosteroids

Corticosteroids are classic examples of immunosuppressive agents. Corticosteroids exhibit an extensive range of effects on elements of both the innate (inflammatory) and adaptive immune response. Corticosteroids are derivatives of the glucocorticoid family of steroid hormones, and after crossing the cell membrane they bind to cytoplasmic glucocorticoid receptors. Once activated, glucocorticoid receptors are transported to the nucleus where they bind to DNA and interact with other transcription factors, resulting in the regulation of as many as 20% of the genes expressed in leukocytes.[209] The useful antiinflammatory effects of corticosteroids are summarized in Table 1.8. The effect on the adaptive immune response is complex.[588] For example, in cases of autoimmune disease such as autoimmune hemolytic anemia, corticosteroids may act by reducing phagocytosis of antibody-coated cells by the reticuloendothelial system rather than decreasing antibody production. Nevertheless corticosteroids have been shown to have effects on antibody production, and in the horse

TABLE **1.8 Antiinflammatory Effects of Corticosteroid Therapy, Mediated by Regulation of Gene Transcription**

Action of Corticosteroids	
Direct Effects	**Physiologic Effects**
↓ IL-1, TNF-α, GM-CSF, IL-3, IL-4, IL-5, CXCL8	↓ Inflammation caused by cytokines
↓ Nitric oxide synthetase	↓ Nitric oxide
↓ Phospholipase A2, cyclooxygenase type 2 ↑ Annexin-1	↓ Protaglandins, leukotrienes
↓ Adhesion molecules	↓ Emigration of leukocytes from vessels
↑ Endonucleases	↑ Apoptosis in lymphocytes and eosinophils

From Murphy KP, *Janeway's immunobiology,* 8th edition, 2012.

they can suppress de novo antigen-specific IgG1 and IgG4/7 responses while sparing IgG3/5 responses.[468] This may be consistent with the action of corticosteroids to suppress T_H1 responses while sparing T_H2 responses.[580,581,589,590] Cell migration is significantly affected by corticosteroids, and in the horse corticosteroids have been shown to suppress neutrophil migration, together with phagocytic and bactericidal activity.[591] The mechanism whereby migration is decreased involves decreased expression of adhesion molecules including selectins and integrins.[592] Experientially, high-dose dexamethasone (1.0 mg/kg intravenously daily for 9 days) profoundly reduces the numbers of B and T lymphocytes in the peripheral blood of horses.[575] Intravenous administration of single lower doses of dexamethasone (0.05, 0.1, or 0.2 mg/kg) to a healthy horse results in neutrophilia and reduction of peripheral blood $CD4^+$ T-cell numbers, with a corresponding increase in the numbers of $CD8^+$ T cells and B cells.[593]

Although corticosteroids are powerful immunosuppressive agents, they may also predispose patients to life-threatening opportunistic infections[579] or recrudesence of viral infection.[575,576,594] Additional extensive undesirable side effects include fluid retention, decreased wound healing, and the concern that their use can be associated with the development of laminitis in some circumstances.[595-598] For this reason prolonged or high-dose corticosteroid therapy must be used judiciously.

Cytotoxic Drugs

The two commonly used immunosuppressive cytotoxic drugs, azathioprine and cyclophosphamide, both interfere with DNA synthesis and act primarily on dividing cells.[209] This activity is useful for treatment of cancer and for suppression of dividing lymphocytes. Cyclophosphamide has been used along with prednisolone in a chemotherapeutic protocol to achieve remission of thoracic lymphoma in a horse.[599] Toxicity limits the use of these drugs, although at lower doses they can be used in combination with corticosteroids. These drugs have been used in horses to treat immune-mediated hemolytic anemia or thrombocytopenia with success in some cases[600-602] and unsuccessfully for treatment of pemphigus vulgaris.[436]

Bacterial and Fungal Derivatives

This class of drugs includes relatively nontoxic alternatives to immunosuppressive cytotoxic drugs. Cyclosporine is a fungal derivative and has emerged as a major immunosuppressive agent for allograft survival. It selectively inhibits proliferation, cytotoxicity, and lymphokine production of T cells by binding to intracellular proteins known as immunophilins and interfering with signaling pathways that are important for clonal expansion of lymphocytes.[209] Cyclosporine is efficacious in suppressing specific immune responses with minimal nonspecific toxic effects on polymorphonuclear leukocytes, monocytes, and macrophages. Thus immunosuppressed patients suffer fewer severe secondary infections. The drug is not hazard free, as in addition to suppressing lymphocyte responses in general, it is also toxic to the kidneys and other organs.

In horses the use of cyclosporine has been limited to topical and intravitreal therapy for ocular inflammatory disease, including keratitis[603] and uveitis.[604,605] In the treatment of uveitis, numbers of infiltrating T lymphocytes and IL-2 and IFN-γ levels were suppressed by cyclosporine treatment.[606] Use of a suprachoroidal cyclosporine implant results in long-term maintenance of vision in horses with recurrent uveitis.[607]

IMMUNOSTIMULANTS

Physiologic Products

Cytokines

Given the central role of cytokines in immunoregulation (see under Equine Immunology), their potential as immunomodulators is obvious, and a discussion of the possible use of cytokines as therapeutic agents in this regard would be extensive although largely hypothetical. Therefore consideration will be limited to the two cytokines that have found clinical application in horses to date.

Interferon-α. The clinical application of human interferon-α in the horse has been extensively reviewed.[608-610] Interferon-α has antiviral and immunostimulant properties, and oral administration of interferon-α has been shown to reduce inflammatory airway disease[611] in racehorses.[612] The treatment employed was low-dose (50–150 IU) natural human interferon-α, and it resulted in decreased BAL cell counts and a noninflammatory cytologic profile.[613] Higher doses (450 IU) were less effective, consistent with results in other species.[410] The efficacy of oral therapy probably depends on effects mediated through oropharyngeal lymphoid tissue, as the agent is destroyed in the stomach. Recombinant human interferon-α (Intron-A, Merck, Kenilworth, NJ) has similar therapeutic effects as natural human interferon-α.[614]

Granulocyte Colony-Stimulating Factor. The clinical application of granulocyte colony-stimulating factor (G-CSF) in the horse has been extensively reviewed.[609,610] G-CSF treatment in neonatal foals results in a sustained, dose-dependent increase in neutrophil count and a left shift,[615,616] by increasing neutrophil production in the bone marrow and shortening the time to release into the circulation while the half-life remains unchanged at 8 hours. Recombinant human G-CSF (Neupogen, Amgen, Thousand Oaks, CA) has been effective for treating persistent neutropenia in foals with alloimmune neonatal neutropenia.[617,618] Canine recombinant G-CSF has been used to treat sepsis and endotoxemia in foals with some success.[609,610]

Bacterial, Viral, and Plant Products

A variety of bacterial and fungal microorganisms or microbial products have been identified that have immunomodulating effects. A common feature of many of these products is a nonspecific immunostimulant effect, purportedly due to macrophage activation and release of cytokines including interferons, IL-1, TNF-α, or IL-6.[609,619] Consequently mild fever and malaise may be associated with this form of treatment. In the horse these treatments are most commonly used in cases of respiratory infection or sarcoids. Extensive reviews of the use of these and other immunostimulants in the horse have been published.[609,610,620]

Mycobacterial Products

A wide range of mycobacterial fractions have been identified with immunomodulating ability.[609,619] The minimal structure with immunologic (adjuvant) activity is muramyl dipeptide, which is a potent adjuvant.[621] Preparations commercially available for use in horses include bacillus Calmette-Guérin (BCG), a modified live human tuberculosis vaccine, and protein-free mycobacterial cell wall extract (MCWE; Equimune, Immunocidin, and Settle, NovaVive, Athens, GA). Efficacy is reported for treatment of equine respiratory disease (Equimune; intravenous), sarcoids (Immunocidin; intralesional), and endometritis (Settle; intravenous or intrauterine). Intravenous administration of MCWE at the time of artificial insemination downregulates IL-1 expression in mares susceptible to postbreeding endometritis and thus might have therapeutic value in this population.[622] Adverse hypersensitivity reactions have been reported after multiple intravenous treatments in horses, resulting in interstitial lung infiltration and progressive pulmonary fibrosis. In horses, a successful application of these products has been in the intralesional treatment of sarcoids, particularly periocular sarcoids,[623] and this is discussed elsewhere in this book.

Propionibacterium acnes

Propionibacterium acnes is a commensal gram-positive anaerobe, marketed for use in horses as a killed preparation under the trade name EqStim (Neogen, Lexington, KY) for treatment of equine respiratory disease. It is recommended for use prophylactically before weaning, transport stress, or comingling, or for the therapy of chronic infectious respiratory disease. After intravenous administration, *P. acnes* is taken up by macrophages in the liver and spleen and degraded slowly, resulting in macrophage activation. In addition, *P. acnes* CpG motifs (see later discussion) result in immune activation by binding TLR9 on macrophages, dendritic cells, and NK cells.[610,620] In healthy horses, *P. acnes* resulted in increased $CD4^+$ T-lymphocyte numbers and LAK cell activity in peripheral blood and BAL fluid, increased nonopsonized phagocytosis in peripheral blood leukocytes, and decreased pulmonary cellularity.[469] Treatment of horses with *P. acnes* has also been shown to increase IFN-γ and antimicrobial peptide expression in PBMCs.[624] In adult horses, two randomized controlled trials have demonstrated that the addition of *P. acnes* treatment to conventional therapy enhances clinical recovery or improvement of spontaneously occurring infectious respiratory disease.[620] Several other studies have shown that *P. acnes* treatment is beneficial in reducing the prevalence of respiratory disease associated with longdistance transport and in resolving endometritis.[620] Although *P. acnes* treatment had no effect on IFN-γ production by stimulated PBMCs or IFN-γ expression in peripheral blood of neonatal foals less than 1 week of age, both were increased following a second series of doses when the foals were 1 month old.[625] Macrophages isolated from foals treated with *P. acnes* beginning at 1 week of age demonstrate less intracellular proliferation of *R. equi* when infected in vitro compared with macrophages from untreated control foals.[626] Overall this agent is one of the most popular immunostimulants marketed for horses. Based on the published literature it appears to be of therapeutic benefit in adult horses but likely less so in neonatal foals.

Parapoxvirus ovis

Parapoxvirus ovis is a DNA virus of small ruminants that produces a variety of modulatory factors that are maintained after virus inactivation. As such, inactivated *Parapoxvirus ovis* (iPPVO) has been shown to activate APC, leading to upregulation of proinflammatory cytokines IL-6, TNF-α, and CXCL8; T_H1-inducing IL-12; and T-cell production of IL-2, IFN-α, and IFN-γ.[610,620] Marketed under the trade name Baypamun (Bayer Animal Health, DE), iPPVO was used extensively in Europe for prophylaxis and treatment of infectious disease in companion animals, horses, and pigs.[609] In the United States iPPVO was marketed as Zylexis (Zoetis, Florham Park, NJ) for enhancing immunity before stressful events or during disease, particularly to improve resolution of viral respiratory disease, including EHV-1 and EHV-4.[620] At the time of this writing, however, Zylexis has been discontinued and is not currently available. Efficacy has been demonstrated against viral and bacterial disease in several species,[609] and there is evidence in horses that prophylactic administration before weaning reduces signs of respiratory disease after weaning.[627] More recently, however, treatment with iPPVO did not affect the decreased IFN-γ, TNF-α, and IL-10 production by PBMCs associated with the stress of abrupt weaning.[628] In a model of transport stress, treatment with Zylexis led to restoration of suppressed antibody responses to influenzavirus infection in horses.[629] More recently, treatment with Zylexis was associated with a moderate reduction in clinical signs and reduced shedding of EHV-1 and *S. equi*.[630] In healthy yearlings, iPPVO treatment resulted in increased IFN-γ expression in peripheral blood.[631] Neutrophils from foals treated with iPPVO at 1 week of age had enhanced phagocytic and oxidative burst activity against *R. equi* in vitro compared with baseline controls and with foals treated with *P. acnes*.[626] In iPPVO-treated foals, TNF-α induction was higher in monocyte-derived macrophages, and IL-12 induction was higher in BAL macrophages infected with *R. equi* in vitro compared with control foals. In a separate study using foals on an *R. equi* endemic farm, iPPVO treatment initiated within the first 2 days of life resulted in a higher number of PBMC producing IFN-γ by 1 to 2 weeks of age compared with control foals, but there was no difference in the proportion of foals that developed *R. equi* pneumonia.[632] Finally, iPPVO has not shown efficacy for treatment of sarcoids.[633]

Imiquimod

Imiquimod (Aldara) is a synthetic imidazoquinoline immunomodulating cream used for topical treatment of actinic keratosis and genital warts in humans and to successfully treat

sarcoids and aural plaques in horses.[610,623] It acts as a TLR7 agonist, resulting in increased proinflammatory cytokine production, and has potent antiviral and antitumor activity. Studies report that 56% to 82% of treated sarcoids achieve complete remission, with 80% having at least a 75% reduction in size. Mean treatment time is 3.7 months.[623]

Cytosine-Phosphate-Guanosine-Oligodeoxynucleotides

In recent years an extensive body of literature has developed describing the immunomodulatory effects of certain unmethylated bacterial DNA motifs.[634] The specific immunostimulatory DNA motifs are called CpG sequences, and appropriate oligodeoxynucleotides (ODN) containing CpG motifs for immunostimulation of domestic species have been identified.[635] Much work has been done on examining the efficacy of CpG-ODN as vaccine adjuvants,[636] as immunomodulators for hyposensitization (see later discussion), or cancer therapy.[637] The value of CpG-ODN in horses as both vaccine adjuvants[638] and as potential nonspecific immunomodulators[340,639,640] has been demonstrated. Recently intramuscular administration of a CpG-ODN formulation to newborn foals resulted in increased IFN-γ production by neutrophils and modulated neutrophil degranulation.[641] Although CpG-ODN hold promise as immunomodulators, commercial products for clinical use are not yet available.

Chemically Defined Agents

Levamisole

Levamisole is synthetic anthelmintic used for treatment of nematode infections, which has also been reported to restore impaired host immune defenses.[609] Levamisole appears to have little effect on the normal immune system, but it appears to stimulate a subnormal response and suppress hyperactive responses. The effects are dose related, and low doses are reported to enhance whereas higher doses suppress responses. In cattle levamisole has been shown to enhance lymphoproliferative responses in vitro, although in vivo coadministration with a vaccine had no immunostimulant effect.[642] Similarly, levamisole did not prevent corticosteroid-mediated immunosuppression in cattle[643] or enhance postpartum lymphoproliferative responses in pigs.[642] Other than anecdotal reports there are no published controlled studies of its value in horses.

Antigen-Specific Immunomodulation

Vaccination and Adjuvants

Vaccination is a critically important tool in preventing infectious disease in humans and animals, and both passive and active vaccination are extensively employed in the horse. Specific equine vaccination strategies are presented elsewhere in this book, and the scientific principles and the practice of equine vaccination have been extensively reviewed.[644-647] Vaccination implies active immunization where an antigen-specific immune response is induced in the vaccinated animal by administration of subunit, whole killed or attenuated live antigen, or DNA in a plasmid or a recombinant viral vector capable of expressing protein antigens in vivo. Each of these strategies has advantages and disadvantages. For example, killed vaccines do not result in efficient MHC I processing and presentation and thus do not elicit robust CTL responses, limiting effectiveness against predominantly intracellular pathogens. Attenuated live vaccines have the potential to cause disease in some individuals or to revert to virulence. Recombinant viral vector vaccines result in intracellular expression of antigens without the risk of causing disease or reversion to virulence. The success of all of these types of vaccines, subunit and killed vaccines in particular, is frequently dependent on use of an effective adjuvant.[648] Adjuvants are compounds capable of potentiating immune responses and include substances that result in depot formation, induce inflammatory cytokines, recruit leukocytes, enhance antigen uptake and presentation, or increase the expression of costimulatory molecules. As such, adjuvants are some of the most important types of immunomodulating agents in use in equine medicine.

Passive immunization is accomplished by administering preformed antibodies either as a plasma transfusion or in a concentrated form, as in commercially available tetanus antitoxin. This strategy can be highly effective in diseases for which there is no available vaccine (e.g., *R. equi*) or in high-risk situations when there is inadequate time for protection to be generated by active immunization. Although USDA-approved commercial equine plasma and serum products are derived from donors screened for routine blood-borne pathogens, passive immunization carries a risk of transmitting previously unidentified infectious agents. Examples include the association with acute hepatic necrosis with a previous administration of tetanus antitoxin[649] and more recently an outbreak of liver disease associated with transmission of Theiler's disease–associated virus (TDAV) via administration of botulinum antitoxin.[202] Equine blood products could contain other newly reported viruses associated with hepatitis in horses, including equine hepacivirus and equine pegivirus.[650] As was the case in the discovery of TDAV,[202] deep sequencing techniques may reveal other infectious pathogens that could contaminate equine blood products.

Hyposensitization

Because horses suffer from a number of hypersensitivity diseases, attempts have been made to perform antigen-specific immunosuppression. Examples include IBH and RAO.[651] The principle of this type of therapy is that the immune response against an allergen can be redirected to reduce hypersensitivity disease.[652] For example, because type 1 hypersensitivity is associated with a T_H2-biased immune response, treatments that cause a shift to a T_H1 response could eliminate or control the hypersensitivity by changing the antibody response from one dominated by IgE to IgG.[209] Typically hyposensitization treatments, also known as allergen-specific immunotherapy (ASIT), use injections of the allergen itself, starting with very small doses, and gradually increasing the dose over time. This form of treatment depends on correct identification of the allergen against which the hypersensitivity is directed, and the difficulty in identifying these allergens using intradermal or IgE ELISA-based serologic testing methodologies[396,653] may provide an explanation for the mixed success of ASIT in horses.[654,655] Prospects for ASIT have been improved recently with the use of pure recombinant protein allergens, which are far superior in terms of sensitivity (up to 89%) and specificity (up to 97%) for detection of allergen-specific IgE.[656,657] A protein microarray containing a large panel of complex extracts along with pure and recombinant proteins and analyzed using mathematical modeling techniques has been recently described

that is capable of high-throughput detection of allergen-specific IgE.[658] This microarray has yielded a sensitivity and specificity of 100% and 97%, respectively, for the diagnosis of IBH. Even when previous allergen identification methods are used, ASIT can be effective for managing atopic skin disease in horses as described in a recent retrospective study involving 54 horses (1991–2008) treated for urticaria, pruritus, or both.[659] Intradermal and/or serologic testing was used to identify allergens, and 84% of owners reported that ASIT reduced clinical signs, with 59% able to manage clinical signs without the use of immunosuppressive drugs. The majority of owners (75%) discontinued ASIT after a mean of 2.2 years, most because of resolution of clinical signs. The availability of increasingly sensitive and specific allergen testing strategies will undoubtedly further improve the success of ASIT. Of considerable promise, a vaccine approach for potentially preventing IBH has recently been described in which small doses of recombinant *Culicoides* allergens are inoculated along with a T_H1-focusing adjuvant.[660] Immunization results in induction of allergen-specific IgG without inducing IgE, does not produce IgE-mediated hypersensitivity, and produces IgG that is capable of partially blocking the binding of IgE to the vaccine allergens.

Administration of CpG DNA has been evaluated for hyposensitization because it promotes the T_H1 immune responses.[661-663] In horses with RAO, inhalation of CpG-ODN nanoparticles resulted in reduced respiratory effort, nasal discharge, tracheal secretion, and viscosity; decreased tracheobronchial neutrophil percentage; and increased arterial oxygen pressure.[664] Administration of CpG-ODN has been evaluated for the potential to reduce vaccine-associated hypersensitivity reactions in horses. Administration of CpG-ODN along with a killed West Nile virus vaccine increased the differentiation of T_{regs} in response to bovine serum albumin (BSA), and an inverse correlation between numbers of T_{regs} and BSA-specific IgE concentration was observed.[665] Taken together, the progress in this field indicates that new and effective prophylactic strategies and therapies will likely be developed in the future.

REFERENCES

1. Sharp SE. Commensal and pathogenic organisms. In: Murray PR, Barron EJ, Pfaller MA, et al., eds. *Manual of Clinical Microbiology*. 7th ed. Washington, D.C.: ASM Press; 1999:23–32.
2. Scott DW. Structure and function of the skin. In: Scott DW, ed. *Large Animal Dermatology*. Philadelphia: W.B. Saunders Company; 1988:1–28.
3. Scott DW. Bacteria and yeast on the surface and within non-inflamed hair follicles of skin biopsies from dogs with non-neoplastic dermatoses. *Cornell Vet*. 1992;82:379–386.
4. Nell A, James SA, Bond CJ, et al. Identification and distribution of a novel *Malassezia* species yeast on normal equine skin. *Vet Rec*. 2002;150:395–398.
5. Bailey GD, Love DN. Oral associated bacterial infection in horses: studies on the normal anaerobic flora from the pharyngeal tonsillar surface and its association with lower respiratory tract and paraoral infections. *Vet Microbiol*. 1991;26:367–379.
6. Thompson H, Rybalka A, Moazzez R, et al. In vitro culture of previously uncultured oral bacterial phylotypes. *Appl Environ Microbiol*. 2015;81:8307–8314.
7. Kennedy R, Lappin DF, Dixon PM, et al. The microbiome associated with equine periodontitis and oral health. *Vet Res*. 2016;47:49.
8. Crane SA, Ziemer EL, Sweeney CR. Cytologic and bacteriologic evaluation of tracheobronchial aspirates from clinically normal foals. *Am J Vet Res*. 1989;50:2042–2048.
9. Moore CP, Heller N, Majors LJ, et al. Prevalence of ocular microorganisms in hospitalized and stabled horses. *Am J Vet Res*. 1988;49:773–777.
10. Koopman JP, Kennis HM, Mullink JW, et al. "Normalization" of germfree mice with anaerobically cultured caecal flora of "normal" mice. *Lab Anim*. 1984;18:188–194.
11. Blumberg RS, Saubermann LJ, Strober W. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol*. 1999;11:648–656.
12. Mackie RI, Wilkins CA. Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. *Appl Environ Microbiol*. 1988;54:2155–2160.
13. Yuki N, Shimazaki T, Kushiro A, et al. Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by lactobacilli. *Appl Environ Microbiol*. 2000;66:5030–5034.
14. Davies MK. Studies on the microbial flora of the large intestine of the horse by continuous culture in an artificial colon. *Vet Sci Communications*. 1979;3:39–44.
15. Julliand V, de Vaux A, Millet L, et al. Identification of *Ruminococcus flavefaciens* as the predominant cellulolytic bacterial species of the equine cecum. *Appl Environ Microbiol*. 1999;65:3738–3741.
16. Daly K, Stewart CS, Flint HJ, et al. Bacterial diversity within the equine large intestine as revealed by molecular analysis of cloned 16S rRNA genes. *FEMS Microbiol Ecol*. 2001;38:141–151.
17. Julliand V, Grimm P. Horse species symposium: the microbiome of the horse hindgut: history and current knowledge. *J Anim Sci*. 2016;94:2262–2274.
18. Orpin CG. Isolation of cellulolytic phycomycete fungi from the caecum of the horse. *J Gen Microbiol*. 1981;123:287–296.
19. Ericsson AC, Johnson PJ, Lopes MA, et al. A Microbiological Map of the Healthy Equine Gastrointestinal Tract. *PLoS One*. 2016;11:e0166523.
20. Traub-Dargatz JL, Garber LP, Fedorka-Cray PJ, et al. Fecal shedding of *Salmonella* spp by horses in the United States during 1998 and 1999 and detection of *Salmonella* spp in grain and concentrate sources on equine operations. *J Am Vet Med Assoc*. 2000;217:226–230.
21. Alinovi CA, Ward MP, Couetil LL, et al. Detection of *Salmonella* organisms and assessment of a protocol for removal of contamination in horse stalls at a veterinary teaching hospital. *J Am Vet Med Assoc*. 2003;223:1640–1644.
22. Ward MP, Alinovi CA, Couetil LL, et al. Evaluation of a PCR to detect *Salmonella* in fecal samples of horses admitted to a veterinary teaching hospital. *J Vet Diagn Invest*. 2005;17:118–123.
23. Weese JS, Staempfli HR, Prescott JF. A prospective study of the roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in equine diarrhoea. *Equine Vet J*. 2001;33:403–409.
24. Nakazawa M, Sugimoto C, Isayama Y. Quantitative culture of *Rhodococcus equi* from the feces of horse. *Natl Inst Anim Health Q (Tokyo)*. 1983;23:67–68.
25. Woolcock JB, Mutimer MD, Farmer AM. Epidemiology of *Corynebacterium equi* in horses. *Res Vet Sci*. 1980;28:87–90.
26. Bordin AI, Suchodolski JS, Markel ME, et al. Effects of administration of live or inactivated virulent *Rhodococccus equi* and age on the fecal microbiome of neonatal foals. *PLoS One*. 2013;8:e66640.
27. Moore BE, Dehority BA. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. *J Anim Sci*. 1993;71:3350–3358.
28. Hinrichs K, Cummings MR, Sertich PL, et al. Clinical significance of aerobic bacterial flora of the uterus, vagina, vestibule, and clitoral fossa of clinically normal mares. *J Am Vet Med Assoc*. 1988;193:72–75.
29. Madsen M, Christensen P. Bacterial flora of semen collected from Danish Warmblood stallions by artificial vagina. *Acta Vet Scand*. 1995;36:1–7.

30. Platt H, Atherton JG, Orskov I. *Klebsiella* and *Enterobacter* organisms isolated from horses. *J Hyg (Lond)*. 1976;77:401–408.
31. Newcombe JR. Comparison of the bacterial flora of three sites in the genital tract of the mare. *Vet Rec*. 1978;102:169–170.
32. Goodson J, Tyznik WJ, Cline JH, et al. Effects of an abrupt diet change from hay to concentrate on microbial numbers and physical environment in the cecum of the pony. *Appl Environ Microbiol*. 1988;54:1946–1950.
33. Owen RA, Fullerton J, Barnum DA. Effects of transportation, surgery, and antibiotic therapy in ponies infected with *Salmonella*. *Am J Vet Res*. 1983;44:46–50.
34. Baverud V, Gustafsson A, Franklin A, et al. *Clostridium difficile* associated with acute colitis in mature horses treated with antibiotics. *Equine Vet J*. 1997;29:279–284.
35. Gustafsson A, Baverud V, Gunnarsson A, et al. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. *Equine Vet J*. 1997;29:314–318.
36. Wilson DA, MacFadden KE, Green EM, et al. Case control and historical cohort study of diarrhea associated with administration of trimethoprim-potentiated sulphonamides to horses and ponies. *J Vet Intern Med*. 1996;10:258–264.
37. Hogenauer C, Hammer HF, Krejs GJ, et al. Mechanisms and management of antibiotic-associated diarrhea. *Clin Infect Dis*. 1998;27:702–710.
38. Peeters T, Matthijs G, Depoortere I, et al. Erythromycin is a motilin receptor agonist. *Am J Physiol*. 1989;257:G470–G474.
39. Lester GD, Merritt AM, Neuwirth L, et al. Effect of erythromycin lactobionate on myoelectric activity of ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res*. 1998;59:328–334.
40. Austin SM, Foreman JH, Hungerford LL. Case-control study of risk factors for development of pleuropneumonia in horses. *J Am Vet Med Assoc*. 1995;207:325–328.
41. Raidal SL, Bailey GD, Love DN. Effect of transportation on lower respiratory tract contamination and peripheral blood neutrophil function. *Aust Vet J*. 1997;75:433–438.
42. Raidal SL, Love DN, Bailey GD. Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 hours of confinement with the head elevated. *Aust Vet J*. 1995;72:45–50.
43. Gaynes R, Richards C, Edwards J, et al. Feeding back surveillance data to prevent hospital-acquired infections. *Emerg Infect Dis*. 2001;7:295–298.
44. Boerlin P, Eugster S, Gaschen F, et al. Transmission of opportunistic pathogens in a veterinary teaching hospital. *Vet Microbiol*. 2001;82:347–359.
45. Colahan PT, Peyton LC, Connelly MR, et al. *Serratia* spp infection in 21 horses. *J Am Vet Med Assoc*. 1984;185:209–211.
46. Weese JS, Baird JD, Poppe C, et al. Emergence of *Salmonella typhimurium* definitive type 104 (DT104) as an important cause of salmonellosis in horses in Ontario. *Can Vet J*. 2001;42:788–792.
47. Begg AP, Johnston KG, Hutchins DR, et al. Some aspects of the epidemiology of equine salmonellosis. *Aust Vet J*. 1988;65:221–223.
48. Ikeda JS, Hirsh DC. Common plasmid encoding resistance to ampicillin, chloramphenicol, gentamicin, and trimethoprim-sulfadiazine in two serotypes of *Salmonella* isolated during an outbreak of equine salmonellosis. *Am J Vet Res*. 1985;46:769–773.
49. Amavisit P, Markham PF, Lightfoot D, et al. Molecular epidemiology of *Salmonella Heidelberg* in an equine hospital. *Vet Microbiol*. 2001;80:85–98.
50. Gerlach RG, Hensel M. Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens. *Int J Med Microbiol*. 2007;297:401–415.
51. Fernandez LA, Berenguer J. Secretion and assembly of regular surface structures in gram-negative bacteria. *FEMS Microbiol Rev*. 2000;24:21–44.
52. Wu H, Fives-Taylor PM. Molecular strategies for fimbrial expression and assembly. *Crit Rev Oral Biol Med*. 2001;12:101–115.
53. van der Flier M, Chhun N, Wizemann TM, et al. Adherence of *Streptococcus pneumoniae* to immobilized fibronectin. *Infect Immun*. 1995;63:4317–4322.
54. Wizemann TM, Moskovitz J, Pearce BJ, et al. Peptide methionine sulfoxide reductase contributes to the maintenance of adhesins in three major pathogens. *Proc Natl Acad Sci U S A*. 1996;93:7985–7990.
55. Gilot P, Andre P, Content J. *Listeria monocytogenes* possesses adhesins for fibronectin. *Infect Immun*. 1999;67:6698–6701.
56. Sinha B, Francois P, Que YA, et al. Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells. *Infect Immun*. 2000;68:6871–6878.
57. Sinha B, Francois PP, Nusse O, et al. Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. *Cell Microbiol*. 1999;1:101–117.
58. Rich RL, Kreikemeyer B, Owens RT, et al. Ace is a collagen-binding MSCRAMM from *Enterococcus faecalis*. *J Biol Chem*. 1999;274:26939–26945.
59. Wizemann TM, Adamou JE, Langermann S. Adhesins as targets for vaccine development. *Emerg Infect Dis*. 1999;5:395–403.
60. Srivastava SK, Barnum DA. The role of lipoteichoic acids on the adherence of *Streptococcus equi* to epithelial cells. *Vet Microbiol*. 1983;8:485–492.
61. Colombo AV, Hirata Jr R, de Souza CM, et al. *Corynebacterium diphtheriae* surface proteins as adhesins to human erythrocytes. *FEMS Microbiol Lett*. 2001;197:235–239.
62. Smyth CJ, Marron MB, Twohig JM, et al. Fimbrial adhesins: similarities and variations in structure and biogenesis. *FEMS Immunol Med Microbiol*. 1996;16:127–139.
63. Zhou D, Mooseker MS, Galan JE. An invasion-associated *Salmonella* protein modulates the actin-bundling activity of plastin. *Proc Natl Acad Sci U S A*. 1999;96:10176–10181.
64. Reddy VM, Kumar B. Interaction of *Mycobacterium avium* complex with human respiratory epithelial cells. *J Infect Dis*. 2000;181:1189–1193.
65. Sangari FJ, Goodman J, Bermudez LE. *Mycobacterium avium* enters intestinal epithelial cells through the apical membrane, but not by the basolateral surface, activates small GTPase Rho and, once within epithelial cells, expresses an invasive phenotype. *Cell Microbiol*. 2000;2:561–568.
66. Srivastava SK, Barnum DA, Prescott JF. Production and biological properties of M-protein of *Streptococcus equi*. *Res Vet Sci*. 1985;38:184–188.
67. Gilmour MI, Park P, Selgrade MK. Ozone-enhanced pulmonary infection with *Streptococcus zooepidemicus* in mice. The role of alveolar macrophage function and capsular virulence factors. *Am Rev Respir Dis*. 1993;147:753–760.
68. Anzai T, Timoney JF, Kuwamoto Y, et al. In vivo pathogenicity and resistance to phagocytosis of *Streptococcus equi* strains with different levels of capsule expression. *Vet Microbiol*. 1999;67:277–286.
69. Timoney JF, Kumar P. Early pathogenesis of equine *Streptococcus equi* infection (strangles). *Equine Vet J*. 2008;40:637–642.
70. Tzianabos AO, Kasper DL, Onderdonk AB. Structure and function of *Bacteroides fragilis* capsular polysaccharides: relationship to induction and prevention of abscesses. *Clin Infect Dis*. 1995;20(suppl 2):S132–S140.
71. Brook I. Encapsulated anaerobic bacteria in clinical infections. *Zentralbl Bakteriol*. 1993;279:443–446.
72. Brook I. The role of encapsulated anaerobic bacteria in synergistic infections. *FEMS Microbiol Rev*. 1994;13:65–74.
73. Patrick S, Lutton DA, Crockard AD. Immune reactions to *Bacteroides fragilis* populations with three different types of capsule in a model of infection. *Microbiology*. 1995;141(Pt 8):1969–1976.
74. Tomas JM, Ciurana B, Benedi VJ, et al. Role of lipopolysaccharide and complement in susceptibility of *Escherichia coli* and *Salmonella typhimurium* to non-immune serum. *J Gen Microbiol*. 1988;134:1009–1016.

75. Morrison DC, Brown DE, Vukajlovich SW, et al. Ganglioside modulation of lipopolysaccharide-initiated complement activation. *Mol Immunol*. 1985;22:1169–1176.
76. Hill HR, Bohnsack JF, Morris EZ, et al. Group B streptococci inhibit the chemotactic activity of the fifth component of complement. *J Immunol*. 1988;141:3551–3556.
77. Jagels MA, Travis J, Potempa J, et al. Proteolytic inactivation of the leukocyte C5a receptor by proteinases derived from Porphyromonas gingivalis. *Infect Immun*. 1996;64:1984–1991.
78. Heffernan EJ, Reed S, Hackett J, et al. Mechanism of resistance to complement-mediated killing of bacteria encoded by the *Salmonella typhimurium* virulence plasmid gene rck. *J Clin Invest*. 1992;90:953–964.
79. Boschwitz JS, Timoney JF. Inhibition of C3 deposition on *Streptococcus equi* subsp. *equi* by M protein: a mechanism for survival in equine blood. *Infect Immun*. 1994;62:3515–3520.
80. Chanter N, Ward CL, Talbot NC, et al. Recombinant hyaluronate associated protein as a protective immunogen against *Streptococcus equi* and *Streptococcus* zooepidemicus challenge in mice. *Microb Pathog*. 1999;27:133–143.
81. Hoe NP, Kordari P, Cole R, et al. Human immune response to streptococcal inhibitor of complement, a serotype M1 group A *Streptococcus* extracellular protein involved in epidemics. *J Infect Dis*. 2000;182:1425–1436.
82. Johnsson E, Berggard K, Kotarsky H, et al. Role of the hypervariable region in streptococcal M proteins: binding of a human complement inhibitor. *J Immunol*. 1998;161:4894–4901.
83. Mitchell TJ. Virulence factors and the pathogenesis of disease caused by *Streptococcus pneumoniae*. *Res Microbiol*. 2000;151:413–419.
84. Boschwitz JS, Timoney JF. Characterization of the antiphagocytic activity of equine fibrinogen for *Streptococcus equi* subsp. *equi*. *Microb Pathog*. 1994;17:121–129.
85. Timoney JF, Artiushin SC, Boschwitz JS. Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. *Infect Immun*. 1997;65:3600–3605.
86. Gao LY, Kwaik YA. The modulation of host cell apoptosis by intracellular bacterial pathogens. *Trends Microbiol*. 2000;8:306–313.
87. Feltis BA, Wiesner SM, Kim AS, et al. *Clostridium difficile* toxins A and B can alter epithelial permeability and promote bacterial paracellular migration through HT-29 enterocytes. *Shock*. 2000;14:629–634.
88. Grassme H, Jendrossek V, Gulbins E. Molecular mechanisms of bacteria induced apoptosis. *Apoptosis*. 2001;6:441–445.
89. Schroeder GN, Jann NJ, Hilbi H. Intracellular type III secretion by cytoplasmic *Shigella flexneri* promotes caspase-1-dependent macrophage cell death. *Microbiology*. 2007;153:2862–2876.
90. Haslinger B, Strangfeld K, Peters G, et al. *Staphylococcus aureus* alpha-toxin induces apoptosis in peripheral blood mononuclear cells: role of endogenous tumour necrosis factor-alpha and the mitochondrial death pathway. *Cell Microbiol*. 2003;5:729–741.
91. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev*. 2000;13:16–34. table of contents.
92. Bulawa CE, Miller DW, Henry LK, et al. Attenuated virulence of chitin-deficient mutants of *Candida albicans*. *Proc Natl Acad Sci U S A*. 1995;92:10570–10574.
93. Buurman ET, Westwater C, Hube B, et al. Molecular analysis of CaMnt1p, a mannosyl transferase important for adhesion and virulence of *Candida albicans*. *Proc Natl Acad Sci U S A*. 1998;95:7670–7675.
94. Fu Y, Rieg G, Fonzi WA, et al. Expression of the *Candida albicans* gene ALS1 in *Saccharomyces cerevisiae* induces adherence to endothelial and epithelial cells. *Infect Immun*. 1998;66:1783–1786.
95. Gale CA, Bendel CM, McClellan M, et al. Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene, INT1. *Science*. 1998;279:1355–1358.
96. Kinneberg KM, Bendel CM, Jechorek RP, et al. Effect of INT1 gene on *Candida albicans* murine intestinal colonization. *J Surg Res*. 1999;87:245–251.
97. Newman SL, Chaturvedi S, Klein BS. The WI-1 antigen of *Blastomyces dermatitidis* yeasts mediates binding to human macrophage CD11b/CD18 (CR3) and CD14. *J Immunol*. 1995;154:753–761.
98. Fries BC, Taborda CP, Serfass E, et al. Phenotypic switching of *Cryptococcus neoformans* occurs in vivo and influences the outcome of infection. *J Clin Invest*. 2001;108:1639–1648.
99. Rotrosen D, Edwards Jr JE, Gibson TR, et al. Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. *J Infect Dis*. 1985;152:1264–1274.
100. Eissenberg LG, Goldman WE. *Histoplasma capsulatum* fails to trigger release of superoxide from macrophages. *Infect Immun*. 1987;55:29–34.
101. Eissenberg LG, Schlesinger PH, Goldman WE. Phagosome-lysosome fusion in P388D1 macrophages infected with *Histoplasma capsulatum*. *J Leukoc Biol*. 1988;43:483–491.
102. Eissenberg LG, Poirier S, Goldman WE. Phenotypic variation and persistence of *Histoplasma capsulatum* yeasts in host cells. *Infect Immun*. 1996;64:5310–5314.
103. Woods JP, Heinecke EL, Luecke JW, et al. Pathogenesis of *Histoplasma capsulatum*. *Semin Respir Infect*. 2001;16:91–101.
104. Hube B. *Candida albicans* secreted aspartyl proteinases. *Curr Top Med Mycol*. 1996;7:55–69.
105. Resnick S, Pappagianis D, McKerrow JH. Proteinase production by the parasitic cycle of the pathogenic fungus *Coccidioides immitis*. *Infect Immun*. 1987;55:2807–2815.
106. Yu JJ, Smithson SL, Thomas PW, et al. Isolation and characterization of the urease gene (URE) from the pathogenic fungus *Coccidioides immitis*. *Gene*. 1997;198:387–391.
107. Yuan L, Cole GT. Isolation and characterization of an extracellular proteinase of *Coccidioides immitis*. *Infect Immun*. 1987;55:1970–1978.
108. Iadarola P, Lungarella G, Martorana PA, et al. Lung injury and degradation of extracellular matrix components by *Aspergillus fumigatus* serine proteinase. *Exp Lung Res*. 1998;24:233–251.
109. Rodriguez E, Boudard F, Mallie M, et al. Murine macrophage elastolytic activity induced by *Aspergillus fumigatus* strains in vitro: evidence of the expression of two macrophage-induced protease genes. *Can J Microbiol*. 1997;43:649–657.
110. Ghannoum MA. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microbiol Rev*. 2000;13:122–143. table of contents.
111. Mitrovic S, Kranjcic-Zec I, Arsic V, et al. In vitro proteinase and phospholipase activity and pathogenicity of *Candida* species. *J Chemother*. 1995;7(suppl 4):43–45.
112. Prakobphol A, Leffler H, Hoover CI, et al. Palmitoyl carnitine, a lysophospholipase-transacylase inhibitor, prevents *Candida* adherence in vitro. *FEMS Microbiol Lett*. 1997;151:89–94.
113. Noverr MC, Toews GB, Huffnagle GB. Production of prostaglandins and leukotrienes by pathogenic fungi. *Infect Immun*. 2002;70:400–402.
114. Mendes-Giannini MJ, Taylor ML, Bouchara JB, et al. Pathogenesis II: fungal responses to host responses: interaction of host cells with fungi. *Med Mycol*. 2000;38(suppl 1):113–123.
115. Golden MC, Hahm SJ, Elessar RE, et al. DNA damage by gliotoxin from *Aspergillus fumigatus*. An occupational and environmental propagule: adduct detection as measured by 32P DNA radiolabelling and two-dimensional thin-layer chromatography. *Mycoses*. 1998;41:97–104.
116. Bock M, Stoye JP. Endogenous retroviruses and the human germline. *Curr Opin Genet Dev*. 2000;10:651–655.
117. Flint SJ, Enquist LW, Krug RM, et al. *Principles of Virology: Molecular Biology, Pathogenesis, and Control*. Washington, D.C.: ASM Press; 1999.

118. Murphy FA, Gibbs EPJ, Horzinek MC, et al. *Veterinary Virology*. 3rd ed. San Diego: Academic Press; 1999.
119. Preston BD, Dougherty JP. Mechanisms of retroviral mutation. *Trends Microbiol*. 1996;4:16–21.
120. Domingo E, Baranowski E, Ruiz-Jarabo CM, et al. Quasispecies structure and persistence of RNA viruses. *Emerg Infect Dis*. 1998;4:521–527.
121. Scholtissek C. Molecular epidemiology of influenza. *Arch Virol Suppl*. 1997;13:99–103.
122. Puvion-Dutilleul F, Besse S, Pichard E, et al. Release of viruses and viral DNA from nucleus to cytoplasm of HeLa cells at late stages of productive adenovirus infection as revealed by electron microscope in situ hybridization. *Biol Cell*. 1998;90:5–38.
123. Thomson BJ. Viruses and apoptosis. *Int J Exp Pathol*. 2001;82:65–76.
124. Carr EA, Theon AP, Madewell BR, et al. Expression of a transforming gene (E5) of bovine papillomavirus in sarcoids obtained from horses. *Am J Vet Res*. 2001;62:1212–1217.
125. Burmeister T. Oncogenic retroviruses in animals and humans. *Rev Med Virol*. 2001;11:369–380.
126. Garcia-Blanco MA, Cullen BR. Molecular basis of latency in pathogenic human viruses. *Science*. 1991;254:815–820.
127. Borchers K, Wolfinger U, Lawrenz B, et al. Equine herpesvirus 4 DNA in trigeminal ganglia of naturally infected horses detected by direct in situ PCR. *J Gen Virol*. 1997;78(Pt 5):1109–1114.
128. Edington N, Bridges CG, Huckle A. Experimental reactivation of equid herpesvirus 1 (EHV 1) following the administration of corticosteroids. *Equine Vet J*. 1985;17:369–372.
129. Welch HM, Bridges CG, Lyon AM, et al. Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co-cultivation from lymphoid tissues. *J Gen Virol*. 1992;73(Pt 2):261–268.
130. Padgett DA, Sheridan JF, Dorne J, et al. Social stress and the reactivation of latent herpes simplex virus type 1. *Proc Natl Acad Sci U S A*. 1998;95:7231–7235.
131. Crawford TB, Wardrop KJ, Tornquist SJ, et al. A primary production deficit in the thrombocytopenia of equine infectious anemia. *J Virol*. 1996;70:7842–7850.
132. Oaks JL, McGuire TC, Ulibarri C, et al. Equine infectious anemia virus is found in tissue macrophages during subclinical infection. *J Virol*. 1998;72:7263–7269.
133. Belshan M, Baccam P, Oaks JL, et al. Genetic and biological variation in equine infectious anemia virus Rev correlates with variable stages of clinical disease in an experimentally infected pony. *Virology*. 2001;279:185–200.
134. Maury W, Perryman S, Oaks JL, et al. Localized sequence heterogeneity in the long terminal repeats of in vivo isolates of equine infectious anemia virus. *J Virol*. 1997;71:4929–4937.
135. Jacob RJ, Price R, Bouchey D, et al. Temperature sensitivity of equine herpesvirus isolates: a brief review. *SAAS Bull Biochem Biotechnol*. 1990;3:124–128.
136. Cravens RL, Ellsworth MA, Sorensen CD, et al. Efficacy of a temperature-sensitive modified-live bovine herpesvirus type-1 vaccine against abortion and stillbirth in pregnant heifers. *J Am Vet Med Assoc*. 1996;208:2031–2034.
137. Youngner JS, Whitaker-Dowling P, Chambers TM, et al. Derivation and characterization of a live attenuated equine influenza vaccine virus. *Am J Vet Res*. 2001;62:1290–1294.
138. Fenner F. Mouse-pox; infectious ectromelia of mice; a review. *J Immunol*. 1949;63:341–373.
139. Calisher CH. Medically important arboviruses of the United States and Canada. *Clin Microbiol Rev*. 1994;7:89–116.
140. Walker C, Love DN, Whalley JM. Comparison of the pathogenesis of acute equine herpesvirus 1 (EHV-1) infection in the horse and the mouse model: a review. *Vet Microbiol*. 1999;68:3–13.
141. Edington N, Smyth B, Griffiths L. The role of endothelial cell infection in the endometrium, placenta and foetus of equid herpesvirus 1 (EHV-1) abortions. *J Comp Pathol*. 1991;104:379–387.
142. Whitwell KE, Blunden AS. Pathological findings in horses dying during an outbreak of the paralytic form of equid herpesvirus type 1 (EHV-1) infection. *Equine Vet J*. 1992;24:13–19.
143. Green SL. Rabies. *Vet Clin North Am Equine Pract*. 1997;13:1–11.
144. Baxi MK, Efstathiou S, Lawrence G, et al. The detection of latency-associated transcripts of equine herpesvirus 1 in ganglionic neurons. *J Gen Virol*. 1995;76(Pt 12):3113–3118.
145. Winer JB. Guillain Barré syndrome. *Mol Pathol*. 2001;54:381–385.
146. Nolte KB, Alakija P, Oty G, et al. Influenza A virus infection complicated by fatal myocarditis. *Am J Forensic Med Pathol*. 2000;21:375–379.
147. Evermann JF, Heeney JL, Roelke ME, et al. Biological and pathological consequences of feline infectious peritonitis virus infection in the cheetah. *Arch Virol*. 1988;102:155–171.
148. Jacobs DE. *A Colour Atlas of Equine Parasites*. Philadelphia: Lea & Febiger; 1986.
149. Slocombe JO. Pathogenesis of helminths in equines. *Vet Parasitol*. 1985;18:139–153.
150. Fox MT. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Vet Parasitol*. 1997;72:285–297. discussion 297–308.
151. Symons LEA. *Pathophysiology of endoparasitic infection compared with ectoparasitic infestation and microbial infection*. London: Academic Press, Inc.; 1989.
152. Herd RP. Epidemiology and control of parasites in northern temperate regions. *Vet Clin North Am Equine Pract*. 1986;2:337–355.
153. Nielsen MK, Jacobsen S, Olsen SN, et al. Nonstrangulating intestinal infarction associated with *Strongylus vulgaris* in referred Danish equine cases. *Equine Vet J*. 2016;48:376–379.
154. McCraw BM, Slocombe JO. *Strongylus vulgaris* in the horse: a review. *Can Vet J*. 1976;17:150–157.
155. Klei TR. Recent observations on the epidemiology, pathogenesis and immunology of equine helminth infections. In: *Proceedings of the 6th International Conference on Equine Infectious Diseases*. Newmarket: R&W Publications; 1991:129–136.
156. Klei TR, Turk MA, McClure JR, et al. Effects of repeated *Strongylus vulgaris* inoculations and concurrent ivermectin treatments on mesenteric arterial lesions in pony foals. *Am J Vet Res*. 1990;51:654–660.
157. Monahan CM, Taylor HW, Chapman MR, et al. Experimental immunization of ponies with *Strongylus vulgaris* radiation-attenuated larvae or crude soluble somatic extracts from larval or adult stages. *J Parasitol*. 1994;80:911–923.
158. Clayton HM. Ascarids. Recent advances. *Vet Clin North Am Equine Pract*. 1986;2:313–328.
159. Lyons ET, Tolliver SC, Ionita M, et al. Evaluation of parasiticidal activity of fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate in horse foals with emphasis on ascarids *(Parascaris equorum)* in field studies on five farms in Central Kentucky in 2007. *Parasitol Res*. 2008;103:287–291.
160. Barclay WP, Phillips TN, Foerner JJ. Intussusception associated with *Anoplocephala perfoliata* infection in five horses. *J Am Vet Med Assoc*. 1982;180:752–753.
161. Beroza GA, Barclay WP, Phillips TN, et al. Cecal perforation and peritonitis associated with *Anoplocephala perfoliata* infection in three horses. *J Am Vet Med Assoc*. 1983;183:804–806.
162. Nielsen MK. Equine tapeworm infections: disease, diagnosis, and control. *Equine Vet Educ*. 2016;28:388–395.
163. Kjaer LN, Lungholt MM, Nielsen MK, et al. Interpretation of serum antibody response to *Anoplocephala perfoliata* in relation to parasite burden and faecal egg count. *Equine Vet J*. 2007;39:529–533.
164. Williamson RM, Gasser RB, Middleton D, et al. The distribution of *Anoplocephala perfoliata* in the intestine of the horse and associated pathological changes. *Vet Parasitol*. 1997;73:225–241.
165. Uhlinger C. Effects of three anthelmintic schedules on the incidence of colic in horses. *Equine Vet J*. 1990;22:251–254.
166. Uhlinger C. Equine small strongyles: epidemiology, pathology and control. *Compend Contin Educ Pract Vet*. 1991;13:863–869.

167. Reid SW, Mair TS, Hillyer MH, et al. Epidemiological risk factors associated with a diagnosis of clinical cyathostomiasis in the horse. *Equine Vet J*. 1995;27:127–130.
168. Love S, Murphy D, Mellor D. Pathogenicity of cyathostome infection. *Vet Parasitol*. 1999;85:113–121. discussion 121-112, 215-125.
169. Murphy D, Love S. The pathogenic effects of experimental cyathostome infections in ponies. *Vet Parasitol*. 1997;70:99–110.
170. Castro GA. Immunophysiology of enteric parasitism. *Parasitol Today*. 1989;5:11–19.
171. Bueno L, Ruckebusch Y, Dorchies P. Disturbances of digestive motility in horses associated with strongyle infection. *Vet Parasitol*. 1979;5:253.
172. Lester GD, Bolton JR, Cambridge H, et al. The effect of *Strongylus vulgaris* larvae on equine intestinal myoelectrical activity. *Equine Vet J Suppl*. 1989:8–13.
173. Berry CR, Merritt AM, Burrows CF, et al. Evaluation of the myoelectrical activity of the equine ileum infected with *Strongylus vulgaris* larvae. *Am J Vet Res*. 1986;47:27–30.
174. Artis D. New weapons in the war on worms: identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. *Int J Parasitol*. 2006;36:723–733.
175. Finkelman FD, Shea-Donohue T, Goldhill J, et al. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol*. 1997;15:505–533.
176. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol*. 2003;3:733–744.
177. Patel N, Kreider T, Urban Jr JF, et al. Characterisation of effector mechanisms at the host:parasite interface during the immune response to tissue-dwelling intestinal nematode parasites. *Int J Parasitol*. 2009;39:13–21.
178. Nielsen MK. Evidence-based considerations for control of *Parascaris* spp. infections in horses. *Equine Vet Educ*. 2016;28:224–231.
179. Soulsby EJL. *Immune Responses in Parasitic Infections: Immunology, Immunopathology and Immunoprophylaxis, Nematodes*. Boca Raton: CRC Press; 1987.
180. Allen JE, Adjei O, Bain O, et al. Of mice, cattle, and humans: the immunology and treatment of river blindness. *PLoS Negl Trop Dis*. 2008;2:e217.
181. King CL, Nutman TB. Regulation of the immune response in lymphatic filariasis and onchocerciasis. *Immunol Today*. 1991;12:A54–A58.
182. Foil LD, Klei TR, Miller RI, et al. Seasonal changes in density and tissue distribution of *Onchocerca cervicalis* microfilariae in ponies and related changes in *Culicoides variipennis* populations in Louisiana. *J Parasitol*. 1987;73:320–326.
183. Ogbourne CP. Studies on the epidemiology of *Strongylus vulgaris* infection of the horse. *Int J Parasitol*. 1975;5:423–426.
184. Murrell KD. Induction of protective immunity to *Strongyloides ransomi* in pigs. *Am J Vet Res*. 1981;42:1915–1919.
185. Klei TR, Chapman MR. Immunity in equine cyathostome infections. *Vet Parasitol*. 1999;85:123–133. discussion 133-126, 215-125.
186. Monahan CM, Chapman MR, Taylor HW, et al. Experimental cyathostome challenge of ponies maintained with or without benefit of daily pyrantel tartrate feed additive: comparison of parasite burdens, immunity and colonic pathology. *Vet Parasitol*. 1998;74:229–241.
187. Chapman MR, French DD, Taylor HW, et al. One season of pasture exposure fails to induce a protective resistance to cyathostomes but increases numbers of hypobiotic third-stage larvae. *J Parasitol*. 2002;88:678–683.
188. Klei TR, Chapman MR, Dennis VA. Role of the eosinophil in serum-mediated adherence of equine leukocytes to infective larvae of *Strongylus vulgaris*. *J Parasitol*. 1992;78:477–484.
189. Dennis VA, Klei TR, Chapman MR, et al. In vivo activation of equine eosinophils and neutrophils by experimental *Strongylus vulgaris* infections. *Vet Immunol Immunopathol*. 1988;20:61–74.
190. Finkelman FD, Pearce EJ, Urban Jr JF, et al. Regulation and biological function of helminth-induced cytokine responses. *Immunol Today*. 1991;12:A62–A66.
191. Maizels RM, Blaxter ML, Scott AL. Immunological genomics of *Brugia malayi*: filarial genes implicated in immune evasion and protective immunity. *Parasite Immunol*. 2001;23:327–344.
192. Maizels RM, Bundy DA, Selkirk ME, et al. Immunological modulation and evasion by helminth parasites in human populations. *Nature*. 1993;365:797–805.
193. Elliott DE, Summers RW, Weinstock JV. Helminths and the modulation of mucosal inflammation. *Curr Opin Gastroenterol*. 2005;21:51–58.
194. Summers RW, Elliott DE, Weinstock JV. Is there a role for helminths in the therapy of inflammatory bowel disease? *Nat Clin Pract Gastroenterol Hepatol*. 2005;2:62–63.
195. Hoerauf A, Satoguina J, Saeftel M, et al. Immunomodulation by filarial nematodes. *Parasite Immunol*. 2005;27:417–429.
196. Jackson JA, Friberg IM, Little S, et al. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? *Immunology*. 2009;126:18–27.
197. van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology*. 2007;212:475–490.
198. Edmonds JD, Horohov DW, Chapmat MR, et al. Altered immune responses to a heterologous protein in ponies with heavy gastrointestinal parasite burdens. *Equine Vet J*. 2001;33:658–663.
199. Nielsen MK, Rubinson EF, Chambers TM, et al. Interaction between anthelmintic treatment and vaccine responses in ponies naturally infected with cyathostomins. *Vet Immunol Immunopathol*. 2015;164:110–117.
200. Sellon D, Long M. *Equine Infectious Diseases*. 2nd ed. Philadelphia, PA: Saunders Elsevier; 2014.
201. Mealey RH. New perspectives in infectious diseases. *Vet Clin North Am Equine Pract*. 2014;30. xv-xvi.
202. Chandriani S, Skewes-Cox P, Zhong W, et al. Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis. *Proc Natl Acad Sci U S A*. 2013;110:E1407–E1415.
203. Li L, Giannitti F, Low J, et al. Exploring the virome of diseased horses. *J Gen Virol*. 2015;96:2721–2733.
204. Ceciliani F, Eckersall D, Burchmore R, et al. Proteomics in veterinary medicine: applications and trends in disease pathogenesis and diagnostics. *Vet Pathol*. 2014;51:351–362.
205. Engleberg N, DiRita V, Dermody T. *Schaechter's Mechanisms of Microbial Disease*. 5th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2013.
206. Horohov DW. The equine immune responses to infectious and allergic disease: a model for humans? *Mol Immunol*. 2015;66:89–96.
207. Abbas A, Lichtman A, Pillai S. *Cellular and Molecular Immunology*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2015.
208. Filippe M. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
209. Murphy K. *Janeway's Immunobiology*. 8th ed. New York, NY: Garland Science; 2012.
210. Tizard I. *Veterinary Immunology*. 9th ed. Philadelphia, PA: Elsevier Saunders; 2013.
211. Coombs SL, Webbon PM. Tracheal mucus transport in the horse following equine influenza vaccination. *Vet Rec*. 1986;119:601–602.
212. Dixon PM. Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. *Vet Rec*. 1992;131:229–235.
213. Oikawa M, Takagi S, Anzai R, et al. Pathology of equine respiratory disease occurring in association with transport. *J Comp Pathol*. 1995;113:29–43.

214. Nordengrahn A, Rusvai M, Merza M, et al. Equine herpesvirus type 2 (EHV-2) as a predisposing factor for *Rhodococcus equi* pneumonia in foals: prevention of the bifactorial disease with EHV-2 immunostimulating complexes. *Vet Microbiol*. 1996;51:55–68.
215. Furr M, Cohen ND, Axon JE, et al. Treatment with histamine-type 2 receptor antagonists and omeprazole increase the risk of diarrhoea in neonatal foals treated in intensive care units. *Equine Vet J Suppl*. 2012:80–86.
216. Buendgens L, Bruensing J, Matthes M, et al. Administration of proton pump inhibitors in critically ill medical patients is associated with increased risk of developing *Clostridium difficile*-associated diarrhea. *J Crit Care*. 2014;29. 696.e611-695.
217. MacKay RJ. Inflammation in horses. *Vet Clin North Am Equine Pract*. 2000;16:15–27. v.
218. Netea MG, van der Graaf C, Van der Meer JWM, et al. Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. *J Leukoc Biol*. 2004;75:749–755.
219. Petersen HH, Nielsen JP, Heegaard PM. Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res*. 2004;35:163–187.
220. Grondahl G, Sternberg S, Jensen-Waern M, et al. Opsonic capacity of foal serum for the two neonatal pathogens *Escherichia coli* and Actinobacillus equuli. *Equine Vet J*. 2001;33:670–675.
221. Camp CJ, Leid RW. Chemotaxis of radiolabeled equine neutrophils. *Am J Vet Res*. 1982;43:397–401.
222. Higgins AJ, Lees P. The acute inflammatory process, arachidonic acid metabolism and the mode of action of anti-inflammatory drugs. *Equine Vet J*. 1984;16:163–175.
223. Gibson KT, Hodge H, Whittem T. Inflammatory mediators in equine synovial fluid. *Aust Vet J*. 1996;73:148–151.
224. Verburg KM, Maziasz TJ, Weiner E, et al. COX-2-specific inhibitors: definition of a new therapeutic concept. *Am J Ther*. 2001;8:49–64.
225. Morton AJ, Campbell NB, Gayle JM, et al. Preferential and non-selective cyclooxygenase inhibitors reduce inflammation during lipopolysaccharide-induced synovitis. *Res Vet Sci*. 2005;78:189–192.
226. Lees P, Landoni MF, Giraudel J, et al. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *J Vet Pharmacol Ther*. 2004;27:479–490.
227. Beretta C, Garavaglia G, Cavalli M. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. *Pharmacol Res*. 2005;52:302–306.
228. Raidal SL, Edwards S, Pippia J, et al. Pharmacokinetics and safety of oral administration of meloxicam to foals. *J Vet Intern Med*. 2013;27:300–307.
229. Walliser U, Fenner A, Mohren N, et al. Evaluation of the efficacy of meloxicam for post-operative management of pain and inflammation in horses after orthopaedic surgery in a placebo controlled clinical field trial. *BMC Vet Res*. 2015;11:113.
230. Barton MH, Darden JE, Clifton S, et al. Effect of firocoxib on cyclooxygenase 2, microsomal prostaglandin E2 synthase 1, and cytosolic phospholipase A2 gene expression in equine mononuclear cells. *Am J Vet Res*. 2015;76:1051–1057.
231. Barton MH, Paske E, Norton N, et al. Efficacy of cyclo-oxygenase inhibition by two commercially available firocoxib products in horses. *Equine Vet J*. 2014;46:72–75.
232. Cook VL, Meyer CT, Campbell NB, et al. Effect of firocoxib or flunixin meglumine on recovery of ischemic-injured equine jejunum. *Am J Vet Res*. 2009;70:992–1000.
233. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91–97.
234. Hovanessian N, Davis JL, McKenzie 3rd HC, et al. Pharmacokinetics and safety of firocoxib after oral administration of repeated consecutive doses to neonatal foals. *J Vet Pharmacol Ther*. 2014;37:243–251.
235. Kvaternick V, Pollmeier M, Fischer J, et al. Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther*. 2007;30:208–217.
236. Letendre LT, Tessman RK, McClure SR, et al. Pharmacokinetics of firocoxib after administration of multiple consecutive daily doses to horses. *Am J Vet Res*. 2008;69:1399–1405.
237. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest*. 2001;108:15–23.
238. Hardy J, Bertone AL, Weisbrode SE, et al. Cell trafficking, mediator release, and articular metabolism in acute inflammation of innervated or denervated isolated equine joints. *Am J Vet Res*. 1998;59:88–100.
239. Gangur V, Birmingham NP, Thanesvorakul S. Chemokines in health and disease. *Vet Immunol Immunopathol*. 2002;86:127–136.
240. Cook VL, Neuder LE, Blikslager AT, et al. The effect of lidocaine on in vitro adhesion and migration of equine neutrophils. *Vet Immunol Immunopathol*. 2009;129:137–142.
241. Brooks AC, Rickards KJ, Cunningham FM. CXCL8 attenuates chemoattractant-induced equine neutrophil migration. *Vet Immunol Immunopathol*. 2011;139:141–147.
242. Marr KA, Lees P, Cunningham FM. Agonist-induced adherence of equine neutrophils to fibronectin- and serum-coated plastic is CD18 dependent. *Vet Immunol Immunopathol*. 1999;71:77–88.
243. Xu J, Cai J, Anderson B, et al. Cloning and functional characterization of recombinant equine P-selectin. *Vet Immunol Immunopathol*. 2007;116:115–130.
244. Jones SL, Sharief Y, Chilcoat CD. Signaling mechanism for equine neutrophil activation by immune complexes. *Vet Immunol Immunopathol*. 2001;82:87–100.
245. Re F, Muzio M, De Rossi M, et al. The type II "receptor" as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J Exp Med*. 1994;179:739–743.
246. Healy DP. New and emerging therapies for sepsis. *Ann Pharmacother*. 2002;36:648–654.
247. Lewis MJ, Wagner B, Woof JM. The different effector function capabilities of the seven equine IgG subclasses have implications for vaccine strategies. *Mol Immunol*. 2008;45:818–827.
248. Wagner B, Miller DC, Lear TL, et al. The complete map of the Ig heavy chain constant gene region reveals evidence for seven IgG isotypes and for IgD in the horse. *J Immunol*. 2004;173:3230–3242.
249. Perkins GA, Wagner B. The development of equine immunity: current knowledge on immunology in the young horse. *Equine Vet J*. 2015;47:267–274.
250. Wagner B. Immunoglobulins and immunoglobulin genes of the horse. *Dev Comp Immunol*. 2006;30:155–164.
251. Lunn DP, Holmes MA, Antczak DF, et al. Report of the Second Equine Leucocyte Antigen Workshop, Squaw valley, California, July 1995. *Vet Immunol Immunopathol*. 1998;62:101–143.
252. McGuire TC, Van Hoosier Jr GL, Henson JB. The complement-fixation reaction in eguine infectious anemia: demonstration of inhibition by IgG (T). *J Immunol*. 1971;107:1738–1744.
253. Sun Y, Wang C, Wang Y, et al. A comprehensive analysis of germline and expressed immunoglobulin repertoire in the horse. *Dev Comp Immunol*. 2010;34:1009–1020.
254. Ford J, Home W, Gibson D. Light chain isotype regulation in the horse. Characterization of Ig kappa genes. *J Immunol*. 1994;153:1099–1111.
255. Home W, Ford J, Gibson D. L chain isotype regulation in horse. I. Characterization of Ig lambda genes. *J Immunnol*. 1992;149:3927–3936.
256. Tschetter JR, Davis WC, Perryman LE, et al. CD8 dimer usage on alpha beta and gama delta T lymphocytes from equine lymphoid tissues. *Immunobiology*. 1998;198:424–438.

257. Alcover A, Alarcon B. Internalization and intracellular fate of TCR-CD3 complexes. *Crit Rev Immunol*. 2000;20:325–346.
258. Kisielow P, Miazek A. Thymic selection and tolerance. *Transplant Proc*. 1996;28:3429–3430.
259. Bailey E. Identification and genetics of horse lymphocyte alloantigens. *Immunogenetics*. 1980;11:499–506.
260. Antczak DF, Allen WR. Maternal immunological recognition of pregnancy in equids. *J Reprod Fertil Suppl*. 1989;37:69–78.
261. Huang T, Lehmann MJ, Said A, et al. Major histocompatibility complex class I downregulation induced by equine herpesvirus type 1 pUL56 is through dynamin-dependent endocytosis. *J Virol*. 2014;88:12802–12815.
262. Ramsay JD, Leib SR, Orfe L, et al. Development of a DNA microarray for detection of expressed equine classical MHC class I sequences in a defined population. *Immunogenetics*. 2010;62:633–639.
263. Tallmadge RL, Campbell JA, Miller DC, et al. Analysis of MHC class I genes across horse MHC haplotypes. *Immunogenetics*. 2010;62:159–172.
264. Tallmadge RL, Lear TL, Antczak DF. Genomic characterization of MHC class I genes of the horse. *Immunogenetics*. 2005;57:763–774.
265. Chung C, Leib SR, Fraser DG, et al. Novel classical MHC class I alleles identified in horses by sequencing clones of reverse transcription-PCR products. *Eur J Immunogenet*. 2003;30:387–396.
266. Tseng CT, Miller D, Cassano J, et al. Identification of equine major histocompatibility complex haplotypes using polymorphic microsatellites. *Anim Genet*. 2010;41(suppl 2):150–153.
267. Brinkmeyer-Langford CL, Cai JJ, Gill CA, et al. Microsatellite variation in the equine MHC. *Anim Genet*. 2013;44:267–275.
268. Zinkernagel RM, Doherty PC. Immunological surveilance against altered self-components by sensitized T lymphocytes in lymphocytic choriomeningitis. *Nature*. 1974;251:547–548.
269. Bjorkman PJ, Saper MA, Samraoui B, et al. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature*. 1987;329:512–518.
270. Braciale TJ, Morrison LA, Sweetser MT, et al. Antigen presentation pathways to class I and class II MHC-restricted T lymphocytes. *Immunol Rev*. 1987;98:95–114.
271. Lechner F, Cuero AL, Kantzanou M, et al. Studies of human antiviral CD8+ lymphocytes using class I peptide tetramers. *Rev Med Virol*. 2001;11:11–22.
272. Mealey RH, Sharif A, Ellis SA, et al. Early detection of dominant Env-specific and subdominant Gag-specific CD8+ lymphocytes in equine infectious anemia virus-infected horses using major histocompatibility complex class I/peptide tetrameric complexes. *Virology*. 2005;339:110–126.
273. Mealey RH, Stone DM, Hines MT, et al. Experimental Rhodococcus equi and equine infectious anemia virus DNA vaccination in adult and neonatal horses: effect of IL-12, dose, and route. *Vaccine*. 2007;25:7582–7597.
274. Mealey RH, Lee JH, Leib SR, et al. A single amino acid difference within the alpha-2 domain of two naturally occurring equine MHC class I molecules alters the recognition of Gag and Rev epitopes by equine infectious anemia virus-specific CTL. *J Immunol*. 2006;177:7377–7390.
275. Yao S, Liu J, Qi J, et al. Structural Illumination of Equine MHC Class I Molecules Highlights Unconventional Epitope Presentation Manner That Is Evolved in Equine Leukocyte Antigen Alleles. *J Immunol*. 2016;196:1943–1954.
276. Bergmann T, Moore C, Sidney J, et al. The common equine class I molecule Eqca-1*00101 (ELA-A3.1) is characterized by narrow peptide binding and T cell epitope repertoires. *Immunogenetics*. 2015;67:675–689.
277. Crepaldi T, Crump A, Newman M, et al. Equine T lymphocytes express MHC class II antigens. *J Immunogenet*. 1986;13:349–360.
278. Lunn DP, Holmes MA, Duffus WP. Equine T-lymphocyte MHC II expression: variation with age and subset. *Vet Immunol Immunopathol*. 1993;35:225–238.
279. Frayne J, Stokes CR. MHC Class II positive cells and T cells in the equine endometrium throughout the oestrous cycle. *Vet Immunol Immunopathol*. 1994;41:55–72.
280. Albright-Fraser DG, Reid R, Gerber V, et al. Polymorphism of DRA among equids. *Immunogenetics*. 1996;43:315–317.
281. Arbanasic H, Galov A, Ambriovic-Ristov A, et al. Extensive polymorphism of the major histocompatibility complex DRA gene in Balkan donkeys: perspectives on selection and genealogy. *Anim Genet*. 2013;44:711–716.
282. Kamath PL, Getz WM. Adaptive molecular evolution of the Major Histocompatibility Complex genes, DRA and DQA, in the genus Equus. *BMC Evol Biol*. 2011;11:128.
283. Fraser DG, Bailey E. Polymorphism and multiple loci for the horse DQA gene. *Immunogenetics*. 1998;47:487–490.
284. Fraser DG, Bailey E. Demonstration of three DRB loci in a domestic horse family. *Immunogenetics*. 1996;44:441–445.
285. Horin P, Matiasovic J. A second locus and new alleles in the major histocompatibility complex class II (ELA-DQB) region in the horse. *Anim Genet*. 2002;33:196–200.
286. Andersson LS, Swinburne JE, Meadows JR, et al. The same ELA class II risk factors confer equine insect bite hypersensitivity in two distinct populations. *Immunogenetics*. 2012;64:201–208.
287. Raphael I, Nalawade S, Eagar TN, et al. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine*. 2015;74:5–17.
288. Meulenbroeks C, van der Lugt JJ, van der Meide NM, et al. Allergen-Specific Cytokine Polarization Protects Shetland Ponies against Culicoides obsoletus-Induced Insect Bite Hypersensitivity. *PloS one*. 2015;10:e0122090.
289. Horohov DW. Equine T-cell cytokines. Protection and pathology. *Vet Clin North Am Equine Pract*. 2000;16:1–14.
290. Aggarwal N, Holmes MA. Characterisation of equine T helper cells: demonstration of Th1- and Th2-like cells in long-term equine T-cell cultures. *Res Vet Sci*. 1999;66:277–279.
291. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014;13:668–677.
292. Ueno A, Ghosh A, Hung D, et al. Th17 plasticity and its changes associated with inflammatory bowel disease. *World J Gastroenterol*. 2015;21:12283–12295.
293. Olofsson KM, Hjertner B, Fossum C, et al. Expression of T helper type 17 (Th17)-associated cytokines and toll-like receptor 4 and their correlation with Foxp3 positive cells in rectal biopsies of horses with clinical signs of inflammatory bowel disease. *Vet J*. 2015;206:97–104.
294. Regan DP, Aarnio MC, Davis WS, et al. Characterization of cytokines associated with Th17 cells in the eyes of horses with recurrent uveitis. *Vet Ophthalmol*. 2012;15:145–152.
295. Korn A, Miller D, Dong L, et al. Differential Gene Expression Profiles and Selected Cytokine Protein Analysis of Mediastinal Lymph Nodes of Horses with Chronic Recurrent Airway Obstruction (RAO) Support an Interleukin-17 Immune Response. *PloS one*. 2015;10:e0142622.
296. Bullone M, Lavoie JP. Asthma "of horses and men"—how can equine heaves help us better understand human asthma immunopathology and its functional consequences? *Mol Immunol*. 2015;66:97–105.
297. Liu M, Bordin A, Liu T, et al. Gene expression of innate Th1-, Th2-, and Th17-type cytokines during early life of neonatal foals in response to *Rhodococcus equi*. *Cytokine*. 2011;56:356–364.
298. Grant CR, Liberal R, Mieli-Vergani G, et al. Regulatory T-cells in autoimmune diseases: challenges, controversies and yet-unanswered questions. *Autoimmun Rev*. 2015;14:105–116.
299. Wagner B, Hillegas JM, Brinker DR, et al. Characterization of monoclonal antibodies to equine interleukin-10 and detection of T regulatory 1 cells in horses. *Vet Immunol Immunopathol*. 2008;122:57–64.

300. de Mestre A, Noronha L, Wagner B, et al. Split immunological tolerance to trophoblast. *Int J Dev Biol*. 2010;54:445–455.
301. Robbin MG, Wagner B, Noronha LE, et al. Subpopulations of equine blood lymphocytes expressing regulatory T cell markers. *Vet Immunol Immunopathol*. 2011;140:90–101.
302. Hamza E, Gerber V, Steinbach F, et al. Equine CD4(+) CD25(high) T cells exhibit regulatory activity by close contact and cytokine-dependent mechanisms in vitro. *Immunology*. 2011;134:292–304.
303. Hamza E, Mirkovitch J, Steinbach F, et al. Regulatory T cells in early life: comparative study of CD4+CD25high T cells from foals and adult horses. *PloS one*. 2015;10:e0120661.
304. Hamza E, Akdis CA, Wagner B, et al. In vitro induction of functional allergen-specific CD4+ CD25high Treg cells in horses affected with insect bite hypersensitivity. *Clin Exp Allergy*. 2013;43:889–901.
305. Hamza E, Steinbach F, Marti E. CD4+CD25+ T cells expressing FoxP3 in Icelandic horses affected with insect bite hypersensitivity. *Vet Immunol Immunopathol*. 2012;148:139–144.
306. Schaffartzik A, Hamza E, Janda J, et al. Equine insect bite hypersensitivity: what do we know? *Vet Immunol Immunopathol*. 2012;147:113–126.
307. Henriquez C, Perez B, Morales N, et al. Participation of T regulatory cells in equine recurrent airway obstruction. *Vet Immunol Immunopathol*. 2014;158:128–134.
308. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. *Blood*. 2013;121:29–37.
309. Lunn DP, Breathnach C, Soboll G. Immunology and immunopathology. In: McGorum BC, Dixon PM, Robinson NE, Schumacher J, eds. *Equine respiratory medicine and surgery*. 1st ed. Edinburgh: Sauders-Elsevier; 2007:71–81.
310. Ganusov VV, De Boer RJ. Tissue distribution of lymphocytes and plasma cells and the role of the gut: response to Pabst et al. *Trends Immunol*. 2008;29:209–210.
311. Pabst R, Russell MW, Brandtzaeg P. Tissue distribution of lymphocytes and plasma cells and the role of the gut. *Trends Immunol*. 2008;29:206–208.
312. Kumar P, Timoney JF, Sheoran AS. M cells and associated lymphoid tissue of the equine nasopharyngeal tonsil. *Equine Vet J*. 2001;33:224–230.
313. Breathnach CC, Yeargan MR, Timoney JF, et al. Detection of equine herpesvirus-specific effector and memory cytotoxic immunity in the equine upper respiratory tract. *Vet Immunol Immunopathol*. 2006;111:117–125.
314. Perryman LE, McGuire TC, Torbeck RL. Ontogeny of lymphocyte function in the equine fetus. *Am J Vet Res*. 1980;41:1197–1200.
315. McGuire TC, Crawford TB. Passive immunity in the foal: measurement of immunoglobulin classes and specific antibody. *Am J Vet Res*. 1973;34:1299–1303.
316. Tallmadge RL, McLaughlin K, Secor E, et al. Expression of essential B cell genes and immunoglobulin isotypes suggests active development and gene recombination during equine gestation. *Dev Comp Immunol*. 2009;33:1027–1038.
317. Sheoran AS, Timoney JF, Holmes MA, et al. Immunoglobulin isotypes in sera and nasal mucosal secretions and their neonatal transfer and distribution in horses. *Am J Vet Res*. 2000;61:1099–1105.
318. Hannant D, Rossdale PD, McGladdery AJ, et al. *Immune responses of the equine foetus to protein antigens. Proceedings of the Sixth International Conference on Equine Infectious Diseases*; 1991:86. Cambridge, UK.
319. Martin BR, Larson KA. Immune response of equine fetus to coliphage T2. *Am J Vet Res*. 1973;34:1363–1364.
320. Morgan DO, Bryans JT, Mock RE. Immunoglobulins produced by the antigenized equine fetus. *J Reprod Fertil Suppl*. 1975:735–738.
321. Blanchard-Channell M, Moore PF, Stott JL. Characterization of monoclonal antibodies specific for equine homologues of CD3 and CD5. *Immunology*. 1994;82:548–554.
322. Lunn DP, Holmes MA, Duffus WP. Three monoclonal antibodies identifying antigens on all equine T lymphocytes, and two mutually exclusive T-lymphocyte subsets. *Immunology*. 1991;74:251–257.
323. Wyatt CR, Davis WC, McGuire TC, et al. T lymphocyte development in horses. I. Characterization of monoclonal antibodies identifying three stages of T lymphocyte differentiation. *Vet Immunol Immunopathol*. 1988;18:3–18.
324. Perryman LE, Wyatt CR, Magnuson NS, et al. T lymphocyte development and maturation in horses. *Anim Genet*. 1988;19:343–348.
325. Giguere S, Polkes AC. Immunologic disorders in neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:241–272.
326. Tallmadge R. The immune system of the young horse. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
327. Wichtel MG, Anderson KL, Johnson TV, et al. Influence of age on neutrophil function in foals. *Equine Vet J*. 1991;23:466–469.
328. Morris DD, Gaulin G, Strzemienski PJ, et al. Assessment of neutrophil migration, phagocytosis and bactericidal capacity in neonatal foals. *Vet Immunol Immunopathol*. 1987;16:173–184.
329. Hietala SK, Ardans AA. Neutrophil phagocytic and serum opsonic response of the foal to *Corynebacterium equi*. *Vet Immunol Immunopathol*. 1987;14:279–294.
330. Bernoco M, Liu IK, Wuest Ehlert CJ, et al. Chemotactic and phagocytic function of peripheral blood polymorphonuclear leucocytes in newborn foals. *J Reprod Fertil Suppl*. 1987;35:599–605.
331. Demmers S, Johannisson A, Grondahl G, et al. Neutrophil functions and serum IgG in growing foals. *Equine Vet J*. 2001;33:676–680.
332. Flaminio MJ, Rush BR, Davis EG, et al. Characterization of peripheral blood and pulmonary leukocyte function in healthy foals. *Vet Immunol Immunopathol*. 2000;73:267–285.
333. McTaggart C, Yovich JV, Penhale J, et al. A comparison of foal and adult horse neutrophil function using flow cytometric techniques. *Res Vet Sci*. 2001;71:73–79.
334. Bernoco MM, Liu IK, Willits NH. Hemolytic complement activity and concentrations of its third component during maturation of the immune response in colostrum-deprived foals. *Am J Vet Res*. 1994;55:928–933.
335. Lavoie JP, Spensley MS, Smith BP, et al. Complement activity and selected hematologic variables in newborn foals fed bovine colostrum. *Am J Vet Res*. 1989;50:1532–1536.
336. Liu IKM, Walsh EM, Bernoco M, et al. Bronchalveolar lavage in the newborn foal. *J Reprod Fert Suppl*. 1987;35:587–592.
337. Smith III R, Chaffin MK, Cohen ND, et al. Age-related changes in lymphocyte subsets of quarter horse foals. *Am J Vet Res*. 2002;63:531–537.
338. Flaminio MJ, Rush BR, Shuman W. Peripheral blood lymphocyte subpopulations and immunoglobulin concentrations in healthy foals and foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med*. 1999;13:206–212.
339. Breathnach CC, Sturgill-Wright T, Stiltner JL, et al. Foals are interferon gamma-deficient at birth. *Vet Immunol Immunopathol*. 2006;112:199–209.
340. Flaminio MJ, Borges AS, Nydam DV, et al. The effect of CpG-ODN on antigen presenting cells of the foal. *J Immune Based Ther Vaccines*. 2007;5:1.
341. Flaminio MJ, Nydam DV, Marquis H, et al. Foal monocyte-derived dendritic cells become activated upon Rhodococcus equi infection. *Clin Vaccine Immunol*. 2009;16:176–183.
342. Sturgill TL, Giguere S, Berghaus LJ, et al. Comparison of antibody and cell-mediated immune responses of foals and adult horses after vaccination with live *Mycobacterium bovis* BCG. *Vaccine*. 2014;32:1362–1367.
343. Ryan C, Giguere S. Equine neonates have attenuated humoral and cell-mediated immune responses to a killed adjuvanted vaccine compared to adult horses. *Clin Vaccine Immunol*. 2010;17:1896–1902.

344. Ryan C, Giguere S, Hagen J, et al. Effect of age and mitogen on the frequency of interleukin-4 and interferon gamma secreting cells in foals and adult horses as assessed by an equine-specific ELISPOT assay. *Vet Immunol Immunopathol*. 2010;133:66–71.
345. Jacks S, Giguere S, Crawford PC, et al. Experimental infection of neonatal foals with Rhodococcus equi triggers adult-like gamma interferon induction. *Clin Vaccine Immunol*. 2007;14:669–677.
346. Wagner B, Burton A, Ainsworth D. Interferon-gamma, interleukin-4 and interleukin-10 production by T helper cells reveals intact Th1 and regulatory TR1 cell activation and a delay of the Th2 cell response in equine neonates and foals. *Vet Res*. 2010;41:47.
347. Harris SP, Hines MT, Mealey RH, et al. Early development of cytotoxic T lymphocytes in neonatal foals following oral inoculation with *Rhodococcus equi*. *Vet Immunol Immunopathol*. 2011;141:312–316.
348. Basha S, Surendran N, Pichichero M. Immune responses in neonates. *Expert Rev Clin Immunol*. 2014;10:1171–1184.
349. Holmes MA, Lunn DP. A study of bovine and equine immunoglobulin levels in pony foals fed bovine colostrum. *Equine Vet J*. 1991;23:116–118.
350. Jeffcott LB. Studies on passive immunity in the foal. 1. Gammaglobulin and antibody variations associated with the maternal transfer of immunity and the onset of active immunity. *J Comp Pathol*. 1974;84:93–101.
351. Lavoie JP, Spensley MS, Smith BP, et al. Absorption of bovine colostral immunoglobulins G and M in newborn foals. *Am J Vet Res*. 1989;50:1598–1603.
352. Wilson WD. Vaccination of foals. In: *British Equine Veterinary Association Conference*. ; 2001. Harrogate.
353. Rouse BT. The immunoglobulins of adult equine and foal sera: a quantitative study. *Vet J*. 1971;127:45–51.
354. Sheoran AS, Sponseller BT, Holmes MA, et al. Serum and mucosal antibody isotype responses to M-like protein (SeM) of *Streptococcus equi* in convalescent and vaccinated horses. *Vet Immunol Immunopathol*. 1997;59:239–251.
355. Nelson KM, Schram BR, McGregor MW, et al. Local and systemic isotype-specific antibody responses to equine influenza virus infection versus conventional vaccination. *Vaccine*. 1998;16:1306–1313.
356. Hoffman AM, Viel L, Juniper E, et al. Clinical and endoscopic study to estimate the incidence of distal respiratory tract infection in thoroughbred foals on Ontario breeding farms. *Am J Vet Res*. 1993;54:1602–1607.
357. Dawson TR, Horohov DW, Meijer WG, et al. Current understanding of the equine immune response to *Rhodococcus equi*. An immunological review of *R. equi* pneumonia. *Vet Immunol Immunopathol*. 2010;135:1–11.
358. Wilson WD, Mihalyi JE, Hussey S, et al. Passive transfer of maternal immunoglobulin isotype antibodies against tetanus and influenza and their effect on the response of foals to vaccination. *Equine Vet J*. 2001;33:644–650.
359. Gibbs E, Wilson J, All B. Studies on Passive Immunity and the Vaccination of foals against Eastern Equine Encephalitis in Florida. In: *Equine infectious Disease V: Proceedings of the Fifth International Conferance*. ;5. ; 1988:201–205.
360. Galan JE, Timoney JF, Lengemann FW. Passive transfer of mucosal antibody to *Streptococcus equi* in the foal. *Infect Immun*. 1986;54:202–206.
361. van Maanen C, Bruin G, de Boer-Luijtze E, et al. Interference of maternal antibodies with the immune response of foals after vaccination against equine influenza. *Vet Q*. 1992;14:13–17.
362. Van Oirschot JT, Bruin G, de Boer-Luytze E, et al. Maternal antibodies against equine influenza virus in foals and their interference with vaccination. *Zentralbl Veterinarmed [B]*. 1991;38:391–396.
363. Davis EG, Bello NM, Bryan AJ, et al. Characterisation of immune responses in healthy foals when a multivalent vaccine protocol was initiated at age 90 or 180 days. *Equine Vet J*. 2015;47:667–674.
364. Merant C, Breathnach CC, Kohler K, et al. Young foal and adult horse monocyte-derived dendritic cells differ by their degree of phenotypic maturity. *Vet Immunol Immunopathol*. 2009;131:1–8.
365. Swiderski CE. Hypersensitivity disorders in horses. *Vet Clin North Am Equine Pract*. 2000;16:131–151. vii.
366. McAleese SM, Halliwell RE, Miller HR. Cloning and sequencing of the horse and sheep high-affinity IgE receptor alpha chain cDNA. *Immunogenetics*. 2000;51:878–881.
367. Watson JL, Jackson KA, King DP, et al. Molecular cloning and sequencing of the low-affinity IgE receptor (CD23) for horse and cattle. *Vet Immunol Immunopathol*. 2000;73:323–329.
368. Wagner B, Hillegas JM, Babasyan S. Monoclonal antibodies to equine CD23 identify the low-affinity receptor for IgE on subpopulations of IgM+ and IgG1+ B-cells in horses. *Vet Immunol Immunopathol*. 2012;146:125–134.
369. Klei TR. Equine immunity to parasites. *Vet Clin North Am Equine Pract*. 2000;16:69–78. vi.
370. Fadok VA. Update on equine allergies. *Vet Clin North Am Equine Pract*. 2013;29:541–550.
371. Brown EM, Arrieta MC, Finlay BB. A fresh look at the hygiene hypothesis: how intestinal microbial exposure drives immune effector responses in atopic disease. *Semin Immunol*. 2013;25:378–387.
372. Kehrli D, Jandova V, Fey K, et al. Multiple hypersensitivities including recurrent airway obstruction, insect bite hypersensitivity, and urticaria in 2 Warmblood horse populations. *J Vet Intern Med*. 2015;29:320–326.
373. Hamza E, Torsteinsdottir S, Eydal M, et al. Increased IL-4 and decreased regulatory cytokine production following relocation of Icelandic horses from a high to low endoparasite environment. *Vet Immunol Immunopathol*. 2010;133:40–50.
374. Olsen L, Ingvast-Larsson C, Brostrom H, et al. Clinical signs and etiology of adverse reactions to procaine benzylpenicillin and sodium/potassium benzylpenicillin in horses. *J Vet Pharmacol Ther*. 2007;30:201–207.
375. Blue JT, Dinsmore RP, Anderson KL. Immune-mediated hemolytic anemia induced by penicillin in horses. *Cornell Vet*. 1987;77:263–276.
376. Banks KL, Henson JB, McGuire TC. Immunologically mediated glomerulitis of horses. I. Pathogenesis in persistent infection by equine infectious anemia virus. *Lab Invest*. 1972;26:701–707.
377. Suter M, Fey H. Further purification and characterization of horse IgE. *Vet Immunol Immunopathol*. 1983;4:545–553.
378. Matthews AG, Imlah P, McPherson EA. A reagin-like antibody in horse serum: 1. Occurrence and some biological properties. *Vet Res Commun*. 1983;6:13–23.
379. Wagner B, Siebenkotten G, Radbruch A, et al. Nucleotide sequence and restriction fragment length polymorphisms of the equine Cvarepsilon gene. *Vet Immunol Immunopathol*. 2001;82:193–202.
380. Navarro P, Barbis DP, Antczak D, et al. The complete cDNA and deduced amino acid sequence of equine IgE. *Mol Immunol*. 1995;32:1–8.
381. Marti E, Szalai G, Bucher K, et al. Partial sequence of the equine immunoglobulin epsilon heavy chain cDNA. *Vet Immunol Immunopathol*. 1995;47:363–367.
382. Halliwell RE, McGorum BC, Irving P, et al. Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Vet Immunol Immunopathol*. 1993;38:201–215.
383. Halliwell RE, Hines MT. Studies on equine recurrent uveitis. I: Levels of immunoglobulin and albumin in the aqueous humor of horses with and without intraocular disease. *Current Eye Research*. 1985;4:1023–1031.
384. Marti E, Peveri P, Griot-Wenk M, et al. Chicken antibodies to a recombinant fragment of the equine immunoglobulin epsilon heavy-chain recognising native horse IgE. *Vet Immunol Immunopathol*. 1997;59:253–270.

385. van der Haegen A, Griot-Wenk M, Welle M, et al. Immunoglobulin-E-bearing cells in skin biopsies of horses with insect bite hypersensitivity. *Equine Vet J*. 2001;33:699–706.
386. Wilson AD, Harwood LJ, Bjornsdottir S, et al. Detection of IgG and IgE serum antibodies to *Culicoides* salivary gland antigens in horses with insect dermal hypersensitivity (sweet itch). *Equine Vet J*. 2001;33:707–713.
387. Eder C, Crameri R, Mayer C, et al. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet Immunol Immunopathol*. 2000;73:241–253.
388. Eder C, Curik I, Brem G, et al. Influence of environmental and genetic factors on allergen-specific immunoglobulin-E levels in sera from Lipizzan horses. *Equine Vet J*. 2001;33:714–720.
389. Wagner B, Radbruch A, Rohwer J, et al. Monoclonal anti-equine IgE antibodies with specificity for different epitopes on the immunoglobulin heavy chain of native IgE. *Vet Immunol Immunopathol*. 2003;92:45–60.
390. Wilson AD, Harwood L, Torsteinsdottir S, et al. Production of monoclonal antibodies specific for native equine IgE and their application to monitor total serum IgE responses in Icelandic and non-Icelandic horses with insect bite dermal hypersensitivity. *Vet Immunol Immunopathol*. 2006;112:156–170.
391. Wagner B, Flaminio JB, Hillegas J, et al. Occurrence of IgE in foals: evidence for transfer of maternal IgE by the colostrum and late onset of endogenous IgE production in the horse. *Vet Immunol Immunopathol*. 2006;110:269–278.
392. Wagner B, Stokol T, Ainsworth DM. Induction of interleukin-4 production in neonatal IgE+ cells after crosslinking of maternal IgE. *Dev Comp Immunol*. 2010;34:436–444.
393. Hanna CJ, Eyre P, Wells PW, et al. Equine immunology 2: immunopharmacology—biochemical basis of hypersensitivity. *Equine Vet J*. 1982;14:16–24.
394. Nielsen IL, Jacobs KA, Huntington PJ, et al. Adverse reaction to procaine penicillin G in horses. *Aust Vet J*. 1988;65 :181–185.
395. Orsini J, Divers T. *Equine Emergencies Treatment and Procedures*. 4th ed. St. Louis, MO: Elsevier Saunders; 2014.
396. Wagner B. Allergy. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
397. Wagner B, Miller WH, Morgan EE, et al. IgE and IgG antibodies in skin allergy of the horse. *Vet Res*. 2006;37:813–825.
398. Lazary S, Marti E, Szalai G, et al. Studies on the frequency and associations of equine leucocyte antigens in sarcoid and summer dermatitis. *Anim Genet*. 1994;25(suppl 1):75–80.
399. Marti E, Gerber H, Lazary S. On the genetic basis of equine allergic diseases: II. Insect bite dermal hypersensitivity. *Equine Vet J*. 1992;24:113–117.
400. Wilson AD. Immune responses to ectoparasites of horses, with a focus on insect bite hypersensitivity. *Parasite Immunol*. 2014;36:560–572.
401. Bullone M, JP L. Recurrent airway obstruction and summer pasture-associated obstructive pulmonart disease. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
402. Leclere M, Lavoie-Lamoureux A, Lavoie JP. Heaves, an asthma-like disease of horses. *Respirology*. 2011;16:1027–1046.
403. Schmallenbach KH, Rahman I, Sasse HH, et al. Studies on pulmonary and systemic *Aspergillus fumigatus*-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Vet Immunol Immunopathol*. 1998;66:245–256.
404. Kunzle F, Gerber V, Van Der Haegen A, et al. IgE-bearing cells in bronchoalveolar lavage fluid and allergen-specific IgE levels in sera from RAO-affected horses. *J Vet Med A Physiol Pathol Clin Med*. 2007;54:40–47.
405. Robinson NE, Derksen FJ, Olszewski MA, et al. The pathogenesis of chronic obstructive pulmonary disease of horses. *Vet J*. 1995;152:283–306.
406. Cordeau M-E, Joubert P, Dewachi O, et al. IL-4, IL-5 and IFN-g mRNA expression in pulmonary lymphocytes in equine heaves. *Vet Immunol Immunopathol*. 2004;97:87–96.
407. Ainsworth DM, Grunig G, Matychak MB, et al. Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. *Vet Immunol Immunopathol*. 2003;96:83–91.
408. Kleiber C, McGorum BC, Horohov DW, et al. Cytokine profiles of peripheral blood and airway CD4 and CD8 T lymphocytes in horses with recurrent airway obstruction. *Vet Immunol Immunopathol*. 2005;104:91–97.
409. Horohov DW, Beadle RE, Mouch S, et al. Temporal regulation of cytokine mRNA expression in equine recurrent airway obstruction. *Vet Immunol Immunopathol*. 2005;108:237–245.
410. Berndt A, Derksen FJ, Venta PJ, et al. Elevated amount of Toll-like receptor 4 mRNA in bronchial epithelial cells is associated with airway inflammation in horses with recurrent airway obstruction. *Am J Physiol*. 2007;292:L936–L943.
411. Traub-Dargatz JL, McClure JJ, Koch C, et al. Neonatal isoerythrolysis in mule foals. *J Am Vet Med Assoc*. 1995;206:67–70.
412. McClure JJ, Koch C, Traub-Dargatz J. Characterization of a red blood cell antigen in donkeys and mules associated with neonatal isoerythrolysis. *Anim Genet*. 1994;25:119–120.
413. Buechner-Maxwell V, Scott MA, Godber L, et al. Neonatal alloimmune thrombocytopenia in a quarter horse foal. *J Vet Intern Med*. 1997;11:304–308.
414. Ramirez S, Gaunt SD, McClure JJ, et al. Detection and effects on platelet function of anti-platelet antibody in mule foals with experimentally induced neonatal alloimmune thrombocytopenia. *J Vet Intern Med*. 1999;13:534–539.
415. Perkins GA, Miller WH, Divers TJ, et al. Ulcerative dermatitis, thrombocytopenia, and neutropenia in neonatal foals. *J Vet Intern Med*. 2005;19:211–216.
416. Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. *J Immunol*. 1985;135:3134–3137.
417. Hunyadi L, Pusterla N. Purpura hemorrhagica. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
418. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118–121.
419. Divers TJ, Timoney JF, Lewis RM, et al. Equine glomerulonephritis and renal failure associated with complexes of group-C streptococcal antigen and IgG antibody. *Vet Immunol Immunopathol*. 1992;32:93–102.
420. Hines MT. Immunologically mediated ocular disease in the horse. *Vet Clin North Am Large Anim Pract*. 1984;6:501–512.
421. Deeg CA, Kaspers B, Gerhards H, et al. Immune responses to retinal autoantigens and peptides in equine recurrent uveitis. *Invest Ophthalmol Vis Sci*. 2001;42:393–398.
422. Davidson MG, Nasisse MP, Roberts SM. Immunodiagnosis of leptospiral uveitis in two horses. *Equine Vet J*. 1987;19:155–157.
423. Faber NA, Crawford M, LeFebvre RB, et al. Detection of Leptospira spp. in the aqueous humor of horses with naturally acquired recurrent uveitis. *J Clin Microbiol*. 2000;38:2731–2733.
424. Parma AE, Fernandez AS, Santisteban CG, et al. Tears and aqueous humor from horses inoculated with Leptospira contain antibodies which bind to cornea. *Vet Immunol Immunopathol*. 1987;14:181–185.
425. Gilger B. Recurrent uveitis. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
426. Dwyer AE, Crockett RS, Kalsow CM. Association of leptospiral seroreactivity and breed with uveitis and blindness in horses: 372 cases (1986–1993). *J Am Vet Med Assoc*. 1995;207:1327–1331.
427. Gilger BC, Malok E, Cutter KV, et al. Characterization of T-lymphocytes in the anterior uvea of eyes with chronic equine recurrent uveitis. *Vet Immunol Immunopathol*. 1999;71: 17–28.

428. Cines DB, Liebman H, Stasi R. Pathobiology of secondary immune thrombocytopenia. *Semin Hematol*. 2009;46: S2–S14.
429. Verma A, Kumar P, Babb K, et al. Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins. *PLoS neglected tropical diseases*. 2010;4:e778.
430. McClure JJ. Equine autoimmunity. *Vet Clin North Am Equine Pract*. 2000;16:153–164.
431. Wilkerson MJ, Davis E, Shuman W, et al. Isotype-specific antibodies in horses and dogs with immune-mediated hemolytic anemia. *J Vet Intern Med*. 2000;14:190–196.
432. Divers T. Immune-mediated cytopenias. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
433. Pfeiffer CJ, Spurlock S, Ball M. Ultrastructural aspects of equine pemphigus foliaceus-like dermatitis. Report of cases. *J Submicrosc Cytol Pathol*. 1988;20:453–461.
434. Vandenabeele SI, White SD, Affolter VK, et al. Pemphigus foliaceus in the horse: a retrospective study of 20 cases. *Vet Dermatol*. 2004;15:381–388.
435. Scott D, Miller W. *Equine Dermatology*. 2nd ed. Philadelphia, PA: Elsevier Saunders; 2011.
436. Winfield LD, White SD, Affolter VK, et al. Pemphigus vulgaris in a Welsh pony stallion: case report and demonstration of antidesmoglein autoantibodies. *Vet Dermatol*. 2013;24. 269–e260.
437. Geor RJ, Clark EG, Haines DM, et al. Systemic lupus erythematosus in a filly. *J Am Vet Med Assoc*. 1990;197:1489–1492.
438. Fordyce PS, Edington N, Bridges GC, et al. Use of an ELISA in the differential diagnosis of cauda equina neuritis and other equine neuropathies. *Equine Vet J*. 1987;19:55–59.
439. Wright JA, Fordyce P, Edington N. Neuritis of the cauda equina in the horse. *J Comp Pathol*. 1987;97:667–675.
440. Kadlubowski M, Ingram PL. Circulating antibodies to the neuritogenic myelin protein, P2, in neuritis of the cauda equina of the horse. *Nature*. 1981;293:299–300.
441. van Galen G, Cassart D, Sandersen C, et al. The composition of the inflammatory infiltrate in three cases of polyneuritis equi. *Equine Vet J*. 2008;40:185–188.
442. Hahn CN. Polyneuritis equi: the role of T-lymphocytes and importance of differential clinical signs. *Equine Vet J*. 2008;40:100.
443. Piercy RJ, Swardson CJ, Hinchcliff KW. Erythroid hypoplasia and anemia following administration of recombinant human erythropoietin to two horses. *J Am Vet Med Assoc*. 1998;212:244–247.
444. Woods PR, Campbell G, Cowell RL. Nonregenerative anaemia associated with administration of recombinant human erythropoetin to a Thoroughbred racehorses. *Equine Vet J*. 1997;29:326–328.
445. Perryman LE. Primary immunodeficiencies of horses. *Vet Clin North Am Equine Pract*. 2000;16:105–116. vii.
446. Sellon DC. Secondary immunodeficiencies of horses. *Vet Clin North Am Equine Pract*. 2000;16:117–130.
447. Crisman MV, Scarratt WK. Immunodeficiency disorders in horses. *Vet Clin North Am Equine Pract*. 2008;24: 299–310. vi.
448. Filippe M. Immunodeficiencies. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
449. Clabough DL, Levine JF, Grant GL, et al. Factors associated with failure of passive transfer of colostral antibodies in Standardbred foals. *J Vet Intern Med*. 1991;5:335–340.
450. Stoneham SJ, Digby NJ, Ricketts SW. Failure of passive transfer of colostral immunity in the foal: incidence, and the effect of stud management and plasma transfusions. *Vet Rec*. 1991;128:416–419.
451. Raidal SL. The incidence and consequences of failure of passive transfer of immunity on a thoroughbred breeding farm. *Aust Vet J*. 1996;73:201–206.
452. Shin EK, Perryman LE, Meek K. A kinase-negative mutation of DNA-PK(CS) in equine SCID results in defective coding and signal joint formation. *J Immunol*. 1997;158:3565–3569.
453. McGuire TC, Poppie MJ, Banks KL. Combined (B- and T-lymphocyte) immunodeficiency: a fatal genetic disease in Arabian foals. *J Am Vet Med Assoc*. 1974;164:70–76.
454. Liepman RS, Dembek KA, Slovis NM, et al. Validation of IgG cut-off values and their association with survival in neonatal foals. *Equine Vet J*. 2015;47:526–530.
455. Leblanc MM. Immunologic considerations. In: Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. 1st ed. Philadelphia: Lea and Febiger; 1990:275–295.
456. Clabough DL, Conboy HS, Roberts MC. Comparison of four screening techniques for the diagnosis of equine neonatal hypogammaglobulinemia. *J Am Vet Med Assoc*. 1989;194: 1717–1720.
457. McCue PM. Evaluation of a turbidimetric immunoassay for measurement of plasma IgG concentration in foals. *Am J Vet Res*. 2007;68:1005–1009.
458. Davis DG, Schaefer DM, Hinchcliff KW, et al. Measurement of serum IgG in foals by radial immunodiffusion and automated turbidimetric immunoassay. *J Vet Intern Med*. 2005;19:93–96.
459. Riley CB, McClure JT, Low-Ying S, et al. Use of Fourier-transform infrared spectroscopy for the diagnosis of failure of transfer of passive immunity and measurement of immunoglobulin concentrations in horses. *J Vet Intern Med*. 2007;21:828–834.
460. Pusterla N, Pusterla JB, Spier SJ, et al. Evaluation of the SNAP foal IgG test for the semiquantitative measurement of immunoglobulin G in foals. *Vet Rec*. 2002;151:258–260.
461. Davis R, Giguere S. Evaluation of five commercially available assays and measurement of serum total protein concentration via refractometry for the diagnosis of failure of passive transfer of immunity in foals. *J Am Vet Med Assoc*. 2005;227:1640–1645.
462. Edwards DF, Parker JW, Wilkinson JE, et al. Plasma cell myeloma in the horse. A case report and literature review. [Review]. *J Vet Intern Med*. 1993;7:169–176.
463. Shin EK, Perryman LE, Meek K. Evaluation of a test for identification of Arabian horses heterozygous for the severe combined immunodeficiency trait. *J Am Vet Med Assoc*. 1997;211:1268–1270.
464. Fraser DG, Mealey RH, McGuire TC. Selecting peptides to optimize Th1 responses to an equine lentivirus using HLA-DR binding motifs and defined HIV-1 Th peptides. *Immunogenetics*. 2003;55:508–514.
465. Yu Y, Arora A, Min W, et al. U incorporation is an alternative non-radioactive assay to [(3)H]thymidine uptake for in vitro measurement of mice T-cell proliferations. *J Immunol Methods*. 2009;350:29–35.
466. McClure JT, Lunn DP, McGuirk SM. Combined immunodeficiency in 3 foals. *Equine Vet Educ*. 1993;5:14–18.
467. Hutchison JM, Garry FB, Belknap EB, et al. Prospective characterization of the clinicopathologic and immunologic features of an immunodeficiency syndrome affecting juvenile llamas. *Vet Immunol Immunopathol*. 1995;49:209–227.
468. Slack JA, Risdahl JM, Valberg S, et al. Effects of corticosteroids on equine IgG sub-isotype responses to vaccination. *Am J Vet Res*. 1997;61:1530–1533.
469. Flaminio MJ, Rush BR, Shuman W. Immunologic function in horses after non-specific immunostimulant administration. *Vet Immunol Immunopathol*. 1998;63:303–315.
470. Zink MC, Yager JA, Prescott JF, et al. In vitro phagocytosis and killing of *Corynebacterium equi* by alveolar macrophages of foals. *Am J Vet Res*. 1985;46:2171–2174.
471. Flaminio MJ, Rush BR, Davis EG, et al. Simultaneous flow cytometric analysis of phagocytosis and oxidative burst activity in equine leukocytes. *Vet Res Commun*. 2002;26:85–92.
472. Hormanski CE, Truax R, Pourciau SS, et al. Induction of lymphokine-activated killer cells of equine origin: specificity for equine target cells. *Vet Immunol Immunopathol*. 1992;32:25–36.

473. Lunn DP, Schram BR, Vagnoni KE, et al. Positive selection of EqCD8+ precursors increases equine lymphokine-activated killing. *Vet Immunol Immunopathol*. 1996;53:1–13.
474. Liu C, Betancourt A, Cohen DA, et al. Granzyme B-mRNA expression by equine lymphokine activated killer (LAK) cells is associated with the induction of apoptosis in target cells. *Vet Immunol Immunopathol*. 2011;143:108–115.
475. Canisso IF, Ball BA, Cray C, et al. Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. *Am J Reprod Immunol*. 2014;72:376–385.
476. Hooijberg EH, van den Hoven R, Tichy A, et al. Diagnostic and predictive capability of routine laboratory tests for the diagnosis and staging of equine inflammatory disease. *J Vet Intern Med*. 2014;28:1587–1593.
477. Leclere M, Lavoie-Lamoureux A, Lavoie JP. Acute phase proteins in racehorses with inflammatory airway disease. *J Vet Intern Med*. 2015;29:940–945.
478. Passamonti F, Vardi DM, Stefanetti V, et al. *Rhodococcus equi* pneumonia in foals: an assessment of the early diagnostic value of serum amyloid A and plasma fibrinogen concentrations in equine clinical practice. *Vet J*. 2015;203:211–218.
479. Pihl TH, Scheepers E, Sanz M, et al. Influence of disease process and duration on acute phase proteins in serum and peritoneal fluid of horses with colic. *J Vet Intern Med*. 2015;29:651–658.
480. Zabrecky KA, Slovis NM, Constable PD, et al. Plasma C-reactive protein and haptoglobin concentrations in critically ill neonatal foals. *J Vet Intern Med*. 2015;29:673–677.
481. Reis KJ. A hemolytic assay for the measurement of equine complement. *Vet Immunol Immunopathol*. 1989;23:129–137.
482. Grondahl G, Johannisson A, Jensen-Waern M, et al. Opsonization of yeast cells with equine iC3b, C3b, and IgG. *Vet Immunol Immunopathol*. 2001;80:209–223.
483. McGuire TC, Poppie MJ. Hypogammaglobulinemia and thymic hypoplasia in horses: a primary combined immunodeficiency disorder. *Infect Immun*. 1973;8:272–277.
484. Perryman LE. Molecular pathology of severe combined immunodeficiency in mice, horses, and dogs. *Veterinary pathology*. 2004;41:95–100.
485. Perryman LE, Torbeck RL. Combined immunodeficiency of Arabian horses: confirmation of autosomal recessive mode of inheritance. *J Am Vet Med Assoc*. 1980;176:1250–1251.
486. Wiler R, Leber R, Moore BB, et al. Equine severe combined immunodeficiency: a defect in V(D)J recombination and DNA-dependent protein kinase activity. In: *Proceedings of the National Academy of Sciences of the United States of America*. ;92. ; 1995:11485–11489.
487. Poppie MJ, McGuire TC. Combined immunodeficiency in foals of Arabian breeding: evaluation of mode of inheritance and estimation of prevalence of affected foals and carrier mares and stallions. *J Am Vet Med Assoc*. 1977;170:31–33.
488. Bernoco D, Bailey E. Frequency of the SCID gene among Arabian horses in the USA. *Anim Genet*. 1998;29:41–42.
489. Ding Q, Bramble L, Yuzbasiyan-Gurkan V, et al. DNA-PKcs mutations in dogs and horses: allele frequency and association with neoplasia. *Gene*. 2002;283:263–269.
490. Perryman LE, McGuire TC, Crawford TB. Maintenance of foals with combined immunodeficiency: causes and control of secondary infections. *Am J Vet Res*. 1978;39:1043–1047.
491. Bjorneby JM, Leach DR, Perryman LE. Persistent cryptosporidiosis in horses with severe combined immunodeficiency. *Infect Immun*. 1991;59:3823–3826.
492. Bue CM, Davis WC, Magnuson NS, et al. Correction of equine severe combined immunodeficiency by bone marrow transplantation. *Transplantation*. 1986;42:14–19.
493. Perryman LE, Bue CM, Magnuson NS, et al. Immunologic reconstitution of foals with combined immunodeficiency. *Vet Immunol Immunopathol*. 1987;17:495–508.
494. Holliman A, Scholes SP. Possible immune deficiency in Fell ponies. *Vet Rec*. 1995;137:176.
495. Scholes SF, Holliman A, May PD, et al. A syndrome of anaemia, immunodeficiency and peripheral ganglionopathy in Fell pony foals. *Vet Rec*. 1998;142:128–134.
496. Fox-Clipsham L, Swinburne JE, Papoula-Pereira RI, et al. Immunodeficiency/anaemia syndrome in a Dales pony. *Vet Rec*. 2009;165:289–290.
497. Fox-Clipsham LY, Brown EE, Carter SD, et al. Population screening of endangered horse breeds for the foal immunodeficiency syndrome mutation. *Vet Rec*. 2011;169:655.
498. Fox-Clipsham LY, Carter SD, Goodhead I, et al. Identification of a mutation associated with fatal Foal Immunodeficiency Syndrome in the Fell and Dales pony. *PLoS genetics*. 2011;7:e1002133.
499. Butler CM, Westermann CM, Koeman JP, et al. The Fell pony immunodeficiency syndrome also occurs in the Netherlands: a review and six cases. *Tijdschr Diergeneeskd*. 2006;131:114–118.
500. Gardner RB, Hart KA, Stokol T, et al. Fell Pony syndrome in a pony in North America. *J Vet Intern Med*. 2006;20:198–203.
501. Richards AJ, Kelly DF, Knottenbelt DC, et al. Anaemia, diarrhoea and opportunistic infections in Fell ponies. *Equine Vet J*. 2000;32:386–391.
502. Tallmadge RL, Stokol T, Gould-Earley MJ, et al. Fell Pony syndrome: characterization of developmental hematopoiesis failure and associated gene expression profiles. *Clin Vaccine Immunol*. 2012;19:1054–1064.
503. Thomas GW, Bell SC, Carter SD. Immunoglobulin and peripheral B-lymphocyte concentrations in Fell pony foal syndrome. *Equine Vet J*. 2005;37:48–52.
504. Thomas GW, Bell SC, Phythian C, et al. Aid to the antemortem diagnosis of Fell pony foal syndrome by the analysis of B lymphocytes. *Vet Rec*. 2003;152:618–621.
505. Bell SC, Savidge C, Taylor P, et al. An immunodeficiency in Fell ponies: a preliminary study into cellular responses. *Equine Vet J*. 2001;33:687–692.
506. Carter SD, Fox-Clipsham LY, Christley R, et al. Foal immunodeficiency syndrome: carrier testing has markedly reduced disease incidence. *Vet Rec*. 2013;172:398.
507. Pellegrini-Masini A, Bentz AI, Johns IC, et al. Common variable immunodeficiency in three horses with presumptive bacterial meningitis. *J Am Vet Med Assoc*. 2005;227:114–122. 187.
508. Flaminio MJ, LaCombe V, Kohn CW, et al. Common variable immunodeficiency in a horse. *J Am Vet Med Assoc*. 2002;221:1296–1302.
509. Flaminio MJ, Tallmadge RL, Salles-Gomes CO, et al. Common variable immunodeficiency in horses is characterized by B cell depletion in primary and secondary lymphoid tissues. *J Clin Immunol*. 2009;29:107–116.
510. Tallmadge RL, Shen L, Tseng CT, et al. Bone marrow transcriptome and epigenome profiles of equine common variable immunodeficiency patients unveil block of B lymphocyte differentiation. *Clin Immunol*. 2015;160:261–276.
511. Tallmadge RL, Such KA, Miller KC, et al. Expression of essential B cell development genes in horses with common variable immunodeficiency. *Mol Immunol*. 2012;51:169–176.
512. Perryman LE, McGuire TC, Hilbert BJ. Selective immunoglobulin M deficiency in foals. *J Am Vet Med Assoc*. 1977;170:212–215.
513. Weldon AD, Zhang C, Antczak DF, et al. Selective IgM deficiency and abnormal B-cell response in a foal. *J Am Vet Med Assoc*. 1992;201:1396–1398.
514. Perryman LE, McGuire TC. Evaluation for immune system failures in horses and ponies. *J Am Vet Med Assoc*. 1980;176:1374–1377.
515. Louis AG, Gupta S. Primary selective IgM deficiency: an ignored immunodeficiency. *Clin Rev Allergy Immunol*. 2014;46:104–111.
516. Perkins GA, Nydam DV, Flaminio MJ, et al. Serum IgM concentrations in normal, fit horses and horses with lymphoma or other medical conditions. *J Vet Intern Med*. 2003;17:337–342.
517. Boy MG, Zhang C, Antczak DF, et al. Unusual selective immunoglobulin deficiency in an arabian foal. *J Vet Intern Med*. 1992;6:201–205.

518. McGuire TC, Poppie MJ, Banks KL. Hypogammaglobulinemia predisposing to infection in foals. *J Am Vet Med Assoc*. 1975;166:71–75.
519. Deem DA, Traver DS, Thacker HL, et al. Agammaglobulinaemia in a horse. *J Am Vet Med Assoc*. 1979;175:469–472.
520. Banks KL, McGuire TC, Jerrells R. Absence of B lymphocytes in a horse with primary agammaglobulinaemia. *Clin Immunol Immunopathol*. 1976;5:282–290.
521. Vetrie D, Vorechovsky I, Sideras P, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature*. 1993;361:226–233.
522. McGuire TC, Crawford TB, Hallowell AL, et al. Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. *J Am Vet Med Assoc*. 1977;170:1302–1304.
523. Koterba AM, Brewer BD, Tarplee FA. Clinical and clinicopathological characteristics of the septicaemic neonatal foal: review of 38 cases. *Equine Vet J*. 1984;16:376–382.
524. Rouse BT, Ingram DG. The total protein and immunoglobulin profile of equine colostrum and milk. *Immunology*. 1970;19:901–907.
525. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol*. 2007;7:715–725.
526. Cervenak J, Kacskovics I. The neonatal Fc receptor plays a crucial role in the metabolism of IgG in livestock animals. *Vet Immunol Immunopathol*. 2009;128:171–177.
527. Kacskovics I. Fc receptors in livestock species. *Vet Immunol Immunopathol*. 2004;102:351–362.
528. Mayer B, Doleschall M, Bender B, et al. Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. *J Dairy Res*. 2005;72:107–112. Spec No.
529. Jeffcott LB. Duration of permeability of the intestine to macromolecules in the newly-born foal. *Vet Rec*. 1971;88:340–341.
530. Jeffcott LB. Passive immunity and its transfer with special reference to the horse. *Biol Rev Camb Philos Soc*. 1972;47:439–464.
531. Perkins GA, Goodman LB, Wimer C, et al. Maternal T-lymphocytes in equine colostrum express a primarily inflammatory phenotype. *Vet Immunol Immunopathol*. 2014;161:141–150.
532. Morris DD, Meirs DA, Merryman GS. Passive transfer failure in horses: incidence and causative factors on a breeding farm. *Am J Vet Res*. 1985;46:2294–2299.
533. McClure JT, DeLuca JL, Miller J. Comparison of five screening tests for detection of failure of passive transfer in foals. *J Vet Intern Med: ACVIM 20th Annual Veterinary Medical Forum Abstract Program*. 2002;16:336.
534. LeBlanc MM, Tran T, Baldwin JL, et al. Factors that influence passive transfer of immunoglobulins in foals. *J Am Vet Med Assoc*. 1992;200:179–183.
535. Le Jan C. Cellular components of mammary secretions and neonatal immunity: a review. *Vet Res*. 1996;27:403–417.
536. Xu RJ. Development of the newborn GI tract and its relation to colostrum/milk intake: a review. *Reprod Fertil Dev*. 1996;8:35–48.
537. Pearson RC, Hallowell AL, Bayly WM, et al. Times of appearance and disappearance of colostral IgG in the mare. *Am J Vet Res*. 1984;45:186–190.
538. Townsend HG, Tabel H, Bristol FM. Induction of parturition in mares: effect on passive transfer of immunity to foals. *J Am Vet Med Assoc*. 1983;182:255–257.
539. Lavoie JP, Spensley MS, Smith BP, et al. Colostral volume and immunoglobulin G and M determinations in mares. *Am J Vet Res*. 1989;50:466–470.
540. Knottenbelt D, Holdstock N, Madigan J. *Equine Neonatology Medicine and Surgery*. Philadelphia, PA: Elsevier Saunders; 2004.
541. Rumbaugh GE, Ardans AA, Ginno D, et al. Measurement of neonatal equine immunoglobulins for assessment of colostral immunoglobulin transfer: comparison of single radial immunodiffusion with the zinc sulfate turbidity test, serum electrophoresis, refractometry for total serum protein, and the sodium sulfite precipitation test. *J Am Vet Med Assoc*. 1978;172:321–325.
542. Korosue K, Murase H, Sato F, et al. Correlation of serum IgG concentration in foals and refractometry index of the dam's pre- and post-parturient colostrums: an assessment for failure of passive transfer in foals. *J Vet Med Sci*. 2012;74:1387–1395.
543. Hines MT, Schott 2nd HC, Bayly WM, et al. Exercise and immunity: a review with emphasis on the horse. *J Vet Intern Med*. 1996;10:280–289.
544. Horohov DW, Dimock A, Guirnalda P, et al. Effect of exercise on the immune response of young and old horses. *Am J Vet Res*. 1999;60:643–647.
545. Horohov DW, Keadle TL, Pourciau SS, et al. Mechanism of exercise-induced augmentation of lymphokine activated killer (LAK) cell activity in the horse. *Vet Immunol Immunopathol*. 1996;53:221–233.
546. Nesse LL, Johansen GI, Blom AK. Effects of racing on lymphocyte proliferation in horses. *Am J Vet Res*. 2002;63:528–530.
547. Raidal SL, Rose RJ, Love DN. Effects of training on resting peripheral blood and BAL-derived leucocyte function in horses. *Equine Vet J*. 2001;33:238–243.
548. Wong CW, Smith SE, Thong YH, et al. Effects of exercise stress on various immune functions in horses. *Am J Vet Res*. 1992;53:1414–1417.
549. Wong CW, Thompson HL, Thong YH, et al. Effect of strenuous exercise stress on chemiluminescence response of equine alveolar macrophages. *Equine Vet J*. 1990;22:33–35.
550. Robson PJ, Alston TD, Myburgh KH. Prolonged suppression of the innate immune system in the horse following an 80 km endurance race. *Equine Vet J*. 2003;35:133–137.
551. Liburt NR, Adams AA, Betancourt A, et al. Exercise-induced increases in inflammatory cytokines in muscle and blood of horses. *Equine Vet J Suppl*. 2010:280–288.
552. Donovan DC, Jackson CA, Colahan PT, et al. Assessment of exercise-induced alterations in neutrophil function in horses. *Am J Vet Res*. 2007;68:1198–1204.
553. Folsom RW, Littlefield-Chabaud MA, French DD, et al. Exercise alters the immune response to equine influenza virus and increases susceptibility to infection. *Equine Vet J*. 2001;33:664–669.
554. Gross DK, Hinchcliff KW, French PS, et al. Effect of moderate exercise on the severity of clinical signs associated with influenza virus infection in horses. *Equine Vet J*. 1998;30:489–497.
555. Horohov DW. Is exercise bad for the immune system? *Equine Vet J*. 2003;35:113–116.
556. Hoffman AM, Viel L, Prescott JF. Microbiologic changes during antimicrobial treatment and rate of relapse of distal respiratory tract infections in foals. *Am J Vet Res*. 1993;54:1608–1614.
557. Hansen S, Baptiste KE, Fjeldborg J, et al. A review of the equine age-related changes in the immune system: comparisons between human and equine aging, with focus on lung-specific immune-aging. *Ageing Res Rev*. 2015;20:11–23.
558. Horohov DW, Adams AA, Chambers TM. Immunosenescence of the equine immune system. *J Comp Pathol*. 2010;142(suppl 1):S78–S84.
559. Boraschi D, Italiani P. Immunosenescence and vaccine failure in the elderly: strategies for improving response. *Immunol Lett*. 2014;162:346–353.
560. Fulop T, Le Page A, Fortin C, et al. Cellular signaling in the aging immune system. *Curr Opin Immunol*. 2014;29:105–111.
561. Horohov DW, Kydd JH, Hannant D. The effect of aging on T cell responses in the horse. *Dev Comp Immunol*. 2002;26:121–128.
562. McFarlane D, Sellon DC, Gibbs SA. Age-related quantitative alterations in lymphocyte subsets and immunoglobulin isotypes in healthy horses. *Am J Vet Res*. 2001;62:1413–1417.
563. Adams AA, Breathnach CC, Katepalli MP, et al. Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. *Mech Ageing Dev*. 2008;129:656–664.
564. Adams AA, Katepalli MP, Kohler K, et al. Effect of body condition, body weight and adiposity on inflammatory cytokine responses in old horses. *Vet Immunol Immunopathol*. 2009;127:286–294.

565. Hansen S, Sun L, Baptiste KE, et al. Age-related changes in intracellular expression of IFN-gamma and TNF-alpha in equine lymphocytes measured in bronchoalveolar lavage and peripheral blood. *Dev Comp Immunol*. 2013;39:228–233.
566. Hansen S, Baptiste KE, Fjeldborg J, et al. A comparison of pro-inflammatory cytokine mRNA expression in equine bronchoalveolar lavage (BAL) and peripheral blood. *Vet Immunol Immunopathol*. 2014;158:238–243.
567. Franzke B, Neubauer O, Wagner KH. Super DNAging-New insights into DNA integrity, genome stability and telomeres in the oldest old. *Mutat Res Rev Mutat Res*. 2015;766:48–57.
568. Katepalli MP, Adams AA, Lear TL, et al. The effect of age and telomere length on immune function in the horse. *Dev Comp Immunol*. 2008;32:1409–1415.
569. Muirhead TL, McClure JT, Wichtel JJ, et al. The effect of age on serum antibody titers after rabies and influenza vaccination in healthy horses. *J Vet Intern Med*. 2008;22:654–661.
570. Adams AA, Sturgill TL, Breathnach CC, et al. Humoral and cell-mediated immune responses of old horses following recombinant canarypox virus vaccination and subsequent challenge infection. *Vet Immunol Immunopathol*. 2011;139:128–140.
571. Perryman LE, Wyatt CR, Magnuson NS. Biochemical and functional characterization of lymphocytes from a horse with lymphosarcoma and IgM deficiency. *Comp Immunol Microbiol Infect Dis*. 1984;7:53–62.
572. Furr MO, Crisman MV, Robertson J, et al. Immunodeficiency associated with lymphosarcoma in a horse. *J Am Vet Med Assoc*. 1992;201:307–309.
573. Dopson LC, Reed SM, Roth JA, et al. Immunosuppression associated with lymphosarcoma in two horses. *J Am Vet Med Assoc*. 1983;182:1239–1241.
574. Buechner-Maxwell V, Zhang C, Robertson J, et al. Intravascular leukostasis and systemic aspergillosis in a horse with subleukemic acute myelomonocytic leukemia. *J Vet Intern Med*. 1994;8:258–263.
575. Tumas DB, Hines MT, Perryman LE, et al. Corticosteroid immunosuppression and monoclonal antibody-mediated CD5+ T lymphocyte depletion in normal and equine infectious anaemia virus-carrier horses. *J Gen Virol*. 1994;75(Pt 5):959–968.
576. Kono Y, Hirasawa K, Fukunaga Y, et al. Recrudescence of equine infectious anemia by treatment with immunosuppressive drugs. *Natl Inst Anim Health Q (Tokyo)*. 1976;16:8–15.
577. Craigo JK, Leroux C, Howe L, et al. Transient immune suppression of inapparent carriers infected with a principal neutralizing domain-deficient equine infectious anaemia virus induces neutralizing antibodies and lowers steady-state virus replication. *J Gen Virol*. 2002;83:1353–1359.
578. Gibson JS, Slater JD, Field HJ. The pathogenicity of Ab4p, the sequenced strain of equine herpesvirus-1, in specific pathogen-free foals. *Virology*. 1992;189:317–319.
579. Mair TS. Bacterial pneumonia associated with corticosteroid therapy in three horses. *Vet Rec*. 1996;138:205–207.
580. DeKruyff RH, Fang Y, Umetsu DT. Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in CD4+ lymphocytes by inhibiting IL-12 production. *J Immunol*. 1998;160:2231–2237.
581. Ramierz F, Fowell DJ, Puklavec M, et al. Glucocorticoids promote a TH2 cytokine response by CD4+ T cells in vitro. *J Immunol*. 1996;156:2406–2412.
582. Allen GP, Kydd JH, Slater JD, et al. Advances in understanding of the pathogenesis, epidemiology, and immunological control of equid herpesvirus) abortion. In: Wernery U, Wade JF, Mumford JA, Kaaden O-R, eds. *Equine infectious diseases VIII. Proceedings of the Eighth International Conference*. Dubai: 23rd-26th March, 1998, 1 ed. Newmarket: R & W Publications; 1999:129–146.
583. Bryans JT, Allen GP. Herpesviral diseases of the horse. In: Wittmann G, ed. *Herpesvirus diseases of cattle, horses, and pigs*. Boston: Kluwer Academic Publishers; 1989:176–229.
584. Newman MJ, Issel CJ, Truax RE, et al. Transient suppression of equine immune responses by equine infectious anemia virus (EIAV). *Virology*. 1991;184:55–66.
585. Murakami K, Sentsui H, Shibahara T, et al. Reduction of CD4+ and CD8+ T lymphocytes during febrile periods in horses experimentally infected with equine infectious anemia virus. *Vet Immunol Immunopathol*. 1999;67:131–140.
586. McClure JJ, Addison JD, Miller RI. Immunodeficiency manifested by oral candidiasis and bacterial septicemia in foals. *J Am Vet Med Assoc*. 1985;186:1195–1197.
587. Freestone JF, Hietala S, Moulton J, et al. Acquired immunodeficiency in a seven-year-old horse. *J Am Vet Med Assoc*. 1987;190:689–691.
588. Wilckens T, De Rijk R. Glucocorticoids and immune function: unknown dimensions and new frontiers. [Review] [74 refs]. *Immunol Today*. 1997;18:418–424.
589. Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. *J Immunol*. 1997;158:5589–5595.
590. Franchimont D, Louis E, Dewe W, et al. Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures. *Regul Pept*. 1998;73:59–65.
591. Morris DD, Strzemienski PJ, Gaulin G, et al. The effects of corticosteroid administration on the migration, phagocytosis and bactericidal capacity of equine neutrophils. *Cornell Vet*. 1988;78:243–252.
592. Burton JL, Kehrli Jr ME, Kapil S, et al. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. *J Leukoc Biol*. 1995;57:317–325.
593. Filippe M. Immunosupressive therapy. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
594. Gibson JS, Slater JD, Awan AR, et al. Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation. *Arch Virol*. 1992;123:351–366.
595. Bathe AP. The corticosteroid laminitis story: 3. The clinician's viewpoint. *Equine Vet J*. 2007;39:12–13.
596. Bailey SR, Elliott J. The corticosteroid laminitis story: 2. Science of if, when and how. *Equine Vet J*. 2007;39:7–11.
597. Dutton H. The corticosteroid laminitis story: 1. Duty of care. *Equine Vet J*. 2007;39:5–6.
598. Johnson PJ, Slight SH, Ganjam VK, et al. Glucocorticoids and laminitis in the horse. *Vet Clin North Am Equine Pract*. 2002;18:219–236.
599. Saulez MN, Schlipf Jr JW, Cebra CK, et al. Use of chemotherapy for treatment of a mixed-cell thoracic lymphoma in a horse. *J Am Vet Med Assoc*. 2004;224:733–738. 699.
600. Messer NTt, Arnold K. Immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc*. 1991;198:1415–1416.
601. Humber KA, Beech J, Cudd TA, et al. Azathioprine for treatment of immune-mediated thrombocytopenia in two horses. *J Am Vet Med Assoc*. 1991;199:591–594.
602. McGurrin MK, Arroyo LG, Bienzle D. Flow cytometric detection of platelet-bound antibody in three horses with immune-mediated thrombocytopenia. *J Am Vet Med Assoc*. 2004;224:83–87. 53.
603. Gratzek AT, Kaswan RL, Martin CL, et al. Ophthalmic cyclosporine in equine keratitis and keratouveitis: 11 cases. *Equine Vet J*. 1995;27:327–333.
604. Gilger BC, Wilkie DA, Davidson MG, et al. Use of an intravitreal sustained-release cyclosporine delivery device for treatment of equine recurrent uveitis. *Am J Vet Res*. 2001;62:1892–1896.
605. Gilger BC, Michau TM, Salmon JH. Immune-mediated keratitis in horses: 19 cases (1998–2004). *Vet Ophthalmol*. 2005;8:233–239.

606. Gilger BC, Malok E, Stewart T, et al. Effect of an intravitreal cyclosporine implant on experimental uveitis in horses. *Vet Immunol Immunopathol*. 2000;76:239–255.
607. Gilger BC, Wilkie DA, Clode AB, et al. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. *Vet Ophthalmol*. 2010;13:294–300.
608. Moore BR. Clinical application of interferons in large animal medicine. *J Am Vet Med Assoc*. 1996;208:1711–1715.
609. Rush BR, Flaminio MJ. Immunomodulation in horses. *Vet Clin North Am Equine Pract*. 2000;16:183–197. viii.
610. Davis E. Immunomodulators. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
611. Moore BR, Krakowka S, Robertson JT, et al. Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. *Am J Vet Res*. 1995;56:562–567.
612. Moore BR, Krakowka S, Cummins JM, et al. Changes in airway inflammatory cell populations in standardbred racehorses after interferon-alpha administration. *Vet Immunol Immunopathol*. 1996;49:347–358.
613. Moore BR, Krakowka S, McVey DS, et al. Inflammatory markers in bronchoalveolar lavage fluid of standardbred racehorses with inflammatory airway disease: response to interferon-alpha. *Equine Vet J*. 1997;29:142–147.
614. Moore I, Horney B, Day K, et al. Treatment of inflammatory airway disease in young Standardbreds with interferon alpha. *Can Vet J*. 2004;45:594–601.
615. Zinkl JG, Madigan JE, Fridmann DM, et al. Haematological, bone marrow and clinical chemical changes in neonatal foals given canine recombinant granulocyte-colony stimulating factor. *Equine Vet J*. 1994;26:313–318.
616. Madigan JE, Zinkl JG, Fridmann DM, et al. Preliminary studies of recombinant bovine granulocyte-colony stimulating factor on haematological values in normal neonatal foals. *Equine Vet J*. 1994;26:159–161.
617. Wong DM, Alcott CJ, Clark SK, et al. Alloimmune neonatal neutropenia and neonatal isoerythrolysis in a Thoroughbred colt. *J Vet Diagn Invest*. 2012;24:219–226.
618. Davis EG, Rush B, Bain F, et al. Neonatal neutropenia in an Arabian foal. *Equine Vet J*. 2003;35:517–520.
619. Rush BR, Lunn DP. Immunomodulation in horses: indications and preparations. In: *Proceeding of the 50th Annual Convention of the American Association of Equine Practitioners*. Denver: Colorado; 2004:454–458.
620. Paillot R. A systematic review of the immune-modulators *Parapoxvirus ovis* and *Propionibacterium acnes* for the prevention of respiratory disease and other infections in the horse. *Vet Immunol Immunopathol*. 2013;153:1–9.
621. Audibert FM, Lise LD. Adjuvants: current status, clinical perspectives and future prospects. *Trends Pharmacol Sci*. 1993;14:174–178.
622. Fumuso E, Giguere S, Wade J, et al. Endometrial IL-1beta, IL-6 and TNF-alpha, mRNA expression in mares resistant or susceptible to post-breeding endometritis. Effects of estrous cycle, artificial insemination and immunomodulation. *Vet Immunol Immunopathol*. 2003;96:31–41.
623. Bergvall KE. Sarcoids. *Vet Clin North Am Equine Pract*. 2013;29:657–671.
624. Davis EG, Rush BR, Blecha F. Increases in cytokine and antimicrobial peptide gene expression in horses by immunomodulation with Propionibacterium acnes. *Vet Ther*. 2003;4:5–11.
625. Sturgill TL, Strong D, Rashid C, et al. Effect of Propionibacterium acnes-containing immunostimulant on interferon-gamma (IFNgamma) production in the neonatal foal. *Vet Immunol Immunopathol*. 2011;141:124–127.
626. Ryan C, Giguere S, Fultz L, et al. Effects of two commercially available immunostimulants on leukocyte function of foals following ex vivo exposure to *Rhodococcus equi*. *Vet Immunol Immunopathol*. 2010;138:198–205.
627. Ziebell KL, Steinmann H, Kretzdorn D, et al. The use of Baypamun N in crowding associated infectious respiratory disease: efficacy of Baypamun N (freeze dried product) in 4-10 month old horses. *Zentralbl Veterinarmedizin Reihe B J Vet Med Series B*. 1997;44:529–536.
628. Adams AA, Horohov DW. The effect of an immunomodulator (parapoxvirus ovis) on cell-mediated immunity (CMI) in abruptly weaned foals. *Vet Immunol Immunopathol*. 2013;153:118–122.
629. Lunn DP, Rush BR. Immunomodulation: Principles and Mechanisms. In: *Proceeding of the 50th Annual Convention of the American Association of Equine Practitioners*. Denver: Colorado; 2004:447–453.
630. Ons E, Van Brussel L, Lane S, et al. Efficacy of a *Parapoxvirus ovis*-based immunomodulator against equine herpesvirus type 1 and *Streptococcus equi equi* infections in horses. *Vet Microbiol*. 2014;173:232–240.
631. Horohov DW, Breathnach CC, Sturgill TL, et al. In vitro and in vivo modulation of the equine immune response by parapoxvirus ovis. *Equine Vet J*. 2008;40:468–472.
632. Sturgill TL, Giguere S, Franklin RP, et al. Effects of inactivated parapoxvirus ovis on the cumulative incidence of pneumonia and cytokine secretion in foals on a farm with endemic infections caused by *Rhodococcus equi*. *Vet Immunol Immunopathol*. 2011;140:237–243.
633. Studer U, Marti E, Stornetta D, et al. [The therapy of equine sarcoid with a non-specific immunostimulator—the epidemiology and spontaneous regression of sarcoids]. Schweizer Archiv fur Tierheilkunde|Sat. *Schweiz Arch Tierheilkd*. 1997;139:385–391.
634. Hacker G, Redecke V, Hacker H. Activation of the immune system by bacterial CpG-DNA. *Immunology*. 2002;105:245–251.
635. Rankin R, Pontarollo R, Ioannou X, et al. CpG motif identification for veterinary and laboratory species demonstrates that sequence recognition is highly conserved. *Antisense Nucleic Acid Drug Dev*. 2001;11:333–340.
636. Sato Y, Roman M, Tighe H, et al. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science*. 1996;273:352–354.
637. Whitmore MM, Li S, Falo Jr L, et al. Systemic administration of LPD prepared with CpG oligonucleotides inhibits the growth of established pulmonary metastases by stimulating innate and acquired antitumor immune responses. *Cancer Immunol Immunother*. 2001;50:503–514.
638. Lopez AM, Hecker R, Mutwiri G, et al. Formulation with CpG ODN enhances antibody responses to an equine influenza virus vaccine. *Vet Immunol Immunopathol*. 2006;114:103–110.
639. Bordin AI, Liu M, Nerren JR, et al. Neutrophil function of neonatal foals is enhanced in vitro by CpG oligodeoxynucleotide stimulation. *Vet Immunol Immunopathol*. 2012;145:290–297.
640. Liu M, Liu T, Bordin A, et al. Activation of foal neutrophils at different ages by CpG oligodeoxynucleotides and *Rhodococcus equi*. *Cytokine*. 2009;48:280–289.
641. Cohen ND, Bourquin JR, Bordin AI, et al. Intramuscular administration of a synthetic CpG-oligodeoxynucleotide modulates functional responses of neutrophils of neonatal foals. *PloS one*. 2014;9:e109865.
642. Babiuk LA, Misra V. Levamisole and bovine immunity: in vitro and in vivo effects on immune responses to herpesvirus immunization. *Can J Microbiol*. 1981;27:1312–1319.
643. Roth JA, Kaeberle ML. Effect of levamisole on lymphocyte blastogenesis and neutrophil function in dexamethasone-treated cattle. *Am J Vet Res*. 1984;45:1781–1784.
644. Lunn DP, Townsend HGG. Equine vaccination. *Vet Clin North Am Equine Pract*. 2000;16:199–226.
645. Bordin A, Cohen N. Types of vaccines. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.
646. Cohen N, Bordin A. Principles of vaccination. In: Filippe M, ed. *Equine Clinical Immunology*. Oxford, UK: John Wiley & Sons, Inc.; 2016.

647. Wilson W, Pusturla N, Long M. Immunoprophylaxis. In: Sellon D, Long M, eds. *Equine Infectious Diseases*. 2nd ed. Philadelphia, PA: Saunders Elsevier; 2014.
648. Horohov DW, Dunham J, Liu C, et al. Characterization of the in situ immunological responses to vaccine adjuvants. *Vet Immunol Immunopathol*. 2015;164:24–29.
649. Savage CJ. Diseases of the liver. In: Moore J, ed. *Equine Medicine and Surgery*. 5th ed. St. Louis: Mosby Inc; 1999:816–833.
650. Ramsay JD, Evanoff R, Wilkinson Jr TE, et al. Experimental transmission of equine hepacivirus in horses as a model for hepatitis C virus. *Hepatology (Baltimore, Md)*. 2015;61:1533–1546.
651. Marti E, Horohov DW, Antzak DF, et al. Advances in equine immunology: Havemeyer workshop reports from Santa Fe, New Mexico, and Hortobagy, Hungary. *Vet Immunol Immunopathol*. 2003;91:233–243.
652. Crameri R. Allergy vaccines: dreams and reality. *Expert Rev Vaccines*. 2007;6:991–999.
653. Jose-Cunilleras E, Kohn CW, Hillier A, et al. Intradermal testing in healthy horses and horses with chronic obstructive pulmonary disease, recurrent urticaria, or allergic dermatitis. *J Am Vet Med Assoc*. 2001;219:1115–1121.
654. Barbet JL, Bevier D, Greiner EC. Specific immunotherapy in the treatment of *Culicoides* hypersensitive horses: a double-blind study. *Equine Vet J*. 1990;22:232–235.
655. Ginel PJ, Hernandez E, Lucena R, et al. Allergen-specific immunotherapy in horses with insect bite hypersensitivity: a double-blind, randomized, placebo-controlled study. *Vet Dermatol*. 2014;25. 29–e10.
656. Peeters LM, Janssens S, Goddeeris BM, et al. Evaluation of an IgE ELISA with Culicoides spp. extracts and recombinant salivary antigens for diagnosis of insect bite hypersensitivity in Warmblood horses. *Vet J*. 2013;198:141–147.
657. van der Meide NM, Savelkoul HF, Meulenbroeks C, et al. Evaluation of a diagnostic ELISA for insect bite hypersensitivity in horses using recombinant Obsoletus complex allergens. *Vet J*. 2014;200:31–37.
658. Marti E, Wang X, Jambari NN, et al. Novel in vitro diagnosis of equine allergies using a protein array and mathematical modelling approach: a proof of concept using insect bite hypersensitivity. *Vet Immunol Immunopathol*. 2015;167:171–177.
659. Stepnik CT, Outerbridge CA, White SD, et al. Equine atopic skin disease and response to allergen-specific immunotherapy: a retrospective study at the University of California–Davis (1991–2008). *Vet Dermatol*. 2012;23:29–35. e27.
660. Jonsdottir S, Hamza E, Janda J, et al. Developing a preventive immunization approach against insect bite hypersensitivity using recombinant allergens: A pilot study. *Vet Immunol Immunopathol*. 2015;166:8–21.
661. Jahn-Schmid B, Wiedermann U, Bohle B, et al. Oligodeoxynucleotides containing CpG motifs modulate the allergic TH2 response of BALB/c mice to Bet v 1, the major birch pollen allergen. *J Allergy Clin Immunol*. 1999;104:1015–1023.
662. Goodman JS, Van Uden JH, Kobayashi H, et al. DNA immunotherapeutics: new potential treatment modalities for allergic disease. *Int Arch Allergy Immunol*. 1998;116:177–187.
663. Broide D, Schwarze J, Tighe H, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol*. 1998;161:7054–7062.
664. Klier J, Lehmann B, Fuchs S, et al. Nanoparticulate CpG immunotherapy in RAO-affected horses: phase I and IIa study. *J Vet Intern Med*. 2015;29:286–293.
665. Behrens NE, Gershwin LJ. Immune modulation of T regulatory cells and IgE responses in horses vaccinated with West Nile virus vaccine combined with a CpG ODN. *Vaccine*. 2015;33:5764–5771.

CHAPTER 2

Pharmacologic Principles

Jennifer L. Davis*

Introduction to Clinical Pharmacology

Drug administration in equine practice relies on selection of a safe and efficacious dosage regimen based on the individual horse's physiology and the nature and formulation of the drug. It is the veterinarian's responsibility to ensure that the selected drug is efficacious with minimal toxicity or adverse reactions in the patient. Individual animals of various ages and species vary widely in their handling of an administered drug. A basic understanding of pharmacokinetics and the effects of pathophysiology on drug disposition in the horse enables the clinician to optimize therapy while minimizing the risk of adverse drug effects.

PHARMACOKINETICS

Pharmacokinetics describes the effect the body has on a drug, including drug absorption and distribution throughout the body, along with metabolism and excretion from the body. Basic pharmacokinetic studies are usually performed in healthy animals. Unfortunately, however, veterinarians do not often administer drugs to normal, healthy animals. Dosage regimens derived from studies in healthy animals may not be accurate for diseased animals. Clinical pharmacokinetics is the study of the effects of disease states or other variables (age, sex, pregnancy) on the pharmacokinetics of drugs. Clinical pharmacokinetics guides veterinarians in adjusting dosage regimens determined in healthy animals to optimize treatment of diseased animals.

PLASMA DRUG CONCENTRATIONS AS THERAPEUTIC GUIDELINES

Most pharmacokinetic information is derived from plasma drug concentrations, even though pharmacologic action depends on drug concentration at a particular effector site, which is often a specific drug receptor. In reality, measuring drug concentrations at the drug receptor site is not practical. Instead, plasma (or serum) drug concentrations are measured and assumed to represent drug concentrations in the target tissues. Most cells in the body are perfused with tissue fluids or plasma, and drug concentrations usually reach equilibrium between tissue fluids and the blood. Therefore for most drugs pharmacologic action correlates well with the plasma drug concentration.

VARIATION BETWEEN DRUG DOSE AND PLASMA DRUG CONCENTRATION

Drug dosages needed for a therapeutic effect differ widely among individuals. The "usual" dose has no effect in some individuals, causes serious toxicity in others, and produces an optimal effect in a few. The relationship between the dosage of a drug and its concentration in plasma is affected by its bioavailability, the animal's body size and fluid composition, variability in drug distribution within the body, and variability in rates of metabolism and excretion. These factors are all influenced by genetic differences in metabolism and excretion, environmental factors, disease alterations in system function, and concurrent administration of other drugs. Therefore the plasma concentration of a drug is not a perfect index of pharmacologic response. However, pharmacologic response is more closely related to plasma drug concentration than to drug dose, and knowledge of plasma drug concentration should always be used in combination with careful medical observation and judgment to determine optimal therapy.

DEFINITIONS IN PHARMACOKINETICS

Pharmacokinetic information is used to determine drug dosage regimens in clinical patients. An understanding of the way in which drug dosage regimens are derived and how they can be adjusted for different disease states requires knowledge of some basic pharmacokinetic terms. Mathematical *models* provide equations to describe drug concentration as a function of time. With an *open model* the drug is eliminated from the body. An open model describes the fate of most drugs. With a *closed model* the drug is recirculated within the body (e.g., a drug that undergoes enterohepatic recirculation). In pharmacokinetic models the body is represented by a series of *compartments* that

* The editors and authors acknowledge and appreciate the contributions of Patricia M. Dowling as a previous contributor to this chapter. Some of her original work has been incorporated into this edition.

communicate reversibly with one another. A *compartment* is a tissue or group of tissues with similar blood flow and drug affinity. A drug is assumed to be uniformly distributed within a compartment and can move dynamically in and out of compartments. Rate constants represent the entry and exit of drugs from each compartment. The *central compartment* is made up of the highly perfused tissues that equilibrate rapidly with the drug. Overall drug elimination occurs mainly from the central compartment, because the kidneys and liver are well-perfused tissues. The *peripheral compartment* is made up of less perfused tissues such as muscle and connective tissues. The *deep compartment* consists of slowly perfused tissues or depot tissues such as fat and bone. The presence of a deep compartment for drug distribution is important for toxins and drug residues. Most drugs in clinical use are described by one- or two-compartment models. Models with more than three compartments are usually not physiologically relevant. Describing drug disposition with compartment models creates differential equations that describe drug concentration changes in each compartment and provides a visual representation of the rate processes among compartments.

RATES AND ORDERS OF REACTIONS

The drug absorption or elimination rate is the speed with which it occurs. If the amount of drug in the body is decreasing over time, the elimination rate is expressed as follows:

$$\Delta C/\Delta t$$

The absorption and elimination rate of a drug is determined experimentally by measuring the plasma drug concentration at given time intervals. *Rate constants* relate the observed rate of a kinetic process to the drug concentration that controls the process. The elimination rate constant *(K)* is equal to the rate of drug elimination divided by the amount of drug in the body. The absorption rate constant (*K*a) describes the rate of drug absorption into the central compartment. *Reaction order* refers to the way that drug concentration influences reaction rate.

With a *zero order reaction* the amount of drug changes at a constant time interval, regardless of the drug concentration. The rate of drug elimination is as follows:

$$\Delta C/\Delta t = -K_0$$

where K_0 is the zero order rate constant in mg/mL/min. A graph of drug concentration versus time on regular graph paper for a zero order reaction produces a straight line (Fig. 2.1), described by the following equation:

$$C = -K_0 t + C_0$$

where C is the drug concentration at any time t, and C_0 is the drug concentration at time zero. For most drugs zero order elimination occurs only when elimination mechanisms become saturated. Renal tubular secretion and bile secretion of drugs are examples of potentially saturable processes. In veterinary medicine, drugs with well-known zero order elimination include phenylbutazone in horses and deracoxib in dogs. Once elimination processes are saturated, increased dosages of such drugs result in wildly unpredictable plasma concentrations and easily result in toxicity.

With a first order reaction, the amount of drug changes at a rate proportional to the amount of drug remaining. The first order elimination rate is expressed as follows:

$$\Delta C/\Delta t = -KC$$

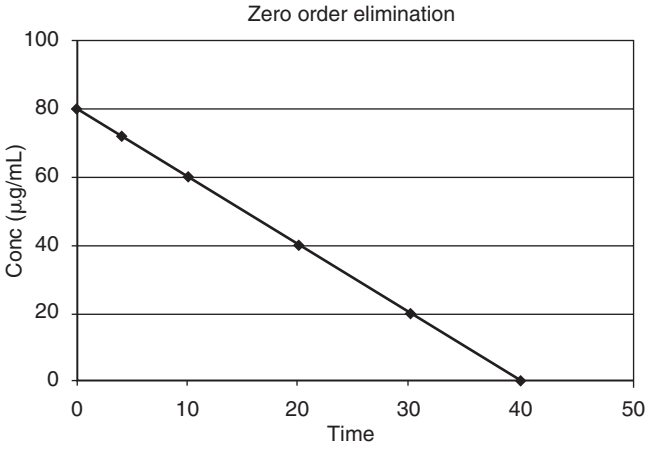


FIG. **2.1** Drug concentration versus time for a zero order reaction produces a straight line on regular graph paper.

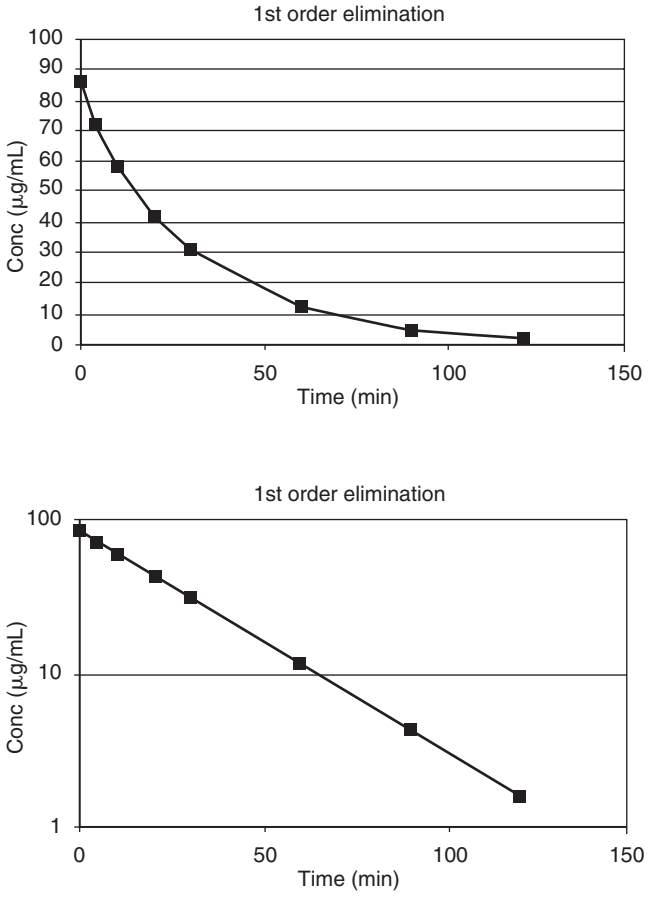


FIG. **2.2** Drug concentration versus time for a first order reaction produces an exponential curve on regular graph paper but produces a straight line on semilogarithmic graph paper.

where K is the first order rate constant, is expressed in units of time^{-1} (min^{-1} or hr^{-1}), and defines the fraction of drug eliminated from the body per unit time; C is the plasma drug concentration at any time t. Although K remains constant, the rate ($>C/>t$) is always changing because C is always decreasing. A graph of drug concentration versus time for a first order reaction produces an exponential curve on regular graph paper

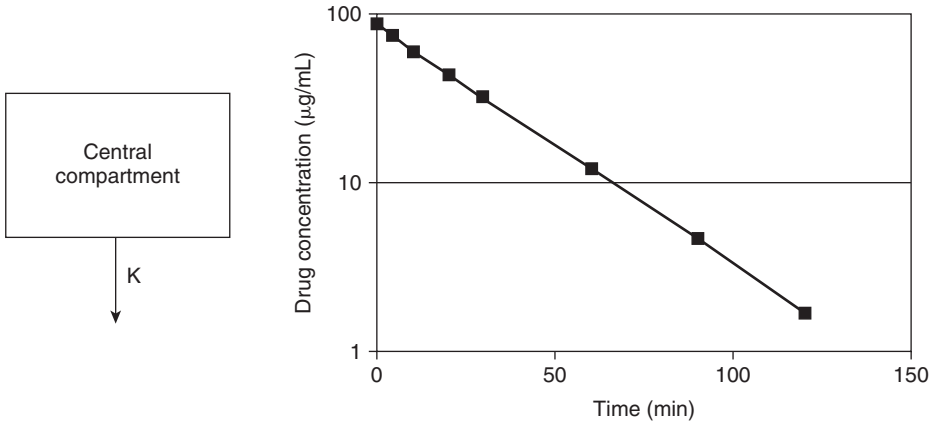


FIG. **2.3** Graphic representation of a one-compartment open model with IV administration and first order elimination.

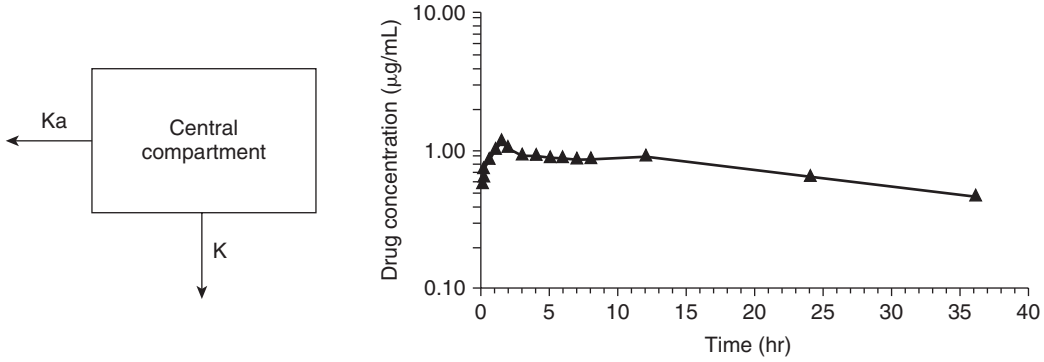


FIG. **2.4** Plasma concentration versus time graph after intramuscular administration of long-acting OTC to a horse, demonstrating a one-compartment open model with first order absorption and elimination.

but produces a straight line on semilogarithmic graph paper (Fig. 2.2) and is described by the following equation:

$$C = C_0 e^{-Kt}$$

where C is drug concentration at any time t, K is the first order rate constant in minutes or hours, and C_0 is the drug concentration at time zero (the moment of injection). Most drugs are absorbed and eliminated by first order processes. Glomerular filtration by the kidney is a first order process.

CLINICAL APPLICATION OF COMPARTMENTAL MODELING, RATES, AND ORDERS OF REACTIONS

The aforementioned concepts can be combined to mathematically describe the changes in the drug concentration in the body over time. Drug disposition described by a one-compartment open model with intravenous (IV) injection and first order elimination (Fig. 2.3) means that the body acts as one homogeneous compartment. A drug's concentration in one part of the body is assumed to be proportional to its concentration in any other part. The movement of drug into and out of the central compartment is described by the rate constants K_{01} and K_{10}.
Many drugs administered by routes other than IV, such as oral (PO), subcutaneous (SC), intramuscular (IM), or intradermal, are described by a one-compartment open model with first order absorption *(Ka)* and elimination *(K)* (Fig. 2.4). With a two-compartment open model with IV injection and first order elimination, the model assumes the body acts as two compartments: the central compartment (blood and highly vascularized tissues) and a peripheral compartment (less vascularized tissues). Many drugs administered in veterinary medicine are described by this model (Fig. 2.5). Elimination is considered to occur only from the central compartment, because the liver and kidneys are highly vascularized tissues. The plasma concentration versus time graph does not produce a straight line on semilogarithmic paper but can be broken into two sections and described by the following biexponential equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the concentration at any time t, A is the y-intercept of the first portion of the curve extrapolated to zero, and α is the slope of the line; B is the y-intercept of the latter portion of the curve extrapolated to zero, and β is its slope. The movement of drug between the central and peripheral compartments is described by the rate constants K_{12} and K_{21}.

For some concentration versus time data, the line can be broken into three or more straight lines and described mathematically with three or more exponential terms. Theoretically, drug distribution in the body can be described by as many compartments as there are different tissues, but for practical purposes more than three-compartment models are not necessary. Drugs that are described by three-compartment models usually have some tissue site where the drug is sequestered

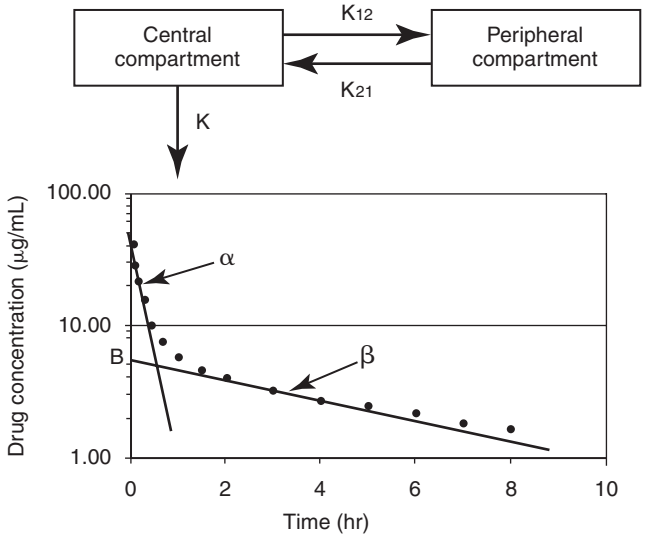


FIG. **2.5** Plasma concentration versus time graph after IV administration of gentamicin to a horse, demonstrating a two-compartment open model with first order elimination from the central compartment. The equation of the line is biexponential, where $C = A\,e^{-\alpha t} + Be^{-\beta t}$.

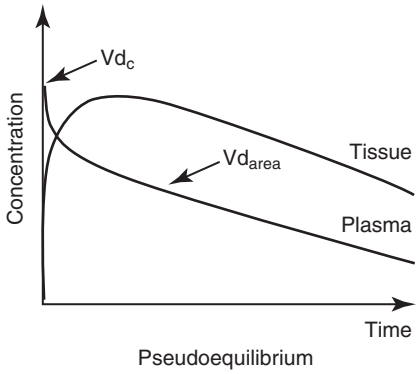


FIG. **2.6** Graphical representation of the difference between the volume of the central compartment and the volume of distribution by area.

and slowly eliminated from the body, such as the aminoglycosides, which sequester in the renal tubular epithelial cells, and oxytetracycline, which sequesters in teeth and bone.

DISTRIBUTION OF DRUGS IN THE BODY

The volume of distribution (Vd) of a drug is the mathematical term used to describe the apparent volume of the body in which a drug is dissolved.[1] The Vd is the parameter used to assess the amount of drug in the body from the measurement of a "snapshot" plasma concentration. The numeric value of Vd can give some indication of the distribution of the drug in the body. A drug's distribution is determined by its ability to cross biologic membranes and reach tissues outside the vascular system. The physical characteristics of the drug molecule, such as ionization, lipid solubility, molecular size, and degree of protein binding, determine its ability to cross biologic membranes.

Three volumes of distribution (Vd) are reported in the veterinary literature: the volume of the central compartment, the steady-state volume of distribution, and the volume of

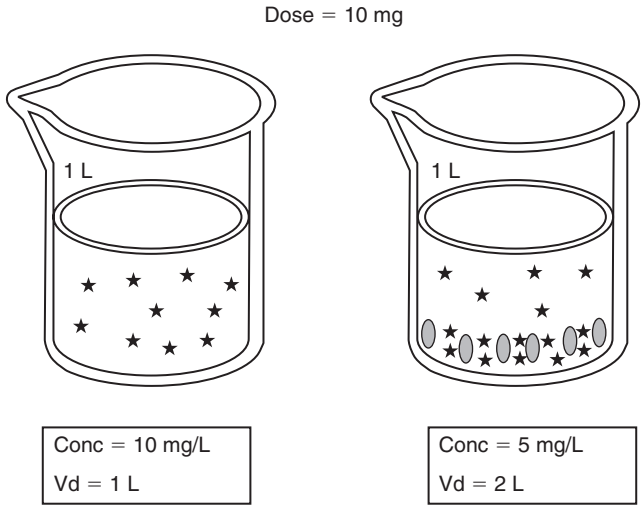


FIG. **2.7** The beaker on the left represents a low Vd drug that is mainly distributed to the extracellular fluid. A sample from the fluid will contain a high concentration of drug and therefore will have a low value for Vd. The beaker on the right represents a high Vd drug that readily crosses membranes and moves out of the extracellular fluid into tissues. A sample from the fluid will contain a low concentration of drug and therefore will have a high value for Vd.

distribution calculated by the area method. Conceptually, the easiest demonstration of the volume of distribution is with the volume of the central compartment (Vd_c). Just after an IV dose, plasma drug concentration is maximal (Fig. 2.6). Assuming that the instant drug concentration (C_0) results from the drug mixing in the blood, the Vd_c is the apparent volume from which drug elimination occurs since the kidneys and liver belong to the central compartment, and is calculated from the following equation:

$$Vd_c = \text{Dose}/C_0$$

where C_0 is the concentration at time zero, extrapolated from the plasma concentration versus time graph. To understand what the Vd_c for a drug represents, consider the body as a beaker filled with fluid (Fig. 2.7). The fluid represents the plasma and other components of extracellular water. If a drug is administered intravenously, it rapidly distributes in the extracellular fluid (ECF). If the drug does not readily cross lipid membranes, it will be confined mainly to the ECF, and a plasma sample therefore will have a high drug concentration. The higher the measured concentration in relation to the original dose, the lower the numeric value for Vd_c. Drugs such as the β-lactam and aminoglycoside antibiotics are poorly lipid soluble and therefore remain predominantly in the ECF and have low values for Vd. In contrast, some drugs readily cross lipid membranes and distribute into tissues. This is represented by the beaker on the right, where the stars at the bottom of the beaker represent drug molecules that have been taken up by tissues. A plasma sample will have a low drug concentration in proportion to the original dose and therefore will have a high numeric value for Vd_c. Given the limitations on measuring drug concentrations at "time zero" and using the aforementioned formula, the measured concentration of highly lipid-soluble drugs can be low enough to result in a value of Vd_c that is greater than 1 L/kg, so it is often referred to as an *apparent volume of distribution*. In the example on

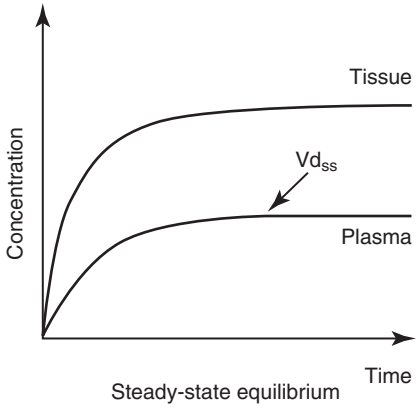


FIG. **2.8** Graphical representation of the volume of distribution when plasma concentrations are measured at steady-state conditions.

the right, the "apparent" Vd is 2 L/kg even though the beaker contains only 1 L of fluid.

For most drugs after a single IV dose, the drug is distributed and begins to be eliminated simultaneously. When concentrations are measured and the data graphed, there is a distribution phase, wherein the plasma drug concentration that is due to elimination and not distribution increases until it reaches an asymptotic value at which pseudoequilibrium is achieved (see Fig. 2.6). When pseudoequilibrium is reached, the movement of drug between the peripheral and central compartments reaches equilibrium, and decreasing plasma concentrations are now due only to irreversible elimination (described by the elimination rate constant, β). The applicable Vd value in this situation is the volume of distribution by area (Vd_{area}):

$$Vd_{area} = Dose \div AUC_{0-\infty}/\beta$$

where $AUC_{0-\infty}$ is the area under the plasma concentration time curve extrapolated to infinity. To be calculated accurately, the amount of drug that enters the systemic circulation must be accurately known, and the terminal phase must be a pure elimination phase. An inaccurate Vd_{area} is frequently published for "long-acting" IM- or SC-administered drugs, where prolonged elimination is due to delayed absorption (flip-flop kinetics).

With an IV infusion or with a multiple-dose regimen, the rate of drug entry into the body is equal to its elimination rate, and the body becomes a closed system with no clearance. In this situation the correct Vd to describe distribution is the Vd at steady-state (Vd_{ss}; Fig. 2.8):

Vd_{ss} = Drug in body at steady state/Concentration in plasma

Clinical Use of the Different Volume of Distribution Values

The Vd_c is used to predict the initial plasma drug concentration after an IV bolus of a drug when a loading dose is needed to rapidly achieve a therapeutic drug concentration. The Vd_{ss} is used to calculate a loading dose when it is clinically necessary to rapidly reach steady-state concentrations. The Vd_{area} is used to predict the amount of drug remaining in the body. For all drugs the value of Vd_{area} is greater than Vd_{ss}, but generally the difference is small and the values are used interchangeably. However, with the IV administration of drugs that are rapidly eliminated into urine (e.g., aminoglycosides), Vd_{area} can be much larger than Vd_{ss} because a large fraction of the drug is eliminated before pseudoequilibrium is reached.

It is useful to compare a drug's Vd to the distribution of water in the body to get an idea of its distribution. Drugs with a Vd value of less than 0.3 L/kg are predominantly confined to the ECF, whereas drugs with a Vd value of greater than 1 L/kg are highly lipid soluble and tend to distribute out of the ECF and into tissue compartments (Box 2.1). Although the value of Vd does not confirm penetration of a drug into specific tissues, in general the higher the value of the Vd, the more likely it is that the drug will reach sequestered sites such as the brain and cerebrospinal fluid (CSF), the prostate and other sex organs, the eye, and the mammary gland. Studies must be performed to confirm that therapeutic concentrations are achieved in such sites.

BOX 2.1 Volume of Distribution (Vd) of Various Drugs

LOW-VD DRUGS (<0.3 L/KG)
Penicillins
Cephalosporins
Aminoglycosides
Nonsteroidal antiinflammatory drugs

MEDIUM-VD DRUGS (>0.3–<1 L/KG)
Phenobarbital
Sulfonamides
Prednisolone
Rifampin

HIGH-VD DRUGS (>1 L/KG)
Macrolides
Tetracyclines
Fluoroquinolones
Chloramphenicol
Metronidazole
Trimethoprim
Dexamethasone
Furosemide
Ketamine
Diazepam
Firocoxib

Conditions That Affect Volume of Distribution

The Vd is constant for any drug and will change only with physiologic or pathologic conditions that change the distribution of the drug. Drugs with high Vd are usually very lipid soluble and typically are not significantly affected by changes in body water status and do not require dosage adjustment. However, there are many medical conditions that affect the disposition of low Vd drugs in a patient (e.g., nonsteroidal antiinflammatory drugs and aminoglycosides), and these drugs do require dosage adjustment because of their narrow therapeutic index. Many conditions in horses, such as colic, are characterized by volume contraction and dehydration and changes in acid-base balance, which affect the ECF volume. Neonatal foals have a higher percentage of body water than adult horses (80% vs. 60% total body water), and the extra 20% is primarily confined to the ECF, so the Vd values of drugs such as gentamicin are higher in neonatal foals than older foals or adult horses.[2] Therefore to achieve equivalent therapeutic

plasma concentrations of gentamicin in a neonatal foal, the dose must be higher than that administered to the older foal or adult horse.

Bioavailability

Bioavailability (F) is a measure of the systemic availability of a drug administered by a route other than IV.[3] Bioavailability is determined by comparing the area under the plasma drug concentration curve versus time *(AUC)* for the extravascular formulation to the *AUC* for the IV formulation. The *AUC* is calculated by computer or by the trapezoidal method, wherein the entire curve is divided into trapezoids, then the area of each trapezoid is calculated and summed to give the *AUC*. For an orally administered drug, use the following equation:

$$F = (AUC_{oral} \div AUC_{IV}) \times 100 = \% \text{ bioavailable}$$

If *F* is significantly less than 100%, the drug dose must be increased to achieve systemic drug concentrations similar to the IV formulation:

$$\text{Adjusted dose} = \text{Dose}_{IV}/F$$

If the oral formulation of a drug has a mean bioavailability of 50%, the drug dose must be doubled to achieve the same concentrations in plasma as achieved using the IV formulation. However, the variability of the bioavailability in the population is more clinically significant than the mean. To make sure that the horse with the poorest absorption is dosed appropriately, the dose must be increased according to the lowest bioavailability, not the mean. For example, if a drug has a mean *F* of 50% with a range of 20% to 70%, then to achieve an exposure of 100% for all the treated horses, the dose must be multiplied by 5, not just 2. However, if this is done, the horses with an *F* of 70% will be overdosed by a factor of 3.5. For a drug with a narrow therapeutic window and a poor bioavailability, there may be no dose that is ideal for all horses in the population. Low bioavailability of antimicrobials and anthelmintics is a major cause of subtherapeutic dosages that promote drug resistance. Poor oral bioavailability is a major limitation of many drugs administered to horses.[4]

Lipid Solubility and Drug Ionization (the pH-Partition Hypothesis)

The degree of lipid solubility determines how readily a drug will cross biologic membranes. Drugs are classified as lipid soluble (or nonpolar) versus water soluble (or polar). Highly lipophilic drugs diffuse easily across almost all tissue membranes. Most of the drugs used in equine practice exist as weak acids or weak bases. Their lipid solubility depends a great deal on their degree of ionization (charged state). An *ionized* drug is hydrophilic and poorly lipid soluble. A *nonionized* drug is lipophilic and can cross biologic membranes. The degree of ionization for a weak acid or weak base depends on the p*K*a of the drug and the pH of the surrounding fluid. At a given pH, there is an equilibrium between the ionized and nonionized proportions of drug. When the pH is equal to the p*K*a of the drug, the drug will be 50% ionized and 50% nonionized (log 1 = 0). As the pH changes, the proportion of ionized to nonionized drug will change according to the *Henderson-Hasselbach* equations:

For a weak acid:

$$\text{pH} = \text{p}Ka + \log(\text{Ionized drug}/\text{Nonionized drug})$$

For a weak base:

$$\text{pH} = \text{p}Ka + \log(\text{Nonionized drug}/\text{Ionized drug})$$

BOX 2.2 Drugs Classified by pH

ACIDIC DRUGS
Penicillins
Cephalosporins
Sulfonamides
Nonsteroidal antiinflammatory drugs

BASIC DRUGS
Macrolides
Trimethoprim
Chloramphenicol
Metronidazole
Aminoglycosides

AMPHOTERIC DRUGS
Fluoroquinonolones
Tetracyclines

Whereas the precise ratios of ionized versus nonionized drug can be calculated from the Henderson-Hasselbalch equations, the relevance of the equations can be understood by simply remembering the sentence "like is nonionized in like." For example, a weak acid will be most nonionized in an acidic environment, so aspirin is most nonionized in the stomach and is readily absorbed. The fluid of most sequestered sites in the body (CSF, accessory sex gland fluid, milk, abscesses) has a pH more acidic than plasma. In cattle with mastitis weak acid antibiotics are typically administered by intramammary infusion, whereas weak bases are administered parenterally. This makes sense according to the pH-partition concept. Weak bases in the plasma are highly nonionized and readily cross into the mammary gland. Then, as the equilibrium shifts, they become "ion trapped" in the more acidic milk, but the fraction of nonionized drug in the mammary gland is available to cross the bacterial cell membrane for antimicrobial action. Weak acids such as penicillins and cephalosporins are highly ionized in plasma and therefore do not penetrate into the mammary gland very well, so these are most effective when administered by local infusion into the udder, where the extremely high local concentrations negate local pH effects.

Typically, drugs that are weak acids will have low Vd values and weak bases will have high values for Vd (Box 2.2). Amphoteric drugs such as the fluoroquinolones and tetracyclines have acidic and basic groups on their chemical structures. These drugs have a pH range where they are maximally nonionized. For example, enrofloxacin is most lipid soluble (nonionized) in the pH range of 6 to 8, so it is lipid soluble at most physiologic pH levels. In acidic urine significant ionization occurs, which reduces enrofloxacin's antibacterial activity. But this reduction in activity is offset by the extremely high concentrations of enrofloxacin achieved in urine, so it is of no clinical importance. Despite being weak bases, the aminoglycosides are very large, hydrophilic molecules and have high p*K*a values, so they are highly ionized at physiologic pH levels. Therefore parenterally administered aminoglycosides do not cross lipid membranes well and do not achieve therapeutic concentrations in milk, accessory sex gland fluids, abscesses, or CSF.

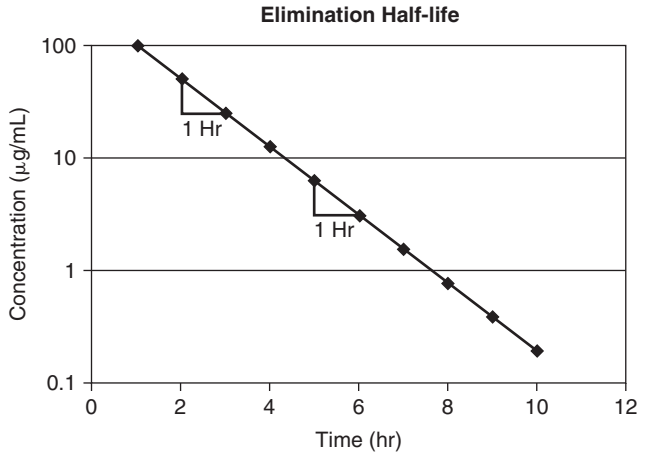


FIG. **2.9** For a drug with first order elimination, the plasma concentration decreases by 50% every hour, so the elimination half-life is 1 hour.

Drug Protein Binding

Protein binding can involve plasma proteins, extracellular tissue proteins, or intracellular tissue proteins. Many drugs in circulation are bound to plasma proteins, and because bound drug is too large to pass through biologic membranes, only free drug is available for delivery to the tissues and to produce the desired pharmacologic action. Therefore the degree of protein binding can greatly affect the pharmacokinetics of drugs. Acidic drugs such as nonsteroidal antiinflammatory drugs (NSAIDs) tend to bind predominantly to albumin.[5] Albumin is the most abundant plasma protein, and it is critical to maintaining the colloidal oncotic pressure in the vascular system. As a negative acute phase protein, albumin concentration decreases during inflammation. Hypoalbuminemia results from decreased production, seen with severe hepatic insufficiency, or by loss through increased rates of urinary excretion, such as in glomerulonephritis or with mucosal damage, as with protein-losing enteropathies. Basic drugs typically bind to α-1 acid glycoprotein, which is an acute phase protein, whose hepatic production increases significantly with inflammatory conditions.[6] Other proteins, including corticosteroid binding globulin, are important for binding of some specific drugs but are less important in overall drug-protein binding.[7] There is equilibrium between free and bound drug, however, just like the relationship of ionized and nonionized drug molecules. Protein binding is most clinically significant for antimicrobial therapy, where a high degree of protein binding serves as a drug "depot," allowing for increased duration of the time the drug concentration remains above the bacterial minimum inhibitory concentration, adding to antimicrobial efficacy.[8] For other drugs changes in plasma protein binding can influence individual pharmacokinetic parameters, but changes in plasma protein binding usually do not influence the clinical exposure of the patient to a drug. Changes in protein binding caused by drug interactions are assumed to instantaneously change free drug concentrations and have been frequently cited as a cause of adverse drug reactions. But the increase in free drug concentration is only transient, because drug distribution and drug elimination change to compensate. The often-cited example of the concurrent administration of phenylbutazone and warfarin leading to bleeding caused by increased free concentrations of warfarin is erroneous. The true interaction is from phenylbutazone-induced inhibition of the hepatic metabolism of warfarin, which results in increased plasma concentrations and increased anticoagulant effect.[7] Therefore adjustments in dosing regimens because of hypoproteinemia or concurrent administration of highly bound drugs are not necessary except in the rare case of a drug with a high hepatic extraction ratio and narrow therapeutic index that is given parenterally (e.g., IV dosing of lidocaine).[9]

DRUG ELIMINATION FROM THE BODY

Drug elimination refers to the irreversible removal of drug from the body by all routes of elimination. Elimination may be divided into two major components: excretion and biotransformation. Drug excretion is the removal of the intact drug. Most drugs are excreted by the kidney into the urine. Other pathways include the excretion of drug into bile, sweat, saliva, or milk. Biotransformation (drug metabolism) converts the drug in the body to a metabolite that is more readily excreted, usually by adding a chemical group to the molecule to make it more water soluble. Enzymes involved in biotransformation are mainly located in the liver. Other tissues, such as the kidney, lung, small intestine, and skin, also contain biotransformation enzymes.

ELIMINATION RATE CONSTANT AND ELIMINATION HALF-LIFE

The rate of elimination for most drugs is a first order process. The elimination rate constant *(K)* represents the sum of drug elimination by excretion and metabolism. Drug elimination is considered always to occur from the central compartment, because the liver and kidney are well-perfused tissues. The elimination rate constant is used to calculate the drug's half-life ($T_{½}$), or the time required for drug concentration to decrease by one half (Fig. 2.9). For first order reactions, $T_{½}$ is constant across the plasma concentration versus time curve and is calculated from

$$T_{1/2} = 0.693/K$$

where 0.693 = ln2 (the natural logarithm of 2). Mean residence time (MRT) is roughly the equivalent of $T_{½}$ when pharmacokinetics is calculated using statistical moment theory. The MRT is an estimate of the average time a drug molecule spends in the body, which typically correlates to the time it takes for drug concentration to decrease by 63.2%. Therefore the MRT value should be slightly greater than $T_{½}$. If the calculated value for MRT is not longer than the $T_{½}$, there was likely an inadequate number of samples in the distribution or elimination phase of the study. Although these parameters are similar, it is the $T_{½}$ that is used to determine the drug dosage interval, how long a toxic or pharmacologic effect will persist, and drug withdrawal times for food animals or performance horses. Notice that it takes 10 $T_{½}$ to decrease the plasma concentration by 99.9% (Table 2.1). Knowing a drug's plasma $T_{½}$ can give the clinician some idea of the drug's withdrawal time for food or performance animals. However, for drugs that undergo hepatic metabolism (e.g., phenylbutazone) or drugs that sequester in specific tissues (e.g., aminoglycosides, isoxsuprine), simply multiplying the $T_{½}$ by a factor of 10 for a withdrawal time may not be sufficient to prevent violative residues. Also note that doubling

TABLE **2.1** Half-Life of Elimination of a Drug

Number of Half-Lives	Fraction of Drug Remaining (%)	Fraction of Drug Eliminated (%)
0	100	0
1	50	50
2	25	75
3	12.5	87.5
4	6.25	93.75
5	3.125	96.875
6	1.56	98.44
7	0.78	99.22
8	0.39	99.61
9	0.195	99.805
10	0.0975	99.9025

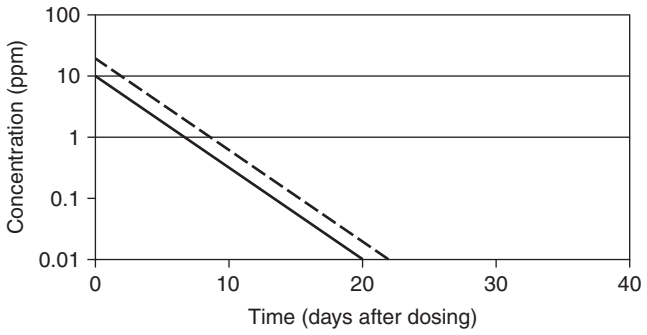


FIG. **2.10** Doubling a drug dose adds only one half-life to its withdrawal time. For this drug with an elimination half-life of 24 hours, if the dose is doubled to reach a plasma concentration of 20 μg/mL, it will take 21 days instead of 20 days to reach an acceptable threshold of 0.01 μg/mL.

a drug dose does not double the withdrawal time; it merely adds 1 half-life to the time it takes to reach the acceptable threshold concentration (Fig. 2.10).

Flip-Flop Kinetics

Long-acting drug formulations are often products whose carriers cause them to be slowly absorbed from the site of administration into the systemic circulation. In these instances, the drug elimination rate is limited by the drug absorption rate. The value for *K* (the elimination rate constant) calculated from the plasma concentration versus time curve is actually the value for *K*a (the absorption rate constant). The easiest way to identify flip-flop kinetics is to compare the plasma concentration versus time curve for the extravascular route of administration to the curve after the drug is given intravenously (Fig. 2.11). If the elimination phases of the curves are not parallel, delayed absorption is prolonging elimination and the flip-flop phenomenon has occurred. Flip-flop kinetics is also one potential reason for a reported bioavailability greater than 100%.

For a two-compartment model, β (from the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$) is the drug elimination rate constant from the entire body once the drug has reached equilibrium between the two compartments. Therefore β is used to calculate the elimination half-life:

$$T_{1/2} = 0.693/\beta$$

Clearance

Clearance is a measure of drug elimination from the body without reference to the mechanism of elimination. It is always reported in pharmacokinetic papers, but its significance is rarely explained in pharmacokinetic studies in horses. Clearance is the most important pharmacokinetic parameter because it is the only parameter that controls overall drug exposure, and it is used to calculate the dosage required to maintain a specific average steady-state concentration.[10]

Clearance (Cl) is the total drug clearance and is the sum of renal clearance (Cl_R), hepatic clearance (Cl_H), and all other elimination mechanisms. By definition, Cl is the volume of fluid containing drug that is cleared of drug per unit of time (mL/kg/min). The most frequent technique for determining plasma Cl is to administer a single IV dose of a drug and then measure plasma concentrations over time. Then,

$$Cl = Dose/AUC$$

where *AUC* is the area under the plasma concentration time curve.

If the body is considered as the whole system clearing the drug, Cl can also be determined by the animal's cardiac output and the extraction ratio *(E),* where *E* is a numeric value between 0 and 1 that is the percentage of the drug that is cleared by a single pass through the clearing organ:

$$Cl = \text{Cardiac output} \times E$$

For a drug with an extraction ratio of 1 (100% removal by the liver and kidney on the first pass), the expected value of Cl is about 50% of the cardiac output, because blood flow to the liver and the kidneys represents approximately half of the cardiac output.

In contrast to Vd values, Cl values have to be interpreted according to the value of cardiac output for the species involved. Given that most drugs are extracted primarily by renal and hepatic mechanisms, the extraction ratio is considered high if *E* is greater than 0.7, medium if *E* equals 0.3, and low if *E* is less than 0.1. Because the liver and kidneys receive about 50% of cardiac output, the overall *E* is high if it is greater than 0.35, medium if it is about 0.15, and low if it is less than 0.05. From this and the cardiac output of the species, breakpoint values can be determined to classify drugs as having high, medium, and low clearance. For the horse with a cardiac output of 55 mL/kg/min, a high Cl value is 19 mL/min/kg, medium Cl is 8.25 mL/min/kg, and low Cl is 3.6 mL/min/kg.

It is sometimes difficult to understand the difference between the elimination half-life and clearance. The relationship is as follows:

$$Cl = (Vd)(K) \text{ or}$$

$$Cl = (Vd)\left(0.693/T_{1/2}\right)$$

Consider the values for clearance and $T_{1/2}$ for four antimicrobial drugs (Table 2.2). Note that the plasma clearance values are similar, but the elimination half-lives are very different. Because the $T_{1/2}$ is influenced by the extent of drug distribution, the drugs have similar clearance, but oxytetracycline has the largest Vd and the longest $T_{1/2}$. Because $T_{1/2}$ is derived from rate constants and does not have a physiologic basis, it is influenced by the sensitivity of the analytic method and by many pharmacokinetic parameters, and it is a poor parameter alone to evaluate physiologic (e.g., age, sex) or pathologic (e.g., renal failure) changes that effect drug disposition.

TABLE **2.2** Comparison of Clearance to Elimination Half-Life

	Penicillin	Gentamicin	Oxytetracycline	Tylosin
Cl (mL/min/kg)	3.5	3.1	4.0	2.2
$T_{½}$ (min)	30	75	360	54

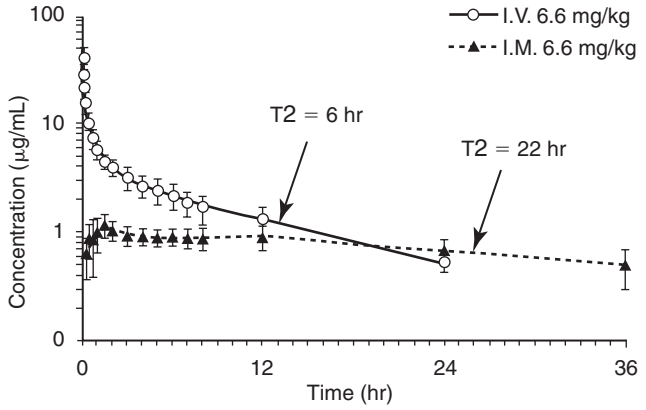


FIG. **2.11** Plasma concentration versus time graph for long-acting oxytetracycline in horses, demonstrating "flip-flop" kinetics. The delayed absorption from the intramuscular injection results in the slow elimination and triples the elimination half-life value.

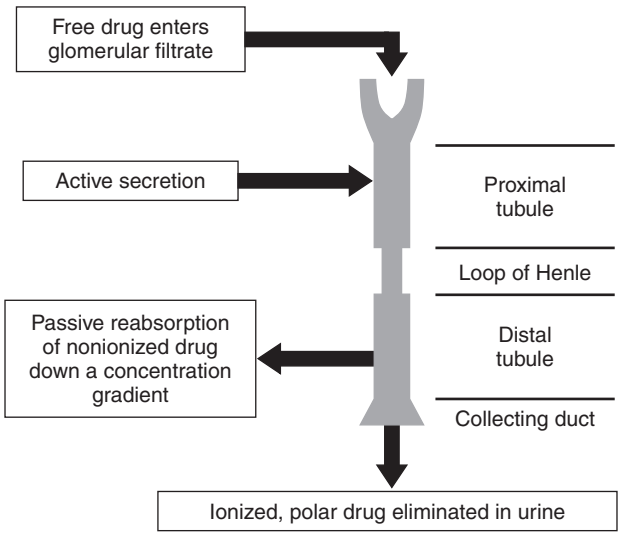


FIG. **2.12** Movement of drugs in the renal tubule.

Renal Clearance of Drugs

Renal excretion is the major route of elimination from the body for most drugs. Drug disposition by the kidneys includes glomerular filtration, active tubular secretion, and tubular reabsorption (Fig. 2.12), such that renal drug clearance is defined by the following equation:

$$Cl_R = Cl_F + Cl_S - FR$$

where Cl_R is total renal clearance, Cl_F is clearance attributed to glomerular filtration, Cl_S is clearance attributed to active tubular secretion, and FR is the fraction of drug reabsorbed from the tubule back to circulation.

TABLE **2.3** Renal Clearance (mL/min/kg) at Different Urinary pHs

			Urine pH		
		p*K*a	4.4	6.4	7.9
Penicillin	Acid	2.4	0.001	0.1	3.0
Oxytetracycline	Base	10.4	1000	10	0.3

Glomerular filtration occurs with small molecules (less than 300 molecular weight) of free (unbound) drug. Large molecules or protein-bound drugs do not get filtered at the glomerulus because of size and electrical hindrance. The kidneys receive approximately 25% of cardiac output, so the major driving force for glomerular filtration is the hydrostatic pressure within the glomerular capillaries. Glomerular filtration rate (GFR) is estimated by measuring a substance or drug that is eliminated only by glomerular filtration, such as creatinine or inulin.

If Cl_R is greater than Cl_F, some degree of tubular secretion is occurring. Active tubular secretion is a carrier-mediated transport system, located in the proximal renal tubule. It requires energy input because the drug is moved against a concentration gradient. Two active tubular secretion systems have been identified: anion secretion for acids and cation secretion for bases. Drugs with similar structures may compete with each other for the same transport system. For example, probenecid competes with penicillin or the fluoroquinolones for the same transport system, effectively decreasing Cl_R of these antimicrobials. In patients with reduced functional renal tissue, remaining transport systems become easily saturated and drug accumulation occurs.

If Cl_R is less than the GFR, tubular reabsorption of drug is occurring. Tubular reabsorption is an active process for endogenous compounds (e.g., vitamins, electrolytes, glucose). It is a passive process for the majority of drugs. It occurs along the entire nephron but primarily in the distal renal tubule. Factors that affect reabsorption include the p*K*a of the drug and urine pH, which in turn influence drug ionization. According to the Henderson-Hasselbach equation, a drug that is a weak base will be mainly nonionized in alkaline urine, and a weak acid will be mainly ionized in alkaline urine. The nonionized form of the drug is more lipid soluble and has greater reabsorption (Table 2.3). The p*K*a of a drug is constant, but urinary pH is highly variable in animals and varies with the diet, drug intake, time of day, and systemic acidosis/alkalosis. Species differences have a major influence on the renal excretion of ionized drugs. Carnivores, with a urine pH of 5.5 to 7.0, will have a greater renal excretion of basic drugs than herbivores, with a urine pH of 7.0 to 8.0, and vice versa. Additional factors affecting drug reabsorption include lipid solubility, drug size, and urine flow.

Hepatic Clearance of Drugs

Nonrenal drug elimination is assumed to be due primarily to biotransformation (hepatic metabolism) and biliary excretion. Clearance of a drug by the liver is determined by hepatic blood flow (Q_H) and the intrinsic ability of the liver to extract the drug (extraction ratio, or ER_H):

$$Cl_H = (Q_H)\,(ER_H)$$

Drugs with a high extraction ratio (approaching 1) have Cl_H equal to the hepatic blood flow. These drugs are called *high*

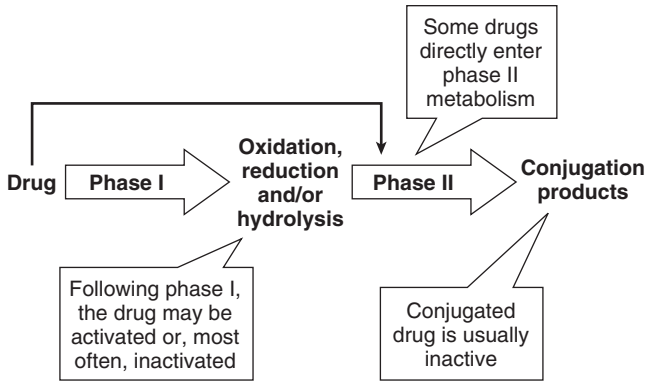


FIG. **2.13** Hepatic metabolism increases the water solubility of drugs, facilitating excretion from the body.

clearance drugs. Examples of drugs with high ER_H are lidocaine, propranolol, and isoproterenol. Clearance of drugs with high ER_H is highly influenced by changes in hepatic blood flow. Drugs that are administered orally and are absorbed across the intestinal mucosa must first pass through the liver via the portal circulation before being distributed to the rest of the body. Most of a drug with a high ER_H will be cleared in one pass through the liver; this is called the *first pass effect,* and it limits the oral administration of such drugs (e.g., morphine). Drugs that have a low hepatic extraction rate ($ER_H \leq 0.2$) are not greatly affected by changes in hepatic blood flow. However, their clearance will be affected by changes in the hepatic microsomal enzyme systems and protein binding. A first pass effect does not interfere with the systemic availability of these drugs. Drugs with a low ER_H include chloramphenicol, phenylbutazone, phenobarbital, and digoxin.

Biotransformation (Hepatic Metabolism) of Drugs

Metabolism is necessary for removal of lipophilic drugs from the body (Fig. 2.13). Biotransformation depends on the chemical composition of the liver, activity of major drug metabolism enzymes, hepatic volume (perfusion rate), drug accessibility to and extraction by hepatic metabolic sites, and physicochemical properties of the drug. Biotransformation of a parent drug results in metabolites that may be active or inactive themselves. A *prodrug* is a drug administered in an inactive form that must be biotransformed to its active form, such as prednisone to prednisolone. Drug metabolic pathways are divided into *phase I* and *phase II* reactions. Phase I reactions (oxidation, reduction, hydrolysis, hydration, dethioacetylation, isomerization) typically add functional groups to the drug molecule necessary for phase II reactions. Phase II reactions (glucuronidation, glucosidation, sulfation, methylation, acetylation, amino acid conjugation, glutathione conjugation, and fatty acid conjugation) typically include conjugation reactions that increase the water solubility of the drug, facilitating excretion from the body.

Among the reactions catalyzed by drug metabolism enzymes, the cytochrome P450 mixed function oxidase system is the most intensively studied. This reaction catalyzes the hydroxylation of hundreds of structurally diverse drugs, whose only common characteristic is high lipid solubility. Species differences in drug metabolic rate are the primary source of variation in drug activity and toxicity. Cats have a poor ability to glucuronidate drugs, pigs are deficient in sulfate conjugation, and dogs are relatively poor acetylators. Compared with other species, the metabolizing pathways of the horse are relatively unknown.

BOX 2.3 Drugs That Affect Enzyme Function

ENZYME INDUCERS
Chlorinated hydrocarbons
Griseofulvin
Omeprazole
Phenobarbital
Phenytoin
Rifampin

ENZYME INHIBITORS
Chloramphenicol
Cimetidine
Dexamethasone
Erythromycin
Fluoroquinolones
Ketoconazole
Phenobarbital
Phenylbutazone
Prednisolone
Quinidine

Induction and Inhibition of Metabolism

Metabolism of drugs can be substantially affected by enzyme induction or inhibition by other drugs or chemicals (Box 2.3). In some cases the drug itself may alter its own metabolic fate by induction or inhibition. Many drugs are capable of inducing enzyme activity, thereby increasing the rate of metabolism and hepatic clearance of concurrently administered drugs, which typically results in a decreased pharmacologic effect. Enzyme induction usually occurs slowly, requiring several weeks to reach maximum effect. Induction is accompanied by increased hepatic ribonucleic acid (RNA) and protein synthesis and increased hepatic weight. Enzyme induction is important in the pathogenesis of hepatotoxicity and therapeutic failure of many drugs. Phenobarbital is a potent enzyme inducer known for hepatotoxicity and for inducing its own metabolism. Rifampin induces the metabolism of azole antifungals; concurrent administration with itraconazole results in subtherapeutic itraconazole concentrations.

Drug-induced enzyme inhibition also occurs and typically results in prolonged clearance of a concurrently administered drug. The potential for toxicity or an exaggerated pharmacologic response is increased. In contrast to induction, inhibition occurs rapidly. Erythromycin and enrofloxacin are known inhibitors of the metabolism of theophylline; concurrent administration can cause central nervous system toxicity and seizures.[11,12]

Kinetics of Drug Metabolism

The enzymes that catalyze drug metabolism typically obey Michaelis-Menten kinetics as a *first order reaction:*

$$V = (V_{max}[C])/(K_m + [C])$$

where V is the rate of drug metabolism, K_m is the Michaelis constant, and C is the drug concentration. In most

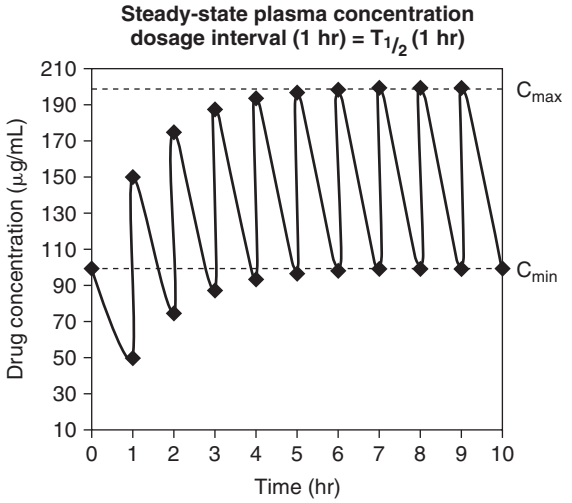


FIG. **2.14** Plasma concentration versus time graph for an IV drug that produces a peak plasma concentration of 100 μg/mL after a single dose. After 6 hours (which equals 6 half-lives), the maximum and minimum drug concentrations become constant, further drug accumulation does not occur, and the drug is steady-state.

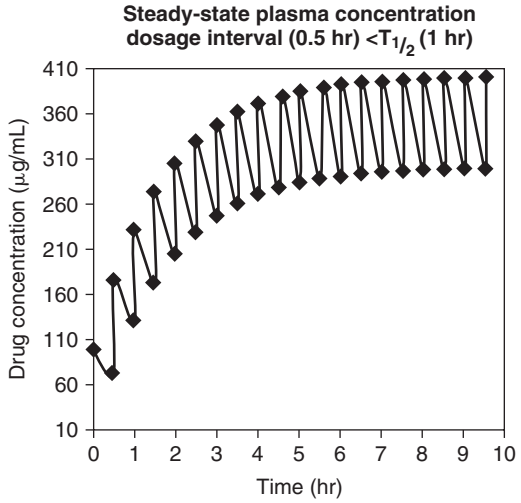


FIG. **2.15** Plasma concentration versus time graph for a drug with a dosage interval (0.5 hour) less than the half-life (1 hour), demonstrating significant drug accumulation at steady-state.

clinical situations the drug concentration is much less than the Michaelis constant, so the equation reduces to the following:

$$V = (V_{max}\,[C]\,)/K_m$$

That is, the rate of drug metabolism is directly proportional to the concentration of free drug, and first order kinetics are observed in that a constant fraction of drug is metabolized per unit of time. With a few drugs, such as phenylbutazone, ethanol, deracoxib, and phenytoin, or if very large doses of a drug are given, the drug concentrations achieved are much greater than K_m and the rate equation is as follows:

$$V = (V_{max}\,[C]\,)/\,[C]\, = V_{max}$$

The enzymes are saturated by the high free drug concentrations, and the rate of metabolism remains constant over time. This is termed *zero order kinetics* or *nonlinear kinetics.*

DRUG ACCUMULATION

Drugs are often given in multiple-dose regimens. To predict plasma drug concentrations, it is necessary to decide whether successive doses of a drug have any effect on the previous dose. The *principle of superposition* assumes that early doses of drug do not affect the pharmacokinetics of subsequent doses. For most drugs, as equal doses are given at a constant dosage interval, the plasma concentration-time curve plateaus and a *steady-state* is reached. At steady-state the plasma drug concentration fluctuates between a maximum concentration (C_{max}, or peak) and minimum concentration (C_{min}, or trough). Once steady-state is reached, C_{max} and C_{min} are constant and remain unchanged from dose to dose (Fig. 2.14). The time to steady-state depends solely on the elimination half-life. It takes approximately 5 to 6 $T_{½}$ to reach 99% steady-state levels. The drug dose and dosage frequency influence the values of C_{max} and C_{min} at steady-state, and the dosage frequency and $T_{½}$ influence the fluctuation between C_{max} and C_{min}.

Clinical Consequences of Dosage Intervals Less Than the Half-Life

Drugs such as phenobarbital, potassium bromide, phenylbutazone, and digoxin are commonly given at dosage intervals much shorter than their $T_{½}$ (Fig. 2.15). This results in drug accumulation and a C_{max} at steady-state that is greater than the peak concentration after a single dose. The predicted accumulation ratio (RA) with multiple-dose administration can be estimated using the following equation:

$$RA = 1/1 - e^{-\lambda z \tau}$$

where λz is the slope of the elimination phase and τ is the dosage interval. There is minimal fluctuation between C_{max} and C_{min}, and missing a single dose will not affect plasma concentrations greatly. There is a lag time to reach the desired plasma concentrations at steady-state, and there will be a lag time for plasma concentrations to change in response to a dose change.

Clinical Consequences of Dosage Intervals Greater Than the Half-Life

Drugs such as IV formulations of penicillin and cephalosporins are administered at dosage intervals that are greater than the $T_{½}$ (Fig. 2.16). As the dosage interval increases, C_{max} at steady-state is closer in value to the peak concentration after a single dose. If the dosage interval is greater than 10 $T_{½}$ (the time required to eliminate 99.9% of the previous dose), drug accumulation essentially does not occur. There is marked fluctuation between C_{max} and C_{min} (peak and trough), and missing a dose will greatly affect plasma concentrations. However, there is minimal lag time to achieve the desired plasma concentration.

DESIGNING DRUG DOSAGE REGIMENS

The success of drug therapy is highly dependent on the dosage regimen design. Not all drugs require rigid individualization

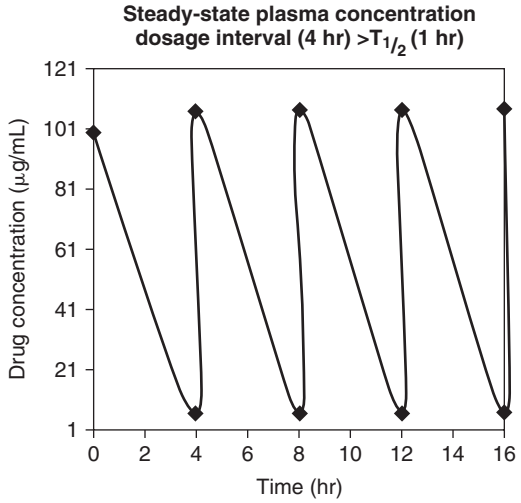


FIG. **2.16** Plasma concentration versus time graph for a drug with a dosage interval (4 hours) greater than the half-life (1 hour), resulting in no significant drug accumulation at steady-state.

of the dosage regimen. In the case of antimicrobials with a broad safety range, such as the penicillins and cephalosporins, the dosage is not titrated precisely but rather determined on the basis of the dose required to maintain an effective plasma concentration above the minimum inhibitory concentration of the bacterial pathogen. For drugs with a narrow therapeutic margin, such as digoxin, the aminoglycosides, and theophylline, the individualization of the dosage regimen is very important. The objective of the dosage regimen for these drugs is to produce a safe, therapeutic plasma drug concentration that does not exceed the minimum toxic concentration. Factors that influence the concentration of drug attained at the drug's site of action include the dose administered, the route of administration, release and absorption of drug from dosage form, the extent of drug distribution, and the rate of drug elimination.

The horse's age may have a profound effect on drug disposition (Table 2.4).[13] The definition of *geriatric* varies among species and may even vary among breeds. Aging may also be influenced by genetics, environment, nutrition, basic health care, and use. In general, horses greater than 18 to 20 years may be considered geriatric. Ponies often age better than horses and can be considered geriatric between 25 and 30 years and older. Body composition and regional blood flow change in geriatric horses. Cardiac output decreases, so regional and organ blood flow also decrease. These changes have an impact on drug absorption, distribution, and elimination. Blood flow is preferentially redistributed to the brain and heart, so there is an increase in risk of drug toxicity in these organs. Gastrointestinal (GI) motility and absorptive capacity are reduced. Hepatocyte number and function decrease along with hepatic and splanchnic blood flow. As renal blood flow decreases, GFR and active secretory capacity of the nephron decrease, resulting in decreased renal clearance of drugs. Lean body mass decreases while fatty tissues increase. The plasma concentrations of water-soluble (low volume of distribution) drugs tend to increase, whereas the plasma concentrations of lipid-soluble (high volume of distribution) drugs tend to decrease. Serum albumin decreases while gamma globulins increase, so total plasma protein concentrations essentially remain the same.

TABLE **2.4** **Age-Related Changes in Geriatric and Pediatric Patients**

Body Part/Function Affected	Geriatric	Pediatric
Organ blood flow	Decreased	Increased
Total body water	Decreased	Greatly increased
Body fat	Increased	Decreased
Serum proteins	Decreased albumin	Decreased albumin
	Increased globulins	Decreased globulins
Hepatic metabolism	Decreased	Greatly decreased

The definition of *neonate* also varies with species and age, but the determinants of drug disposition may be altered as the foal matures.[14] Blood flow to the heart and brain is greater and faster, making the foal more susceptible to drug-induced cardiotoxicity and neurotoxicity. GI absorption may be decreased as a result of decreased gastric emptying and decreased intestinal peristalsis. Absorption from the GI tract may also be affected by the differences in gastric pH between neonates and adults. Gastric pH in foals is highly variable (pH 1.5–7.0) and dependent on feeding status. Absorption from IM and SC sites changes as muscle mass and blood flow change. Neonates have less fat and greater total body water (primarily ECF) than adults. Therefore low-Vd drugs (e.g., gentamicin, ketoprofen) distribute into a larger volume, making it necessary to increase the dose to avoid therapeutic failure. Because of low body fat stores, lipid-soluble drugs will have higher plasma concentrations in foals. For example, moxidectin is much more lipid soluble than ivermectin, so it is more easily overdosed in foals.[15] Drug elimination by both hepatic metabolism and renal excretion is limited in neonates, so drug dosing intervals may need to be increased for some drugs, such as aminoglycosides and nonsteroidal antiinflammatory drugs. Not all drugs behave this way, however, and differences in pharmacokinetics result in a shortened dosing interval for some drugs, such as firocoxib and meloxicam.

Finally, disease states may alter drug pharmacokinetics. The sick horse usually has impaired drug detoxifying and elimination capability. Hepatic damage reduces drug metabolism and may increase drug action, whereas renal damage and impaired excretion decrease drug clearance. Alterations in GI motility affect drug absorption and are a concern in horses with postoperative ileus. Peripheral circulation is decreased in shock, resulting in decreased absorption of intramuscularly and subcutaneously administered drugs.

Pharmacokinetic-Based Dosage Regimens

A drug dose regimen is composed of a dose and a dosing frequency. Some drugs are given as single doses, so a specific plasma concentration will be targeted. When multiple doses of a drug are given, the dosage frequency and drug accumulation must be considered. There are simple calculations that can be used to design a patient-specific dose regimen.

Single-Dose Regimen

A single drug dose has its duration of action determined by the size of the dose, its elimination rate, and the volume of distribution. To calculate a single dose of a drug, or when

assuming a once-a-day dosing, only the Vd and the desired plasma concentration are needed:

$$\text{Dose} = (\text{Vd})(C_0)$$

For drugs administered other than intravenously, the dose must be corrected for bioavailability:

$$\text{Dose} = (\text{Vd})(C_0)/F$$

Continuous Rate Infusion

When the desired response needs to be constant, the drug may be infused intravenously at a constant rate (R_0) following the initial IV dose:

$$R = (C)(\text{Vd})(K)$$

This equation can also be written as

$$R_0 = C_{ss} \times \text{Cl}$$

The infusion rate is essentially the rate of drug loss from the body (mg/min or mg/hr). Therefore to maintain the established concentration of drug in the body, it is necessary to infuse drug at the rate equal to its loss (K = elimination rate). For example, the vasopressive drugs dopamine and dobutamine have extremely short elimination half-lives, so they must be administered by constant infusion.

Multiple-Dose Regimen

Continuous IV infusion offers the most precise control of drug levels in the body and is essential for precise control of drugs with a narrow safety margin or very rapid elimination, as well as for those drugs with a time-dependent effect (e.g., β-lactam antibiotics). This method is not feasible for most drugs in veterinary medicine. It is possible to maintain an average desired plasma concentration by repeat dosing at constant dosing intervals. Obviously, the highest plasma concentrations will occur soon after drug administration, and the lowest concentrations will be just before the next dose is administered. As long as the lowest concentration is acceptable for therapy and the highest concentration does not cause toxicity, these variations in plasma concentration are acceptable:

$$\text{Dose} = \frac{(C_{ave})(\text{Vd})(\tau)}{(1.44)\,T_{1/2}}$$

where τ = dosage interval and 1.44 is a constant to correct for log scale.

Loading Dose Followed by Maintenance Dose Regimen

Drugs that have long elimination half-lives, such as phenobarbital and fluconazole, have a long lag time to acceptable drug concentrations and therefore are usually given by a large loading dose (Dose_L) followed by maintenance doses (Dose_M).

$$\text{Dose}_L = \frac{\text{Dose}_M}{1 - e^{-K\tau}}$$

THERAPEUTIC DRUG MONITORING

Monitoring plasma drug concentrations is valuable if there is a relationship between the plasma drug concentration and the desired clinical effect or an adverse effect. It is particularly helpful in horses with systemic diseases that may affect pharmacokinetics. It is also useful when many drugs are being administered at the same time when interactions may occur. Therapeutic drug monitoring (TDM) is often valuable for regulating the dosage of drugs used chronically or prophylactically. For those drugs in which plasma concentration and clinical effect are not related, other pharmacodynamic parameters may be monitored. For example, clotting times may be measured in patients on anticoagulant therapy.

The drugs for which TDM is most commonly used are characterized by serious toxicity (e.g., digoxin, phenobarbital, aminoglycosides); a steep dose-response curve, wherein a small increase in dose can cause a marked increase or decrease in response (e.g., theophylline); marked pharmacokinetic variability among individual patients, so dose is poorly predictive of plasma drug concentration (e.g., cyclosporine); easily saturable elimination mechanisms that lead to nonlinear kinetics; or when the cost of therapy justifies confirming a desired plasma drug concentration (Table 2.5).

Performing Therapeutic Drug Monitoring

Samples for TDM should not be submitted until plasma drug concentrations have reached steady-state in the patient (after approximately 5–6 elimination half-lives have passed). For conditions in which steady-state concentrations must be reached immediately, administer a loading dose. The risk of adverse drug reactions is obviously increased, so TDM can be used to proactively determine the proper maintenance dose. When a loading dose is being administered, TDM should be done after the loading dose to establish a baseline. The second TDM should be 1 drug half-life later to ensure that the maintenance dose is able to maintain the concentrations achieved by the loading dose. If the drug concentrations at the second sample do not match those of the first sample, the maintenance dose can be adjusted at this time rather than waiting for steady-state, with the risk of therapeutic failure or toxicity. The third time to do TDM is at steady-state to ensure an appropriate dosage regimen.

The number of samples collected for TDM depends on the drug, its $T_{½}$, and the reason for monitoring. To determine optimal aminoglycoside therapy, peak and trough samples are needed. To allow for the distribution phase, blood sampling for the peak concentration is done 0.5 to 1 hour after administration, and the trough sample is usually taken before the next dose. With very long elimination half-lives and twice-daily dosing, there are no statistically significant differences between C_{max} and C_{min} values for phenobarbital or potassium bromide. Therefore a single sample can be collected for TDM at any time during the dosing interval. However, C_{max} and C_{min} samples should be collected in any horse that is not responding as expected to therapy in order to determine whether the horse has a shorter- or longer-than-normal elimination time for the drug (Box 2.4).

Adjustment of Dosage Regimens

Adjustment of the dosage regimen is frequently required when drugs are administered to diseased horses, due to the fact that most dosage regimens have been established in a small number of normal horses. Adjustment is indicated when the drug's elimination or distribution is significantly altered in the animal. In general, the following rules are true:

- If the volume of distribution changes, change the drug dose.

TABLE **2.5 Recommendations for Therapeutic Drug Monitoring**

Drug	Therapeutic Range	Vd (L/kg)	$T_{½}$	Time to Steady State	Sample Collection
Amikacin	40 μg/mL peek <3 μg/mL trough	0.25	1–2 hr	1 day	Peak: 0.5–1 hr after administration Trough: just before next dose or 8 hr after dosing if using once-daily administration; collect in plastic only
Bromide	Monotherapy: 2–3 μg/mL (20–30 mmol/L) W/ phenobarb: 1–2 –g/mL (10–20 mmol/L)		24 days	4 mo	Any time
Digoxin	0.9–2.0 ng/mL	6.7	23 hr	7 days	2–5 hr after dose, collect in glass only
Gentamicin	20 μg/mL peek <2 μg/mL trough	0.3	1 hr	1 day	Peak: 0.5–1 hour after administration Trough: just before next dose or 8 hr after dosing if using once-daily administration; collect in plastic only
Phenobarbital	15–40 μg/mL (70–170 mmol/L)	0.96	24 hr	6 days	Any time
Quinidine	2–6 μg/mL	3.10	6.65 hr	40 hr	Trough
Theophylline	10–20 μg/mL	0.8	13 hr	3 days	1–2 hr after dose

BOX 2.4 Interpreting the Results of Therapeutic Drug Monitoring

PLASMA CONCENTRATIONS LOWER THAN ANTICIPATED

Poor compliance with regimen
Error in dosage regimen
Wrong product (e.g., controlled release instead of immediate release)
Poor bioavailability
Rapid elimination
Increased apparent volume of distribution
Poor timing of blood sample

PLASMA CONCENTRATIONS HIGHER THAN ANTICIPATED

Poor compliance with regimen
Dose incorrect
Very rapid drug absorption
Decreased apparent volume of distribution
Slow elimination

PLASMA CONCENTRATION CORRECT BUT POOR PATIENT RESPONSE TO THERAPY

Diagnosis incorrect
Altered tissue receptor sensitivity (tolerance)
Drug interaction at receptor site

- If the elimination of the drug changes, change the dosing interval.

For drug dosage regimens determined by TDM, modifications are made on a percentage basis:

$$\text{New dose} = \text{Old dose} \times (\text{Target} + \text{Concentration} \div \text{Measured concentration})$$

DOSAGE ADJUSTMENTS IN RENAL FAILURE

Renal disease can have a profound impact on the disposition of drugs administered to animals in renal failure. With reduced renal clearance, the parent drug, its metabolites, or both may accumulate in the patient and cause toxicity. Loss of proteins and electrolytes in urine and the alterations in acid-base balance associated with renal failure affect the pharmacokinetics and pharmacodynamics of drugs. Enhanced drug activity or toxicity can occur as a result of synergy with uremic complications. Altogether, these effects make it difficult to determine safe and effective drug dosages for veterinary patients in renal failure.

The goal of dosage adjustment is to provide a drug concentration–time profile in the horse with renal failure that is as similar as possible to that of a normal horse. The best approach to modifying drug therapy in horses with renal failure would be to carry out therapeutic drug monitoring and adjust the dosage for each patient. This is possible with some drugs, such as phenobarbital and digoxin, but it is impractical and cost prohibitive for most drugs used in veterinary practice. The best approach for most drugs is to estimate a corrected dose from available renal function tests and then to monitor the patient closely for evidence of efficacy or toxicity. For drugs that are eliminated primarily by renal mechanisms, creatinine (Cr) clearance correlates well with drug clearance. Cr is an endogenous product of creatinine phosphate metabolism in muscle. It is removed by glomerular filtration, and serum concentrations are relatively constant in healthy people and animals. The elimination half-life of a drug that is eliminated in urine remains stable until Cr clearance is reduced to 30% to 40% of normal, which is why drug dosage regimens are typically not adjusted until two thirds of

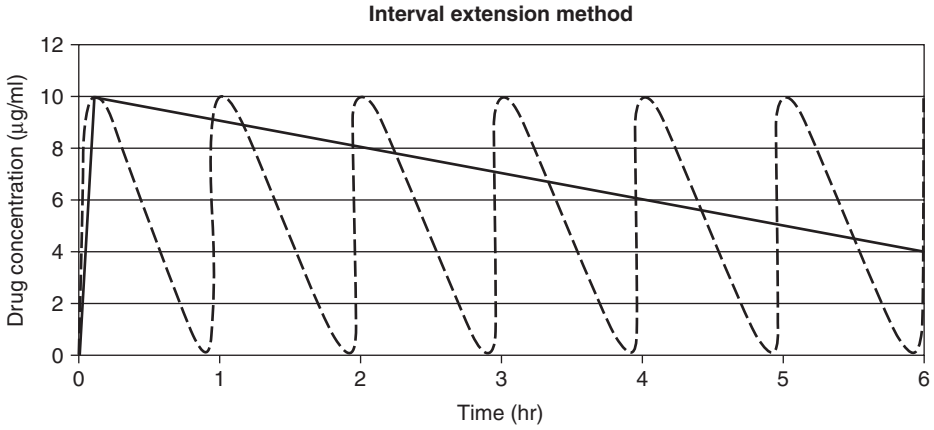


FIG. **2.17** Comparison of an interval-extension dosage regimen *(solid line)* in a renal failure patient with a normal dosage regimen in a healthy patient *(dotted line)*. Normal elimination half-life was 15 minutes; in the patient with renal failure, it increased to 8 hours.

renal function has been lost.[16] In human patients Cr clearance is quantified by determining urinary Cr excretion over a 24-hour period. The measured Cr clearance is then used in formulas to make drug dosage adjustments. Unlike in human medicine, values for Cr clearance are not usually available for veterinary patients. When Cr clearance is not available, a single value of the patient's serum creatinine can be substituted in the formulas. However, the relationship between serum creatinine is not linear once serum creatinine is above 4 mg/dL, so the adjustment formulas are even less accurate for predicting an ideal dose adjustment.[16] These formulas do not account for changes in the volume of distribution, degree of protein binding, and nonrenal clearance mechanisms of the drug that may be caused by the renal dysfunction. Therefore these dosage adjustments must be regarded as preliminary estimations to be followed by adjustments based on observed clinical response.

Dose-Reduction Method

With the dose-reduction method the normal dosage regimen is adjusted by reducing the drug dose and maintaining the drug dosing interval.

$$\text{Adjusted dose} = \text{Normal dose} \times (\text{Patient's Cr clearance} \div \text{Normal Cr clearance})$$

or

$$\text{Adjusted dose} = \text{Normal dose} \times (\text{Normal Cr} \div \text{Patient's Cr})$$

Interval-Extension Method

With the interval-extension method the drug dose is maintained and the drug dosing interval is extended.

$$\text{Adjusted interval} = \text{Normal interval} \left[1/(\text{Patient's Cr clearance}/\text{Normal Cr clearance})\right]$$

or

$$\text{Adjusted interval} = \text{Normal interval} \left[1/(\text{Normal serum Cr}/\text{Patient's serum Cr})\right]$$

Both methods attempt to keep the average plasma drug concentrations constant. The interval-extension method produces C_{max} and C_{min} values similar to those seen in healthy patients (Fig. 2.17), but it does produce substantial periods of time during which drug concentrations may be subtherapeutic. This is the preferred method with aminoglycosides, which have a long postantibiotic effect and in which a low trough concentration is desirable to reduce the risk of nephrotoxicity. Depending on the relationship of the elimination half-life to the dosage interval, significant drug accumulation may occur with the dose-reduction method (Fig. 2.18), but at steady-state there are no periods of time during which concentrations are subtherapeutic. This is the preferred method for the penicillin and cephalosporin antibiotics, wherein maintaining the plasma concentration above the pathogen's minimum inhibitory concentration (MIC) correlates with efficacy and the drugs are relatively nontoxic even if accumulation occurs. To decide which method to use, the practitioner should determine whether drug efficacy and toxicity are related to peak, trough, or average plasma concentrations and then select the method that best balances efficacy against potential toxicity. The interval-extension method is more convenient for the client because the normal recommended dose is simply administered less frequently. In addition, if the drugs are available only in fixed dosage forms (e.g., capsules, unbreakable tablets), it is easier to adjust the dosage interval.

Because the elimination half-life is prolonged in patients with renal disease and it always takes 6 elimination half-lives to reach 99% of steady-state concentrations, there is a delay in reaching steady-state in horses with renal failure compared with horses with normal renal function. Therefore it may be necessary to administer a loading dose to rapidly achieve therapeutic drug concentrations. If the dose-reduction method is used, this is achieved by giving the usual dose initially, followed by the reduced dose at the next time. If the interval-extension method is used, this is accomplished by giving a double dose initially.

For renal failure patients in general, the practitioner must consider the following:

1. Avoid using any drugs at all, unless there are definite therapeutic indications. If a drug is absolutely necessary, try to select one that is hepatically metabolized and excreted in bile rather than eliminated by the kidneys (e.g., doxycycline).
2. If therapeutic drug monitoring is available, tailor the drug dosage regimen to that specific patient.

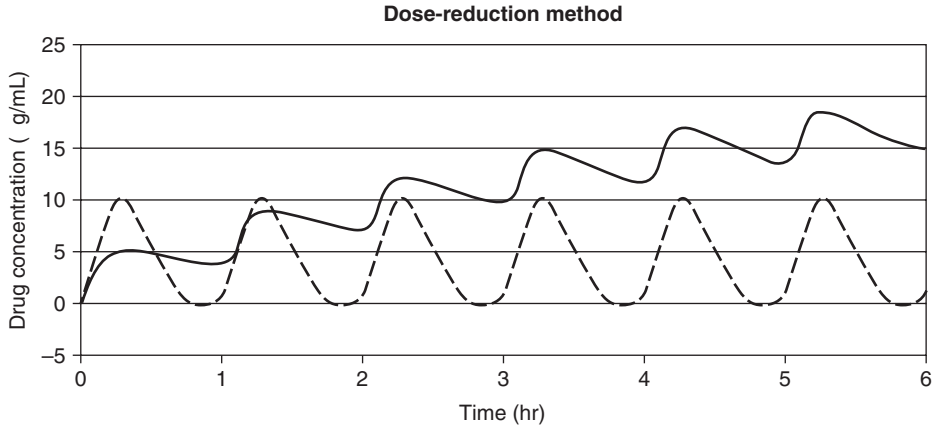


FIG. **2.18** Comparison of a dose-reduction dosage regimen in a renal failure patient *(solid line)* compared with a normal dosage regimen in a healthy patient *(dotted line).* Normal elimination half-life was 15 minutes; in the renal failure patient, it increased to 8 hours.

3. If therapeutic drug monitoring is unavailable, determine whether there are clinically proven, adjusted dosage regimens for specific drugs. The package insert that accompanies pharmaceuticals intended for human use often gives guidelines for adjusting dosages.
4. If the drug has not been sufficiently studied to have dosage adjustment recommendations, determine whether there is sufficient information about its kinetics to estimate the proper drug dose in renal failure.
5. Carefully monitor treated patients for signs of efficacy and toxicity.

Antimicrobial Therapy

Successful antimicrobial therapy relies on administering sufficient doses so that pathogens at the site of infection are killed or suppressed to the extent that they can be eliminated by the host's immune system. There are complex pharmacokinetic (PK) and pharmacodynamic (PD) relationships among the host, the bacteria, and the antimicrobial. Pharmacokinetics is what the body does to a drug; the processes of absorption, distribution to the various organs and tissues, metabolism, and elimination. Pharmacodynamics is what the antimicrobial does to the bacteria. It describes the drug action and responses of the bacteria. Pharmacokinetics and pharmacodynamics are interrelated in that PK effects determine the amount of drug that reaches the site of action, and the intensity of a PD effect is associated with the drug concentration at the site of action. New information on the PK/PD relationships of veterinary pathogens and antimicrobials is rapidly emerging and changing the way in which the dosage of antimicrobials is determined in equine practice.

RATIONAL USE OF ANTIMICROBIAL AGENTS

The following questions must be considered when developing an antimicrobial regimen:

1. *Does the diagnosis warrant antimicrobial therapy?* Using antimicrobials to treat minor infections or purely viral or inflammatory diseases is irrational, expensive, and potentially hazardous to the patient; it also encourages antimicrobial resistance. Clients have come to expect antimicrobials for trivial infections or in the event that an infection may develop. Equine practitioners must resist client pressure to use or prescribe unnecessary drugs.
2. *What organism(s) are likely to be involved?* For many infections the likely organism can be successfully predicted on the basis of the history and clinical signs (e.g., *Streptococcus zooepidemicus* and respiratory infections).
3. *What is the in vitro antimicrobial susceptibility of the organism?* For many pathogens the in vitro susceptibility can be reliably predicted. For example, *Streptococcus* species are typically susceptible to penicillin. However, many gram-negative bacteria have unpredictable susceptibilities, and susceptibility testing is essential for determining appropriate drug therapy.
4. *In what part of the body or tissue is the infection located? Will the antimicrobial penetrate to the infection?* Consideration of the pathophysiology of the infection will help the practitioner select an effective therapy. Treatment of sequestered infections such as mastitis or meningitis requires antimicrobials that readily cross membrane barriers. Antimicrobials characterized by low values for volume of distribution (Vd) are unlikely to reach therapeutic concentrations in such sites.
5. *Will the antimicrobial be effective in the local environment of the organism?* The local infection environment reduces the efficacy of some antimicrobials. Sulfonamides are ineffective in purulent debris because para-aminobenzoic acid (PABA) released from decaying neutrophils serves as a PABA source for bacteria and reduces the competitive effect of the sulfonamide. Aminoglycosides are ineffective in an abscess because of the acidic, anaerobic environment along with the presence of nucleic acid material from decaying cells, which inactivates this class of drug.
6. *What drug formulation and dose regimen will maintain the appropriate antimicrobial concentration for the proper duration of time?* Label doses apply only to label pathogens. For off-label uses the dosage regimen must be adjusted depending on the antimicrobial susceptibility of the specific pathogen.

7. *What adverse drug reactions or toxicities might be expected? Do the benefits outweigh the risks?* The risks of adverse reactions from antimicrobials are often underappreciated. A serious adverse reaction may complicate treatment of the original problem and even be fatal. Failure to communicate the risks of adverse drug reactions to clients is a common cause of litigation.
8. *Can you choose an approved product? If using an antimicrobial in an off-label manner, can you determine appropriate withdrawal times for food animals? Can you determine appropriate withdrawal times for performance horses?* The antimicrobials used in horses are not approved for horses intended for food, and information on appropriate withdrawal times is lacking. Also, competitive horses are subject to the rules of the sport's governing association regarding drugs, which vary according to the organization, state or province, and country. Understanding the principles of drug elimination allows the practitioner to determine appropriate withdrawal times for competitive or slaughter horses.

Documenting the Infection

A diagnosis must be established before any therapy can be administered. It is not always necessary to culture samples from all patients with infectious diseases to identify the organism involved. Often the practitioner can base a diagnosis on clinical experience from similar cases. The signs of some infectious diseases are so obvious that the need for microbiologic identification is minimal; however, for those infectious diseases of unknown cause or for those attributable to organisms with irregular antimicrobial susceptibility, there is no substitute for isolation and identification of the causative agent. For these organisms initial therapy while waiting for culture results must include an antimicrobial with a broad spectrum of activity.

Whenever possible, the veterinarian should obtain a representative sample of material from a clinical patient. Beware of sampling grossly contaminated sites such as purulent nasal discharges. Treatment failure may also be related to sampling of an inappropriate area. For instance, in cases of pleuropneumonia, failure to culture both transtracheal wash fluid and pleural fluid may result in an inability to culture all associated organisms. An immediate Gram stain can be performed from a direct smear and will direct initial therapy. Samples are submitted for appropriate culture and identification. If the identified organism has unpredictable susceptibility patterns, the practitioner should request a susceptibility test, such as Kirby-Bauer, E test, or MIC method.

In some clinical cases identification of the pathogen may be made by serologic demonstration of antibodies (e.g., anaplasmosis, leptospirosis, brucellosis).

Antimicrobial Dosage Regimen Design

The relationship among the host, the bacteria, and the drug may be very complex. High plasma antimicrobial concentrations are *assumed* to be advantageous in that a large concentration of drug will diffuse into the ECF of various tissues. Drug concentration at the site of infection is assumed to be of major importance in determining drug efficacy. In many infections the site of infection is the ECF; however, infections may also occur within cells or other protected sites. Drug movement from the plasma to extravascular tissues depends on molecular size, lipid solubility, drug p*K*a, local pH, specific cellular transport mechanisms, and degree of protein binding. The relationship between bacteria and drug in the laboratory is described by the following:

MIC: The lowest drug concentration that inhibits bacterial growth. Often expressed as the concentration that inhibits 50% (MIC_{50}) or 90% (MIC_{90}) of the bacterial growth.
Minimum bactericidal concentration (MBC): The lowest drug concentration that kills 99.9% of bacteria.
Mutant prevention concentration (MPC): Threshold concentration above which the selective proliferation of resistant mutants is expected to occur only rarely.

The MICs are used to determine the drug dose, in an attempt to achieve blood and tissue concentrations that exceed the in vitro MIC for the pathogen.

Interpreting Minimum Inhibitory Concentrations

According to the Clinical Laboratory Standards Institute (CLSI; formerly called the National Committee for Clinical Laboratory Standards [NCCLS]) definition, the MIC values are derived as serially doubled concentrations (in μg/mL). Susceptible (S), intermediate (I), and resistant (R) designations are derived from *breakpoints* assigned by laboratory-based PK and PD data, pathogen population MIC distribution, and clinical trial results.[17] A CLSI-approved breakpoint is specific for the animal species, disease, pathogen, antimicrobial, and dosage regimen. When a pathogen is reported as *susceptible,* it means that the recommended dosage of the antimicrobial will reach plasma or tissue concentrations that will inhibit bacterial growth in vivo. When a pathogen is reported as *resistant,* inhibitory antimicrobial concentrations are not safely attainable in the patient. If the pathogen is reported as *intermediate,* administering the antimicrobial at higher-than-recommended doses may result in effective therapy.[18] Pathogens with intermediate susceptibility may also be successfully treated if the infection is located in an area of high drug concentration (e.g., urine for renally excreted drugs) or by treatment with local routes of administration (e.g., topical administration to the eye for corneal infections).

Susceptibility testing results predict which bacteria have intrinsic or acquired resistance mechanisms to a particular antimicrobial. In vitro tests do this because bacterial susceptibility usually clusters around a small range of MICs. In Fig. 2.19 the bacterial inhibition of *E. coli* by amoxicillin has a bimodal distribution. The large cluster of *Escherichia coli* inhibited by amoxicillin concentrations of 0.5 to 16 μg/mL are considered the *normal range* and 16 μg/mL is considered the breakpoint of susceptibility. *E. coli* requiring an amoxicillin concentration of 16 μg/mL to be inhibited are likely to have intrinsic or acquired resistance mechanisms, and amoxicillin is unlikely to be a successful treatment for patients infected with these isolates. In vitro susceptibility tests predict treatment outcome fairly well, considering that many variables in the host-pathogen relationship are not taken into account.[17] Antimicrobial susceptibility data may not account for the following:

1. *Host defenses:* The interaction between the host and the pathogen is complex and not predicted by in vitro tests. Antimicrobial drug action takes place in concert with host defenses such as humoral and cell-mediated immunity; complement components; and nonspecific antibacterial factors such as lactoferrin, lactoperoxidase, and lysozyme.[19]

2. *Drug distribution in the body:* The S, I, and R designations assigned by the microbiology laboratory are typically based on safely achievable plasma concentrations. This does not necessarily take into account extremely high concentrations of antimicrobials achieved in organs and fluids of excretion (kidney, urine, bile) or with local administration of high drug concentrations (e.g., ophthalmic ointments). Macrolide antimicrobials are characterized by negligible plasma concentrations despite very high lung and intracellular concentrations.[20] Pathophysiology may alter drug distribution, and some antimicrobials, such as the tetracyclines, accumulate in pneumonic lung tissues.[21]
3. *Growth rates and size of inoculum:* The incubator of the microbiology laboratory is an ideal setting for bacterial growth. Conditions are managed to promote rapid growth, and rapidly dividing bacteria are more susceptible to antimicrobial drugs. Replication rates may be much slower at the infection site, and MICs are generally unreliable for slow-growing bacteria. Standardized inoculums used in the laboratory may overrepresent or underrepresent pathogen numbers in infected tissues.[19]
4. *Mixed infections:* Separate susceptibility testing of pathogens in a mixed infection does not account for the pathologic synergism between bacteria. The byproducts of one bacterium may facilitate the establishment and growth of another.[22]
5. *Infection environment:* Many antimicrobials are inactive in purulent exudate, which is typically anaerobic, acidic, and hyperosmolar. Some antimicrobials have different activity in body fluids (e.g., plasma, milk, bile) than in nutrient-rich laboratory media. Deposition of fibrin may alter tissue penetration of antimicrobials. Many bacteria are capable of producing a polysaccharide slime capsule to protect them from host factors. Mastitis pathogens typically increase their replication rate when incubated in mastitic milk.[19]
7. *Topically administered antimicrobials are not tested:* Veterinary microbiology laboratories may not routinely do susceptibility testing for antimicrobials that are used only topically. Polymixin B is one of the most effective antimicrobials for superficial *Pseudomonas* infections,[23] but because it causes neurotoxicity and nephrotoxicity if administered systemically, it is rarely included in susceptibility testing. Bacitracin and mupirocin are other examples of topical antimicrobials rarely tested by diagnostic laboratories.
8. *In vivo synergism may occur with antimicrobial combinations:* Despite predictions of resistance from susceptibility testing, therapy may be successful because of synergistic combinations of antimicrobials. Synergism between penicillins and aminoglycosides has been recognized for streptococcal, enterococcal, and staphylococcal infections.[24] The synergism is attributed to increased cellular uptake of the aminoglycoside after cell wall damage from the penicillin.
9. *The CLSI breakpoints may be inappropriate:* The CLSI breakpoints were originally established with bacterial isolates from humans, using human PK data and clinical trials. A veterinary subcommittee was established in 1993 and has proposed veterinary-specific guidelines for susceptibility tests for a limited number of antimicrobials.[25] Currently, only ceftiofur, ampicillin, amikacin, and gentamicin have CLSI-approved breakpoints for equine pathogens. Diagnostic laboratories use human-derived breakpoints for other bacteria-drug combinations. It is important to know if your laboratory uses the equine-specific breakpoints, as these can differ widely among species. For example, the amikacin breakpoint in humans is 16 μg/mL, in adult horses 4 μg/mL, and in foals 2 μg/mL.

Therefore the true relevance of any in vitro MIC in predicting the in vivo results of drug therapy is questionable. The MIC results are not a positive order to use a particular antimicrobial. Selection of the optimal antimicrobial must take into consideration other factors, such as the site of infection, pharmacokinetics of the drugs, and effect of underlying diseases. Considering all of these factors, most veterinarians determine a drug dosage regimen that uses a target plasma drug concentration based on a multiple of the in vitro MIC (usually 2–10).

Bactericidal Versus Bacteriostatic Antimicrobials

It is common to classify antimicrobials as *bactericidal* or *bacteriostatic* (Box 2.5). If the ratio of the MBC to MIC is small (less than 4–6), a drug is considered bactericidal, and it is possible to obtain drug concentrations that will kill 99.9% of the organisms exposed. If the ratio of MBC to MIC is large, it may not be

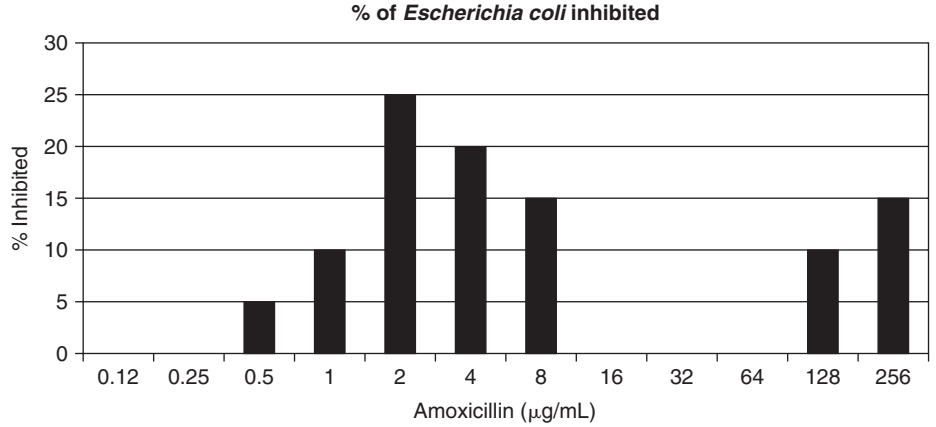


FIG. **2.19** The percentage of *Escherichia coli* inhibited by increasing concentrations of amoxicillin has a bimodal distribution. *E. coli* requiring more than 16 μg/mL of amoxicillin to be inhibited are likely to have intrinsic or acquired resistance mechanisms. Amoxicillin therapy in patients with this pathogen is unlikely to be effective.

possible to safely administer dosages of the drug to kill 99.9% of the bacteria, and the drug is considered bacteriostatic.

For many drugs the distinction between bactericidal and bacteriostatic is not exact and depends on the drug concentration attained in the target tissue and the pathogen involved. Specific situations in which a bactericidal drug is preferred over a bacteriostatic drug include immunocompromised patients such as neonates, life-threatening infections such as bacterial endocarditis and meningitis, and surgical prophylaxis.

Postantibiotic Effect

For some bacteria-antimicrobial interactions, bacterial growth remains suppressed for a period after drug concentration has decreased below the MIC.[26] This postantibiotic effect (PAE) may be the reason that dosage regimens that fail to maintain drug concentration above the MIC are still efficacious. The PAE depends on the antimicrobial and the bacterial pathogen (Table 2.6).

Pharmacokinetic-Pharmacodynamic Relationships

The PK-PD relationship between an antimicrobial and a pathogen determines the way that the dosage regimen is calculated.[27] The PK parameters used in drug dosage design are the area under the plasma concentration versus time curve from 0 to 24 hours (AUC_{0-24}), the maximum plasma concentration (C_{max}), and the time the antimicrobial concentration exceeds a defined PD threshold ($T >$ MIC). In relating the PK and PD parameters to clinical efficacy, antimicrobial drug action is classified as either concentration dependent or time dependent (Box 2.6; Figs. 2.20 and 2.21). For antimicrobials whose efficacy is concentration dependent, high plasma concentrations relative to the MIC of the pathogen (C_{max}:MIC) and the area under the plasma concentration-time curve that is above the bacterial MIC during the dosage interval (AUC_{0-24}:MIC) are the major determinants of clinical efficacy. These drugs also have prolonged PAEs, thereby allowing once-a-day dosing while maintaining maximum clinical efficacy. For fluoroquinolones (e.g., enrofloxacin, orbifloxacin, marbofloxacin), clinical efficacy is associated with achieving either an AUC_{0-24}:MIC greater than 100 to 125 for gram-negative organisms and AUC_{0-24}:MIC greater than 55 for gram-positive organisms or a C_{max}:MIC greater than 10. For aminoglycosides (e.g., gentamicin, amikacin), achieving a C_{max}:MIC greater than 10 is considered optimal for efficacy. Other antimicrobials that appear to have concentration-dependent activity include metronidazole (C_{max}:MIC > 10–25) and azithromycin (AUC_{0-24}:MIC > 25). For some pathogens with very high MIC values, such as *Pseudomonas aeruginosa,* achieving the optimum PK/PD ratios may be impossible with label or even higher-than-label dosages. In such cases underdosing is ineffective and merely contributes to antimicrobial resistance.

For antimicrobials with time-dependent efficacy, the drug concentration at the site of infection must be maintained above the MIC for some portion of the dosing interval. How much above the MIC and for what percentage of the dosing interval concentrations should be above the MIC are still being debated and are likely specific for individual bacteria-drug combinations. Typically, exceeding the MIC by 1 to 5 multiples for between 40% and 100% of the dosage interval is appropriate for time-dependent antibiotics. The $T >$ MIC should be closer to 100% for bacteriostatic antimicrobials and in patients that are immunosuppressed. These drugs typically require frequent dosing or constant rate infusions for appropriate therapy. In sequestered infections penetration of the antimicrobial to the site of infection may require high plasma concentrations to achieve a sufficient concentration gradient. In such cases the AUC_{0-24}:MIC and/or C_{max}:MIC may also be important in determining efficacy of otherwise time-dependent antimicrobials.

BOX 2.5 Classification of Antimicrobials

BACTERICIDAL
Aminoglycosides
β-lactams
Fluoroquinolones
Trimethoprim/sulfonamides

BACTERIOSTATIC
Chloramphenicol
Macrolides
Sulfonamides
Tetracyclines

DESIGNING THE DRUG DOSAGE REGIMEN

When designing specific antimicrobial dosage regimens, the practitioner targets a specific plasma drug concentration. High plasma antimicrobial concentrations are *assumed* to be advantageous in that a large concentration of drug will diffuse into various tissues and body fluids. In

TABLE 2.6 Length of Postantibiotic Effect for Selected Antimicrobials and Antibiotics

Microbe	Long PAE (>3 hr)	Intermediate PAE	Short PAE (<1 hr)
Gram positive	Fluoroquinolones	Aminoglycosides	—
	Macrolides	Penicillins	—
	Chloramphenicol	Cephalosporins	—
	Tetracycline	Carbapenems	
Gram negative	Fluoroquinolones	Carbapenems	Penicillins
	Aminoglycosides	—	Cephalosporins
	—	—	Trimethoprim/sulfonamides
Anaerobes	Metronidazole	—	—

PAE, Postantibiotic effect.

light of the previous information, antimicrobial dosage regimens are designed in one of two ways: either to maximize plasma concentration or to provide a plasma concentration above the bacterial MIC for some percentage of the dosage interval.

For *concentration-dependent antibiotics* with a prolonged PAE whose PK-PD relationship is to have an ideal C_{max}:MIC, and if the volume of distribution (Vd) of the antimicrobial is known, a precise drug dosage regimen for the pathogen can be calculated from the following equation:

$$\text{Dose} = (\text{Vd})(\text{Desired plasma concentration})$$

where the desired plasma concentration is some multiple of the MIC (usually 8–10) and once-daily dosing is assumed.

For *concentration-dependent antibiotics* whose PK-PD relationship is to have an ideal AUC_{0-24}:MIC, the following equation can be used to calculate a daily dose:

$$\text{Dose} = (AUC_{0-24}\text{:MIC})(\text{MIC})(\text{Cl})(F)(24\text{ hr})$$

where AUC_{0-24}:MIC is ≥ 100, Cl is clearance (volume of blood cleared of drug per day in mL/kg/day), and F is bioavailability.

For *time-dependent antibiotics* the objective is to keep the average plasma drug concentration above the pathogen's MIC for the duration of the dosage interval. Again, by using Vd and elimination half-life information, the practitioner can precisely calculate a dosing regimen:

$$\text{Dose} = \frac{(\text{Desired avg plasma concentration})(\text{Vd})(\text{Dosage interval})1.44}{\left(T_{1/2}\right)}$$

BOX 2.6 Classification of Antimicrobials Based on Means of Effect

CONCENTRATION-DEPENDENT ANTIMICROBIALS

Aminoglycosides
Fluoroquinolones
Metronidazole

TIME-DEPENDENT ANTIMICROBIALS

Cephalosporins
Chloramphenicol
Macrolides
Penicillins
Sulfonamides
Tetracyclines

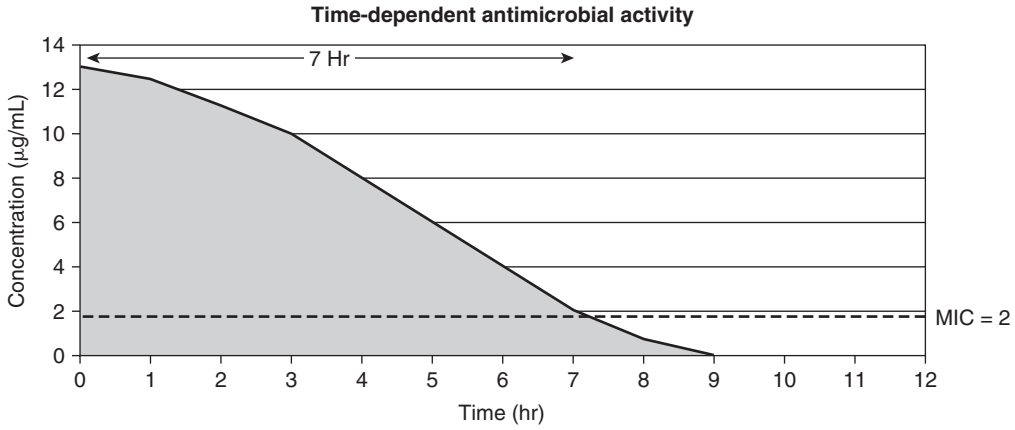


FIG. **2.20** For time-dependent antimicrobials the time during which the antimicrobial concentration exceeds the minimum inhibitory concentration of the pathogen determines clinical efficacy.

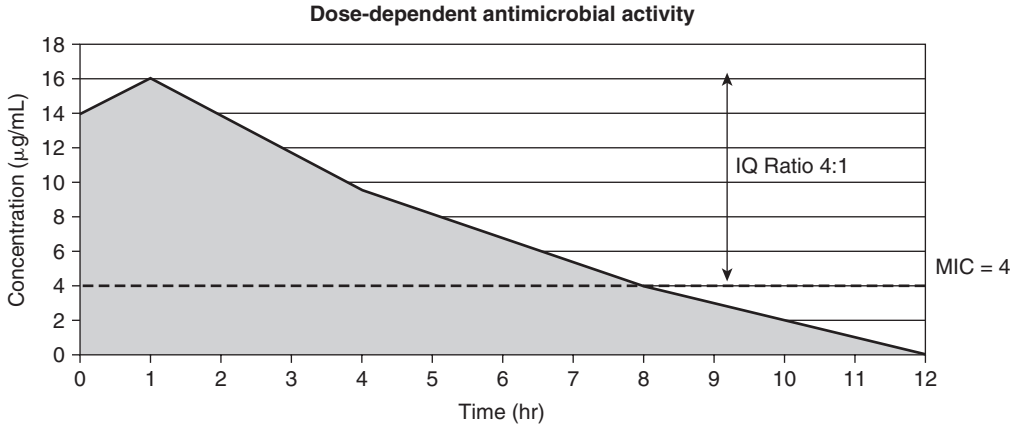


FIG. **2.21** For concentration-dependent antimicrobials, the inhibitory quotient *(IQ)* and the area under the inhibitory curve are the major determinants of clinical efficacy.

CONCURRENT USE OF ADDITIONAL ANTIMICROBIALS

Combination antimicrobial therapy is commonplace in veterinary medicine, but combination therapy has rarely been demonstrated as superior to single-drug therapy through clinical trials. Use of multiple antimicrobial drugs should be limited to the following cases:

1. Known synergism against specific organisms[24]
2. Prevention of rapid development of bacterial resistance[28]
3. To extend antimicrobial spectrum of initial therapy of life-threatening conditions[29]
4. To treat known mixed bacterial infections[30]

Multiple antimicrobial therapy is implicated as a cause of diarrhea in horses, likely from the additive antibacterial effects against normal GI flora.[31] Antagonistic combinations such as chloramphenicol and a macrolide should be avoided; both these classes of drugs competitively bind to the same binding site on bacterial ribosomes, thus effectively battling each other for the site of action.

PROPHYLACTIC USE OF ANTIMICROBIALS

There are few reports examining the efficacy of prophylactic antimicrobials in veterinary medicine in general and in horses specifically.[32] Because of the lack of controlled veterinary studies, information on prophylaxis is largely extrapolated from human studies. The relative risk of infection must warrant the use of prophylactic antimicrobials. The risks of adverse effects from the prophylactic drug must be less than the risk of development of disease and its consequences. In veterinary medicine most of the risk of infection depends on the skill of the surgeon and handling practices in the hospital.[33] The organism or organisms that are likely to cause the infection and their antimicrobial susceptibility should be known or accurately predicted. The antimicrobial should be bactericidal and must be administered and distributed to the site of potential infection before the onset of infection. The veterinarian should consider drugs that can be given intravenously and have a high volume of distribution. Drugs used prophylactically should not be those that would be used therapeutically. The duration of antimicrobial prophylaxis should be as abbreviated as possible. Most of the time a single preoperative dose is sufficient and cost effective.[34]

A comprehensive review of the subject has resulted in several recommendations being made regarding the use of antimicrobials in the absence of culture and sensitivity data or a definitive diagnosis.[35] These include the following:

1. Commonly used antimicrobial combinations remain the mainstay for treating purulent infections. Appropriate choice of an antibiotic that is not inactivated in a purulent environment is recommended.
2. Antimicrobial treatment for compromised foals should be instituted until sepsis is proven or disproven; however, treatment should not extend beyond recovery. Prophylactic administration of antimicrobials to healthy foals is not recommended.
3. Continuation of prophylactic antimicrobials for more than 3 days is likely unnecessary after colic surgery; shorter durations might be equally effective. Exceptions may include large colon volvulus, where an increased risk of septic peritonitis is present.
4. Antimicrobial prophylaxis in adult horses with diarrhea is unlikely to be beneficial, unless suspicion of a specific etiologic agent is extremely high based on prior use of antimicrobials *(Clostridium difficile/perfringens),* season of the year *(Neorickettsia risiticii),* and age *(Lawsonia extracellularis).*

β-LACTAMS: PENICILLINS AND CEPHALOSPORINS

The β-lactam antibiotics include the penicillins, cephalosporins, and carbapenems. They have excellent activity against most gram-positive bacteria and very few associated side effects. They are considered bactericidal and time dependent. Postantibiotic effects have been associated with some drugs in this class. In general, the β-lactam antibiotics have low plasma protein binding, distribute well to the ECF in most tissues, and are excreted renally. With a few exceptions, they have a very short half-life and require frequent dosing. β-Lactams do not distribute well to protected sites, such as the central nervous system, the eye, or the prostate.

Mechanism of Action

β-Lactam antibiotics act on enzymes called *penicillin-binding proteins (PBPs)* responsible for building the bacterial cell wall.[18] Therefore they are active only against rapidly multiplying organisms in which the binding of penicillin within the cell wall interferes with production of cell wall peptidoglycans and results in cell lysis in a hypo-osmotic or iso-osmotic environment. There may be anywhere from two to eight PBPs in a bacterium. When β-lactam antibiotics bind covalently and irreversibly to the PBPs, the bacterial cell wall is disrupted and lysis occurs. Differences in the spectrum and activity of β-lactam antibiotics are due to their relative affinity for different PBPs. To bind to the PBPs, the β-lactam antibiotic must first diffuse through the bacterial cell wall. Gram-negative organisms have an additional lipopolysaccharide layer that decreases antibiotic penetration. Therefore gram-positive bacteria are usually more susceptible to the action of β-lactams than gram-negative bacteria. Because the penicillins poorly penetrate mammalian cells, they are ineffective in the treatment of intracellular pathogens.

Resistance Mechanisms

Resistance mechanisms to the β-lactams include failure of the antibiotic to penetrate the outer bacterial cell layers and alteration of PBPs that decrease the affinity of the PBP for the antibiotic.[36-38] Alterations of the PBPs to a low-affinity PBP2a occur with methicillin-resistant staphylococci and are mediated by the presence of the chromosomal gene mecA.[39] The presence of this gene precludes therapy with any of the currently available β-lactam antibiotics and predicts resistance to several classes of antibiotics. A third mechanism of resistance is from production of β-lactamase enzymes.[40,41]

There may be as many as 50 β-lactamase enzymes (penicillinases, cephalosporinases, and carbapenemases) produced by bacteria. These enzymes hydrolyze the cyclic amide bond of the β-lactam ring and inactivate the antibiotic. Staphylococcal β-lactamases are produced by coagulase-positive *Staphylococcus* spp. The synthesis of these enzymes is plasmid encoded, and the enzymes are exocellular. These enzymes typically do not inactivate cephalosporins and antistaphylococcal penicillins.

Most of these β-lactamases can be inactivated by inhibitors such as clavulanic acid and sulbactam. The β-lactamase inhibitors are a specific class of drugs that inhibit bacterial β-lactamase, so they are administered in combination with β-lactam antibiotics.[42] These drugs combine with β-lactamase enzymes produced by gram-negative and some gram-positive bacteria. An inactive enzyme complex is formed, and the coadministered antibiotic is then able to exert its effect. New evidence suggests that β-lactamase inhibitors, once thought to have little antimicrobial activity of their own, bind to different PBPs, affecting autolysis and contributing to the activity of the concomitantly administered β-lactam antibiotic.[43] The majority of clinically isolated *B. fragilis* spp. are β-lactamase producers. Most of the enzymes are chromosomally mediated cephalosporinases, with activities against many narrow- and broad-spectrum penicillins and cephalosporins.

The gram-negative β-lactamases are a diverse group of enzymes that can be chromosomal coded, plasmid coded, or both. Chromosomal-mediated lactamases hydrolyze penicillin, cephalosporins, or both. In the past few decades, bacteria (mainly *E. coli* and *Klebsiella* spp.) have been discovered that produce what are known as extended-spectrum β-lactamases (ESBLs). These enzymes hydrolyze the extended-spectrum cephalosporins that have an oxyimino side chain, which include third- and fourth-generation drugs such as cefotaxime, ceftriaxone, and ceftazidime. The expression of ESBLs is plasmid mediated, and these plasmids often encode for other genes that infer resistance to antimicrobials of other classes (i.e., aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones). ESBL-producing strains are particularly feared as they are resistant to all penicillins, cephalosporins, and aztreonam.

PENICILLIN G (BENZYLPENICILLIN)

Penicillin G was the first antibiotic developed and remains one of the most effective antibiotics available. It is still the initial drug of choice for many bacterial infections.

Spectrum of Activity

Aerobic bacteria susceptible to penicillin G include most β-hemolytic streptococci, β-lactamase–negative staphylococci, *Actinomyces* spp., some *Bacillus anthracis, Corynebacterium* spp., and *Erysipelothrix rhusiopathiae.*[44] Most species of anaerobes are susceptible, excluding β-lactamase–producing *Bacteroides* spp.[45] Penicillin G is easily inactivated by β-lactamases and has little efficacy against organisms that can produce these enzymes. In addition, penicillin G is ineffective against those bacteria that are resistant by other mechanisms, such as having a relatively impermeable cell wall. Therefore penicillin G has little activity against many staphylococci and most gram-negative bacteria.[40]

Pharmacokinetics

Absorption

Because penicillin is a weak acid with a p*K*a of 2.7, it is highly ionized in plasma. Gastric absorption of penicillin G is poor because it is rapidly hydrolyzed in the acid environment of the stomach. Phenoxymethyl penicillin (penicillin V) can be given orally to horses and has a half-life of absorption of 0.2 hours.[46] The sodium and potassium salts of penicillin G are the only dosage forms that are suitable for IV administration and are quickly absorbed from IM or SC sites of administration.[47] Procaine penicillin G is more slowly absorbed from IM administration than are the sodium or potassium salts and so produces lower but more sustained plasma concentrations. The rate of absorption from IM injections of procaine penicillin G varies depending on the injection site, with injections into the neck muscle producing more rapid absorption and higher plasma concentrations than with injections into the hindquarters.[48] Benzathine penicillin G is the least soluble of the dosage forms; it is very slowly absorbed, producing sustained but subtherapeutic plasma concentrations of penicillin G.[47] Because the benzathine fraction is poorly absorbed and plasma concentrations are below the MICs of most pathogens, use of these products is not recommended in horses.[49]

Distribution

The Vd of sodium penicillin G is 0.7 L/kg,[50] and there is a moderate degree of protein binding (52%–54%).[35] After absorption penicillin is distributed mainly in ECF and may not reach therapeutic concentrations in sequestered infections.[49]

Elimination

Elimination of penicillin is primarily renal, with unchanged drug being excreted by glomerular filtration and active renal tubular secretion. The elimination half-life of penicillin G is 1 hour,[49,51] and total clearance is 8.5 ± 1.33 mL/min/kg.[50] Oral penicillin V has an elimination half-life of 3.7 hours.[46]

Adverse Effects and Drug Interactions

Immune-Mediated Reactions

Penicillin is associated with immune-mediated hemolytic anemia (type II hypersensitivity)[52,53] and anaphylaxis (type I hypersensitivity) in horses.[54] The immune-mediated anemia usually resolves with discontinuation of penicillin therapy. Anaphylaxis usually occurs after previous exposure to penicillin and can be fatal. IV epinephrine and oxygen administration and respiratory support are indicated. Although it is widely assumed that penicillin and cephalosporins are cross-reactive in sensitive individuals, the actual incidence in humans is extremely low.[55]

Procaine Reactions and Use in Performance Horses

When procaine penicillin G products are accidentally administered intravascularly, they cause extreme central nervous system stimulation.[56,57] Most horses will survive unless they fatally traumatize themselves during the reaction. Diazepam will attenuate the reaction if given before procaine administration but has no effect if given afterward.[56] Veterinary formulations contain higher concentrations of procaine than human formulations, and high temperatures increase the solubility of procaine. Therefore penicillin procaine G should be kept refrigerated and administered by careful IM injection. Even with careful IM injection, adverse reactions are still reported in horses. Repeated injections increase the chance of intravascular administration and may increase the sensitivity to procaine as a result of neuronal sensitization. Once absorbed into the circulation, procaine is hydrolyzed by plasma esterases to the nontoxic metabolites PABA and diethylaminoethanol.[58] The presence of PABA may inactivate sulfonamide antibiotics.[59] Horses that have had documented adverse reactions to

IM procaine penicillin G injections had significantly lower procaine hydrolyzing capacity compared with horses that did not have a history of adverse reactions.[60] Procaine is slowly eliminated in urine, is easily detectable by regulatory laboratories, and commonly causes violative residues in racehorses and performance horses given penicillin procaine G.[61]

Electrolyte Imbalances

The sodium or potassium content of IV formulations can contribute to electrolyte imbalances associated with congestive heart failure and renal function impairment. A million units of potassium penicillin contain 1.7 mEq of potassium, so it should be administered by slow IV injection. Potassium penicillin is not recommended for use in horses with hyperkalemic periodic paralysis (HYPP).

Phenylbutazone

Concurrent administration with phenylbutazone in horses increases plasma concentrations of penicillin G but lowers tissue concentrations. The effect is likely due to a lower peripheral distribution.[50]

Formulations

Only procaine penicillin G and combination procaine/benzathine penicillin products are currently labeled for use in horses. These formulations are labeled for a dose of 3000 IU/lb (6600 IU/kg), which is a much lower dose than that considered to be therapeutic. Procaine penicillin products are labeled for IM dosing once daily. Combination products are labeled for IM or SC dosing once every other day. Na^+ and K^+ penicillin (also called *crystalline penicillin*) are human formulation drugs used off-label intravenously, intramuscularly, or subcutaneously. They achieve rapid plasma concentrations but have very short elimination half-lives and therefore must be administered frequently or by continuous rate infusion. Potassium penicillin is usually less expensive than sodium penicillin but must be administered more carefully because rapid IV administration can cause cardiac arrhythmias. Procaine penicillin G is a poorly soluble salt that is slowly absorbed after IM injection. It is the most commonly used formulation of penicillin in horses. Benzathine penicillin G is a very insoluble salt that is used in long-acting penicillin preparations, which contain a 50:50 mixture of procaine penicillin G and benzathine penicillin G.

Clinical Use

Penicillin is still the antimicrobial of choice for many diseases in horses, such as streptococcal and anaerobic infections. Traumatic wounds are frequently infected with *Streptococcus zooepidemicus,* which is routinely susceptible to penicillin.[29] However, iatrogenic wounds are frequently infected with penicillinase-producing *Staphylococcus aureus* or other staphylococci, so culture and susceptibility testing is necessary before initiating penicillin therapy.[29,62]

The dosage of penicillin G is typically 22,000 to 44,000 IU/kg. Dosage frequency varies greatly depending on the formulation and on the disease being treated. Procaine penicillin G should be administered every 12 to 24 hours. Every-24-hour dosing is only recommended for treatment of streptococci. Potassium or sodium formulations are administered every 6 hours, although every-3-hour or constant rate infusion can be used in immunosuppressed patients or those with life-threatening sepsis.

AMINOPENICILLINS

Spectrum of Activity

The aminopenicillins are able to penetrate the outer layer of gram-negative bacteria better than penicillin G; therefore they have activity against many of the gram-negative bacteria (*E. coli, Salmonella, Pasteurella* spp.) as well as gram-positive bacteria.[18] However, resistance to the aminopenicillins is easily acquired by gram-negative bacteria, so they are not usually effective against *Klebsiella, Proteus, Pseudomonas,* and *S. aureus.* Most anaerobes are sensitive, except β-lactamase–producing strains of *Bacteroides.*[45] Amoxicillin penetrates the gram-negative cell more easily than does ampicillin; therefore it has greater activity against gram-negative bacteria.[44]

Pharmacokinetics

Absorption

In horses ampicillin sodium is well absorbed after IM or SC administration; however, oral dosage forms are poorly absorbed by adult horses.[63] The IM administration of ampicillin trihydrate produces lower ampicillin blood concentrations that extend over a longer period of time than does IM ampicillin sodium.[63] Oral absorption of amoxicillin is between 5.3% and 10.4% in adult horses[64,65] but between 36% and 42% in foals.[66] The ampicillin prodrugs are converted to ampicillin as they are absorbed from the GI tract. Compared with the bioavailability of oral ampicillin (2%), the ampicillin esters have improved oral bioavailabilities in adult horses: pivampicillin (31%), bacampicillin (39%), and talampicillin (23%).[51,67] The low oral bioavailability of ampicillin esters is due to chemical hydrolysis in the high pH of equine ileal contents.

Distribution

The aminopenicillins are rapidly and widely distributed into most body fluids; distribution into CSF is low unless the meninges are inflamed. The Vd of amoxicillin in adult horses is 0.19[68] and 0.27 L/kg in neonatal foals.[66] The Vd of ampicillin in horses ranges from 0.18 to 0.7 L/kg.[69,70] Peak serum concentrations of ampicillin are 6.2 to 9.7 μg/mL 16 minutes after an IM dose of 10 mg/kg of ampicillin sodium.[63] Penetration into synovial fluid is high,[63,71] and concentrations are increased and persist in infected joints.[72] In a subcutaneous tissue chamber model in ponies, concentrations of IV ampicillin sodium, oral pivampicillin, and IM procaine penicillin G remained above the MIC of *S. zooepidemicus* for 8, 12, and 24 hours, respectively.[73] The protein binding of amoxicillin is moderate (37%–38%),[74] whereas it is low for ampicillin (6%–8%).[51]

Elimination

Amoxicillin and ampicillin are primarily excreted unchanged in the urine. The elimination half-life of amoxicillin is approximately 1 hour in horses and foals.[65,66,72,74] The elimination half-life of ampicillin ranges from 0.5 hour to 2.3 hours.[63,69,70,75,76]

Adverse Effects and Drug Interactions

Amoxicillin and ampicillin have the same adverse effects as penicillin G. Their spectrum of action is greatly enhanced when combined with β-lactamase inhibitors such as clavulanic acid and sulbactam.

Formulations

Sodium ampicillin is available as a human aqueous formulation for IV, IM, and SC injection. The reconstituted aqueous formulations are unstable after more than a few hours. Ampicillin trihydrate is a poorly soluble, slow-release aqueous suspension approved for use in large animals. Absorption is erratic, and it produces prolonged but low plasma concentrations.

Clinical Use

Indications for ampicillin or amoxicillin are few insofar as they offer little advantage over benzylpenicillins because of acquired resistance in gram-negative bacteria. Based on a tissue cage inflammation model, an IM dosage of 15 mg/kg of ampicillin sodium given every 6 hours would be required to treat ampicillin-susceptible bacteria.[70] Sodium ampicillin may be substituted for potassium penicillin as a choice for surgical prophylaxis in horses with current hyperkalemia or HYPP.

ANTIPSEUDOMONAL PENICILLINS

Spectrum of Activity

Carbenicillin, ticarcillin, and piperacillin are the antipseudomonal penicillins.[18] This group of penicillins can penetrate the outer cell wall of *Pseudomonas* spp. and other gram-negative bacteria. They are susceptible to β-lactamase inactivation by *Klebsiella* spp. For this reason, these drugs are often combined with β-lactamase inhibitors. The drug combination most frequently used in equine medicine is ticarcillin and clavulanic acid (CA). This group has activity against gram-negative bacteria at the expense of activity against gram-positive bacteria. They retain activity against anaerobic bacteria and are synergistic when administered with aminoglycosides.

Pharmacokinetics

Absorption

Bioavailability of IM ticarcillin is 65%, and the elimination half-life is less than 1 hour in horses.[77,78] Absorption of ticarcillin CA in foals is age dependent; neonatal foals have a higher systemic bioavailability after IM administration than older foals (100% and 88% vs. 100% and 27%, respectively).[79] When administered intramuscularly in combination with clavulanic acid, ticarcillin demonstrates flip-flop kinetics, wherein the elimination half-life is longer after IM than IV injection as a result of slow absorption from the injection site.[80] Systemic absorption of both ticarcillin and CA is poor after intrauterine administration.[81]

Distribution

Peak endometrial concentrations of ticarcillin after IV administration were 12.9 μg/g but were greater than 150 μg/g when 6 g were diluted in 250 mL of saline and infused into the uterus.[77] The Vd of ticarcillin in older foals was 0.24 L/kg, and the Vd of clavulanic acid was 0.48 L/kg. In neonatal foals the Vd was higher at 0.69 L/kg, as would be expected for a low-Vd drug in a neonate, with its increased ECF volume.[79] In mares the Vd of ticarcillin was 0.13 L/kg, and the Vd of clavulanic acid was 0.18 L/kg.[81]

Elimination

Ticarcillin CA demonstrates age-dependent elimination in foals. In neonatal foals the Vd and clearance of ticarcillin were approximately double those reported for older foals and mares.[79] The renal elimination mechanism of ticarcillin appears immature in a 3-day-old foal but near normal adult function by 28 days of age. In mares the elimination half-life of clavulanic acid is 0.4 hour.[81]

Adverse Effects and Drug Interactions

When ticarcillin is administered intramuscularly into the hindquarters at a concentration of 400 mg/mL with 13.2 mg/mL CA, foals showed signs of significant local discomfort.[79] A lower concentration of drug did not cause signs of discomfort in neonatal foals.

Formulations

Ticarcillin sodium is approved in horses as a 240-mg/mL solution for intrauterine infusion for the treatment of endometritis caused by β-hemolytic streptococci. Ticarcillin disodium–clavulanic acid formulations are available for human use.

Clinical Use

Because of its strong antipseudomonal activity, intrauterine administration of ticarcillin is used to treat endometritis in mares. The ticarcillin β-lactamase inhibitor drugs can be used in combination with aminoglycosides for a synergistic effect against the pathogens commonly encountered in septicemic foals.[82]

CEPHALOSPORINS

Spectrum of Activity

The cephalosporins are a very large group of β-lactam antibiotics. They are most commonly grouped according to "generations," primarily on the basis of their antibacterial activity, as well as their susceptibility to β-lactamases (Table 2.7).[18] By convention, cephalosporins discovered before 1975 are spelled with a *ph* and after 1975 with an *f*.

Cephalosporins are broad-spectrum antibiotics with a wide range of antimicrobial activity.[18,83] They are usually active against β-hemolytic streptococci and against β-lactamase–producing staphylococci but not against methicillin-resistant (oxacillin-resistant) *Staphylococcus aureus* (MRSA) or mycobacteria. Most enterococci are resistant. In the absence of acquired resistance, *E. coli* and *Salmonella* are usually susceptible, as are some *Proteus* and *Klebsiella* spp. Fourth-generation cephalosporins are effective against Enterobacteriaceae and other gram-negative bacteria that are resistant to earlier generations of cephalosporins because of acquired β-lactamase resistance. Common gram-negative aerobic respiratory pathogens such as *Haemophilus* and *Pasteurella,* including β-lactamase producers, are usually susceptible to cephalosporins. Although most corynebacteria are susceptible, *Rhodococcus equi* is usually resistant. Only antipseudomonal cephalosporins (some third and fourth generation) are effective against *P. aeruginosa.* Activity against non–spore-forming anaerobic bacteria is variable and similar to that of the aminopenicillins. Cefoxitin is notably resistant to β-lactamase–producing anaerobes, including *Bacteroides fragilis.* Ceftiofur is active against respiratory pathogens such as streptococci, *Pasteurella* spp., and *Histophilus* spp. and most

TABLE **2.7 Pharmacokinetics of Cephalosporins in Horses**

Classification of Cephalosporins

	Characteristics	Examples
First generation	Effective against almost all gram-positive bacteria, including β-lactamase–positive staphylococci; enterococci are resistant; effective against some gram-negative bacteria,[a] although sensitive to enterobacterial β-lactamase	Cephacetrile, cephaloridine, cephalothin, cephapirin, cefazolin,[a] cefadroxil, cephadrine, cephalexin
Second generation	Greater activity against gram-negative bacteria due to increased resistance to β-lactamases[b]; similar gram-positive spectrum to first-generation drugs	Cefaclor, cefotetan,[b] cefoxitin,[b] cefuroxime, cefuroxime axetil, cefamandole, cefmetazole, cefonocid, cefprozil
Third generation	Greater activity against gram-negative organisms; some drugs active against *Pseudomonas aeruginosa*[c]; resistant to many β-lactamases; less active against gram-positive cocci, with a few exceptions[d]	Cefoperazone,[c] cefsulodin, ceftazidime,[c] cefotaxime,[d] ceftizoxime proxetil, ceftriaxone, ceftiofur,[d] cevofecin, cefdinir, latamoxef (moxalaxtam), cefetamet, cefixime, cefpodoxime, cefovicin
Fourth generation	Broad spectrum of activity against gram-positive and gram-negative bacteria; resistant to staphylococcal, enterobacterial, and pseudomonal β-lactamases	Cefepime, cefquinome, cefpirome

Relative Activity of Cephalosporins against Common Bacteria[e]

Drug	Generation	*S. aureus*[f]	*E. coli, Klebsiella, Proteus*	*Enterobacter*	*Pseudomonas aeruginosa*	*Bacteroides*	Other Anaerobes
Cephalothin	1	+++	++	–	–	–	+
Cefuroxime	2	++	+++	–	–	–	+
Cefoxitin	2	+	+++	+	–	++	++
Cefotaxime	3	++	+++	+	–	+	++
Ceftriaxone	3	+	+++	+	–	–	+
Ceftazidime	3	+	+++	++	+++	–	–
Ceftiofur	3	++	++	+	–	–	+
Cefepime	4	++	+++	+++	+++	–	+

Drug	Vd (L/kg)	$T_{½}$ or MRT (hr)	Protein Binding (%)	Clearance (mL/min/kg)	*F* (%)	Dose (mg/kg)
FIRST GENERATION						
Cefazolin[111]	0.19	0.6–0.8	8	5.51		IV: 11
Cephalothin[112]	0.15	0.25	18	13.6		IV: 11
Cephapirin[113]	0.17	0.9		10	95	IV, IM: 20
Cephradine[114]	0.4	1.6		6.7		IV, PO: 25
CEFADROXIL						
Adults[87]	0.46	0.8		7		IV: 25
Foals[86]	0.52	1.4			37–100	IV: 5, PO: 5–20
SECOND GENERATION						
Cefoxitin[115]	0.12	0.8		4.32	77	IV, IM: 20
THIRD GENERATION						
Ceftriaxone[98]	0.15	0.81		2.81		IV: 14
Cefotaxime[99]	0.29	0.6		5.2		IV: 40
CEFTIOFUR						
Adults[116]	0.43	5.11	99		42	IM: 2.2
Foals[91]	0.76			3		
Cefoperazone[117]	0.68	IV: 0.77 IM: 1.52		12	42	IV, IM: 30

Continued

TABLE **2.7 Pharmacokinetics of Cephalosporins in Horses—cont'd**

Drug	Vd (L/kg)	$T_{½}$ or MRT (hr)	Protein Binding (%)	Clearance (mL/min/kg)	*F* (%)	Dose (mg/kg)
FOURTH GENERATION CEFEPIME						
Adults[100]	0.23	2.1			100	IV, IM: 2.2
Foals[118]	0.18	1.65		1.33		IV: 14

[a]Most activity against gram-negative bacteria in this generation.
[b]Most activity against *Bacteroides fragilis*.
[c]Most activity against *Pseudomonas aeruginosa*.
[d]Most activity against gram-positive cocci in this generation.
[e]+++, Highly active; ++, moderately active; +, limited activity; –, no clinical activity. Susceptibilities for individual isolates may vary.
[f]Methicillin-susceptible *Staphylococcus aureus*.
IM, Intramuscular; *IV,* intravenous.

anaerobes but has less activity against *S. aureus* and Enterobacteriaceae than other third-generation drugs. *Bacteroides* spp. and *Pseudomonas* spp. are resistant to ceftiofur. When administered, ceftiofur is rapidly metabolized to the active metabolite desfuroylceftiofur. Desfuroylceftiofur is less active than ceftiofur against *S. aureus* and *Proteus* spp.[84] Diagnostic laboratories use a ceftiofur disk for susceptibility testing because of the instability of desfuroylceftiofur, so susceptibility testing results for staphylococci and *Proteus* spp. may not reliably predict the efficacy of ceftiofur therapy.

The broad-spectrum antibacterial activity of the cephalosporins may cause overgrowth ("superinfection") by inherently resistant bacteria, including *C. difficile,* which no longer have to compete with the normal microbial flora. Nosocomial infection with vancomycin-resistant enterococci (VRE) has become a serious problem in human hospitals. One of the highest risk factors for contracting a VRE infection in a human hospital is treatment with a cephalosporin.[85]

Pharmacokinetics

Absorption, Distribution, and Elimination

Numerous pharmacokinetic studies have been done on horses using multiple different cephalosporin antibiotics (see Table 2.7). In general, IM and SC administration of cephalosporins results in rapid drug absorption, but the extent varies with the drug and the species. Oral bioavailability is acceptable in neonates but rapidly becomes too low to be practical for older foals or adults.[86,87] An ester formulation of cefpodoxime, cefpodoxime proxetil, has been examined for use in horses. In a study in foals, oral absorption was good enough that a dose of 10 mg/kg every 6 to 12 hours produced plasma concentrations that would potentially treat infections.[88] The values for Vd in horses for the cephalosporins are typically low (less than 0.3 L/kg), indicating distribution primarily to ECF. However, they have good distribution into the ECF of most tissues, including pleural, pericardial, and synovial fluid. Penetration into cortical and cancellous bone is usually adequate.[89] Cephalosporins penetrate poorly into the ocular humor and, except for some third-generation drugs, do not achieve therapeutic concentrations in the central nervous system.[18] In general the third-generation cephalosporins have an increased ability to penetrate the central nervous system.[90] Higher Vd values for foals reflect the increased ECF compartment of the neonate.[86,91] Most cephalosporins are rapidly eliminated as the unchanged drug in the urine. Renal excretion of cephalosporins occurs through a combination of glomerular filtration and active tubular secretion. Therefore it may be necessary to modify the dosage regimen of most cephalosporins for patients in renal failure, although there is a low risk of adverse effects at high concentrations. For the cephalosporins that undergo hepatic metabolism, hepatic insufficiency may decrease metabolism and increase drug accumulation.

The two most recent cephalosporins to be approved for veterinary use are exceptions to the general pharmacokinetic attributes of this drug class. Ceftiofur crystalline free acid (CCFA) and cefovicin are sustained release formulations. Of these, CCFA is labeled for use in horses. This drug provides therapeutic concentrations (based on an MIC of ≤0.2 μg/mL for streptococci) for up to 4 days following a single dose and for 10 days using a two-dose regimen. Compared with ceftiofur sodium, it has a lower C_{max} and a longer T_{max}, with an absolute bioavailability of 100%.[92] The half-life of CCFA is approximately 4 times longer than that of ceftiofur sodium (81 hours vs. 21.5 hours), which is consistent with flip-flop kinetics. Pharmacokinetics are not significantly different when the dose is divided and administered in two different sites.[93] CCFA penetrates well into the endometrium and can be used for treatment of endometritis caused by *Streptococcus equi* subsp. *zooepidemicus.* However, it does not appear to cross the blood-placental barrier as administration to mares with streptococcal placentitis did not improve foal survival or prevent septicemia in foals.[94] Investigations in healthy neonatal foals showed that subcutaneous administration of CCFA had a lower *AUC,* shorter observed time to maximum concentration, and higher observed C_{max} compared with adult horses.[95] Weanling age foals (4–6 months old) also exhibit different pharmacokinetics, including higher peak plasma concentrations and *AUC* compared with adults; however, these differences were not significant enough to necessitate a difference in dosing regimen.[96]

Adverse Effects and Drug Interactions

In general cephalosporins have a high therapeutic index. The adverse effects of the cephalosporins are similar to those reported for the penicillins, including hypersensitivity allergic reactions. Bleeding disorders have been reported in humans but not in animals.[97] The reaction appears to be related to vitamin K antagonism and/or platelet dysfunction and is usually only an issue with drugs that contain an *N*-methylthiotetrazole side chain (cefoperazone, cefotetan, cefamandole). High doses of ceftiofur and cefotaxime

may cause anemia and thrombocytopenia. Ceftriaxone and cefepime cause GI disturbances after administration to foals and horses.[98-100] The currently available cephalosporins are considered to be potentially nephrotoxic by way of deposition of immune complexes in the glomerular basement membrane or from a direct toxic effect leading to acute tubular necrosis, although this does not seem to be an issue at clinically relevant doses.[101] Like the penicillins, cephalosporins can be synergistic with aminoglycosides against many pathogens.[102] In human medicine it is recommended that cephalosporins not be used in conjunction with aminoglycosides; however, animal studies demonstrate a protective effect of the cephalosporins against nephrotoxicity.[103]

Ceftiofur is associated with diarrhea caused by altered GI flora in horses.[104,105] Toxicity studies have shown that horses tolerate ceftiofur doses up to 11 mg/kg/day IM, with pain at the injection site and decreased feed consumption as the most common adverse effect at the highest dose. The main adverse effect associated with CCFA has been injection site reactions, which can be minimized by splitting the dose into two different injection sites, injecting no more than 10 mL per site, drawing up the drug with a separate needle and using a new needle for each injection site, and using a 16-g, 1.5-in needle. Subcutaneous administration of CCFA in adult horses appeared to increase the risk for injection site reactions, although this route is considered safe in neonatal foals.[95,106] Reported consequences of injection site reactions include fever (up to 103.5°F), abscessation, and clostridial myositis.

Formulations

Cefadroxil is available as veterinary-formulated tablets or suspension for small animals. Cephalexin is available as human tablets and suspensions for oral administration. Cefpodoxime proxetil is available as veterinary-formulated tablets (100 or 200 mg) for dogs. Cefepime (human) and ceftiofur (food animal "ready to use") are available as hydrochloride formulations. Ceftiofur sodium is labeled for horses and is available as a 1-g or 4-g sterile powder to be reconstituted in sterile water to a final concentration of 50 mg/mL. Once reconstituted, the product is stable for up to 12 hours at room temperature, 7 days when refrigerated, and 6 months when frozen. CCFA is available as sterile suspension equivalent to 200 mg/mL of ceftiofur in a caprylic/capric triglyceride and cottonseed oil base.

Clinical Use

Ceftiofur sodium is approved for treatment of respiratory disease caused by *S. equi* subsp. *zooepidemicus* at a dose of 2.2 to 4.4 mg/kg q24hr IM. Higher doses or more frequent intervals have been recommended for treating gram-negative organisms (e.g., *Klebsiella, Enterobacter, Salmonella*). Because these organisms are inherently more resistant, higher plasma concentrations are needed for efficacy. The susceptibility breakpoint for ceftiofur use in horses is very low (less than 0.25 μg/mL), and organisms other than streptococci may be classified in vitro as resistant. In septic neonatal foals, doses as high as 10 mg/kg IV q6hr have been used. However, more recent studies in foals have shown lower doses (5 mg/kg IV, SC q12hr) are sufficient for the treatment of most bacteria cultured from septic neonates (MIC_{90} < 0.5 μg/mL).[107] Constant rate infusion of ceftiofur has also been shown to be safe in foals at doses up to 20 mg/kg/day.[108] This dose rate is adequate for the treatment of bacteria with MICs up to 4 μg/mL.

CCFA will provide 10 days' worth of therapeutic concentrations against streptococci (MIC < 0.2 μg/mL) following a two-dose regimen (6.6 mg/kg IM, repeated 96 hours later). Extralabel dosing of the drug has been used in clinical practice, mainly through the use of prolonged dosing regimens. For treatment of streptococci, the recommended dosing regimen for CCFA is 6.6 mg/kg IM on day 1 and 4, then every 7 days after.[109] For more resistant bacteria (MIC ≤ 1 μg/mL), the recommended dosing regimen is 6.6 mg/kg IM every 4 days. Similar dosing regimens are recommended in weanling foals. For neonatal foals, administration at a dose of 6.6 mg/kg body weight SC every 72 hours would be expected to provide protection against bacteria isolated from neonatal foals, based on an MIC of 0.5 μg/mL.[95]

CARBAPENEMS

The carbapenems are the newest class of β-lactam antimicrobials. They have an extended spectrum of activity, including many gram-negative bacteria. The only notable exceptions include MRSA and resistant *Enterococcus faecium*. The high activity of these drugs is due to the ability to withstand the β-lactamases (including ESBL) and the ability to penetrate porin channels that usually exclude other drugs. Pharmacokinetic properties are similar to other β-lactam drugs in horses, including poor oral bioavailability, a short half-life, and low to moderate Vd, although carbapenems have a long postantibiotic effect. Clinical use in horses is typically limited to septic neonates because of cost and the need for appropriate antibiotic stewardship, as these are considered critically important drugs in human medicine. Drugs in this class include imipenem and meropenem.

Imipenem has been used in neonatal foals at a dose of 5 mg/kg IV infused over 20 minutes q6–8hr. A pharmacokinetic study in adult horses suggests a dose of 10 to 20 mg/kg imipenem by slow IV infusion q6hr would be necessary to maintain adequate plasma concentrations.[110] Imipenem is available in human injectable formulations in combination with cilastatin. Cilastatin inhibits renal brush border enzymes that would otherwise convert imipenem into a nephrotoxic metabolite. Prevention of this metabolism not only decreases toxicity of the drug, but it also increases the concentration of active parent drug into the urine.

Meropenem is a slightly less expensive than imipenem and is less irritating when injected IM or SC. Pharmacokinetic data on meropenem are lacking; however, based on therapeutic drug monitoring in clinical cases, the clearance was high (5.2 mL/min/kg) and the half-life was short (34.5 min). Due to its short half-life, a constant rate infusion of 10 μg/kg/min (15 mg/kg/day) is recommended. This dose will be adequate for bacteria with MICs ≤ 2 μg/mL. Pharmacokinetics appear to be linear over a range of 10 to 30 μg/kg/min with no noted adverse effects in the small number of clinical cases for which it has been used.

AMINOGLYCOSIDES

The aminoglycoside antibiotics include streptomycin, neomycin, gentamicin, amikacin, tobramycin, and kanamycin. They have a chemical structure of amino sugars joined by a glycoside linkage. The importance of this group in veterinary medicine is to treat serious infections caused by aerobic gram-negative bacteria and staphylococci. Amikacin and tobramycin

have excellent activity against *P. aeruginosa.* Nevertheless, the aminoglycosides remain important drugs in the treatment of severe gram-negative sepsis, although their highly cationic, polar nature means that distribution across membranes is limited. Single daily dosing is now recommended for most dosage regimens because it maximizes efficacy and reduces toxicity.

Mechanism of Action

The aminoglycosides are large molecules with numerous amino acid groups, making them basic polycations that are highly ionized at physiologic pHs. Aminoglycosides must penetrate the bacteria to assert their effect. Susceptible, aerobic gram-negative bacteria actively pump the aminoglycoside into the cell. This is initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. This interaction displaces divalent cations (Ca^{++}, Mg^{++}), which affects membrane permeability.[119] Once inside the bacterial cell, aminoglycosides bind to the 30S ribosomal subunit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. This changes the cell membrane permeability, resulting in additional antibiotic uptake, further cell disruption, and ultimately cell death.

Aminoglycoside action is bactericidal and concentration dependent. For example, concentrations of gentamicin in the range of 0.5 to 5.0 μg/mL are bactericidal for gram-positive and some gram-negative bacteria. At 10 to 15 μg/mL, gentamicin is effective against the more resistant bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Proteus mirabilis.*[120] The clinical implication is that high initial doses of aminoglycosides increase ionic bonding, enhancing the initial concentration-dependent phase of rapid antibiotic internalization, which leads to greater immediate bactericidal activity. Human clinical studies demonstrate that proper initial therapeutic doses of aminoglycosides are critical in reducing mortality from gram-negative septicemia. For antimicrobials whose efficacy is concentration dependent, high plasma concentration levels relative to the MIC of the pathogen (C_{max}:MIC ratio, also known as the *inhibitory quotient,* or *IQ*) and the area under the plasma concentration–time curve that is above the bacterial MIC during the dosage interval (area under the inhibitory curve, AUIC = AUC/MIC) are the major determinants of clinical efficacy. For the aminoglycosides a C_{max}:MIC ratio of 10 is suggested to achieve optimal efficacy.[27]

The aminoglycosides have a significant PAE, meaning the period of time during which antimicrobial concentrations are below the bacterial MIC but the antimicrobial-damaged bacteria are more susceptible to host defenses. The duration of the PAE tends to increase as the initial aminoglycoside concentration increases.[121]

Antimicrobial activity of aminoglycosides is enhanced in an alkaline environment (pH of 6–8). They bind to and are inactivated by the nucleic acid material released by decaying white blood cells. Therefore they are usually ineffective in the acidic, hyperosmolar, anaerobic environment of abscesses.

Spectrum of Activity

The aminoglycosides are effective against most aerobic gram-negative bacteria, including *Pseudomonas.*[18] They are somewhat effective against staphylococci, although resistance can occur. They are often effective against enterococci, but therapy against streptococci is more effective when combined with a β-lactam antibiotic. *Salmonella* and *Brucella* spp. are intracellular pathogens and are often resistant. Some mycobacteria, spirochetes, and mycoplasma are susceptible. Aminoglycosides are ineffective against anaerobic bacteria because aminoglycoside penetration into the bacteria requires an oxygen-dependent transport mechanism. Amikacin was developed from kanamycin and has the broadest spectrum of activity of the aminoglycosides. It is effective against strains not susceptible to other aminoglycosides because it is more resistant to bacterial enzymatic inactivation and is considered the least nephrotoxic.

Resistance Mechanisms

Aminoglycoside resistance is primarily due to enzymes encoded by genes located on bacterial plasmids. These phosphotransferases, acetyltransferases, and adenyltransferases act internally to alter the aminoglycoside and prevent it from binding to ribosomes.[18] Amikacin is least susceptible to enzyme inactivation. Plasmid-mediated resistance to the aminoglycosides is transferable between bacteria. A single type of plasmid may confer cross-resistance to multiple aminoglycosides and to other unrelated antimicrobials. Bacteria may also utilize other methods that reduce the efficacy of aminoglycosides. Some strains of bacteria are less permeable to aminoglycosides, requiring much higher concentrations of aminoglycosides to kill them and therefore can be selected for during treatment. Chromosomal-mediated resistance is minimal and develops slowly for most of the aminoglycosides, with the exception of streptomycin or dihydrostreptomycin; resistance to streptomycin can occur from a single-step mutation.

First Exposure Adaptive Resistance

Both subinhibitory and inhibitory aminoglycoside concentrations produce resistance in bacterial cells surviving the initial ionic binding.[122] This adaptive resistance is due to decreased aminoglycoside transport into the bacteria. Exposure to one dose of an aminoglycoside is sufficient to produce resistant variants of an organism with altered metabolism and impaired aminoglycoside uptake. In vitro animal and clinical studies show that the resistance occurs within 1 or 2 hours of the first dose. The duration of adaptive resistance relates directly to the elimination half-life of the aminoglycoside. With normal aminoglycoside pharmacokinetics, the resistance may be maximal for up to 16 hours after a single dose of aminoglycoside, followed by partial return of bacterial susceptibility at 24 hours and complete recovery at approximately 40 hours.[123] If the aminoglycoside is dosed multiple times a day or the drug concentration remains constant, as with a continuous infusion, adaptive resistance persists and increases. Adaptive resistance is likely to persist in peripheral compartments, which are often the site of infection because of the persistence of aminoglycosides at these sites. Dose administration at 24-hour intervals, or longer, may increase efficacy by allowing time for adaptive resistance to reverse.[122-124]

Pharmacokinetics

Absorption. See Table 2.8 for the pharmacokinetics of gentamicin, amikacin, and tobramycin in horses. Amikacin and gentamicin are rapidly and well absorbed from IM and SC routes of administration but are not absorbed orally.[119]

Distribution. The aminoglycosides are polar antibiotics; therefore distribution is limited to the ECF space. The Vd in most species ranges between 0.15 and 0.3 L/kg.[125,126] After parenteral administration, effective concentrations are

TABLE **2.8 Pharmacokinetics of Aminoglycosides in Horses**

Drug	Volume of Distribution (L/kg)	Half-Life or MRT (hr)	Clearance (mL/min/kg)	Dose (mg/kg)
AMIKACIN				
Foals, 3-day-old[2]	0.42	2.7	1.92	7
Foals, premature, hypoxic[161]	0.60	5.4	1.9	7
Neonatal, high sepsis score[126]	0.34	4.10	1.17	IV: 6.6
Horses[187]	0.14–0.2	1.14–1.57	1.28–1.49	IV: 4.4, 6.6, 11
Horses[188]	0.13	1.34	1.25	IV: 10
GENTAMICIN				
Foals, 1-day-old[125]	0.31	2.2	1.75	4
Foals, 1-month-old[125]	0.24	3.07	0.9	IV: 4
Mares, late pregnancy[128]	0.15	2.26	1.06	—
Horses[130]	0.17	1.66	1.41	IV: 3
Horses, endotoxic[130]	0.14	1.54	1.17	IV: 3
Horses[158]	0.12	0.78	—	IV: 6.6
Horses[127]	0.27	2.17	1.56	IV: 2.2
Horses[159]	0.14	3.0	—	IV, IM: 6.6
Ponies[129]	0.19	1.82	1.27	IV, IM: 5
Horses, intravenous fluids[131]	0.15	1.96	1.04	IV: 2.2
Horses, postoperative[132]	0.17	1.47	1.27	IV: 6.6
TOBRAMYCIN				
Horses[173]	0.55	4.02	2.17	IV: 4
Horses[174]	0.18	4.61	1.18	IV, IM: 4

IM, Intramuscular; *IV,* intravenous; *MRT,* mean residence time.

obtained in synovial, perilymph, pleural, peritoneal, and pericardial fluid.[127] Therapeutic concentrations are not achieved in bile, CSF, respiratory secretions, and prostatic and ocular fluids.[119] Gentamicin does not cross the placenta of late-term mares; however, endometrial tissue concentrations of gentamicin were higher than plasma concentrations after 7 days of IM therapy with a dose of 5 mg/kg every 8 hours.[128,129] The predominant site of drug accumulation is the renal cortex in most species. In endotoxemia, gentamicin concentrations increase in serum as a result of a fever-induced decrease in the volume of the ECF compartment.[130] The administration of therapeutic fluids, colic surgery, or peritoneal lavage does not significantly change the pharmacokinetics of concurrently administered gentamicin.[131-133]

Gentamicin distributed into synovial fluid in normal horses and reached a peak of 6.4 µg/mL at 2 hours with a single 4.4-mg/kg IV dose.[134] Intraarticular administration or regional perfusion techniques are excellent methods to deliver aminoglycosides locally and prevent the adverse effects of systemic therapy.[135-141] When intraosseous perfusion was compared with IV perfusion, each technique produced mean peak concentrations of amikacin ranging from 5 to 50 times that of recommended peak serum concentrations for therapeutic efficacy.[142] Gentamicin-impregnated polymethylmethacrylate beads or collagen sponges may also be used to achieve extremely high local concentrations of drug, while avoiding systemic toxicity.[143,144]

Elimination

The aminoglycosides are almost exclusively eliminated in urine by glomerular filtration.[119] The plasma elimination half-lives range from 1 to 3 hours in adult animals but are increased in animals with renal dysfunction.[126] Renal accumulation results in extended withdrawal times for food animals.

Foals

The pharmacokinetics of aminoglycosides in foals is different from that in adults. The most consistent difference is in the Vd, which is higher in neonatal foals due to a larger volume of ECF. Clearance is usually unaffected, except for slight increases in septic or compromised neonates. This results in an increased elimination half-life in animals less than 2 weeks of age.[126] It also results in a C_{max} lower than that in adult horses, necessitating higher doses and possibly longer dosing intervals.

Adverse Effects and Drug Interactions

Nephrotoxicity

The aminoglycosides enter the renal tubule after filtration through the glomerulus (Fig. 2.22). From the luminal fluid the cationic aminoglycoside molecules bind to anionic phospholipids on the proximal tubular cells.[145] The aminoglycoside is taken into the cell by way of carrier-mediated pinocytosis and translocated into cytoplasmic vacuoles, which fuse with lysosomes.[146] The drug is sequestered unchanged in the lysosomes. With additional pinocytosis drug continues to accumulate within the lysosomes. The accumulated aminoglycoside interferes with normal lysosomal function, and eventually the overloaded lysosomes swell and rupture. Lysosomal enzymes, phospholipids, and the aminoglycoside are released into the cytosol of the proximal tubular cell, disrupting other organelles and causing cell death.[147]

The risk factors for aminoglycoside toxicity include prolonged therapy (longer than 7–10 days), acidosis and electrolyte

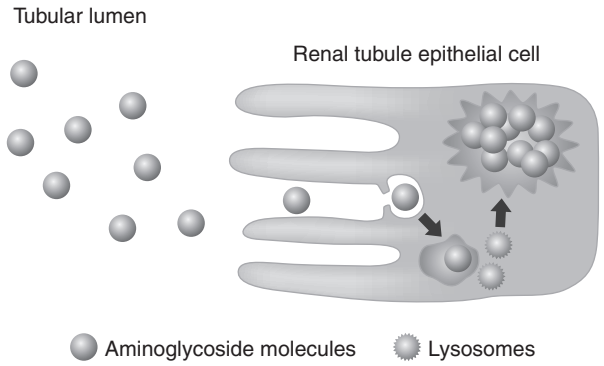


FIG. **2.22** Nephrotoxicity occurs from ionic binding of aminoglycoside molecules to polysaccharide cations on the proximal tubular epithelium, followed by pinocytosis and accumulation within lysosomes.

disturbances (hypokalemia, hyponatremia), volume depletion (shock, endotoxemia), concurrent nephrotoxic drug therapy, preexisting renal disease, and elevated plasma trough concentrations.[147-153] Calcium supplementation can reduce the risk of nephrotoxicity.[154] Administration of antioxidants such as silymarin and vitamin E may decrease aminoglycoside nephrotoxicity.[155] The risk of nephrotoxicity can also be decreased by feeding the patient a diet high in protein and calcium, such as alfalfa hay, because protein and calcium cations compete with aminoglycoside cations for binding to renal tubular epithelial cells.[156] High dietary protein also increases GFR and renal blood flow, reducing aminoglycoside accumulation.[157]

Uptake and accumulation of aminoglycosides into renal tubular epithelium demonstrate saturable kinetics. Because nephrotoxicity is related to aminoglycoside accumulation in the renal proximal tubular cells, it is logical that peak concentrations are not related to toxicity and that longer dose intervals result in less total drug exposure to the renal brush border membrane. High-dose, once-daily dosing of aminoglycosides has now become common in human and veterinary medicine; it takes advantage of the concentration-dependent killing and long PAE of these drugs and prevents first exposure adaptive resistance and toxicity.[132,158-160]

Individual horses can differ widely in the serum concentrations produced by the same aminoglycoside dosage regimen. There is a tendency to underdose neonatal patients, especially those that are receiving aggressive fluid therapy.[161] To maximize efficacy and minimize toxicity, therapeutic drug monitoring is recommended to ensure a C_{max}:MIC ratio of 10 is acheived.[27,119,162] Because the trough concentration is associated with nephrotoxicity, it is recommended that trough concentrations be less than 2 μg/mL for gentamicin and less than 5 μg/mL for amikacin before the next dose is administered.[119,162] To allow for the distribution phase, blood sampling for the peak concentration is performed 0.5 to 1 hour after IV administration, and the trough sample is usually taken before the next dose. The peak and trough concentrations can then be used to estimate the elimination half-life for the individual patient. An increase in the elimination half-life during therapy is a very sensitive indicator of early tubular insult.[163] If a once-daily regimen is used, a blood sample just before the next dose will be well below the recommended trough concentrations and may even be below the limit of detection of the assay. For these patients an 8-hour postdose sample will provide a more accurate estimate of the elimination half-life.

If therapeutic drug monitoring is unavailable, then once-daily high-dose therapy is recommended; the development of nephrotoxicity is detected by an increase in urine gamma glutamyl transferase (UGGT) enzyme and an increase in the UGGT:urine creatinine (UCr) ratio. The UGGT:UCr may increase to 2 to 3 times baseline within 3 days of a nephrotoxic dose.[148,163,164] If these tests are not available, the development of proteinuria is the next best indicator of nephrotoxicity and is easily determined in a practice setting.[163,164] Elevations in serum urea nitrogen and Cr confirm nephrotoxicity but are not seen for 7 days after significant renal damage has occurred.[126] Elimination half-lives of 24 to 45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug.[151] Although peritoneal dialysis is useful in lowering creatinine and serum urea nitrates, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside.[151] The horse's ability to recover most likely depends on the type of medication exposure and the amount of healthy renal tissue remaining to compensate.

Ototoxicity

Aminoglycoside ototoxicity occurs from the same accumulation mechanisms as nephrotoxicity. In the inner ear aminoglycosides appear to generate free radicals that subsequently do permanent damage to sensory cells and neurons.[165] Gentamicin damages the cochlear division of the eighth cranial nerve, resulting in vertigo, and amikacin damages the auditory division of the eighth cranial nerve, resulting in permanent deafness. This drug-specific toxicity may be due to the distribution characteristics of each drug and concentration achieved in each sensory organ.[166]

Neuromuscular Blockade

Neuromuscular blockade is a rare effect, related to blockade of acetylcholine at the nicotinic cholinergic receptor.[167] It is most often seen when anesthetic agents are administered concurrently with aminoglycosides.[168,169] Diseases that affect the neuromuscular junction, such as botulism, can also precipitate blockade. Affected patients should be treated promptly with parenteral calcium chloride at 10 to 20 mg/kg intravenously or calcium gluconate at 30 to 60 mg/kg intravenously to reverse dyspnea from muscle response depression. IV edrophonium at 0.5 mg/kg will also reverse neuromuscular blocking effects.[168]

Drug Interactions

Aminoglycosides are inactivated if combined in vitro with other drugs because of pH incompatibilities. In vitro, penicillins interact chemically with the aminoglycoside antibacterials to form biologically inactive amides by a reaction between the amino groups on the aminoglycosides and the β-lactam ring on the penicillins, inactivating both antibacterials. In vivo however, the aminoglycosides are synergistic against streptococci, *Pseudomonas,* and other gram-negative bacteria if combined with β-lactam antibiotics as a result of the bacterial cell wall being disrupted by the β-lactam antibiotic. Halothane anesthesia causes significant changes in the pharmacokinetics of gentamicin; total body clearance and volume of distribution decrease, and half-life of elimination increases.[169] A longer gentamicin dosing interval after anesthesia may help correct for the changes, but the practitioner should seriously consider selecting another antimicrobial. Neuromuscular blocking agents or drugs with neuromuscular blocking activity should not be used concurrently with aminoglycosides because they

can increase the risk of neuromuscular blockade, particularly during anesthesia.[168] Other nephrotoxic drugs should be avoided when possible during aminoglycoside therapy. Concurrent administration of phenylbutazone with gentamicin decreases the elimination half-life of gentamicin by 23% and decreases the Vd by 26%; the pharmacokinetics of phenylbutazone are not affected.[170] Flunixin has no effect on the pharmacokinetics of gentamicin when administered concurrently to adult horses.[132]

Formulations

Gentamicin and amikacin are available as brand name and generic solutions for intrauterine infusion in mares. Human and small animal labeled products for gentamicin, amikacin, and tobramycin are administered by IV, IM, SC, intraarticular, and intraosseous routes to horses. Gentamicin is also available in ophthalmic formulations for the treatment of gram-negative keratitis.

Clinical Use

Gentamicin and amikacin are commonly used to treat serious gram-negative infection in horses and septicemia in foals, often combined with β-lactam antimicrobials. Amikacin or tobramycin is used when antimicrobial resistance to gentamicin has developed in gram-negative pathogens, but gentamicin has greater activity against streptococci than amikacin.[29] The use of aminoglycosides in horses had previously been limited because of toxicity concerns, but high-dose once-daily dosing greatly reduces the risks. Use of the aminoglycosides is also limited by their poor penetration of cellular membranes and inactivation in purulent environments.

AMPHENICOLS

Chloramphenicol (CHPC) was isolated in 1947 from a soil actinomycete from Venezuela. Florfenicol (FLF) is a fluorinated derivative of CHPC. Although CHPC is well tolerated by horses, FLF alters fecal consistency, enteric flora, and susceptibility of *Salmonella* spp., *E. coli,* and *Clostridium perfringens*, and its use is not recommended.

Mechanism of Action

Chloramphenicol is a bacteriostatic antibiotic that inhibits protein synthesis by binding to ribosomal subunits of susceptible bacteria, leading to the inhibition of peptidyl transferase and thereby preventing the transfer of amino acids to growing peptide chains and subsequent protein formation.[175] There is a very wide spectrum of activity, including streptococci, staphylococci (including many MRSA), anaerobes, *Haemophilus, Salmonella, Pasteurella, Mycoplasma,* and *Brucella* spp., as well as rickettsia, chlamydia, and hemobartonella.[2]

Mechanisms of Resistance

Bacterial resistance to CHPC results from plasmid-mediated bacterial production of acetylase enzymes. Acetylation of hydroxyl groups prevents drug binding to the 50S ribosomal subunit.

Pharmacokinetics

Absorption

CHPC is rapidly absorbed after oral administration. The oral bioavailability of CHPC in foals is 83%[176] but only 40% after a single administration in mares, and bioavailability declines to 20% after five doses.[177] Due to the high lipid solubility of CHPC, even topical administration in the eye can result in significant systemic absorption.

Distribution

Because of high lipid solubility and low protein binding, CHPC is widely distributed throughout the body. Highest drug levels are attained in the liver and kidney, but therapeutic drug concentrations are attained in most tissues and fluids, including abscesses, ocular humor, and synovial fluid.[18] CHPC may achieve CSF concentrations of up to 50% of plasma concentrations when the meninges are normal, and more if inflammation is present.[178] The Vd of CHPC is 2.83 L/kg in horses[179] and 1.6 L/kg in neonatal foals.[160] The degree of protein binding of CHPC in horses is 30%.[180]

Elimination

In most species CHPC is eliminated by renal excretion of parent drug and by hepatic glucuronide conjugation and elimination in feces. The elimination half-life of CHPC in foals older than 7 days and adult horses is less than 1 hour.[176,179-182] In 1- and 3-day-old foals the elimination half-life is 5.3 and 1.4 hours, respectively, indicating the immaturity of the foal's hepatic metabolism capacity.[181] The elimination half-life is greatly prolonged in premature foals, and this drug should be used with caution.

Adverse Effects and Drug Interactions

Insofar as these drugs are protein synthesis inhibitors, dose-related anemia and pancytopenia are associated with chronic therapy (longer than 14 days), causing a decrease in protein synthesis in the bone marrow. FLF is more likely than CHPC to cause reversible bone marrow suppression with chronic dosing or overdose.[183] In humans an idiosyncratic aplastic anemia occurs with exposure to CHPC.[184] The reaction is rare (1 in 30,000) and not dose related. Toxic effects are related to the presence of the para-nitro group on the CHPC molecule. If CHPC is dispensed for client use at home, appropriate client education should always accompany the prescription, including dissolving the drug rather than crushing tablets and wearing gloves during and washing hands after administration.

CHPC is a hepatic microsomal enzyme inhibitor. It decreases the clearance of other drugs metabolized by the same cytochrome P450 enzymes, including phenytoin, phenobarbital, pentobarbital, phenylbutazone, xylazine, and cyclophosphamide.[185,186]

In general, CHPC should not be administered concurrently with penicillins, macrolides, aminoglycosides, or fluoroquinolones. CHPC may antagonize the activity of penicillins or aminoglycosides, and they act on the same ribosomal site as the macrolides.[187,188] Inhibition of protein synthesis by CHPC interferes with the production of autolysins necessary for cell lysis after fluoroquinolones interfere with DNA supercoiling.[189]

Formulations

CHPC sodium succinate is a water-soluble formulation for IV use and is hydrolyzed to CHPC in the liver. CHPC free base and CHPC-palmitate are available for oral administration. The CHPC-palmitate is hydrolyzed in the GI tract to CHPC. Ophthalmic formulations of CHPC are also available.

Clinical Use

CHPC is banned for use in any type of food animal because it can lead to idiosyncratic aplastic anemia in humans. In countries where its use is permitted in horses, it is used for a variety of bacterial infections, especially when penetration into abscesses (e.g., umbilical abscesses) and the central nervous system is desired, but appropriate precautions should be taken in handling the product to prevent human exposure. Because of the short half-life, high doses administered frequently are recommended (50 mg/kg PO q6–8hr).

POTENTIATED SULFONAMIDES

The sulfonamides are a group of organic compounds with chemotherapeutic activity (hence they are antimicrobials, not antibiotics). They have a common chemical nucleus that is closely related to *p*-aminobenzoic acid, an essential component in the folic acid pathway of nucleic acid synthesis. Sulfonamides are combined with diaminopyrimidines such as trimethoprim (TMP) and pyrimethamine (PYM), which inhibit an essential step farther along the folate pathway. Because the potentiated sulfonamides are remarkably synergistic and nontoxic, they are commonly used in equine medicine. Their use is complicated by differences in pharmacokinetics among TMP and PYM and the various sulfonamides used in the combinations.

Mechanism of Action

The sulfonamides inhibit the bacterial enzyme dihydropteroate synthetase (DPS) in the folic acid pathway, thereby blocking bacterial nucleic acid synthesis. Sulfonamides substitute for PABA, preventing its conversion to dihydrofolic acid. Alone, this action is considered bacteriostatic. Because the activity is by competitive substitution, the sulfonamide tissue concentration must be kept high enough to prevent bacterial access to PABA. Therefore the sulfonamides are ineffective in pus and necrotic tissue, which provide additional sources of PABA to the bacteria. The sulfonamides are nontoxic to mammalian cells because they use dietary folate for synthesis of dihydrofolic acid and do not require PABA. The addition of TMP or PYM to a sulfonamide creates a bactericidal combination. TMP inhibits bacterial folic acid synthesis at the next step in the folic acid sequence, inhibiting the conversion of dihydrofolic acid to tetrahydrofolic acid by inhibiting dihydrofolate reductase. This enzyme is present in both bacteria and mammalian cells, but the bacterial enzyme is inhibited at much lower concentrations than necessary to inhibit the mammalian enzyme.[190] The MICs against specific susceptible bacteria for each drug are generally lowered when the antimicrobials are administered in the potentiated sulfonamide combination. The resistance developed to the potentiated sulfonamides is lower than that to each individual drug; this is an important benefit because resistance to sulfonamides is very common and resistance develops rapidly to diaminopyrimidines when used alone.[191]

The potentiated sulfonamides have a broad spectrum of activity. The following bacteria are usually susceptible: *Streptococcus, Proteus, E. coli, Pasteurella, Haemophilus,* and *Salmonella* spp. Staphylococci, anaerobes, *Nocardia, Corynebacterium, Klebsiella,* and *Enterobacter* are susceptible but may become resistant. *Pseudomonas* spp., *Bacteroides* spp., and enterococci are usually resistant.[29,62,192] Other significant organisms that are susceptible to potentiated sulfonamides include protozoa *(Toxoplasma gondii, Sarcocystis neurona)* and coccidia. PYM is more effective than TMP against protozoa.[191] The potentiated sulfonamides are formulated at a fixed ratio of 1:5 of TMP to sulfonamide. The optimum concentration for bactericidal action is 1:20.[190] When and where this optimum concentration is achieved are difficult to predict in vivo, but a 1:20 ratio is used in susceptibility testing.[193]

Mechanisms of Resistance

Bacterial resistance to the sulfonamides is common and may be from chromosomal mutations or mediated by plasmids. Chromosomal mutations can lead to bacterial hyperproduction of PABA, which overcomes the competitive substitution of the sulfonamides. Plasmid-encoded resistance results in a bypass of the drug-sensitive step by production of altered forms of the DPS enzyme with a lower affinity for sulfonamides. Resistance to TMP usually occurs by plasmid-encoded production of TMP-resistant dihydrofolate reductase. Other resistance mechanisms include excessive bacterial production of dihydrofolate reductase (DHFR) and reduction in the ability of the drug to penetrate the bacterial cell wall. Cross-resistance among sulfonamides is considered complete and often occurs with pyrimidines.[190] Resistance to the TMP-sulfonamide combinations develops slowly but is now common among equine bacterial isolates.[29,194]

Pharmacokinetics

Pharmacokinetics of the potentiated sulfonamides are complicated by the distinct differences between disposition of TMP and PYM and the various sulfonamides. When sulfonamides and diaminopyrimidines are administered concurrently to horses, the pharmacokinetics of each drug appears to be unaffected by the presence of the other. Table 2.9 outlines the pharmacokinetics of specific potentiated sulfonamide combinations in horses. Although the potentiated sulfonamides are frequently used interchangeably, pharmacokinetic studies show that they are not bioequivalent in horses.

Absorption

In general, potentiated sulfonamides are readily absorbed from the GI tract of horses, but absorption may be affected by feeding.[195-197] For TMP and sulfachlorpyridazine, peak plasma concentrations and bioavailabilities are significantly reduced when the drug is mixed with concentrate compared with nasogastric administration.[198] Both drugs also demonstrate a biphasic absorption pattern, and it appears that this is due to a portion of the TMP and sulfachlorpyridazine dose binding to feed, with a second absorption phase occurring in the large intestine.[199] Bioavailability following intrauterine administration was 23% to 43% for TMP and 29% to 34% for sulfadoxine, and both were detected in the milk of lactating mares.[200] The oral bioavailability of PYM is 56% in horses.[201]

Distribution

Because the sulfonamides are weak acids and relatively hydrophilic, they distribute well in ECF and typically have values for Vd of 0.3 to 0.7 L/kg. Concentrations of sulfonamides in tissues are generally lower than those in plasma. The diaminopyrimidines are lipophilic weak bases and penetrate intracellularly better than sulfonamides, resulting in values for Vd of 1.5 to 2.7 L/kg and higher tissue concentrations than plasma

TABLE **2.9 Pharmacokinetics of Trimethoprim, Pyrimethamine, and Sulfonamides in Horses**

Drug	Volume of Distribution (L/kg)	Half-Life or MRT (hr)	Protein Binding (%)	Clearance (mL/min/kg)	Dose (mg/kg)
Trimethoprim	—	2.4	—	—	PO: 5
Sulfadiazine[196]		7.4			PO: 25
Trimethoprim	2.0	2.8	35	8.8	IV: 2.5
Sulfadiazine[218]	0.5	4.6	20	1.5	IV: 12.5
Trimethoprim	1.5	3	50	—	IV: 8
Sulfadoxine[206]	0.39	14	14–72		IV: 40
Trimethoprim	2.8	3.4	—	11	IV: 7.5
Sulfamethoxazole[222]	0.5	4.8	—	1.4	IV: 36.5
Trimethoprim	1.6	1.9	—	13	IV: 2.5
Sulfamethoxazole[202]	0.33	3.5	—	1.3	IV: 12.5
Trimethoprim	1.5	2.6	—	7.7	IV: 5
Sulfachlorpyridazine[198]	0.26	3.8	—	2.6	IV: 25
Pyrimethamine[201]	1.5	12	—	1.6	IV, PO: 1
Sulfadiazine[207]	0.4	3.8	43	2.3	IV: 20
Sulfamerazine[207]	0.49	3.2	44	1.8	IV: 20
Sulfamethazine[197]	0.63	11.4	—	0.8	IV: 160
Sulfamethazine[207]	0.33	5.4	69	0.9	IV: 20

IV, Intravenous; *MRT,* mean residence time; *PO,* oral.

concentrations.[190] Distribution of potentiated sulfonamides has been broadly investigated in the horse. Sulfadiazine and TMP and sulfamethoxazole and TMP are all well distributed into peritoneal fluid, CSF, synovial fluid, and urine.[202-204] Inflammation in the meninges or synovium does not significantly affect distribution into the respective fluids. After repeated doses sulfamethoxazole, unlike TMP, accumulates in the CSF.[202] CSF concentrations of PYR reach 25% to 50% of serum concentrations but do not appear to accumulate in horses with daily dosing.[205]

Sulfonamides can be highly bound to plasma proteins, but the extent of binding depends on species, drug, and concentration. In the horse the degree of protein binding varies from 33% for sulfaphenazole to 93% for sulfamethoxine.[190] Approximately 50% of TMP is protein bound, and binding is independent of plasma concentration.[206]

Metabolism

Diaminopyrimidines and sulfonamides are metabolized by the liver, usually by acetylation, aromatic hydroxylation, and glucuronidation.[190] The acetylated, hydroxylated, and conjugated forms of the sulfonamides are significantly less microbiologically active than their parent compounds. The precise metabolic pathways for TMP or PYR have not been elucidated. Metabolites may compete with the parent drug for involvement in folic acid synthesis. They have little detrimental effect on bacteria, so their presence can decrease the activity of the remaining parent drug.[207,208]

Elimination

Sulfonamides are primarily excreted in urine, but excretion in feces, bile, milk, sweat, and tears also occurs. Renal excretion of unchanged drug and metabolites occurs by glomerular filtration and active tubular secretion.[190] Reabsorption occurs in the distal tubule by passive diffusion. Because most sulfonamides are weak acids, alkaline urine increases their ionization and elimination. Renal excretion of TMP occurs by glomerular filtration, active tubular secretion, and reabsorption. In horses it appears that a large percentage of TMP is metabolized before elimination in urine (46%) and feces (52%). The clearance of the diaminopyrimidines is affected by urine pH, plasma concentration, and extent of diuresis. In contrast to the sulfonamides, alkaline urine increases the reabsorption of the basic TMP.[206]

Adverse Effects and Drug Interactions

The potentiated sulfonamides are noted for their widely varying adverse effects. Crystalluria, hematuria, and renal tubular obstruction can result from poorly soluble sulfonamides, especially in dehydrated patients with acidic urine.[190] However, the lower doses of sulfonamide used in the potentiated sulfonamide combinations make crystallization less likely than with sulfonamides administered alone. Local infusion of potentiated sulfonamides into the uterus of mares caused irritation of the endometrium and a decreased pregnancy rate.[200] Intramuscular administration is not recommended because of tissue irritation from the organic solvents, high concentration, and high pH of the formulations. IV administration must be done by slow and careful injection. Rapid administration is associated with thrombophlebitis and anaphylaxis.[190,209] The concurrent use of IV potentiated sulfonamides with detomidine is contraindicated because it appears that the potentiated sulfonamide sensitizes the myocardium and results in cardiac dysrhythmias and hypotension that may be fatal.[210,211] The procaine in procaine penicillin G is a PABA analog and may reduce efficacy if used concurrently with potentiated sulfonamides.[18]

Folate Antagonism Effects

Anemia, thrombocytopenia, and even pancytopenia may be seen in response to long-term, high-dose administration, such as in the treatment of equine protozoal myeloencephalitis (EPM). Anemia may be related to folate deficiency due to inhibition of folate production by intestinal bacteria or inhibition of its conversion to tetrahydrofolate and dihydrofolate. Coadministration of TMP and PYR does not increase the efficacy against protozoa and is suspected to increase the incidence of adverse effects caused by folate reduction. Supplementation with oral folic acid is often recommended for horses on long-term potentiated sulfonamide therapy.[212] The administration of oral folic acid to pregnant mares being treated for EPM may not protect the fetus from the effects of folate deficiency. Mares have delivered foals with congenital defects after oral administration of potentiated sulfonamides, despite supplementation with oral folic acid during treatment.[213] Treatment with TMP-sulfamethoxazole and PYM does not affect semen quality, testicular volume, sperm production efficiency, erection, or libido of healthy stallions. However, treatment may induce changes in copulatory form and agility and alter the pattern and strength of ejaculation.[214] Stallions that develop neurologic signs during treatment should be bred with caution. TMP-sulfamethoxazole has been associated with immune-mediated hemolytic anemia in a horse.[215]

Effects on GI Flora

The effects of potentiated sulfonamides on normal GI flora are controversial. In some studies potentiated sulfonamides alone or with concurrent penicillin or aminoglycoside therapy are associated with diarrhea in horses.[216,217] Other studies show little effect on fecal flora.[218,219] The likelihood of any antimicrobial therapy causing diarrhea in a horse depends on several factors, including the antibacterial spectrum of the drug and the drug concentrations achieved in the GI tract. The presence or absence of potential pathogens in the composition of the individual horse's microflora and the presence of antimicrobial-resistant pathogens in the hospital or clinic environment are also important factors in the incidence of GI disturbances. Despite the low incidence of colitis in horses treated with potentiated sulfonamides, the client and clinician should be aware of the potential, as cases can be rapidly fatal.

Formulations

TMP-sulfadiazine is available as a 48% injectable solution for IV administration in horses. It is also available as oral suspension, paste and powder formulations for horses. Generic tablets of TMP-sulfamethoxazole available for human use are commonly administered to horses. A commercially available PYR-sulfadiazine formulation is marketed for the treatment of equine protozoal myelitis in horses.

Clinical Use

It is very difficult to apply pharmacokinetic principles to determine drug dosage regimens for the potentiated sulfonamides. Different pathogens have varying MIC values, and the optimal ratio of TMP or PYM to sulfonamide also varies among bacteria and protozoa. The most important component of the formulation for efficacy appears to be the diaminopyrimidine, and the choice of sulfonamide may not be nearly as important. Therefore there is considerable controversy regarding the dosage regimen of these combinations. Most veterinary products are labeled for once-daily administration, but studies indicate that twice-daily dosing is better for attaining therapeutic plasma concentrations.[218,220] However, the newest formulation approved in the United States is a suspension labeled for dosing at 24 mg/kg PO q12hr. Pharmacokinetic data available from this product showed that this dosing strategy produced free drug concentrations that exceeded the MIC_{90} for *Streptococcus equi* subsp. *zooepidemicus* (SDZ-TMP: 4.75/0.25) for the entire 24-hour dosing interval. Despite frequent clinical use for equine streptococcal infections, even prophylactic administration failed to prevent abscessation caused by *Streptococcus equi* subsp. *zooepidemicus.*[221]

TETRACYCLINES

Tetracycline was discovered after a team of workers examined 100,000 soil samples from around the world. Tetracycline derivatives include *oxytetracycline, chlortetracycline, doxycycline,* and *minocycline.* Oxytetracycline (OTC), doxycycline (DXC), and minocycline (MNC) are used in horses.

Mechanism of Action

The tetracyclines bind to the 30S ribosomal subunit and interfere with bacterial protein synthesis. They are bacteriostatic at usual therapeutic concentrations but bactericidal at high concentrations. Drug entry into the bacteria is by an energy-dependent mechanism. Mammalian cells do not possess the tetracycline transport mechanism. Tetracyclines are most active at an acidic pH. The tetracyclines are broad-spectrum in activity; they are effective against gram-positive and gram-negative bacteria, as well as *Chlamydia, Mycoplasma,* and *Rickettsia* spp. and some protozoa (*Haemobartonella, Anaplasma* spp.). Their activity against staphylococci is limited, and they are not active against enterococci. *Pseudomonas, E. coli, Klebsiella,* and *Proteus* are usually resistant. Most anaerobes are susceptible to DXC.[18] In vitro concentrations of OTC above 0.01 μg/mL effectively suppress growth of *Neorickettsia risticii.*[223] Tetracyclines are also effective against intracellular pathogens of foals, such as *Lawsonia intracellularis* and *Rhodococcus equi.*[224] Multiple other actions have been attributed to DXC and MNC, including anticollagenolytic activity through inhibition of matrix metalloproteinases (MMPs), antiinflammatory activity, and ability to enhance corneal repair.[225,226]

Mechanisms of Resistance

Widespread acquired resistance in many pathogens limits the clinical usefulness of the tetracyclines. Resistance occurs from plasmid-mediated failure in the active transport of the drug into the bacterial cell and increased efflux from the cell. Another major mechanism of resistance involves cytoplasmic production of a protein that protects the ribosome from tetracycline action.[18]

Pharmacokinetics

Absorption

Oral absorption of OTC is erratic, and oral administration is not recommended in horses because of its adverse effects on GI flora.[219] The long-acting formulation of OTC in polyethylene glycol has a bioavailability of 83% after IM injection in horses.[227]

At an oral dose of 10 mg/kg in fed horses, DXC produced serum, synovial fluid, peritoneal fluid, and endometrial tissue concentrations above 0.25 μg/mL, suggesting effective therapy

for gram-positive infections.[228] At an oral dose of 20 mg/kg in fasted horses DXC produced mean maximum plasma concentration of 0.91 μg/mL following a single dose and 1.74 μg/mL after multiple doses using a 12-hour dosing interval.[229] A precise bioavailability cannot be determined because IV administration of DXC causes cardiac toxicity.[230] However, using allometric scaling, the estimated systemic absorption for DXC after oral administration in horses is only 2.7%.[229] Oral absorption of DXC in foals is higher, with a single oral dose of 10 mg/kg producing mean maximum plasma concentrations of 2.54 μg/mL and 4.05 μg/mL after multiple doses using a 12-hour dosing interval.[231] Steady-state mean plasma concentrations of minocycline following administration of 4 mg/kg PO q12hr were 0.67 μg/mL, and concentrations were above the MIC_{50} and MIC_{90} for many gram-positive equine pathogens (0.25 μg/mL).[232] Although it has not been studied directly, based on data from different studies, MNC oral bioavailability is approximately 23%.

Distribution

The tetracyclines are well distributed to most tissues, except the central nervous system. Therapeutic levels may be achieved, however, when the meninges are inflamed. Tetracyclines readily diffuse into milk.[18] OTC reaches 50% of plasma concentrations in synovial fluid and peritoneal fluid. Urine OTC concentrations are relatively high, with peak concentrations above 1500 μg/mL.[233] The Vd of OTC in neonatal foals is 2 L/kg[234] and 0.34 to 0.95 L/kg in adult horses.[227,235] The apparent Vd of DXC in horses after oral absorption is 25 L/kg, indicating the high lipid solubility and tissue penetration of DXC. Synovial and peritoneal fluids achieve the same DXC concentrations as plasma, and endometrial tissue concentrations are more than twice plasma concentrations. DXC is not detectable in CSF after oral administration.[228] DXC concentrates intracellularly, with mean C_{max} in polymorphonuclear cells approximately 17 times higher than concentrations found in plasma.[229] In foals peritoneal fluid, synovial fluid, and bronchoalveolar lavage cell activity of DXC were similar to plasma concentrations; however, activity in the CSF was significantly lower, and activity in pulmonary epithelial lining fluid and urine was significantly higher.[231] Minocycline has a Vd of 1.53 L/kg in adults. Synovial fluid concentrations of minocycline were approximately 74% of the corresponding trough plasma concentrations. Minocycline penetrates the CSF to a greater extent than OTC and DXC with a mean CSF concentration of 0.39 μg/mL at steady state, which was 69.5% of the corresponding plasma concentration.

Ocular penetration of systemic DXC and MNC has been studied. Vitreal drug concentrations of DXC were 0.17 μg/mL after multiple doses of 10 mg/kg.[236] Aqueous humor concentrations were approximately 0.11 μg/mL after multiple doses of 20 mg/kg, which represented 7.5% of corresponding plasma concentrations.[229] DXC was also detectable in the preocular tear film of horses without ocular disease after once-daily administration of 20 mg/kg.[225] As with the CSF, MNC penetrated into the aqueous humor of horses to a greater extent, with concentrations being 17% to 20% that of plasma concentration at steady-state.[232]

OTC is 50% protein bound in horses.[237] Plasma protein binding of DXC is high (82%), which is similar to that of other species.[229] This impairs distribution of DXC into the ECF. Plasma protein binding of MNC is 68% in horses.[238] The increased free fraction of drug may explain the better penetration into the eye and CSF of MNC compared with DXC.

Elimination

The tetracyclines are not known to be biotransformed to any significant extent before elimination. OTC is eliminated in urine unchanged primarily by glomerular filtration. Unmetabolized drug is also eliminated with bile into the GI tract and may undergo enterohepatic recirculation, prolonging its effects.[18] DXC is primarily excreted into the feces by way of nonbiliary routes in an inactive form. Therefore DXC does not accumulate in patients with renal insufficiency.[239] The clearance of OTC in foals is 3.3 mL/min/kg[234] and 2.2 mL/min/kg in adult horses.[227] When administered intravenously, the elimination half-life is 7 hours in foals[234] and 6 hours in horses.[227] Because of flip-flop kinetics, the elimination half-life is 22 hours after IM administration of OTC in polyethylene glycol.[227] DXC clearance cannot be accurately determined from oral dosing.[229] The half-life of MNC in adult horses is 8 to 11 hours, and the plasma clearance is 2.7 mL/kg/min.[232,238]

Adverse Effects and Drug Interactions

GI Effects and Interactions

Calcium-containing products (e.g., milk, antacids) or other divalent cations will chelate with tetracyclines and interfere with GI absorption.[18] Because DXC is less likely than the older tetracyclines to form chelation complexes with divalent and trivalent metals, there is less interference with oral absorption by calcium or other substances.[239]

The clinical use of OTC in horses is controversial because of reports of adverse GI effects. However, adverse effects were also associated with excessive dosage,[240] concomitant use of other antimicrobials, and stressors such as surgery and transport.[241-244] Anecdotally, OTC therapy has been used successfully in equine practice, and recognition of the equine rickettsial diseases has increased OTC use in horses.[245-247] In a chronic dosing study using a long-acting formulation of OTC, no deleterious effects on fecal flora were detected, and treated horses remained clinically normal.[248] DXC is less likely to cause adverse GI effects because it is bound in an inactive form in the intestines; however, at higher doses the risk of adverse effects may be increased. One study reported that doses of 20 mg/kg orally twice daily produced signs of abdominal discomfort in one horse and a severe enterocolitis in another. The other four horses in that study were unaffected.[229] DXC administered to foals at 10 mg/kg orally twice daily for 8 to 17 days for treatment of *Lawsonia intracellularis* infection did not produce any adverse effects.[224] No adverse GI effects of minocycline have been reported, although clinical use has been limited compared with other drugs in this class.

Renal Effects

Renal tubular necrosis caused by OTC is associated with high doses, outdated parenteral products, endotoxemia, dehydration and hypovolemia, and concurrent pigment nephropathy.[18,249] In normal foals single high-dose IV OTC administration for the correction of flexural deformities does not cause renal toxicity.[250] Multiple doses administered at dosing intervals less than every 48 hours may increase the risk of nephrotoxicity in foals. Oliguric renal failure developed in a foal that had concurrent neonatal isoerythrolysis given 70 mg/kg of IV OTC for a flexural deformity.[249]

Cardiovascular Effects

Rapid IV administration of OTC results in hypotension and collapse. This is attributed to intravascular chelation of calcium, decreased blood pressure from the drug vehicle (propylene glycol), or both. Pretreatment with IV calcium borogluconate prevents collapse.[251,252] Both rapid IV bolus and slow constant rate infusion of DXC to horses causes tachycardia, arrhythmias, systemic arterial hypertension, collapse, and death.[230,253] It is suggested that this reaction is due to chelation of intracellular calcium, resulting in neuromuscular blockade of the myocardium. Plasma total and ionized calcium concentrations are not affected by IV DXC administration.

Musculoskeletal Effects

Intramuscular injection of long-acting OTC formulations causes localized pain and swelling at the injection site.[18,248] OTC causes flexor tendon relaxation; this effect has been used to treat foals with flexural deformities.[254,255] OTC induces a dose-dependent inhibition of collagen gel contraction by equine myofibroblasts. Inhibition of normal collagen organization may provide the mechanistic explanation for the results seen after the pharmacologic treatment of flexural deformities by OTC administration.[256] Because of the neuromuscular blocking effects, tetracyclines are not recommended for the treatment of diseases affecting the neuromuscular junction, such as botulism.

Doxycycline and Rifampin Combination Therapy

Doxycycline is a possible treatment for foals that develop macrolide-resistant *R. equi* infections. However, a study examining the use of DXC combined with rifampin in foals with naturally occurring clinical disease noted that some foals developed hemolytic anemia and icterus or increased liver enzymes 17 to 20 days after initiating the treatment. The cause of these reactions is unknown, and similar clinical findings have not been seen in foals treated with doxycycline alone or with rifampin combined with macrolides.[257] At this time, this combination should only be used with caution and not as a first-line treatment for *R. equi*.

Formulations

Injectable OTC products are formulated as short- or long-acting products. The short-acting solutions are in propylene glycol and have concentrations of 50 or 100 mg/mL. The long-acting solutions are in 2-pyrrolidone or polyethylene glycol and have a concentration of 200 mg/mL. The polyethylene glycol formulation is less irritating than the 2-pyrrolidone formulation. The long-acting formulations may be administered by slow IV injection, but the long-acting effect is lost.[210] DXC and MNC tablets are available in multiple generic and proprietary formulations.

Clinical Use

OTC is the drug of choice for treatment of Potomac horse fever *(Neorickettsia risticii)* and equine anaplasmosis *(Anaplasma phagocytophilum)*. It is also used to treat contracted flexor tendons in foals. Its use for other microbial infections in horses is controversial because of concerns related to adverse GI effects and widespread antimicrobial resistance. DXC and MNC are also indicated for rickettsial diseases in horses and may be a suitable oral alternative to OTC. Tetracyclines are commonly used for treatment of proliferative enteropathy caused by *Lawsonia intracellularis*. DXC is also used for the treatment of keratomalacic diseases in horses because it may decrease corneal proteinases such as MMP-2 and MMP-9.[225]

MACROLIDES AND AZALIDES

The macrolide antibiotics include erythromycin, clarithromycin, tylosin, tilmicosin, and tiamulin. Azalides, such as azithromycin and gamithromycin, have a similar mechanism of action but have a methylated nitrogen in the macrocyclic ring. Triamilides, such as tulathromycin, are semisynthetic macrolides prepared by fermentation followed by organic synthesis. Because of their adverse GI effects, these drugs are typically contraindicated in adult horses; however, erythromycin, clarithromycin, and azithromycin are commonly used in foals.

Mechanism of Action

The macrolides and their derivatives bind to the 50S ribosomal subunit in a manner similar to that of CHPC, and they interfere with protein synthesis. They are usually considered bacteriostatic but may be bactericidal at high concentrations. Macrolides are not effective against gram-negative bacteria, except some strains of *Pasteurella* and *Haemophilus.*[18] Azithromycin is more active than the macrolides against gram-negative bacteria and anaerobes.[223,258] Susceptible bacteria include staphylococci, streptococci, *Campylobacter jejuni, Clostridium* spp., *Rhodococcus equi, Lawsonia intracellularis, Mycoplasma* spp., and *Chlamydia* spp.[259]Antimicrobial activity of these weak bases is optimum at an alkaline pH; therefore they have reduced activity in acidic environments (e.g., pus, abscesses) but may be clinically effective because of high concentrations due to ion trapping. Erythromycin has nonantimicrobial effects on host cell metabolism, inflammatory mediators, and GI motility.[260,261]

Mechanisms of Resistance

The routine use of the macrolides is limited because bacterial resistance develops quickly after repeated exposure.[262] Mechanisms of resistance include decreased drug entry into bacteria, inability to bind to the bacterial 50S ribosomal subunit, and plasmid-mediated production of esterases.[18] Resistance to erythromycin has been reported in 3.9% of *R. equi* isolates.[263] Extensive cross-resistance occurs among the macrolides.[18]

Pharmacokinetics

Absorption

Erythromycin is available for oral administration as enteric-coated erythromycin base, erythromycin esters (ethylsuccinate or estolate), and erythromycin salts (phosphate or stearate). Because of expense, many practitioners administer the drug as crushed enteric-coated tablets of erythromycin base. However, erythromycin base is degraded in the stomach by gastric acid. The esterified formulations are absorbed intact and must be hydrolyzed to the active erythromycin A. The erythromycin salts are absorbed unchanged.[260] The oral bioavailability of erythromycin base is 17% in fasted foals, with most of the drug being degraded and absorbed as the microbiologically inactive anhydroerythromycin A.[264] Microencapsulation of the base improves the oral bioavailability of erythromycin base to 26% in fasted foals, but it remains only 7.7% in fed foals.[265] The oral bioavailability of erythromycin estolate in fasted foals is

36% but only 16% for erythromycin phosphate.[266] The estolate formulation appears to have the best pharmacokinetic profile in foals. Because of injection site irritation, IM administration of erythromycin is not recommended in horses. The reported oral bioavailability of azithromycin in foals ranges between 39% and 56%.[267,268] The oral bioavailability of clarithromycin in foals is similar (57%).[269] Telithromycin, an oral ketolide antibiotic used for treatment of macrolide-resistant *R. equi* in humans, has also been studied in foals. Bioavailability has not been determined; however, although absorption was adequate for treatment of susceptible *R. equi* isolates, it was not high enough for treatment of most macrolide-resistant *R. equi* isolates in foals.[270] The bioavailability of tulathromycin has not been determined; however, after IM administration the drug is rapidly detected in the plasma of foals, with maximum concentrations of 0.41 μg/mL 4 hours after administration.[271] Gamithromycin has maximum concentrations of 0.33 μg/mL 1 hour after IM administration in foals.[272]

Distribution

The macrolides concentrate in leukocytes, making them very effective against intracellular pathogens such as *R. equi*.[273] Concentrations of azithromycin and clarithromycin in bronchoalveolar lavage cells were significantly higher than erythromycin, and azithromycin had a significantly longer elimination half-life from the cells.[274] Bronchoalveolar cell and pulmonary epithelial lining fluid concentrations of azithromycin are 15- to 170-fold and 1- to 16-fold higher than concurrent serum concentrations, respectively, and did not decrease for up to 48 hours after administration.[267] Clarithromycin concentrations in bronchoalveolar cell and pulmonary epithelial lining fluid are 91- to 105-fold and 324- to 585-fold higher than concurrent serum concentrations, respectively; however, in contrast to azithromycin, clarithromycin concentrations significantly decreased within 12 hours of administration.[269] Tulathromycin concentrates in bronchoalveolar cells, and concentrations are detectable for at least 8 days after a single administration, although they are still lower than the MIC for *R. equi* (greater than 64 μg/mL).[271] The relative distribution of macrolide antibiotics into bronchoalveolar cell and pulmonary epithelial lining fluid has been reviewed in depth.[275]

Because they are weak bases, macrolides are ion trapped in milk, CSF, and gastric fluids. The Vd for erythromycin is 2.7 L/kg in foals.[264] Azithromycin is known for its high degree of lipid solubility, and the Vd of azithromycin in foals is 11.6 to 18.6 L/kg.[267,268] Clarithromycin also exhibits a large volume of distribution, with reported values of 10.4 L/kg.[269] Peritoneal and synovial fluid concentrations of azithromycin and clarithromycin parallel serum concentrations.[269] The apparent Vd for tulathromycin after IM administration is 15.2 L/kg.[271]

Elimination

Erythromycin is extensively metabolized, with much of the parent drug and active metabolite excreted into the bile, resulting in an elimination half-life of 1 to 2 hours.[264-266,276] Erythromycin inhibits the metabolism of a number of other drugs by interfering with cytochrome P450 enzymes.[18] In addition, the degradation product anhydroerythromycin A is a potent inhibitor of cytochrome P450–mediated metabolism.[277] Erythromycin undergoes enterohepatic cycling.[18] Azithromycin is not highly metabolized and is primarily eliminated in bile. The elimination half-life is 16 to 20.3 hours in foals.[267,268] Clarithromycin is extensively metabolized in humans, and the active metabolite, 14-hydroxy-clarithromycin, is produced in foals, although the pharmacokinetics have not been quantitated.[269] The elimination half-life of clarithromycin in foals is intermediate between erythromycin and azithromycin at 5.4 hours.[269] Tulathromycin has the longest elimination half-life, reported at 117 hours in foals.[271] Gamithromycin also has an extended elimination phase, with a half-life of 39.1 hours.[272]

Adverse Effects and Drug Interactions

The use of erythromycin in horses is associated with a number of adverse effects. Since erythromycin came into clinical use in the 1950s, it was apparent that therapy was frequently accompanied by adverse GI effects. Macrolide antibiotics, including erythromycin and clarithromycin, are motilin receptor agonists. They also appear to stimulate motility via cholinergic and noncholinergic neuronal pathways. At microbially ineffective doses they stimulate migrating motility complexes and antegrade peristalsis in the GI tract.[261,276,278,279] When used at antimicrobial doses, erythromycin is associated with potentially fatal colitis from *Clostridium* spp.[280-282]

Erythromycin is associated with acute respiratory distress syndrome, gastroenteritis, and hepatotoxicity in foals.[277,283,284] Erythromycin also interferes with host cell metabolism and decreases inflammatory responses in airways, but the clinical significance of this has not been determined.[277]

Hyperthermia following administration of macrolides is most associated with erythromycin, although it also associated with azithromycin and clarithromycin to a lesser degree. The mechanism of macrolide-induced hyperthermia has been shown to be drug-induced anhidrosis, as evidenced by significantly reduced sweating in response to an intradermal sweat tested in treated versus placebo control foals.[285] Anhidrosis begins shortly after initiation of treatment, partially recovers during treatment, and can take at least 10 days after stopping treatment to fully recover. To minimize the risks of macrolide-induced hyperthermia, it is recommended to decrease access to direct sunlight, provide ample shade, avoid turnout during the hottest parts of the day, and provide access to clean, fresh water at all times. Erythromycin is commonly administered in conjunction with rifampin to take advantage of antimicrobial synergism and reduce the chance of resistance development.[28] Erythromycin may interact with other drugs metabolized by the same cytochrome P450 enzyme system. Concurrent administration of erythromycin with theophylline results in a doubling of plasma theophylline concentrations and can result in seizures in foals.[18]

Clarithromycin is also frequently administered in combination with rifampin due to synergistic effects against *R. equi*.[286] However, coadministration of rifampin reduces clarithromycin bioavailability by as much as 90%, resulting in plasma concentrations of clarithromycin that are below the MIC_{90} of *R. equi*.[287] Bronchoalveolar lavage cell and pulmonary epithelial lining fluid concentrations were also decreased, but they remained above therapeutic concentrations. The exact cause of the decrease is unknown. It does not appear to be due to induction of hepatic metabolism by rifampin, as the pharmacokinetics of clarithromycin and its metabolite remain unchanged. It is speculated that the decreased bioavailability is secondary to changes in oral absorption, likely through inhibition of an unknown intestinal uptake transporter.[288] Concentrations of tulathromycin in the lung of foals was also significantly lowered by comedication with rifampin. Because tulathromycin

is only administered via the IM route, the mechanism of this interaction also remains unknown.[289]

Azithromycin is associated with fewer adverse effects than erythromycin in human beings. No adverse reactions were detected during or after repeated intragastric administration of azithromycin or clarithromycin in foals.[273,275] A multiple-dose study of oral azithromycin in adults (10 mg/kg PO q24hr for 5 days) showed mild decreases in appetite and alterations in fecal consistency in some horses.[290] There are anecdotal reports of antimicrobial-associated colitis in older weanlings, particularly those treated with compounded formulations of azithromycin.

In a study that evaluated the use of tulathromycin in foals with evidence of pulmonary abscessation, self-limiting diarrhea, fever, and injection site reactions were the only adverse effects noted. In one foal in that study, the injection site reaction was severe and resulted in temporary lameness and severe swelling.[288] Injection site reactions and colic have been reported following IM gamithromycin administration and may limit the use of this antibiotic in foals.[291]

Formulations

Because the base of erythromycin is unstable in gastric acid, it is formulated as enteric-coated erythromycin tablets. Erythromycin stearate and erythromycin phosphate are insoluble salt formulations that disassociate in the intestine, allowing absorption of the free erythromycin base. Erythromycin ethylsuccinate and erythromycin estolate are ester formulations that are absorbed intact from the intestine, and then plasma esterases release active drug. Erythromycin is available in an IM formulation approved for cattle. Because of its highly irritating nature, this formulation is not recommended for IM use in horses, and it can be fatal if injected intravenously. A human-labeled formulation of erythromycin lactobionate is available for IV use. Tablet formulations are available for azithromycin in 250-, 500-, and 600-mg strengths. Clarithromycin is available as an immediate and extended release tablet. The extended release formulations have not been studied in horses. Tulathromycin is available as a 100-mg/mL injectable solution for cattle and swine. Gamithromycin is available as a 150-mg/mL injectable solution for cattle.

Clinical Use

On account of its association with potentially fatal adverse effects, erythromycin is usually limited to treatment of *R. equi* infections in foals. It also has been shown to be an effective therapy for Potomac horse fever[292] and equine proliferative enteropathy caused by *Lawsonia intracellularis.*[293] Its motilin-like activity is exploited for use as a treatment for adynamic ileus in horses.[261] Azithromycin has become an attractive alternative to erythromycin for the treatment of *R. equi* infections in foals because of its pharmacokinetic profile, which allows once-daily or every-other-day dosing and an apparent reduced incidence of adverse effects.[294] However, in a retrospective study of 81 foals treated for naturally occurring *R. equi* infection, the combination of clarithromycin (10 mg/kg q24hr) and rifampin was shown to be superior to combinations of azithromycin (7.5 mg/kg q12hr) and rifampin and erythromycin (25 mg/kg q6hr, 25 mg/kg q8hr, or 37.5 mg/kg q12hr) and rifampin.[273] Given the drug interactions noted between clarithromycin and rifampin, further work is needed to determine whether monotherapy with clarithromycin alone would be as effective as or more effective than combination therapy. Azithromycin alone has been shown to be as effective as azithromycin and rifampin combined.[295] Tulathromycin is not recommended for treatment of *R. equi* in foals, due to the high MICs (greater than 64 μg/mL). Gamithromycin offers more promise for treatment of *R. equi.* A once-weekly IM dosing regimen (6 mg/kg) may provide a more affordable treatment alternative, although adverse reactions may limit the use of this drug in horses.

Although most frequently used for *R. equi* pneumonia, the macrolide anitbiotics are also effective against *Lawsonia intracellularis,* although the risk of adverse GI effects in weanling age foals limits their use and tetracyclines have replaced them in most cases. Some drugs in this class, including azithromycin and gamithromycin, have good activity against streptococci and may be useful for treatment of abscesses caused by *Streptococcus* spp. in foals.

FLUOROQUINOLONES

The quinolones are a group of synthetic antimicrobials. The first was *nalidixic acid,* which was introduced in 1964. It had good activity against gram-negative bacteria but had a low Vd and numerous adverse effects and was limited to treatment of urinary tract infections. Further chemical manipulation resulted in development of the fluorinated quinolones, which had extended antimicrobial activity with improved safety. Included in this group are ciprofloxacin, enrofloxacin, danofloxacin, difloxacin, orbifloxacin, marbofloxacin, fleroxacin, moxifloxacin, and levofloxacin. No fluoroquinolones are approved for use in horses, but because of their pharmacokinetics and antimicrobial activity, they are commonly used for serious gram-negative infections.

Mechanism of Action

The fluoroquinolones have a unique mechanism of action for bacterial killing. The fluoroquinolones inhibit bacterial deoxyribonucleic acid (DNA) gyrase (also known as *topisomerase II*). Bacteria have a single chromosome consisting of double-stranded DNA. Within the bacterial cell the chromosome is folded around an RNA core, and each fold is supercoiled. DNA gyrase, which has been found in every organism examined, is responsible for supercoiling the strand of bacterial DNA. The DNA gyrase structure has four subunits: two A monomers and two B monomers. The enzyme forms a heart-shaped molecule, with the A monomers forming the atria and the B monomers forming the ventricles. The bacterial DNA binds to the gyrase in the cleft between the A and B subunits. The DNA gyrase nicks double-stranded DNA, introduces negative supercoils, and seals the nicked DNA. The fluoroquinolones bind to the DNA-DNA gyrase complex and inhibit the DNA resealing, resulting in abnormal spatial DNA configuration, which leads to DNA degradation by exonucleases.[296]

Fluoroquinolone activity is concentration dependent, and clinical efficacy is most associated with achieving either an AUC_{0-24}:MIC greater than 125 or a C_{max}:MIC greater than 10. These PK/PD relationships are related, insofar as increasing the dose to increase the peak plasma concentration will also increase the *AUC* value.[18]

All of the fluoroquinolones are bactericidal, although these drugs have an optimum concentration for bacterial killing. Higher or lower drug concentrations result in reduced bactericidal activity. It is thought that the DNA-DNA gyrase complex has two binding sites for fluoroquinolones. At low

drug concentrations only one binding site is occupied, resulting in single-stranded nicks in the DNA. Reduced killing at high concentrations is thought to be due to dose-dependent inhibition of RNA or protein synthesis. RNA and protein synthesis are required for production of bacterial autolysins, which are responsible for the fluoroquinolone-induced cell lysis.[18,296]

The fluoroquinolones are broad spectrum in activity, with activity against most gram-negative bacteria; some gram-positive bacteria; and *Mycoplasma, Chlamydia,* and *Rickettsia* spp. They are particularly effective against the enteric gram-negative pathogens, including some strains resistant to aminoglycosides and cephalosporins. Reported MICs are very low, and MBCs are 1 to 2 times the MIC for most pathogens. They are usually active against staphylococci but have variable activity against streptococci and are not effective against enterococci. Most diagnostic laboratories use ciprofloxacin or enrofloxacin to determine pathogen susceptibility; however, MIC values vary among the fluoroquinolones. Ciprofloxacin has the greatest activity against *Pseudomonas* spp. Orbifloxacin has lower MIC values than enrofloxacin for the gram-negative bacteria *Actinobacillus equuli, E. coli, Pasteurella* spp., and *Salmonella* spp. Enrofloxacin has lower MIC values for the gram-positive bacteria and *Pseudomonas* spp.[18] Even with in vitro evidence of susceptibility, however, enrofloxacin is not a good choice for *Pseudomonas* spp. as resistance develops rapidly.

Most fluoroquinolones are not active against anaerobic bacteria.[297] This susceptibility pattern may be a therapeutic advantage in the treatment of enteric infections in horses, because GI anaerobes rarely cause disease and usually are protective by competitively inhibiting colonization by pathogenic aerobic organisms.

The fluoroquinolones concentrate within phagocytic cells. Uptake occurs by simple diffusion, and intracellular concentrations may be several times greater than plasma concentrations. Intracellular drug is microbiologically active against intracellular pathogens such as *Brucella* spp., *Mycoplasma* spp., and *Mycobacterium* spp.[18] Exposure of gram-negative bacteria to fluoroquinolones at concentrations several times the MIC for 1 to 2 hours results in a PAE with a recovery period of 1 to 6 hours. This effect suggests that fluoroquinolone dosage regimens can tolerate plasma concentrations below the pathogen's MIC for extended periods of time without a reduction in efficacy.[298]

Mechanisms of Resistance

Microbial resistance to the fluoroquinolones is primarily due to chromosomal mutations that alter bacterial DNA gyrase, decrease cell wall permeability, or increase fluoroquinolone efflux from the cell. Plasmid-mediated resistance has only recently been documented for the fluoroquinolones. Fluoroquinolone-resistance plasmids can be transmitted horizontally and provide low-level resistance that facilitates the emergence of higher level resistance at therapeutic levels.[299] The fluoroquinolones must penetrate bacteria to reach the target DNA gyrase. They do so by diffusing through porin channels in the outer membrane of gram-negative bacteria. *Pseudomonas* spp. resistance is associated with alterations in a wide range of outer-membrane proteins. From these mutations the increase in the MICs of the fluoroquinolones is relatively low (2- to 32-fold). However, there is cross-resistance with unrelated antibiotics, most frequently TMP, tetracycline, CHPC, and cefoxitin.[300]

Since the fluoroquinolones have been used intensively in human medicine in the past 3 decades, high-level resistance has emerged in some pathogens. Chronic fluoroquinolone use encourages the development of chromosomally mediated resistance. In high-level resistant bacterial strains, one resistance mechanism alone is usually not responsible; rather, two or three mechanisms of resistance operate in conjunction. In resistant *S. pneumoniae* increased efflux is often coupled with a gyrase mutation.[301] In resistant *E. coli* gyrase mutations are usually associated with changes in the outer membrane proteins.[302]

Pharmacokinetics

Absorption

The fluoroquinolones are rapidly and well absorbed from the GI tract of monogastrics and preruminant calves. Enrofloxacin is more lipid soluble than ciprofloxacin and has a higher oral bioavailability than ciprofloxacin in horses and small animals. The oral bioavailability of ciprofloxacin is only 6.8% to 10.5% in adult horses and ponies.[303,304] The oral bioavailability of enrofloxacin is approximately 60% in adult horses and 42% in foals.[305-307] The moderate oral absorption of enrofloxacin has been determined to be due to hepatic first pass effect.[308] Oral absorption of enrofloxacin is not affected by feeding.[309] Antacids containing divalent cations (calcium, magnesium) chelate fluoroquinolones and reduce oral bioavailability.[18] Oral bioavailability of marbofloxacin and orbifloxacin is 62% and 68%, respectively.[308,310] Administration of the injectable formulation of danofloxacin intragastrically to horses had a bioavailability of only 35.8%.[311] Bioavailability from IM injection is nearly 100% for all fluoroquinolones, but IM injections of enrofloxacin are irritating to tissues.[312] For economic reasons and client convenience, practitioners have attempted to administer the bovine injectable solution of enrofloxacin orally to horses. This results in a bioavailability of approximately 65% in horses when administered intragastrically.[313] Unfortunately, the injectable formulation is highly irritating to the oral mucosa and may cause ulceration with repeated use. A methylcellulose gel formulation compounded from the injectible liquid has better oral absorption but may also cause oral ulceration.[314]

Distribution

The fluoroquinolones are generally lipid soluble and well distributed to most tissues. The Vd of the fluoroquinolones studied in horses ranges from 1.2 L/kg for marbofloxacin[315] to 4.9 L/kg for ciprofloxacin.[304] Tissue concentrations typically exceed plasma concentrations during therapy. Extremely high concentrations are achieved in the kidney, urine, liver, and bile. Therapeutic concentrations for gram-negative bacteria may be achieved in cerebrospinal and ocular fluids.[303,306,316,317] Aqueous humor concentrations of enrofloxacin after IV administration of 3 daily doses of 7.5 mg/kg were 0.32 μg/mL.[318] Plasma protein binding of fluoroquinolones is low to moderate in most species. Protein binding data are available for ciprofloxacin, enrofloxacin, orbifloxacin, and levofloxacon in the horse. Binding is similar across drugs and ranges between 21% and 28%.[310,319]

Elimination

The fluoroquinolones are predominantly excreted in the urine by glomerular filtration and active tubular secretion.[18] For marbofloxacin approximately 40% is excreted unchanged in

the urine, whereas with enrofloxacin only 3.4% is excreted unchanged in the urine.[308] Ciprofloxacin undergoes some sulfoxidation, and its metabolites also have antimicrobial activity. Enrofloxacin is metabolized (de-ethylated) to ciprofloxacin in horses and donkeys,[320] with serum ciprofloxacin concentrations reaching 20% to 35% of enrofloxacin concentrations.[312] Metabolism of enrofloxacin to ciprofloxacin is negligible in foals, and ciprofloxacin was not detected in the plasma of foals in which IV or oral enrofloxacin was administered.[305] The elimination half-life of ciprofloxacin in ponies is 2.5 hours and 5 hours in horses.[303] Enrofloxacin has an elimination half-life of 4.4 hours after IV administration and 10 hours after IM administration, indicating flip-flop kinetics.[312] With oral administration the elimination half-life of enrofloxacin is 8 hours.[317] The elimination half-lives of danofloxacin, orbifloxacin, and marbofloxacin are 8, 5.1, and 4.14 hours, respectively.

Adverse Effects and Drug Interactions

Toxicity of fluoroquinolones is mild in most species, and GI irritation is the most common side effect.[18] Both ciprofloxacin and moxifloxacin have been associated with GI effects in horses.[304,321] These effects with ciprofloxacin ranged from mild transient diarrhea to severe colitis, endotoxemia, and laminitis necessitating euthanasia of three horses on humane grounds. Adverse effects were seen regardless of the route of administration and the high incidence of adverse events precludes oral and rapid IV bolus administration of ciprofloxacin.

The effect of most clinical concern with most fluoroquinolones is cartilage damage with subsequent arthropathy. Arthropathies have been reported in humans, dogs, and foals, but not calves, swine, or poultry.[322-325] The risk of arthropathy is age dependent, with younger animals being at higher risk. Weight bearing and exercise are also associated with increased severity of disease. Arthropathies have been documented in 2-week-old foals after receiving 10 mg/kg of enrofloxacin orally.[325] Damage was characterized by synovial joint effusion and lameness and erosion and cleft formation in articular cartilage. Arthropathies were not seen in adult horses that were given up to 25 mg/kg of IV enrofloxacin daily for 3 weeks or 15 mg/kg orally every 12 hours for 3 weeks.[316,326] Although they are not recommended for use in pregnant humans or animals, the fluoroquinolones appear to have little effect on the developing fetus.[327] Enrofloxacin was successfully used to treat chronic pleuritis in a pregnant mare with no apparent detrimental effects on the foal.[328]

Ciprofloxacin and enrofloxacin interfere with the cytochrome P450 system metabolism of methylxanthines such as theophylline. Serum theophylline concentrations may double and result in central nervous system and cardiac toxicity, so concentrations must be monitored during therapy.[329] Coadministration of nonsteroidal antiinflammatory drugs and enrofloxacin may influence the pharmacokinetics of either drug depending on the species studied and the specific drugs administered. Coadministration of firocoxib and enrofloxacin did not influence firocoxib concentrations in the horse.[330]

The fluoroquinolones may cause adverse central nervous system effects in humans and animals as a result of a γ-aminobutyric acid (GABA) receptor antagonism. This has been associated with an increase in the incidence of seizures in human beings and dogs. Administration of enrofloxacin to human beings results in hallucinations.[18] Rapid IV administration of high doses of enrofloxacin to horses causes transient neurologic signs, including excitability and seizure-like activity.[326] This can be prevented by slow injection or dilution of the dose. Dilution of the dose should be performed in sterile saline solution, because the cations found in other fluids, such as lactated Ringer's solution, may chelate and inactivate the drug. Photosensitivity reactions and Achilles tendon rupture have been associated with fluoroquinolone use in humans but have not been reported in animals.[296]

The fluoroquinolones have been used in combination with other antimicrobial agents to expand the therapeutic spectrum, suppress emergence of drug-resistant bacterial populations, or exploit inhibitory or bactericidal synergism against drug-resistant populations. Minimal synergy occurs between fluoroquinolones and β-lactams or aminoglycosides against gram-negative enteric bacteria because of the already high susceptibility of these organisms. Combinations with aminoglycosides, β-lactams, or vancomycin are additive or indifferent against staphylococci. Antagonism between fluoroquinolones and CHPC or rifampin appears to be due to the inhibition of bacterial autolysin synthesis from concurrent administration of bacterial protein synthesis inhibitors.[18]

Formulations

Ciprofloxacin is available as human-labeled tablets, a diluted solution for IV administration, and a solution for ophthalmic use. Enrofloxacin is available as oral tablets and a 50-mg/mL injectable solution for IM injection in dogs and as a 100-mg/mL injectable solution for the SC treatment of cattle. Both injectable solutions can be administered intravenously to horses. Orbifloxacin and marbofloxacin are available as oral tablets for small animals. Danofloxacin is available as a 180-mg/mL injectable formulation for cattle.

Clinical Use

The use of fluoroquinolones in horses has been limited because of the risk of arthropathies; however, enrofloxacin has been successfully used in clinical cases, and fluoroquinolones may be the only viable option for treating some infections.[328,331-333] Informed consent from the client should always be obtained before using fluoroquinolones in young horses. Because the fluoroquinolones are concentration-dependent killers with a long PAE, the ideal dosage regimen is once-daily high-dose therapy. Enrofloxacin is commonly administered at doses of 7.5 to 10 mg/kg orally or intravenously, once daily.

RIFAMPIN

Mechanism of Action

The rifamycins are antibiotics produced from *Streptomyces mediterranei.* Rifampin inhibits DNA-dependent RNA polymerase in susceptible organisms, suppressing RNA synthesis. It has no effect on the mammalian enzyme. Its action is bacteriostatic or bactericidal, depending on the susceptibility of the bacteria and the concentration of the drug. Rifampin is effective against a variety of mycobacterium species and *S. aureus, Haemophilus,* and *R. equi.* Rifampin is considered especially active in the treatment of staphylococcal and rhodococcal infections and in the eradication of pathogens located in difficult-to-reach target areas, such as inside phagocytic cells. It is active at an acid pH, making it a rational choice for the treatment of septic foci and granulomatous infections. It has moderate activity against *Actinobacillus suis, Actinobacillus equuli, Bordetella bronchiseptica,*

and *Pasteurella* spp. Equine isolates of *P. aeruginosa, E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Proteus* spp., and *Salmonella* spp. are resistant. The ability of rifampin to reach intracellular bacteria can make it difficult to predict in vivo therapy results on the basis of in vitro sensitivity tests.[334] Because bacterial resistance to rifampin develops rapidly, it is usually administered with another antimicrobial.[335] Although it is commonly combined with erythromycin for the treatment of *R. equi,*[28] resistance to the combination has been reported.[262,336] Rifampin is also commonly administered with newer macrolide and macrolide derivative drugs, such as clarithromycin and azithromycin.[273]

Pharmacokinetics

Absorption

The oral bioavailability of rifampin varies from 70% in fasted horses to 26% when administered with feed.[48,334,337] Because rifampin is usually administered with feed, recommended dosages compensate for the decreased bioavailability. Bioavailability after IM injection is 60%.[338] In foals, maximum plasma concentrations reach approximately 18 µg/mL at approximately 3.5 hours.

Distribution

Rifampin is highly lipophilic and penetrates most tissues, as well as milk, bone, abscesses, and the central nervous system. The Vd of rifampin in horses is 0.6 to 0.9 L/kg.[337,338] Rifampin is 78% bound to plasma proteins.[337]

Elimination

In other species rifampin is deacetylated in the liver to a metabolite that also has antibacterial activity. Desacetylrifampin was not detected in equine serum samples after an IV dose of 10 mg/kg or oral doses of 10 mg/kg every 12 hours for seven doses.[337] Desacetylrifampin was measured in urine, but rifampin was much more predominant. However, only 6.82% of the total dose was recovered in the urine as either rifampin or desacetylrifampin. In foals, metabolite concentrations were no higher than 0.1 µg/mL in plasma following a single oral dose. The elimination half-life of rifampin is 6 to 8 hours after IV administration and 12 to 14 hours after oral administration.[334,337] Because of immature hepatic metabolism, elimination of rifampin is delayed in very young foals.[339,340] Plasma clearance ranges between 1.14 and 1.34 mL/min/kg.[337,338] As a hepatic enzyme inducer, rifampin induces its own metabolism so that multiple oral dosing significantly decreases the elimination half-life. Enzyme induction is typically not seen with fewer than 5 days of therapy, but once it occurs, the increase in enzyme activity may last for more than 2 weeks after discontinuation of treatment.[341]

Adverse Effects and Drug Interactions

Rifampin stains everything it touches red, and treated animals may produce red urine, tears, sweat, and saliva. There are no harmful consequences from this effect. Most horses object to the taste of rifampin, so care must be taken to deposit a dose quite far back on the tongue and rinse the horse's mouth afterward. Combination therapy of rifampin and doxycycline in foals with *R. equi* pneumonia resulted in adverse effects severe enough to warrant stopping treatment, including icterus and elevated liver enzymes.

Microsomal enzyme induction from rifampin may shorten the elimination half-life and decrease plasma drug concentrations of CHPC, corticosteroids, theophylline, itraconazole, ketoconazole, warfarin, and barbiturates.[342] Rifampin has a significant effect on clarithromycin bioavailability, decreasing clarithromycin plasma concentrations by 70% to 90%.[288]

Formulations

Rifampin is available as human-labeled capsules or suspension for oral administration or as a diluted solution for IV use.

Clinical Use

Rifampin is primarily used in the treatment of pulmonary abscess caused by *R. equi* and *L. intracellularis* proliferative enteropathy in foals in combination with a macrolide or a macrolide derivative.

METRONIDAZOLE

Mechanism of Action

Metronidazole is rapidly taken up by bacteria, where it is metabolized by a reduction process to cytotoxic derivatives (short-lived free radical compounds). These cytotoxic compounds damage DNA and other critical intracellular macromolecules. Aerobic bacteria lack the reductive pathway necessary to produce the radical compounds.[343] Metronidazole is highly effective against anaerobic bacteria, including *Bacteroides fragilis* (penicillin resistant), *Fusobacterium,* and *Clostridium* spp. Metronidazole-resistant *C. difficile* may cause diarrhea in foals.[344] Metronidazole has good activity against protozoa, including *Giardia* and *Trichomonas* spp.[345] Metronidazole has antiinflammatory effects in human beings, particularly in the GI tract, and has been used for the treatment of chronic inflammatory bowel diseases.[346]

Pharmacokinetics

Absorption

Metronidazole is rapidly and well absorbed after oral administration in horses, with an oral bioavailability of 75% to 85%.[345,347] In horses with GI ileus, metronidazole may be administered rectally and is rapidly absorbed; however, the bioavailability is only 30%.[348] Bioavailability in foals 1 to 2.5 days of age is 100%.[349]

Distribution

Metronidazole is lipophilic and widely distributed in tissues. It penetrates bone, abscesses, and the central nervous system. The Vd in mares is 0.7 to 1.7 L/kg.[345,347] In foals less than 2 weeks of age, the Vd is 0.87 L/kg.

Elimination

Metronidazole is primarily metabolized in the liver. Both metabolites and unchanged drug are eliminated in urine and feces. Plasma clearance is 2.8 mL/min/kg, and elimination half-life is 3 to 4 hours in horses.[345,347,348,350] Age-related differences in clearance have been noted in foals within the neonatal period.[349] At 1 to 2.5 days of age, clearance is 0.84 mL/min/kg and increases to 1.14 mL/min/kg in foals 10 to 12 days of age. This results in a significantly longer half-life in very young foals, with values of 11.8 and 9.1 hours for foals 1 to 2.5 and 10 to 12 days old, respectively.

Adverse Effects and Drug Interactions

With clinical use in horses, anorexia and salivation are the only adverse effects associated with the oral use of metronidazole.[351] Histopathologic evidence of peripheral neuropathy and hepatopathy have been seen in horses administered high doses for prolonged periods of time (30 mg/kg PO q12hr for 30 days). Metronidazole produces mutations in bacteria, and carcinogenicity occurs in laboratory mice with prolonged exposure. Therefore metronidazole is banned from use in food animals. Because it has been implicated as a teratogen in laboratory animals, use in pregnant animals is not recommended.[18] In neonatal foals, central nervous system excitement can be seen with high or frequent dosing. If seen, dosing should be stopped immediately. This appears to occur more commonly with IV dosing, therefore slow administration over 30 minutes is recommended.

Formulations

Metronidazole is available only as human-labeled formulations. It is most commonly administered orally as tablets and capsules. Because it is poorly soluble, the IV formulation must be diluted in a large volume for administration, and it is cost prohibitive in adult horses.

Clinical Use

Metronidazole is used to treat anaerobic infections, especially pleuropneumonia and lung abscesses caused by penicillin-resistant *Bacteroides fragilis* and clostridial enterocolitis.[30,351-353] Although rectal absorption is inferior to oral absorption, it is a viable option for treatment when oral administration is not feasible. Metronidazole has also been used for the treatment of infiltrative bowel diseases in the horse.[354]

Nonsteroidal Antiinflammatory Drugs

The most commonly used drugs for treatment of pain and inflammation in horses are the nonsteroidal antiinflammatory drugs (NSAIDs). NSAIDs inhibit the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandins, thromboxane, and prostacyclin (Fig. 2.23). Blocking these ecosanoids results in antiinflammatory, analgesic, antipyretic, and antithrombotic effects.[355]

MECHANISM OF ACTION

Cyclooxygenase Inhibition

Two different, distinct forms of COX have been identified. A third form (COX-3) has been identified in other species, but its significance in the horse is currently unknown. Of the two forms known to exist in the horse, COX-1 is considered to be the constitutively expressed form and is necessary for normal homeostatic mechanisms in the body, whereas COX-2 is the inducible form produced in response to injury.[356] COX-1 is found in platelets, the kidneys, and the GI tract; COX-2 is identified in fibroblasts, chondrocytes, endothelial cells, macrophages, and mesangial cells. COX-2 is induced by exposure to various cytokines, mitogens, and endotoxin and is upregulated at sites of inflammation.[357]

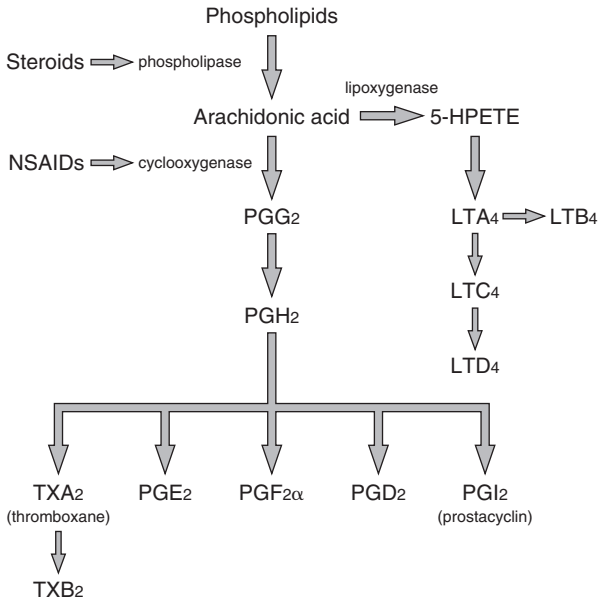


FIG. **2.23** In the arachidonic acid cascade, cyclooxygenase works on arachidonic acid to produce prostaglandins *(PG),* thromboxanes *(TX),* and prostacyclin, whereas lipoxygenase works on arachidonic acid to produce leukotrienes *(LT). NSAIDs,* Nonsteroidal antiinflammatory drugs; *HPETE,* hydroperoxyeicosatetraenoic acid.

Unfortunately, this classification of "good" versus "bad" COX proved too simplistic to explain the roles of the different forms of COX.[358] COX-2 is produced constitutively in the brain, spinal cord, kidney, ovary, uterus, placenta, thymus, bone, cartilage, synovia, endothelia, prostate, and lung. Additionally, COX-2 is involved in cellular processes such as gene expression, differentiation, mitogenesis, apoptosis, bone remodeling, wound healing, and neoplasia[359] but can also be induced by hormones, nitric oxide, cytokines, and lipoxygenase products. The prostaglandins produced in the GI tract and kidney that maintain mucosal integrity in the upper GI tract and renal perfusion, respectively, originally appeared to be derived only from COX-1. It is now widely accepted that both COX-1 and COX-2 are involved in mucosal defense in the GI tract.[360] Likewise, both COX-1 and COX-2 are involved in normal renal function, insofar as prostaglandins affect renal circulation through vasodilation, renin secretion, and sodium and water excretion. As in the GI tract, it was thought that COX-1–derived prostaglandins were involved in regulating homeostatic functions and COX-2–derived prostaglandins were involved only in inflammation or tissue damage. However, constitutive expression has been demonstrated for both isoforms in the kidney. NSAIDs also reduce sodium excretion and may cause acute renal failure when maintenance of adequate renal perfusion is prostaglandin dependent.[361]

Nonselective Versus COX-2–Selective Inhibitors

Newer NSAID products have targeted selective inhibition of COX-2 to supposedly increase the safety profile of these drugs. However, as previously stated, the theory behind this may be oversimplified. Either of the COX enzymes is capable of producing any of the prostanoids. The profile of the mediators produced depends mainly on the cell types present. Additionally, both enzymes are responsible in

some part for the inflammatory response. COX-1, because it is constitutively expressed, is present in the early stages of inflammation. Production of COX-2 will increase as the inflammatory process progresses, because it is an inducible enzyme. COX-2 is also involved in mucosal healing, particularly in the case of gastric and colonic ulceration, and is constitutively expressed in some tissues, such as the kidney. Renal responses to furosemide, dobutamine, and exercise were similar between meloxicam, a COX-2 preferential agent, and phenylbutazone treatment, which suggested that COX-2 was the mediator of prostanoid-induced changes to renal function in horses.[362] Therefore the safety of COX-2 selective inhibitors versus nonselective inhibitors has been called into doubt.[363,364] Nevertheless, there does appear to be a benefit to the use of these selective inhibitors in veterinary practice.

COX-2 inhibitors exert their specificity by taking advantage of the differences in protein structure between COX-1 and COX-2. There is a valine-leucine substitution in COX-2 that is not present in COX-1. This creates a side pocket in the tertiary structure of the molecule, and it is this side pocket that COX-2 inhibitors preferentially bind to. The selectivity of the drug for COX-2 versus COX-1 therefore depends on the affinity a drug has for this site. Regardless of affinity, all COX inhibitors bind both COX-1 and COX-2 to some extent; therefore there are no truly COX-2–specific drugs available at this time. Some drugs that have been described as COX-2 selective include etodolac, meloxicam, deracoxib, carprofen, and firocoxib. Of these, firocoxib is the only drug labeled for use in the horse that has COX-2–selective activity at therapeutic concentrations.

Affinity, or specificity, for COX-1 and COX-2 is generally assessed by in vitro techniques using whole blood assays. The activity against COX-1 is most commonly determined by measurement of thromboxane B_2 (TXB_2), the stable metabolite of TXA_2, which is thought to be specific for COX-1 production in platelets. For COX-2 activity, macrophages are stimulated with lipopolysaccharide (LPS), and production of prostaglandin E_2 (PGE_2) is measured. Enzyme-linked immunosorbent assays (ELISAs) are used for measurement of mediators. These tests are most valuable as comparative assays performed within the same laboratory, by the same investigators, within one class of drugs, as a measure of the potency of the drugs against the specific COX enzymes.

Antiinflammatory and Analgesic Effects

The NSAIDs are primarily antiinflammatory as a result of their inhibition of prostaglandin production. Therefore NSAIDs do not resolve inflammation but prevent its ongoing occurrence. Whereas prostaglandin production will rapidly diminish, any previously present prostaglandin must be removed before inflammation will subside. From tissue cage work, it has been shown that phenylbutazone, ketoprofen, and carprofen have delayed peak concentrations at the site of inflammation and persist in inflammatory exudates for long periods of time after plasma concentrations are negligible.[365-367] This explains the delayed onset and prolonged duration of antiinflammatory action that does not correlate with plasma pharmacokinetics of the NSAIDs.

The NSAIDs are commonly used in horses to attenuate the prostaglandin-mediated effects of endotoxin.[368-370] Flunixin meglumine administered at doses as low as one fourth the label dose will block prostaglandin production when administered before endotoxin exposure without obscuring the signs of colic pain, and with a lesser risk of adverse effects. However, at low doses it does not alter endotoxin-induced leukopenia, and with ongoing inflammation there is little benefit in using the low dose over the recommended dose.[371-377] Flunixin and phenylbutazone significantly inhibit movement of polymorphonuclear and mononuclear cells and antagonize the effects of endotoxin on bowel motility.[368,378]

COX inhibition does not explain all of the antiinflammatory activity of NSAIDs. NSAIDs are more lipophilic at a low pH, such as is found in inflamed tissues. Some antiinflammatory action appears to be related to their ability to insert into the lipid bilayer of cells and disrupt normal signals and protein-protein interactions in cell membranes. In the cell membrane of neutrophils, NSAIDs inhibit neutrophil aggregation, decrease enzyme release and superoxide generation, and inhibit lipoxygenase.[379,380]

The NSAIDs act as analgesics by inhibiting COX and preventing the production of prostaglandins that sensitize the afferent nociceptors at peripheral sites of inflammation. However, increasing evidence suggests that some NSAIDs have a central mechanism of action at the level of the spinal cord for analgesia unrelated to COX inhibition.[381] This action is synergistic with opioids and β_2-receptor–adrenergic drugs.[381,382]

For managing pain and inflammation in horses, NSAIDs are more effective as analgesics when inflammation is a part of the pain process and when they are given before the onset of the inflammatory process or insult. The time to onset and duration of analgesia of NSAIDs does not correlate well with their antiinflammatory properties. Because the analgesic effect has a more rapid onset and shorter duration of action than the antiinflammatory action, dosage regimens for effective analgesia may need to be different than those for antiinflammatory effects, or adjunct analgesics may need to be used.

CHIRALITY

Many of the NSAIDs are *stereoisomers.* Stereoisomers consist of enantiomers with the same molecular formula; however, due to asymmetrically oriented chemical groups on a central carbon, they form three-dimensional, nonsuperimposable mirror images and are known as *chiral* compounds.[383] For the NSAIDs it is common to use the *S (sinister)* and *R (rectus)* designations for each of a pair of enantiomers.[384] Although each member of a pair of enantiomers differs in three-dimensional orientation, their physical properties (e.g., melting and boiling points, refractive index, solubility) are identical. Biologic systems are highly chiral environments, and the pharmacokinetics and pharmacodynamic effects of each of a pair of enantiomers may be very different. Stereospecificity may occur in the pharmacokinetic processes of absorption, distribution, metabolism, and excretion, especially if the process involves a carrier protein.[385] If the fit of a drug molecule into the binding site on a protein, enzyme, or receptor involves the chiral center, the affinity for attachment will be different for each of a pair of enantiomers. Therapeutic efficacy, toxicity, or both may be related specifically to one enantiomer. Work with the specific enantiomers of some NSAIDs has shown the S enantiomers to have good COX-inhibitory effects, whereas R forms can have weak activity against COX yet still produce analgesia.[383,386,387]

Most chiral drugs are formulated as racemic mixtures, containing equal amounts of each enantiomer, because pure

enantiomeric compounds are difficult and expensive to manufacture. All of the propionic acid NSAIDs (i.e., ketoprofen, carprofen, vedoprofen, naproxen) are chiral compounds, and, except for naproxen, they are formulated as racemic mixtures. After administration, some enantiomers undergo *chiral inversion,* as hepatic enzymes convert one form of the enantiomer to the other form. When chiral inversion of the propionic acid derivatives occurs, it is almost invariably unidirectional, from R to S.[383] The degree of chiral inversion varies among species and cannot be predicted from one species to another, making extrapolating dosages for NSAIDs extremely hazardous.

PHYSICAL PROPERTIES

Almost all NSAIDs are weak acids and highly bound to plasma proteins such as albumin.[355] Therefore they are well absorbed from the stomach, and most of the drug in the plasma is protein bound. Because of protein binding, they are predominantly distributed in the ECF, and only low concentrations of NSAIDs are found in normal tissues and joint fluid. In damaged tissues and joints, however, total (bound and unbound) NSAID concentrations increase to therapeutic levels because of increased blood flow, vascular permeability, and acute phase protein penetration into sites of inflammation. Most NSAIDs undergo hepatic metabolism either through oxidation or glucuronide conjugation before being eliminated in the urine.[355]

DRUG INTERACTIONS

The occurrence and potential hazards of drug interactions must be considered before initiating therapeutic use of the NSAIDs. In general, any two NSAIDs administered together will be additive in their effect.[388-391] The downside to this practice is the increased risk of toxicity that occurs when NSAIDs are administered concurrently. Horses administered a combination of phenylbutazone and flunixin at the recommended doses for 5 days had a higher incidence of gastric ulceration, as well as a significant decrease in blood protein concentrations, compared with placebo-treated horses and horses receiving phenylbutazone alone.[391] It is likely that the benefits of giving two NSAIDs at once are far outweighed by the risks. Because most NSAIDs act by similar mechanisms of COX inhibition, a higher dose of a single NSAID should produce the same response.

Antacids, mucoprotective agents, and adsorbent antidiarrheal drugs can interfere with the absorption of NSAIDs.[346] Concurrent use of corticosteroids is generally contraindicated because corticosteroids increase the secretion of gastric acid, pepsin, and trypsin; alter the structure of gastric mucin; and decrease mucosal cell proliferation. This action is synergistic with NSAID-induced GI mucosal damage, but the specific risks of concurrent NSAID and steroid administration are unknown.[392] Several antibiotics have been shown to alter phenylbutazone disposition. Chloramphenicol decreases the elimination rate, and rifampin significantly increases the elimination rate of phenylbutazone.[393] Alternatively, phenylbutazone may affect the disposition of other antibiotics. Coadministration of phenylbutazone and procaine penicillin G results in higher serum concentrations of penicillin because of decreased tissue distribution.[394] Concurrent administration of gentamicin and phenylbutazone results in increased distribution and delayed elimination of gentamicin.[395] Phenylbutazone also decreases urinary excretion of furosemide and attenuates furosemide-induced increases in urinary excretion of sodium and chloride.[396,397]

ADVERSE EFFECTS OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

The adverse effects of the NSAIDs are primarily related to COX inhibition in tissues where prostaglandins are beneficial and protective. The NSAIDs have a higher incidence of toxicity in neonates because kidney and liver function is not fully developed.[398-400] When indicated in neonates, NSAIDs should be administered at the lowest possible doses and at extended dosing intervals. NSAIDs should be administered very cautiously to dehydrated animals.[401,402] Because they mostly distribute in extracellular water, plasma concentrations will be greater than normal in the dehydrated animal and more likely to cause toxicity. Decreased feed intake may also contribute to an increase in toxicity by increasing mucosal contact of drugs that may be directly cytotoxic. Common adverse drug reactions associated with NSAID use in the horse include renal papillary necrosis (medullary crest necrosis) and oral and GI ulceration.[397,401-408]

The renal toxicity of NSAIDs is a major concern, particularly in the perioperative period. NSAIDs typically have little effect on renal function in normal adult animals.[409] However, they decrease renal blood flow and GFR in patients with congestive heart failure, those that are hypotensive or hypovolemic (especially during anesthesia and surgery), or those that have chronic renal disease.[361] A more severe dose-dependent toxicity associated most commonly with phenylbutazone is renal papillary necrosis.[397,401,408] Although attributed to impaired renal blood flow, other mechanisms, such as direct nephrotoxicity of the drug or its metabolites, also may be involved. Risk for nephrotoxicity is increased when NSAIDs are coadministered with other nephrotoxic drugs, such as polymyxin B or aminoglycoside antibiotics.

Ulceration of the GI tract occurs most commonly in the oral cavity, gastric glandular mucosa, and duodenum. With duodenal involvement, stricture formation is possible, and surgical bypass of the proximal duodenum may be required for successful treatment. Ulceration in the right dorsal colon may also occur and can result in severe disease. The treatment of right dorsal colitis is intensive and mainly symptomatic.[403] The hypoproteinemia that results from loss of plasma proteins into the ulcerated GI tract can be corrected with IV infusions of plasma. The fluid and electrolyte losses that accompany the diarrhea are managed with commercially available IV fluids. Broad-spectrum antimicrobials are indicated when bacterial septicemia appears to exist. Pain must be managed with opioid analgesics. Antiulcer medications may be beneficial and speed recovery. Surgical removal of damaged sections of the colon may be necessary in some cases.[403] Recovery is usually slow, and in severe cases the prognosis is always guarded.

Less common adverse drug reactions include hemostatic effects and altered proteoglycan synthesis. NSAIDs classically inhibit platelet aggregation by preventing thromboxane production via the COX-1 pathway.[355] Recovery of platelet function is dependent on the pharmacokinetics of the NSAID and the mechanism of COX inhibition.[410-414] Aspirin permanently modifies COX, so platelet function is restored only by

the production of new platelets.[410,414] The effects of NSAIDs on proteoglycan synthesis should be considered in their clinical use for equine joint disease. Many NSAIDs affect cartilage anabolism in addition to their antiinflammatory actions.[415,416] A few NSAIDs, such as carprofen, increase proteoglycan synthesis.[415,417] Phenylbutazone does not affect proteoglycan synthesis or chondrocyte viability, but it is protective against chondrocyte-mediated catabolism.[416] The effects of NSAIDs on bone healing are currently controversial, with some laboratory animal studies showing detrimental effects and others showing little or no effect on bone healing.[418] Currently the only NSAID that has shown negative bone healing effects in horses is phenylbutazone.[419] Clinicians should consider the clear benefits of analgesia in equine patients against the potential adverse effects.

ASPIRIN

Aspirin (sodium salicylate) is available only in oral forms. Because it is a weak acid, it is best absorbed in the acidic environment of the upper GI tract, although overall bioavailability is low via the oral route (6%). Absorption is higher when given via the rectal route (17%).[420] During absorption aspirin is partially hydrolyzed to salicylic acid and distributed throughout the body. The highest concentrations are attained in the liver, heart, lungs, renal cortex, and plasma. Protein binding is moderate (about 60%) and depends on species and drug and albumin concentrations.[355] Aspirin is hepatically metabolized by glycine and glucuronide conjugation. Salicylates and their metabolites are rapidly excreted in urine by way of glomerular filtration and active tubular excretion, with an elimination half-life in the horse of approximately 1 hour.[420] In the horse salicylic acid is the primary salicyl compound found in urine.[421] Significant tubular reabsorption occurs, which depends greatly on pH.[355]

Aspirin is the most effective NSAID for antiplatelet therapy.[410,422,423] Aspirin irreversibly acetylates the COX present in platelets, which inhibits the formation of thromboxane A_2, which is responsible for vasoconstriction and platelet aggregation. [410,422,423] Because platelets do not contain a nucleus, they are incapable of synthesizing additional COX; therefore platelet thromboxane production will be inhibited for the life of any circulating platelets. Antiplatelet therapy may be beneficial in the management of equine laminitis, disseminated intravascular coagulation, and equine verminous arteritis. A precise antiplatelet dose has not been established, but a dose of 12 mg/kg prolongs bleeding time for 48 hours.[410]

CARPROFEN

Carprofen is a propionic acid derivative formulated as a racemic mixture that is currently available for use in horses in Europe. The Vd is 0.1 L/kg for the R enantiomer and 0.29 L/kg for the S enantiomer.[365] At the recommended dose of 0.7 mg/kg, carprofen has a longer elimination half-life in horses than most other NSAIDs, at 21 hours for the R enantiomer and 17 hours for the S enantiomer following IV administration. The R enantiomer predominates in plasma and exudates because of the hepatic stereospecificity for glucuronidation of the S enantiomer, leading to its more rapid clearance.[424] Chiral inversion of carprofen does not occur in the horse.[424] Like other NSAIDs, carprofen accumulates in inflammatory exudate but produces only modest reductions in the concentrations of ecosanoids compared with flunixin or phenylbutazone.[386,425] Despite this, carprofen produces significant analgesia, probably owing to the central actions of the R enantiomer.[425] Carprofen may also have other beneficial antiinflammatory effects, including inhibition of matrix metalloproteinases in cartilage explants and inhibition of the activation of NfκB-dependent genes such as inducible nitric oxide synthase.[426,427]

DICLOFENAC

Diclofenac is available as a 1% liposomal suspension cream for topical application in horses. It is used to control pain and inflammation associated with osteoarthritis in the tarsal, carpal, metacarpophalangeal, metatarsophalangeal, and proximal interphalangeal joints in horses. A 5-inch ribbon of diclofenac topical cream can be applied twice daily over the affected joint for up to 10 days. Owners should wear rubber gloves to prevent absorption into the hands. A single topical application of diclofenac cream produced measurable concentrations of diclofenac in transudate within 6 hours and significantly attenuated carrageenan-induced local production of PGE_2.[428] However, in an amphotericin B acute synovitis model, there was no overall difference between the diclofenac-treated group and the control group.[429] In a controlled field study in horses with osteoarthritis, average lameness scores showed statistically significant improvement after treatment with diclofenac topical cream.[430] Topical application over the sites of injection for regional limb perfusion has also been found to decrease inflammation at the site, as well as provide analgesia, potentially allowing extended use of this procedure.[431] The limited systemic absorption of diclofenac makes it useful in the treatment of acute lameness in competitive horses.[432] However, as a result of central sensitization and windup, locally administered diclofenac will be less effective than systemically administered NSAIDs for therapy of chronic pain. Topical diclofenac can be used simultaneously with systemically administered NSAIDs for maximal effect.

FIROCOXIB

Firocoxib is an NSAID in the coxib class introduced in the United States in 2005. It is labeled for use in the horse as a paste preparation for oral administration at a dose of 0.1 mg/kg for 14 days for the treatment of osteoarthritis. In 2011 it was also approved as an injectable formulation for IV administration at 0.09 mg/kg for 5 days. In clinical trials of horses with naturally occurring osteoarthritis, overall clinical efficacy of firocoxib was comparable to that of a paste formulation of phenylbutazone after 14 days of administration.[433] A canine tablet formulation is also available, and data suggest that the canine formulation is absorbed in the horse and may also be used as an effective antiinflammatory agent.[434,435] The use of the canine formulation is controversial, as it is often administered because of the decreased cost compared with the paste. The American Veterinary Medical Association has stated that it will not support the practice and this extralabel use is not justified.[436]

Firocoxib is highly COX-2 selective, with COX-1:COX-2 IC_{50} ratios of 263 to 643 demonstrated in vitro.[437] Several studies have confirmed this in ex vivo models of the horse, with minimal to no COX-1 inhibition noted following oral or IV administration.[434,438] On the basis of in vitro whole blood experiments, the concentration of firocoxib that inhibits 50%

(IC_{50}) or 80% (IC_{80}) of COX-2 activity in LPS-stimulated equine whole blood is approximately 30 ng/mL and 67 ng/mL, respectively.[439] Adjusting for plasma concentrations by assuming a plasma concentration that is 65% of whole blood, the IC_{80} equates to 103 ng/mL.[435] Concentrations of 100 ng/mL firocoxib have been shown to reduce COX-2 and mPGES1 gene expression in isolated equine peripheral blood mononuclear cells.[440] When modeled using plasma concentration profiles in ex vivo models, similar results were found, with an IC_{80} reported between 96 and 110 ng/mL.[434,435] This IC_{80} is similar to what was achieved following IV administration of a single dose[434] and oral administration of multiple doses[435] of either the paste or tablet formulation in the other study.

In safety studies, oral ulcers were detected at 3× and 5× the label dose, but no other adverse effects were noted. However, adverse effects may still occur, particularly in patients with preexisting disease, or those that receive NSAID stacking. A study examining the effects of coadministration of firocoxib with phenylbutazone showed significantly increased serum creatinine, significantly decreased total protein, and a significantly lower urine specific gravity after 10 days of treatment at commonly used doses.[441]

In horses firocoxib is well absorbed, with an oral bioavailability of 79%, although the time to maximum concentration following the first dose is often prolonged. Firocoxib is slowly eliminated, and Vd values are approximately 2 L/kg. Reported elimination half-lives are as long as 2 days.[437,439] Based on the long half-life, it may take multiple days for steady concentrations and maximum efficacy to be reached. To overcome this, a loading dose of 0.3 mg/kg once has been recommended,[442] which allows achievement of average steady-state drug concentrations within 24 hours. After the loading dose, maintenance doses following label directions maintain a relatively constant average drug concentration that produces less variability in onset of action and efficacy.

Foals 4 to 6 weeks of age have similar pharmacokinetics to adult horses[443]; however, the pharmacokinetics differs significantly in neonatal foals. In neonates, firocoxib is more rapidly absorbed, with a higher maximum concentration but a shorter half-life, resulting in less drug accumulation.[444] Steady-state is achieved after approximately 3 doses. In healthy neonates, no clinically apparent adverse effects were noted after 9 days of oral firocoxib at 0.1 mg/kg. Intravenous data in neonates suggest that the difference in pharmacokinetics is due to an increased clearance. Even following multiple doses, IC_{80} levels were not reached in neonates at horse label doses. Further work is needed to determine the IC_{80} necessary for foals and whether or not the use of higher doses or shorter dose intervals is safe and effective; however, current clinical practice is to use 0.2 mg/kg PO or IV q12–24hr.

FLUNIXIN MEGLUMINE

Flunixin meglumine is a very potent inhibitor of COX that is approved for use in horses and is available in injectable and oral formulations. Flunixin is rapidly absorbed after oral administration, with a bioavailability of 86% and peak serum levels within 30 minutes.[445] Absorption is delayed by feeding.[446] The Vd is 0.1 to 0.3 L/kg in horses, and the plasma elimination half-life is 1 to 2 hours.[445-447] In newborn foals the elimination half-life is prolonged at 13.4 hours. The Vd is also increased in the newborn foal, as is expected with a low-Vd drug in the neonate.[448] It is highly protein bound (86%) but appears to readily partition into tissues, hence the relatively high volume of distribution.[449,450] The elimination half-life in inflammatory exudate is 16 hours.[451] The onset of antiinflammatory action is within 2 hours, peak response occurs between 12 and 16 hours, and duration of action is 36 hours. Analgesic effects have a more rapid onset and shorter duration. Only 14% of a dose is excreted in urine, but otherwise little is known about the metabolism of flunixin.[355]

Flunixin is used in horses for a variety of inflammatory and painful conditions, including colic, colitis, exertional rhabdomyolysis, endotoxic shock, respiratory disease, ocular disease, general surgery, and laminitis.[355] Flunixin is more effective than phenylbutazone in preventing the clinical signs of endotoxemia but appears equivalent to ketoprofen.[452] Flunixin may prevent abortion in endotoxic mares.[453] Flunixin is dosed at 1.1 mg/kg every 12 to 24 hours for musculoskeletal pain but may be administered more frequently at lower doses (0.5 mg/kg q6–12hr) for colic pain. Therapy at 0.25 mg/kg every 6 to 8 hours is used as the lowest dose that will provide antiprostaglandin effects, but this dose will not provide much analgesia. Extremely high doses of flunixin may mask signs of surgical colic pain and interfere with treatment decisions.

Flunixin has a good safety profile, but high doses or chronic dosing can cause anorexia, depression, and GI ulcers.[406,454] In normal foals the label dose of flunixin administered for 5 days did not produce adverse effects, but 6 times the label dose resulted in GI ulcers.[400] In another study foals were administered flunixin at the label dose for 30 days, and all treated foals developed gastric ulcers.[398] Area under the curve, clearance, and half-life of flunixin are significantly greater in newborn foals compared with adult horses.[448] The half-life increases from 1 to 2 hours in adults to 6 to 8 hours in neonates. Based on this, higher doses with prolonged dosing intervals should be used in neonatal foals.

IM injections of flunixin are highly irritating to muscle and have been incriminated in cases of clostridial myonecrosis in horses; they should therefore be avoided when possible, despite the label directions.[455,456] If not treated promptly and aggressively, clostridial myonecrosis causes severe tissue damage and may be rapidly fatal. Flunixin treatment of horses with ischemic GI disease may cause prolonged permeability defects in recovering mucosa.[495]

KETOPROFEN

Ketoprofen is a chiral propionic acid derivative approved for horses as a racemic solution for IV or IM injection. The label dose is 2.2 mg/kg administered once daily. Oral and rectal bioavailability is too poor for these routes to be used clinically.[367,457,458] Ketoprofen is 92.8% protein bound in horses.[459] Ketoprofen has a moderate Vd for both enantiomers of approximately 0.5 L/kg and short plasma elimination half-lives of 1 to 1.5 hours.[367,387,451,459,460] Ketoprofen is hepatically metabolized by conjugation reactions, with only 25% of a dose eliminated as unchanged drug in urine.[459] The S enantiomer is associated with antiprostaglandin activity and toxicity, whereas the R enantiomer is associated with analgesia and does not produce GI ulceration.[367,461] Because of chiral inversion, the S enantiomer predominates in horses.[367] Ketoprofen accumulates in inflammatory exudates in the horse, where the elimination half-life of the S enantiomer is 23 hours and the R enantiomer is 20 hours. The maximum antiinflammatory effects of ketoprofen occur at 4 hours after a dose and last for

24 hours, illustrating that the antiinflammatory effects are not related to plasma concentrations.[451] Similar to flunixin, ketoprofen pharmacokinetics are different in neonatal foals, and higher doses with longer dosing intervals are recommended.[462] In studies of noninfectious arthritis, endotoxemia, and colic, ketoprofen is clinically similar to flunixin meglumine in efficacy.[451,452,461] In an experimentally induced synovitis model, phenylbutazone was more effective in reducing lameness and synovial fluid prostaglandin concentrations.[463] In horses with chronic laminitis, ketoprofen was more effective than phenylbutazone at relieving pain but only at a higher-than-label dose (3.63 mg/kg).[464] In comparative toxicity studies in both horses and donkeys, ketoprofen at the label dose had less potential for toxicity than flunixin meglumine or phenylbutazone.[406,465] In drug tolerance studies using 25 times the label dose for 5 days, horses developed depression, icterus, nephritis, hepatitis, and hemorrhagic necrosis of the adrenal glands.[466]

MELOXICAM

Meloxicam is an enolic acid belonging to the oxicam class of NSAIDs. It is slightly COX-2 selective in the horse with an IC_{50} COX-1:IC_{50} COX-2 of 3.8.[467] Meloxicam is labeled for use in Europe as an antiinflammatory drug in the horse for both oral and injectable formulations. Oral bioavailability is nearly complete, and absorption is not affected by feeding.[468] The Vd is 0.12 L/kg, with most drug eliminated unchanged in the urine. The elimination half-life is 5.2 to 8.5 hours.[468,469] Clearance differs significantly between horses and donkeys, with values of 34.7 mL/kg/hr and 187.9 mL/kg/hr, respectively.[470] Pharmacokinetic-pharmacodynamic analysis has shown that a maximum effect is achieved at plasma concentrations of approximately 0.2 μg/mL, which translates into a daily dose of 0.6 mg/kg intravenously or orally.

This dose has proven effective in lameness models, as well as experimental models of colic and abdominal pain. Direct comparison of flunixin and meloxicam in postoperative cases of small intestinal strangulating obstructions showed that meloxicam was as effective as flunixin when comparing most major clinical variables, although more horses in the meloxicam group showed gross signs of pain.[471] Additionally, meloxicam treatment did not impair mucosal recovery after ischemic injury in a small intestinal strangulation model.[472] Meloxicam also had no detectable difference in sucrose permeability following intragastric administration, indicating less compromise to gastric mucosal permeability compared with phenylbutazone.[473] However, chronic dosing with higher doses (1.8–3.0 mg/kg) was associated with dose-dependent adverse effects typical of this class of drugs, including hypoproteinemia and hypoalbuminemia, GI disorders, renal damage, and bone marrow dyscrasias.[474] Administration of meloxicam IV before surgery followed by once-daily oral administration for 4 consecutive days is efficacious in controlling postoperative pain and inflammation in horses undergoing orthopedic surgery.[475]

Meloxicam pharmacokinetics in neonatal foals differs from adults.[476] Similar to firocoxib, meloxicam exhibits a higher clearance in neonates, with an elimination half-life of approximately 2.5 hours. Based on a multiple-dose study, 0.6 mg/kg orally q12hr to foals less than 7 weeks of age produced concentrations thought to be effective for treatment of pain and inflammation. Adverse effects, including gastric ulceration, changes in white blood cell counts, and changes in serum albumin and protein concentrations, were not noted in foals receiving meloxicam at these doses for 21 consecutive days.

Another NSAID in this group, piroxicam, has received some attention in equine medicine, not for treatment of lameness or colic, but for the treatment of squamous cell carcinoma (SCC). Some tumors, particularly SCC, have been shown to produce COX-2.[477] Treatment with piroxicam has produced long-term remissions of SCC in the bladder, urethra, and periocular structures.[478] Adverse effects, even at the low doses used (0.2 mg/kg PO q24hr), have been noted, and include diarrhea and abdominal pain. It is not known at this time whether or not meloxicam would have a similar effect, with a higher safety profile.

PHENYLBUTAZONE

Phenylbutazone is the most widely used NSAID in the horse and is available in many generic IV and oral formulations. After oral administration, phenylbutazone is well absorbed, but time to peak concentration may be delayed by feeding.[479-481] The Vd is 0.15 L/kg, with highest concentrations in the liver, heart, kidney, lungs, and plasma.[482] The elimination half-life is 3.5 to 7 hours.[483] In neonatal foals the Vd is higher (0.27 L/kg) and the elimination half-life is longer (6.4–22.1 hours) than in adult horses.[484] Plasma protein binding in horses is greater than 99%.[483] Phenylbutazone is metabolized in the liver to oxyphenbutazone, an active metabolite that is eliminated more slowly from the body than phenylbutazone. Oxyphenbutazone inhibits the metabolism of phenylbutazone. Phenylbutazone and its metabolite cross the placenta and are excreted in milk. Less than 2% is excreted in the urine as unchanged drug. The capacity of the liver to metabolize phenylbutazone becomes overwhelmed at relatively low drug doses, resulting in dose-dependent kinetics.[485] The elimination half-life increases with increasing dose rates and increasing age.[485-487] The elimination half-life from exudate is 24 hours.[366] Therapeutic efficacy lasts for more than 24 hours as a result of the irreversible binding of phenylbutazone to COX, slow elimination from inflamed tissues, and long elimination half-life of oxyphenbutazone.[483] Therefore high or frequent doses of phenylbutazone result in disproportionately increasing plasma concentrations, which easily result in toxicity.

Phenylbutazone is used extensively in horses for a variety of musculoskeletal disorders. Although phenylbutazone also antagonizes the disruptive effects of endotoxin on bowel motility, flunixin meglumine is generally preferred for treatment of colic in horses.[368] Phenylbutazone appears to inhibit prostaglandin synthesis at low plasma concentrations in the horse (5–15 μg/mL), whereas much higher drug concentrations are needed in human beings (50–150 μg/mL).[483] This discrepancy is probably due to a species difference in the structure of COX. An initial dose of 4.4 mg/kg every 12 hours on the first day of therapy is followed by a decreased dose and increased dosing interval for subsequent therapy. Because of the accumulation from the long elimination half-life of phenylbutazone and oxyphenbutazone, chronic therapy should be at the lowest possible dose and the longest possible dosing interval that still controls pain.

Phenylbutazone has a narrow safety margin, especially in foals and ponies and dehydrated horses.[400,401] Phenylbutazone toxicity most commonly results in adverse GI effects, including oral, esophageal, gastric, cecal, and right dorsal colonic ulcerations, and accompanying protein-losing enteropathy,

hypoproteinemia, leukopenia, and anemia.[401-405,488] Renal papillary necrosis (renal medullary crest necrosis) results from inhibition of prostaglandins that maintain renal blood flow and direct toxicity of phenylbutazone and metabolites.[408] Ulcerative cystitis associated with prolonged phenylbutazone administration has also been reported[489] and may also relate to direct cytotoxic effects of drug secreted in the urine. Because phenylbutazone can mask symptoms of lameness in horses for several days after therapy, it may be used to disguise lameness during soundness examinations or for competitive purposes.[483] Extravascular administration results in severe tissue necrosis. Phenylbutazone may have a negative effect on bone healing in horses.[419] Phenylbutazone significantly suppresses total T_4 and free T_4 concentrations in horses for 10 days.[490]

VEDAPROFEN

Vedaprofen is structurally related to ketoprofen and carprofen and also is formulated as a racemic mixture of S and R enantiomers. It is available in some countries as an IV injectable solution and as a palatable gel for oral administration with a loading dose of 2 mg/kg followed by 1 mg/kg every 12 hours. Oral bioavailability is approximately 100%, and it is highly protein bound (99%). Within 2 hours after IV administration, the mean R:S plasma concentration ratio is 95:5. This is due to marked distribution and elimination differences between the enantiomers, and not chiral inversion. The R enantiomer has an elimination half-life of 2.2 hours and a Vd of 0.23 L/kg, and the S enantiomer has an elimination half-life of 0.76 hours and a volume of distribution of 0.5 L/kg.[491] Both enantiomers accumulate in inflammatory exudate and are more slowly cleared from exudate than from plasma. In an equine acute nonimmune inflammation model, vedaprofen produced significant inhibition of inflammatory swelling and partially inhibited leukocyte migration into the exudate. Inhibition of leukocyte migration was not seen in this model with other NSAIDs.[491] Based on in vitro models, vedaprofen is moderately COX-1 selective.

OTHER DRUGS

Numerous other NSAIDs have been studied in the horse, although their clinical use is sporadic and no formulations are labeled for use in the horse. Etodolac is a relatively selective COX-2 inhibitor in the horse. It has been used clinically in horses with a previous history of gastric or colonic ulceration, as well as in a few horses with active GI disease, with apparent benefits. Doses of 20 mg/kg PO q12–24hr have been reported, although the COX-2 selectivity may be lost at these high doses.[492-495] Deracoxib is a moderately selective COX-2 inhibitor in the horse with a long elimination half-life and average maximum plasma concentration of 0.54 µg/mL following oral administration. Doses of 2 mg/kg PO q12hr would maintain concentrations above the IC_{80} for COX-2.[496] Acetaminophen, although not considered a classic NSAID due to its weak inhibitory effects on COX-1 and COX-2, is another drug occasionally used as an antiinflammatory, analgesic, and antipyretic in horses. It is well absorbed with a bioavailability of approximately 91%[497] and has been used successfully in laminitis cases with no specific adverse effects.[498] Doses range from 20 to 25 mg/kg PO q12–24hr.

REFERENCES

1. Toutain PL, Bousquet-Melou A. Volumes of distribution. *J Vet Pharmacol Ther*. 2004;27:441–453.
2. Baggot JD, Love DN, Stewart J, et al. Gentamicin dosage in foals aged one month and three months. *Equine Vet J*. 1986;18:113–116.
3. Toutain PL, Bousquet-Melou A. Bioavailability and its assessment. *J Vet Pharmacol Ther*. 2004;27:455–466.
4. Baggot JD. Bioavailability and bioequivalence of veterinary drug dosage forms, with particular reference to horses: an overview. *J Vet Pharmacol Ther*. 1992;15:160–173.
5. Bertucci C, Domenici E. Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. *Curr Med Chem*. 2002;9:1463–1481.
6. Bailey DN, Briggs JR. The binding of selected therapeutic drugs to human serum alpha-1 acid glycoprotein and to human serum albumin in vitro. *Ther Drug Monit*. 2004;26:40–43.
7. Toutain PL, Bousquet-Melou A. Free drug fraction vs free drug concentration: a matter of frequent confusion. *J Vet Pharmacol Ther*. 2002;25:460–463.
8. Craig WA, Ebert SC. Protein binding and its significance in antibacterial therapy. *Infect Dis Clin North Am*. 1989;3:407–414.
9. Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. *Clin Pharmacol Ther*. 2002;71:115–121.
10. Toutain PL, Bousquet-Melou A. Plasma clearance. *J Vet Pharmacol Ther*. 2004;27:415–425.
11. Intorre L, Mengozzi G, Maccheroni M, et al. Enrofloxacin-theophylline interaction: influence of enrofloxacin on theophylline steady-state pharmacokinetics in the beagle dog. *J Vet Pharmacol Ther*. 1995;18:352–356.
12. von Rosensteil NA, Adam D. Macrolide antibacterials. Drug interactions of clinical significance. *Drug Saf*. 1995;13:105–122.
13. Boothe DM. *Drug disposition and extrapolation of dosing regimens*. St. Louis: Saunders; 2001.
14. Baggot JD, Short CR. Drug disposition in the neonatal animal, with particular reference to the foal. *Equine Vet J*. 1984;16:364–367.
15. Johnson PJ, Mrad DR, Schwartz AJ, et al. Presumed moxidectin toxicosis in three foals. *J Am Vet Med Assoc*. 1999;214:678–680.
16. Riviere JE. *Comparative pharmacokinetics: principles, techniques and applications*. Hoboken, NJ: Wiley-Blackwell; 2003.
17. Lorian V, Burns L. Predictive value of susceptibility tests for the outcome of antibacterial therapy. *J Antimicrob Chemother*. 1990;25:175–181.
18. Giguere S, Prescott JF, Baggot JD, et al. *Antimicrobial therapy in veterinary medicine*. 4th ed. Ames: Blackwell Publishing; 2006.
19. Sandholm M, Kaartinen L, Pyorala S. Bovine mastitis: why does antibiotic therapy not always work? An overview. *J Vet Pharmacol Ther*. 1990;13:248–260.
20. Clark C, Dowling PM, Ross S, et al. Pharmacokinetics of tilmicosin in equine tissues and plasma. *J Vet Pharmacol Ther*. 2008;31:66–70.
21. Ames TR, Patterson EB. Oxytetracycline concentrations in plasma and lung of healthy and pneumonic calves, using two oxytetracycline preparations. *Am J Vet Res*. 1985;46:2471–2473.
22. Kuriyama T, Nakagawa K, Kawashiri S, et al. The virulence of mixed infection with *Streptococcus constellatus* and *Fusobacterium nucleatum* in a murine orofacial infection model. *Microbes Infect*. 2000;2:1425–1430.
23. Hariharan H, McPhee L, Heaney S, et al. Antimicrobial drug susceptibility of clinical isolates of *Pseudomonas aeruginosa*. *Can Vet J*. 1995;36:166–168.
24. Fantin B, Carbon C. In vivo antibiotic synergism: contribution of animal models. *Antimicrob Agents Chemother*. 1992;36:907–912.
25. Marshall SA, Jones RN, Wanger A, et al. Proposed MIC quality control guidelines for National Committee for Clinical Laboratory Standards susceptibility tests using seven veterinary antimicrobial agents: ceftiofur, enrofloxacin, florfenicol, penicillin G-novobiocin, pirlimycin, premafloxacin, and spectinomycin. *J Clin Microbiol*. 1996;34:2027–2029.

26. Vogelman BS, Craig WA. Postantibiotic effects. *J Antimicrob Chemother*. 1985;15:A37–46.
27. McKellar QA, Sanchez Bruni SF. Jones DG: Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *J Vet Pharmacol Ther*. 2004;27:503–514.
28. Prescott JF, Nicholson VM. The effects of combinations of selected antibiotics on the growth of *Corynebacterium equi. J Vet Pharmacol Ther*. 1984;7:61–64.
29. Clark C, Greenwood S, Boison JO, et al. Bacterial isolates from equine infections in western Canada. *Can Vet J*. 1998-2003;2008(49):153–160.
30. Sweeney CR, Holcombe SJ, Barningham SC, et al. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *J Am Vet Med Assoc*. 1991;198:839–842.
31. Cohen ND, Woods AM. Characteristics and risk factors for failure of horses with acute diarrhea to survive: 122 cases (1990-1996). *J Am Vet Med Assoc*. 1999;214:382–390.
32. Raidal SL, Taplin RH, Bailey GD, et al. Antibiotic prophylaxis of lower respiratory tract contamination in horses confined with head elevation for 24 or 48 hours. *Aust Vet J*. 1997;75:126–131.
33. Whittem TL, Johnson AL, Smith CW, et al. Effect of perioperative prophylactic antimicrobial treatment in dogs undergoing elective orthopedic surgery. *J Am Vet Med Assoc*. 1999;215:212–216.
34. Haven ML, Wichtel JJ, Bristol DG, et al. Effects of antibiotic prophylaxis on postoperative complications after rumenotomy in cattle. *J Am Vet Med Assoc*. 1992;200:1332–1335.
35. Dunkel B, Johns IC. Antimicrobial use in critically ill horses. *J Vet Emerg Crit Care (San Antonio)*. 2015;25:89–100.
36. Dever LA, Dermody TS. Mechanisms of bacterial resistance to antibiotics. *Arch Intern Med*. 1991;151:886.
37. Gold HS, Moellering RC. Antimicrobial-drug resistance. *N Engl J Med*. 1996;335:1445–1453.
38. Ayala J, Quesada A, Vadillo S, et al. Penicillin-binding proteins of *Bacteroides fragilis* and their role in the resistance to imipenem of clinical isolates. *J Med Microbiol*. 2005;54:1055–1064.
39. de Lencastre H, Oliveira D, Tomasz A. Antibiotic resistant *Staphylococcus aureus:* a paradigm of adaptive power. *Curr Opin Microbiol*. 2007;10:428–435.
40. Geddes AM, Klugman KP, Rolinson GN. Introduction: historical perspective and development of amoxicillin/clavulanate. *Int J Antimicrob Agents*. 2007;30(suppl 2):S109–112.
41. Essack SY. The development of beta-lactam antibiotics in response to the evolution of beta-lactamases. *Pharm Res*. 2001;18:1391–1399.
42. Sandanayaka VP, Prashad AS. Resistance to beta-lactam antibiotics: structure and mechanism based design of beta-lactamase inhibitors. *Curr Med Chem*. 2002;9:1145–1165.
43. Finlay J, Miller L, Poupard JA. A review of the antimicrobial activity of clavulanate. *J Antimicrob Chemother*. 2003;52:18–23.
44. Papich MG. The beta-lactam antibiotics: clinical pharmacology and recent developments. *Compend Contin Educ Pract Vet*. 1987;9:68–74.
45. Falagas ME, Siakavellas E. *Bacteroides, Prevotella,* and *Porphyromonas* species: a review of antibiotic resistance and therapeutic options. *Int J Antimicrob Agents*. 2000;15:1–9.
46. Schwark WS, Ducharme NG, Shin SJ, et al. Absorption and distribution patterns of oral phenoxymethyl penicillin (penicillin V) in the horse. *Cornell Vet*. 1983;73:314–322.
47. Schipper IA, Filipovs D, Ebeltoft H, et al. Blood serum concentrations of various benzyl penicillins after their intramuscular administration to cattle. *J Am Vet Med Assoc*. 1971;158:494–500.
48. Baggot JD. Bioavailability and bioequivalence of veterinary drug dosage forms, with particular reference to horses: an overview. *J Vet Pharmacol Ther*. 1992;15:160–173.
49. Love DN, Rose RJ, Martin IC, et al. Serum concentrations of penicillin in the horse after administration of a variety of penicillin preparations. *Equine Vet J*. 1983;15:43–48.
50. Firth EC, Nouws JF, Klein WR, et al. The effect of phenylbutazone on the plasma disposition of penicillin G in the horse. *J Vet Pharmacol Ther*. 1990;13:179–185.
51. Durr A. Comparison of the pharmacokinetics of penicillin G and ampicillin in the horse. *Res Vet Sci*. 1976;20:24–29.
52. McConnico RS, Roberts MC, Tompkins M. Penicillin-induced immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc*. 1992;201:1402–1403.
53. Wilkerson MJ, Davis E, Shuman W, et al. Isotype-specific antibodies in horses and dogs with immune-mediated hemolytic anemia. *J Vet Intern Med*. 2000;14:190–196.
54. Nielsen IL, Jacobs KA, Huntington PJ, et al. Adverse reaction to procaine penicillin G in horses. *Aust Vet J*. 1988;65:181–185.
55. Romano A, Mayorga C, Torres MJ, et al. Immediate allergic reactions to cephalosporins: cross-reactivity and selective responses. *J Allergy Clin Immunol*. 2000;106:1177–1183.
56. Chapman CB, Courage P, Nielsen IL, et al. The role of procaine in adverse reactions to procaine penicillin in horses. *Aust Vet J*. 1992;69:129–133.
57. Tobin T, Blake JW. The pharmacology of procaine in the horse: relationships between plasma and urinary concentrations of procaine. *J Equine Med Surg*. 1977;1:188–194.
58. Tobin T, Blake JW, Sturma L, et al. Pharmacology of procaine in the horse: procaine esterase properties of equine plasma and synovial fluid. *Am J Vet Res*. 1976;37:1165–1170.
59. Fischbach H, Welch H, King EQ, et al. Procaine penicillin and sulfonamide antagonism. *J Am Pharm Assoc Am Pharm Assoc*. 1949;38:544–546.
60. Olsen L, Ingvast-Larsson C, Brostrom H, et al. Clinical signs and etiology of adverse reactions to procaine benzylpenicillin and sodium/potassium benzylpenicillin in horses. *J Vet Pharmacol Ther*. 2007;30:201–207.
61. Stevenson AJ, Weber MP, Todi F, et al. Plasma elimination and urinary excretion of procaine after administration of different products to standardbred mares. *Equine Vet J*. 1992;24:118–124.
62. Adamson PJ, Wilson WD, Hirsh DC, et al. Susceptibility of equine bacterial isolates to antimicrobial agents. *Am J Vet Res*. 1985;46:447–450.
63. Firth EC, Klein WR, Nouws JF, et al. Effect of induced synovial inflammation on pharmacokinetics and synovial concentration of sodium ampicillin and kanamycin sulfate after systemic administration in ponies. *J Vet Pharmacol Ther*. 1988;11:556–562.
64. Ensink JM, Moi A, Vulto AG, et al: Bioavailability of pivampicillin and ampicillin trihydrate administered as an oral paste in horses. *Vet Q* 18:2s117–120.
65. Wilson WD, Spensley MS, Baggot JD, et al. Pharmacokinetics and estimated bioavailability of amoxicillin in mares after intravenous, intramuscular, and oral administration. *Am J Vet Res*. 1988;49:1688–1694.
66. Baggot JD, Love DN, Stewart J, et al. Bioavailability and disposition kinetics of amoxicillin in neonatal foals. *Equine Vet J*. 1988;20:125–127.
67. Ensink JM, Vulto AG, van Miert AS, et al. Oral bioavailability and in vitro stability of pivampicillin, bacampicillin, talampicillin, and ampicillin in horses. *Am J Vet Res*. 1996;57:1021–1024.
68. Ensink JM, Klein WR, Mevius DJ, et al. Bioavailability of oral penicillins in the horse: a comparison of pivampicillin and amoxicillin. *J Vet Pharmacol Ther*. 1992;15:221–230.
69. Sarasola P, McKellar QA. Pharmacokinetics and applications of ampicillin sodium as an intravenous infusion in the horse. *J Vet Pharmacol Ther*. 1993;16:63–69.
70. van den Hoven R, Hierweck B, Dobretsberger M, et al. Intramuscular dosing strategy for ampicillin sodium in horses, based on its distribution into tissue chambers before and after induction of inflammation. *J Vet Pharmacol Ther*. 2003;26:405–411.
71. Bowman KF, Dix LP, Riond JL, et al. Prediction of pharmacokinetic profiles of ampicillin sodium, gentamicin sulphate, and combination ampicillin sodium-gentamicin sulphate in serum and synovia of healthy horses. *Am J Vet Res*. 1986;47:1590–1596.

72. Errecalde JO, Carmely D, Marino EL, et al. Pharmacokinetics of amoxycillin in normal horses and horses with experimental arthritis. *J Vet Pharmacol Ther*. 2001;24:1–6.
73. Ensink JM, Klein WR, Barneveld A, et al. Distribution of penicillins into subcutaneous tissue chambers in ponies. *J Vet Pharmacol Ther*. 1996;19:439–444.
74. Montesissa C, Carli S, Sonzogni O, et al. Pharmacokinetics of sodium amoxicillin in horses. *Res Vet Sci*. 1988;44:233–236.
75. Beech J, Leitch M, Kohn CW, et al. Serum and synovial fluid levels of sodium ampicillin and ampicillin trihydrate in horses. *J Equine Med Surg*. 1979;3:3503–3504.
76. Traver DS, Riviere JE. Ampicillin in mares: a comparison of intramuscular sodium ampicillin or sodium ampicillin-ampicillin trihydrate injection. *Am J Vet Res*. 1982;43:402–404.
77. Spensley MS, Baggot JD, Wilson WD, et al. Pharmacokinetics and endometrial tissue concentrations of ticarcillin given to the horse by intravenous and intrauterine routes. *Am J Vet Res*. 1986;47:2587–2590.
78. Sweeney CR, Soma LR, Beech J, et al. Pharmacokinetics of ticarcillin in the horse after intravenous and intramuscular administration. *Am J Vet Res*. 1984;45:1000–1002.
79. Wilson WD, Spensley MS, Baggot JD, et al. Pharmacokinetics and bioavailability of ticarcillin and clavulanate in foals after intravenous and intramuscular administration. *J Vet Pharmacol Ther*. 1991;14:78–89.
80. Sweeney RW, Beech J, Simmons RD, et al. Pharmacokinetics of ticarcillin and clavulanic acid given in combination to adult horses by intravenous and intramuscular routes. *J Vet Pharmacol Ther*. 1988;11:103–108.
81. Van Camp SD, Papich MG, Whitacre MD. Administration of ticarcillin in combination with clavulanic acid intravenously and intrauterinely to clinically normal oestrous mares. *J Vet Pharmacol Ther*. 2000;23:373–378.
82. Hoffman AM, Viel L, Muckle CA, et al. Evaluation of sulbactam plus ampicillin for treatment of experimentally induced *Klebsiella pneumoniae* lung infection in foals. *Am J Vet Res*. 1992;53:1059–1067.
83. Hornish RE, Kotarski SF. Cephalosporins in veterinary medicine: ceftiofur use in food animals. *Curr Top Med Chem*. 2002;2:717–731.
84. Salmon SA, Watts JL, Yancey RJ. In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J Vet Diagn Invest*. 1996;8:332–336.
85. Rice LB. Emergence of vancomycin-resistant enterococci. *Emerg Infect Dis*. 2001;7:183–187.
86. Duffee NE, Christensen JM, Craig AM. The pharmacokinetics of cefadroxil in the foal. *J Vet Pharmacol Ther*. 1989;12:322–326.
87. Wilson WD, Baggot JD, Adamson PJ, et al. Cefadroxil in the horse: pharmacokinetics and in vitro antibacterial activity. *J Vet Pharmacol Ther*. 1985;8:246–253.
88. Carrillo NA, Giguère S, Gronwall RR, et al. Disposition of orally administered cefpodoxime proxetil in foals and adult horses and minimum inhibitory concentration of the drug against common bacterial pathogens of horses. *Am J Vet Res*. 2005;66:30–35.
89. Lovering AM, Walsh TR, Bannister GC, et al. The penetration of ceftriaxone and cefamandole into bone, fat and haematoma and relevance of serum protein binding to their penetration into bone. *J Antimicrob Chemother*. 2001;47:483–486.
90. Cunha BA. Third-generation cephalosporins: a review. *Clin Ther*. 1992;14:616–652, discussion 615.
91. Meyer JC, Brown MP, Gronwall RR, et al. Pharmacokinetics of ceftiofur sodium in neonatal foals after intramuscular injection. *Equine Vet J*. 1992;24:485–486.
92. Collard WT, Cox SR, Lesman SP, et al. Pharmacokinetics of ceftiofur crystalline-free acid sterile suspension in the equine. *J Vet Pharmacol Ther*. 2011;34:476–481.
93. Giguère S, Sturgill TL, Berghaus LJ, et al. Effects of two methods of administration on the pharmacokinetics of ceftiofur crystalline free acid in horses. *J Vet Pharmacol Ther*. 2011;34:193–196.
94. Macpherson ML, Giguère S, Hatzel JN, et al. Disposition of desfuroylceftiofur acetamide in serum, placental tissue, fetal fluids, and fetal tissues after administration of ceftiofur crystalline free acid (CCFA) to pony mares with placentitis. *J Vet Pharmacol Ther*. 2013;36:59–67.
95. Hall TL, Tell LA, Wetzlich SE, et al. Pharmacokinetics of ceftiofur sodium and ceftiofur crystalline free acid in neonatal foals. *J Vet Pharmacol Ther*. 2011;34:403–409.
96. Credille BC, Giguère S, Berghaus LJ, et al. Plasma and pulmonary disposition of ceftiofur and its metabolites after intramuscular administration of ceftiofur crystalline free acid in weanling foals. *J Vet Pharmacol Ther*. 2012;35:259–264.
97. Strom BL, Schinnar R, Gibson GA, et al. Risk of bleeding and hypoprothrombinaemia associated with NMTT side chain antibiotics: using cefoperazone as a test case. *Pharmacoepidemiol Drug Saf*. 1999;8:81–94.
98. Gardner SY, Aucoin DP. Pharmacokinetics of ceftriaxone in mares. *J Vet Pharmacol Ther*. 1994;17:155–156.
99. Gardner SY, Sweeney RW, Divers TJ. Pharmacokinetics of cefotaxime in neonatal pony foals. *Am J Vet Res*. 1993;54:576–579.
100. Guglick MA, MacAllister CG, Clarke CR, et al. Pharmacokinetics of cefepime and comparison with those of ceftiofur in horses. *Am J Vet Res*. 1998;59:458–463.
101. Fanos V, Cataldi L. Renal transport of antibiotics and nephrotoxicity: a review. *J Chemother*. 2001;13:461–472.
102. Miranda-Novales G, Leanos-Miranda BE, Vilchis-Perez M, et al. In vitro activity effects of combinations of cephalothin, dicloxacillin, imipenem, vancomycin and amikacin against methicillin-resistant *Staphylococcus* spp. strains. *Ann Clin Microbiol Antimicrob*. 2006;5:25.
103. Beauchamp D, Theriault G, Grenier L, et al. Ceftriaxone protects against tobramycin nephrotoxicity. *Antimicrob Agents Chemother*. 1994;38:750–756.
104. Foreman JH: Does ceftiofur cause diarrhea? AAEP 44th Annual Convention Proceedings. 1994;146–147.
105. Mahrt CR. Safety of ceftiofur sodium administered intramuscularly in horses. *Am J Vet Res*. 1992;53:2201–2205.
106. Fultz L, Giguère S, Berghaus LJ, et al. Comparative pharmacokinetics of desfuroylceftiofur acetamide after intramuscular versus subcutaneous administration of ceftiofur crystalline free acid to adult horses. *J Vet Pharmacol Ther*. 2013;36:309–312.
107. Meyer S, Giguère S, Rodriguez R, et al. Pharmacokinetics of intravenous ceftiofur sodium and concentration in body fluids of foals. *J Vet Pharmacol Ther*. 2009;32:309–316.
108. Wearn JM, Davis JL, Hodgson DR, et al. Pharmacokinetics of a continuous rate infusion of ceftiofur sodium in normal foals. *J Vet Pharmacol Ther*. 2013;36:99–101.
109. Fultz L, Giguère S, Berghaus LJ, et al. Plasma and pulmonary pharmacokinetics of desfuroylceftiofur acetamide after weekly administration of ceftiofur crystalline free acid to adult horses. *Equine Vet J*. 2014;46:252–255.
110. Orsini JA, Moate PJ, Boston RC, et al. Pharmacokinetics of imipenem-cilastatin following intravenous administration in healthy adult horses. *J Vet Pharmacol Ther*. 2005;28:355–361.
111. Sams RA, Ruoff WW. Pharmacokinetics and bioavailability of cefazolin in horses. *Am J Vet Res*. 1985;46:348–352.
112. Ruoff WW, Sams RA. Pharmacokinetics and bioavailability of cephalothin in horse mares. *Am J Vet Res*. 1985;46:2085–2090.
113. Brown MP, Gronwall RR, Houston AE. Pharmacokinetics and body fluid and endometrial concentrations of cephapirin in mares. *Am J Vet Res*. 1986;47:784–788.
114. Henry MM, Morris DD, Lakritz J, et al. Pharmacokinetics of cephradine in neonatal foals after single oral dosing. *Equine Vet J*. 1992;24:242–243.
115. Brown MP, Gronwall RR, Houston AE. Pharmacokinetics and body fluid and endometrial concentrations of cefoxitin in mares. *Am J Vet Res*. 1986;47:1734–1738.
116. Jaglan PS, Roof RD, Yein FS, et al. Concentration of ceftiofur metabolites in the plasma and lungs of horses following intramuscular treatment. *J Vet Pharmacol Ther*. 1994;17:24–30.

117. Soraci AL, Mestorino ON, Errecalde JO. Pharmacokinetics of cefoperazone in horses. *J Vet Pharmacol Ther*. 1996;19:39–43.
118. Gardner SY, Papich MG. Comparison of cefepime pharmacokinetics in neonatal foals and adult dogs. *J Vet Pharmacol Ther*. 2001;24:187–192.
119. Brown SA, Riviere JE. Comparative pharmacokinetics of aminoglycoside antibiotics. *J Vet Pharmacol Ther*. 1991;14:1–35.
120. Barclay ML, Begg EJ, Hickling KG. What is the evidence for once-daily aminoglycoside therapy? *Clin Pharmacokinet*. 1994;27:32–48.
121. Nestaas E, Bangstad HJ, Sandvik L, et al. Aminoglycoside extended interval dosing in neonates is safe and effective: a meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2005;90:F294–300.
122. Barclay ML, Begg EJ. Aminoglycoside toxicity and relation to dose regimen. *Adverse Drug React Toxicol Rev*. 1994;13:207–234.
123. Daikos GL, Jackson GG, Lolans VT, et al. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. *J Infect Dis*. 1990;162:414–420.
124. Daikos GL, Lolans VT, Jackson GG. First-exposure adaptive resistance to aminoglycoside antibiotics in vivo with meaning for optimal clinical use. *Antimicrob Agents Chemother*. 1991;35:117–123.
125. Cummings LE, Guthrie AJ, Harkins JD, et al. Pharmacokinetics of gentamicin in newborn to 30-day-old foals. *Am J Vet Res*. 1990;51:1988–1992.
126. Wichtel MG, Breuhaus BA, Aucoin D. Relation between pharmacokinetics of amikacin sulfate and sepsis score in clinically normal and hospitalized neonatal foals. *J Am Vet Med Assoc*. 1992;200:1339–1343.
127. Anderson BH, Firth EC, Whittem T. The disposition of gentamicin in equine plasma, synovial fluid and lymph. *J Vet Pharmacol Ther*. 1995;18:124–131.
128. Santschi EM, Papich MG. Pharmacokinetics of gentamicin in mares in late pregnancy and early lactation. *J Vet Pharmacol Ther*. 2000;23:359–363.
129. Haddad NS, Pedersoli WM, Ravis WR, et al. Pharmacokinetics of gentamicin at steady-state in ponies: serum, urine, and endometrial concentrations. *Am J Vet Res*. 1985;46:1268–1271.
130. Wilson RC, Moore JN, Eakle N. Gentamicin pharmacokinetics in horses given small doses of *Escherichia coli* endotoxin. *Am J Vet Res*. 1983;44:1746–1749.
131. Jones SL, Wilson WD, Milhalyi JE. Pharmacokinetics of gentamicin in healthy adult horses during intravenous fluid administration. *J Vet Pharmacol Ther*. 1998;21:247–249.
132. Tudor RA, Papich MG, Redding WR. Drug disposition and dosage determination of once daily administration of gentamicin sulfate in horses after abdominal surgery. *J Am Vet Med Assoc*. 1999;215:503–506.
133. Easter JL, Hague BA, Brumbaugh GW, et al. Effects of postoperative peritoneal lavage on pharmacokinetics of gentamicin in horses after celiotomy. *Am J Vet Res*. 1997;58:1166–1170.
134. Beech J, Kohn C, Leitch M, et al. Therapeutic use of gentamicin in horses: concentrations in serum, urine, and synovial fluid and evaluation of renal function. *Am J Vet Res*. 1977;38:1085–1087.
135. Lloyd KC, Stover SM, Pascoe JR, et al. Effect of gentamicin sulfate and sodium bicarbonate on the synovium of clinically normal equine antebrachiocarpal joints. *Am J Vet Res*. 1988;49:650–657.
136. Lescun TB, Adams SB, Wu CC, et al. Continuous infusion of gentamicin into the tarsocrural joint of horses. *Am J Vet Res*. 2000;61:407–412.
137. Murphey ED, Santschi EM, Papich MG. Regional intravenous perfusion of the distal limb of horses with amikacin sulfate. *J Vet Pharmacol Ther*. 1999;22:68–71.
138. Whitehair KJ, Blevins WE, Fessler JF, et al. Regional perfusion of the equine carpus for antibiotic delivery. *Vet Surg*. 1992;21:279–285.
139. Whitehair KJ, Bowersock TL, Blevins WE, et al. Regional limb perfusion for antibiotic treatment of experimentally induced septic arthritis. *Vet Surg*. 1992;21:367–373.
140. Errico JA, Trumble TN, Bueno AC, et al. Comparison of two indirect techniques for local delivery of a high dose of an antimicrobial in the distal portion of forelimbs of horses. *Am J Vet Res*. 2008;69:334–342.
141. Parra-Sanchez A, Lugo J, Boothe DM, et al. Pharmacokinetics and pharmacodynamics of enrofloxacin and a low dose of amikacin administered via regional intravenous limb perfusion in standing horses. *Am J Vet Res*. 2006;67:1687–1695.
142. Butt TD, Bailey JV, Dowling PM, et al. Comparison of 2 techniques for regional antibiotic delivery to the equine forelimb: intraosseous perfusion vs. intravenous perfusion. *Can Vet J*. 2001;42:617–622.
143. Booth TM, Butson RJ, Clegg PD, et al. Treatment of sepsis in the small tarsal joints of 11 horses with gentamicin-impregnated polymethylmethacrylate beads. *Vet Rec*. 2001;148:376–380.
144. Ivester KM, Adams SB, Moore GE, et al. Gentamicin concentrations in synovial fluid obtained from the tarsocrural joints of horses after implantation of gentamicin-impregnated collagen sponges. *Am J Vet Res*. 2006;67:1519–1526.
145. Kaloyanides GJ. Antibiotic-related nephrotoxicity. *Nephrol Dial Transplant*. 1994;9:4130–4134.
146. Tulkens PM. Nephrotoxicity of aminoglycoside antibiotics. *Toxicol Lett*. 1989;46:107–123.
147. Kaloyanides GJ. Drug-phospholipid interactions: role in aminoglycoside nephrotoxicity. *Ren Fail*. 1992;14:351–357.
148. van der Harst MR, Bull S, Laffont CM, et al. Gentamicin nephrotoxicity—a comparison of in vitro findings with in vivo experiments in equines. *Vet Res Commun*. 2005;29:247–261.
149. Molitoris BA, Meyer C, Dahl R, et al. Mechanism of ischemia-enhanced aminoglycoside binding and uptake by proximal tubule cells. *Am J Physiol*. 1993;264:F907–916.
150. Riviere JE, Coppoc GL, Hinsman EJ, et al. Species dependent gentamicin pharmacokinetics and nephrotoxicity in the young horse. *Fundam Appl Toxicol*. 1983;3:448–457.
151. Sweeney RW, MacDonald M, Hall J, et al. Kinetics of gentamicin elimination in two horses with acute renal failure. *Equine Vet J*. 1988;20:182–184.
152. Matzke GR, Frye RF. Drug administration in patients with renal insufficiency. Minimising renal and extrarenal toxicity. *Drug Saf*. 1997;16:205–231.
153. Thatte L, Vaamonde CA. Drug-induced nephrotoxicity: the crucial role of risk factors. *Postgrad Med*. 1996;100:83–84. 87–88, 91 passim.
154. Brashier MK, Geor RJ, Ames TR, et al. Effect of intravenous calcium administration on gentamicin-induced nephrotoxicosis in ponies. *Am J Vet Res*. 1998;59:1055–1062.
155. Varzi HN, Esmailzadeh S, Morovvati H, et al. Effect of silymarin and vitamin E on gentamicin-induced nephrotoxicity in dogs. *J Vet Pharmacol Ther*. 2007;30:477–481.
156. Schumacher J, Wilson RC, Spano JS, et al. Effect of diet on gentamicin-induced nephrotoxicosis in horses. *Am J Vet Res*. 1991;52:1274–1278.
157. Behrend EN, Grauer GF, Greco DS, et al. Effects of dietary protein conditioning on gentamicin pharmacokinetics in dogs. *J Vet Pharmacol Ther*. 1994;17:259–264.
158. Godber LM, Walker RD, Stein GE, et al. Pharmacokinetics, nephrotoxicosis, and in vitro antibacterial activity associated with single versus multiple (three times) daily gentamicin treatments in horses. *Am J Vet Res*. 1995;56:613–618.
159. Magdesian KG, Hogan PM, Cohen ND, et al. Pharmacokinetics of a high dose of gentamicin administered intravenously or intramuscularly to horses. *J Am Vet Med Assoc*. 1998;213:1007–1011.
160. Magdesian KG, Wilson WD, Mihalyi J. Pharmacokinetics of a high dose of amikacin administered at extended intervals to neonatal foals. *Am J Vet Res*. 2004;65:473–479.
161. Green SL, Conlon PD, Mama K, et al. Effects of hypoxia and azotaemia on the pharmacokinetics of amikacin in neonatal foals. *Equine Vet J*. 1992;24:475–479.

162. Barclay ML, Begg EJ. Aminoglycoside adaptive resistance: importance for effective dosage regimens. *Drugs*. 2001;61:713–721.
163. Brown SA, Garry FB. Comparison of serum and renal gentamicin concentrations with fractional urinary excretion tests as indicators of nephrotoxicity. *J Vet Pharmacol Ther*. 1988;11:330–337.
164. Whiting PH, Brown PA. The relationship between enzymuria and kidney enzyme activities in experimental gentamicin nephrotoxicity. *Ren Fail*. 1996;18:899–909.
165. Bates DE. Aminoglycoside ototoxicity. *Drugs Today (Barc)*. 2003;39:277–285.
166. Selimoglu E, Kalkandelen S, Erdogan F. Comparative vestibulotoxicity of different aminoglycosides in the Guinea pigs. *Yonsei Med J*. 2003;44:517–522.
167. Paradelis AG, Triantaphyllidis C, Giala MM. Neuromuscular blocking activity of aminoglycoside antibiotics. *Methods Find Exp Clin Pharmacol*. 1980;2:45–51.
168. Hildebrand SV, Hill 3rd T. Interaction of gentamycin and atracurium in anaesthetised horses. *Equine Vet J*. 1994;26:209–211.
169. Smith CM, Steffey EP, Baggot JD, et al. Effects of halothane anesthesia on the clearance of gentamicin sulfate in horses. *Am J Vet Res*. 1988;49:19–22.
170. Whittem T, Firth EC, Hodge H, et al. Pharmacokinetic interactions between repeated dose phenylbutazone and gentamicin in the horse. *J Vet Pharmacol Ther*. 1996;19:454–459.
171. Orsini JA, Soma LR, Rourke JE, et al. Pharmacokinetics of amikacin in the horse following intravenous and intramuscular administration. *J Vet Pharmacol Ther*. 1985;8:194–201.
172. Pinto N, Schumacher J, Taintor J, et al. Pharmacokinetics of amikacin in plasma and selected body fluids of healthy horses after a single intravenous dose. *Equine Vet J*. 2011;43:112–116.
173. Hubenov H, Bakalov D, Krastev S, et al. Pharmacokinetic studies on tobramycin in horses. *J Vet Pharmacol Ther*. 2007;30:353–357.
174. Newman JC, Prange T, Jennings S, et al. Pharmacokinetics of tobramycin following intravenous, intramuscular, and intraarticular administration in healthy horses. *J Vet Pharmacol Ther*. 2013;36:532–541.
175. Cannon M, Harford S, Davies J. A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. *J Antimicrob Chemother*. 1990;26:307–317.
176. Brumbaugh GW, Martens RJ, Knight HD, et al. Pharmacokinetics of chloramphenicol in the neonatal horse. *J Vet Pharmacol Ther*. 1983;6:219–227.
177. Gronwall R, Brown MP, Merritt AM, et al. Body fluid concentrations and pharmacokinetics of chloramphenicol given to mares intravenously or by repeated gavage. *Am J Vet Res*. 1986;47:2591–2595.
178. Nau R, Sorgel F, Prange HW. Pharmacokinetic optimisation of the treatment of bacterial central nervous system infections. *Clin Pharmacokinet*. 1998;35:223–246.
179. Brown MP, Kelly RH, Gronwall RR, et al. Chloramphenicol sodium succinate in the horse: serum, synovial, peritoneal, and urine concentrations after single-dose intravenous administration. *Am J Vet Res*. 1984;45:578–580.
180. Sisodia CS, Kramer LL, Gupta VS, et al. A pharmacological study of chloramphenicol in horses. *Can J Comp Med*. 1975;39:216–223.
181. Adamson PJ, Wilson WD, Baggot JD, et al. Influence of age on the disposition kinetics of chloramphenicol in equine neonates. *Am J Vet Res*. 1991;52:426–431.
182. Varma KJ, Powers TE, Powers JD. Single- and repeat-dose pharmacokinetic studies of chloramphenicol in horses: values and limitations of pharmacokinetic studies in predicting dosage regimens. *Am J Vet Res*. 1987;48:403–406.
183. Tuttle AD, Papich MG, Wolfe BA. Bone marrow hypoplasia secondary to florfenicol toxicity in a Thomson's gazelle *(Gazella thomsonii)*. *J Vet Pharmacol Ther*. 2006;29:317–319.
184. Page SW. Chloramphenicol 1. Hazards of use and the current regulatory environment. *Aust Vet J*. 1991;68:1–2.
185. Burrows GE, MacAllister CG, Tripp P, et al. Interactions between chloramphenicol, acepromazine, phenylbutazone, rifampin and thiamylal in the horse. *Equine Vet J*. 1989;21:34–38.
186. Grubb TL, Muir WW, Bertone AL, et al. Use of yohimbine to reverse prolonged effects of xylazine hydrochloride in a horse being treated with chloramphenicol. *J Am Vet Med Assoc*. 1997;210:1771–1773.
187. Asmar BI, Prainito M, Dajani AS. Antagonistic effect of chloramphenicol in combination with cefotaxime or ceftriaxone. *Antimicrob Agents Chemother*. 1988;32:1375–1378.
188. Ruiz NM, Ramirez-Ronda CH. Tetracyclines, macrolides, lincosamides and chloramphenicol. Bol Asoc Med P R. 82:8–17.
189. Neu HC. Synergy of fluoroquinolones with other antimicrobial agents. *Rev Infect Dis*. 1989;11(suppl 5):S1025–1035.
190. Van Duijkeren E, Vulto AG, Van Miert AS. Trimethoprim/sulfonamide combinations in the horse: a review. *J Vet Pharmacol Ther*. 1994;17:64–73.
191. van Miert AS. The sulfonamide-diaminopyrimidine story. *J Vet Pharmacol Ther*. 1994;17:309–316.
192. van Duijkeren E, van Klingeren B, Vulto AG, et al. In vitro susceptibility of equine *Salmonella* strains to trimethoprim and sulfonamide alone or in combination. *Am J Vet Res*. 1994;55:1386–1390.
193. Grace ME, Bushby SR, Sigel CW. Diffusion of trimethoprim and sulfamethoxazole from susceptibility disks into agar medium. *Antimicrob Agents Chemother*. 1975;8:45–49.
194. Marsh PS, Palmer JE. Bacterial isolates from blood and their susceptibility patterns in critically ill foals: 543 cases. *J Am Vet Med Assoc*. 1991-1998;218:1608–1610. 2001.
195. Bogan JA, Galbraith A, Baxter P, et al. Effect of feeding on the fate of orally administered phenylbutazone, trimethoprim and sulphadiazine in the horse. *Vet Rec*. 1984;115:599–600.
196. Sigel CW, Byars TD, Divers TJ, et al. Serum concentrations of trimethoprim and sulfadiazine following oral paste administration to the horse. *Am J Vet Res*. 1981;42:2002–2005.
197. Wilson RC, Hammond LS, Clark CH, et al. Bioavailability and pharmacokinetics of sulfamethazine in the pony. *J Vet Pharmacol Ther*. 1989;12:99–102.
198. van Duijkeren E, Vulto AG, Sloet van Oldruitenborgh-Oosterbaan MM, et al. Pharmacokinetics of trimethoprim/sulphachlorpyridazine in horses after oral, nasogastric and intravenous administration. *J Vet Pharmacol Ther*. 1995;18:47–53.
199. Van Duijkeren E, Kessels BG, Sloet van Oldruitenborgh-Oosterbaan MM, et al. In vitro and in vivo binding of trimethoprim and sulphachlorpyridazine to equine food and digesta and their stability in caecal contents. *J Vet Pharmacol Ther*. 1996;19:281–287.
200. Boyd EH, Allen WE. Absorption of two trimethoprim/sulphonamide combinations from the uterus of pony mares. *J Vet Pharmacol Ther*. 1989;12:438–443.
201. Clarke CR, Burrows GE, MacAllister CG, et al. Pharmacokinetics of intravenously and orally administered pyrimethamine in horses. *Am J Vet Res*. 1992;53:2292–2295.
202. Brown MP, Gronwall R, Castro L. Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares. *Am J Vet Res*. 1988;49:918–922.
203. Brown MP, Kelly RH, Stover SM, et al. Trimethoprim-sulfadiazine in the horse: serum, synovial, peritoneal, and urine concentrations after single-dose intravenous administration. *Am J Vet Res*. 1983;44:540–543.
204. Brown MP, McCartney JH, Gronwall R, et al. Pharmacokinetics of trimethoprim-sulphamethoxazole in two-day-old foals after a single intravenous injection. *Equine Vet J*. 1990;22:51–53.
205. Clarke CR, MacAllister CG, Burrows GE, et al. Pharmacokinetics, penetration into cerebrospinal fluid, and hematologic effects after multiple oral administrations of pyrimethamine to horses. *Am J Vet Res*. 1992;53:2296–2299.
206. Rasmussen F, Gelsa H, Nielsen P. Pharmacokinetics of sulphadoxine and trimethoprim in horses. Half-life and volume of distribution of sulphadoxine and trimethoprim and cumulative excretion of [14C]-trimethoprim. *J Vet Pharmacol Ther*. 1979;2:245–255.

207. Nouws JF, Firth EC, Vree TB, et al. Pharmacokinetics and renal clearance of sulfamethazine, sulfamerazine, and sulfadiazine and their N4-acetyl and hydroxy metabolites in horses. *Am J Vet Res*. 1987;48:392–402.
208. Nouws JF, Vree TB, Baakman M, et al. Disposition of sulfadimidine and its N4-acetyl and hydroxy metabolites in horse plasma. *J Vet Pharmacol Ther*. 1985;8:303–311.
209. Gray AK, Kidd AR, O'Brien J, et al. Suspected adverse reactions to medicines during 1988. *Vet Rec*. 1989;124:286–287.
210. Dick IG, White SK. Possible potentiated sulphonamide-associated fatality in an anaesthetised horse. *Vet Rec*. 1987;121:288.
211. Taylor PM, Rest RJ, Duckham TN, et al. Possible potentiated sulphonamide and detomidine interactions. *Vet Rec*. 1988;122:143.
212. Fenger CK, Granstrom DE, Langemeier JL, et al. Epizootic of equine protozoal myeloencephalitis on a farm. *J Am Vet Med Assoc*. 1997;210:923–927.
213. Toribio RE, Bain FT, Mrad DR, et al. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc*. 1998;212:697–701.
214. Bedford SJ, McDonnell SM. Measurements of reproductive function in stallions treated with trimethoprim-sulfamethoxazole and pyrimethamine. *J Am Vet Med Assoc*. 1999;215:1317–1319.
215. Thomas HL, Livesey MA. Immune-mediated hemolytic anemia associated with trimethoprim-sulphamethoxazole administration in a horse. *Can Vet J*. 1998;39:171–173.
216. Ensink JM, Klein WR, Barneveld A, et al. Side effects of oral antimicrobial agents in the horse: a comparison of pivampicillin and trimethoprim/sulphadiazine. *Vet Rec*. 1996;138:253–256.
217. Wilson DA, MacFadden KE, Green EM, et al. Case control and historical cohort study of diarrhea associated with administration of trimethoprim-potentiated sulphonamides to horses and ponies. *J Vet Intern Med*. 1996;10:258–264.
218. Gustafsson A, Baverud V, Franklin A, et al. Repeated administration of trimethoprim/sulfadiazine in the horse—pharmacokinetics, plasma protein binding and influence on the intestinal microflora. *J Vet Pharmacol Ther*. 1999;22:20–26.
219. White G, Prior SD. Comparative effects of oral administration of trimethoprim/sulphadiazine or oxytetracycline on the faecal flora of horses. *Vet Rec*. 1982;111:316–318.
220. Bertone AL, Jones RL, McIlwraith CW. Serum and synovial fluid steady-state concentrations of trimethoprim and sulfadiazine in horses with experimentally induced infectious arthritis. *Am J Vet Res*. 1988;49:1681–1687.
221. Ensink JM, Bosch G, van Duijkeren E. Clinical efficacy of prophylactic administration of trimethoprim/sulfadiazine in a *Streptococcus equi* subsp. zooepidemicus infection model in ponies. *J Vet Pharmacol Ther*. 2005;28:45–49.
222. Green SL, Mayhew IG, Brown MP, et al. Concentrations of trimethoprim and sulfamethoxazole in cerebrospinal fluid and serum in mares with and without a dimethyl sulfoxide pretreatment. *Can J Vet Res*. 1990;54:215–222.
223. Rikihisa Y, Jiang BM. In vitro susceptibilities of *Ehrlichia risticii* to eight antibiotics. *Antimicrob Agents Chemother*. 1988;32:986–991.
224. Sampieri F, Hinchcliff KW, Toribio RE. Tetracycline therapy of *Lawsonia intracellularis* enteropathy in foals. *Equine Vet J*. 2006;38:89–92.
225. Baker A, Plummer CE, Szabo NJ, et al. Doxycycline levels in preocular tear film of horses following oral administration. *Vet Ophthalmol*. 2008;11:381–385.
226. Fortier LA, Motta T, Greenwald RA, et al. Synoviocytes are more sensitive than cartilage to the effects of minocycline and doxycycline on IL-1alpha and MMP-13-induced catabolic gene responses. *J Orthop Res*. 2010;28:522–528.
227. Dowling PM, Russell AM. Pharmacokinetics of a long-acting oxytetracycline-polyethylene glycol formulation in horses. *J Vet Pharmacol Ther*. 2000;23:107–110.
228. Bryant JE, Brown MP, Gronwall RR, et al. Study of intragastric administration of doxycycline: pharmacokinetics including body fluid, endometrial and minimum inhibitory concentrations. *Equine Vet J*. 2000;32:233–238.
229. Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue distribution of doxycycline after oral administration of single and multiple doses in horses. *Am J Vet Res*. 2006;67:310–316.
230. Riond JL, Riviere JE, Duckett WM, et al. Cardiovascular effects and fatalities associated with intravenous administration of doxycycline to horses and ponies. *Equine Vet J*. 1992;24:41–45.
231. Womble A, Giguere S, Lee EA. Pharmacokinetics of oral doxycycline and concentrations in body fluids and bronchoalveolar cells of foals. *J Vet Pharmacol Ther*. 2007;30:187–193.
232. Schnabel LV, Papich MG, Divers TJ, et al. Pharmacokinetics and distribution of minocycline in mature horses after oral administration of multiple doses and comparison with minimum inhibitory concentrations. *Equine Vet J*. 2012;44:453–458.
233. Brown MP, Stover SM, Kelly RH, et al. Oxytetracycline hydrochloride in the horse: serum, synovial, peritoneal and urine concentrations after single dose intravenous administration. *J Vet Pharmacol Ther*. 1981;4:7–10.
234. Papich MG, Wright AK, Petrie L, et al. Pharmacokinetics of oxytetracycline administered intravenously to 4- and 5-day-old foals. *J Vet Pharmacol Ther*. 1995;18:375–378.
235. Horspool LJ, McKellar QA. Disposition of oxytetracycline in horses, ponies and donkeys after intravenous administration. *Equine Vet J*. 1990;22:284–285.
236. Gilmour MA, Clarke CR, Macallister CG, et al. Ocular penetration of oral doxycycline in the horse. *Vet Ophthalmol*. 2005;8:331–335.
237. Pilloud M. Pharmacokinetics, plasma protein binding and dosage of oxytetracycline in cattle and horses. *Res Vet Sci*. 1973;15:224–230.
238. Nagata S, Yamashita S, Kurosawa M, et al. Pharmacokinetics and tissue distribution of minocycline hydrochloride in horses. *Am J Vet Res*. 2012;71:1062–1066.
239. Shaw DH, Rubin SI. Pharmacologic activity of doxycycline. *J Am Vet Med Assoc*. 1986;189:808–810.
240. Andersson G, Ekman L, Mansson I, et al. Lethal complications following administration of oxytetracycline in the horse. *Nord Vet Med*. 1971;23:9–22.
241. Baker JR, Leyland A. Diarrhoea in the horse associated with stress and tetracycline therapy. *Vet Rec*. 1973;93:583–584.
242. Cook W. Diarrhoea in the horse associated with stress and tetracycline therapy. *Vet Rec*. 1973;93:15–17.
243. Owen R. Post stress diarrhoea in the horse. *Vet Rec*. 1975;96:267–270.
244. Owen RA, Fullerton J, Barnum DA. Effects of transportation, surgery, and antibiotic therapy in ponies infected with *Salmonella*. *Am J Vet Res*. 1983;44:46–50.
245. Palmer JE. Potomac horse fever. *Vet Clin North Am Equine Pract*. 1993;9:399–410.
246. Palmer JE, Benson CE, Whitlock RH. Effect of treatment with oxytetracycline during the acute stages of experimentally induced equine ehrlichial colitis in ponies. *Am J Vet Res*. 1992;53:2300–2304.
247. Palmer JE, Whitlock RH, Benson CE. Equine ehrlichial colitis: effect of oxytetracycline treatment during the incubation period of *Ehrlichia risticii* infection in ponies. *J Am Vet Med Assoc*. 1988;192:343–345.
248. Dowling PM: Long-acting oxytetracycline in horses, 17th Annual ACVIM Forum 217–219, 1999.
249. Vivrette S, Cowgill LD, Pascoe J, et al. Hemodialysis for treatment of oxytetracycline-induced acute renal failure in a neonatal foal. *J Am Vet Med Assoc*. 1993;203:105–107.
250. Wright AK, Petrie L, Papich MG, et al. Effect of high dose oxytetracycline on renal parameteres in neonatal foals: recommended dose for treatment of flexural limb deformities. *Proc Am Assoc Equine Practitioners*. 1993;38:297–298.

251. Gyrd-Hansen N, Rasmussen F, Smith M. Cardiovascular effects of intravenous administration of tetracycline in cattle. *J Vet Pharmacol Ther*. 1981;4:15–25.
252. Smith M, Gyrd-Hansen N, Rasmussen F. Tetracycline intravenously to cattle: cardiovascular side-effects. *Nord Vet Med*. 1981;33:272–273.
253. Riond JL, Duckett WM, Riviere JE, et al. Concerned about intravenous use of doxycycline in horses. *J Am Vet Med Assoc*. 1989;195(846):848.
254. Kasper CA, Clayton HM, Wright AK, et al. Effects of high doses of oxytetracycline on metacarpophalangeal joint kinematics in neonatal foals. *J Am Vet Med Assoc*. 1995;207:71–73.
255. Madison JB, Garber JL, Rice B, et al. Effect of oxytetracycline on metacarpophalangeal and distal interphalangeal joint angles in newborn foals. *J Am Vet Med Assoc*. 1994;204:246–249.
256. Arnoczky SP, Lavagnino M, Gardner KL, et al. In vitro effects of oxytetracycline on matrix metalloproteinase-1 mRNA expression and on collagen gel contraction by cultured myofibroblasts obtained from the accessory ligament of foals. *Am J Vet Res*. 2004;65:491–496.
257. Venner M, Astheimer K, Lämmer M, et al. Efficacy of mass antimicrobial treatment of foals with subclinical pulmonary abscesses associated with *Rhodococcus equi*. *J Vet Intern Med*. 2013;27:171–176.
258. Neu HC. Clinical microbiology of azithromycin. *Am J Med*. 1991;91:12S–18S.
259. Jacks SS, Giguere S, Nguyen A. In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother*. 2003;47:1742–1745.
260. Lakritz J. Erythromycin: clinical uses, kinetics and mechanism of action. *15th Annual ACVIM Forum*. 1997:368–370.
261. Lester GD, Merritt AM, Neuwirth L, et al. Effect of erythromycin lactobionate on myoelectric activity of ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res*. 1998;59:328–334.
262. Kenney DG, Robbins SC, Prescott JF, et al. Development of reactive arthritis and resistance to erythromycin and rifampin in a foal during treatment for *Rhodococcus equi* pneumonia. *Equine Vet J*. 1994;26:246–248.
263. Giguère S, Lee E, Williams E, et al. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R equi*. *J Am Vet Med Assoc*. 2010;237:74–81.
264. Lakritz J, Wilson WD, Mihalyi JE. Comparison of microbiologic and high-performance liquid chromatography assays to determine plasma concentrations, pharmacokinetics, and bioavailability of erythromycin base in plasma of foals after intravenous or intragastric administration. *Am J Vet Res*. 1999;60:414–419.
265. Lakritz J, Wilson WD, Marsh AE, et al. Effects of prior feeding on pharmacokinetics and estimated bioavailability after oral administration of a single dose of microencapsulated erythromycin base in healthy foals. *Am J Vet Res*. 2000;61:1011–1015.
266. Lakritz J, Wilson WD, Marsh AE, et al. Pharmacokinetics of erythromycin estolate and erythromycin phosphate after intragastric administration to healthy foals. *Am J Vet Res*. 2000;61:914–919.
267. Jacks S, Giguere S, Gronwall PR, et al. Pharmacokinetics of azithromycin and concentration in body fluids and bronchoalveolar cells in foals. *Am J Vet Res*. 2001;62:1870–1875.
268. Davis JL, Gardner SY, Jones SL, et al. Pharmacokinetics of azithromycin in foals after i.v. and oral dose and disposition into phagocytes. *J Vet Pharmacol Ther*. 2002;25:99–104.
269. Womble AY, Giguere S, Lee EA, et al. Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals. *Am J Vet Res*. 2006;67:1681–1686.
270. Javsicas LH, Giguère S, Womble AY. Disposition of oral telithromycin in foals and in vitro activity of the drug against macrolide-susceptible and macrolide-resistant *Rhodococcus equi* isolates. *J Vet Pharmacol Ther*. 2010;33:383–388.
271. Scheuch E, Spieker J, Venner M, et al. Quantitative determination of the macrolide antibiotic tulathromycin in plasma and broncho-alveolar cells of foals using tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;850:464–470.
272. Berghaus LJ, Giguère S, Sturgill TL, et al. Plasma pharmacokinetics, pulmonary distribution, and in vitro activity of gamithromycin in foals. *J Vet Pharmacol Ther*. 2012;35:59–66.
273. Giguere S, Jacks S, Roberts GD, et al. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med*. 2004;18:568–573.
274. Suarez-Mier G, Giguere S, Lee EA. Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals. *J Vet Pharmacol Ther*. 2007;30:109–115.
275. Villarino N, Martín-Jiménez T. Pharmacokinetics of macrolides in foals. *J Vet Pharmacol Ther*. 2013;36:1–13.
276. Prescott JF, Hoover DJ, Dohoo IR. Pharmacokinetics of erythromycin in foals and in adult horses. *J Vet Pharmacol Ther*. 1983;6:67–73.
277. Lakritz J, Wilson WD, Watson JL, et al. Effect of treatment with erythromycin on bronchoalveolar lavage fluid cell populations in foals. *Am J Vet Res*. 1997;58:56–61.
278. Steiner A, Roussel AJ. Drugs coordinating and restoring gastrointestinal motility and their effect on selected hypodynamic gastrointestinal disorders in horses and cattle. *Zentralbl Veterinarmed A*. 1995;42:613–631.
279. Nieto JE, Rakestraw PC, Snyder JR, et al. In vitro effects of erythromycin, lidocaine, and metoclopramide on smooth muscle from the pyloric antrum, proximal portion of the duodenum, and middle portion of the jejunum of horses. *Am J Vet Res*. 2000;61:413–419.
280. Baverud V, Franklin A, Gunnarsson A, et al. *Clostridium difficile* associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for *Rhodococcus equi* pneumonia. *Equine Vet J*. 1998;30:482–488.
281. Gustafsson A, Baverud V, Gunnarsson A, et al. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. *Equine Vet J*. 1997;29:314–318.
282. Larsen J, Dolvik NI, Teige J. Acute post-treatment enterocolitis in 13 horses treated in a Norwegian surgical ward. *Acta Vet Scand*. 1996;37:203–211.
283. Stratton-Phelps M, Wilson WD, Gardner IA. Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases. *J Am Vet Med Assoc*. 1986-1996;217:68–73. 2000.
284. Traub-Dargatz J, Wilson WD, Conboy HS, et al. Hyperthermia in foals treated with erythromycin alone or in combination with rifampin for respiratory disease during hot environmental conditions. *Proc Am Assoc Equine Pract*. 1996;42:243–244.
285. Stieler AL, Sanchez LC, Mallicote MF, et al. Macrolide-induced hyperthermia in foals: role of impaired sweat responses. *Equine Vet J*. 2016;48:590–594.
286. Berghaus LJ, Giguère S, Guldbech K. Mutant prevention concentration and mutant selection window for 10 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol*. 2013;166:670–675.
287. Peters J, Block W, Oswald S, et al. Oral absorption of clarithromycin is nearly abolished by chronic comedication of rifampicin in foals. *Drug Metab Dispos*. 2011;39:1643–1649.
288. Peters J, Eggers K, Oswald S, et al. Clarithromycin is absorbed by an intestinal uptake mechanism that is sensitive to major inhibition by rifampicin: results of a short-term drug interaction study in foals. *Drug Metab Dispos*. 2012;40:522–528.
289. Venner M, Peters J, Höhensteiger N, et al. Concentration of the macrolide antibiotic tulathromycin in broncho-alveolar cells is influenced by comedication of rifampicin in foals. *Naunyn Schmiedebergs Arch Pharmacol*. 2012;381:161–169.
290. Leclere M, Magdesian KG, Cole CA, et al. Pharmacokinetics and preliminary safety evaluation of azithromycin in adult horses. *J Vet Pharmacol Ther*. 2012;35:541–549.

291. Hildebrand F, Venner M, Giguère S. Efficacy of gamithromycin for the treatment of foals with mild to moderate bronchopneumonia. *J Vet Intern Med*. 2015;29:333–338.
292. Palmer JE, Benson CE. Effect of treatment with erythromycin and rifampin during the acute stages of experimentally induced equine ehrlichial colitis in ponies. *Am J Vet Res*. 1992;53:2071–2076.
293. Lavoie JP, Drolet R, Parsons D, et al. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine Vet J*. 2000;32:418–425.
294. Chaffin MK, Cohen ND, Martens RJ. Chemoprophylactic effects of azithromycin against *Rhodococcus equi*–induced pneumonia among foals at equine breeding farms with endemic infections. *J Am Vet Med Assoc*. 2008;232:1035–1047.
295. Venner M, Credner N, Lämmer M, et al. Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with *Rhodococcus equi*. *Vet Rec*. 2013;173:397.
296. Brown SA. Fluoroquinolones in animal health. *J Vet Pharmacol Ther*. 1996;19:1–14.
297. Appelbaum PC. Quinolone activity against anaerobes. *Drugs*. 1999;58(suppl 2):60–64.
298. Nicolau DP. Predicting antibacterial response from pharmacodynamic and pharmacokinetic profiles. *Infection*. 2001;29 (suppl 2):11–15.
299. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis*. 2006;6:629–640.
300. Hooper DC. Mechanisms of fluoroquinolone resistance. *Drug Resist Updat*. 1999;2:38–55.
301. Schmitz FJ, Perdikouli M, Beeck A, et al. Molecular surveillance of macrolide, tetracycline and quinolone resistance mechanisms in 1191 clinical European *Streptococcus pneumoniae* isolates. *Int J Antimicrob Agents*. 2001;18:433–436.
302. Webber M, Piddock LJ. Quinolone resistance in *Escherichia coli*. *Vet Res*. 2001;32:275–284.
303. Dowling PM, Wilson RC, Tyler JW, et al. Pharmacokinetics of ciprofloxacin in ponies. *J Vet Pharmacol Ther*. 1995;18:7–12.
304. Yamarik TA, Wilson WD, Wiebe VJ, et al. Pharmacokinetics and toxicity of ciprofloxacin in adult horses. *J Vet Pharmacol Ther*. 2010;33:587–594.
305. Bermingham EC, Papich MG, Vivrette SL. Pharmacokinetics of enrofloxacin administered intravenously and orally to foals. *Am J Vet Res*. 2000;61:706–709.
306. Giguere S, Belanger M. Concentration of enrofloxacin in equine tissues after long-term oral administration. *J Vet Pharmacol Ther*. 1997;20:402–404.
307. Giguere S, Sweeney RW, Belanger M. Pharmacokinetics of enrofloxacin in adult horses and concentration of the drug in serum, body fluids, and endometrial tissues after repeated intragastrically administered doses. *Am J Vet Res*. 1996;57:1025–1030.
308. Peyrou M, Bousquet-Melou A, Laroute V, et al. Enrofloxacin and marbofloxacin in horses: comparison of pharmacokinetic parameters, use of urinary and metabolite data to estimate first-pass effect and absorbed fraction. *J Vet Pharmacol Ther*. 2006;29:337–344.
309. Steinman A, Britzi M, Levi O, et al. Lack of effect of diet on the pharmacokinetics of enrofloxacin in horses. *J Vet Pharmacol Ther*. 2006;29:67–70.
310. Davis JL, Papich MG, Weingarten A. The pharmacokinetics of orbifloxacin in the horse following oral and intravenous administration. *J Vet Pharmacol Ther*. 2006;29:191–197.
311. Lopez BS, Giguère S, Berghaus LJ, et al. Pharmacokinetics of danofloxacin and *N*-desmethyldanofloxacin in adult horses and their concentration in synovial fluid. *J Vet Pharmacol Ther*. 2015;38:123–129.
312. Kaartinen L, Panu S, Pyorala S. Pharmacokinetics of enrofloxacin in horses after single intravenous and intramuscular administration. *Equine Vet J*. 1997;29:378–381.
313. Boeckh CBC, Boeckh A, Wilkie S, Davis C, Buchanan T, Boothe D. Pharmacokinetics of the bovine formulation of enrofloxacin (Baytril 100) in horses. *Vet Ther*. 2001;2:129–134.
314. Epstein K, Cohen N, Boothe D, et al. Pharmacokinetics, stability and retrospective analysis of use of an oral get formulation of the bovine injectable enrofloxacin in horses. *Vet Ther*. 2004;5:155–167.
315. Carretero M, Rodríguez C, San Andrés MI, et al. Pharmacokinetics of marbofloxacin in mature horses after single intravenous and intramuscular administration. *Equine Vet J*. 2002;34:360–365.
316. Giguere S, Sweeney RW, Habecker PL, et al. Tolerability of orally administered enrofloxacin in adult horses: a pilot study. *J Vet Pharmacol Ther*. 1999;22:343–347.
317. Langston VC, Sedrish S, Boothe DM. Disposition of single-dose oral enrofloxacin in the horse. *J Vet Pharmacol Ther*. 1996;19:316–319.
318. Divers TJ, Irby NL, Mohammed HO, et al. Ocular penetration of intravenously administered enrofloxacin in the horse. *Equine Vet J*. 2008;40:167–170.
319. Goudah A, Abo El-Sooud K, Shim JH, et al. Characterization of the pharmacokinetic disposition of levofloxacin in stallions after intravenous and intramuscular administration. *J Vet Pharmacol Ther*. 2008;31:399–405.
320. Sekkin S, Gokbulut C, Kum C, et al. Plasma disposition of enrofloxacin following intravenous and intramuscular administration in donkeys. *Vet Rec*. 2012;171:447.
321. Gardner SY, Davis JL, Jones SL, et al. Moxifloxacin pharmacokinetics in horses and disposition into phagocytes after oral dosing. *J Vet Pharmacol Ther*. 2004;27:57–60.
322. Alghasham AA, Nahata MC. Clinical use of fluoroquinolones in children. *Ann Pharmacother*. 2000;34:344–413, 347–359; quiz.
323. Burkhardt JE, Hill MA, Turek JJ, et al. Ultrastructural changes in articular cartilages of immature beagle dogs dosed with difloxacin, a fluoroquinolone. *Vet Pathol*. 1992;29:230–238.
324. Beluche LA, Bertone AL, Anderson DE, et al. In vitro dose-dependent effects of enrofloxacin on equine articular cartilage. *Am J Vet Res*. 1999;60:577–582.
325. Vivrette SL, Bostian A, Bermingham EC, et al. Quinolone-induced arthropathy in neonatal foals. *47th Annual American Association of Equine Practitioners Convention*. 2001:376–377.
326. Bertone AL, Tremaine WH, Macoris DG, et al. Effect of long-term administration of an injectable enrofloxacin solution on physical and musculoskeletal variables in adult horses. *J Am Vet Med Assoc*. 2000;217:1514–1521.
327. Larsen H, Nielsen GL, Schonheyder HC, et al. Birth outcome following maternal use of fluoroquinolones. *Int J Antimicrob Agents*. 2001;18:259–262.
328. Heath SE. Chronic pleuritis in a horse. *Can Vet J*. 1989;30:69.
329. Intorre L, Mengozzi G, Maccheroni M, et al. Enrofloxacin-theophylline interaction: influence of enrofloxacin on theophylline steady-state pharmacokinetics in the beagle dog. *J Vet Pharmacol Ther*. 1995;18:352–356.
330. Cox S, Dudenbostel L, Sommardahl C, et al. Pharmacokinetics of firocoxib and its interaction with enrofloxacin in horses. *J Vet Pharmacol Ther*. 2012;35:615–617.
331. Dechant J. Combination of medical and surgical therapy for pleuropneumonia in a horse. *Can Vet J*. 1997;38:499–501.
332. MacDonald DG, Bailey JV, Fowler JD. Arthrodesis of the scapulohumeral joint in a horse. *Can Vet J*. 1995;36:312–315.
333. Rodger LD, Carlson GP, Moran ME, et al. Resolution of a left ureteral stone using electrohydraulic lithotripsy in a thoroughbred colt. *J Vet Intern Med*. 1995;9:280–282.
334. Wilson WD, Spensley MS, Baggot JD, et al. Pharmacokinetics, bioavailability, and in vitro antibacterial activity of rifampin in the horse. *Am J Vet Res*. 1988;49:2041–2046.
335. Fines M, Pronost S, Maillard K, et al. Characterization of mutations in the rpoB gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. *J Clin Microbiol*. 2001;39:2784–2787.

336. Takai S, Takeda K, Nakano Y, et al. Emergence of rifampin-resistant *Rhodococcus equi* in an infected foal. *J Clin Microbiol*. 1997;35:1904–1908.
337. Kohn CW, Sams R, Kowalski JJ, et al. Pharmacokinetics of single intravenous and single and multiple dose oral administration of rifampin in mares. *J Vet Pharmacol Ther*. 1993;16:119–131.
338. Burrows GE, MacAllister CG, Beckstrom DA, et al. Rifampin in the horse: comparison of intravenous, intramuscular, and oral administrations. *Am J Vet Res*. 1985;46:442–446.
339. Burrows GE, MacAllister CG, Ewing P, et al. Rifampin disposition in the horse: effects of age and method of oral administration. *J Vet Pharmacol Ther*. 1992;15:124–132.
340. Castro LA, Brown MP, Gronwall R, et al. Pharmacokinetics of rifampin given as a single oral dose in foals. *Am J Vet Res*. 1986;47:2584–2586.
341. Burrows GE, MacAllister CG, Ewing P, et al. Rifampin disposition in the horse: effects of repeated dosage of rifampin or phenylbutazone. *J Vet Pharmacol Ther*. 1992;15:305–308.
342. Frank LA. Clinical pharmacology of rifampin. *J Am Vet Med Assoc*. 1990;197:114–117.
343. Baggot JD, Wilson WD, Hietala S. Clinical pharmacokinetics of metronidazole in horses. *J Vet Pharmacol Ther*. 1988;11:417–420.
344. Magdesian KG, Hirsh DC, Jang SS, et al. Characterization of *Clostridium difficile* isolates from foals with diarrhea: 28 cases (1993-1997). *J Am Vet Med Assoc*. 2002;220:67–73.
345. Sweeney RW, Sweeney CR, Soma LR, et al. Pharmacokinetics of metronidazole given to horses by intravenous and oral routes. *Am J Vet Res*. 1986;47:1726–1729.
346. Rubin DT, Kornblunth A. Role of antibiotics in the management of inflammatory bowel disease: a review. *Rev Gastroenterol Disord*. 2005;5(suppl 3):S10–S15.
347. Steinman A, Gips M, Lavy E, et al. Pharmacokinetics of metronidazole in horses after intravenous, rectal and oral administration. *J Vet Pharmacol Ther*. 2000;23:353–357.
348. Garber JL, Brown MP, Gronwall RR, et al. Pharmacokinetics of metronidazole after rectal administration in horses. *Am J Vet Res*. 1993;54:2060–2063.
349. Swain EA, Magdesian KG, Kass PH, et al. Pharmacokinetics of metronidazole in foals: influence of age within the neonatal period. *J Vet Pharmacol Ther*. 2015;38:227–234.
350. Specht TE, Brown MP, Gronwall RR, et al. Pharmacokinetics of metronidazole and its concentration in body fluids and endometrial tissues of mares. *Am J Vet Res*. 1992;53:1807–1812.
351. Sweeney RW, Sweeney CR, Weiher J. Clinical use of metronidazole in horses: 200 cases (1984-1989). *J Am Vet Med Assoc*. 1991;198:1045–1048.
352. Jones RL. Clostridial enterocolitis. *Vet Clin North Am Equine Pract*. 2000;16:471–485.
353. McGorum BC, Dixon PM, Smith DG. Use of metronidazole in equine acute idiopathic toxaemic colitis. *Vet Rec*. 1998;142:635–638.
354. Barr BS. Infiltrative intestinal disease. *Vet Clin North Am Equine Pract*. 2006;22:e1–e7.
355. Lees P, Higgins AJ. Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. *Equine Vet J*. 1985;17:83–96.
356. Wallace JL. How do NSAIDs cause ulcer disease? *Baillieres Best Pract Res Clin Gastroenterol*. 2000;14:147–159.
357. Wallace JL. Distribution and expression of cyclooxygenase (COX) isoenzymes, their physiological roles, and the categorization of nonsteroidal anti-inflammatory drugs (NSAIDs). *Am J Med*. 1999;107:11S–16S, discussion 16S-17S.
358. Wallace JL, Ma L. Inflammatory mediators in gastrointestinal defense and injury. *Exp Biol Med (Maywood)*. 2001;226:1003–1015.
359. Wallace JL. Selective cyclooxygenase-2 inhibitors: after the smoke has cleared. *Dig Liver Dis*. 2002;34:89–94.
360. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol Rev*. 2008;88:1547–1565.
361. Giovanni G, Giovanni P. Do non-steroidal anti-inflammatory drugs and COX-2 selective inhibitors have different renal effects? *J Nephrol*. 2002;15:480–488.
362. Raidal SL, Hughes KJ, Charman AL, et al. Effects of meloxicam and phenylbutazone on renal responses to furosemide, dobutamine, and exercise in horses. *Am J Vet Res*. 2014;75:668–679.
363. Wallace JL, Reuter BK, McKnight W, et al. Selective inhibitors of cyclooxygenase-2: are they really effective, selective, and GI-safe? *J Clin Gastroenterol*. 1998;27(suppl 1):S28–S34.
364. Wallace JL, Muscara MN. Selective cyclo-oxygenase-2 inhibitors: cardiovascular and gastrointestinal toxicity. *Dig Liver Dis*. 2001;33(suppl 2):S21–S28.
365. Armstrong S, Tricklebank P, Lake A, et al. Pharmacokinetics of carprofen enantiomers in equine plasma and synovial fluid—a comparison with ketoprofen. *J Vet Pharmacol Ther*. 1999;22:196–201.
366. Higgins AJ, Lees P, Sedgwick AD. Development of equine models of inflammation. The Ciba-Geigy Prize for Research in Animal Health. *Vet Rec*. 1987;120:517–522.
367. Landoni MF, Lees P. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse. *J Vet Pharmacol Ther*. 1996;19:466–474.
368. King JN, Gerring EL. Antagonism of endotoxin-induced disruption of equine bowel motility by flunixin and phenylbutazone. *Equine Vet J*. 1989;21(suppl 7):38–42.
369. Moses VS, Hardy J, Bertone AL, et al. Effects of anti-inflammatory drugs on lipopolysaccharide-challenged and unchallenged equine synovial explants. *Am J Vet Res*. 2001;62:54–60.
370. Danek J. Effects of flunixin meglumine on selected clinicopathologic variables, and serum testosterone concentration in stallions after endotoxin administration. *J Vet Med A Physiol Pathol Clin Med*. 2006;53:357–363.
371. Dunkle NJ, Bottoms GD, Fessler JF, et al. Effects of flunixin meglumine on blood pressure and fluid compartment volume changes in ponies given endotoxin. *Am J Vet Res*. 1985;46:1540–1544.
372. Olson NC, Meyer RE, Anderson DL. Effects of flunixin meglumine on cardiopulmonary responses to endotoxin in ponies. *J Appl Physiol*. 1985;59:1464–1471.
373. Semrad SD. Comparison of flunixin, prednisolone, dimethyl sulfoxide, and a lazaroid (U74389F) for treating endotoxemic neonatal calves. *Am J Vet Res*. 1993;54:1517–1522.
374. Semrad SD, Hardee GE, Hardee MM, et al. Flunixin meglumine given in small doses: pharmacokinetics and prostaglandin inhibition in healthy horses. *Am J Vet Res*. 1985;46:2474–2479.
375. Semrad SD, Hardee GE, Hardee MM, et al. Low dose flunixin meglumine: effects on eicosanoid production and clinical signs induced by experimental endotoxaemia in horses. *Equine Vet J*. 1987;19:201–206.
376. Semrad SD, Moore JN. Effects of multiple low doses of flunixin meglumine on repeated endotoxin challenge in the horse. *Prostaglandins Leukot Med*. 1987;27:169–181.
377. Templeton CB, Bottoms GD, Fessler JF, et al. Endotoxin-induced hemodynamic and prostaglandin changes in ponies: effects of flunixin meglumine, dexamethasone, and prednisolone. *Circ Shock*. 1987;23:231–240.
378. Dawson J, Lees P, Sedgwick AD. Actions of non-steroidal anti-inflammatory drugs on equine leucocyte movement in vitro. *J Vet Pharmacol Ther*. 1987;10:150–159.
379. Pillinger MH, Capodici C, Rosenthal P, et al. Modes of action of aspirin-like drugs: salicylates inhibit erk activation and integrin-dependent neutrophil adhesion. *Proc Natl Acad Sci U S A*. 1998;95:14540–14545.
380. Weissmann G, Montesinos MC, Pillinger M, et al. Non-prostaglandin effects of aspirin III and salicylate: inhibition of integrin-dependent human neutrophil aggregation and inflammation in COX 2- and NF kappa B (P105)-knockout mice. *Adv Exp Med Biol*. 2002;507:571–577.

381. Chambers JP, Waterman AE, Livingston A. The effects of opioid and alpha 2 adrenergic blockade on non-steroidal anti-inflammatory drug analgesia in sheep. *J Vet Pharmacol Ther*. 1995;18:161–166.
382. Johnson CB, Taylor PM, Young SS, et al. Postoperative analgesia using phenylbutazone, flunixin or carprofen in horses. *Vet Rec*. 1993;133:336–338.
383. Landoni MF, Soraci AL, Delatour P, et al. Enantioselective behaviour of drugs used in domestic animals: a review. *J Vet Pharmacol Ther*. 1997;20:1–16.
384. Landoni MF, Lees P. Chirality: a major issue in veterinary pharmacology. *J Vet Pharmacol Ther*. 1996;19:82–84.
385. Lapicque F, Muller N, Payan E, et al. Protein binding and stereoselectivity of nonsteroidal anti-inflammatory drugs. *Clin Pharmacokinet*. 1993;25:115–123.
386. Armstrong S, Lees P. Effects of R and S enantiomers and a racemic mixture of carprofen on the production and release of proteoglycan and prostaglandin E_2 from equine chondrocytes and cartilage explants. *Am J Vet Res*. 1999;60:98–104.
387. Verde CR, Simpson MI, Frigoli A, et al. Enantiospecific pharmacokinetics of ketoprofen in plasma and synovial fluid of horses with acute synovitis. *J Vet Pharmacol Ther*. 2001;24:179–185.
388. Brouwers JR, de Smet PA. Pharmacokinetic-pharmacodynamic drug interactions with nonsteroidal anti-inflammatory drugs. *Clin Pharmacokinet*. 1994;27:462–485.
389. Semrad SD, Sams RA, Harris ON, et al. Effects of concurrent administration of phenylbutazone and flunixin meglumine on pharmacokinetic variables and in vitro generation of thromboxane B_2 in mares. *Am J Vet Res*. 1993;54:1901–1905.
390. Keegan KG, Messer NT, Reed SK, et al. Effectiveness of administration of phenylbutazone alone or concurrent administration of phenylbutazone and flunixin meglumine to alleviate lameness in horses. *Am J Vet Res*. 2008;69:167–173.
391. Reed SK, Messer NT, Tessman RK, et al. Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. *Am J Vet Res*. 2006;67:398–402.
392. Peng S, Duggan A. Gastrointestinal adverse effects of nonsteroidal anti-inflammatory drugs. *Expert Opin Drug Saf*. 2005;4:157–169.
393. Burrows GE, MacAllister CG, Tripp P, et al. Interactions between chloramphenicol, acepromazine, phenylbutazone, rifampin and thiamylal in the horse. *Equine Vet J*. 1989;21:34–38.
394. Firth EC, Nouws JF, Klein WR, et al. The effect of phenylbutazone on the plasma disposition of penicillin G in the horse. *J Vet Pharmacol Ther*. 1990;13:179–185.
395. Whittem T, Firth EC, Hodge H, et al. Pharmacokinetic interactions between repeated dose phenylbutazone and gentamicin in the horse. *J Vet Pharmacol Ther*. 1996;19:454–459.
396. Dyke TM, Hinchcliff KW, Sams RA. Attenuation by phenylbutazone of the renal effects and excretion of furosemide in horses. *Equine Vet J*. 1999;31:289–295.
397. Hinchcliff KW, McKeever KH, Muir 3rd WW, et al. Pharmacologic interaction of furosemide and phenylbutazone in horses. *Am J Vet Res*. 1995;56:1206–1212.
398. Carrick JB, Papich MG, Middleton DM, et al. Clinical and pathological effects of flunixin meglumine administration to neonatal foals. *Can J Vet Res*. 1989;53:195–201.
399. Traub JL, Gallina AM, Grant BD, et al. Phenylbutazone toxicosis in the foal. *Am J Vet Res*. 1983;44:1410–1418.
400. Traub-Dargatz JL, Bertone JJ, Gould DH, et al. Chronic flunixin meglumine therapy in foals. *Am J Vet Res*. 1988;49:7–12.
401. Gunson DE, Soma LR. Renal papillary necrosis in horses after phenylbutazone and water deprivation. *Vet Pathol*. 1983;20:603–610.
402. Karcher LF, Dill SG, Anderson WI, et al. Right dorsal colitis. *J Vet Intern Med*. 1990;4:247–253.
403. Cohen ND, Carter GK, Mealey RH, et al. Medical management of right dorsal colitis in 5 horses: a retrospective study. *J Vet Intern Med*. 1987-1993;1995(9):272–276.
404. Collins LG, Tyler DE. Experimentally induced phenylbutazone toxicosis in ponies: description of the syndrome and its prevention with synthetic prostaglandin E_2. *Am J Vet Res*. 1985;46:1605–1615.
405. Hough ME, Steel CM, Bolton JR, et al. Ulceration and stricture of the right dorsal colon after phenylbutazone administration in four horses. *Aust Vet J*. 1999;77:785–788.
406. MacAllister CG, Morgan SJ, Borne AT, et al. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc*. 1993;202:71–77.
407. Meschter CL, Gilbert M, Krook L, et al. The effects of phenylbutazone on the morphology and prostaglandin concentrations of the pyloric mucosa of the equine stomach. *Vet Pathol*. 1990;27:244–253.
408. Read WK. Renal medullary crest necrosis associated with phenylbutazone therapy in horses. *Vet Pathol*. 1983;20:662–669.
409. Held JP, Daniel GB. Use of nonimaging nuclear medicine techniques to assess the effect of flunixin meglumine on effective renal plasma flow and effective renal blood flow in healthy horses. *Am J Vet Res*. 1991;52:1619–1621.
410. Cambridge H, Lees P, Hooke RE, et al. Antithrombotic actions of aspirin in the horse. *Equine Vet J*. 1991;23:123–127.
411. Hardee MM, Moore JN, Hardee GE. Effects of flunixin meglumine, phenylbutazone and a selective thromboxane synthetase inhibitor (UK-38,485) on thromboxane and prostacyclin production in healthy horses. *Res Vet Sci*. 1986;40:152–156.
412. Heath MF, Evans RJ, Poole AW, et al. The effects of aspirin and paracetamol on the aggregation of equine blood platelets. *J Vet Pharmacol Ther*. 1994;17:374–378.
413. Lees P, Ewins CP, Taylor JB, et al. Serum thromboxane in the horse and its inhibition by aspirin, phenylbutazone and flunixin. *Br Vet J*. 1987;143:462–476.
414. Baxter GM, Moore JN. Effect of aspirin on ex vivo generation of thromboxane in healthy horses. *Am J Vet Res*. 1987;48:13–16.
415. Frean SP, Cambridge H, Lees P. Effects of anti-arthritic drugs on proteoglycan synthesis by equine cartilage. *J Vet Pharmacol Ther*. 2002;25:289–298.
416. Jolly WT, Whittem T, Jolly AC, et al. The dose-related effects of phenylbutazone and a methylprednisolone acetate formulation (Depo-Medrol) on cultured explants of equine carpal articular cartilage. *J Vet Pharmacol Ther*. 1995;18:429–437.
417. Frean SP, Abraham LA, Lees P. In vitro stimulation of equine articular cartilage proteoglycan synthesis by hyaluronan and carprofen. *Res Vet Sci*. 1999;67:183–190.
418. Pountos I, Georgouli T, Blokhuis TJ, et al. Pharmacological agents and impairment of fracture healing: what is the evidence? *Injury*. 2008;39:384–394.
419. Rohde C, Anderson DE, Bertone AL, et al. Effects of phenylbutazone on bone activity and formation in horses. *Am J Vet Res*. 2000;61:537–543.
420. Broome TA, Brown MP, Gronwall RR, et al. Pharmacokinetics and plasma concentrations of acetylsalicylic acid after intravenous, rectal, and intragastric administration to horses. *Can J Vet Res*. 2003;67:297–302.
421. Murdick PW, Ray RS, Noonan JS. Salicylic acid concentration in plasma and urine of medicated and nonmedicated horses. *Am J Vet Res*. 1968;29:581–585.
422. Judson DG, Barton M. Effect of aspirin on haemostasis in the horse. *Res Vet Sci*. 1981;30:241–242.
423. Trujillo O, Rios A, Maldonado R, et al. Effect of oral administration of acetylsalicylic acid on haemostasis in the horse. *Equine Vet J*. 1981;13:205–206.
424. Soraci A, Benoit E, Jaussaud P, et al. Enantioselective glucuronidation and subsequent biliary excretion of carprofen in horses. *Am J Vet Res*. 1995;56:358–361.
425. Lees P, McKellar Q, May SA, et al. Pharmacodynamics and pharmacokinetics of carprofen in the horse. *Equine Vet J*. 1994;26:203–208.

426. Williams A, Smith JR, Allaway D, et al. Carprofen inhibits the release of matrix metalloproteinases 1, 3, and 13 in the secretome of an explant model of articular cartilage stimulated with interleukin 1. *Arthritis Res Ther*. 2013;15:R223.
427. Bryant CE, Farnfield BA, Janicke HJ. Evaluation of the ability of carprofen and flunixin meglumine to inhibit activation of nuclear factor kappa B. *Am J Vet Res*. 2003;64:211–215.
428. Caldwell FJ, Mueller PO, Lynn RC, et al. Effect of topical application of diclofenac liposomal suspension on experimentally induced subcutaneous inflammation in horses. *Am J Vet Res*. 2004;65:271–276.
429. Schleining JA, McClure SR, Evans RB, et al. Liposome-based diclofenac for the treatment of inflammation in an acute synovitis model in horses. *J Vet Pharmacol Ther*. 2008;31:554–561.
430. Lynn RC, Hepler DI, Kelch WJ, et al. Double-blinded placebo-controlled clinical field trial to evaluate the safety and efficacy of topically applied 1% diclofenac liposomal cream for the relief of lameness in horses. *Vet Ther*. 2004;5:128–138.
431. Levine DG, Epstein KL, Neelis DA, et al. Effect of topical application of 1% diclofenac sodium liposomal cream on inflammation in healthy horses undergoing intravenous regional limb perfusion with amikacin sulfate. *Am J Vet Res*. 2009;70:1323–1325.
432. Anderson D, Kollias-Baker C, Colahan P, et al. Urinary and serum concentrations of diclofenac after topical application to horses. *Vet Ther*. 2005;6:57–66.
433. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91–97.
434. Holland B, Fogle C, Blikslager AT, et al. Pharmacokinetics and pharmacodynamics of three formulations of firocoxib in healthy horses. *J Vet Pharmacol Ther*. 2015;38:249–256.
435. Barton MH, Paske E, Norton N, et al. Efficacy of cyclo-oxygenase inhibition by two commercially available firocoxib products in horses. *Equine Vet J*. 2014;46:72–75.
436. American Veterinary Medical Foundation: Clarification regarding substitution of Previcox® for Equioxx®. May 11, 2010. Available at https://www.avma.org/KB/Resources/Reference/Pages/Previcox-for-Equioxx.aspx.
437. Letendre LT, Tessman RK, McClure SR, et al. Pharmacokinetics of firocoxib after administration of multiple consecutive daily doses to horses. *Am J Vet Res*. 2008;69:1399–1405.
438. Duz M, Parkin TD, Cullander RM, et al. Effect of flunixin meglumine and firocoxib on ex vivo cyclooxygenase activity in horses undergoing elective surgery. *Am J Vet Res*. 2015;76:208–215.
439. Kvaternick V, Pollmeier M, Fischer J, et al. Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther*. 2007;30:208–217.
440. Barton MH, Darden JE, Clifton S, et al. Effect of firocoxib on cyclooxygenase 2, microsomal prostaglandin E_2 synthase 1, and cytosolic phospholipase A_2 gene expression in equine mononuclear cells. *Am J Vet Res*. 2015;76:1051–1057.
441. Kivett L, Taintor J, Wright J. Evaluation of the safety of a combination of oral administration of phenylbutazone and firocoxib in horses. *J Vet Pharmacol Ther*. 2014;37:413–416.
442. Cox S, Villarino N, Sommardahl C, et al. Disposition of firocoxib in equine plasma after an oral loading dose and a multiple dose regimen. *Vet J*. 2013;198:382–385.
443. McConnico RS, Khodadad JL. Pharmacokinetics and clinical safety of oral paste firocoxib, a COX-1 sparing NSAID, in foals. *J Vet Int Med*. 2011;25:665.
444. Hovanessian N, Davis JL, McKenzie 3rd HC, et al. Pharmacokinetics and safety of firocoxib after oral administration of repeated consecutive doses to neonatal foals. *J Vet Pharmacol Ther*. 2014;37:243–251.
445. Soma LR, Behrend E, Rudy J, et al. Disposition and excretion of flunixin meglumine in horses. *Am J Vet Res*. 1988;49:1894–1898.
446. Welsh JC, Lees P, Stodulski G, et al. Influence of feeding schedule on the absorption of orally administered flunixin in the horse. *Equine Vet J*. 1992;11(suppl 1):62–65.
447. Coakley M, Peck KE, Taylor TS, et al. Pharmacokinetics of flunixin meglumine in donkeys, mules, and horses. *Am J Vet Res*. 1999;60:1441–1444.
448. Crisman MV, Wilcke JR, Sams RA. Pharmacokinetics of flunixin meglumine in healthy foals less than twenty-four hours old. *Am J Vet Res*. 1996;57:1759–1761.
449. Galbraith EA, McKellar QA. Protein binding and in vitro serum thromboxane B_2 inhibition by flunixin meglumine and meclofenamic acid in dog, goat and horse blood. *Res Vet Sci*. 1996;61:78–81.
450. Higgins AJ, Lees P, Sharma SC, et al. Measurement of flunixin in equine inflammatory exudate and plasma by high performance liquid chromatography. *Equine Vet J*. 1987;19:303–306.
451. Landoni MF, Lees P. Comparison of the anti-inflammatory actions of flunixin and ketoprofen in horses applying PK/PD modelling. *Equine Vet J*. 1995;27:247–256.
452. Jackman BR, Moore JN, Barton MH, et al. Comparison of the effects of ketoprofen and flunixin meglumine on the in vitro response of equine peripheral blood monocytes to bacterial endotoxin. *Can J Vet Res*. 1994;58:138–143.
453. Daels PF, Stabenfeldt GH, Hughes JP, et al. Effects of flunixin meglumine on endotoxin-induced prostaglandin F_2 alpha secretion during early pregnancy in mares. *Am J Vet Res*. 1991;52:276–281.
454. MacAllister CG, Sangiah S. Effect of ranitidine on healing of experimentally induced gastric ulcers in ponies. *Am J Vet Res*. 1993;54:1103–1107.
455. Brehaus BA, Brown CM, Scott EA, et al. Clostridial muscle infections following intramuscular injections. *Equine Vet Sci*. 1983;3:42–46.
456. Rebhun WC, Shin SJ, King JM, et al. Malignant edema in horses. *J Am Vet Med Assoc*. 1985;187:732–736.
457. Corveleyn S, Deprez P, Van der Weken G, et al. Bioavailability of ketoprofen in horses after rectal administration. *J Vet Pharmacol Ther*. 1996;19:359–363.
458. Corveleyn S, Henrist D, Remon JP, et al. Bioavailability of racemic ketoprofen in healthy horses following rectal administration. *Res Vet Sci*. 1999;67:203–204.
459. Sams R, Gerken DF, Ashcraft SM. Pharmacokinetics of ketoprofen after multiple intravenous doses to mares. *J Vet Pharmacol Ther*. 1995;18:108–116.
460. Landoni MF, Lees P. Influence of formulation on the pharmacokinetics and bioavailability of racemic ketoprofen in horses. *J Vet Pharmacol Ther*. 1995;18:446–450.
461. Landoni MF, Foot R, Frean S, et al. Effects of flunixin, tolfenamic acid, R(−) and S(+) ketoprofen on the response of equine synoviocytes to lipopolysaccharide stimulation. *Equine Vet J*. 1996;28:468–475.
462. Wilcke JR, Crisman MV, Scarratt WK, et al. Pharmacokinetics of ketoprofen in healthy foals less than twenty-four hours old. *Am J Vet Res*. 1998;59:290–292.
463. Owens JG, Kamerling SG, Stanton SR, et al. Effects of pretreatment with ketoprofen and phenylbutazone on experimentally induced synovitis in horses. *Am J Vet Res*. 1996;57:866–874.
464. Owens JG, Kamerling SG, Stanton SR, et al. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse. *Equine Vet J*. 1995;27:296–300.
465. Mozaffari AA, Derakhshanfar A, Alinejad A, et al. A comparative study on the adverse effects of flunixin, ketoprofen and phenylbutazone in miniature donkeys: haematological, biochemical and pathological findings. *N Z Vet J*. 2010;58:224–228.
466. Ketofen. In: Arrioja A, ed. *Compendium of veterinary products*. 10th ed. Hensall, ON: North American Compendiums Ltd; 2007. p 1673.
467. Beretta C, Garavaglia G, Cavalli M. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. *Pharmacol Res*. 2005;52:302–306.

468. Toutain PL, Reymond N, Laroute V, et al. Pharmacokinetics of meloxicam in plasma and urine of horses. *Am J Vet Res*. 2004;65:1542–1547.
469. Toutain PL, Cester CC. Pharmacokinetic-pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. *Am J Vet Res*. 2004;65:1533–1541.
470. Sinclair MD, Mealey KL, Matthews NS, et al. Comparative pharmacokinetics of meloxicam in clinically normal horses and donkeys. *Am J Vet Res*. 2006;67:1082–1085.
471. Naylor RJ, Taylor AH, Knowles EJ, et al. Comparison of flunixin meglumine and meloxicam for postoperative management of horses with strangulating small intestinal lesions. *Equine Vet J*. 2014;46:427–434.
472. Little D, Brown SA, Campbell NB, et al. Effects of the cyclooxygenase inhibitor meloxicam on recovery of ischemia-injured equine jejunum. *Am J Vet Res*. 2007;68:614–624.
473. D'Arcy-Moskwa E, Noble GK, Weston LA, et al. Effects of meloxicam and phenylbutazone on equine gastric mucosal permeability. *J Vet Intern Med*. 2012;26:1494–1499.
474. Noble G, Edwards S, Lievaart J, et al. Pharmacokinetics and safety of single and multiple oral doses of meloxicam in adult horses. *J Vet Intern Med*. 2012;26:1192–1201.
475. Walliser U, Fenner A, Mohren N, et al. Evaluation of the efficacy of meloxicam for post-operative management of pain and inflammation in horses after orthopaedic surgery in a placebo controlled clinical field trial. *BMC Vet Res*. 2015;11:113.
476. Raidal SL, Edwards S, Pippia J, et al. Pharmacokinetics and safety of oral administration of meloxicam to foals. *J Vet Intern Med*. 2013;27:300–307.
477. Thamm DH, Ehrhart 3rd EJ, Charles JB, et al. Cyclooxygenase-2 expression in equine tumors. *Vet Pathol*. 2008;45:825–828.
478. Moore AS, Beam SL, Rassnick KM, et al. Long-term control of mucocutaneous squamous cell carcinoma and metastases in a horse using piroxicam. *Equine Vet J*. 2003;35:715–718.
479. Rose RJ, Kohnke JR, Baggot JD. Bioavailability of phenylbutazone preparations in the horse. *Equine Vet J*. 1982;14:234–237.
480. Smith PB, Caldwell J, Smith RL, et al. The bioavailability of phenylbutazone in the horse. *Xenobiotica*. 1987;17:435–443.
481. Sullivan M, Snow DH. Factors affecting absorption of non-steroidal anti-inflammatory agents in the horse. *Vet Rec*. 1982;110:554–558.
482. Soma LR, Gallis DE, Davis WL, et al. Phenylbutazone kinetics and metabolite concentrations in the horse after five days of administration. *Am J Vet Res*. 1983;44:2104–2109.
483. Tobin T, Chay S, Kamerling S, et al. Phenylbutazone in the horse: a review. *J Vet Pharmacol Ther*. 1986;9:1–25.
484. Wilcke JR, Crisman MV, Sams RA, et al. Pharmacokinetics of phenylbutazone in neonatal foals. *Am J Vet Res*. 1993;54:2064–2067.
485. Tobin T, Blake JW, Valentine R. Drug interactions in the horse: effects of chloramphenicol, quinidine, and oxyphenbutazone on phenylbutazone metabolism. *Am J Vet Res*. 1977;38:123–127.
486. Lees P, Maitho TE, Taylor JB. Pharmacokinetics of phenylbutazone in two age groups of ponies: a preliminary study. *Vet Rec*. 1985;116:229–232.
487. Piperno E, Ellis DJ, Getty SM, et al. Plasma and urine levels of phenylbutazone in the horse. *J Am Vet Med Assoc*. 1968;153:195–198.
488. Hunt JM, Lees P, Edwards GB. Suspected non-steroidal anti-inflammatory drug toxicity in a horse. *Vet Rec*. 1985;117:581–582.
489. Aleman M, Nieto JE, Higgins JK. Ulcerative cystitis associated with phenylbutazone administration in two horses. *J Am Vet Med Assoc*. 2011;239:499–503.
490. Ramirez S, Wolfsheimer KJ, Moore RM, et al. Duration of effects of phenylbutazone on serum total thyroxine and free thyroxine concentrations in horses. *J Vet Intern Med*. 1997;11:371–374.
491. Lees P, May SA, Hoeijmakers M, et al. A pharmacodynamic and pharmacokinetic study with vedaprofen in an equine model of acute nonimmune inflammation. *J Vet Pharmacol Ther*. 1999;22:96–106.
492. Davis JL, Papich MG, Morton AJ, et al. Pharmacokinetics of etodolac in the horse following oral and intravenous administration. *J Vet Pharmacol Ther*. 2007;30:43–48.
493. Symonds KD, MacAllister CG, Erkert RS, et al. Use of force plate analysis to assess the analgesic effects of etodolac in horses with navicular syndrome. *Am J Vet Res*. 2006;67:557–561.
494. Morton AJ, Campbell NB, Gayle JM, et al. Preferential and non-selective cyclooxygenase inhibitors reduce inflammation during lipopolysaccharide-induced synovitis. *Res Vet Sci*. 2005;78:189–192.
495. Tomlinson JE, Wilder BO, Young KM, et al. Effects of flunixin meglumine or etodolac treatment on mucosal recovery of equine jejunum after ischemia. *Am J Vet Res*. 2004;65:761–769.
496. Davis JL, Marshall JF, Papich MG, et al. The pharmacokinetics and in vitro cyclooxygenase selectivity of deracoxib in horses. *J Vet Pharmacol Ther*. 2011;34:12–16.
497. Neirinckx E, Vervaet C, De Boever S, et al. Species comparison of oral bioavailability, first-pass metabolism and pharmacokinetics of acetaminophen. *Res Vet Sci*. 2010;89:113–119.
498. West E, Bardell D, Morgan R, et al. Use of acetaminophen (paracetamol) as a short-term adjunctive analgesic in a laminitic pony. *Vet Anaesth Analg*. 2011;38:521–522.

CHAPTER 3

Recognizing and Treating Pain in Horses

Rachel C. Hector and Khursheed R. Mama

INTRODUCTION

Following a brief review of general concepts related to pain and its assessment in horses, this chapter will focus on available pharmacologic and to a lesser extent complementary therapeutic options. The intent is to provide information that veterinarians can directly apply to management of acute and chronic pain in horses.

Pathophysiology of Pain

The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Box 3.1).[1] Nociception refers to the activation of high threshold receptors (nociceptors) located at the distal ends of unmyelinated (C) or poorly myelinated (Aδ) nerve fibers. Electrical impulses conducted by these afferent fibers reach the dorsal horn of the spinal cord and via ascending pathways (spinothalamic and spinoreticular thalamic) are transmitted to the brain in which they may be perceived or recognized as painful (Fig. 3.1). Nociception may occur in the absence of perception (as in the anesthetized horse) and can trigger neuroendocrine (cortisol and catecholamines), metabolic (hyperglycemia), and physiologic (heart rate and respiratory rate) responses. Perception, which is influenced by external and animal-related factors, will trigger similar responses and behaviors (e.g., avoidance, lameness) intended to protect the horse from further insult. Comprehensive assessment of these responses is useful when evaluating the severity, stress, and impact of pain on the horse's quality of life.

Acutely painful states may resolve with discontinuation of the stimulus and appropriate intervention. Neuronal input may also be modulated by descending inhibitory pathways originating from the brain and by use of alternate nonpainful afferent neural input (e.g., ice at the site of injury). In the absence of these, continued nociceptive input can lead to chronic nervous system pathology resulting from peripheral and central sensitization. Clinically these conditions are associated with hypersensitivity (or a reduction in the intensity of the stimulus required to cause pain) and allodynia (in which a typically nonpainful stimulus is now perceived as pain). Exaggerated responses to a noxious stimulus (hyperalgesia) and persistence of pain after removal of a noxious stimulus (hyperpathia) may also occur.

Peripheral sensitization is caused by the local production, release, and accumulation of chemicals (prostaglandins, leukotrienes, neuropeptides, and nerve growth factors) that sensitize peripheral nerve fibers and activate additional (silent) nociceptors at the terminal ends of Aδ and C fibers.[2,3] Central sensitization results from the cumulative effects of repetitive and sustained nociceptive input on the dorsal horn of the spinal cord and a subsequent release of glutamate and neuropeptides (substance P and neurokinin A) that activate *N*-methyl-D-aspartate (NMDA) and tachykinin receptors, resulting in a gradual "windup" of central neural pathways.[2-4]

Visceral pain (e.g., pain from intestine, liver, spleen, kidney, or bladder), unlike somatic pain, is transmitted by parasympathetic (principally vagal) and sympathetic splanchnic afferent nerve fibers.[5,6] The autonomic fibers involved in transmitting noxious

BOX 3.1 Definitions of Terms Used to Describe Pain

1. **Pain:** Unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage.
2. **Noxious stimulus:** Stimulus (mechanical, chemical, and thermal) of sufficient intensity to threaten or overtly cause tissue damage.
3. **Nociception:** Process of pain perception via pain receptors (nociceptors) and transmission of noxious (painful) stimuli, including transduction, transmission, modulation, and perception.
4. **Hyperalgesia:** Increased response (hypersensitivity) to a noxious stimulus, either at the site of injury (primary) or in surrounding undamaged tissue (secondary).
5. **Hyperesthesia:** Increased sensitivity to *non*noxious stimuli.
6. **Hyperpathia:** Greatly exaggerated pain sensation to nociceptive stimuli.
7. **Allodynia:** Pain produced by *non*noxious stimuli.
8. **Preemptive analgesia:** Prevention or minimization of pain by the administration of analgesics before the production of pain or before the introduction of a noxious stimulus (surgery) if pain already exists. The goal of preemptive analgesia is to provide a therapeutic intervention in advance of pain to prevent or minimize the central nervous system response to a noxious stimulus.
9. **Multimodal therapy:** Administration of multiple drugs that act by different mechanisms of action to produce the desired (analgesic) effect.

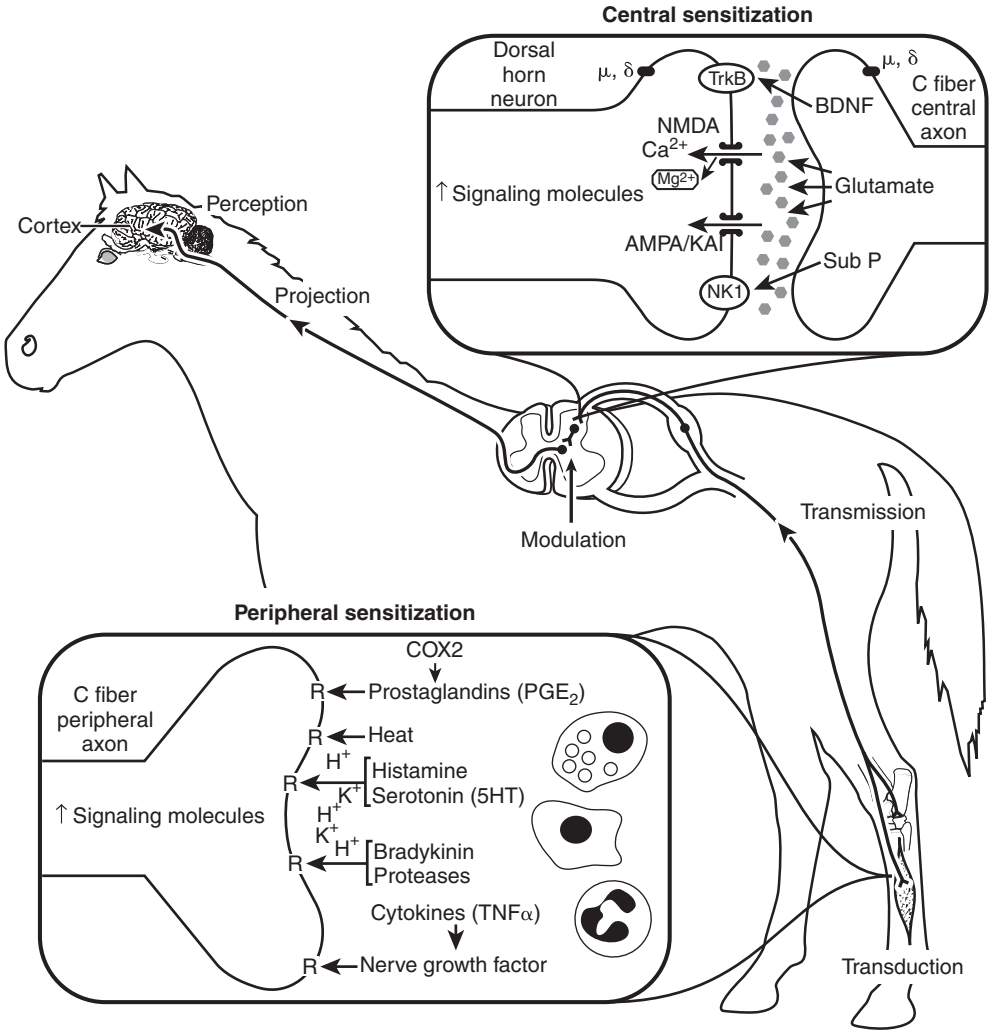


FIG. **3.1** Pain nociception and perception. Painful thermal, mechanical, and chemical stimuli are transduced to electric potentials (action potentials) that are transmitted to the spinal cord, in which they are modulated and then projected to the brain (perception). The major excitatory neurotransmitter in the spine is glutamate, which normally activates α-amino-3-hydroxy-5-isoxazole proprionic acid (AMPA) and kainate (KAI) receptors. NMDA, *N*-methyl-D-aspartate; NK1, neurokinin.

inputs from visceral organs are diffuse and overlap extensively, resulting in difficulty in localization of the site of pain. Neuropathic pain, which is yet another form of pain, may result from injury to the peripheral or central nervous system. For additional details regarding the pathophysiology of pain the reader is referred to McMahon et al. (2013) and Almeida et al. (2004).[7,8]

Pain Assessment

Although horses will demonstrate a variety of behavioral indicators of pain (Box 3.2), assessment of pain in horses has historically been considered challenging.[9] This could be because horses, as prey animals, do not wish to appear vulnerable and so do not fully exhibit signs of pain in unfamiliar or threatening environments until pain becomes severe or unrelenting. Consequently, horses may not be appropriately treated for painful conditions by the inexperienced observer. Although early reports suggest an emphasis on physiologic or limited behavioral parameters to assess pain, today there is the recognition that evaluation of pain requires a multifaceted approach and should be assessed in light of the cause (e.g., gastrointestinal, orthopedic, dental). Additionally, it is recognized that the pain experience is likely to be influenced by animal and environmental factors.[10-12] Among physiologic assessments, stress hormones (e.g., cortisol, β-endorphins), and catecholamines (e.g., epinephrine, norepinephrine) have been measured in both clinical and laboratory settings in an attempt to correlate these "objective data" with pain states.[10,13] Their usefulness has, however, been mixed because shock, stress, and medications influence them like they do other physiologic measurements, such as heart rate. Further, they are not contemporaneous to the pain assessment; thus, they only have retrospective value. Measurement of inflammatory mediators such as prostaglandin E2 and substance P from joints are similarly not consistently reliable as indicators of pain, and results are not immediately available.[14,15]

In an attempt to overcome these challenges, there has been significant recent work on the development of multifaceted pain assessment tools that consider physiologic (e.g., heart rate, blood pressure) and behavioral (both spontaneous and in response to interaction) indices developed specifically for

BOX 3.2 Potential Behavioral Indicators of Pain in Horses

- Considerable restlessness, agitation, and anxiety
- Rigid stance and reluctance to move
- Lowered head carriage
- Fixed stare and dilated nostrils, clenched jaw
- Aggression toward its own foal
- Aggression toward handlers, horses, objects, and self
- Decreased interactions with handlers
- Vocalization (deep groaning and grunting)
- Rolling
- Kicking at abdomen
- Flank watching
- Stretching
- Dullness and depression
- Weight-shifting among limbs
- Limb guarding
- Abnormal weight distribution
- Pointing, hanging, and rotating limbs
- Abnormal movement
- Reluctance to move
- Arched back
- Head shaking
- Abnormal bit behavior
- Altered eating; anorexia, quidding

horses in different clinical scenarios (e.g., postarthroscopy, colic). Each parameter on many of these scales is further categorized in an attempt to indicate severity of pain; for example, the parameter "kicking at the abdomen" is characterized by frequency of the same over a fixed interval, with greater frequency assigned a worse score (greater degree of pain). Hence, these scales serve not only to assess the presence or absence of pain but also the severity (two such scales are included in Figs. 3.2 and 3.3).[16-21] One of these—namely, the horse grimace scale—presents a somewhat unique approach and is worth highlighting. This scale, which was modeled after similar work in rodents, correlates six specific facial expressions with the degree of pain; for example, ears stiffly backward can indicate a greater degree of pain versus none if ears face forward. Although this was developed in horses undergoing castration, it may have applicability or provide a good adjunctive assessment tool in horses with other conditions.[19] Photographic examples of horses demonstrating facial expressions of pain are included in Fig. 3.4.

Note that the majority of these pain scales are targeted toward acute or nociceptive pain in adult horses. Assessment of chronic pain may require additional and different tools (e.g., force plate, gait analysis), owner or trainer input, and greater reliance on response to therapy. Similarly, the authors are not aware of ethograms or pain scales for foals, but based on the author's impressions and in talking with veterinarians who routinely manage foals, additional behavioral signs should be considered. For example, grinding of the teeth is commonly associated with pain from stomach ulcers in foals. Additionally, unwillingness to lie down despite buckling the front limbs, stamping hind feet toward the belly, and lying down in lateral recumbency with one or both legs cranially toward or over the head has been associated with pain in foals.

Although methodology may need to vary based on the circumstance, the goal of pain assessment is to provide objective, repeatable, and consistent categorization of pain, such that therapies can be both targeted and monitored. If multiple individuals are to be involved in assessing the animal, training in use of the preferred pain scale or behaviors is helpful to minimize interobserver variation on parameters that are open to interpretation. Accurate assessment of pain will allow veterinarians to continue to improve analgesic efforts in horses.

Development of a Treatment Plan

Given the multifaceted nature of pain, the many individual breeds of horses, and additional equid species (e.g., donkeys, mules), as well as consideration of a wide age range of individual equids in which veterinarians may be faced with managing pain, a systematic approach to developing and modifying a pain treatment plan should be considered. Because of the significance of well-being, pain is considered the "5th vital sign" in human patients. In addition to the potential emotional association and subsequent avoidance behavior horses exhibit to locations in which they may have experienced pain, physiologic consequences such as negative energy balance, poor wound healing, and altered gastrointestinal motility may be significant.[22]

Management of nociceptive pain such as surgical pain or that associated with acute injury is a common scenario. Nociceptive pain is considered an adaptive or protective strategy, and the associated inflammatory response is considered essential to the healing process. The goal of therapy is to normalize pain sensitivity and not allow it to progress to a maladaptive state or one that is no longer proportional to the noxious stimulus and healing tissue.[23] Nonsteroidal antiinflammatory drugs (NSAIDs) help normalize the inflammatory response generated by tissue injury and are commonly used in horses. Although there have been mixed reports of the overall advantage of preemptive versus postprocedural use of NSAIDs, it is most common to administer these preemptively to limit inflammation. Additionally, medications used in the horse for sedation (e.g., α_2-adrenergic agonists) and general anesthesia (e.g., ketamine) are also analgesic and affect different sites in the nociceptive pathway (e.g., α_2-adrenergic agonist drugs mediate their analgesia through descending inhibition), providing a multimodal approach. Regional techniques (e.g., intraarticular, epidural, perineural) further facilitate this multimodal approach to analgesia and minimize the adverse effects (e.g., gastrointestinal stasis) associated with systemic medications.

Although foals are similarly deserving of a multimodal approach in treating pain, the safety of NSAIDs (e.g., gastrointestinal ulcers) and the cardiovascular side effects of α_2-adrenergic agents when used at doses similar to the adult horse deserve consideration. Conversely, some regional techniques (e.g., lumbosacral epidural) are more feasible, and certain drugs (e.g., opioids) may not be limited by behavioral side effects to the same degree as in adult horses.

The goal in treating pain early and using a multimodal approach is to minimize development of a chronic condition that can be more challenging to evaluate and treat. The subsequent text includes information on pain treatment modalities and medications that the veterinarian may select to best manage individual equine patients.

DRUGS USED TO TREAT PAIN

Almost all types of drugs used to treat pain in small animals have also been used in horses, although there are fewer controlled trials evaluating their efficacy. This may

Physiological Data	Criteria	Score /12
Heart Rate (Compared to Baseline)	<10% increase	0
	>11-30% increase	1
	>31-50% increase	2
	>50% increase	3
Respiratory Rate (Compared to Baseline)	<10% increase	0
	>11-30% increase	1
	>31-50% increase	2
	>50% increase	3
Digestive Sounds	Normal motility	0
	Decreased motility	1
	No motility	2
	Hypermotility	3
Rectal Temperature (Compared to Baseline)	<0.5°C variation	0
	<1°C variation	1
	<2°C variation	2
	>2°C variation	3
Response To Interaction	**Criteria**	**Score /6**
Interactive Behavior	Pays attention to people	0
	Exaggerated response to auditory stimuli	1
	Excessive to aggressive response to auditory stimuli	2
	Stupor, prostration, no response to auditory stimuli	3
Response To Palpation Of Painful Area	No reaction to palpation	0
	Mild reaction to palpation	1
	Resistance to palpation	2
	Violent reaction to palpation	3
Behavior	**Criteria**	**Score /21**
Appearance (reluctance to move, restlessness, agitation, anxiety)	Bright, lowered head and ears, no reluctance to move	0
	Bright, alert, occasional head movement, no reluctance to move	1
	Restless, pricked up ears, abnormal facial expressions, dilated pupils	2
	Excited, continuous body movements, abnormal facial expression	3
Sweating	No obvious signs of sweat	0
	Damp to the touch	1
	Wet to the touch, beads of sweat apparent over body	2
	Excessive sweating, beads of sweat running off the animal	3
Kicking At Abdomen	Quietly standing, no kicking	0
	Kicking at abdomen 1 - 2 times/5 mins	1
	Kicking at abdomen 3 - 4 times/5 mins	2
	Kicking at abdomen >5 times/5 mins, intermittent attempts to roll	3
Pawing On Floor (includes pointing or hanging limb)	Quietly standing, no pawing	0
	Occasionally pawing (1 - 2 times/5 mins)	1
	Frequent pawing (3 - 4 times/5 mins)	2
	Excessive pawing (>5 times/5 mins)	3
Posture (weight distribution and comfort)	Stands quietly, normal walk	0
	Occasional weight shift, slight muscle tremors	1
	Non-weight bearing, abnormal weight distribution	2
	Analgesic posture, attempts to urinate, prostration, muscle tremors	3
Head Movement (lateral or vertical head movements)	No evidence of discomfort, head straight ahead mostly	0
	Intermittent head movement, looking at flanks or lip curl 1 - 2 times /5 mins	1
	Intermittent, rapid head movement, looking at flanks or lip curl >5 times/5 mins	2
	Continuous head movements, looking at flanks or lip curl >5 times/5 mins	3
Appetite	Eats hay readily	0
	Hesitates to eat hay	1
	Little interest in eating hay, takes hay but doesn't chew or swallow	2
	Neither shows interest in nor eats hay	3
Total CPS		39

FIG. **3.2** Multifactorial numeric rating composite pain scale for evaluating pain in horses. (Reprinted with permission from Elsevier. Bussières G, Jacques C, Troncy E, et al. Development of a composite orthopedic pain scale in horses. *Res Vet Sci.* 2008; 85:294.)

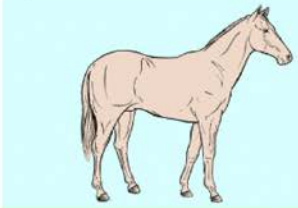
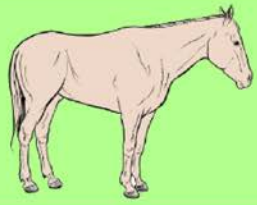
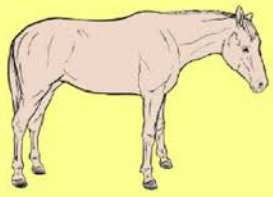
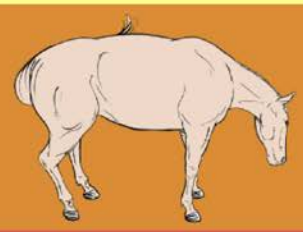
Colorado State University
Veterinary Medical Center

Date _____

Time _____

Equine Comfort Assessment Scale

*This scale is designed to be used in the context of the clinical presentation of each animal. If you do not believe the pain scoring criteria to be accurate for this patient, please explain in the comments section below.

Pain Score		Behavior	Clinical Assessment	Postural Features
0		❑ Responds with interest to gate opening, approach by observer ❑ Takes care in movements around people ❑ Head above withers ❑ Attentive ❑ Moving freely, calmly ❑ Resting comfortably	❑ HR:_____ (usually ≤ 40 bpm) ❑ Eyes: relaxed, normally responsive ❑ Normal muscle tension ❑ No focal areas of heat ❑ Palpation not aversive	❑ No lameness perceptible, bears weight equally ❑ Moves with ease of stride
1		❑ Head at or above withers ❑ Facing forward and watching ❑ Performs normal behaviors less frequently than expected ❑ Responds with quiet interest to gate opening, approach by observer ❑ Takes care in movements around people	❑ HR: _____ (may be ≤ 40 bpm) ❑ Mild muscle tension ❑ Mild focal areas of heat ❑ Slightly steps, leans or pulls away from palpation, +/- muscle twitching	❑ Lameness difficult to observe, inconsistently apparent ❑ Mild injury or stiffness in movement
2		❑ Head level with withers ❑ Moving slowly about with bedding undisturbed ❑ Mild but more frequent restlessness ❑ Responds to approach ❑ Less enthusiastic, less interested, less interactive ❑ Less careful about movements around people	❑ HR:_____(may be ≥ 48 bpm) ❑ Tachypnea +/- RR:_____ ❑ Moderate muscle tension ❑ Increasing areas of heat ❑ Palpation more aversive	❑ Lameness apparent only under certain circumstances, favors leg(s) occasionally ❑ Obvious stiffness in movement
3		❑ Head level or below withers ❑ May face back or corner of stall ❑ More vigorous signs of restlessness ❑ Eyes distracted, far away, weary ❑ Minimally reacts to interaction ❑ Stands in one position ❑ Beginning to become internalized ❑ Less careful about movements around people	❑ HR:_____(may be ≥ 60 bpm) ❑ Tachypnea +/- RR:_____ ❑ Sweating ❑ Severe muscle tension ❑ Widespread areas of heat ❑ Vigorously aversive response to palpation	❑ Moderate lameness, able to bear weight but clearly favors one or more limbs ❑ Obvious discomfort, weight shifting ❑ Arched back ❑ Very stiff movements ❑ Abnormal standing posture
4		❑ Head often below withers ❑ Stands in corner or faces wall ❑ Ears back, eyes weary ❑ Frequent signs of severe agitation ❑ Extremely uncomfortable, panicky OR ❑ Extremely internalized/withdrawn ❑ Unwilling to rise ❑ Careless about movements around people	❑ HR:_____(may be ≥ 70 bpm) ❑ Tachypnea +/- RR:_____ ❑ Profuse sweating ❑ Extreme muscle tension / rigidity +/- fasciculation ❑ Widespread areas of heat ❑ Extremely aversive response to palpation, possibly aggressive	❑ Unable or unwilling to bear weight ❑ May not be able to move ❑ Constant shifting of weight ❑ Very abnormal standing posture OR ❑ In sternal or lateral recumbency

Current Pain Treatment(s):

Comments: _____

© 2007 JE Blossom, PW Hellyer, PM Mich, NG Robinson, BD Wright.

FIG. **3.3** A multidimensional, nonvalidated pain scale that is used at the authors' institution.



FIG. **3.4** Examples of equids demonstrating facial expressions of pain. (A) Note the horse has partially closed eyes and drawn back upper lip. (B) The donkey shows ears pointed back and strained nostrils.

be one reason why there are limited validated pharmacologic options for analgesia in horses compared with those for dogs and cats. Practically, there may also be limitations to the use of certain drugs in horses with respect to drug volume or cost. As in any species, analgesic drugs also have potential adverse effects that may limit their use. Pharmacologic approaches tend to provide the greatest benefit with fewer or less severe adverse effects when drugs are selected for a specific purpose, used preemptively, or as a part of a multimodal approach, and the dose is scaled to the severity of pain.

Classes of drugs most commonly used in the horse include antiinflammatories, opioids, α_2-adrenergic agonists, and local anesthetics. There are, however, several additional drugs that have a place in equine pain management, and these have been included here. Greater detail about some of these drugs may be found in Chapter 2 and numerous additional resources.[24-26] Dosing information is provided in Table 3.1.

Antiinflammatory Drugs

Antiinflammatory drugs used in horses include corticosteroids and NSAIDs. They are often the first-line choice in the management of pain related to inflammatory conditions (e.g., synovitis and osteoarthritis)[27-29] and are administered by various routes, including systemically, intraarticularly, and topically.[30-32] Generally, NSAIDs are the most commonly used drugs in horses.[33] They are readily available and relatively inexpensive and do not require precise record keeping.

Corticosteroids act on the enzyme phospholipase A2 to prevent the breakdown of membrane phospholipids to arachidonic acid, which is the precursor to inflammatory prostaglandins and leukotrienes.[26] NSAIDs act to block cyclooxygenase (COX), the enzyme that produces prostaglandins from arachidonic acid.[34] Prostaglandins and leukotrienes have important housekeeping functions in the body and play a significant role in the development of pain, inflammation, and fever.

There are at least two isoforms of cyclooxygenase, and the most important ones are COX-1 and COX-2. COX-1 is considered constitutively expressed in most body tissues and plays an important role in maintaining renal blood flow, protecting gastric mucosa, and facilitating platelet aggregation (among other processes). The COX-2 isoform has a greater role in the development of inflammation and pain, and traditionally therapeutic effects of NSAIDs are attributed to inhibition of COX-2 effects.[35,36] However, COX-2 can also have housekeeping functions in the gastrointestinal tract and kidney, with greater effects in diseased states,[37] and the traditional absolute separation into constitutive with respect to COX-1 and inducible with respect to COX-2 is an oversimplification of the roles of the COX isoforms.[38,39] The existence of a COX-3 isoform, originally discovered as a variant of COX-1 in dogs, is reported in canine, rodent, and human literature.[40] COX-3 is the purported target of acetaminophen (paracetamol), although considerable debate exists regarding this topic.[41,42] Whether a COX-3 isoform exists in horses is unknown. Regardless, important species differences exist in the expression of different COX isoforms in various tissues, and extrapolation of what works in a dog or a human is not uniformly applicable to the horse.[43,44]

The most common adverse effects associated with blocking the COX pathways are seen in the gastrointestinal and renal systems. In horses gastric ulceration, right dorsal ulcerative colitis, and renal papillary/crest necrosis are reported with NSAID use.[45-47] Renal effects may be worsened by concurrent dehydration.[48] The reader should be aware that although the practice of combining multiple NSAIDs or corticosteroids and NSAIDs may result in increased analgesia, this practice is also associated with increased frequency and severity of adverse effects.[49-52] In circumstances in which pain is best managed by

TABLE **3.1** **Drugs Used for Analgesia in Horses**

Drug	Route	Dose	Comments
NONSTEROIDAL ANTIINFLAMMATORY DRUGS			
Phenylbutazone[28]	IV, PO	2.2–4.4 mg/kg q12–24 h	Lower doses suggested for chronic use
Flunixin meglumine[230]	IV, PO	1.1 mg/kg q 12–24 h	IM route on label but not recommended because of potential for local tissue irritation
Ketoprofen[28,231]	IV	1.1–2.2 mg/kg q 12–24 h	—
Firocoxib[57]	IV	0.09 mg/kg q 24 h	Nonaqueous solution should not be mixed with aqueous flush solutions in IV lines
	PO	Loading dose: 0.3 mg/kg Then: 0.1 mg/kg q 24 h	—
Meloxicam[55,56]	PO	0.6 mg/kg q 24 h	Increased dose frequency may be required for foals (q 12 h)
Diclofenac[32]	Topical	1% liposomal cream applied to bare skin over area of interest	—
OPIOIDS			
Can be combined with acepromazine, α_2-adrenergic agonists. Dilute epidural dose to desired volume.			
Butorphanol[62,63,179,232-235]	IV, IM, SC	0.01–0.22 mg/kg	Common clinically used doses are lower than reported analgesic doses
	IV CRI	CRI: 0.013–0.025 mg/kg/h	Loading dose recommended
Nalbuphine[236]	IV, IM	0.02–0.15 mg/kg	—
Buprenorphine[83,237-239]	IV, sublingual	0.005–0.01 mg/kg	—
	Epidural	0.005 mg/kg	—
Morphine[87-89,91,93,240,241]	IV, IM	0.05–0.2 mg/kg	—
	IV CRI	0.1 mg/kg/h	Loading dose recommended
	Epidural	0.1–0.2 mg/kg	—
	Intraarticular	0.1–0.2 mg/kg	—
Fentanyl[81,201,242]	IV CRI	Loading dose: 0.005 mg/kg CRI: 0.006 mg/kg/h	Less effective analgesic in horses compared with other species
	Transdermal	20- to 30-mg adhesive patch applied to bare skin per approximately 500-kg adult horse	As an adjunct to other analgesics
Remifentanil[240,243,244]	IV CRI	Loading dose: 0.0005 mg/kg CRI: 0.002–0.006 mg/kg/h	As an adjunct to general anesthesia
Methadone[133,245,246]	IV, IM, PO	0.05–0.15 mg/kg	—
	Epidural	0.1 mg/kg	—
Meperidine (pethidine)[62,247-249]	IV, IM	1 mg/kg	—
	Epidural	0.3–0.8 mg/kg	—
Hydromorphone[250]	Epidural	0.04 mg/kg	—
α_2-ADRENERGIC AGONISTS			
Doses provided will cause sedation. Lower doses may be effective for analgesia, especially in combination with opioids. CRI doses are used for standing procedures or as an adjunct to general anesthesia.			
Xylazine[251,252-255]	IV, IM	0.2–1.1 mg/kg	—
	IV CRI	0.016–0.69 mg/kg/h	—
	Epidural	0.17–0.22 mg/kg	Hind limb weakness/recumbency is a possible side effect
Detomidine[93,244,252,256-258]	IV, IM	0.01–0.03 mg/kg	—
	IV CRI	0.005–0.02 mg/kg/h	—
	Epidural	0.015–0.03 mg/kg	Systemic absorption is likely
Romifidine[235,252,259,260]	IV, IM	0.02–0.08 mg/kg	—
	IV CRI	0.03–0.04 mg/kg/h	—
	Epidural	0.03–0.06 mg/kg	—

TABLE **3.1 Drugs Used for Analgesia in Horses—cont'd**

Drug	Route	Dose	Comments
Dexmedetomidine[240,241,261,262]	IV, IM	0.003–0.005 mg/kg	—
	IV CRI	0.001–0.008 mg/kg/h	—
Medetomidine[251,263,264]	IV, IM	0.007–0.01 mg/kg	—
	IV CRI	0.003–0.005 mg/kg/h	—
LOCAL ANESTHETICS			
Epidural doses may be combined with opioids or α_2-adrenergic agonists; recommended volume when local anesthetics are used is not to exceed 5–7 mL in an adult horse.			
Lidocaine[133,247,265,266]	IV CRI	Loading dose: 1.3–1.5 mg/kg CRI: 0.05 mg/kg/min	Loading dose given over 10–20 min
	Sacrococcygeal epidural	0.2–0.35 mg/kg	—
Mepivacaine[267,268]	Sacrococcygeal epidural	0.16–0.2 mg/kg	—
Ropivacaine[269,270]	Sacrococcygeal epidural	0.08–0.15 mg/kg	—
Bupivacaine[271,272]	Sacrococcygeal epidural	0.02–0.06 mg/kg	—
OTHER DRUGS			
Ketamine[150-152]	IV CRI	0.4–0.8 mg/kg/h	In conscious horses higher doses can be used as an adjunct to general anesthesia
Gabapentin[160,161]	IV, PO	5–20 mg/kg q 8–12 h	Low oral bioavailability; higher dosing may be needed; titrate dose to effect
Methocarbamol[236]	PO	40–60 mg/kg q 12–24 h	—
N-butylscopolamine[179]	IV	0.3 mg/kg	Lower doses may achieve desired clinical effect

IV, Intravenously; *PO,* orally; *IM,* intramuscularly; *SC,* subcutaneously; *CRI,* constant rate infusion.

a combination of drugs within these two classes, concurrent use of gastrointestinal protectants is suggested.

Most NSAIDs approved for use in horses (e.g., aspirin, phenylbutazone, ketoprofen, flunixin meglumine, diclofenac) work by inhibiting both COX-1 and COX-2. However, COX-2–selective NSAIDs (firocoxib and meloxicam) have been developed for horses, with the primary goal of reducing gastrointestinal side effects,[53-55] especially because NSAIDs may be used chronically in some horses. Meloxicam specifically may offer further benefit in foals, in which traditional NSAIDs have a higher risk of adverse effects because of prolonged clearance in foals compared with adult horses. Meloxicam is cleared more rapidly in foals than adults, which may result in improved tolerance to longer treatment durations.[56]

The use of a canine chewable tablet formulation of firocoxib has become popular with horse owners because of low cost and ease of use; however, the canine tablet has reduced bioavailability and reduced COX-2 inhibition in horses compared with the oral equine formulation.[57] Additionally, practitioners should be aware that use of the canine formulation may represent extralabel drug use depending on their country of practice. The same is true of the COX-2–selective NSAID carprofen, which is licensed for dogs but has been administered to foals in place of phenylbutazone because of perceived efficacy and lower toxicity.

Opioids

Opioids produce their effects by activating mu (μ), kappa (κ), or delta (δ) opioid receptors.[58] Available opioids include pure μ-agonists (e.g., morphine, methadone, fentanyl), partial μ-agonists (e.g., buprenorphine), and κ-agonist/μ-antagonists (e.g., butorphanol). Morphine is the prototypic μ-agonist with which all other opioids are compared.[59,60] Although traditionally pure μ-agonist opioids are considered to provide the most potent analgesic effects, butorphanol is reported to be equally as or more effective (with potentially fewer adverse effects) than μ-agonists in the treatment of gastrointestinal pain in horses.[60-63] Opioids, especially morphine, produce profound sedative effects when combined with α_2-agonists. This is commonly termed *neuroleptanalgesia* and can be useful in animals that are refractory to analgesia or when standing interventions are necessary.[64,65]

Opioid use in horses is often limited by the potential for adverse effects. μ-Agonists in particular are known to cause excitement, agitation, and increased locomotor activity in horses.[58,66,67] This may be explained in part by the location, density, and binding affinity of μ-opioid receptors in the equine brain.[68] Although this effect is well documented, the clinical importance is confounded by multiple variables, including potential bias in the studies because they were conducted using healthy, nonpainful horses. Dosages used in investigational studies may also be higher than dosages used clinically, contributing to excitatory effects.[69] As mentioned previously, coadministration with sedative drugs ameliorates the potential excitatory effects of opioids and augments sedation.[58,68] In painful horses, low to moderate doses of opioids have a calming effect and in some studies are associated with

improved anesthesia recovery quality,[70,71] but variability exists with respect to behavioral responses to opioids in horses. In other species for which extensive research exists, differences have been attributed to opioid receptor polymorphisms. A μ-opioid receptor polymorphism has recently been shown to significantly affect locomotor responses to fentanyl in adult horses.[72] Continued research in this area may help to clarify varying clinical observations regarding opioid use in this species.

A further untoward effect of opioids is that they can delay gastric emptying and prolong intestinal transit time. Because of this, opioids can predispose horses to constipation and colonic impaction, because water is absorbed from the slow-moving contents of the intestinal lumen.[73,74] Although it has been the authors' experience that horses receiving large doses of opioids such as morphine can show gastrointestinal stasis necessitating passage of a nasogastric tube to relieve gas, studies on the risk of colic associated with morphine use in horses undergoing general anesthesia are conflicting.[69,75-77] Most show no relationship between the perioperative use of morphine and gastrointestinal complications. In addition, pain itself may predispose horses to ileus.[78,79] Although the potential for adverse effects should not to be ignored, it should be noted that complications are most likely when opioids are used systemically at large doses.

Experimental studies on the use of opioids in horses continue to emerge as potential clinical benefits of this class of drugs are increasing recognized. Several new studies describe the pharmacology of specific opioids in horses, but the need for additional evidence to support specific clinical treatment protocols is great. For example, intravenous fentanyl appears to be a relatively ineffective analgesic with undesirable behavioral effects at the doses studied in adult horses,[80,81] but it has a sedative effect in foals at relatively low doses.[82] Buprenorphine shows promise as an analgesic because of its long plasma half-life in horses and its efficacy via sublingual administration.[83] Reports of significant buprenorphine-induced excitement or increases in locomotor activity exist in adult horses and foals, however, even when the drug is given in combination with a sedative.[84-86] The use of systemic opioids in horses will, therefore, continue to be challenged by the need for appropriate case selection and dose titration such that clinical benefits are achieved without undesired effects.

Systemic adverse effects can be mitigated via the use of alternative routes of administration. Routes that have been studied in horses with promising results include intraarticular, transdermal, perineural, and epidural administration. Intraarticular morphine, for example, has a profound and lasting analgesic and antiinflammatory effect and produces a more significant reduction in lameness than intravenous morphine in horses with synovitis.[87-90] Epidural morphine provides useful and prolonged analgesia for a variety of surgical procedures,[91-93] although dose-dependent decreases in gastrointestinal transit time are reported.[79,91]

α_2-Adrenoreceptor Agonists

α_2-Adrenoreceptor agonists (e.g., xylazine, detomidine, romifidine, medetomidine, dexmedetomidine) produce sedation, muscle relaxation, and analgesia via activation of central and peripheral α_2-receptors. Although drug effects are broadly the same, individual differences exist with respect to α_1:α_2-receptor selectivity, chemical structure, drug metabolism, and elimination. Sedation is attributed to activation of central nervous system α_2-receptors in areas of the brain that are responsible for awareness, arousal, and vigilance. Activation of α_2-receptors in the brain and spinal cord decreases the release of excitatory neurotransmitters and interferes with sensory processing and transmission, producing analgesia.[94]

α_2-Adrenoceptor agonists are profound visceral analgesics. This property is crucial in the management of moderate to severe colic. The use of α_2-adrenoceptor agonists can prevent injury to both practitioner and horse and allow critical diagnostic and treatment procedures to be performed. These drugs, however, may also mask signs of clinical deterioration,[95,96] especially if higher doses or drugs with longer half-lives are administered (e.g., detomidine).[97] Behavioral and possibly analgesic effects may be improved when α_2-adrenoceptor agonists are administered with opioids (e.g., butorphanol or morphine).[63]

The use of α_2-adrenoceptor agonists is not without significant adverse effects. Clinically, the administration of α_2-agonists for the purpose of analgesia is accompanied by varying levels of sedation, which may be undesirable. α_2-Adrenoceptor agonists also cause peripheral vasoconstriction and subsequent baroreceptor-mediated reflex bradycardia and bradyarrhythmias (e.g., second degree atrioventricular block).[98,99] The increase in afterload associated with α_2-adrenoceptor agonist administration causes a profound reduction in cardiac output,[99-101] which may be detrimental in a horse with cardiovascular compromise. As the vasoconstrictive effect wanes, a decrease in arterial blood pressure follows, which is caused by a decrease in central nervous system sympathetic output and continued bradycardia.[94,102] Decreases in cardiac output are relatively independent of dose.[103] In horses with severe hemodynamic compromise (e.g., strangulating colic), the benefits of the use of α_2-adrenoceptor agonists must be weighed carefully with the risk of cardiovascular collapse.

Other adverse effects of α_2-adrenoceptor agonists include occasional paradoxic excitement or aggression, changes in respiratory rate and tidal volume, upper airway obstruction caused by relaxation of pharyngeal muscles, hyperglycemia, increased urine production, and immediate and pronounced decreases in gastrointestinal tone and motility that can last well beyond the duration of the drug.[104-110] These effects are dose dependent but can be related to postoperative ileus and colic.[111] Increased urine production could exacerbate dehydration in a debilitated horse if supplemental fluids are not provided. Tachypnea is sometimes observed when febrile horses receive any of this class of drugs.

α_2-Adrenoceptor agonists can be administered as bolus doses or via constant rate infusion.[110] Like opioids, they can also be administered intraarticularly, perineurally, and epidurally. Intraarticular use of α_2-adrenoceptor agonists provides analgesia by an undetermined mechanism; regional and systemic effects (from drug absorption), however, are possible.[112] The potential for chondrotoxic effects of α_2-adrenoceptor agonists are currently under investigation, with early reports indicating the potential for chondrotoxicity with some but not all drugs in this class.[113,114] α_2-Adrenoceptor agonists have been shown to improve duration of analgesia provided by perineural local anesthetics.[115] Epidural administration of α_2-adrenoceptor agonists provides beneficial analgesia for surgical procedures.[93,116] Some α_2-adrenoceptor agonists (e.g., detomidine) demonstrate systemic effects, however, because they are rapidly absorbed from the epidural space.[116,117]

The effects of α_2-adrenoceptor agonists can be reversed with α_2-adrenoceptor antagonists (e.g., atipamezole, tolazoline, yohimbine, idazoxan).[111,118] When using these reversal agents, all α_2 effects, including analgesia, are abolished. If α_2-adrenoceptor antagonists are used in a situation in which pain is anticipated, the practitioner should plan to provide analgesia by an alternate method. The use of α_2-adrenoceptor antagonists has been associated with significant adverse effects in horses, including behavioral changes, tachycardia, hypotension, and death. Adverse effects are more often associated with high doses and rapid (e.g., intravenous) administration.[119] Assessment of peripheral α_2-adrenoceptor antagonists that retain beneficial central effects and reduce the negative cardiovascular effects of α_2-adrenoceptor agonists is ongoing.[120,121]

Local Anesthetics

Local anesthetics (e.g., lidocaine, mepivacaine, bupivacaine, ropivacaine) are frequently used alone or in combination with other analgesic drugs to produce loss of sensation. They may be administered topically, regionally (perineurally and epidurally), or systemically. Analgesia results from a blockade of sodium ion channels, which prevents the initiation and conduction of electrical activity (action potentials) in small-diameter (C, Aδ) sensory nerve fibers. Larger (clinical) doses of local anesthetics also block conduction in large fibers (Aβ), and when administered at certain sites (e.g., epidural, perineural to a mixed nerve) produce a loss of motor function and result in temporary motor paralysis.

The local anesthetic lidocaine when administered intravenously produces mild central nervous depression and has antiarrhythmic, antiinflammatory, free radical scavenging, and gastrointestinal promotility effects.[122-127] It also provides analgesia, decreases the requirement for inhalant anesthetics, and potentiates the analgesic actions of opioids or α_2-adrenoceptor agonists.[122,123,128,129] Because of variable cardiac toxicity, administration of other local anesthetic agents by this route is currently not recommended. Lidocaine produces minimal cardiovascular and respiratory effects in otherwise normal, healthy horses but can decrease cardiac output, arterial blood pressure, and heart rate when administered intravenously because of decreases in central nervous system sympathetic output, myocardial contractile force, and venous return.[123,128] Lidocaine infusion does not improve cardiovascular function in anesthetized horses despite decreases in inhalant anesthetic dose, and in certain circumstances it may predispose horses to a poorer quality of recovery from general anesthesia.[129-131]

Significant differences in metabolism, elimination, and the potential to produce central nervous system toxicity (disorientation, ataxia, and seizures) are seen among different local anesthetics.[125] Horses appear to be more sensitive to the neurotoxic effects than some other species, with large doses producing central nervous stimulation typified by nervousness, agitation, sedation, ataxia, and collapse.[132] Seizures have also been observed by the authors in clinical patients. Although less likely to occur in full-size adult horses, toxic doses may also be reached when these drugs are administered locally or regionally.

Local anesthetics can be administered epidurally to decrease the need for additional analgesics.[133] However, as mentioned previously, they have the potential to produce a loss of motor function (paralysis), which can become problematic in an animal as large as a horse. The horse may injures itself while unable to control its limbs or may becomes recumbent until the local anesthetic effect has worn off. Local anesthetic drugs can also cause respiratory paralysis when administered by epidural or spinal (subarachnoid) routes. Although unlikely unless excessive drug volume is administered inadvertently, the migration of local anesthetic cranially to the cervical region can paralyze the diaphragm, resulting in hypoventilation and apnea. To avoid these adverse effects of epidural local anesthetics, the authors recommend these drugs generally not be placed in epidural catheters and that the volume of local anesthetic drug when using the caudal epidural space be limited to 5 to 7 mL for an adult horse.

Intraarticular use of local anesthetics is common in equine practice for the diagnosis and management of lameness and surgery of the joints. Of concern is the potential for local anesthetic-induced chondrotoxicity, which has been clinically recognized in humans with the use of bupivacaine.[134] An *in vitro* investigation in the horse showed chondrotoxicity associated with bupivacaine, lidocaine, and mepivacaine, with mepivacaine considered the least toxic to cartilage and safest for clinical use.[135] A subsequent *in vitro* human study showed that chondrotoxic effects were worse in arthritic joints.[136] In contrast, a single injection of bupivacaine and lidocaine produced no increases in collagen degradation biomarkers in an *in vivo* study in healthy horses.[137] More data are necessary before the issue is clarified in horses, and practitioners should weigh potential risks with benefits in individual clinical scenarios.

Ketamine

Ketamine is commonly used as an anesthetic induction and maintenance agent in horses and also possesses important analgesic properties. NMDA receptor activation is critical to the development of hyperalgesia and central sensitization mediated through interaction with a number of other receptors.[138,139] Ketamine's antagonism at this receptor helps mitigate these effects. Analgesia may also be partly mediated via the monoaminergic pathway through ketamine-induced release of norepinephrine, dopamine, and serotonin.[138]

At anesthetic doses, ketamine increases heart rate, cardiac output, and arterial blood pressure in horses.[140] When used with a goal of preventing central sensitization as an adjunct to inhalant anesthesia, ketamine infusion both reduces inhalant dose requirement and improves cardiac output.[141] Beneficial cardiovascular effects are attributed to increased circulating catecholamines, even though ketamine in isolation is a direct myocardial depressant.[142,143] Nonsystemic routes of ketamine administration have also been used with some success. For example, short-term local anesthesia is observed when used perineurally in horses.[144] Similarly when administered epidurally, ketamine produces analgesia, but evidence of localized neuronal toxicity, especially with chronic use, limits its use in clinical patients.[145,146]

Behavioral side effects associated with ketamine use in horses must be considered. In humans, ketamine produces hallucinations[138]; in horses, the potential for excitement or delirium during ketamine use in anesthesia is a reality minimized by the provision of other drugs (e.g., α_2-adrenoceptor agonists).[140,147] Subanesthetic doses of ketamine used to prevent central sensitization do not seem to be associated with adverse behavioral effects in people, dogs, and cats.[148,149] In horses, low-dose infusions can be used with minimal to no behavioral effects.[150] Evidence of antinociceptive effect with this dosing of ketamine in horses is conflicting and clinical data are sparse, but subanesthetic infusions appear to be an

effective adjunct for the management of laminitic pain.[150-153] Prolonged infusions of ketamine lead to an increase in gastrointestinal transit time and decreased fecal output in healthy horses,[154] but similar data from clinical patients are lacking.

Magnesium

Like ketamine, magnesium owes its analgesic effects largely to its NMDA receptor blockade. Magnesium is also a calcium channel blocker, and although this attribute has historically been used in the treatment of hypertension and arrhythmias, it is also an important mechanism by which systemic administration of magnesium is used to help treat pain.[155] Although magnesium has not been studied as an analgesic in horses, it has been extensively studied in human medicine. Some data are conflicting, but overall it appears that magnesium reduces postoperative pain and opioid requirements after a variety of surgical procedures.[156,157] In an experimental setting, magnesium also potentiates analgesia provided by morphine and delays the development of morphine tolerance.[158] Adverse effects appear to be minimal with appropriate dosing but include hypotension, bradycardia, and sedation.[157]

Anticonvulsants

Gabapentin is an anticonvulsant drug that also has analgesic effects, which is likely caused by its interaction with the $\alpha_2\delta$-subunit on voltage-gated calcium channels, which is upregulated in chronic and neuropathic pain. In humans, gabapentin is considered a first-line drug for the treatment of neuropathic pain.[159] It has low oral bioavailability in horses.[160] Oral gabapentin given for 2 weeks at common clinical dosages (5–10 mg/kg every 8 hours) failed to reduce the lameness level of chronically lame horses compared with placebo treatment.[161] However, a relatively low oral dose appeared effective for treating neuropathic pain in a horse in one case report.[162]

When given intravenously, 20 mg/kg of gabapentin was associated only with very mild sedation,[160] suggesting this higher dosage could be used without significant adverse effects. In humans, appropriate dose titration may take several weeks to achieve.[163] More clinical data are necessary to determine whether this is a factor in the efficacy of gabapentin in horses. The primary dose-limiting adverse effect of gabapentin in humans is somnolence.[163]

Pregabalin is a similar drug with the same mechanism of action used as both an anticonvulsant and analgesic in humans. When given via nasogastric tube to healthy horses, bioavailability was high and sedation was the most common adverse effect.[164] Use of pregabalin in equine clinical patients has not been reported.

Tramadol

Tramadol is a synthetic weak μ-opioid receptor agonist and serotonin and norepinephrine reuptake inhibitor. Tramadol is not a controlled drug in many countries. There are several metabolites of tramadol, but *O*-desmethyl-tramadol (M1) is considered the primary analgesic metabolite because it has increased affinity for the μ-receptor compared with the parent compound.[165,166]

In pharmacokinetic studies, horses show wide individual variability in production of M1, and this metabolite has either not been detectable or measured in very low concentrations.[167-170] It is possible that horses efficiently glucuronidate the M1 metabolite, affecting M1 concentrations.[167] Regardless, tramadol did not have an antinociceptive effect in horses in multiple research models and caused excitement with increases in dose.[171-173] In clinical patients, tramadol was mildly effective at reducing pain in laminitic horses in multimodal approaches to pain control,[151, 174] and no adverse effects were seen when it was used as an adjunct to acepromazine, detomidine, ketamine, diazepam, and flunixin meglumine for castration.[175] Further study is necessary to determine the clinical utility of tramadol in horses, but it is currently not promising as a sole analgesic in this species.

Soluble Epoxide Hydrolase Inhibitors

Oxidative metabolism of polyunsaturated fatty acids is a critical step in the production of mediators important to the development of inflammation and pain associated with tissue damage. The most well-known group of drugs blocking this process is the NSAIDs, which prevent arachidonic acid metabolism via COX inhibition, as discussed earlier. However, the COX pathway is only part of a much more complex set of processes responsible for inflammatory and neuropathic pain.

Recently inhibition of the cytochrome P450–mediated transformation of polyunsaturated fatty acids into epoxides has been explored as a potential analgesic treatment for horses with laminitis.[176] Soluble epoxide hydrolase activity measured in the digital laminae is increased in laminitic horses, and experimental treatment with the soluble epoxide hydrolase inhibitor *t*-TUCB provided moderate improvement in clinical signs (e.g., limb lifting) and pain scores in a small group of laminitic horses in a recent clinical trial.[177] The novel nature of this treatment means that further research is needed before widespread use is adopted, but it represents a promising addition to a multimodal approach to pain control in horses with laminitis.

Acepromazine

Acepromazine is a phenothiazine tranquilizer that does not have inherent analgesic properties.[178,179] As such, it should not be used as a sole agent with the intent to provide analgesia, nor should its calming effects be interpreted as analgesic efficacy. Although not licensed for such use, acepromazine is used by veterinarians in combination with α_2-adrenoceptor agonists or opioids to augment sedation. The literature also suggests that acepromazine administration improves undesired locomotor activity associated with the use of opioids in horses.[58]

N-Butylscopolamine

Butylscopolamine is an anticholinergic and antispasmodic drug that has a short duration of effect on gastrointestinal pain and is used to treat spasmodic colic,[179-181] and it can be given in combination with other analgesic drugs. Because its duration of action is very short (20–30 minutes), a perceived benefit is that it will not mask pain from a deteriorating, nonspasmodic colic while still providing relief to horses with uncomplicated colic pain. It should be noted that the anticholinergic effect of this drug will increase the heart rate, which may complicate physiologic assessment of pain.

Methocarbamol

Methocarbamol is a skeletal muscle relaxant that has been used in horses for several years to treat discomfort associated with muscle spasms, and it is moderately orally bioavailable in horses.[182] Although there is considerable anecdotal evidence of its use to treat equine back pain, scientific data are lacking.

Bisphosphonates

Tiludronate and clodronate are bisphosphonate drugs used to treat bone-related pain in horses. Bisphosphonates distribute primarily to bone and inhibit bone resorption primarily via negative effects on osteoclasts and macrophages.[183,184] These drugs are given intravenously and intramuscularly, respectively, with a duration of effect of several months.

Small clinical studies with tiludronate have shown some improvement in lameness caused by navicular disease and distal hock arthritis,[185] and a larger double-blind, placebo-controlled trial for hock arthritis also demonstrated this drug's efficacy.[186] Tiludronate has also proven efficacious in the treatment of thoracolumbar vertebral pain in horses.[187] Although it has been administered during regional limb perfusion, its safety and the efficacy of this technique are not known.[188] Colic has been associated with the use of tiludronate in horses,[185] and varying degrees of renal damage have been demonstrated in humans with the use of different bisphosphonates.[189] Extensive clinical data regarding renal toxicity in horses do not exist. However, the potential for adverse effects should be strongly considered in horses before treatment, because anecdotal reports of renal damage are increasing. The practice of pretreating with flunixin meglumine to prevent tiludronate-associated colic signs is discouraged because of the concern for compounding nephrotoxicity.

Autologous Conditioned Serum (Interleukin-1 Receptor Antagonist Protein)

Interleukin-1 receptor antagonist protein (IRAP) is an antiinflammatory cytokine used in the treatment of equine osteoarthritis. Whole blood is collected and incubated with beads that enhance the production of IRAP.[190] Serum is then separated and injected intraarticularly. Clinically, IRAP improves lameness scores in horses with osteoarthritis compared with placebo.[191] Because the product is autologous and includes no drugs, it can be used in performance horses before competition.

SPECIFIC ANALGESIC TECHNIQUES

Epidural Catheters

Epidural catheters (Fig. 3.5) can be placed in horses to provide a safe and efficient way to administer repeated doses of analgesic drugs (e.g., opioids, α_2-adrenoceptor agonists) into the epidural space. Epidural catheter kits that can be used in horses are available commercially (Fig. 3.6). These catheters can be maintained successfully for several days to weeks. In clinical cases, patient-related complications (e.g., inflammation at the catheter insertion site) appear to be very infrequent when proper aseptic technique is used in the placement and maintenance of epidural catheters. The most common catheter-related complications in horses include dislodgement of the catheter or filter, loss of patency of the catheter, or catheter leakage.[192] Indwelling catheters do cause epidural inflammation and fibrosis, which may be responsible for loss of patency over time.[192,193] These issues do not preclude replacement with a new catheter but may make it more challenging.

Perineural Catheters

Continuous peripheral nerve block via the use of perineural catheters has been successfully used in human medicine for a variety of applications, including postoperative analgesia.



FIG. **3.5** An epidural catheter secured with a bandage, which prevents debris (such as straw or shavings) from contaminating the catheter.

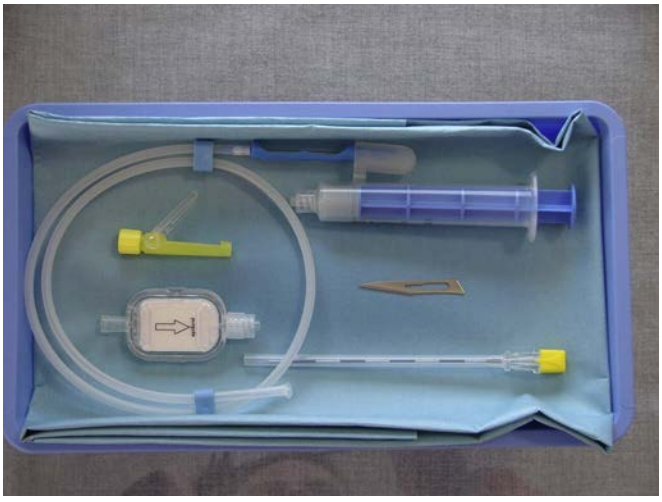


FIG. **3.6** Components of a commercially available epidural catheter kit, including scalpel blade, catheter, connector, filter, plastic loss of resistance syringe, and Tuohy needle for placement. (Photo courtesy Gregg Griffenhagen, DVM, DACVAA.)

This technique involves continuous infusion (or intermittent, low-volume boluses) of local anesthetics in catheters placed adjacent to peripheral nerves. A significant advantage of this technique is the ability to reduce or discontinue systemically administered analgesics, thus avoiding the systemic side effects of those drugs.[194-197]

In horses, this technique has been developed for use in the distal limb, in which a perineural catheter can be placed in a standing, sedated patient and maintained under a bandage thereafter (Fig. 3.7). Possible complications include inadvertent vascular or synovial structure puncture, local infection, nerve injury, and swelling or hematoma. Complications can be avoided by adhering to proper landmarks and using strict aseptic technique.[194,198]

Topical and Transdermal Drug Administration

Topical administration of analgesic drugs offers advantages to systemic drug administration. Application is directed over

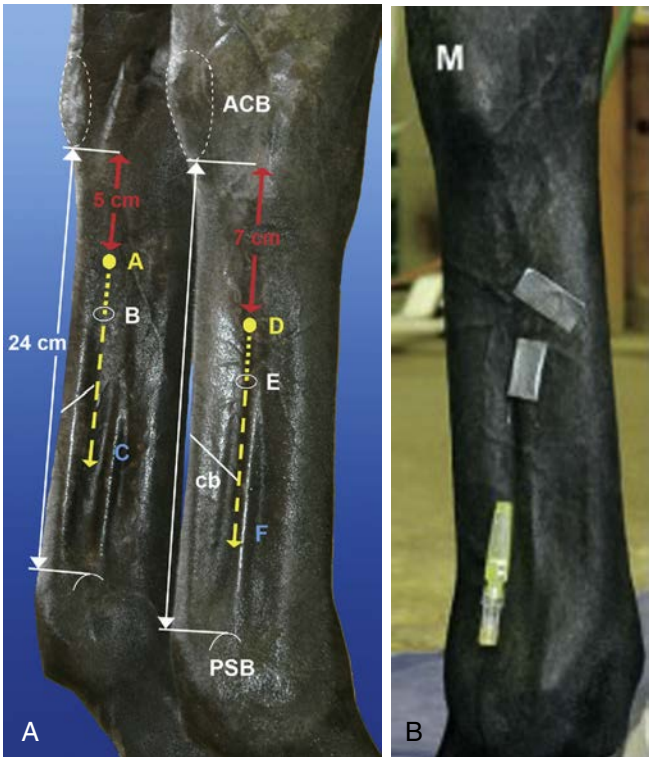


FIG. **3.7** (A) Anatomic diagram showing proper placement of a distal limb perineural catheter in the horse, 5 cm distal to the accessory carpal bone (ACB) on the medial aspect of the limb, and 7 cm distal to ACB on the lateral aspect. Dotted lines between points A and B and D and E represent the intended path for subcutaneous tunneling of the catheter. Dashed lines between points B and C and E and F represent the intended path for the subfascial placement of the catheter, ending distal to the communicating branch (cb) between the medial and lateral palmar nerves and proximal to the proximal sesamoid bones (PSB). (B) Perineural catheter secured in place on the lateral aspect of the left front limb of a horse. (Reprinted with permission from Elsevier. Driessen B, Scandella M, Zarucco L. Development of a technique for continuous perineural blockade of the palmar nerves in the distal equine thoracic limb. Vet Anaesth Analg. 2008; 35:432.)

the desired site of action such that the dose (and therefore side effects) may be reduced. The NSAID diclofenac is available in the United States in a liposomal formulation that has been shown to reduce clinical signs of lameness in horses with osteoarthritis, providing beneficial disease-modifying effects compared with systemically administered phenylbutazone.[199] This equine formulation is not available worldwide, and the use of topical diclofenac products formulated for humans has thus been adopted in many places. These formulations, however, do not offer similar absorption and efficacy, which is likely caused by differences between human and equine skin for which these products were not designed.[200]

Transdermal drug administration offers a method for continuously delivering systemic drugs over several hours or days. This is especially beneficial for drugs with a short duration of effect, such that multiple bolus doses can be avoided. The opioid fentanyl, for example, is widely available as a transdermal patch designed to provide controlled fentanyl release for 72 hours. Intravenous administration of fentanyl has been associated with adverse behavioral effects and questionable analgesic efficacy in horses. In contrast, transdermal fentanyl has not been associated with untoward side effects, and there are promising reports of clinical efficacy in horses in pain.[201] Pharmacologic data, however, show widely variable absorption in both adult horses and foals, in which effective plasma concentrations were not reached in all individuals.[202,203]

Several factors may affect patch efficacy, including improper patch placement and premature patch removal. Conditions under which the patch is applied (e.g., whether hair is clipped and the area cleaned) or anatomic location of the patch on the horse might also affect absorption. Location-dependent differences in absorption could be related to differences in the thickness of the stratum corneum or differences in cutaneous blood flow. *In vitro* work with horse skin suggests that absorption through the groin or thorax is superior to absorption through skin on the distal limb.[204]

COMPLEMENTARY THERAPIES

Complementary techniques may play an important role in multimodal analgesic management of horses. Acupuncture, chiropractic manipulations, massage, and extracorporeal shock wave therapy have been adopted and successfully used by equine veterinarians.[205] At their introduction, these therapies were used in horses with primarily anecdotal evidence, but scientific data on these modalities are emerging, especially as they become increasingly popular with horse owners.

Acupuncture

Acupuncture is purported to act by restoration of energy flow via stimulation of certain points on channels or "meridians" within the body.[206] Points can be stimulated with needles alone, injected substances, or electrically ("electroacupuncture").[207] Modern scientific research has shown that analgesia is produced by the stimulation of specific (acupuncture) points and not produced by similar stimulation of other points on the body.[208] The analgesic effect results from modulation of nociceptive input at the level of the dorsal horn of the spinal cord through activation of antinociceptive pathways and the release of endogenous opioids.[209] Acupuncture is an appealing modality because of its relative safety and lack of systemic drug levels, which allows its use in performance horses during competition.

In horses, acupuncture and electroacupuncture have positive effects on endorphin release and cutaneous analgesia.[207,210] Electroacupuncture has also been used to increase pain threshold in an experimental rectal distention model.[211] Clinical results, however, have been inconsistent. In one study, electroacupuncture may have provided clinical improvement in horses with chronic lameness, but differences from controls were not significant.[212] In a more recent study of naturally occurring palmar heel pain, acupuncture failed to consistently affect pain in those horses.[213] Further research into the use of acupuncture as a clinical modality is needed to define which conditions might benefit the most from this treatment.

Extracorporeal Shock Wave Therapy

Extracorporeal shock wave therapy is used to treat a variety of musculoskeletal injuries in horses, including osteoarthritis, back pain, stress fractures, tendonitis, and desmitis.[214] Although the exact mechanism of action is not known, the basic principle is that when focused, pulsed, high-energy waves are applied to the injured area, they lead to neovascularization and tissue remodeling.[214,215] In some studies, shock

wave therapy produced acute local analgesia for 48 to 72 hours after treatment,[216,217] which has raised concern about potential catastrophic injury in performance horses subjected to work after minor injury and subsequent treatment. However, in further studies with specific lesions, shock wave therapy did not reduce lameness in the immediate posttreatment period, and its acute analgesic effect in these cases has been questioned.[15,218] Nevertheless, shock wave remains a viable potential adjunct therapy in a multimodal approach to analgesia.

Chiropractic Manipulations

Chiropractic manipulations rely on "high-velocity, low-amplitude" manual thrusts that attempt to restore normal joint motion and improve tissue function of the equine spine.[219] These factors might be negatively altered by an underlying pathologic condition, such as limb lameness.[220] Addressing this additional potential source of pain could theoretically improve the overall clinical condition, especially if the underlying condition is also managed. Chiropractic manipulations change the kinematics of horses with back pain, increase mechanical nociceptive thresholds in healthy horses compared with controls, and reduce cervical muscle activity.[219,221,222] The significance of these findings with respect to acute or chronic pain control is unknown.

Low-Level Laser Therapy

Low-level lasers ("cold lasers") have become a popular therapeutic adjunct in the equine veterinary world. Cold lasers are typically relatively low power and nonthermal, such that tissue damage is avoided while beneficial effects are achieved. There is anecdotal support from performance horse owners regarding the use of laser therapy in the treatment of acute and chronic musculoskeletal pain. Despite enthusiasm for this modality, strong scientific data supporting the use of lasers in the horse are sparse.[223] Evidence of laser therapy as an analgesic, antiinflammatory, and aid to wound healing is emerging in human medicine and research models.[224-226] Studies suggest that possible mechanisms of action include modulation of local inflammatory mediators (e.g., prostaglandins, tumor necrosis factor-α [TNF-α]) and reduction or alteration in inflammatory cell infiltration or distribution.[227]

A wide variety of cold lasers are marketed that can be sold directly to horse owners.[228] Lasers are classified on a 1 to 4 scale by the U.S. Food and Drug Administration or by the International Electrotechnical Commission based on the level of potential operating hazard, with higher classes being more powerful and also more likely to cause injury if improperly operated.[229] Lasers available for equine therapy come from one of the two highest classes: class 3B or class 4. Class 4 lasers are typically more expensive, more powerful and more efficient and can treat larger or deeper areas. Protocols and laser types used in human medicine vary across studies, and there is no scientific consensus as to the most appropriate laser or protocol for use in equine therapy. Positive results from human studies, however, should encourage further research on laser therapy in horses, which will help to clarify the place of cold laser therapy in management of equine pain.

SUMMARY

Equine practitioners have more tools now than ever before to assess and manage pain. Detailed pain scales improve clinical pain assessment, and promising clinical studies have emerged showing benefits of many different drugs and nontraditional analgesic techniques. In addition, the importance of treating pain and the use of multimodal techniques is more and more widely accepted. Although pain management in the horse continues to be challenging, the increasing availability of pharmacologic and complementary options provides a reason for optimism.

REFERENCES

1. Merskey HR, Bogduk N. *Classification of chronic pain, descriptions of chronic pain syndromes and definitions of pain terms*. 2nd ed. Seattle, WA: IASP Press; 1994.
2. Muir WW, Woolf CJ. Mechanisms of pain and their therapeutic implications. *J Am Vet Med Assoc*. 2001;219:1346.
3. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science*. 2000;288:1765.
4. Woolf CJ, Decosted I. Implications of recent advances in the understanding of pain pathophysiology for the assessment of pain in patients. *Pain Suppl*. 1999;6:S141.
5. Blackshaw LA, Gabhart GF. The pharmacology of gastrointestinal nociceptive pathways. *Curr Opin Pharmacol*. 2002;2:642.
6. Bueno L, Fioramonti J, Delvaux M, et al. Mediators and pharmacology of visceral sensitivity: from basic to clinical investigations. *Gastroenterology*. 1997;112:1714.
7. McMahon S, Koltzenburg M, Tracey I, et al. *Wall and Melzack's textbook of pain*. 6th ed. Philadelphia: Saunders; 2013.
8. Almeida TF, Roizenblatt S, Tufik S. Afferent pain pathways: a neuroanatomical review. *Brain Res*. 2004;1000:40.
9. Love EJ, Taylor PM, Clark C, et al. Analgesic effect of butorphanol in ponies following castration. *Equine Vet J*. 2009;41:552.
10. Bussières G, Jacques C, Troncy E, et al. Development of a composite orthopaedic pain scale in horses. *Res Vet Sci*. 2008;85:294.
11. Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. *Equine Vet J*. 2005;37:565.
12. Van Loon JPAM, Back W, Hellebrekers LJ, et al. Application of a composite pain scale to objectively monitor horses with somatic and visceral pain under hospital conditions. *J Equine Vet Sci*. 2010;30:641.
13. Raekallio M, Taylor PM, Bennett RC. Preliminary investigations of pain and analgesia assessment in horses administered phenylbutazone or placebo after arthroscopic surgery. *Vet Surg*. 1997;26:150.
14. deGraue JC, van de Lest CH, van Weeren R, et al. Arthrogenic lameness of the fetlock: synovial fluid markers or inflammation and cartilage turnover in relation to clinical joint pain. *Equine Vet J*. 2006;38:305.
15. Kirker-Head CA, Chandna VK, Agarwal RK, et al. Concentrations of substance P and prostaglandin E2 in synovial fluid of normal and abnormal joints of horses. *Am J Vet Res*. 2000;61:714.
16. Taylor D, Hood DM, Wagner IP. Short-term effect of therapeutic shoeing on severity of lameness in horses with chronic laminitis. *Am J Vet Res*. 2002;63:1629.
17. Price J, Catriona S, Welsh EM, et al. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. *Vet Anaesth Analg*. 2003;30:124.
18. Graubner C, Gerber V, Doherr M, et al. Clinical application and reliability of a post abdominal surgery pain assessment scale (PASPAS) in horses. *Vet J*. 2011;188:178.
19. Dalla Costa E, Minero M, Lebelt D, et al. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS One*. 2014;9:e92281.
20. van Loon JP, Jonckheer-Sheehy VSM, Back W, et al. Monitoring equine visceral pain with a composite pain scale score and correlation with survival after emergency gastrointestinal surgery. *Vet J*. 2014;200:109.

21. Sutton GA, Dahan R, Turner D, et al. A behavior based pain scale for horses with acute colic: scale construction. *Vet J*. 2013;196:394.
22. Taylor PM, Pascoe PJ, Mama KR. Diagnosing and treating pain in the horse. Where are we today? *Vet Clin North Am Equine Pract*. 2002;18:1.
23. Woolf CJ. Pain: moving from symptom control toward mechanism-specific pharmacologic management. *Ann Internal Med*. 2004;140:441.
24. Riviere JE, Papich MG. *Veterinary pharmacology and therapeutics*. 9th ed. Somerset: John Wiley & Sons; 2013.
25. Cunningham FM. *Comparative and veterinary pharmacology*. 1st ed. Heidelberg: Springer; 2010.
26. Papich MG. *Saunders handbook of veterinary drugs: small and large animal*. 3rd ed. Philadelphia: Elsevier/Saunders; 2011.
27. Owens JG, Kamerling SG, Stanton SR, et al. Effects of pretreatment with ketoprofen and phenylbutazone on experimentally induced synovitis in horses. *Am J Vet Res*. 1996;57:866.
28. Soma LR, Uboh CE, Maylin GM. The use of phenylbutazone in the horse. *J Vet Pharmacol Ther*. 2012;35:1.
29. Hu HH, MacAllister CG, Payton ME, et al. Evaluation of the analgesic effects of phenylbutazone administered at a high or low dosage in horses with chronic lameness. *J Am Vet Med Assoc*. 2005;226:414.
30. Soma LR, Uboh CE, Liu Y, et al. Pharmacokinetics of dexamethasone following intra-articular, intravenous, intramuscular, and oral administration in horses and its effects on endogenous hydrocortisone. *J Vet Pharmacol Ther*. 2013;36:181.
31. de Grauw JC, Visser-Meijer MC, Lashley F, et al. Intra-articular treatment with triamcinolone compared with triamcinolone with hyaluronate: a randomised open-label multicentre clinical trial in 80 lame horses. *Equine Vet J*. 2016;48:152.
32. Levine DG, Epstein KL, Neelis DA, et al. Effect of topical application of 1% diclofenac sodium liposomal cream on inflammation in healthy horses undergoing intravenous regional limb perfusion with amikacin sulfate. *Am J Vet Res*. 2009;70:1323.
33. Goodrich LR, Nixon AJ. Medical treatment of osteoarthritis in the horse–a review. *Vet J*. 2006;171:51.
34. Lees P, May SA, McKellar QA. Pharmacology and therapeutics of non-steroidal anti-inflammatory drugs in the dog and cat: 1. General pharmacology. *J Small Anim Pract*. 1991;32:183.
35. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. *J Pain Res*. 2015;8:105.
36. Lees P, Landoni MF, Giraudel J, et al. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *J Vet Pharmacol Ther*. 2004;27:479.
37. DeMaria AN, Weir MR. Coxibs—beyond the GI tract: renal and cardiovascular issues. *J Pain Symptom Manage Suppl*. 2003;25:41.
38. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol Rev*. 2004;56:387.
39. KuKanich B, Bidgood T, Knesl O. Clinical pharmacology of nonsteroidal anti-inflammatory drugs in dogs. *Vet Anaesth Analg*. 2012;39:69.
40. Kis B, Snipes JA, Busija DW. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. *J Pharmacol Exp Ther*. 2005;315:1.
41. Botting R, Ayoub SS. COX-3 and the mechanism of action of paracetamol/acetaminophen. *Prostaglandins Leukot Essent Fatty Acids*. 2005;72:85.
42. Davies NM, Good RL, Roupe KA, et al. Cyclooxygenase-3: axiom, dogma, anomaly, enigma or splice error? Not as easy as 1, 2, 3. *J Pharm Pharm Sci*. 2004;7:217.
43. Radi ZA. Pathophysiology of cyclooxygenase inhibition in animal models. *Toxicol Pathol*. 2009;37:34.
44. Marshall JF, Blikslager AT. The effect of nonsteroidal anti-inflammatory drugs on the equine intestine. *Equine Vet J Suppl*. 2011;39:140.
45. McConnico RS, Morgan TW, Williams CC, et al. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *Am J Vet Res*. 2008;69:1496.
46. MacAllister CG, Morgan SJ, Borne AT, et al. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc*. 1993;202:71.
47. Read WK. Renal medullary crest necrosis associated with phenylbutazone therapy in horses. *Vet Pathol*. 1983;20:662.
48. Gunson DE, Soma LR. Renal papillary necrosis in horses after phenylbutazone and water deprivation. *Vet Pathol*. 1983;20:603.
49. Keegan KG, Messer NT, Reed SK, et al. Effectiveness of administration of phenylbutazone alone or concurrent administration of phenylbutazone and flunixin meglumine to alleviate lameness in horses. *Am J Vet Res*. 2008;69:167.
50. Boston SE, Moens NMM, Kruth SA, et al. Endoscopic evaluation of the gastroduodenal mucosa to determine the safety of short-term concurrent administration of meloxicam and dexamethasone in healthy dogs. *Am J Vet Res*. 2003;64:1369.
51. Kivett L, Taintor J, Wright J. Evaluation of the safety of a combination of oral administration of phenylbutazone and firocoxib in horses. *J Vet Pharmacol Ther*. 2014;37:413.
52. Reed SK, Messer NT, Tessman RK, et al. Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. *Am J Vet Res*. 2006;67:398.
53. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91.
54. Orsini JA, Ryan WG, Carithers DS, et al. Evaluation of oral administration of firocoxib for the management of musculoskeletal pain and lameness associated with osteoarthritis in horses. *Am J Vet Res*. 2012;73:664.
55. D'Arcy-Moskwa E, Noble G, Weston L, et al. Effects of meloxicam and phenylbutazone on equine gastric mucosal permeability. *J Vet Intern Med*. 2012;26:1494.
56. Raidal SL, Edwards S, Pippia J, et al. Pharmacokinetics and safety of oral administration of meloxicam to foals. *J Vet Intern Med*. 2013;27:300.
57. Holland B, Fogle C, Blikslager AT, et al. Pharmacokinetics and pharmacodynamics of three formulations of firocoxib in healthy horses. *J Vet Pharmacol Ther*. 2015;38:249.
58. Combie J, Shults T, Nugent EC, et al. Pharmacology of narcotic analgesics in the horse: selective blockade of narcotic-induced locomotor activity. *Am J Vet Res*. 1981;42:716.
59. Combie J, Blake JW, Ramey BE, et al. Pharmacology of narcotic analgesics in the horse: quantitative detection of morphine in equine blood and urine and logit-log transformations of this data. *Am J Vet Res*. 1981;42:1523.
60. Combie J, Nugent TE, Tobin T. Pharmacokinetics and protein binding of morphine in horses. *Am J Vet Res*. 1983;44:870.
61. Kalpravidh M, Lumb WV, Wright M, et al. Effects of butorphanol, flunixin, levorphanol, morphine, and xylazine in ponies. *Am J Vet Res*. 1984;45:217.
62. Muir WW, Robertson JT. Visceral analgesia: effects of xylazine, butorphanol, meperidine, and pentazocine in horses. *Am J Vet Res*. 1985;46:2081.
63. Sellon DC, Roberts MC, Blikslager AT, et al. Effects of continuous rate intravenous infusion of butorphanol on physiologic and outcome variables in horses after celiotomy. *J Vet Intern Med*. 2004;18:555.
64. Robertson JT, Muir WW. A new analgesic drug combination in the horse. *Am J Vet Res*. 1983;44:1667.
65. Muir WW, Skarda RT, Sheehan W. Hemodynamic and respiratory effects of xylazine-morphine sulfate in horses. *Am J Vet Res*. 1979;40:1417.

66. Mama KR, Pascoe PJ, Steffey EP. Evaluation of the interaction of mu and kappa opioid agonists on locomotor behavior in the horse. *Can J Vet Res*. 1993;57:106.
67. Nugent TE, Combie JD, Weld JM, et al. Effects of enkephalins versus opiates on locomotor activity of the horse. *Res Commun Chem Pathol Pharmacol*. 1982;35:405.
68. Hellyer PW, Bai L, Supon J, et al. Comparison of opioid and alpha-2 adrenergic receptor binding in horse and dog brain using radioligand autoradiography. *Vet Anaesth Analg*. 2003;30:172.
69. Andersen MS, Clark L, Dyson SJ, et al. Risk factors for colic in horses after general anaesthesia for MRI or nonabdominal surgery: absence of evidence of effect from perianaesthetic morphine. *Equine Vet J*. 2006;38:368.
70. Taylor PM. Effect of postoperative pethidine on the anaesthetic recovery period in the horse. *Equine Vet J*. 1986;18:70.
71. Clark L, Clutton RE, Blissitt KJ, et al. The effects of morphine on the recovery of horses from halothane anaesthesia. *Vet Anaesth Analg*. 2005;35:22.
72. Wetmore L, Pascoe P, Shilo-Benjamini Y, et al. Effects of fentanyl administration on locomotor response in horses with the G57C μ-opioid receptor polymorphism. *Am J Vet Res*. 2016;77:828.
73. Boscan P, Van Hoogmoed LM, Farver TB, et al. Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. *Am J Vet Res*. 2006;67:992.
74. Sojka JE, Adams SB, Lamar CH, et al. Effect of butorphanol, pentazocine, meperidine, or metoclopramide on intestinal motility in female ponies. *Am J Vet Res*. 1988;49(4):527.
75. Senior JM, Pinchbeck GL, Dugdale AHA, et al. Retrospective study of the risk factors and prevalence of colic in horses after orthopaedic surgery. *Vet Rec*. 2004;155:321.
76. Nelson BB, Lordan EE, Hassel DM. Risk factors associated with gastrointestinal dysfunction in horses undergoing elective procedures under general anaesthesia. *Equine Vet J Suppl*. 2013;45:8.
77. Mircica E, Clutton RE, Kyles KW, et al. Problems associated with perioperative morphine in horses: a retrospective case analysis. *Vet Anaesth Analg*. 2003;30:147.
78. Koenig J, Cote N. Equine gastrointestinal motility—ileus and pharmacological modification. *Can Vet J*. 2006;47:551.
79. Sano H, Martin-Flores M, Santos LCP, et al. Effects of epidural morphine on gastrointestinal transit in unmedicated horses. *Vet Anaesth Analg*. 2011;38:121.
80. Sanchez L, Robertson S, Maxwell L, et al. Effect of fentanyl on visceral and somatic nociception in conscious horses. *J Vet Intern Med*. 2007;21:1067.
81. Ohta M, Wakuno A, Okada J, et al. Effects of intravenous fentanyl administration on end-tidal sevoflurane concentrations in thoroughbred racehorses undergoing orthopedic surgery. *J Vet Med Sci*. 2010;72:1107.
82. Knych HK, Steffey EP, Casbeer HC, et al. Disposition, behavioural and physiological effects of escalating doses of intravenously administered fentanyl to young foals. *Equine Vet J*. 2015;47:592.
83. Messenger KM, Davis JL, LaFevers DH, et al. Intravenous and sublingual buprenorphine in horses: pharmacokinetics and influence of sampling site. *Vet Anaesth Analg*. 2011;38:374.
84. Davis JL, Messenger KM, LaFevers DH, et al. Pharmacokinetics of intravenous and intramuscular buprenorphine in the horse. *J Vet Pharmacol Ther*. 2012;35:52.
85. Potter JJ, MacFarlane PD, Love EJ, et al. Preliminary investigation comparing a detomidine continuous rate infusion combined with either morphine or buprenorphine for standing sedation in horses. *Vet Anaesth Analg*. 2016;43:189.
86. Risberg ÅI, Spadavecchia C, Ranheim B, et al. Antinociceptive effect of buprenorphine and evaluation of the nociceptive withdrawal reflex in foals. *Vet Anaesth Analg*. 2015;42:329.
87. Santos LCP, De Moraes AN, Saito ME. Effects of intraarticular ropivacaine and morphine on lipopolysaccharide-induced synovitis in horses. *Vet Anaesth Analg*. 2009;36:280.
88. Lindegaard C, Thomsen MH, Larsen S, et al. Analgesic efficacy of intra-articular morphine in experimentally induced radiocarpal synovitis in horses. *Vet Anaesth Analg*. 2010;37:171.
89. van Loon JP, De Grauw JC, van Dierendonck M, et al. Intra-articular opioid analgesia is effective in reducing pain and inflammation in an equine LPS induced synovitis model. *Equine Vet J*. 2010;42:412.
90. Lindegaard C, Frost AB, Thomsen MH, et al. Pharmacokinetics of intra-articular morphine in horses with lipopolysaccharide-induced synovitis. *Vet Anaesth Analg*. 2010;37:186.
91. Martin-Flores M, Campoy L, Kinsley MA, et al. Analgesic and gastrointestinal effects of epidural morphine in horses after laparoscopic cryptorchidectomy under general anesthesia. *Vet Anaesth Analg*. 2014;41:430.
92. Van Hoogmoed LM, Galuppo LD. Laparoscopic ovariectomy using the endo-GIA stapling device and endo-catch pouches and evaluation of analgesic efficacy of epidural morphine sulfate in 10 mares. *Vet Surg*. 2005;34:646.
93. Goodrich LR, Nixon AJ, Fubini SL, et al. Epidural morphine and detomidine decreases postoperative hindlimb lameness in horses after bilateral stifle arthroscopy. *Vet Surg*. 2002;31:232.
94. England GCW, Clarke KW. Alpha2 adrenoceptor agonists in the horse: a review. *Br Vet J*. 1996;152:641.
95. Jochle W, Moore JN, Brown J, et al. Comparison of detomidine, butorphanol, flunixin meglumine and xylazine in clinical cases of equine colic. *Equine Vet J Suppl*. 1989;7:111.
96. Lowe JE, Hilfiger J. Analgesic and sedative effects of detomidine compared to xylazine in a colic model using IV and IM routes of administration. *Acta Vet Scand*. 1986;82:85.
97. Lowe JE, Hilfiger J. Analgesic and sedative effects of detomidine in a colic model: blind studies on efficacy and duration of effects. *Proc Annu Conv Am Equin*. 1984;30:225.
98. Muir WW, Skarda RT, Sheehan W. Hemodynamic and respiratory effects of a xylazine-acetylpromazine drug combination in horses. *Am J Vet Res*. 1979;40:1518.
99. Wagner AE, Muir WW, Hinchcliff KW. Cardiovascular effects of xylazine and detomidine in horses. *Am J Vet Res*. 1991;52:651.
100. Still J, Serteyn D, Van der Merwe CA. Cardiovascular and respiratory effects of detomidine in isoflurane-anaesthetised horses. *J S Afr Vet Assoc*. 1996;67:199.
101. Bettschart-Wolfensberger R, Freeman SL, Bowen I, et al. Cardiopulmonary effects and pharmacokinetics of i.v. dexmedetomidine in ponies. *Equine Vet J*. 2005;37:60.
102. Kamerling SG, Cravens WMT, Bagwell CA. Dose-related effects of detomidine on autonomic responses in the horse. *J Auton Pharmacol*. 1988;8:241.
103. Pypendop BH, Verstegen JP. Hemodynamic effects of medetomidine in the dog: a dose titration study. *Vet Surg*. 1998;27:612.
104. Lester GD, Merritt AM, Neuwirty L, et al. Effect of α2-adrenergic, cholinergic, and nonsteroidal anti-inflammatory drugs on myoelectric activity of ileum, cecum, and right ventral colon and on cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res*. 1998;58:320.
105. Merritt AM, Burrows JA, Hartless CS. Effect of xylazine, detomidine, and a combination of xylazine and butorphanol on equine duodenal motility. *Am J Vet Res*. 1998;59:619.
106. Sutton DGM, Preston T, Christley RM, et al. The effects of xylazine, detomidine, acepromazine and butorphanol on equine solid phase gastric emptying rate. *Equine Vet J*. 2002;34:486.
107. Nuñez E, Steffey EP, Ocampo L, et al. Effects of alpha2-adrenergic receptor agonists on urine production in horses deprived of food and water. *Am J Vet Res*. 2004;65:1342.
108. Valdes-Vazquez MA, Aguilera-Tejero E, Mayer-Valor R. Effect of xylazine during endoscopic evaluation of functional upper respiratory disorders in horses. *J Equine Vet Sci*. 1993;13:84.
109. Ducharme NG, Hackett RP, Fubini SL, et al. The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses. Part II. Influence of side of examination, reexamination, and sedation. *Vet Surg*. 1991;20:180.

110. Ringer SK, Schwarzwald CC, Portier KG, et al. Effects on cardiopulmonary function and oxygen delivery of doses of romifidine and xylazine followed by constant rate infusions in standing horses. *Vet J*. 2013;195:228.
111. Grubb TL, Muir WW, Bertone AL, et al. Use of yohimbine to reverse prolonged effects of xylazine hydrochloride in a horse being treated with chloramphenicol. *J Am Vet Med Assoc*. 1997;210:1771.
112. Di Salvo A, Della Rocca G, Bazzica C, et al. A pharmacokinetic/clinical approach to postulate a local action of intra-articular xylazine administration in the horse: a preliminary study. *J Vet Pharmacol Ther*. 2014;37:464.
113. Ullmer J, Mama KR, Lee C, et al. *The effects of xylazine on normal and interleukin-1 conditioned equine articular cartilage explants*. CA: Proc 38th Am College Vet Anesth San Diego; 2013.
114. Mama KR, Ullmer J, King M, et al. *The effects of xylazine, dexmedetomidine and clonidine on normal and interleukin-1 conditioned equine cartilage explants*. Japan: Proc 12th World Congress of Vet Anaesth Kyoto; 2015.
115. Abdallah FW, Brull R. Facilitatory effects of perineural dexmedetomidine on neuraxial and peripheral nerve block: a systematic review and meta-analysis. *Br J Anaesth*. 2013;110(915):2013.
116. Skarda RT, Muir WW. Comparison of antinociceptive, cardiovascular, and respiratory effects, head ptosis, and position of pelvic limbs in mares after caudal epidural administration of xylazine and detomidine hydrochloride solution. *Am J Vet Res*. 1996;57:1338.
117. Skarda RT, Muir WW. Caudal analgesia induced by epidural or subarachnoid administration of detomidine hydrochloride solution in mares. *Am J Vet Res*. 1994;55:670.
118. Hubbell JAE, Muir WW. Antagonism of detomidine sedation in the horse using intravenous tolazoline or atipamezole. *Equine Vet J*. 2006;38:238.
119. Carroll GL, Matthews NS, Hartsfield SM, et al. The effect of detomidine and its antagonism with tolazoline on stress-related hormones, metabolites, physiologic responses, and behavior in awake ponies. *Vet Surg*. 1997;26:69.
120. Pakkanen SAE, Raekallio MR, Mykkänen AK, et al. Detomidine and the combination of detomidine and MK-467, a peripheral alpha-2 adrenoceptor antagonist, as premedication in horses anaesthetized with isoflurane. *Vet Anaesth Analg*. 2015;42:527.
121. Vainionpää MH, Raekallio MR, Pakkanen SA, et al. Plasma drug concentrations and clinical effects of a peripheral alpha-2-adrenoceptor antagonist, MK-467, in horses sedated with detomidine. *Vet Anaesth Analg*. 2013;40:257.
122. Doherty TJ, Frazier DL. Effect of intravenous lidocaine on halothane minimum alveolar concentration in ponies. *Equine Vet J*. 1998;30:300.
123. Robertson SA, Sanchez LC, Merritt AM, et al. Effect of systemic lidocaine on visceral and somatic nociception in conscious horses. *Equine Vet J*. 2005;37:122.
124. Harkins JD, Mundy GD, Woods WE, et al. Lidocaine in the horse: its pharmacological effects and their relationship to analytical findings. *J Vet Pharmacol Ther*. 1998;21:462.
125. Harkins JD, Stanley S, Mundy GD, et al. A review of the pharmacology, pharmacokinetics, and regulatory control in the US of local anaesthetics in the horse. *J Vet Pharmacol Ther*. 1995;18:397.
126. Brianceau P, Chevalier H, Karas A, et al. Intravenous lidocaine and small-intestinal size, abdominal fluid, and outcome after colic surgery in horses. *J Vet Intern Med*. 2002;16:736.
127. Guschlbauer M, Feige K, Geburek F, et al. Effects of in vivo lidocaine administration at the time of ischemia and reperfusion on in vitro contractility of equine jejunal smooth muscle. *Am J Vet Res*. 2011;72:1449.
128. Rezende ML, Wagner AE, Mama KR, et al. Effects of intravenous administration of lidocaine on the minimum alveolar concentration of sevoflurane in horses. *Am J Vet Res*. 2011;72:446.
129. Wagner AE, Mama KR, Steffey EP, et al. Comparison of the cardiovascular effects of equipotent anesthetic doses of sevoflurane alone and sevoflurane plus an intravenous infusion of lidocaine in horses. *Am J Vet Res*. 2011;72:452.
130. Valverde A, Gunkel C, Doherty TJ, et al. Effect of a constant rate infusion of lidocaine on the quality of recovery from sevoflurane or isoflurane general anaesthesia in horses. *Equine Vet J*. 2005;37:559.
131. Schuhbeck MM, Kuhn M, Spadavecchia C, et al. Continuous intravenous lidocaine infusion during isoflurane anaesthesia in horses undergoing surgical procedures. *Pferdeheilkunde*. 2012;28:252.
132. Meyer GA, Lin HC, Hanson RR, et al. Effects of intravenous lidocaine overdose on cardiac electrical activity and blood pressure in the horse. *Equine Vet J*. 2001;33:431.
133. Olbrich VH, Mosing M. A comparison of the analgesic effects of caudal epidural methadone and lidocaine in the horse. *Vet Anaesth Analg*. 2003;30:156.
134. Rapley JH, Beavis RC, Barber FA, et al. Glenohumeral chondrolysis after shoulder arthroscopy associated with continuous bupivacaine infusion. *Arthroscopy*. 2009;25:1367.
135. Park J, Sutradhar BC, Hong G, et al. Comparison of the cytotoxic effects of bupivacaine, lidocaine, and mepivacaine in equine articular chondrocytes. *Vet Anaesth Analg*. 2011;38:127.
136. Breu A, Rosenmeier K, Kujat R, et al. The cytotoxicity of bupivacaine, ropivacaine, and mepivacaine on human chondrocytes and cartilage. *Anesth Analg*. 2013;117:514.
137. Piat P, Richard H, Beauchamp G, et al. In vivo effects of a single intra-articular injection of 2% lidocaine or 0.5% bupivacaine on articular cartilage of normal horses. *Vet Surg*. 2012;41:1002.
138. Mion G, Villevieille T. Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). *CNS Neurosci Ther*. 2013;19:370.
139. De Kock MF, Lavand'homme PM. The clinical role of NMDA receptor antagonists for the treatment of postoperative pain. *Best Pract Res Clin Anaesthesiol*. 2007;21:85.
140. Muir WW, Skarda RT, Milne DW. Evaluation of xylazine and ketamine hydrochloride for anesthesia in horses. *Am J Vet Res*. 1977;38:195.
141. Muir WW, Sams R. Effects of ketamine infusion on halothane minimal alveolar concentration in horses. *Am J Vet Res*. 1802;53:1992.
142. Lin HC, Passler T, Wilborn RR, et al. A review of the general pharmacology of ketamine and its clinical use for injectable anaesthesia in horses. *Equine Vet Educ*. 2015;27:146.
143. Takki S, Nikki P, Jäättelä A, et al. Ketamine and plasma catecholamines. *Br J Anaesth*. 1972;44:1318.
144. López-Sanromán FJ, Cruz JM, Santos M, et al. Evaluation of the local analgesic effect of ketamine in the palmar digital nerve block at the base of the proximal sesamoid (abaxial sesamoid block) in horses. *Am J Vet Res*. 2003;64:475.
145. Gómez de Segura IA, De Rossi R, Santos M, et al. Epidural injection of ketamine for perineal analgesia in the horse. *Vet Surg*. 1998;27:384.
146. Braun S, Gaza N, Werdehausen R, et al. Ketamine induces apoptosis via the mitochondrial pathway in human lymphocytes and neuronal cells. *Br J Anaesth*. 2010;105:347.
147. Taylor PM, Bennett RC, Brearley JC, et al. Comparison of detomidine and romifidine as premedicants before ketamine and halothane anesthesia in horses undergoing elective surgery. *Am J Vet Res*. 2001;62:359.
148. Wagner AE, Walton JA, Hellyer P, et al. Use of low doses of ketamine administered by constant rate infusion as an adjunct for postoperative analgesia in dogs. *J Am Vet Med Assoc*. 2002;221:72.
149. Carstensen M, Møller AM. Adding ketamine to morphine for intravenous patient-controlled analgesia for acute postoperative pain: a qualitative review of randomized trials. *Br J Anaesth*. 2010;104:401.
150. Fielding CL, Brumbaugh GW, Matthews NS, et al. Pharmacokinetics and clinical effects of a subanesthetic continuous rate infusion of ketamine in awake horses. *Am J Vet Res*. 2006;67:1484.

151. Guedes AGP, Matthews NS, Hood DM. Effect of ketamine hydrochloride on the analgesic effects of tramadol hydrochloride in horses with signs of chronic laminitis-associated pain. *Am J Vet Res*. 2012;73:610.
152. Peterbauer C, Larenza PM, Knobloch M, et al. Effects of a low dose infusion of racemic and S-ketamine on the nociceptive withdrawal reflex in standing ponies. *Vet Anaesth Analg*. 2008;35:414.
153. Wagner AE, Mama KR, Contino EK, et al. Evaluation of sedation and analgesia in standing horses after administration of xylazine, butorphanol, and subanesthetic doses of ketamine. *J Am Vet Med Assoc*. 2011;238:1629.
154. Elfenbein JR, Robertson SA, Corser AA, et al. Systemic effects of a prolonged continuous infusion of ketamine in healthy horses. *J Vet Intern Med*. 2011;25:1134.
155. Tramer MR, Schneider J, Marti RA, et al. Role of magnesium sulfate in postoperative analgesia. *Anesthesiology*. 1997;84:340.
156. Stomatology FM, Yan Q. Effects of systemic magnesium on post-operative analgesia: is the current evidence strong enough? *Pain Physician*. 2015;18:405.
157. Albrecht E, Kirkham KR, Liu SS, et al. Peri-operative intravenous administration of magnesium sulphate and postoperative pain: a meta-analysis. *Anaesthesia*. 2013;68:79.
158. McCarthy RJ, Kroin JS, Tuman KJ, et al. Antinociceptive potentiation and attenuation of tolerance by intrathecal co-infusion of magnesium sulfate and morphine in rats. *Anesth Analg*. 1998;86(830):1998.
159. Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol*. 2015;14:162.
160. Terry RL, McDonnell SM, Van Eps AW, et al. Pharmacokinetic profile and behavioral effects of gabapentin in the horse. *J Vet Pharmacol Ther*. 2010;33:485.
161. Caldwell FJ, Taintor J, Waguespack RW, et al. Effect of PO administered gabapentin on chronic lameness in horses. *J Equine Vet Sci*. 2015;35:536.
162. Davis JL, Posner LP, Elce Y. Gabapentin for the treatment of neuropathic pain in a pregnant horse. *J Am Vet Med Assoc*. 2007;231:755.
163. Dworkin RH, O'Connor AB, Backonja M, et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain*. 2007;132:237.
164. Mullen KR, Schwark W, Divers TJ. Pharmacokinetics of single-dose intragastric and intravenous pregabalin administration in clinically normal horses. *Am J Vet Res*. 2013;74:1043.
165. Grond S, Sablotzki A. Clinical pharmacology of tramadol. *Clin Pharmacokinet*. 2004;43:879.
166. Vettorato E, Zonca A, Cagnardi P, et al. Pharmacokinetics and efficacy of intravenous and extradural tramadol in dogs. *Vet J*. 2010;183:310.
167. Knych HK, Corado CR, McKemie DS, et al. Pharmacokinetics and selected pharmacodynamic effects of tramadol following intravenous administration to the horse. *Equine Vet J*. 2013;45:490.
168. Stewart AJ, Boothe DM, Cruz-Espindola C, et al. Pharmacokinetics of tramadol and metabolites *O*-desmethyltramadol and *N*-desmethyltramadol in adult horses. *Am J Vet Res*. 2011;72:967.
169. Shilo Y, Britzi M, Eytan B, et al. Pharmacokinetics of tramadol in horses after intravenous, intramuscular and oral administration. *J Vet Pharmacol Ther*. 2008;31:60.
170. Giorgi M, Soldani G, Manera C, et al. Pharmacokinetics of tramadol and its metabolites M1, M2 and M5 in horses following intravenous, immediate release (fasted/fed) and sustained release single dose administration. *J Equine Vet Sci*. 2007;27:481.
171. Dhanjal JK, Wilson DV, Robinson E, et al. Intravenous tramadol: effects, nociceptive properties, and pharmacokinetics in horses. *Vet Anaesth Analg*. 2009;36:581.
172. Carregaro AB, Freitas GC, Ribeiro MH, et al. Physiological and analgesic effects of continuous-rate infusion of morphine, butorphanol, tramadol or methadone in horses with lipopolysaccharide (LPS)-induced carpal synovitis. *BMC Vet Res*. 2014;10:1.
173. Milaré AS, De Oliveira FA, Luna SPL, et al. Intravenous tramadol injection has no antinociceptive effect in horses undergoing electrical and thermal stimuli. *J Equine Vet Sci*. 2013;33:823.
174. Guedes A, Knych H, Hood D. Plasma concentrations, analgesic and physiological assessments in horses with chronic laminitis treated with two doses of oral tramadol. *Equine Vet J*. 2016;48:528.
175. Cagnardi P, Ferraresi C, Zonca A, et al. Clinical pharmacokinetics of tramadol and main metabolites in horses undergoing orchiectomy. *Vet Q*. 2014;34:143.
176. Guedes A, Galuppo L, Hood D, et al. Soluble epoxide hydrolase activity and pharmacologic inhibition in horses with chronic severe laminitis. *Equine Vet J*. 2016.
177. Guedes AG, Morrisseau C, Sole A, et al. Use of a soluble epoxide hydrolase inhibitor as an adjunctive analgesic in a horse with laminitis. *Vet Anaesth Analg*. 2013;40:440.
178. Love EJ, Taylor PM, Murrell J, et al. Effects of acepromazine, butorphanol and buprenorphine on thermal and mechanical nociceptive thresholds in horses. *Equine Vet J*. 2012;44:221.
179. Sanchez LC, Elfenbein JR, Robertson SA. Effect of acepromazine, butorphanol, or *N*-butylscopolammonium bromide on visceral and somatic nociception and duodenal motility in conscious horses. *Am J Vet Res*. 2008;69:579.
180. Roelvink ME, Goossens L, Kalsbeek HC, et al. Analgesic and spasmolytic effects of dipyrone, hyoscine-*N*-butylbromide and a combination of the two in ponies. *Vet Rec*. 1991;129:378.
181. Boatwright CE, Fubini SL, Grohn YT, et al. A comparison of *N*-butylscopolammonium bromide and butorphanol tartrate for analgesia using a balloon model of abdominal pain in ponies. *Can J Vet Res*. 1996;60:65.
182. Rumpler MJ, Colahan P, Sams RA. The pharmacokinetics of methocarbamol and guaifenesin after single intravenous and multiple-dose oral administration of methocarbamol in the horse. *J Vet Pharmacol Ther*. 2014;37:25.
183. Cremers S, Papapoulos S. Pharmacology of bisphosphonates. *Bone*. 2011;49:42.
184. Catterall JB, Cawston TE. Drugs in development: bisphosphonates and metalloproteinase inhibitors. *Arthritis Res Ther*. 2003;5:12.
185. Kamm L, McIlwraith W, Kawcak C. A review of the efficacy of tiludronate in the horse. *J Equine Vet Sci*. 2008;28:209.
186. Gough MR, Thibaud D, Smith RKW. Tiludronate infusion in the treatment of bone spavin: a double blind placebo-controlled trial. *Equine Vet J*. 2010;42:381.
187. Coudry V, Thibaud D, Riccio B, et al. Efficacy of tiludronate in the treatment of horses with signs of pain associated with osteoarthritic lesions of the thoracolumbar vertebral column. *Am J Vet Res*. 2007;68:329.
188. Hunter BG, Duesterdieck-Zellmer KF, Larson MK. Tiludronate concentrations and cytologic findings in synovial fluid after intravenous regional limb perfusion with tiludronate in horses. *Peer J*. 2015;3:e889.
189. Body JJ, Pfister T, Bauss F, et al. Preclinical perspectives on bisphosphonate renal safety. *Oncologist*. 2005;10(suppl 1):3.
190. Meijer H, Reinecke J, Becker C, et al. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm Res*. 2003;52:404.
191. Frisbie DD, Kawcak CE, Werpy NM, et al. Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis. *Am J Vet Res*. 2007;68:290.
192. Martin CA, Kerr CL, Pearce SG, et al. Outcome of epidural catheterization for delivery of analgesics in horses: 43 cases (1998–2001). *J Am Vet Med Assoc*. 2003;222:1394.

193. Sysel AM, Pleasant RS, Jacobson JD, et al. Systemic and local effects associated with long-term epidural catheterization and morphine-detomidine administration in horses. *Vet Surg*. 1997;26:141.
194. Zarucco L, Driessen B, Scandella M, et al. Continuous perineural block of the palmar nerves: a new technique for pain relief in the distal equine forelimb. *Clin Tech Equine Pract*. 2007;6:154.
195. Chelly JE, Greger J, Casati A, et al. Continuous lateral sciatic blocks for acute postoperative pain management after major ankle and foot surgery. *Foot Ankle*. 2002;23:749.
196. Ganesh A, Rose JB, Wells T, et al. Continuous peripheral nerve blockade for inpatient and outpatient postoperative analgesia in children. *Anesth Analg*. 2007;105:1234.
197. Boezaart AP. Perineural infusion of local anesthetics. *Anesthesiology*. 2006;104:872.
198. Driessen B, Scandella M, Zarucco L. Development of a technique for continuous perineural blockade of the palmar nerves in the distal equine thoracic limb. *Vet Anaesth Analg*. 2008;35:432.
199. Frisbie DD, McIlwraith CW, Kawcak CE, et al. Evaluation of topically administered diclofenac liposomal cream for treatment of horses with experimentally induced osteoarthritis. *Am J Vet Res*. 2009;70:210.
200. Andreeta A, Verde C, Babusci M, et al. Comparison of diclofenac diethylamine permeation across horse skin from five commercial medical human formulations. *J Equine Vet Sci*. 2011;31:502.
201. Thomasy SM, Slovis N, Maxwell LK, et al. Transdermal fentanyl combined with nonsteroidal anti-inflammatory drugs for analgesia in horses. *J Vet Intern Med*. 2004;18:550.
202. Eberspächer E, Stanley SD, Rezende M, et al. Pharmacokinetics and tolerance of transdermal fentanyl administration in foals. *Vet Anaesth Analg*. 2008;35:249.
203. Orsini JA, Moate PJ, Kuersten K, et al. Pharmacokinetics of fentanyl delivered transdermally in healthy adult horses–variability among horses and its clinical implications. *J Vet Pharmacol Ther*. 2006;29:539.
204. Mills PC, Cross SE. Regional differences in transdermal penetration of fentanyl through equine skin. *Res Vet Sci*. 2007;82:252.
205. Hubbell JAE, Saville WJA, Bednarski RM, et al. The use of sedatives, analgesic and anaesthetic drugs in the horse: an electronic survey of members of the American Association of Equine Practitioners (AAEP). *Equine Vet J*. 2010;42:487.
206. Vincent CA, Richardson PH. The evaluation of therapeutic acupuncture: concepts and methods. *Pain*. 1986;24:1.
207. Skarda RT, Tejwani GA, Muir WW. Cutaneous analgesia, hemodynamic and respiratory effects, and beta-endorphin concentration in spinal fluid and plasma of horses after acupuncture and electroacupuncture. *Am J Vet Res*. 2002;63:1435.
208. Takeshige C, Oka K, Mizuno T, et al. The acupuncture point and its connecting central pathway for producing acupuncture analgesia. *Brain Res Bull*. 1993;30:53.
209. Tobaldini G, Aisengart B, Lima MMS, et al. Ascending nociceptive control contributes to the antinociceptive effect of acupuncture in a rat model of acute pain. *J Pain*. 2014;15:422.
210. Bossut DFB, Leshin LS, Stromberg MW, et al. Plasma cortisol and beta-endorphin in horses subjected to electro-acupuncture for cutaneous analgesia. *Peptides*. 1983;4:501.
211. Skarda RT, Muir WW. Comparison of electroacupuncture and butorphanol on respiratory and cardiovascular effects and rectal pain threshold after controlled rectal distention in mares. *Am J Vet Res*. 2003;64:137.
212. Steiss JE, White NA, Bowen JM. Electroacupuncture in the treatment of chronic lameness in horses and ponies: a controlled clinical trial. *Can J Vet Res*. 1989;53:239.
213. Robinson KA, Manning ST. Efficacy of a single-formula acupuncture treatment for horses with palmar heel pain. *Can Vet J*. 2015;56:1257.
214. Waguespack RW, Burba DJ, Hubert JD, et al. Effects of extracorporeal shock wave therapy on desmitis of the accessory ligament of the deep digital flexor tendon in the horse. *Vet Surg*. 2011;40:450.
215. Imboden I, Waldern NM, Wiestner T, et al. Short term analgesic effect of extracorporeal shock wave therapy in horses with proximal palmar metacarpal/plantar metatarsal pain. *Vet J*. 2009;179:50.
216. McClure SR, Sonea IM, Evans RB, et al. Evaluation of analgesia resulting from extracorporeal shock wave therapy and radial pressure wave therapy in the limbs of horses and sheep. *Am J Vet Res*. 2005;66:1702.
217. Dahlberg JA, McClure SR, Evans RB, et al. Force platform evaluation of lameness severity following extracorporeal shock wave therapy in horses with unilateral forelimb lameness. *J Am Vet Med Assoc*. 2006;229:100.
218. Brown KE, Nickels FA, Caron JP, et al. Investigation of the immediate analgesic effects of extracorporeal shock wave therapy for treatment of navicular disease in horses. *Vet Surg*. 2005;34:554.
219. Gomez Alvarez CBG, L'ami JJ, Moffat D, et al. Effect of chiropractic manipulations on the kinematics of back and limbs in horses with clinically diagnosed back problems. *Equine Vet J*. 2008;40:153.
220. Landman M, De Blaauw JA, Van Weeren PR, et al. Field study of the prevalence of lameness in horses with back problems. *Vet Rec*. 2004;155:165.
221. Langstone J, Ellis J, Cunliffe C. A preliminary study of the effect of manual chiropractic treatment on the splenius muscle in horses when measured by surface electromyography. *Equine Vet J*. 2015;47(18):2015.
222. Sullivan KA, Hill AE, Haussler KK. The effects of chiropractic, massage and phenylbutazone on spinal mechanical nociceptive thresholds in horses without clinical signs. *Equine Vet J*. 2008;40:14.
223. Klide AM. Treatment of chronic back pain in horses stimulation of acupuncture points with a low powered infrared laser. *Vet Surg*. 1987;16:106.
224. Hagiwara S, Iwasaka H, Okuda K, et al. GaAlAs (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. *Lasers Surg Med*. 2007;39:797.
225. Ginani F, Soares DM, Barboza CAG. Effect of low-level laser therapy on mesenchymal stem cell proliferation: a systematic review. *Lasers Med Sci*. 2015;30:2189.
226. Smoot B, Chiavola-Larson L, Lee J, et al. Effect of low-level laser therapy on pain and swelling in women with breast cancer-related lymphedema: a systematic review and meta-analysis. *J Cancer Surviv*. 2014;9:287.
227. Bjordal JM, Johnson MI, Iversen V, et al. Low-level laser therapy in acute pain: a systematic review of possible mechanisms of action and clinical effects in randomized placebo-controlled trials. *Photomed Laser Surg*. 2006;24:158.
228. ColdLasers.org: A guide to equine lasers. http://www.coldlasers.org/guide-to-equine-lasers; 2016.
229. U.S. Food and Drug Administration. Laser products and instruments. http://www.fda.gov/radiation-emittingproducts/radiationemittingproductsandprocedures/homebusinessandentertainment/laserproductsandinstruments/default.htm; 2016.
230. Lee CD, Maxwell LK. Effect of body weight on the pharmacokinetics of flunixin meglumine in miniature horses and quarter horses. *J Vet Pharmacol Ther*. 2014;37:35.
231. Landoni MF, Lees P. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse. *J Vet Pharmacol Ther*. 1996;19:466.
232. Spadavecchia C, Arendt-Nielsen L, Spadavecchia L, et al. Effects of butorphanol on the withdrawal reflex using threshold, suprathreshold and repeated subthreshold electrical stimuli in conscious horses. *Vet Anaesth Analg*. 2007;34:48.

233. Dias BP, Araújo MA, Deschk M, et al. Effects of a continuous rate infusion of butorphanol in isoflurane-anesthetized horses on cardiorespiratory parameters, recovery quality, gastrointestinal motility and serum cortisol concentrations. *Acta Cir Bras*. 2014;29:801.
234. Chiavaccini L, Claude AK, Lee JH, et al. Pharmacokinetics and pharmacodynamics comparison between subcutaneous and intravenous butorphanol administration in horses. *J Vet Pharmacol Ther*. 2015;38:365.
235. Marly C, Bettschart-Wolfensberger R, Nussbaumer P, et al. Evaluation of a romifidine constant rate infusion protocol with or without butorphanol for dentistry and ophthalmologic procedures in standing horses. *Vet Anaesth Analg*. 2014;41:491.
236. Orsini JA. *Equine emergencies: treatment and procedures*. 4th ed. St. Louis: Elsevier/Saunders; 2014.
237. Cruz FSF, Carregaro AB, Machado M, et al. Sedative and cardiopulmonary effects of buprenorphine and xylazine in horses. *Can J Vet Res*. 2011;75:35.
238. Taylor P, Coumbe K, Henson F, et al. Evaluation of sedation for standing clinical procedures in horses using detomidine combined with buprenorphine. *Vet Anaesth Analg*. 2014;41:14.
239. Fischer BL, Ludders JW, Asakawa M, et al. A comparison of epidural buprenorphine plus detomidine with morphine plus detomidine in horses undergoing bilateral stifle arthroscopy. *Vet Anaesth Analg*. 2009;36:67.
240. Benmansour P, Husulak ML, Bracamonte JL, et al. Cardiopulmonary effects of an infusion of remifentanil or morphine in horses anesthetized with isoflurane and dexmedetomidine. *Vet Anaesth Analg*. 2014;41:346.
241. Gozalo-Marcilla M, Steblaj B, Schauvliege S, et al. Comparison of the influence of two different constant-rate infusions (dexmedetomidine versus morphine) on anaesthetic requirements, cardiopulmonary function and recovery quality in isoflurane anaesthetized horses. *Res Vet Sci*. 2013;95:1186.
242. Thomasy SM, Steffey EP, Mama KR, et al. The effects of iv fentanyl administration on the minimum alveolar concentration of isoflurane in horses. *Br J Anaesth*. 2006;97:232.
243. Benmansour P, Duke-Novakovski T. Prolonged anesthesia using sevoflurane, remifentanil and dexmedetomidine in a horse. *Vet Anaesth Analg*. 2013;40:521.
244. Lamuraglia R, Kirkby P, Funcia JP. Cardiopulmonary effects and recovery quality of remifentanil–isoflurane anesthesia in horses. *J Equine Vet Sci*. 2015;35:271.
245. Nilsfors L, Kvart C, Kallings P, et al. Cardiorespiratory and sedative effects of a combination of acepromazine, xylazine and methadone in the horse. *Equine Vet J*. 1988;20:364.
246. Linardi RL, Stokes AM, Keowen ML, et al. Bioavailability and pharmacokinetics of oral and injectable formulations of methadone after intravenous, oral, and intragastric administration in horses. *Am J Vet Res*. 2012;73:290.
247. DeRossi R, Medeiros U, de Almeida RG, et al. Meperidine prolongs lidocaine caudal epidural anaesthesia in the horse. *Vet J*. 2008;178:294.
248. Foreman JH, Ruemmler R. Efficacy of intramuscular meperidine hydrochloride versus placebo in experimental foot lameness in horses. *Equine Vet J Suppl*. 2013;45:48.
249. Skarda RT, Muir WW. Analgesic, hemodynamic, and respiratory effects induced by caudal epidural administration of meperidine hydrochloride in mares. *Am J Vet Res*. 2001;62:1001.
250. Natalini CC, Linardi RL. Analgesic effects of epidural administration of hydromorphone in horses. *Am J Vet Res*. 2006;67:11.
251. Yamashita K, Muir WW, Tsubakishita S, et al. Clinical comparison of xylazine and medetomidine for premedication of horses. *J Am Vet Med Assoc*. 2002;221:1144.
252. López-Sanromán FJ, Holmbak-Petersen R, Varela M, et al. Accelerometric comparison of the locomotor pattern of horses sedated with xylazine hydrochloride, detomidine hydrochloride, or romifidine hydrochloride. *Am J Vet Res*. 2013;74:828.
253. Neto PIN, Luna SP, Queiroz-Williams P, et al. Cardiorespiratory and antinociceptive effects of two different doses of lidocaine administered to horses during a constant intravenous infusion of xylazine and ketamine. *BMC Vet Res*. 2013;9:1.
254. Moens Y, Lanz F, Doherr MG, et al. A comparison of the antinociceptive effects of xylazine, detomidine and romifidine on experimental pain in horses. *Vet Anaesth Analg*. 2003;30:183.
255. LeBlanc PH, Caron JP, Patterson JS, et al. Epidural injection of xylazine for perineal analgesia in horses. *J Am Vet Med Assoc*. 1988;193:1405.
256. Wilson DV, Bohart GV, Evans AT, et al. Retrospective analysis of detomidine infusion for standing chemical restraint in 51 horses. *Vet Anaesth Analg*. 2002;29:54.
257. da Silva Serpa PB, Natalini CC, Cavalcanti RL, et al. Effects of detomidine constant rate infusion on blood glucose and lactate in sevoflurane anesthetized horses. *Acta Sci Vet*. 2012;40:1051.
258. Serpa PB, Garbade P, Natalini CC, et al. Energy imbalance and physiological changes during detomidine hydrochloride constant rate infusion in standing horses. *J Equine Vet Sci*. 2015;35:232.
259. Ringer SK, Portier K, Torgerson PR, et al. The effects of a loading dose followed by constant rate infusion of xylazine compared with romifidine on sedation, ataxia and response to stimuli in horses. *Vet Anaesth Analg*. 2013;40:157.
260. Devisscher L, Schauvliege S, Dewulf J, et al. Romifidine as a constant rate infusion in isoflurane anaesthetized horses: a clinical study. *Vet Anaesth Analg*. 2010;37:425.
261. Rezende ML, Grimsrud KN, Stanley SD, et al. Pharmacokinetics and pharmacodynamics of intravenous dexmedetomidine in the horse. *J Vet Pharmacol Ther*. 2015;38:15.
262. Marcilla MG, Schauvliege S, Segaert S, et al. Influence of a constant rate infusion of dexmedetomidine on cardiopulmonary function and recovery quality in isoflurane anaesthetized horses. *Vet Anaesth Analg*. 2012;39:49.
263. Creighton CM, Lemke KA, Lamont LA, et al. Comparison of the effects of xylazine bolus versus medetomidine constant rate infusion on cardiopulmonary function and depth of anesthesia in horses anesthetized with isoflurane. *J Am Vet Med Assoc*. 2012;240:991.
264. Kempchen S, Kuhn M, Spadavecchia C, et al. Medetomidine continuous rate intravenous infusion in horses in which surgical anaesthesia is maintained with isoflurane and intravenous infusions of lidocaine and ketamine. *Vet Anaesth Analg*. 2012;39:245.
265. Nannarone S, Cenani A, Gialletti R, et al. Clinical comparison of two regimens of lidocaine infusion in horses undergoing laparotomy for colic. *Vet Anaesth Analg*. 2015;42:150.
266. Peiró JR, Barnabe PA, Cadioli FA, et al. Effects of lidocaine infusion during experimental endotoxemia in horses. *J Vet Intern Med*. 2010;24:940.
267. Skarda RT, Muir WW. Segmental epidural and subarachnoid analgesia in conscious horses: a comparative study. *Am J Vet Res*. 1870;44:1983.
268. Hendrickson DA, Wilson DG. Laparoscopic cryptorchid castration in standing horses. *Vet Surg*. 1997;26:335.
269. Skarda RT, Muir WW. Analgesic, hemodynamic and respiratory effects of caudal epidurally administered ropivacaine hydrochloride in mares. *Vet Anaesth Analg*. 2001;28:61.
270. van Loon JP, Menke ES, Doornenbal A, et al. Antinociceptive effects of low dose lumbosacral epidural ropivacaine in healthy ponies. *Vet J*. 2012;193:240.
271. DeRossi R, Módolo TJ, Pagliosa RC, et al. Comparison of analgesic effects of caudal epidural 0.25% bupivacaine with bupivacaine plus morphine or bupivacaine plus ketamine for analgesia in conscious horses. *J Equine Vet Sci*. 2012;32:190.
272. DeRossi R, Miguel GLS, Frazílio FO, et al. L-Bupivacaine 0.5% vs. racemic 0.5% bupivacaine for caudal epidural analgesia in horses. *J Vet Pharmacol Ther*. 2005;28:293.

CHAPTER 4

Critical Care

Samuel D. Hurcombe*

INTRODUCTION

The development of critical care in equine medicine has truly evolved over the past 20 years to deliver high-level care to the sickest patients, both adult horses and foals. To this end, critical care has developed into a specialty stream of medicine in its own right in which many of the leading hospitals and foundation criticalists have pushed the envelope in advancing care to our equine patients with improved outcomes. The development of the American College of Veterinary Emergency and Critical Care (ACVECC) large-animal group was formed and has somewhat legitimized equine critical care as a specialty in its own right. Both members of ACVECC, many of whom are also specialists in internal medicine or surgery, and other like-minded clinicians around the world, are skilled to run an intensive care unit (ICU) and deliver urgent and immediate care. However, there are still significant challenges and clinical obstacles to overcome, yet this exciting field of study is changing rapidly and will surely continue to do so.

Critical care is provided to an animal whose condition is typically severe, life-threatening, and acute. Early recognition of a disease state or complex condition is needed to execute a diagnostic or therapeutic plan, which oftentimes has a significant impact on the outcome. For example, in human emergency departments, the administration of antimicrobials within 60 to 180 minutes of sepsis diagnosis is significantly associated with improved survival.[1]

Intensive care is a dynamic process. Patient condition can change minute to minute, and oftentimes these patients require fine-tuning of a therapeutic plan over several days. As such, this is time-consuming, detail oriented, tiring, and often physically demanding. Therefore a team of personnel including clinicians, technicians, and barn staff is best suited to the ICU setting to optimize patient care.

Patients in the ICU setting are better suited segregated from a general hospital population. There is a twofold positive effect. First, ICU patients are often sick, immunocompromised, and may be at risk of developing opportunistic infection. Second, certain ICU populations may be at higher risk of shedding infectious organisms like salmonella. For example, juvenile horses admitted for gastrointestinal disease, administration of antibiotics, and fasting are such examples.[2]

In addition to dedicated stalls and personnel, some key equipment for diagnostic and therapeutic purposes are necessary to deliver optimal care. Fluid pumps, electrocardiographic (ECG) capabilities, ultrasonography, and point-of-care clinicopathologic testing capabilities are a few examples. However, there is no greater asset in an ICU than methodical, detail-oriented personnel that serially evaluate their patients and are in tune with even subtle changes.

There are no randomized, controlled studies that advocate for the provision of critical care medicine and improved survival outcomes. The precedent has been set time and again in human medicine where, in many instances, dedicated personnel, personalized care, early goal-directed therapies, and novel therapies result in greater survival, shorter duration of stay in the hospital, decreased morbidity, and lower hospital costs. More studies are needed in veterinary medicine to support similar outcomes in equine practice.

The provision of critical care to horses is at the purview of equine clinicians; however, certain criteria appear to be universal among specialists, which include horses with parenteral fluid requirement to support the cardiovascular system, systemic inflammatory response syndrome ([SIRS]; both septic and nonseptic causes), (poly)trauma, hemorrhage, cardiovascular collapse, malignant dysrhythmia, respiratory distress (notably lower respiratory dysfunction), acute abdominal disease (surgical and medical), seizure disorder, and acute neurologic decompensation, to name a few.

Therapeutic goals for all patients include providing appropriate care for the primary problems, anticipating complications and initiating appropriate preventive therapy, and providing appropriate supportive care for all vital body systems.

In this chapter, salient features of the critically ill patient profile, diagnostic options, and immediate life-saving therapeutics for specific body systems will be provided. Additional specific information on select medical conditions will be covered in Chapter 20.

EQUINE INTENSIVE CARE UNIT

Equine ICUs are becoming increasingly prevalent in clinical practice. Common features include housing of patients according to type or severity of illness with appropriate biosecurity precautions; readily available equipment for diagnosing,

*The editors and authors acknowledge and appreciate the contributions of J. Hardy, P. Marsh, P. Mooresey, B. Barr, B. Waldridge, and Y. Nout as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

monitoring, and treating the seriously compromised horse; and 24-hour staffing with professionals who have the knowledge and experience to treat these patients. The goals of the ICU are to provide comprehensive care and improve patient outcomes while using tools and resources that promote efficiency.

Several studies describe the general case population and commonly performed procedures and treatments of patients admitted on an emergency basis to large university referral centers.[3,4] Because equine emergencies are relatively common among patients requiring critical care, such information provides an initial database for understanding population dynamics and the required expertise when staffing the ICU. Colic or acute abdominal pain is the most common type of case encountered in emergency/critical care centers, and studies show that expertise is required that can handle both medical and surgical needs. However, the fact remains that a variety of other problems present as emergencies, and the required skills to deal with these include experience with dysfunction of most all body systems. Each ICU population will reflect the local horse population type, and regular review of hospital populations is useful to appropriately allocate resources (staff and equipment) to meet the unique needs of the region.

Human ICU treatment requires a multidisciplinary team that includes intensivists (i.e., physicians who specialize in critical illness care), nurses, respiratory care therapists, dieticians, pharmacists, and other consultants from a broad range of specialties, such as surgery, internal medicine, and anesthesiology. Because of patient numbers, costs of therapy, and limitations of certain aspects in equine practice, the range of specialists is often consolidated to fewer, multiskilled, broadly trained individuals.

Integral to the ICU are licensed veterinary technicians who provide intensive and continuous care. Generally, good nursing care is pivotal to successful outcomes. The technical staff are the "eyes and ears" of the ICU and are trained to identify early subtle changes in patient status and feel comfortable using a range of equipment. The ability to perform common techniques and to recognize changes in condition early is essential.

Box 4.1 lists basic, intermediate, and advanced equipment that may be used in the ICU. Again, based on patient populations, if a practice rarely sees critically ill foals, purchase of a mechanical ventilator would be a poor economic choice. Regular review of equipment to ensure it is in working order as well as training for all personnel on new equipment are essential.

Commonly performed procedures include patient monitoring, fluid administration, and attention to and provision of appropriate analgesia. Monitoring includes close, astute observation and serial physical examinations. Use of diagnostic techniques and laboratory support are adjunctive measures that complement competent observation to monitor the systemic health of the patient. Parenteral fluid administration encompasses routine administration of a wide range of products, including crystalloids, synthetic colloids, blood, and blood components. Choosing analgesia in the ICU will depend on the type and severity of pain experienced by the patient (e.g., inflammatory pain versus neuropathic pain). The clinician needs to consider potential adverse effects that also may influence the primary disorder (e.g., limiting morphine use in the abdominal patient to minimize ileus). Multimodal analgesia techniques are being used that have the purported benefits of analgesia being delivered through several mechanisms of action while using lower total doses to the patient.

Emergency drugs should be readily available and accessible within seconds to minutes in any hospital. Mobile crash carts and drug bags/packs strategically located around the hospital are advisable. Drug expiration dates should be regularly monitored and expired medications replaced. Table 4.1 lists several emergency drugs and dosages used in adult horses. Having emergency drug cheat sheets located in several key areas with volumes, routes, and indications listed helps all ICU personnel deliver safe, appropriate care in an emergency situation.

In addition, assembling packs for specific anticipated emergency situations, such as all the necessary items for delivering supplemental oxygen or supplies to perform an urgent tracheotomy, and placing these packs in key areas is recommended.

Essential monitoring equipment commonly used in the ICU includes an ECG, a blood pressure monitor, a point-of-care glucometer and lactate meter, a point-of-care electrolyte and chemistry analyzer, an ultrasound unit, a centrifuge for hematocrit determination, a refractometer for determining total protein and urine specific gravity (SG), and urine test strips for urinalysis. Other monitoring tools to consider are a hemocytometer, microscope with 100× magnification, equipment for cytologic examination (including Gram stains), and a blood gas and electrolyte monitoring unit. A colloid osmometer is useful for determining colloid osmotic pressure (COP) in hypoproteinemic horses.

Advanced imaging is becoming more common, and the use of ultrasound has become an essential component of diagnosing and monitoring critically ill patients. Ultrasound can be used for identifying and monitoring effusions, intestinal distention, and motility; identifying umbilical structures; monitoring pregnancy; and visualizing ocular structures, among other anatomic areas. To enable imaging of a wide variety of

BOX 4.1 Equipment List for Different Levels of Equine Intensive Care Units

BASIC

- Fluid administration system: coil sets and fluid hooks
- Electrocardiogram
- Centrifuge
- Refractometer
- Glucose meter
- Lactate meter
- Urinalysis strips
- Microscope for cytologic examination/Gram stain
- Ultrasound including Doppler capability
- Oxygen tank and regulator
- Biosecurity equipment and personnal protective equipment

INTERMEDIATE

- Blood gas/electrolyte/glucose analyzer
- Complete blood count capability
- Coagulation profile testing
- Blood pressure monitor (direct and indirect)
- Intravenous fluid pump delivery systems
- Sling and hoist for down horses

ADVANCED

- Pulse oximeter
- Mechanical ventilator
- Colloid osmometer
- Capnograph
- Continuous electrocardiogram telemetry
- Syringe infusion pumps

TABLE **4.1 Emergency Drugs Used for Adult Horses**

Drug	Dose	Dose per 1000 lb	Route	Comment
Atropine	0.01–0.02 mg/kg	0.3–0.45 mL	IV	Bradycardia; rescue therapy for severe bronchoconstriction
Dobutamine (positive inotrope)	2–10 μg/kg/min (1 vial [250 mg] in 1000 mL = 250 μg/mL)	900–4500 μg/min	IV	Use diluted solution within 24 h; it is compatible with most IV fluids; do not mix with alkaline solution calcium chloride/gluconate
Doxapram (respiratory stimulant)	0.2 mg/kg	4.5 mL	IV or topical under tongue	Do not mix with alkaline drugs/ fluid
Epinephrine For anaphylaxis For asystole	 0.01–0.02 mg/kg 0.1–0.5 mg/kg	4.5–9 mL	IV/IM/SC/ Intratracheal	Do not give with bicarbonate, hypertonic saline, or aminophylline It does not need to be diluted when given IV to adults Double volumes when given intratracheally
Furosemide	0.5–1.1 mg/kg	5–10 mL	IV	Pulmonary edema, diuresis, congestive heart failure
Glycopyrrolate (for bronchodilation and bradycardia)	0.001–0.005 mg/kg	2.25–11.25 mL	IV/IM/SC	Do not mix with alkaline drugs/ fluids
Lidocaine (treatment of ileus)	1.3 mg/kg loading slowly over 5 minutes 0.05-mg/kg/min infusion	Loading: 30 mL Infusion: 67 mL/h	IV	Ensure that product does not contain epinephrine
Lidocaine (treatment of arrhythmias)	Bolus: 0.25–0.5 mg/kg (slowly) Infusion: 20–50 μg/kg/min	Bolus: 5–10 mL Infusion: 30–60 mL/h	IV	Ensure that product does not contain epinephrine
Aminophylline	5 mg/kg	2.5 g	Nebulized	

IV, Intravenously; *IM,* intramuscularly; *SC,* subcutaneously.

structures, access to a variety of transducers ranging from 2.5 to 10 MHz, as well as a rectal probe, is recommended.

Oxygen provision for supplementation through nasal insufflation, demand valve access, or for mechanical ventilation should be available. Compressed gas cylinders can be used but must be stored and handled appropriately to prevent injury. Oxygen cylinders can be fitted with a diameter index safety system for connection to a demand valve. At peak flow, a demand valve can provide up to 160 L/min of oxygen. For each cylinder type, knowledge of the capacity of the cylinder and the flow rate enables calculation of the amount of time provided. The small portable E cylinders contain 655 L of oxygen when full and can provide oxygen for 260 minutes when set at a flow rate of 5 L/min. Adult horses may require flow rates of 10 to 15 L/min to have any significant effect on the fraction of oxygen inspired (Fio_2). Larger G or H cylinders containing 5290 or 6910 L, respectively, allow oxygen supplementation for longer periods.

The design of the ICU should accommodate the care of horses with a wide variety of problems. All stalls should have the equipment necessary for hanging large-volume fluid bags. Stalls should be free of objects that could cause injury. In addition, the design should include one or two stalls for easy unloading of down horses, and the structure must be able to withstand hoisting. A track system to hoist horses off a trailer and transport to a surgical table or ICU stall would be a welcome feature. Commercially made glides are also essential to facilitate safe movement of down horses. If care of neonatal patients is expected to be routine, large foaling stalls should be available. These stalls may feature mobile separators to facilitate treatment of foals while allowing them access to their dams.

A central office facilitates oversight of the entire unit. A central viewing station with either cameras or direct visualization of stalls is ideal. The ICU should have adequate storage and be uncluttered. Hand sanitizer, wash sinks, and boxes of gloves should be easily accessible. Hand washing between patients is essential to prevent spread of nosocomial infection.

Food preparation areas should be separate, and operators should be diligent in washing hands to prevent cross-contamination of pathogens in feed.

In the ICU, strict disinfection and isolation protocols should be in place to prevent nosocomial infections and the spread of infectious disease. In addition to hand washing, hand rubbing with an alcohol-based solution is more effective than hand washing with an antiseptic, probably because it does not require rinsing and drying of hands.[5]

Guidelines and standard operating procedures (SOPs) can be applied to a variety of aspects of the ICU; for example, guidelines for the use of antibiotics, guidelines on how to handle multidrug-resistant pathogens, SOPs for personnel injuries, and so forth. It is recommended that each ICU establish "best practice regimens" to optimize care, minimize complications and mistakes, and maximize patient survival and personnel safety.

The overarching goal of critical care is to support and treat the patient such that normal homeostatic mechanisms

become fully functional and independent of exogenous support. Treating and preventing shock, in all its various forms, is a common theme among patients. Provision of care that maintains oxygen and cellular metabolic substrate delivery to meet the unique demands of the patient is key. The clinician often focuses on a specific disease process or even organ system. However, because of the systemic consequences of several pathologic processes (e.g., inflammation, neoplasia), methodical and serial evaluations of the entire patient are important. This allows the practitioner to identify problems early with hopes of instituting early intervention and avoid morbidity. Severely ill patients require special attention. Their condition is dynamic and can change quickly. These patients may require rechecking key indices such as coagulation panels or ionized electrolyte concentrations as frequently as each hour. Cardiac rhythm reassessment or direct blood pressure measurement may be required on a minute-by-minute basis (continuous telemetry is particularly beneficial in these patients).

Expected and unexpected problems can be encountered in the ICU patient. Horses with focal disease (e.g., injection site abscess) that by itself is unlikely to be life-threatening can develop multisystemic consequences that jeopardize the patient (e.g., hemolytic anemia, fever, anemic hypoxia, and pigment nephropathy). Oftentimes, the development of serious problems can be a consequence of a ramped up immune system and SIRS. In health, inflammatory pathways can be regulated by antiinflammatory pathways. When the balance tips in favor of inflammatory pathways, overwhelming activated systemic inflammation ensues and downstream effects can be observed, such as coagulopathy or acute respiratory distress syndrome (ARDS), which may be seemingly unrelated to the primary disease process.

Medication mistakes can be categorized in two main categories: therapeutic-related unexpected adverse effects in the patient and administration mistakes made by the ICU staff. An example of an adverse effect would be the development of antibiotic-associated colitis (AAC). Horses with acute gastrointestinal disease represent the most common admission to the ICU. In many instances, these patients are stressed, inappetent (often forcibly), and often require intestinal surgery, all of which are considered to be selection pressures for intestinal dysbiosis.[2] These horses also are frequently treated with antibiotics in the perioperative period, which, with preexisting pressures, can lead to life-threatening colitis. It is prudent for the clinician to exercise good judgment on antibiotic usage and try to institute early enteral feeding where possible to help avoid AAC.

Medication administration mistakes can also occur. A recent article highlighted that poor compliance to medication recommendations occurs more frequently than anticipated.[6] Mistakes can be minimized by having clear medical records that list the generic name of the medication, the dose (e.g., number of milligrams), the amount of units delivered (e.g., milliliters), route of administration, and frequency of dosing: for example, gentamicin, 3000 mg (30 mL), intravenously (IV), q 24 h.

ICU orders should be reviewed by a supervising clinician and signed off on as a check and balance, especially when multiple users may be following and carrying out these orders. Daily rounds with ICU personnel on the patient condition, current treatment and monitoring plan, and a discussion of potential complications is highly informative for ICU staff and helps maintain an open line of communication to ensure that the orders are clearly understood.

Adult Horse: Basic Procedures in Critical Care

Samuel D. Hurcombe

Expedient acquisition of important physiologic and physical findings in the critically ill horse is essential to formulating an initial diagnostic and/or therapeutic plan. Regardless of the nature of the particular condition presented to the clinician, expertise in a variety of technical procedures is required to facilitate diagnostic and therapeutic efforts.

VASCULAR ACCESS AND ADMINISTRATION OF FLUIDS

Establishing venous access is often crucial in critical care. Generally, using large veins (i.e., jugular veins, lateral thoracic veins, cephalic veins, and rarely saphenous veins) for access makes it easier to catheterize and deliver medications, fluids, parenteral nutrition (PN), and perform blood draws for clinicopathologic monitoring. IV catheters are available in varying materials, constructions, lengths, and diameters (Tables 4.2 and 4.3). In choosing a catheter, the practitioner should consider the desired fluid rate, fluid viscosity, the length of time the catheter will remain in the vein, the severity of the systemic illness, and the size of the animal. Determinants of catheter flow rate follow the Poiseuille-Hagen law: larger lumen diameter (r), shorter length (L), and higher pressure differences (ΔP) between flow into the catheter versus flow out of the catheter result in greater volumes per unit time through the catheter (Poiseuille-Hagen equation of flow through a tube [e.g., catheter]):

$$\text{Flow } \alpha \text{ Pressure gradient } (\Delta P) \times \text{radius } (r)^4/\text{viscosity } (\eta) \times \text{length } (L)$$

In cases in which volume provision is critical, placement of short, large-bore catheters using a pressurized fluid bag would optimize fluid delivery. Viscosity (η) of the fluid administered also modulates a flow rate in which higher viscosity fluids (i.e., natural colloids, blood, blood products) result in slower rates of fluid administration. Cautious use of high flow rates may be more traumatic to the vascular endothelium, increasing thrombotic risk.

Catheters are generally made of Teflon or polyurethane. The thrombogenic properties of each material should be taken into consideration with the patient's clinical condition. Polyurethane catheters (i.e., MILA International) are considered to be less thrombogenic than Teflon; therefore, it is recommended to use this material in patients with clinical coagulopathy or "at risk for" clinical coagulopathy. Typically these patients are horses with hypoproteinemia; SIRS; those requiring blood products, PN provision, endotoxemic/hemorrhagic shock, septicemia/bacteremia; and horses with inherited or acquired coagulopathic disease. Teflon catheters should be changed every 3 days, and polyurethane catheters can remain in the vein for up to 2 weeks. Regardless of the type of catheter, the site of vascular access should be closely monitored several times daily for signs of flow disturbance, thrombosis, and/or infection.

For most applications, over-the-needle catheters are easiest and faster to place and achieve therapeutic goals in adult

TABLE **4.2 List of Available Catheters (by Material Type)**

Material	Example	Comment
Polypropylene	Polyethylene tubing, Medicut	Highly thrombogenic not recommended
Teflon	Angiocath	Less thrombogenic
Polyurethane	MILA	Much less thrombogenic
Silastic	Centrasil	Least thrombogenic

TABLE **4.3 Commercially Available Catheters (by Construction Type)**

Type	Description	Advantage	Disadvantage
Butterfly	Needle attached to tubing	Ease of use	Laceration of vessel; vessel puncture and extravascular administration
Over-the-needle	Stylet inside catheter for venipuncture	Available in large diameter, ease of insertion	Limited length of catheter, not flexible, break at catheter and hub junction
Through-the-needle	Short needle inserted; catheter threaded through needle	All lengths available, flexible, peel-away needle	Technically more difficult to insert
Over-the-wire	Needle serving as guide to insert wire, which serves as guide for catheter	Flexible, long catheters available, ensures proper catheter placement	More technical expertise required to place catheter, expensive

horses. Over-the-wire (OTW) catheters are more technically challenging to place; however, they are often better tolerated long term and are less traumatic to the vascular endothelium. OTW catheters come in single-lumen and multilumen models and are often placed in foals and adult horses at high risk for thrombotic complications or patients in which multiple solutions may be administered, especially hyperosmolar/hypertonic solutions. OTW catheters are recommended for lateral thoracic vein catheterization caused by the acute angulation that the vessel travels into the thorax. Ultrasound guidance can assist in catheter placement at this location, particularly in obese and edematous horses.

Short- and long-extension sets are available as well as small-bore and large-bore diameters. Using an extension set with a Luer lock that screws into the hub of the catheter is best to prevent dislodgment. In horses with low central venous pressures (CVPs), disconnection of the line can result in significant aspiration of air and cardiovascular collapse, notably from jugular venous catheters. Moreover, one-way hemostatic valves are also available to help prevent backflow of blood and aspiration of air.

Horses receiving multiple infusions (i.e., crystalloid fluids and continuous rate infusions [CRI's]), may benefit from double extensions in which each fluid/medication type has an individual port entering a central line catheter rather than "piggybacking" fluids into each other.

Catheter Maintenance and Care

Before using any placed catheter, drawing back on the catheter to ensure intravascular placement is advised. Catheters should be flushed frequently with heparinized saline (10 units/mL) before and after each medication administered and every 4 to 6 hours after catheter placement. For some catheters that are infrequently used, a "heparin lock" comprised of 20 units/mL heparinized saline instilled in the catheter, followed by line clamps, may help preserve the integrity of flow. Provision of an antithrombotic such as clopidogrel (4 mg/kg PO once, then 2 mg/kg PO every 24 hours) has been used to help prevent thrombotic catheter complications despite proven efficacy.

Injection caps should be changed daily or sooner if excessively perforated with multiple injections. Before inserting a needle, injection caps should be wiped with 70% isopropyl alcohol and allowed to dry.

Catheters and extension sets may be secured using adhesive glue for short-term catheters or directly sutured to the skin using 2-0 nonabsorbable suture material. Care should be taken to ensure that no part of the catheter is exposed when being secured.

Each line can be labeled and the utmost care should be exercised to never break the circuit of any solution containing glucose (e.g., supplemented crystalloid solutions or PN) to help prevent catheter and line sepsis. Some practitioners apply triple antibiotic ointment to insertion sites to prevent infection, although evidence is lacking about the efficacy of this practice. If infection is suspected, the catheter should be removed and the catheter tip should be cultured (aerobic and anaerobic).

Coil sets are used for stall-side fluid administration for most adult horses. These are advantageous because they allow the horse to move around, lie down, and eat without restraint. An overhead pulley system with a rotating hook prevents fluid lines from becoming tangled.

Solution administration sets are used for short-term fluid or drug administration and typically deliver 10 drops/mL. Long coiled extension sets can then be used to connect fluids to the horse.

Calibrated pumps allow continuous even delivery of solutions at designated flow rates (CRI). When using a calibrated fluid pump, one should take care to use the appropriate set calibrated for the brand of pump. These pumps have alarms that signal air in the line, an empty fluid bag, or catheter problems.

TABLE **4.4 Parameters Used for Estimation of Dehydration in the Horse**

% Estimated Dehydration	Heart Rate (Beats per Minute)	CRT (Seconds)	PCV/TP (%/g/dL)	Creatinine (mg/dL)
6	40–60	2	40/7	1.5–2
8	61–80	3	45/7.5	2–3
10	81–100	4	50/8	3–4
12	>100	>4	>50/>8	>4

CRT, Capillary refill time; *PCV,* packed cell volume; *TP,* total protein.

The maximal fluid rate that most commercial pumps can deliver is 999 mL/h, which is insufficient for crystalloid delivery in most adult horses. Infusion pumps are useful for PN, multimodal analgesia, prokinetic therapies, and cardiovascular drugs, to name a few. Some clinicians advocate CRI antibiotic administration for time-dependent drugs (i.e., β-lactams) to minimize peak-trough pharmacodynamics and potential periods of subtherapeutic (sub–minimum inhibitory concentration) drug delivery.

For large-volume fluid delivery, peristaltic or oscillation infusion pumps are available that can deliver up to 40 L/h. One must supervise these pumps constantly when in use because they continue to run even if fluids run out. Large-bore catheters should be used to prevent trauma resulting from the jet effect on the endothelium of the vein.

FUNDAMENTALS OF FLUID THERAPY

The provision of fluid therapy to adult horses is a pivotal modality in treating the critically ill equine patient. Dehydration is detectable clinically when the deficit comprises 5% or greater. Fluids can be delivered in several methods but most commonly enterally and IV. Intraperitoneal and intraosseous delivery is less commonly used but possible with specific indications. Parenteral fluids should be administered to horses who cannot tolerate enteral fluids (i.e., gastrointestinal obstruction, esophageal trauma, dysphagia, and/or dehydration estimates exceeding 5%). Enteral fluids are often more cost-effective and indicated in cases with mild volume deficits (≤5%) and may be more effective for certain conditions than parenteral fluid delivery (e.g., colonic impaction) and maintenance therapy.

Rational Fluid Therapy Planning

An understanding of fluid types and properties as well as the needs of the patient are essential to design a fluid therapy plan. The clinician should consider several key questions that facilitate developing a fluid regimen:

1. What volume of fluid is required?
2. What rate of fluid is required?
3. What type of fluid is required?
4. What specific circumstances (physical and physiologic) do you need to consider?

The volume of fluid required is determined based on the clinical and clinicopathologic data gleaned from a thorough clinical examination and minimum database laboratory work. Skin turgor, heart rate, mucous membrane color and texture, eye position, neurologic status, serum creatinine concentration, and packed cell volume with total protein estimates help the clinician determine a deficit, often expressed as a percentage of body weight. Maintenance fluid requirements should be combined along with either quantification (i.e., number of liters of enterogastric reflux) or estimates of ongoing fluid losses to calculate a total volume deficit/requirement for the first 24 hours.

The rate of fluid administration depends on the severity of the critical illness. Horses with profound deficits and cardiovascular compromise require expedient delivery of resuscitative fluids.

The type of fluid also varies, depending on the critical nature. Replacement crystalloids typically have higher sodium and chloride concentrations than maintenance solutions that have lower sodium and higher calcium, potassium, and magnesium concentrations. Hyperosmolar solutions and/or colloid solutions may also be indicated, and, together with isotonic crystalloids, all three types should be considered.

Special circumstances to consider when delivering fluid therapy include patients with renal failure. Both volume and content of fluid choice should be considered depending on the clinical disease process—for example, anuric renal failure versus acute tubular necrosis of the proximal convoluted tubule (PCT) and abnormal fractional electrolyte clearances. Horses with decreased oncotic pressure may not tolerate high-volume crystalloid therapy without adjunctive colloid support.

Cardiopulmonary arrest, while dire in nature, is the ultimate form of congestive heart failure, and judicious limited fluid therapy to allow for enough circulation of cardioactive therapies is indicated without excess loading of the vascular circuit.

Maintenance Requirements

In the adult horse, maintenance fluid requirements have been estimated at 40 to 60 mL/kg per day. This volume likely overestimates the actual needs of a resting, fasted animal but appears to be safe in most situations. Smaller breeds may be more appropriately dosed at 60 mL/kg per day, and heavier breeds (e.g., draft horses) are more appropriately dosed at 40 mL/kg per day. In horses with renal failure, monitoring of body weight, CVP, and ideally urine output is indicated. If weight gain, edema, or increased CVP (>12 mm Hg) is evident, the fluid administration rate should be decreased if not stopped and other renal replacement therapy (RRT) considered.

Fluid Deficit

Practical evaluation of hypovolemia caused by dehydration is a subjective estimate using both clinical and clinicopathologic findings. Table 4.4 lists useful parameters for evaluating acute, extracellular dehydration.

After obtaining an estimate of dehydration, the clinician can calculate the amount of fluids to be given as follows (Table 4.5):

$$\text{Volume (Liters)} = \text{Body Weight (kilograms)} \times \text{Percent Dehydrated (expressed as a decimal)}$$

TABLE **4.5 Commonly Used Equations in Critical Care**

Maintenance fluids requirement (mL)	40–60 mL/kg/day
Fluid deficit calculation (L)	Body weight (kg) × percent dehydrated (expressed as a decimal)
Electrolyte deficit for monovalent electrolytes in abundance in the ECF space (e.g., bicarbonate)	Body weight (kg) × 0.3 (ECF coefficient) × ($[Electrolyte]_{desired}$ – $[Electrolyte]_{measured}$)
Shock rate of isotonic crystalloid (mL)	80–100 mL/kg in the first 60–90 min
Blood transfusion volume (L)	($[PCV_{desired}$ – $PCV_{patient}]$ × body weight [kg] × 0.08 [blood volume coefficient])/PCV_{donor}
Osmolarity (mOsm)	2 × [Na] + glucose/18 + BUN/2.8 (when glucose and BUN are measured in mg/dL)
Mean arterial pressure (mm Hg)	⅓ × (SAP – DAP) + DAP
Pulse pressure (mm Hg)	SAP – DAP
Cerebral perfusion pressure (mm Hg)	MAP – ICP
Abdominal perfusion pressure (mm Hg)	MAP – IAP
Content of oxygen in arterial blood (Ca_{O_2} [mL O_2/dL])	(1.34 × [hemoglobin] × Sa_{O_2}) + (0.0031 × Pa_{O_2})
Delivery of oxygen (mL/minute)	Q (cardiac output) × Ca_{O_2}
Oxygen extraction ratio	(Ca_{O_2} – Cv_{O_2})/Ca_{O_2} or (Sa_{O_2} – Sv_{O_2})/Sa_{O_2}
Alveolar P_{O_2} (Pa_{O_2})	Fi_{O_2} × ($P_{atmosphere}$ – P_{H_2O}) – Pa_{CO_2}/0.8
A-a gradient	PA_{O_2} – Pa_{O_2}

ECF, Extracellular fluid; *PCV,* packed cell volume; *BUN,* blood urea nitrogen; *SAP,* systolic arterial pressure; *DAP,* diastolic arterial pressure; *MAP,* mean arterial pressure; *ICP,* intracranial pressure; *IAP,* intraabdominal pressure.

Continued Ongoing Losses

Objective quantification of ongoing losses is ideal but seldom truly reflective of all volume deficits. Even measuring enterogastric reflux volume still does not account for the third spaced volume of fluid within the bowel that remains. This remaining volume does not contribute to effective circulating volume and, as such, quantification of reflux in the bucket underestimates the true volume deficit. Horses with high-volume diarrhea can also lose significant volumes as well as third space volume within the large reservoir of the large colon and cecum.

Regardless of the nature of ongoing losses, frequent clinical and clinicopathologic reassessment of the patient is imperative to minimize further deterioration. Heart rate, measurement of packed cell volume, total protein, and L-lactate concentration are clinically useful every 2 to 6 hours, depending on the nature of critical illness.

Daily creatinine concentration assessment and urinalysis are indicated in patients with azotemia until laboratory values normalize. Additional means of monitoring adequate fluid delivery may include measurement of CVP, arterial blood pressure, and urine output.

The fundamental basic principle of establishing a fluid therapy regimen centers on choosing an appropriate volume as follows (see Table 4.5):

$$\text{Fluid requirement (initial 24 hours)} = \sum (\text{fluid deficit} + \text{estimated losses} + \text{maintenance needs})$$

This is a starting point and subject to change based on clinical response of the patient.

Fluid Choices

Isotonic crystalloid fluids almost always constitute the foundation of any parenteral fluid therapy regimen. The type of crystalloid fluid to administer depends on the results of a chemistry evaluation and disease of the patient. Generally, an appropriate volume is most critical. However, when several options are available, one should decide between a balanced electrolyte solution (BES) or isotonic saline (0.9% NaCl) as the baseline fluid. Additives can then be added à la carte to treat specific electrolyte and/or metabolic derangements.

Table 4.6 lists the composition of various commercially available fluids. Generally, BESs are chosen when serum electrolytes are close to normal or when chemistry analysis is unavailable, because BESs are generally considered more physiologic.

Physiologic saline might be preferable in conditions in which potassium restriction is indicated (e.g., hyperkalemic states, such as hyperkalemic periodic paralysis, uroperitoneum, renal failure) because BESs all contain some potassium. BESs, by their nature, also have buffering ability. Lactate in lactated Ringer's solution (LRS) requires hepatic metabolism; thus, LRS may not be an ideal choice in horses with liver dysfunction. In cases of long-term fluid maintenance therapy (greater than 4–5 days), if the oral route is not available, the practitioner should consider half-strength basic fluids (e.g., 0.45% NaCl and 2.5% dextrose) to which potassium and calcium are added. Long-term fluid therapy with routine BESs can result in hypernatremia, hypokalemia, hypomagnesemia, and hypocalcemia.

Routine electrolyte supplementation often includes calcium, potassium, and magnesium provision. This is particularly important when the horse receives limited or no enteral intake (e.g., gastrointestinal disease). These electrolytes are important for smooth muscle function, vascular tone, and intestinal smooth muscle activity.[7,8]

Suggested doses of calcium depend on ionized calcium concentrations. Routine supplementation with 10 mL of 23% calcium gluconate per liter of fluids is used by the author as a starting point. Potassium chloride can be supplemented at up to 0.5 mEq/kg per hour maximum parenteral rate. The author starts at 0.05 mEq/kg per hour for routine supplementation and 1 g magnesium sulfate per liter of fluids.

Horses with high anion gap metabolic acidosis (e.g., lactic acidosis) are often hypobicarbonatemic because of increased buffering of organic acid production. In most instances, this represents a type A lactic acidosis, such as perfusion-mediated hyperlactatemia. As such, volume restoration to optimize systemic perfusion should be the first

TABLE **4.6 Crystalloid Solutions Available for Fluid Therapy**

Product	Approximate pH	mOsm/L	Na (mEq/L)	K (mEq/L)	Ca (mEq/L)	Mg (mEq/L)	Cl (mEq/L)	Buffer (mEq/L)
Lactated Ringer's solution	6.5	273	130	4	3	—	109	Lactate 28
Lactated Ringer's solution and 5% dextrose	5	525	130	4	3	—	109	Lactate 28
Plasma-Lyte A	7.4	294	140	5	—	3	98	Acetate 27 Gluconate 23
Plasma-Lyte 148	7.4	294	140	5	—	3	98	Acetate 27 Gluconate 23
Plasma-Lyte 148	5.5	294	140	5	—	3	98	Acetate 27 Gluconate 23
Plasma-Lyte 56 and 5% dextrose	5	362	40	13	—	3	40	Acetate 16
5% dextrose	4	252	—	—	—	—	—	—
0.9% NaCl	5	308	154	—	—	—	154	—
7% NaCl	—	2400	1196	—	—	—	1196	—
5% dextrose and 0.9% NaCl	4	560	154	—	—	—	154	—
5% dextrose and 0.45% NaCl	4	280	77	—	—	—	77	—
5% $NaHCO_3$	8	1190	595	—	—	—	595	—
8.4% $NaHCO_3$	—	2000	1000	—	—	—	1000	—
1.3% $NaHCO_3$ (must be mixed)	—	308	154	—	—	—	1541	—

step in treating the acidosis; however, in extreme cases, supplemental bicarbonate ($NaHCO_3$) treatment may also be required.

Horses receiving bicarbonate therapy should have their respiratory function assessed because carbon dioxide (CO_2) generated from carbonic anhydrase activity requires normal alveolar ventilation for effective CO_2 removal. Respiratory compromise will result in worsening of acidemia caused by mixed metabolic and respiratory acidosis.

For horses with a pH ≤7.2 caused by metabolic derangements (nonrespiratory) following shock crystalloid dosing (80 mL/kg in 1 h), isotonic bicarbonate (1.3%) therapy is indicated. First, administer 50% of the bicarbonate deficit (see later) rapidly over 1 to 2 hours followed by the remaining 50% over 12 to 24 hours.

Note that parenteral bicarbonate solutions are incompatible with calcium-containing solutions. The 1.3% sodium bicarbonate solutions are isotonic, and 8.4% sodium bicarbonate solutions are hypertonic (1 mEq/mL). To make a 1.3% $NaHCO_3$ solution, remove 130 mL of water from a 1-L sterile water bag. Add 130 mL (130 mEq) of 8.4% $NaHCO_3$ to the sterile water bag.

$$\text{Bicarbonate deficit} = (\text{Bicarbonate}_{\text{desired}} - \text{Bicarbonate}_{\text{measured}}) \times \text{body weight (kilograms)} \times 0.3 \text{ (extracellular fluid [ECF] compartment coefficient; see Table 4.5)}$$

For ongoing losses of bicarbonate not associated with excessive plasma buffering (e.g., hypersecretory diarrhea, renal tubular acidosis), enteral supplementation is a suitable option. The deficit is calculated and the number of milliequivalents converted to a weight (in grams):

$$1 \text{ g } NaHCO_3 = 12 \text{ mEq } NaHCO_3$$

The total number of grams is divided into several administrations per day. Orally administered $NaHCO_3$ may cause minor caustic injury to the gingival mucosa. Mixing $NaHCO_3$ with Karo syrup or molasses and washing the mouth after the horse swallows may help prevent injury.

Rate of Administration

For patients in severe shock, the clinician should strive to administer a shock dose of isotonic crystalloid fluids in the first hour (80 mL/kg), which can be done only with pressurized bags, a fluid pump, and/or multiple catheters.

In other situations, the volume calculated accounting for losses, replacement, and maintenance represents a volume for the 24-hour requirements and estimates as a volume per hour. Accurate record keeping of a running tally of fluids provided is important to ensure that the correct amount is administered.

ORALLY ADMINISTERED FLUIDS

Oral fluids are indicated in horses with only mild volume deficits and who can tolerate enteral fluids (e.g., no intestinal obstruction or reflux). Oral fluid therapy should be administered using the fluid composition shown in Table 4.7. One should administer calcium separately because it causes

TABLE **4.7 Fluid Composition for Orally Administered Fluid Therapy**

For Every L of Water, add:	FOR EVERY 21 L OF WATER
Electrolyte	**Amount (g)**
NaCl	10
$NaHCO_3$	15
KCl	75
K_2HPO_4	60

precipitation of the solution. This electrolyte solution meets daily needs for an adult horse and can be given through a small, preplaced nasogastric feeding tube or by intermittent intubation.

Parenteral Fluids

Crystalloids

The IV administration of isotonic crystalloids immediately reconstitutes effective circulating volume. Crystalloids (all types) rapidly redistribute out of the vascular space (VS) to the entire ECF space. The ECF is roughly 30% of body weight, and the VS is roughly 8% of body weight. As such, to maintain a desired expanded intravascular volume, 3 to 3.75 times as much volume is required to account for redistribution among the entire ECF. BESs are typically chosen such as LRS. Doses of BESs for rapid volume expansion (not maintenance fluid requirements) are 70 to 90 mL/kg per hour with frequent (every 20 minutes) reassessment of additional volume needs.

Replacement Solutions

Lactated Ringer's Solution

LRS has a lower sodium concentration and higher chloride concentration than equine plasma. LRS should be avoided in cases of hyperkalemia because of low levels of potassium and when administering products containing citrate or bicarbonate, because the calcium in LRS may precipitate. Lactate in LRS is metabolized by the liver and, in theory, may accumulate and contribute to metabolic acidosis in patients with hepatic dysfunction.

Plasma-Lyte 148 and Normosol-R

Plasma-Lyte 148 and Normosol-R are similar to LRS but have a more physiologic sodium:chloride ratio and contain magnesium rather than calcium. They may be administered concurrently with blood products and bicarbonate but should be avoided in cases where neuromuscular blockade is a concern (e.g., botulism). The alkalinizing agents in these fluids are acetate and gluconate, respectively.

Normal Saline (0.9% NaCl)

Normal saline consists solely of sodium and chloride in concentrations significantly higher than that of plasma. Saline is acidifying and is useful in the treatment of patients with hyperkalemia and metabolic alkalosis. The use of normal saline in cases of hyponatremia is cautioned because overly rapid correction of sodium concentrations may lead to excessive osmotic draw of water from cells.

Hypertonic Saline Solution (7.2% NaCl)

Hypertonic saline solution (HSS) is approximately eight times the tonicity of plasma and ECF, and the immediate effect is to rapidly expand the vascular volume by redistribution of fluid from the interstitial and intracellular spaces. The rapid increase in vascular volume improves cardiac output (CO) and tissue perfusion with rapid administration of only a relatively small volume of fluid. Initially, this is a roughly 1:3 ratio—that is, for every liter of HSS administered, the patient recruits up to 3 L of volume expansion. This effect is short-lived (estimated to be ~20 minutes). Because the electrolytes redistribute across the ECF, fluid redistribution occurs again and the patient becomes hypovolemic again. Because the principal effect is fluid redistribution, a total body deficit still exists and it must be replaced. For this reason, concurrent or immediately following HSS administration, isotonic crystalloids must be provided to correct the total body volume deficit. The duration of effect of hypertonic solutions is directly proportional to the distribution constant, which is the indexed CO. The dose is 2 to 4 mL/kg, administered as rapidly as possible. Iatrogenic metabolic acidosis, hypernatremia, and hypokalemia may occur after hypertonic saline administration; therefore, plasma electrolyte concentrations should ideally be evaluated in the patient before and after the administration of hypertonic saline.

Maintenance Solutions

Crystalloid maintenance fluids are polyionic isotonic or hypotonic fluids formulated for long-term use in patients needing chronic fluid support beyond fluid volume resuscitation. They contain lower sodium and higher potassium, calcium, and magnesium concentrations. Examples include Plasma-Lyte 56, Normosol-M, and 0.45% NaCl/2.5% dextrose, and these fluids should not be used for rapid volume expansion.

Colloids

Colloid fluids contain large molecular weight particles that do not readily diffuse across healthy cellular barriers, maintaining or increasing COP within the intravascular space. These molecules do redistribute to the ECF but at a much slower rate than crystalloids; thus, the duration of effect is prolonged compared with that of crystalloids. Table 4.8 describes the different colloids available. Critically ill patients often develop endothelial dysfunction, and the associated increased vascular permeability may ameliorate some of the beneficial effects of colloids, dependent, in part, on the average molecular size of the colloid administered. For example, hetastarch (6% hydroxyethyl starch) has an average molecular size of 450 kDa, and albumin in plasma has a molecular size of 70 kDa. Further, meta-analyses in humans failed to support the theory of improved resuscitation through the use of colloids over crystalloids[9]; however, colloids may be indicated in patients with hypoproteinemia, severe blood loss, or those in need of clotting factors or immunoglobulins.

A disadvantage of natural colloids (plasma or albumin) is that they are more antigenic and can cause allergic reactions. Synthetic colloids have a much lower antigenicity, but they can cause bleeding disorders because of their tendency to coat platelets or by causing a decrease in coagulation factors. In the horse Dextran 40 can cause anaphylactoid reactions. Hetastarch administration can cause a decrease in coagulation factors and prolong clotting times, particularly at high doses (20 mL/kg).[10]

TABLE **4.8 Characteristics of Colloid Solutions Available for Fluid Therapy**

Characteristics	5% Albumin	25% Albumin	Dextran 40	Dextran 70	Hetastarch (600/0.75) 6%	Tetrastarch (130/0.4) 6%
Molecular weight (d)	—	—	—	—	—	—
Average	69,000	69,000	40,000	70,000	450,000	130,000
Number average	69,000	69,000	25,000	39,000	70,000	
Range	—	—	10,000–80,000	15,000–160,000	10,000–3,400,000	110,000–150,000
Solvent	—	—	0.9% saline or 5% dextrose	0.9% saline or 5% dextrose	0.9% saline or balanced electrolyte solution	0.9% saline
Maximum water binding (mL/g)	18	18	37	29	20	—
Concentration (%)	5	25	10	6	6	6
Half-life	14–16 days	14–16 days	2.5 h	6 h	25 h	12 h (human)
Plasma percentage (after 24 h)	—	—	18	29	38	—
Extravascular percentage (after 24 h)	—	—	22	33	39	—
Overall survival in blood	—	—	44 h	4–6 weeks	17–26 weeks	—
Colloid osmotic pressure (mm Hg)	20	100	40	—	30	36

Synthetic colloids do not register on a refractometer. Accurate evaluation of oncotic pressure requires the use of a colloid osmometer. If one is not available, the clinician must use clinical evaluation (e.g., observing the presence of edema and poor circulatory volume and pressure).

Synthetic Colloids

Hetastarch is a more cost-effective synthetic colloid. Advantages of hetastarch include maintenance of plasma oncotic effect for up to 24 hours following administration and the ability to give as a rapid bolus.[10,11] However, unlike plasma, there are no functional proteins present in hydroxyethyl starch solutions and a negative effect on coagulation has been demonstrated in healthy equids.[10,12] Further, a 2013 review of the use of colloids as volume replacement in critically ill humans found an increased risk of mortality and acute kidney injury in patients receiving hetastarch.[13] As such, the use of hetastarch in horses with underlying renal insufficiency is cautioned, and its use is restricted to those with clinical signs of acute hypoproteinemia. Recently tetrastarch (6%) was evaluated and shown to have a more sustained effect on COP with fewer adverse coagulopathic effects than hetastarch.[14] Both hetastarch and tetrastarch can be dosed at 10 mL/kg.

Natural Colloids: Plasma

Plasma contains not only osmotically active albumin but a variety of essential proteins, including clotting factors, immunoglobulins, and antithrombin III. The advantage of plasma over synthetic colloids is that these proteins provide carrier sites for exogenous (pharmaceuticals) and endogenous (hormones) compounds. Disadvantages of plasma as a resuscitation fluid in horses include the prolonged time required for administration, a relatively short duration of oncotic effect, the potential for adverse reactions (10% incidence),[15] and the relatively high cost if given for large-volume resuscitation.

Whole Blood

Whole blood can be considered the ultimate colloid replacement fluid when indicated in cases of hemorrhage.[16] Blood transfusion may restore circulating volume, improve oncotic activity, increase oxygen-carrying capacity, is a supply vehicle for therapeutic drug transport, and replenishes coagulation factors. Whole blood is the ideal fluid for blood loss or platelet loss, provided that it is fresh blood and has been cross-matched. It is important to remember that stored blood loses its oxygen-carrying capacity and it can take several hours to restore it after administration.

Access to whole-blood donors that are free of erythrocyte antigenic determinants, particularly Aa and Qa, and of isoantibodies are important hores to have available to ones practice. The practitioner can perform a major and minor cross-match to select an appropriate donor, provided that complement is added to the test for hemolysin detection. Interpretation of the minor cross-match may be difficult if autoagglutination is present. If cross-matching is unavailable, use of a non-Thoroughbred gelding is advised. Up to 20 mL/kg can be safely collected every 3 weeks in adult horses, and whole blood can be collected in sodium citrate using sterile technique provided the product will be used immediately.

Commercial blood collection kits are also available. The reported incidence of transfusion reactions is 16%,[17] and a recent study showed a marked decrease in transfused erythrocyte half-life with an incompatible cross-match (4.7 days versus 33.5 days).[18] Complications of blood transfusion include acute anaphylactic reactions, allergic reactions, hemolysis, fever, tachypnea, and hypocalcemia caused by citrate chelation.

Critical Care Techniques

Samuel D. Hurcombe

MONTORING ARTERIAL BLOOD PRESSURE

The goal of blood pressure measurement is to identify hypotension and follow the response to therapy after interventions. Mean arterial blood pressures (MAPs) of greater than 70 mm Hg are targeted in the adult, and pressures greater than 60 mm Hg are targeted in neonates. Table 4.9 shows expected blood pressures in healthy conscious horses. Hypertension is uncommon in horses. Pheochromocytoma, pain, and transient hypertensive states associated with pressor agents have been described.

Arterial blood pressure can be measured by direct catheterization of a peripheral artery or by indirect measurements that depend on a cuff placed over an artery and cuff inflation until blood flow is occluded. Blood pressure can also be calculated as the product of CO and systemic vascular resistance (SVR). The MAP is a surrogate estimate of tissue perfusion but does not necessarily correlate with blood flow through the tissue. For example, a hypovolemic patient with high SVR may have a MAP within an acceptable range; yet, the actual volume of blood per unit time flowing through the tissue (e.g., perfusion volume) might be inadequate. Horses can have low MAP as a result of hypovolemia, SIRS, heart failure, or any of a wide variety of disorders.

Most monitoring equipment provides an estimation of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and MAP. MAP is a calculated value determined by integrating the area under the pressure waveform and dividing this by the duration of the cardiac cycle. MAP can also be calculated by MAP = ⅓ × (SAP − DAP) + DAP (see Table 4.5).

Pulse pressure is the difference between systolic and diastolic pressure (SAP − DAP; see Table 4.5). A bounding pulse pressure results from an increased systolic pressure, a decreased diastolic pressure, or both. Conversely, a poor or weak pulse quality indicates little difference between SAP and DAP. Ultimately, pulse pressure is not a good surrogate of perfusion, and clinical markers of perfusion or quantitative methods of CO are required for improved accuracy.

TABLE **4.9 Hemodynamic Parameters in Adult Horses**

Type of Pressure	Blood Pressure Reading
Arterial pressure	
Mean arterial pressure (indirect)	>60 mm Hg
Mean arterial pressure (direct)	106 ± 12 mm Hg
Systolic (indirect)	111.8 ± 13.3 mm Hg
Systolic (direct)	142 ± 18 mm Hg
Diastolic (indirect)	67.7 ± 13.8 mm Hg
Diastolic (direct)	82 ± 12 mm Hg
Central venous pressure	8–12 mm Hg (6–18 cm H_2O)

Data from Magdesian KG. Monitoring the critically ill equine patient. *Vet Clin North Am Equine Pract*. 2004; 20:11-39; Hurcombe SD, Scott VHL. Direct intraabdominal pressures and abdominal perfusion pressures in unsedated normal horses. *J Vet Emerg Crit Care*. 2012; 22(4):441-446.

Oxygen delivery to tissues depends on adequate perfusion of functional capillary density (FCD) of the tissues. FCD has recently become a research interest in veterinary species in which dark-field microscopy has been used to evaluate gingival and colonic perfusion.[19] Clinically, FCD quantification techniques are impractical; thus, blood pressure is used to estimate blood flow and tissue perfusion. MAP as a perfusion estimate does not account for local regulation of tissue bed blood flow. Regional shunting, regional vasomotor tone differences, microthrombi, and interstitial edema may all affect local blood flow. Blood flow through a vessel is also dependent on the viscosity of the fluid (blood) and the radius of the vessel. At high viscosity blood flow may be impaired. Severe vasoconstriction, although maintaining blood pressure, may impair flow.

Direct (Invasive) Blood Pressure Measurement

A 20-gauge or 22-gauge over-the-needle catheter is most commonly used to perform direct or invasive blood pressure measurement. Suitable sites for catheterization include the transverse facial, facial, and greater metatarsal arteries (Fig. 4.1). In the standing horse the transverse facial or the facial artery are most practical. Catheters are placed toward the direction of blood flow (i.e., toward the heart), and noncompliant tubing filled with heparinized saline is used to connect the catheter to a calibrated pressure transducer. It is important to flush all air from the circuit to avoid dampening of the waveform.

Blood pressures are measured in millimeters of mercury (mm Hg), which measures the force exerted by the blood against an area of the vessel wall to raise a column of mercury by a certain number of millimeters. Occasionally, centimeters of water (cm H_2O) are used where 1 mm Hg = 1.36 cm H_2O. Continuous readings of blood pressure are most useful clinically and can be graphically displayed using an electronic transducer that converts a pressure signal to an electronic output. The transducer and flush device (Pressure



FIG. **4.1** Adult horse with an arterial catheter in the transverse facial artery for direct blood pressure monitoring.

Monitoring Kit with TruWave disposable pressure transducer; Edwards Lifesciences, Irvine, CA) then can be used for continuous blood pressure measurement. The transducer should be maintained at the level of the heart base, which is estimated to be at the point of the shoulder. Pressure recording systems should be zeroed to atmosphere before reading from the patient.

When the waveform appears flat or dampened, the line and catheter should be flushed to yield a "square wave." When the flush is stopped, the arterial waveform should resume. This test also evaluates the dampening status of the circuit. Immediately after flushing, wave oscillations are observed before beginning the next arterial waveform. Two oscillations are considered normal. An overdamped system (e.g., clot in the catheter, air bubble in connection tubing) results in fewer oscillations and lower magnitude. An underdamped system (e.g., long stiff tubing) results in a greater number of oscillations.

Indirect (Noninvasive) Blood Pressure Measurement

Indirect blood pressure measurements depend on a cuff placed around the tail (coccygeal artery) or the metatarsus (metatarsal artery). The diameter of the cuff influences the accuracy of the measurement, and cuffs that are too wide result in underestimation of the blood pressure. An ideal cuff width-to-circumference ratio of 0.25 to 0.35 has been recommended for use on the tail or limbs of horses. Cuff widths are available for neonate, pediatric, and adult horses.



FIG. **4.2** Indirect oscillometric blood pressure cuff applied to the tail (coccygeal artery) of this 27-year-old Miniature Horse. Lower left inset is a close-up of the tail cuff. Lower right inset is the electronic indirect blood pressure reading. This horse had apparent blindness and was found to have hypertensive cardiomyopathy on echocardiography.

Doppler (Systolic Arterial Blood Pressure)

Doppler uses a small ultrasound probe placed on a peripheral artery, and a piezoelectric crystal within the probe converts the pulse wave into an audible signal. The probe must be placed over a shaved area (usually under the tail of the horse) after application of a coupling gel. Doppler only measures systolic arterial blood pressure.

Oscillometric Sphygmomanometry

Oscillometric sphygmomanometry relies on detecting changes in oscillations generated by changes in blood flow during gradual deflation of a cuff. Oscillations are initially detected once the cuff pressure decreases to be equal to the systolic pressure. Maximal oscillations are detected when cuff pressure is equal to the mean pressure and then disappear when the cuff reaches diastolic pressure. The meter records and displays systolic, diastolic, and mean pressures (Fig. 4.2).

Photoplethysmography

Photoplethysmography relies on the detection of arterial volume by attenuation of infrared radiation. Photoplethysmography originally was designed for use in human fingers and has been validated for use in small dogs and cats but has not been evaluated for use in horses.

CENTRAL VENOUS PRESSURE

Monitoring Central Venous Pressure

CVP is the hydrostatic pressure within a large central vein, typically the cranial vena cava, and is an estimate of right atrial filling pressure. The CVP is affected by vascular volume, venous tone, and cardiac function. Monitoring CVP is most useful for patients in which the veterinarian is attempting to maintain adequate vascular volume without fluid overload. For example, assessment of CVP may be useful in horses with oliguric or anuric renal failure, horses with large-volume gastric reflux, and horses predisposed to edema formation. The venous system has high volume capacitance, which makes CVP quite insensitive to subclinical volume overload. By the time CVP measurements increase, transvenous fluid shifts, and interstitial edema and volume overload have likely occurred.

Conversely, negative CVP values may reflect hypovolemia, indicating a need for fluid therapy.[20] As with measurements of arterial blood pressure, the trend in CVP seen through repeated monitoring is most informative.

CVP is measured by using an IV catheter placed in the jugular vein and terminating in the intrathoracic portion of the cranial vena cava. The catheter is attached via an extension set to a water manometer positioned such that the pressure is at the level of the base of the heart (point of the shoulder), or it can be connected to an electronic transducer. The latter is preferred by the author because the typical CVP waveform can be visualized to confirm accurate location of the catheter tip. Normal CVP in adult horses is 8 to 12 mm Hg (6–18 cm H_2O).

EMERGENCY TRACHEOSTOMY

Acute respiratory distress referable to obstruction of the upper respiratory tract represents a true emergency and need to establish a patent airway. It is important for the clinician to make a rapid assessment of that patient to determine the anatomic location (upper versus lower respiratory tract) of obstruction

BOX 4.2 Materials Needed for an Emergency Tracheostomy Pack

Local anesthetic (2% without epinephrine)
20-gauge needle and 10-mL syringe
Sterile, disposable no. 10 surgical blade
Metzenbaum scissors
Hemostats
J-type tracheostomy tube or self-retaining tube

and to decide if a tracheostomy is indicated. Box 4.2 shows the typical equipment needed for a tracheostomy.

When possible, the clinician should clip and prepare the planned incision site and infiltrate it with 2% local anesthetic. In cases of true acute respiratory obstruction, the patient may be in such distress that surgical preparation is unnecessary. At this time, the operator should not waste time and immediately perform the tracheostomy without preparation. The clinician makes an 8- to 10-cm longitudinal incision at midline at the junction of the proximal and middle third of the neck, just above the V made by the junction of the sternothyrohyoideus muscles. Blunt dissection with Metzenbaum scissors is used to expose the tracheal rings. Next, an incision is made between two tracheal rings, taking care not to damage the tracheal cartilages. This incision should be no more than 50% of the circumference of the trachea. In emergency situations a J-type tracheostomy tube is used because of its ease of insertion. When the horse is calm, or if the situation is not critical, a metal self-retaining tube is preferable for maintenance because J-tubes tend to fall out. Silicone-cuffed tubes are also available for closed-system ventilation.

The tracheostomy tube should be cleaned daily and changed as needed. The surgical site should be cleaned with dilute antiseptic, and saline with petroleum gel applied around the incision prevents skin scalding. Generally, but particularly in foals, tracheostomy tubes should be removed as early as possible to avoid permanent tracheal deformity. To help decide when to remove the tube, the clinician can occlude the tube temporarily to see if the horse can breathe without it. After the tube is removed, the site should be cleaned of exudate twice daily and allowed to heal by second intention. It is the author's opinion that broad-spectrum antibiotics, such as potentiated sulfonamides, should be administered to help prevent local cellulitis and dissecting infection down fascial planes of the neck. The wound generally closes in 10 to 14 days and heals in 3 weeks.

THORACOCENTESIS

Pleural fluid drainage is integral in cases of effusive disease and can be life-saving. Prolonged increased pleural pressures can lead to pulmonary collapse; associated respiratory dysfunction; dyspnea; and in severe cases, cardiac tamponade. Auscultation and percussion of the chest, an increased field of cardiac auscultation, and transthoracic ultrasound can identify pleural effusion. Different tools can be used to facilitate drainage depending on the volume, shock status of the patient, quality and tenacity of the fluid, and likelihood of the need for continued drainage. Small volumes can be drained using a teat cannula or 14-gauge catheter with the stylet removed. Larger volumes, hyperechoic fluid (e.g., pyothorax), proteinaceous fluid, and horses with infectious pleuritis usually require placement of chest tubes. Indwelling tubes used are typically 20 to 32 Fr and sterile. The location for tube placement is ideally identified by ultrasound; however, a rough guide for safe placement is a hand span caudal to the olecranon and a hand span dorsal to the lateral thoracic vein. Where possible, the most ventrally dependent location that minimizes trauma to the heart and lateral thoracic vein is ideal.

The clinician clips and prepares the site with antiseptic, infiltrating it with 2% local anesthetic. An incision is made in the desensitized skin slightly cranial to the proposed point of entry, and the trocar is inserted through the skin. The clinician moves the incision in a caudal direction to the cranial margin of the nearest rib. Using controlled pressure, the trocar is advanced perpendicular to the chest wall and in a slight caudal direction. Fluid is seen to fill the tube, and the tube is fed off the trocar. A unidirectional flow apparatus should be placed on the free end of the tube (e.g., Heimlich valve, latex glove finger, condom) to allow egress of pleural fluid but preventing air entry into the chest. The tube is secured at the entry point using 0 or 2-0 suture with a purse-string and Chinese finger trap pattern.

Normal pleural fluid has a protein concentration <2.5 g/dL and total nucleated cell count <5000/μL.

Pneumothorax is classified as open (e.g., external wound) or closed (e.g., bronchopleural fistula). As pleural pressure equilibrates with atmospheric pressure, lung collapse occurs and can be seen as a cranioventral retraction of the lung field on radiographs. Tension pneumothorax develops when air continuously enters the chest without evacuation and represents a most serious and life-threatening condition in which supraatmospheric pleural pressures causes rapid cardiorespiratory demise.

With open pneumothorax, rapid recognition, sealing of the open wound, and evacuation of air must occur. In open pneumothorax sealing of the chest must occur, followed by evacuation of air. Clear plastic wrap (e.g., Saran wrap) makes an ideal temporary sealant that conforms to the chest and can be wrapped around the thorax. Application of silver sulfadiazine cream around the wound before plastic wrap application provides a secure air-tight seal.

Pleural air is then evacuated via placement of a cannula or 14-gauge catheter in the caudodorsal lung field. The 12th or 13th intercostal space is a suitable location. Air is gradually removed using 60-mL syringes. Reexpansion pulmonary edema is a concern and can be avoided by slow, gradual air evacuation with evacuation pressures <25 mm Hg. As the lung expands, it begins to "tickle" the cannula end, which is seen as a twitching of the cannula during inspiration. The cannula is removed once the air has been evacuated satisfactorily. In closed pneumothorax or tension pneumothorax, the catheter must be kept in place until the source of air entry can be sealed.

NASOGASTRIC INTUBATION

Nasogastric intubation is an essential and possibly life-saving procedure performed in cases of equine colic. Unless contraindicated (e.g., dangerous patient; head, neck, or esophageal trauma), failing to pass a tube may be construed as negligence.

The patient should be adequately restrained, with a twitch and sedation if needed. The clinician should stand on the side of the horse, with the hand closest to the horse placed on the nose, and the thumb in the nostril. With the other hand, the clinician passes the tube in the ventral meatus, using the

thumb to keep it directed ventromedially. If any resistance is met or a hard structure is encountered (possibly ethmoid turbinates), the user should stop, retract, and redirect ventrally. On reaching the pharynx, the practitioner should feel a soft resistance. The tube can be turned 180 degrees to direct its curvature dorsally. The clinician stimulates the horse to swallow by gentle to-and-fro movement or by blowing in the tube. Keeping the head of the horse flexed at the poll is helpful to direct esophageal passage. Once the horse swallows, the clinician pushes the tube into the esophagus. Blowing into the tube to dilate the esophagus facilitates insertion. If the horse coughs, the clinician withdraws the tube and repeats the procedure until the tube is positioned correctly. Determining correct anatomic placement in the esophagus and stomach is imperative. It is signaled by gentle suction, which should elicit a negative pressure; shaking of the trachea, which should not elicit a rattle; and visual confirmation of correct placement (typically just dorsal to the left jugular furrow). Direct observation is the safest method. The tube is advanced until it is in the stomach (14th rib). If the clinician encounters difficulty in passing the cardia, 60 mL of lidocaine may be injected into the tube.

Once placed, spontaneous egress of fluid may occur. If not, creation of a water siphon to empty the stomach can be achieved by filling the tube with water using a pump or funnel under gravity and then immediately directing the end of the tube downward. One sign of an empty stomach is retrieving froth or foam that can sit on the surface of accumulated gastric fluid. It is not uncommon to lose fluid during the refluxing process in horses in which no appreciable fluid accumulation is present.

Medication or intragastric fluids should never be administered by nasogastric tube to a horse with colic with any net positive volume of fluid accumulation.

To remove the tube, creating a fluid lock is achieved by occluding the tube (putting a thumb on the end or folding it) to prevent its contents from spilling out in the pharynx and possibly the trachea as it is withdrawn. Gentle traction is then applied in a direction parallel to the nose. If bleeding occurs, a towel can be placed over the horse's nose. Epistaxis, even if severe, is typically self-limiting. In rare cases of severe hemorrhage, ε-aminocaproic acid administration, nasal packing, and intranasal phenylephrine (10 mg in 10 mL saline) can be used to help resolve bleeding.

Nasogastric reflux is not normal. Occasionally, a small amount of reflux (≤1 L) is obtained if a horse has had a tube in place for a long time.[21] When reflux occurs, the clinician should note the amount, character, and timing in relation to the onset of colic. In addition, the clinician should note the response to gastric decompression. Reflux originating from the small intestine is alkaline, whereas reflux composed of gastric secretions is acidic. Typically, *reflux* refers to small intestinal ileus, functional or mechanical. Lesions of the proximal small intestine produce large amounts of reflux early in the onset of the colic. Inflammatory lesions (e.g., duodenitis proximal jejunitis) are typically associated with copious volumes of reflux caused by the hypersecretory nature of the disease process. With lesions of the distal small intestine (ileum) and often mechanical obstructions, one initially obtains no reflux, and as the condition persists, one obtains reflux but usually several hours after the onset of the colic. Occasionally, large colon disease can be associated with reflux, notably left dorsal displacement/nephrosplenic entrapment of the large colon. Colonic distention can exert extraluminal pressure on the stomach and/or duodenum.

The clinician should note the amount of reflux obtained because this factors into ongoing losses, and the volume of fluids given IV must be adjusted accordingly. Horses with functional ileus need gastric decompression usually every 2 to 4 hours. The nasogastric tube should be left in place only as long as required, because some horses develop pharyngeal and laryngeal irritation associated with its presence.[22] These horses may show pain on swallowing when they resume feeding. Sinusitis can also occur because of nasogastric tube placement.[23] When tubes are left in place, a small-bore tube that still allows effective gastric emptying should be used.

ABDOMINOCENTESIS

Abdominocentesis is important for evaluating abdominal disease, whether it is colic, weight loss, or postoperative problems. Box 4.3 provides the materials needed to perform this procedure.

The clinician clips a 2 × 2-inch area approximately 3 cm caudal to the xiphoid and 1 to 2 cm to the right of midline or using transabdominal ultrasound to guide fluid localization.[24] Various methods are described with different instruments. Regardless, the procedure is performed under sterile technique. Use of a sterile 18-gauge 1.5-inch needle does not require local anesthetic infiltration of the skin and subcutis. If a teat cannula or bitch catheter is used, local anesthesia infiltration is necessary. The operator makes a stab incision with a no. 15 scalpel blade before cannula entry. Two points of resistance will be encountered: as the device goes through the abdominal wall and as it goes through the peritoneum. Horses may become agitated when the peritoneum is "tented" before penetration.

Fluid should be collected into ethylenediaminetetraacetic acid (EDTA) and sterile culture containers. Low-yield samples should be collected in EDTA tubes in which the anticoagulant is shaken out to avoid misinterpretation of fluid analysis.[25] Obtaining fluid from very dehydrated horses is often difficult, especially in cases of simple obstruction.

Normal values and characteristics for abdominal fluid are as follows: clear, yellow in color (able to read newsprint through), low turbidity, total protein concentration should be less than 2.5 g/dL, and total white blood cell count should be less than 5000 cells/μL. On cytologic examination, neutrophils make up approximately 40% to 50% of cells, with the remainder being lymphocytes, macrophages,

BOX 4.3 Materials Needed for Abdominocentesis

- 18-gauge, 1½-inch needle
- Local anesthetic (2% without epinephrine)
- Teat cannula
- Bitch catheter
- Peritoneal dialysis catheter
- Sterile gloves
- Ethylenediaminetetraacetic acid and culture tubes
- Sterile, disposable no. 15 scalpel blade
- Sterile gauze

and peritoneal cells. L-Lactate concentration should be equal to that of systemic circulation and in normal horses, with <2 mmol/L is appropriate.

With intestinal strangulation, total protein increases initially (in the first 1–2 hours) such that the fluid is clear but more yellow. After 3 to 4 hours of strangulation, red blood cells also leak, and the fluid is more orange or pink. After 6 hours or more, white blood cells increase gradually, with the progression of intestinal necrosis. Serosanguineous fluid is indicative of small bowel strangulation and, in concert with the rest of the examination, likely indicates a need for bowel resection if possible. L-Lactate concentration in horses with strangulating small bowel lesions is greater than systemic concentrations. An L-lactate of ≥4 mmol/L or an increase in L-lactate concentration on repeat assessment is highly associated with small bowel strangulation.[26]

Inadvertent enterocentesis can occur, particularly in cases with increased abdominal pressure and/or large bowel impactions. Enterocentesis must be differentiated from intestinal rupture. Cytologic features of enterocentesis include plant material, bacteria, and debris but no polymorphonuclear cells. Moreover, the clinical condition of the horse is not consistent with rupture (septic shock). With early rupture, clinical evidence may not be evident for several hours. Cytologic examination of abdominal fluid with intestinal rupture shows neutrophils, bacteria, and bacteria that have been phagocytized by neutrophils.

Peritonitis can be classified as primary or secondary. Primary peritonitis is often idiopathic or associated with *Actinobacillus equuli* and covered in more detail elsewhere.[27] Secondary peritonitis is often associated with polymicrobial species when fluid is evaluated cytologically and on Gram stain. Degenerate neutrophils (>90%), the presence of multiple bacteria, and certainly gram-negative enteric species should alert the clinician to the possibility of compromised bowel and may warrant surgical exploration.

Blood contamination must be differentiated from internal hemorrhage or severely devitalized bowel. Blood from skin or body wall vessels and inadvertent splenocentesis can cause a bloody "tap." When centrifuged, the sample often spins clear with minor blood contamination from skin. Inadvertent splenocentesis will result in a higher packed cell volume than peripheral blood, which is caused by the erythrocyte reservoir of the spleen. All fresh blood contamination shows platelets, which are not present with blood older than 12 hours. In internal hemorrhage blood is hemolyzed such that the supernatant is reddish after centrifugation; the sample has no platelets and shows erythrophagocytosis. Ultrasonography also reveals fluid swirling in the abdomen, especially with acute internal hemorrhage of various causes.

Excess EDTA in a low-volume sample will falsely increase the total protein on refractometry (often by 0.9–1.0 g/dL higher). When performing an abdominocentesis, the clinician should shake out the EDTA from the tube to avoid this sampling error.

Abdominal surgery increases the total protein level and white blood cell count postoperatively.[28] Typically, if no enterotomy was performed, the white blood cell count increases for 4 to 7 days and returns to normal by 14 days. The total protein level may remain elevated for 3 to 4 weeks after surgery. Neutrophils appear to be nondegenerate. After an enterotomy or an anastomosis, degenerate neutrophils and occasional bacteria may be seen in the first 12 to 24 hours.[29] Subsequently, the white blood cell count remains elevated for approximately 2 weeks, but on cytologic examination the neutrophils appear to be nondegenerate and no bacteria are apparent. The total protein level remains elevated for 1 month after surgery.

TROCARIZATION

Trocarization of the cecum, or occasionally ascending colon, is useful to decompress the bowel and improve abdominal perfusion pressure ([APP]; see Table 4.5). By decreasing intraabdominal pressure, APP can be improved in cases where obstructive disorders of the large intestine are diagnosed. Trocarization provides analgesic relief by reducing visceral distention and may relieve dyspnea where significant abdominal bloating is apparent. Box 4.4 lists the materials needed for this procedure.

Trocarization is only indicated for large bowel distention. Rectal palpation, abdominal auscultation and percussion, and transabdominal ultrasound can help decipher which segment of bowel is distended. Segments of large bowel adjacent to the body wall are suitable cases. The right paralumbar fossa and cecal trocarization are ideally suited because of the fixed location of the cecal cupula in the right dorsal abdomen. Cecal tympany commonly accompanies obstructions of the ascending colon because of cecal outflow obstruction. These are ideal candidates for trocarization.

The clinician clips and prepares the site with antiseptic, infiltrating a 4 × 4-cm area of skin with a local anesthetic. With gloved hand, the clinician inserts a 14-gauge catheter with an extension tube perpendicular to the skin. The clinician places the end of the extension in water so that gas bubbles are visible when the tip of the catheter is positioned correctly. When gas is obtained, the trochar part of the catheter should be withdrawn slightly or removed to keep from lacerating the bowel. It may be necessary to reposition the catheter several times when gas is not obtained. Connecting the catheter to an extension set in water can help visualize gas egress (Fig. 4.3). Occasionally, a second operator can apply gentle pressure per rectum to facilitate gas egress. After decompression, the clinician removes the trocar and infuses an antibiotic (e.g., gentamicin or procaine penicillin) while withdrawing the catheter through the body wall.

Complications with trocarization include peritonitis and local abscessation. The horse should be observed for 24 hours for signs of peritonitis. If the practitioner suspects peritonitis, confirmation is with abdominocentesis, and systemic broad-spectrum antibiotics should be administered to the horse until the condition resolves. Serum amyloid A concentrations may be useful for monitoring the response to treatment and optimal time to cease therapy. If a local abscess develops, percutaneous external drainage with local lavage of sterile fluids is recommended.

BOX 4.4 Materials Needed for Trocarization

14-gauge, 5¼-inch catheter
Local anesthetic (2% without epinephrine)
Sterile gloves
Extension tubing
Small water container (syringe case works)

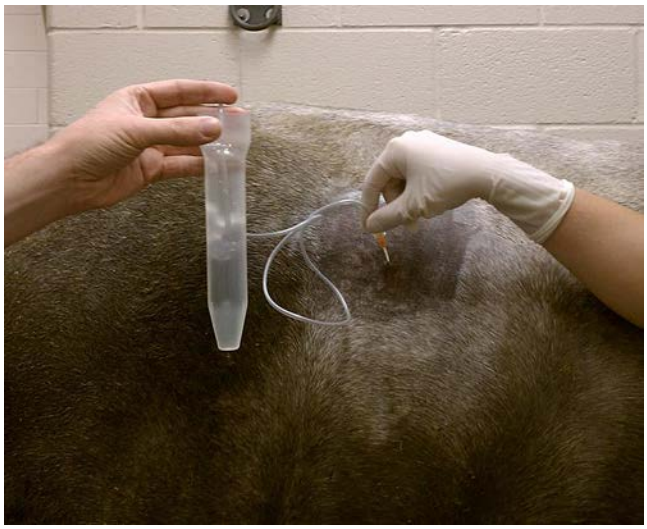


FIG. **4.3** Cecal trocarization using a 14-gauge catheter connected to an extension set in a syringe case of water.

URINARY CATHETERIZATION

Micturition disorders (lower motor neuron bladder, upper motor neuron bladder, and urethral detrusor dyssynergy) may require manual evacuation to relieve the patient and provide comfort. Measurement of urine output in adult horses is indicated in horses with oliguric renal failure or when 24-hour volumetric measurements are needed. Thorough cleansing of the urethral fossa and glans penis in males and the vulva and perineal region in mares is indicated before catheter placement. Gentle soapy water or saline with roll cotton are suitable options. In mares, urine is easily collected by placing a Foley catheter connected to collection tubing. A closed system can be fashioned by using a solution administration set and empty fluid bag. In geldings one can insert a male urinary catheter and suture it in place using a Chinese finger trap pattern. If the horse is recumbent and thrashing, leaving as little as possible of the catheter protruding to keep it from being pulled out is important. Normal horses produce 1 to 2 mL/kg per hour of urine.

Cardiovascular Critical Care

Tiffany L. Hall

REVIEW OF CARDIOVASCULAR PHYSIOLOGY

The cardiovascular system functions to deliver oxygen and nutrients to tissues throughout the body, as well as to deliver carbon dioxide to the lungs for elimination and other wastes from the tissues for detoxification and excretion from the body. It is comprised of the heart, the pulmonary circulation, and the systemic circulation, which act in coordination to deliver blood and its constituents to tissues. Through a complex interplay of neuroendocrine pathways, the cardiovascular system is able to adapt to different physiologic and disease states to meet the body's oxygen and nutrient requirements. When the cardiovascular system fails to meet tissue oxygen demands, a state of shock ensues. Support of cardiovascular function can be achieved through the administration of intravenous fluids, antiarrhythmics, inotropes, and/or vasopressors, depending on the nature of dysfunction.

The volume of blood, and therefore the amount of oxygen, delivered to tissues depends on arterial oxygen content and CO, which is defined as the amount of blood pumped out of the heart per minute and is the product of heart rate and stroke volume. Heart rate may be influenced by a number of neurologic, endocrine, and physical factors. Stroke volume is the amount of blood ejected from the heart with each contraction and is influenced by the venous return of blood to the heart (preload), the force/duration of contraction of the heart (inotropy), and the resistance to forward flow (afterload).

CO is not evenly distributed to all tissues in the body as a result of varying resistance to arterial blood flow dependent on tissue demands. Contraction of arteriole smooth muscle maintains a pressure gradient that allows blood to flow from arterioles through tissue bed capillaries, to venules, and back to the heart. SVR is related to blood pressure and is determined by vasomotor tone, or degree of vessel constriction, in the systemic circulation. The greater the SVR, the less blood flow from the heart through the systemic circulation and the greater the heart must work to achieve the same CO achieved at a lower SVR.

CARDIOVASCULAR INSUFFICIENCY: SHOCK STATES

When the cardiovascular system fails to supply sufficient oxygen to meet tissue demands, shock develops and cells shift to anaerobic metabolism. If left uncorrected, the shock state can result in irreversible cellular dysfunction. There are four major categories of shock that are differentiated by underlying pathophysiology: hypovolemic, cardiogenic, obstructive, and maldistributive.[30]

Hypovolemic shock is the most common type of shock recognized in equine patients and results from decreases in effective circulating blood volume, which may occur through external loss (e.g., diarrhea, reflux), third spacing of plasma volume, or hemorrhage. Decreased blood volume results in decreased venous return to the heart (decreased preload), reduced stroke volume, and decreased CO if heart rate cannot compensate. The mainstay of intervention is through restoration of circulating volume via intravascular fluid administration.

Cardiogenic shock is associated with impaired ventricular function as a result of myocarditis, ventricular dysrhythmias, or valvular pathology resulting in cardiac chamber enlargement.[31] A decrease in contractility (inotropy) results in reduced stroke volume and decreased CO. Therapy is directed at improving cardiac pumping through treatment of the underlying condition and use of inotropes when indicated.

Obstructive shock results from lack of blood flow through the heart or great vessels as a result of thrombosis (atrial or pulmonary), constrictive pericarditis, cardiac tamponade, or pleural space disease (e.g., pneumothorax).[32] This condition is characterized by decreased preload or increased afterload and results in circulatory failure.[33] Therapy is directed at addressing the underlying cause.

Maldistributive (vasodilatory) shock is the result of abnormal shunting of blood through microcirculation in the face of

normal CO, leading to tissue hypoxia and metabolic derangement. This may be caused by septic, anaphylactic, or neurogenic causes.[33] Central to the pathogenesis of maldistributive shock is endothelial dysfunction in response to neutrophil-generated cytokines, proteases, lipid mediators, and oxygen-derived free radicals.[34] Therapy is directed at addressing the underlying cause, reducing systemic inflammation, and use of vasopressors to improve SVR.

Regardless of the inciting cause, if shock is prolonged and severe, the terminal physiologic disturbance will manifest as maldistributive shock.[35] Successful management of shock depends on early recognition and aggressive intervention.[36]

CARDIOVASCULAR SUPPORT THERAPIES

Principles of Management

The goal of therapy in patients with cardiovascular compromise is to restore tissue perfusion and oxygen delivery. Therapies include fluid resuscitation to restore circulating blood volume, administration of vasoactive medications (inotropes/vasopressors) to improve CO and vascular tone, and directed therapies at the underlying disease condition. Fluid therapy has been discussed earlier, and the remainder of this section will focus on cardiovascular-specific therapies.

Vasoactive Drugs

Vasoactive drugs (e.g., inotropes and/or vasopressors) may be administered if tissue perfusion is inadequate following IV fluid resuscitation. Desirable qualities of vasoactive drugs include a short onset of action and rapid metabolism, so they may be quickly titrated to effect in response to changes in patient condition and administered as a CRI. Vasopressors and inotropes exert their effects through interaction with adrenergic and nonadrenergic receptors.

Adrenergic receptors targeted by vasoactive therapy include α_1-, α_2-, β_1-, and β_2-adrenergic receptors and dopaminergic receptors. α_1-Adrenergic receptor stimulation results in peripheral arterial vasoconstriction and altered metabolism as well as increased cardiac contractility.[37-39] Postsynaptic α_2-receptor stimulation results in vasodilation.[40] β_1-Adrenergic receptor stimulation results primarily in cardiac effects with increases in both heart rate (chronotropic effect) and contractility (inotropic effect). β_2-Receptor stimulation results in vasodilation of the arteries of coronary vessels, visceral organs, and skeletal muscle. Slight chronotropic and inotropic improvement after β_2-stimulation is also seen.[41] Dopaminergic stimulation improves myocardial contractility, increases heart rate, and results in peripheral vasoconstriction.[42] Nonadrenergic mechanisms include activation of vasopressin-specific receptors (chiefly V1) and effects on phosphodiesterase activity.[31] Vasopressin receptors (V1) are present throughout the vascular system and stimulation results in vasoconstriction, especially at peripheral arterioles.[43,44] During hypovolemic shock, markedly increased levels of V1 stimulation lead to significant increases in vascular resistance, which is an important mechanism for restoration of arterial blood pressure.[45]

Inotropes

Inotropic drugs increase myocardial contractility, increasing stroke volume and CO. Cardiac workload and oxygen consumption are increased, which is of concern in hypoxemic or dysoxic patients. If response to fluid resuscitation is inadequate, inotropes provide another means to increase tissue perfusion; however, these medications increase cardiac oxygen consumption, so oxygenation should be monitored.[31]

Dobutamine

Dobutamine is a synthetic catecholamine that has primarily β_1-adrenergic agonist activity with weak α- and β_2-affinity. Dobutamine is useful in patients with diminished CO or decreased central venous oxygen tension despite adequate fluid volume restoration. Beneficial effects include increased stroke volume and decreased heart rate with improved splanchnic perfusion and urine output. Dobutamine administered to foals with anesthesia-induced hypotension resulted in increased cardiac index, increased blood pressure, decreased oxygen consumption, decreased oxygen extraction ratio, and apparent maintenance of splanchnic perfusion.[46]

Vasopressors

Vasopressors increase vasomotor tone (SVR), which is reflected as an increase in MAP. Further, decreased venous compliance/capacitance increases venous return (preload), which improves CO.[37,38] Careful monitoring and titration of these medications must be performed to avoid excessive vasoconstriction, which would increase SVR (afterload), thus increasing cardiac workload and potentially decreasing stroke volume and CO.

Norepinephrine

Norepinephrine (NE) has predominantly α_1-adrenergic agonist activity, and it improves organ perfusion pressure during maldistributive and vasodilatory shock in patients nonresponsive to fluid resuscitation and inotrope administration. Resulting vasoconstriction increases cardiac afterload, potentially decreasing CO; however, this effect is countered by its β_1-effects, which facilitate increased stroke volume. Compared with dopamine, NE appears to have increased benefits in sepsis, demonstrating improved outcomes and increased splanchnic perfusion in humans and increased urine output in septic foals when combined with dobutamine.[47,48]

Epinephrine

Epinephrine is a strong α- and β-adrenergic agonist, making it a potent vasopressor. Splanchnic, renal, and coronary blood flow is decreased compared with NE.[49] Increased risk of dysrhythmia is associated with an increase in myocardial irritability, and postresuscitation myocardial depression with increased myocardial oxygen consumption is also reported.[50]

Dopamine

Dopamine is active at adrenergic (α and β) and dopaminergic receptors in a dose-dependent manner. High infusion rates of dopamine tend to result in a predominance of α-adrenergic effects and marked vasopressor response, whereas β-adrenergic effects (inotropy) predominate at lower infusion rates. Considerable controversy exists regarding the utility of dopamine, with mixed experimental evidence regarding its "renal protective" effects and meta-analysis data showing increased mortality in some subsets of patients.[3,51-53]

Phenylephrine

Phenylephrine is primarily an α-agonist resulting in increased SVR, MAP, and decreased CO. When phenylephrine is used as

a vasopressor, concurrent use of a β-agonist (e.g., NE, dobutamine) is often beneficial.

Vasopressin

Vasopressin, or antidiuretic hormone, is a regulatory peptide integral in regulating body water balance through promotion of water conservation in the collecting tubule of nephrons (V2 receptor) in response to centrally mediated detection of dehydration (osmoreceptors). In addition, vasopressin induces vasoconstriction via V1 receptors, particularly during sepsis, and human research demonstrates beneficial effects of vasopressin when used concurrently with catecholamines, such as NE.[37,50,54,55] Vasopressin works through nonadrenergic mechanisms, even in the face of moderate acidosis. This is in stark contrast to catecholamines that do not function as optimally under similar conditions. As with other vasopressors, V1 agonists may be unsuitable for use in horses that have not been volume resuscitated before administration because of a reduction in splanchnic perfusion.[56] Research in clinical equine practice indicates that endogenous vasopressin concentrations are increased in sick or septic animals compared with healthy controls, and that nonsurvivors have higher concentrations at admission compared with survivors.[55,57-62]

CARDIOVASCULAR SUPPORT STRATEGIES

Practical Approach to Fluid Resuscitation

The goal of fluid resuscitation is the restoration of tissue perfusion through increased stroke volume and improved blood pressure by correcting blood volume deficits. The key to fluid resuscitation is, therefore, balancing rapid volume expansion with monitoring to prevent fluid overload and avoiding the negative effects of oversupplementation.

There is little evidence to support one fluid resuscitation protocol over another in horses. Therefore selection of fluid type is highly subjective in most situations, and recommendations are extrapolated from human critical care. Most clinicians select a polyionic isotonic crystalloid fluid (e.g., LRS or Plasma-Lyte A) administered alone, or in combination with hypertonic saline in the presence of clinical shock symptoms. The use of hypertonic saline should be cautiously considered or avoided in cases of uncontrolled hemorrhage or where marked interstitial dehydration is likely; however, it has demonstrated a more rapid return of normal heart rate and urination in endurance horses requiring IV fluid support compared with normal saline.[63,64] Considering the risks/benefits of colloid administration, the concurrent administration of colloids with crystalloids may be indicated where symptoms of decreased plasma oncotic pressure (e.g., edema, effusions) are present or where a critical decrease in oxygen-carrying capacity is detected.

Ideally, volumes to be administered and administration rates are based on estimates of fluid deficits; however, clinical interpretation of percentage dehydration and degree of hypovolemia is insensitive and may result in oversupplementation or undersupplementation. Therefore a fluid challenge method is more commonly used in practice where a bolus of 10 to 20 mL/kg of isotonic crystalloid is administered over 30 to 60 minutes with monitoring of perfusion parameters between sequential boluses. Administration of boluses is stopped when normalization or plateau of perfusion parameters is observed or when limits to fluid resuscitation (e.g., 60–80 mL/kg total volume, CVP >15 mm Hg, declining Pao_2, peripheral edema) are reached. In most situations, an increase in circulating volume to achieve subnormal values for blood pressure are adequate to balance critical tissue perfusion while limiting the risk of fluid overload, secondary compartment syndrome, and tissue edema. Once hypovolemia is corrected, fluids may be administered at 1 to 2 times maintenance to correct the remaining fluid deficits and account for ongoing needs/losses. If limits of fluid resuscitation efforts are reached before perfusion parameters improve, vasoactive medications may be administered to increase cardiac contractility or SVR.

Practical Use of Vasoactive Medications

When normalization of fluid volume fails to correct perfusion abnormalities or limits to volume resuscitation are reached, the clinician should consider the use of vasoactive medications (Table 4.10). Inotropes and vasopressors should not be administered in patients who are hypovolemic because of a risk of tachycardia, increased myocardial oxygen demand in the face of decreased coronary perfusion, and marked vasoconstriction resulting in increased afterload and decreased CO.[53,65] The choice of which medication is most appropriate is case dependent and sometimes patient dependent; however, all medications should be titrated to effect based on cardiovascular monitoring, including serial heart rate/ECG, blood pressure, urine volume, and, when possible, CO determination. Scientific data regarding inotrope/vasopressor use under clinical conditions is limited in horses; therefore, our knowledge is based on human data and limited experimental research in horses.

Administration of an inotrope, such as dobutamine, is currently the first-line choice of vasoactive medication in equine patients, particularly when adequate CO cannot be confirmed. Administration of a vasopressor in the face of inadequate cardiac function would result in a further decrease in CO associated with increased SVR; however, if administration of low-dose dobutamine (2–5 μg/kg/min) fails to improve perfusion indices then a vasopressor may be added. Clinically, this situation is generally limited to neonatal foals with sepsis or adults with vasodilatory shock. NE appears to be the best vasopressor for use in the horse based on the available data and clinical experience, and data would suggest that concurrent administration with dobutamine has a favorable effect on blood pressure and renal perfusion while preserving splanchnic circulation.[47,53,66] If the patient's cardiovascular status remains poor, administration of vasopressin may be considered, especially in the case of septic shock, during which the body's responsiveness to catecholamines may be reduced.[37,67] Research regarding the administration of exogenous vasopressin in the equine patient is limited, but preliminary data support its use.[68]

Therapeutic Goals and Monitoring

The primary goal of the criticalist related to the cardiovascular system is to restore tissue perfusion and oxygen delivery. Early goal-directed therapy (EGDT) outlines management procedures to optimize global tissue perfusion and oxygenation in critical patients and was once considered revolutionary in human sepsis treatment to improved patient outcomes; however, recent clinical trials show no benefit over routine care.[69] Research related to EGDT in veterinary patients is limited.[70,71]

Dysfunction of the cardiovascular system may be identified clinically through abnormal mentation or weakness,

increased heart rate, weak peripheral pulse quality, cool extremities, prolonged jugular refill time, altered mucous membrane color or capillary refill time, and/or decreased urine production. Clinicopathologic abnormalities reflecting poor tissue perfusion include elevated L-lactate concentrations, increases in cardiac troponin I activity, decreased central venous oxygenation, and increased urine SG. The evaluation of each of these and other cardiac monitoring modalities are described in this section.

Physical Examination Parameters

Positive clinical response to therapies are indicated by improved patient mentation and responsiveness to the environment, normalization of the heart rate, and the presence of pink mucous membranes with a capillary refill time of 1 to 2 seconds. Further clinical indicators of adequate tissue perfusion include warming of the extremities, palpation of strong peripheral pulses, and improved jugular refill. In the normal standing horse, the jugular vein should fill quickly in response to occlusion of the vein in the middle of the neck. The presence of jugular pulsation may be a crude indicator of elevated CVP, and thus fluid overload, when occlusion of the vein eliminates jugular pulsation.[72] Other clinical evidence of fluid overload includes elevated respiratory rate, weight gain, and subcutaneous-dependent edema.

Urine Production

Urine production is considered a reflection of renal blood flow and hence CO and serves as an indication of adequate organ and tissue perfusion.[73] Therefore when urine production remains low (< two thirds of fluid volume being administered) in a euvolemic hypotensive horse, the administration of inotropes/vasopressors should be considered. However, the urine output and glomerular filtration rate are unreliable indicators of renal function in humans receiving vasopressors. Please see the section Renal Critical Care later in this chapter for additional information.

L-Lactate

L-Lactate is the terminal product of anaerobic glycolysis, and increases in blood lactate concentration in the horse are most often the result of inadequate delivery of oxygen to peripheral tissues resulting from hypovolemia, hypoxemia, anemia, or decreased perfusion pressure (type A lactic acidosis).[74,75] Other causes of hyperlactatemia include abnormal tissue utilization of oxygen, including mitochondrial dysfunction, or abnormal clearance of lactate (type B lactic acidosis).

Lactate measurement is useful clinically in monitoring the response to fluid therapy and vasoactive drugs in patients with cardiovascular dysfunction. Decreases in plasma lactate concentration temporally follow improvements in cardiovascular performance; therefore, the trend in serial plasma lactate concentration is most clinically useful. In situations of appropriate volume restoration in which lactate concentration remains increased, unresolved inflammatory stimulus or uncontrolled sepsis should be considered.

Arterial Blood Pressure

Arterial blood pressure is the product of CO and SVR. Mean blood pressure, not systolic or diastolic blood pressure, is considered the driving perfusing pressure for tissue and organs.[73] Arterial blood pressure can be measured by direct (invasive) and indirect (noninvasive) means, as described earlier in this chapter. Trends in arterial blood pressure, as opposed to individual readings, combined with other indicators of perfusion (examination and urination) should be used to guide therapy.

Central Venous Pressure

CVP is determined by central venous blood volume, muscular tone of the venous system, and the balance between venous

TABLE **4.10** **Useful Drugs in Cardiovascular Critical Care**

Type	Dose	Comments
Vasopressors		
Norepinephrine	0.05–1 μg/kg/min IV	—
Epinephrine	0.02–0.05 mg/kg IV	—
Dopamine	1–5 μg/kg/min IV 5–10 μg/kg/min IV >10 μg/kg/min IV	Renal dopaminergic-1 receptors β_1-Adrenergic stimulation α-Adrenergic stimulation
Phenylephrine	3 μg/kg/min IV over 15 min	—
Vasopressin	0.4–0.8 units/kg IV	—
Inotropes		—
Dobutamine	1–20 μg/kg/min IV	—
Plasma volume expansion		—
Crystalloids		
Isotonic	50–100 mL/kg IV	—
Hypertonic saline (7.2%)	4–8 mL/kg IV	—
Colloids		—
Plasma	5–10 mL/kg IV	—
Hetastarch 6% Tetrastarch 6%	5–10 mL/kg IV	—

IV, intravenously.

return and CO. CVP is an estimate of right atrial pressure and cardiac preload. It is used to guide fluid therapy, because normalization of CVP may reflect success of fluid volume resuscitation, whereas elevations in CVP may reflect volume overload and limits of fluid resuscitation. As with measurements of arterial blood pressure, the trend in CVP seen through repeated monitoring is most informative.

Venous Blood Gas

Arterial blood gas values are required to assess pulmonary gas exchange, as described in the later section Respiratory Critical Care; however, in patients with cardiovascular compromise in which severe hypoperfusion may be present, tissue level hypoxemia, hypercapnia, and acidemia are more accurately represented in central venous blood. Central venous oxygen saturation is a reflection of oxygen delivery and consumption by tissues and is used in monitoring the efficacy of the cardiovascular system. Decreases in venous oxygen saturation may reflect decreased respiratory function, decreased CO/tissue perfusion, or increased oxygen demand. In goal-directed therapy, normalization of central venous oxygenation serves as a therapeutic target.

Colloid Osmotic Pressure

COP, also referred to as oncotic pressure, results from the osmotic force created by macromolecules within the intravascular compartment and is essential to retaining appropriate intravascular volume. Proteins and colloid molecules are sufficiently large in mass to limit permeability across the vascular endothelium and retain water within the vasculature by virtue of their osmotic draw. In addition, the Gibbs-Donnan effect, in which sodium cations are attracted to negative residues on protein (albumin), adds further to the intravascular osmotic draw.

COP can be measured or calculated, with reasonable agreement in healthy but not hospitalized horses. Because albumin is the major contributor to COP, alterations in the albumin-to-globulin ratio alter COP at any given total plasma protein concentration. Direct measurement of COP is necessary after synthetic colloid (hetastarch) administration because these starch molecules have considerable osmotic effect but are not measurable by refractometry.

Electrocardiogram

Indications for ECG monitoring of equine patients with cardiovascular compromise include dysrhythmias, marked electrolyte abnormalities (e.g., hyperkalemia), and during vasoactive drug therapy. The base-apex lead system is most commonly used in equine medicine, and telemetry units allow remote monitoring of horses free in stalls. A more complete review of the cardiovascular system and its monitoring is available in Chapter 9.

Cardiac Output Measurements

The use of advanced technologies such as lithium dilution, indicator dilution, bioimpedance, and pulse contour analysis for assessment of CO has been extensively reviewed elsewhere.[76] These techniques are impractical in the clinical setting or have not been validated for the horse; however, echocardiography (see Chapter 9) may be used in calculating CO. Volumetric determination (four-chamber and bullet methods) and right ventricular outflow tract Doppler are reliable, accurate, and noninvasive means of determining CO in horses.[77]

Respiratory Critical Care

Tiffany L. Hall

REVIEW OF RESPIRATORY PHYSIOLOGY

The major function of the respiratory system is gas exchange and, in conjunction with the cardiovascular system, to deliver oxygen to the tissues and facilitate carbon dioxide elimination. Table 4.11 shows expected arterial blood gas indices from normal horses. The respiratory control center maintains Pao_2 and $Paco_2$ within a narrow homeostatic range despite the body's wide variety of demands. Under normal conditions the primary driving force of alveolar ventilation is changes in $Paco_2$, which are sensed by chemoreceptors in the brainstem.[78] Changes in Pao_2 are sensed by peripheral chemoreceptors in the carotid and aortic bodies and also have an impact on alveolar ventilation but typically alter respiratory rate or effort only in states of marked hypoxemia.[78]

The basic components of the respiratory system are the upper airways, the sequentially branching respiratory passageways, and the alveolar-capillary membrane. The upper airways and respiratory passageways are not involved in gas exchange; therefore, they are referred to as *anatomic dead space*.[78] The alveolar-capillary membrane is the primary region that participates in gas exchange, although some areas may not be equally perfused and ventilated, resulting in *physiologic dead space*.[78] The alveolar-capillary membranes form a dense network within the alveoli, allowing for maximum exchange of oxygen and carbon dioxide. Factors that affect the rate of gas diffusion include thickness and surface area of the membranes, diffusion coefficient of the gas, and the gas pressure difference between the two sides of the membrane.[78,79]

In the body, oxygen diffuses down a pressure gradient from the alveolar space to the pulmonary capillaries to release within tissue capillary beds. Oxygen in the blood is either bound to hemoglobin (Sao_2) in the erythrocyte or dissolved (Pao_2) in plasma, with approximately 97% of total blood oxygen content (Cao_2) bound to hemoglobin under normal conditions.[78,79] The oxygen-hemoglobin dissociation curve depicts the association between partial pressure of oxygen in the blood (Pao_2) plotted on the *x*-axis and percentage (%) oxygen saturation of hemoglobin (Sao_2) on the *y*-axis. Factors such as pH, temperature, carbon dioxide levels, and the concentration of 2,3-diphosphoglycerate alter the hemoglobin affinity for oxygen and, therefore, the ability of oxygen to be

TABLE **4.11** **Normal Range of Arterial Blood Gas Values for Adult Horses**

Variable	Normal Range
pH	7.4 ± 0.2
$Paco_2$	40 ± 3 mm Hg
Pao_2	94 ± 3 mm Hg
Base excess	0 ± 1 mm Hg
Spo_2	98–99%

From Aguilera-Tejero E, Estepa JC, Lopez I, et al. Arterial blood gases and acid-base balance in healthy young and old horses. *Equine Vet J*. 1998;30:352.

carried to and unloaded at the tissue level.[78,79] Disorders that affect the binding of oxygen to hemoglobin have a tremendous impact on oxygen delivery to the tissues associated with a marked decrease in total oxygen content (Cao_2). As such, Pao_2 is primarily an indicator of pulmonary gas exchange within the lungs, whereas oxygen extraction ratios and venous oxygen indices are better predictors of tissue oxygenation.

RESPIRATORY INSUFFICIENCY: HYPOXEMIA AND HYPERCAPNIA

Respiratory insufficiency leading to hypoxemia, a decrease in oxygen content in arterial blood (low Pao_2), occurs for one of five reasons: decreased inspired oxygen content (Fio_2), diffusion barrier impairment within the lungs, alveolar hypoventilation, right-to-left shunting of blood, and/or ventilation-perfusion (V/Q) mismatch. Hypercapnia (elevated $Paco_2$) results from insufficient elimination of carbon dioxide from the blood to the alveoli, or alveoli to the atmosphere, and is commonly observed in cases of hypoventilation and V/Q mismatch. For practical purposes decreased inspired oxygen is a very rare primary cause of hypoxemia because horses breathing room air inspire sufficient quantities of oxygen to maintain adequate dissolved oxygen fractions.

Diffusion impairment is an uncommon cause of hypoxemia or hypercapnia in horses at rest because pulmonary disease must be very severe before gas exchange is limited, which is caused by a high functional residual capacity of the lungs. Diffusion is impaired because of decreased surface area or increased thickness of the alveolar-capillary membrane, as might occur in pulmonary fibrosis. Carbon dioxide diffuses more rapidly than oxygen (20-fold). Thus, hypoxemia is observed before hypercapnia.

Hypoventilation results in both hypoxemia and hypercapnia and occurs when there is a reduction in the volume of air entering and exiting the alveoli per minute (decreased alveolar ventilation), resulting in decreased gas exchange. Metabolic production of CO_2 is fairly constant under normal metabolic circumstances, so $Paco_2$ is primarily controlled by its rate of elimination through the lungs and, therefore, is an accurate reflection of alveolar ventilation. Hypoventilation may occur with disorders of the central nervous system ([CNS]; e.g., trauma, HIE, pharmaceutical drugs), thoracic cavity (e.g., pleural space disease, pain), respiratory tract (e.g., obstruction), or neuromuscular disease (e.g., botulism).[78,79]

Hypoxemia caused by a right-to-left shunt occurs when blood bypasses the pulmonary circulation and is, therefore, not oxygenated within the lungs. This deoxygenated blood mixes with oxygenated blood in the left heart resulting in hypoxemia. Shunting may be extrapulmonary, such as a right-to-left cardiac shunt (e.g., tetralogy of Fallot), or intrapulmonary, such as occurs with severe V/Q mismatch (e.g., ARDS).

A V/Q mismatch is the most common cause of hypoxemia in the horse and occurs when alveolar ventilation and blood flow are not closely matched, resulting in inefficient gas exchange. V/Q mismatch may result from all forms of pulmonary disease. A high V/Q mismatch occurs when regions of the lung are ventilated but not perfused, such as occurs with pulmonary thromboembolism.[78,79] Conversely, a low V/Q mismatch occurs when regions of the lung are perfused but not ventilated, as with bronchopneumonia and consolidation.[78,79] If V/Q mismatch is severe, the result is a right-to-left intrapulmonary shunting of blood.[78,79]

Hypoxemia is only one of several possible causes of oxygen deficiency at the tissue level, such as a condition referred to as *hypoxia*. Tissue hypoxia can occur in the face of normal respiratory function as a result of any condition that causes decreased oxygen delivery to the tissues or abnormal oxygen utilization within the tissues, including decreased CO, decreased blood oxygen-carrying capacity, peripheral arteriovenous shunting, mitochondrial dysfunction, increased peripheral oxygen consumption, and/or hypermetabolic conditions (e.g., hyperthermia, seizures, sepsis).[78,79]

APPROACH TO THE PATIENT WITH RESPIRATORY DISTRESS

Respiratory dysfunction may be the primary reason that a veterinarian is consulted, or it may occur while a horse is treated for another problem. *Respiratory distress* is defined as an inappropriate degree of breathing effort based on an assessment of respiratory rate, rhythm, and character. Causes of respiratory distress may be respiratory or nonrespiratory in origin. The physiology of this condition is discussed elsewhere (Chapter 8).

Upper respiratory disorders that result in respiratory distress usually do so because of respiratory tract obstruction (Box 4.5). Complete upper airway obstruction is a true emergency because inspiration against a closed airway results in the inability to inspire and marked negative intrathoracic pressures leading to pulmonary edema, which can be fatal.[84] Diagnosis is usually based on physical examination and endoscopy of the upper airway. Horses with upper airway obstruction may require an emergency tracheotomy and additional therapies, depending on the primary cause of the obstruction.

Lower airway diseases that may be associated with respiratory distress are listed in Box 4.6. Diagnosis is based on a thorough physical examination, thoracic imaging, transtracheal aspirate, and arterial blood gas analysis. Treatment depends on the nature of the primary disorder and may include antimicrobial agents, antiinflammatory agents, oxygen administration, or thoracic drainage as indicated. Diagnosis and treatment of specific disorders are described in Chapter 8.

Nonpulmonary causes of respiratory distress include anemia, compensation for metabolic acidosis, pain, anxiety, and hyperthermia. Evaluation of nonpulmonary causes of respiratory distress include a thorough history, physical examination, and blood work. After the initial evaluation further diagnostic testing might include endoscopy, ultrasound, or radiographs.

Oxygen Therapy

Nasopharyngeal administration of oxygen is the most common form of respiratory support in horses suffering from hypoxemia (Fig. 4.4). Oxygen supplementation is indicated when the Pao_2 falls below 60 mm Hg or $Sao_2 < 90\%$.[80] Research demonstrates a significant and dose-dependent increase in Pao_2 and Sao_2 with increasing flow rates of oxygen to a maximum of 10 to 20 L/min, beyond which airway irritation is observed.[81,82] Although adequate delivery of oxygen to tissues is essential, oxygen is also toxic when in excess through production of reactive oxygen species. Serial arterial blood gas measurements will aide in determining the response to treatment and ability to decrease supplementation, with the goal of providing

BOX 4.5 Upper Airway Disorders That May Be Associated with Respiratory Distress in Adult Horses

NASAL DISORDERS
- Trauma, foreign body
- Hemorrhage or hematoma
- Neoplasia
- Abscess
- Granuloma
- Amyloidosis

PHARYNGEAL DISORDERS
- Neoplasia
- Subepiglottic or pharyngeal cyst
- Persistent dorsal displacement of the soft palate
- Trauma, foreign body
- Abscess

LARYNGEAL DISORDERS
- Laryngeal paralysis
- Laryngeal hemiplegia
- Trauma, foreign body
- Edema
- Arytenoid chondritis
- Epiglottitis and epiglottic granuloma

MISCELLANEOUS
- Guttural pouch enlargement
  - Empyema
  - Tympany
  - Mycosis and hemorrhage
- Head swelling: edema, cellulitis, anaphylactic shock, bilateral jugular occlusion

BOX 4.6 Lower Airway Disorders That May Be Associated with Respiratory Distress in Adult Horses

PULMONARY DISORDERS
- Pulmonary edema (cardiogenic or neurogenic)
- Acute interstitial pneumonia
- Chronic interstitial pneumonia including equine multinodular pulmonary fibrosis (equine herpesvirus 5) infection
- Aspiration pneumonia
- Abscessing or coalescing bronchopneumonia
- Acute respiratory distress syndrome/acute lung injury
- Silicosis
- Smoke inhalation
- Tracheal/bronchial foreign body

EXTRAPULMONARY DISORDERS
- Pleural effusion (hydrothorax, pyothorax, chylothorax, and hemothorax)
- Thoracic trauma
- Pneumothorax (open, closed, and tension)

the lowest flow rates (lowest Fio_2) possible to achieve adequate oxygenation.

Inhalational Therapy

Inhalational therapy is beneficial in respiratory disorders of the lower airways, including bronchopneumonia and inflammatory airway conditions. Mucolytics, bronchodilators, and antimicrobials (including amikacin, gentamicin, and sodium ceftiofur [Naxcel]) may be administered by ultrasonic nebulization.[83,84] Inhalers may be used for the administration of bronchodilators, mast cell stabilizers, and corticosteroids (CSs) in horses with inflammatory airway conditions such as inflammatory airway disease or recurrent airway obstruction (RAO).[85,86] Advantages of inhalation therapy include a localized delivery to the target site allowing for higher concentrations at lower doses administered, as well as reduced incidence of adverse systemic effects.

Pharmacologic Ventilation

Hypoxemia and hypercapnia resulting from hypoventilation may respond to the administration of medications that stimulate respiration centrally. Caffeine, doxapram, and theophylline are three such medications that may benefit equine patients with hypoventilation secondary to head trauma, encephalitis, or hypoxic-ischemic encephalopathy. Caffeine is most frequently used in practice because of ease of administration, cost, and wide therapeutic index. Doxapram has a short duration of action requiring frequent or CRI administration, which limits use despite evidence of improved ventilation compared with caffeine.[87] Disadvantages of doxapram use include an increase in myocardial oxygen consumption and cost. Theophylline is similar to caffeine; however, it has a narrow therapeutic index with adverse effects including colic, seizure, tachycardia, and sudden death.[80]



FIG. **4.4** Horse with a nasal oxygen cannula. Suturing to the nostril and affixing the tubing to the halter helps maintain the cannula in place.

Acute bronchoconstriction and decompensation may be seen with severe RAO, inhalation of noxious gases, or other chemical irritants and can be managed with bronchodilating agents. β_2-Agonists (e.g., inhaled albuterol [720 µg per 450-kg horse]) and anticholinergics (e.g., intravenous glycopyrrolate) are beneficial. Atropine may be used as a rescue therapy only. Bronchial inflammation can both trigger and perpetuate

bronchoconstriction and warrants the justified use of inhaled or systemic glucocorticoids in some cases.

Mechanical Ventilation

Mechanical ventilation, when administered in a hospital setting by experienced clinicians, has resulted in improved outcomes in foals[80] and may be beneficial in adults.[88] Indications for mechanical ventilation include marked hypoxemia or hypoventilation (pHa <7.3 with $Paco_2$ >65 mm Hg or Pao_2 <60 mm Hg) despite maximal medical therapy, and fatigue or excessive work of breathing. Clinical diagnoses in equine patients that may benefit from mechanical ventilation include botulism, hypoxemic-ischemic encephalopathy, or ARDS. Long-term mechanical ventilation techniques in adult horses are poorly described and represent a significant challenge for equine critical care.

Therapeutic Goals and Monitoring

The goals in supporting respiratory function are recognition of improved respiratory rate and effort, improved oxygen indices, and normalization of oxygen and carbon dioxide blood concentrations.

Blood Gas Analysis

Blood gas analysis is the most common method for assessment of pulmonary function and acid-base status in horses that are critically ill. Arterial samples may be obtained from the facial artery, transverse facial artery, or lateral metatarsal artery.[89] In the clinical setting, venous samples are best collected from a catheter placed in the cranial vena cava or jugular vein. Correct interpretation of the information obtained from blood gas analysis allows the clinician to make appropriate decisions regarding diagnosis, therapy, and prognosis related to respiratory function and acid-base status.

Samples for blood gas analysis must be collected and handled appropriately to ensure accurate results. A small amount of heparin is used to coat the hub of the needle before aspiration of the sample, which should be stored anaerobically after collection by removing the needle and placing a syringe cap over the tip of the syringe. Prolonged exposure to air or bubbles in the syringe can result in a decrease in $Paco_2$ and an increase in Pao_2 as the sample equilibrates with room air in the bubble.[79,89] A sample stored at room temperature should be analyzed within 10 to 15 minutes. Delayed analysis may result in an increased $Paco_2$, decreased pH, decreased glucose, and increased lactate as blood cells continue to metabolize nutrients.[90]

Blood Gas Interpretation

Respiratory Function

A systematic evaluation of blood gas values includes consideration of pH, Pao_2, arterial and venous oxygen saturation of hemoglobin (Sao_2, Svo_2), $Paco_2$, and bicarbonate concentration. The partial pressure of oxygen dissolved in arterial blood (Pao_2) is a reflection of pulmonary oxygenating capability and is not affected by hemoglobin-bound oxygen. Causes of low Pao_2 (hypoxemia) are discussed in the preceding sections. The partial pressure of CO_2 dissolved in arterial blood ($Paco_2$) is a reflection of the balance between alveolar minute ventilation and metabolic CO_2 production.[78,79,89]

The first step in interpreting the blood gas information with respect to pulmonary function is to assess ventilatory status. Increased $Paco_2$ indicates hypoventilation, whereas decreased $Paco_2$ indicates hyperventilation, the presence of which cannot be accurately assessed based on physical examination alone. Hypoventilation may be caused by central apnea (CNS disease), obstructive apnea (upper respiratory tract disease), increased dead space ventilation (shunt and decreased CO), reduced lung compliance (low lung volume), respiratory muscle weakness or fatigue, poor control when mechanical ventilation is used, thoracic pain, or abdominal distention. Hyperventilation, with decreased $Paco_2$, may result from pain, hypoxemia of any cause, pulmonary disease, or neurologic disease.

Oxygenation status is then determined by the assessment of Pao_2 and Sao_2 in light of the partial pressure of alveolar oxygen (PAo_2) and hemoglobin levels. PAo_2 is influenced by the fraction of inspired oxygen (Fio_2), atmospheric pressure, and $Paco_2$. Pao_2 is typically five times the Fio_2 in the healthy standing horse; therefore, in a patient breathing room air at sea level (Fio_2 = 21%), expected Pao_2 concentration is approximately 100 mm Hg with an Sao_2 >93%.

Calculation of the alveolar-arterial oxygen gradient (A-a gradient) is helpful in evaluating causes of hypoxemia and hypercapnia.[78,79] In the normal horse breathing room air, the Pao_2 should be only slightly less than PAo_2 with an A-a difference of 5 to 10 mm Hg. In patients with a normal A-a gradient, hypoxemia and/or hypercapnia are the result of alveolar hypoventilation. Hypoxemia and hypercapnia in patients with an increased A-a gradient may be associated with V/Q mismatch, right-to-left shunt, or diffusion impairment; however, nonpulmonary causes may also be present, including an imbalance of oxygen demand and delivery, hypermetabolism, or overfeeding.

The causes of hypoxemia can be further differentiated through the administration of supplemental oxygen and evaluation of oxygen saturation and extraction ratios. When the administration of oxygen fails to increase Pao_2 to 100 mm Hg, right-to-left shunting or massive pulmonary thromboembolism should be suspected and appropriate diagnostic testing used. Improvement of Pao_2 above 100 mm Hg with oxygen therapy suggests hypoxemia associated with V/Q mismatch or a diffusion disturbance. Venous blood gas analysis has limited usefulness for evaluation of respiratory tract disease; however, Svo_2 may be used in calculating the oxygen extraction ratio, which provides some information about tissue oxygenation in the horse. Oxygen extraction ratios greater than 30% may reflect increased oxygen consumption (e.g., exercise, hypermetabolism) or inadequate oxygen delivery to tissues associated with hypoxemia, anemia, or cardiovascular dysfunction.

Acid-Base Status

An interpretation of blood gas analysis is not complete without assessment of the patient's acid-base status. In a simplified approach to acid-base status, the clinician first determines whether the blood pH is normal (approximately 7.40) or whether acidemia (pH <7.36) or alkalemia (pH >7.44) exist. There are four primary acid-base disturbances that may occur in a patient based on interpretation of the blood gas: respiratory acidosis, resulting from hypoventilation and reflected by increased $Paco_2$; respiratory alkalosis, resulting from hyperventilation and reflected by decreased $Paco_2$; metabolic acidosis (decreased HCO_3^-); and metabolic alkalosis (increased HCO_3^-). These may exist as sole disorders or in combination (mixed disorders). A complete discussion of acid-base disorders and the approach to critically evaluating acid-base status is beyond the scope of this text, and the reader is referred to

additional resources regarding the physiochemical approach to evaluation of acid-base status in animals.[91,92]

Pulse Oximetry

A useful adjunct to arterial blood gas analysis is pulse oximetry, which is a technique that relies on the reflection of different wavelengths of light to differentiate oxygenated and deoxygenated hemoglobin reported as a percentage of oxygenated hemoglobin (Spo_2). Generally, an Spo_2 greater than 91% is considered indicative of an arterial oxygen level within physiologically normal limits.[79,93] Pulse oximetry has a limited sensitivity for determining changes in pulmonary gas exchange at ranges above 90% hemoglobin saturation; however, Spo_2 below 91% represents increasingly severe reductions in arterial oxygen levels.[79,93] The accuracy of Spo_2 may be influenced by several factors including pigmented skin, hypoperfusion at the measurement site, anemia, hypothermia, and motion. False readings of Spo_2 may occur in the presence of carboxyhemoglobin and methemoglobin, in which cooximetry is the preferred analytical method. Because of the limitations of pulse oximetry, response to treatment in a patient with critical hypoxemia is best monitored with arterial blood gas analysis.

Gastrointestinal Critical Care

Tiffany L. Hall

The primary role of the gastrointestinal tract is digestion of food so that nutrients including proteins, vitamins, minerals, and fluids may be absorbed into systemic circulation for use throughout the body. Normal function of the gastrointestinal tract includes appropriate motility, balanced microbiota, digestion, and absorption through complex interactions among the nervous system, enteric nervous system, endocrine system, and musculature of the gastrointestinal tract. The gastrointestinal mucosa forms a barrier between the body and a luminal environment, which not only contains nutrients but also is laden with potentially hostile microorganisms and toxins. Thus normal function also requires that nutrients be transported across the epithelium while excluding passage of harmful molecules and organisms.

GASTROINTESTINAL DYSFUNCTION

Complications related to the gastrointestinal tract are common in the critically ill horse and include inappetence, pain, endotoxemia/SIRS, ileus, enteritis or colitis, malassimilation (malabsorption/maldigestion), and protein-losing enteropathy. The details of many of these conditions are discussed in Chapter 12. Inappetence is probably the most common complication in the critical equine patient and will be the focus of the remainder of this section. Decreased feed intake may be caused by a mechanical inability to eat (dysphagia) or a loss of appetite associated with a variety of conditions and diseases including pain, stress, systemic infection, or loss of palatability associated with medications. The regulation of appetite is an immensely complex process involving the gastrointestinal tract, central and autonomic nervous systems, and many hormones.

Severe illness or injury has been associated with hypermetabolism characterized by a pronounced catabolic state that is made worse by a patient's inappetence. Inflammatory mediators increase basal metabolic rate, contributing to the inefficient use of oxygen and calories, and may also modulate the body's response to starvation.[94,95] Negative energy balance, in which nutritional intake does not meet the energy demands of the body, depletes the body of structural and functional proteins, impairing wound healing, decreasing immune function, and altering many other normal physiologic functions. The effects of an ongoing, profound negative energy balance may manifest in musculoskeletal weakness, disruption of the gastrointestinal barrier (villous blunting), sepsis, multiorgan dysfunction, and even death.

EVALUATION OF GASTROINTESTINAL FUNCTION

Methods used to recognize gastrointestinal dysfunction in the critically ill horse include changes in the physical examination, abdominal ultrasonography, and clinicopathologic testing. Serial physical examinations will identify changes in vital parameters, mentation, behavior, fecal production, and abdominal size. Transabdominal ultrasound (2.5- to 3.5-mHz transducer) can be used to evaluate the anatomic location, contents, wall thickness, and motility of various regions of the intestine. Classic patterns identified by ultrasound are useful in narrowing a list of differential diagnoses for gastrointestinal disorders. The identification of peritoneal fluid by ultrasound warrants abdominocentesis and fluid analysis to better evaluate the disease process and can help guide therapy and decisions regarding prognosis. Clinical pathologic abnormalities are often nonspecific regarding gastrointestinal disorders but may reflect the presence of systemic inflammation, major fluid shifts or deficits, decreased tissue perfusion, acute protein loss, negative energy balance (e.g., disorders of glucose and triglycerides), and/or electrolyte disturbances. See Chapter 12 for a more in-depth discussion of the use of complete blood count (CBC), biochemistry, ultrasonography, and radiography for disorders of the gastrointestinal tract.

Assessment of the nutritional status of the critically ill equine patient at presentation and at regular intervals during hospitalization is necessary to identify patients that would benefit from early nutritional support. The most practical methods for evaluation of nutritional status and body fat composition in horses are monitoring of body weight (e.g., scale or weight tape) and body condition score (BCS), which is a subjective, semiquantitative method of evaluating body fat and muscle mass by visual inspection and palpation of certain areas of the body. BCS is positively correlated to body fat but not to weight or height. A nine-point scale was described by Henneke in which 1 is extreme emaciation, 9 is obese, and 4 to 6 are ideal.[96] The appropriate assessment and monitoring of nutritional status would include both the body weight and BCS.

NUTRITIONAL SUPPORT

Early nutritional support is indicated in underweight or obese horses, those with highly catabolic disease states (e.g., pleuropneumonia), or in patients that are unable to eat (e.g., dysphagia, ileus). Healthy horses at rest may have feed withheld for 2 to 3 days without significant consequence; however, all hospitalized horses may benefit from early nutritional intervention. Numerous reports in human critical care document

the benefits of early nutritional support because it hastens recovery, diminishes complications of the critically ill, and decreases length of hospitalization.[94,95,97] The goals of early nutritional support are to maintain host defenses and preserve lean body mass while avoiding oversupplementation.

The nutritional requirements of the critically ill adult horse have not been determined and are influenced by size, age, disease state, and metabolic stress. Maintenance requirements for healthy adult horses at rest are estimated to be 30 to 40 kcal/kg per day.[98-100] More research is available in critically ill foals whose resting energy requirements are approximately 50 kcal/kg per day and increase as the foal's clinical condition improves.[101]

Enteral feeding is preferred to PN if the gastrointestinal tract is even partially functional because local delivery of feed bulk and nutrients improves intestinal motility, maintains mucosal integrity, protects against bacterial translocation, and supports organ and immune function.[94,95,97] Even if a horse cannot tolerate enteral feeding sufficient to meet maintenance energy demands, lesser amounts of enteral nutrition are shown to be beneficial while the remainder of energy requirements are met through parenteral means (sometimes termed *trophic feeding*). It can be difficult to determine caloric intake for a horse on a voluntary feeding plan; however, weighing the feed and hay offered compared with that left behind by the patient allows one to estimate the number of ingested calories. If consumption is below 75% of the maintenance requirements for 48 hours, or if the horse is anorexic or dysphagic, feeding by nasogastric tube is recommended.[99,100] See Chapter 5 for a discussion of different enteral diet regimens and methods of administration. It is recommended that enteral feeding begin gradually, with a goal of reaching maintenance requirements over 3 to 7 days, because rapid changes may result in colic or diarrhea.[99,100]

PN is indicated for aged animals off feed, pregnant or lactating mares off feed, horses with a BCS <4, critically ill breeds at risk for hyperlipidemia/hyperlipemia, and critical patients who cannot tolerate enteral feeding because of gastrointestinal dysfunction.[102-104] PN is a combination of nutrients designed to meet the patient's energy requirements through IV administration and may consist of supplementation with dextrose alone or in combination with amino acids and/or lipids. Other components added to PN solutions include vitamins (A, D, E, and B complex) and minerals. Macrominerals such as potassium, calcium, and magnesium are best added to crystalloid fluids. The reader is referred to Chapter 5 for a complete discussion of PN.

Complications associated with PN administration include hyperglycemia, hyperlipidemia, hypokalemia, hypophosphatemia, hypomagnesemia, and thrombophlebitis.[105,106] Hyperglycemia is the most common complication reported in horses and foals administered PN and may be avoided through slow introduction of the fluid, beginning at 25% of the target rate. This allows an appropriate endogenous insulin response to occur in response to dextrose supplementation. Blood glucose levels should be monitored every 4 to 6 hours to avoid hyperglycemia, glucosuria, and osmotic diuresis. A sudden onset of hyperglycemia in a patient that had been tolerating PN can indicate the presence of complications such as infection or sepsis. If hyperglycemia persists, exogenous insulin therapy should be considered. Parenteral formulations containing lipids should be avoided in horses with hyperlipidemia, hyperlipemia, or severe liver disease and used with caution in horses with severe endotoxemia or SIRS. Hypokalemia can result from decreased intake of food and the delivery of a high concentration of dextrose causing pancreatic insulin release; thus, potassium should be supplemented in horses receiving PN.

GASTROINTESTINAL MOTILITY SUPPORT

The promotion of gastrointestinal motility and function in the postoperative patient is discussed elsewhere in this text (see Chapter 12). However, maintenance of normal blood volume, physiologic electrolyte concentrations, and gastrointestinal blood flow will aide in maintaining gastrointestinal integrity and function. The reader is referred to other sections of this text for further information regarding support of normal gastrointestinal tract function.

Renal Critical Care

Samuel D. Hurcombe

Primary renal disease in horses may be underdiagnosed in adult horses. Standard testing using blood work (blood urea nitrogen [BUN] and creatinine concentrations) may only increase once advanced or severe acute disease is established. Horses with critical illness are superb candidates for secondary renal disease, which in many cases critically ill horses may be underperfused, in various shock states, and frequently administered nephrotoxic therapies.

Horses with fluid deficits, severe myopathies, hemolysis, and septic/endotoxemic shock are at high risk of renal disease. Optimizing renal perfusion, glomerular perfusion, and tubular flow should be of highest importance in supporting renal function. Electrolyte imbalances, acid-base disorders, urine output volumes, and neurohormonal regulation of splanchnic blood flow should be evaluated and considered in the development of all fluid therapy strategies in the renal patient.

Evaluation of renal function through clinicopathologic testing of blood and urine are best performed before fluid therapy. Provision of IV fluids may lead to changes in blood electrolyte composition and SG findings.[107]

GENERAL PRINCIPLES

Horses with renal disease should be assessed for their ability to produce urine before fluid therapy. Low-volume resuscitation (<40 mL/kg/day) should be used until it is known that the patient can tolerate maintenance and the previously mentioned maintenance IV fluid rates. Monitoring body weight and/or urine output volumes is the most practical method for determining volume infusion tolerance.

Caution should be exercised in the anuric patient and those with low-volume oliguric renal failure. Patients with uremic encephalopathy and intolerance to IV fluids have a grave prognosis.

If the patient demonstrates volume tolerance, maintenance rates (40–60 mL/kg/day) of isotonic crystalloids and/or bolus infusions of hyperosmolar solutions (7.2% hypertonic saline [2–4 mL/kg]; mannitol 20% solution [0.25–1 g/kg]) may be useful to promote renal perfusion, tubular flow, and nephron recruitment.

Specific treatment and diagnosis of acute and chronic renal failure are discussed in greater detail elsewhere in this text.

COMMON NEPHROTOXIC SUBSTANCES IN THE INTENSIVE CARE UNIT

Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are commonly used in equine practice. Renal perfusion, notably to the inner medulla and medullary pyramids, relies on perfusion of the vasa recta. Intrinsic flow through the vasa recta is affected by both volume status of the patient and vasomotor tone. Vasomotor tone is regulated, in part, by prostaglandins. Prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) promote vasodilation, perfusion, and health of the inner tissues. These are constitutively produced via metabolism of arachidonic acid shunting through cyclooxygenase-1 (COX-1) activity.[108] With the exception of firocoxib, other NSAIDs used in horses are not selective for COX-2, which is induced by inflammatory stimuli.[108] Therefore most NSAIDs inhibit activity of both enzymes to some degree, which blocks renal production of prostaglandins and prevents the compensatory increase in renal blood flow during dehydration, potentially leading to vasomotor nephropathy and renal papillary necrosis (also termed *renal ischemic necrosis*). The mechanism of action and toxicity for NSAIDs is discussed elsewhere in this text.

Aminoglycoside Antibiotics

Aminoglycoside antibiotics (e.g., gentamicin, amikacin) are freely filtered at the glomerulus and excreted into the urine.[109] In low tubular flow states (e.g., dehydration), aminoglycosides are actively taken up by proximal convoluted tubular cells and stored in lysosomes. Drug accumulation exceeds drug disposition and leads to rupture of PCT cells and acute tubular necrosis.[109] Alternative drugs should be considered over systemic aminoglycoside in the hypovolemic and/or azotemic patient.

Endogenous Toxins: Myoglobin and Hemoglobin

Liberation of myoglobin (myopathies, e.g., rhabdomyolysis, myositis) or hemoglobin (intravascular hemolysis, e.g., immune-mediated hemolytic anemia, red maple leaf toxicity) may lead to acute kidney injury via several mechanisms including physical plugging of nephron tubules via polymerization, reflexive renal vasoconstriction causing ischemia, and free radical injury via reduction-oxidation reactions from iron-containing pigments and genesis of reactive species (e.g., hydroxyl radical). free radicals.

IDENTIFYING RENAL DYSFUNCTION

Recognition of renal dysfunction is easily overlooked in critical care patients. It is advisable to monitor serum BUN and creatinine concentrations at least every 48 hours to assess glomerular filtration and renal function. Regular measurement of BUN and creatinine concentrations and assessment of results in light of physical examination findings (e.g., evidence of dehydration) and results of urinalysis are the most readily available methods for monitoring renal function in equine critical care patients. Increases in serum creatinine concentration as small as 0.3 mg/dL can be significant.[110]

Diagnostic samples to assess renal function would include blood (CBC, serum chemistry, and leptospirosis microagglutination serology), urine (cytology, cast assessment, darkfield microscopy, SG, and quantitative urine culture), and renal tissue (histopathology with or without special stains and culture).

Dilute urine (SG ≤1.012) concurrent with azotemia generally indicates decreased renal function. Azotemia with isosthenuria (SG 1.008–1.012) indicates renal failure. Urinary indicators of tubular damage include granular casts in urine, an increased urinary γ-glutamyl transferase-to-creatinine ratio (>25), abnormal fractional clearance of electrolytes such as sodium and potassium,[110] and increased urinary glucose. The presence of white blood cells, bacteria, and excess protein concentration as determined by dipstick or other methods as well as increased urine to protein ratio are abnormal findings in urine and should prompt more in-depth investigation.

To understand and best prognosticate the severity of renal damage and chances of tissue restitution, ultrasound-guided renal biopsy and histopathology are recommended.

A more complete discussion of laboratory methods of renal function assessment is found in Chapter 14.

THERAPY FOR RENAL DYSFUNCTION

Many cases of acute kidney injury respond favorably to fluid therapy and avoidance of nephrotoxic drugs. In some instances, establishing a polyuric state can be beneficial when horses are treated with the previously mentioned maintenance fluid rates and can tolerate IV fluids. High tubular flow rate is essential in establishing endogenous waste clearance (e.g., creatinine) and other noxious substances (e.g., myoglobin). Diuresis may be induced through increased fluid rates above the maintenance requirements (>60 mL/kg/day) of polyionic isotonic solutions, administration of hypertonic solutions, and use of diuretic therapies. Azotemia that decreases by ≥50% in 24 hours in response to fluid therapy (usually at rates >80–100 mL/kg/day) indicates prerenal azotemia.

If fluid therapy does not produce the expected increase in urine output, then diuretics (furosemide, 1 mg/kg, IV) can be administered. Urination should be expected within 30 minutes of furosemide administration. CRIs of furosemide may yield a more consistent diuretic response.[111] Bags and infusion lines should be covered to prevent exposure to light because furosemide is light sensitive.

Volume replacement should always be attempted before diuretic therapy because the resultant increase in urination at blood volume expense will contribute to severe dehydration and reduce systemic perfusion and exacerbate renal damage.

Active treatment of refractory hypotension (hypotension unresponsive to fluid therapy) is important to treat or prevent perfusion-mediated causes of acute kidney injury. Autoregulation begins to fail at a MAP <60 mm Hg. Maintaining an MAP ≥65 mm Hg is recommended to minimize reduced urine output and kidney injury.[53]

Vasoactive therapies such as dobutamine (1–5 μg/kg/min) have been advocated to improve blood pressure. Low-dose dopamine therapy (2–5 μg/kg/min) and fenoldopam (0.04–0.4 μg/kg/min) have purported benefits in healthy subjects on renal blood flow. The effects of dopamine or fenoldopam on renal blood flow in critically ill equine patients is

unknown.[110,53] Blood pressure should be measured in patients at risk for hypotension and those that fail to produce urine despite appropriate therapy.

In horses with oliguric renal dysfunction and certainly anuric renal dysfunction, the CVP may be monitored to assess the potential for fluid overload and edema formation. Normal CVP in the standing horse is <12 mm Hg. Values >12 mm Hg should prompt the clinician to reassess the patient's' tolerance to fluid therapy.

Aminoglycoside toxicity is best prevented by ensuring appropriate dosing regimens and limiting the use of aminoglycosides to hydrated animals without preexisting renal dysfunction.[111] Administering aminoglycoside antibiotics every 24 hours decreases the amount of time that renal tubular cells are exposed to higher concentrations of aminoglycosides. The peak concentration (C_{max}) dictates the efficacy of the drug; however, the trough concentration dictates the safety of the drug. Therapeutic drug monitoring allows for the detection of elevated trough (usually 24 hours after the previous dose) concentrations, which are more likely to cause toxicity. Drug-dosing adjustments should be based on trough concentrations, either as a lower dose administered or a prolonged dosing interval.[109]

Renal Replacement Therapy

RRT such as peritoneal dialysis (PD) or hemodialysis is technically possible in horses. PD is technically easier but less effective than hemodialysis. Recently venovenous hemodiafiltration was described in healthy adult horses and well tolerated.[112] The need for specialized equipment and familiarity with creatinine clearance kinetics, etc., makes the application of hemodialysis in clinical patients somewhat limited currently.

PD has been described in horses as either intermittent or continuous. The concept involves using the peritoneum as an accessory kidney to dialyze blood. In one clinical report, continuous flow PD (CFPD) was more effective than intermittent PD.[113] LRS with 1.5% dextrose and 1 unit of unfractionated heparin per milliliter is an easy dialysate solution to obtain and use. For intermittent PD, 40 mL/kg of dialysate is infused and left for 30 to 60 minutes and then allowed to drain. This is repeated 2 to 6 times per day. With CFPD, an ingress catheter is placed in the left flank and an egress catheter on ventral midline connected to a closed bag collection system for volumetric assessment. Dialysate fluid ingress is provided at ~3 L/h. Eighty percent of ingress volume should be recovered in the collection system. Marked improvements in azotemia have been observed within the first 24 hours of starting CFPD, and it is continued based on creatinine clearance responses.

RRT should be considered in cases where azotemia is unresponsive to fluid therapy and/or diuretic medications or the horse is severely oliguric to anuric. Acute kidney injury would likely yield potentially favorable outcomes using RRT compared with chronic disease states.

Neurologic Critical Care

Samuel D. Hurcombe

Any neurologic condition can represent a true emergency requiring expedient assessment, stabilization, and targeted therapy. Severe manifestations of common diseases can be challenging cases to treat, and horses that present acutely and/or deteriorate in the face of therapy require immediate veterinary attention. Disorders of the forebrain (seizure activity and changes in behavior), spinal cord (ataxia and recumbency), peripheral nervous system (inability to ambulate or severe generalized weakness), and autonomic nervous system (urinary/fecal incontinence) may all require emergency care. Extracranial causes of neurologic dysfunction can also be challenging and represent a multisystemic disorder (e.g., hepatic and other metabolic encephalopathies).

Certain disorders may present as a neurologic disorder but may be caused by other systemic causes. For example, cardiovascular or respiratory diseases and gait abnormalities caused by musculoskeletal diseases such as bone fractures, rhabdomyolysis, or laminitis.

Peripheral nerve damage in horses is not uncommon and most often involves the suprascapular, radial, ulnar, femoral, and selected cranial nerves. Occasionally, the brachial plexus can be injured after a collision accident. Peripheral nerve injury is commonly traumatic and associated with additional musculoskeletal abnormalities (e.g., fracture).

Traumatic head injury with traumatic brain injury (TBI) represents a dynamic neurologic emergency. Depending on the nature of the injury, primary and secondary TBI can cause the clinical presentation of the patient to change frequently as processes such as edema, inflammation, and changes in intracranial pressure (ICP) occur.

Abnormal neurologic function can cause secondary excitement, agitation, and stress in the patient and worry for the owner or caretaker. Horses with alterations in the content of consciousness and behavioral changes may be unpredictable, vacillating between somnolence and maniacal rage. It is prudent to ensure safety of not only the patient to prevent additional injury but also any handler or personnel. In cases of unexplained neurologic deterioration, notably of inapparent causes, a thorough understanding of vaccination history of the patient and use of barrier protection strategies (e.g., medical examination gloves, coveralls) should be performed. Taking note of any "'in-contact" personnel is important if potential zoonotic disease is suspected (i.e., rabies).

DIAGNOSTICS

Performing a thorough neurologic examination is critical. To come to a diagnosis, the practitioner performs a careful neurologic examination followed by appropriate ancillary diagnostics. Ancillary diagnostics used in emergency situations typically include hematology and serum chemistries, radiographs, and potentially cerebrospinal fluid analysis. When available, these patients may require further diagnostic tests, notably imaging such as computed tomography, magnetic resonance imaging, endoscopy and electrodiagnostics, or a combination of tests.

A neuroanatomic localization of the disease should be made after a thorough examination,[114,115] as described in (Chapter 11). The neuroanatomic localization can be simplified by determining whether there is CNS, peripheral nerve, or multifocal nerve disease. In the case of CNS disease, it is helpful to determine whether the lesion is localized cranial or caudal to the foramen magnum (i.e., whether there is brain involvement). If there is brain involvement, it may be possible to further differentiate among cerebral, cerebellar, vestibular, or brainstem disease. If disease is localized caudal to the foramen magnum, the disease may be located in the cervical spinal cord or in the spinal cord caudal to T2.

Stabilization and symptomatic treatment are the primary goals of emergency management of neurologic disease. A definitive diagnosis may not be known for several days following the initial presentation of the emergent neurologic patient—for example, waiting for diagnostics for infectious agents (e.g., protozoal myelitis, West Nile virus, etc.). As such, the clinician's thorough neurologic examination provides a framework for a list of likely differential diagnoses. Empiric treatments, often for several differential diagnoses at once, are often provided until further testing supports a definitive diagnosis.

TREATMENT

The goal of therapy should be to optimize CNS perfusion and delivery of neurotropic substrates. Perfusion to the CNS is modulated by both the perfusing pressure (MAP) and local ICP. To promote global CNS perfusion, increasing MAP and/or decreasing ICP are desirable.

Judicious use of sedation may be needed to facilitate safe examination of the neurologic patient. Low-dose, short-acting drugs such as xylazine hydrochloride are recommended. However, for more fractious and refractory patients, longer acting continuous infusions and anesthetic agents may be required. Stabilization of hemorrhagic and hypovolemic conditions is necessary in trauma patients. Medical treatment of specific primary conditions is described in detail in Chapter 11. This section discusses select groups of drugs that are commonly used in horses with acute neurologic disease.

Analgesics

Analgesic drugs are indicated if horses experience pain (e.g., in traumatic disease). NSAIDs are the mainstay drugs in equine medicine because of their antiinflammatory and analgesic properties. Flunixin meglumine (0.5–1 mg/kg, IV, every 12–24 hours) is suitable. Opioids should be considered when additional analgesia is required, or when analgesia is required in situations of suspected NSAID toxicity, or when the risk of developing NSAID toxicity is high (e.g., dehydration). Opioids do not have specific antiinflammatory effects and in theory, κ-agonists over μ-agonists are advisable. Naloxone, μ-antagonist, has been shown to improve spinal cord blood flow. Multimodal analgesia, including the use of ketamine, may offer advantages in blocking neuroexcitation through *N*-methyl-D-aspartate blockade.

Corticosteroids

Corticosteroids (CSs) are potent antiinflammatory medications and may be indicated in certain types of neurologic diseases. Known inflammatory disease and acute trauma still appear to be indications for short-term corticosteroid therapy. Dexamethasone sodium phosphate is commonly administered (0.05–0.1 mg/kg, IV, every 12–24 hours) for up to 48 hours. A favorable response is expected within 4 to 8 hours after CS administration. Horses being given corticosteroid therapy should be monitored closely for the development of laminitis or secondary infection. If improvement in clinical signs is observed, transition to oral prednisolone therapy (0.5–1.0 mg/kg, PO, daily tapered over 3–5 days) to decrease the chance of adverse effects is indicated. The neuroprotective effect of CSs is thought primarily to be mediated by free radical scavenging.[116] Recently, it has been shown that, similar to methylprednisolone, dexamethasone decreases apoptosis-related cell death in rats that were subjected to traumatic spinal cord injury.[117] Other potential beneficial effects of CSs include reduction in the spread of morphologic damage, prevention of the loss of axonal conduction and reflex activity, preservation of vascular membrane integrity, and stabilization of white matter neuronal cell membranes in the presence of central hemorrhagic lesions. Furthermore, their antiinflammatory properties may be useful in reducing edema and fibrin deposition, as well as their ability to reverse sodium and potassium imbalance caused by edema and necrosis.

Methylprednisolone sodium succinate (MPSS) is a synthetic glucocorticoid with four times more antiinflammatory activity and 0.8 times less mineralocorticoid action compared with cortisol. Beneficial effects of MPSS on neural tissue include inhibition of lipid peroxidation, eicosanoid formation, and lipid hydrolysis, including arachidonic acid release, maintenance of tissue blood flow and aerobic energy metabolism, improved elimination of intracellular calcium accumulation, reduced neurofilament degradation, and improved neuronal excitability and synaptic transmission. The dose of MPSS used in human trials (30 mg/kg) exceeds that necessary for activation of steroid receptors, which suggests that MPSS acts through mechanisms that are unrelated to steroid receptors. Investigators have concluded that high-dose MPSS treatment within 8 hours of spinal cord injury improved neurologic recovery.[118,119] Controversy regarding the beneficial effects of MPSS and other steroids remains.[120,121] A suggested dose for horses is 25 mg/kg, IV bolus, then 5 to 8 mg/kg per hour for 23 hours.

Intracranial Pressure Reduction

Osmotic diuretics such as 20% mannitol (0.25–2.0 mg/kg, administered IV over 20 minutes) and 7.2% hypertonic saline (2–4 mL/kg, administered IV as a bolus) are effective in combating cerebral edema and increased ICP. These have a rapid onset of action (10–20 minutes) and are ideal therapies because they reduce ICP by drawing volume into the vascular compartment and they increase MAP. Animals receiving osmotic diuretics should be adequately hydrated. Mannitol administration is very effective in reducing ICP, although despite some technical limitations to the administration of this osmotic diuretic, multiple doses of mannitol can lead to intravascular dehydration, hypotension, reduction of cerebral blood flow (CBF), and elevation of spinal fluid osmolarity.[122,123]

Hypertonic saline administered early in the treatment of shock associated with head trauma may enhance the return of CBF and cell membrane function. Hypertonic saline may be the maintenance fluid of choice in head injury.[124] It is associated with significant decreases in ICP and cerebral water content compared with isotonic fluid treatment. Another study comparing the effects of a hypertonic saline + solution and mannitol on increased ICP found that the hypertonic saline hydroxyethyl starch reduced the ICP more effectively than mannitol alone.[125] Induction of prolonged hypernatremia using 3% hypertonic saline administered as a continuous infusion appears to be a promising therapy for the control of cerebral edema.[126]

Head and neck elevation at least 30 degrees above shoulder height is also advised to minimize venous pooling in the head. Increased venous pressures lead to increases in ICP via Queckenstedt's phenomenon. Use of a wire and pulley system connected to a halter is useful for the technique (Fig. 4.5).

Dimethyl sulfoxide (DMSO) 1 g/kg, administered IV as a 10% to 20% solution for 3 consecutive days, followed by three



FIG. **4.5** This horse sustained head (and body) trauma. The head and neck are kept elevated to reduce central nervous system venous pooling and intracranial pressure.

treatments every other day may be of benefit in acute neurologic disease. Reported benefits of DMSO include increased brain and spinal cord blood flow, decreased brain and spinal cord edema, increased vasodilating PGE_1, decreased platelet aggregation, decreased PGE_2 and PGF_2, protection of cell membranes, and trapping of hydroxyl radicals. The exact mechanism of DMSO remains unknown and remains controversial because purported positive effects are unsubstantiated.[127] Antioxidants such as vitamin E and selenium have been shown to be beneficial in certain neurologic diseases. Their purported beneficial effects include reduced lipid peroxidation and free radical scavenging[116]; however, they have not proved beneficial in cases of acute injury because of the length of time required to achieve therapeutic concentrations in the CNS.

Antibiotics

Antimicrobial drugs may be required to treat the primary disease process (e.g., in bacterial meningitis) or to treat/prevent secondary complications such as deep decubital ulceration, pneumonia, and cystitis. Horses with prolonged recumbency or dysphagia are at high risk of aspiration pneumonia and warrant broad-spectrum antibiotic therapy.

Ideally, the choice of antibiotic should be based on culture and sensitivity testing; however, empiric coverage while awaiting results should include drugs that can penetrate the site of action desired (e.g., meninges) and be tolerated by the patient (e.g., parenteral drugs in patients with dysphagia).

For CNS penetration, parenteral fluoroquinolones (enrofloxacin 7.5 mg/kg, IV, every 24 hours) or enteral chloramphenicol (50 mg/kg, PO, every 6 hours) appears to be a good choice. Potentiated sulfonamides and third-generation cephalosporins also achieve therapeutic cerebrospinal fluid concentrations. For empiric therapy of secondary sites of infection, trimethoprim-sulfamethoxazole (20–30 mg/kg, PO, every 12 hours) or procaine penicillin (22 mg/kg, IM, every 12 hours) or crystalline penicillin (22,000 IU/kg, IV, every 6 hours) in combination with gentamicin (6.6 mg/kg, IV, every 24 hours) can be used. For anaerobic coverage, metronidazole (15 mg/kg, PO, every 8 hours) is indicated. Gentamicin and tetracyclines have poor cerebrospinal fluid penetration.

SUPPORTING THE DOWN HORSE

Establishing venous access, controlling pain and anxiety of the patient, and performing a thorough examination and frequent repeat examinations should be done. Caution should be exercised and safe handling practices are imperative when dealing with a fractious patient and/or rabies is a concern. Management of a recumbent horse requires intensive supportive care and specific treatment aimed at the underlying or complicating disease processes.[128,129] Supportive care is mainly directed at protection of the animal and maintenance of adequate hydration and nutritional status. Care of down horses is time-consuming and more difficult if the horse is very large.

Down horses should be safely turned every 4 to 6 hours to help prevent muscle compartmental injury, peripheral nerve injury, and decubital ulcers. Treatment and prevention of decubital ulcers are critical in recumbent animals,[130] because prolonged or repeated unrelieved pressures to skin can result in damage and ischemia to underlying tissues and subsequent tissue ulceration.[131] Pressure ulcers usually occur over bony prominences, and in horses the most common sites of decubital ulcers are the tuber coxae, the points of the shoulder, and the caudal to the eye protuberance. Furthermore, pressure sores can occur along the distal extremities. For horses preventive strategies include appropriate padding and bedding, frequent changes in body positioning (i.e., turning), efforts to improve mobility, frequent examination of skin, maintenance of a well-balanced diet, and skin that is kept dry. Pressure sores generally heal well once the horse is able to stand. Rarely, infection of underlying bone can occur with prolonged severe decubitus and require debridement and local therapy.

Surgical intervention may be required in some cases—for example, traumatic skull injury with unstable fragmentation. Successful treatment of such cases has been reported in horses.[132]

Bedding should be absorbent, nonabrasive, and conformable. It may be necessary to adjust the type of bedding when the horse makes (successful) attempts to stand or is standing in the sling. Once the patient begins to make attempts to stand, bedding with good footing is helpful. Deep bedding in those situations may hinder the horse's attempts to stand or ambulate, and straw may be slippery. Where possible, keeping the down horse in sternal recumbency will help V/Q mismatch and respiratory effort. Straw bales can assist in patient positioning. Padded helmets and head bandages are advised to help protect the head and eyes. Damage to the eyes can occur directly by pressure to the eye or from bedding material. Moreover, specific diseases may result in a horse's inability to blink (e.g., botulism, Horner's syndrome, facial nerve paralysis). The most common ophthalmic disorders seen in recumbent horses are corneal ulcers and keratitis. Eyes should be examined daily and assessed and treated carefully. Triple antibiotic without steroid should be applied to the patients' eyes daily to help prevent keratopathy.

Nutrition and Hydration Maintenance

Parenteral fluids are indicated in cases where swallowing is impossible or the patient is in critical condition. Maintenance

fluids (40–60 mL/kg/day) are typically appropriate. Where enteral therapy can be tolerated, intragastric fluids may be preferable and more physiologic. Intermittent nasogastric tube passage or an indwelling small-bore enteral feeding tube placement is a good choice. Depending on the dietary protocol used, electrolytes and glucose may be added to the fluids. Recumbent animals lose body condition rapidly when no nutrition is provided. Dramatic changes are often noticeable within 1 week because of protein-calorie malnutrition. To prevent weight loss and maintain muscle strength and immunity, nutrition provision is essential. A more thorough discussion of nutrition in the critically ill horse can be found in Chapters 5 and 20.

Down horses are prone to developing impactions of the cecum, large colon, or small colon. Horses with botulism are notable cases. Reduced fecal output is an early sign, and judicious use of laxatives (magnesium sulfate or sodium sulfate; 1 g/kg, intragastrically) and/or lubricants (mineral oil 2–4 L per 500-kg horse, intragastrically) is indicated.

Additional Considerations

Horses with autonomic dysfunction (i.e., urine and/or fecal retention) require frequent emptying of the rectum and bladder. Use of stool softeners and frequent manual rectal evacuation should be performed several times each day. Special caution should be made when performing rectal evacuation, especially in the down horse, to avoid traumatic rectal tearing.

An indwelling urinary catheter with either a one-way valve or closed collection system should be placed to assist bladder emptying. Closed systems are recommended to avoid ascending cystitis. Empiric use of potentiated sulfonamides can assist in preventing catheter-related urinary bladder sepsis.

Physiotherapy is advocated to facilitate the rehabilitative process of injured horses. Physiotherapy can be provided in the recumbent animal by manipulating limbs, depending on the horse's attitude, or assisting the horse to stand with a sling. Controlled exercise allows the unaffected parts of the nervous system to compensate for the affected parts by increasing strength and conscious proprioception. Exercise is especially helpful in improving spinal cord deficits (e.g., weakness, ataxia, and spasticity).

Sling usage in the management of the down horse can be very helpful in reducing recumbency-related concerns. Placing the neurologic patient with normal mentation may be useful to facilitate a more thorough examination of individual limb deficits. The presence of lameness, weakness, and ataxia as well as the number of affected limbs is much more easily assessed when the horse is in an upright position. In this position the horse may be able to stand freely and demonstrate clinical signs that may help in determining a diagnosis.

Deterioration or improvement of disease may be determined relatively easily by daily sling assistance to provide short-term recommendations and prognosis. For long-term management the sling can be useful, depending on the horse's primary disease and compliance. Horses may become used to using the sling and can be comfortably managed until they have regained sufficient control and strength to ambulate freely. Use of the sling may also help with physiotherapy during which periodic sessions of partial or assisted standing can facilitate manipulation of limbs.

REFERENCES

1. Dellinger RP, Levy MM, Carlet JM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock. *Crit Care Med*. 2008;36:296–327.
2. Ernst NS, Hernandez JA, MacKay RJ, et al. Risk factors associated with fecal *Salmonella* shedding among hospitalized horses with signs of gastrointestinal tract disease. *J Am Vet Med Assoc*. 2004;225:275–281.
3. Hardy J, Burkhardt HA, Beard W. Equine emergency and intensive care: case survey and assessment of needs (1992–1994). *Proc Am Assoc Equine Pract*. 1996;42:182–183.
4. Dolente B, Lindberg S, Russell G, et al. Emergency case admissions at a large tertiary university referral hospital during a 12-month period. *J Vet Emerg Crit Care*. 2008;18:298–305.
5. Girou E, Loyeau S, Legrand P, et al. Efficacy of handrubbing with alcohol based solution versus standard handwashing with antiseptic soap: randomized clinical trial. *Br Med J*. 2002;325:362.
6. Dallap-Shaer BL, Linton JK, Aceto H: Antimicrobial use in horses undergoing colic surgery. *J Vet Intern Med*. 2012, Nov-Dec; 26:1449–1456.
7. Sevinga M, Barkema HW, Hesselink JW. Serum calcium and magnesium concentrations and the use of a calcium-magnesium-borogluconate solution in the treatment of Friesian mares with retained placenta. *Theriogenology*. 2002;57:941–947.
8. Garcia-Lopez JM, Provost PJ, Rush JE, et al. Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. *Am J Vet Res*. 2001;62:7–12.
9. Perel P, Roberts I. Colloids versus crystalloids for fluid resuscitation in critically ill patients. *Cochrane Database Syst Rev*. 2012;13(6). CD000567.
10. Jones PA, Tomasic M, Gentry PA. Oncotic, hemodilutional, and hemostatic effects of isotonic saline and hydroxyethyl starch solutions in clinically normal ponies. *Am J Vet Res*. 1997;58: 541–548.
11. Jones PA, Bain FT, Byars TD, et al. Effect of hydroxyethyl starch infusion on colloid oncotic pressure in hypoproteinemic horses. *J Am Vet Med Assoc*. 2001;218:1130–1135.
12. Reickhoff K, Forster H, Weidhase R, et al. Administration of 10% hydroxyethyl starch 200/0.5 solution in normovolaemic horses, *Scientific Proc. 7th International Equine Colic Research Symposium*. Manchester, UK. 2002.
13. Zarychanski R, About-Setta AH, Turgeon AF, et al. Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation: a systematic review and metanalysis. *J Amer Med Assoc*. 2013;309:678–688.
14. Epstein KL, Bergren A, Giguere S, et al. Cardiovascular, colloid osmotic pressure, and hemostatic effects of 2 formulations of hydroxyethyl starch in healthy horses. *J Vet Intern Med*. 2014;28:223–233.
15. Wilson EM, Holcombe SJ, Lamar A, et al. Incidence of transfusion reactions and retention of procoagulant and anticoagulant factor activities in equine plasma. *J Vet Intern Med*. 2009;23:323–328.
16. Mugde MC. Acute hemorrhage and blood transfusion in horses. *Vet Clin North Am Equine Pract*. 2014;30:427–436.
17. Hurcombe SD, Mudge MC, Hinchcliff KW. Clinical and clinicopathologic variables in adult horses receiving blood transfusions: 31 cases (1999–2005). *J Am Vet Med Assoc*. 2007;231: 267–274.
18. Tomlinson JE, Taberner E, Boston RC, et al. Survival time of cross-match incompatible red cells in adult horses. *J Vet Intern Med*. 2015;29:1683–1688.
19. Hurcombe SD, Welch BR, Williams JM, et al. Dark-field microscopy in the assessment of large colon microperfusion and mucosal injury in naturally occurring surgical disease of the equine large colon. *Equine Vet J*. 2014;46:674–680.
20. Fielding L. Crystalloid and colloid therapy. *Vet Clin North Am Equine Pract*. 2014;30:415–425.

21. Cruz AM, Li R, Kenney DG, et al. Effects of indwelling nasogastric intubation on gastric emptying of a liquid marker in horses. *Am J Vet Res*. 2006;67:1100–1104.
22. Hardy J, Stewart RH, Beard WL, et al. Complications of nasogastric intubation in horses: nine cases (1987-1989). *J Am Vet Med Assoc*. 1992;201:483–486.
23. Nieto JE, Yamout S, Dechant JE. Sinusitis associated with nasogastric intubation in 3 horses. *Can Vet J*. 2014;55:554–558.
24. Beccati F, Nannarone S, Gialletti R, et al. Evaluation of transabdominal ultrasound as a tool for predicting the success of abdominocentesis in horses. *Vet Rec*. 2014;174:251.
25. Estepa JC, Lopez I, Mayer-Valor R, et al. The influence of anticoagulants on the measurement of total protein concentration in equine peritoneal fluid. *ResVet Sci*. 2006;80:5–10.
26. Peloso JG, Cohen ND. Use of serial measurements of peritoneal fluid lactate concentration to identify strangulating intestinal lesions in referred horses with signs of colic. *J Am Vet Med Assoc*. 2012;240:1208–1217.
27. Matthews S, Dart AJ, Dowling BA, et al. Peritonitis associated with *Actinobacillus equuli* in horses: 51 cases. *Aust Vet J*. 2001;79:536–539.
28. Santschi EM, Grindem CB, Tate Jr LP, et al. Peritoneal fluid analysis in ponies after abdominal surgery. *Vet Surg*. 1988;17:6–9.
29. Hanson RR, Nixon AJ, Gronwall R, et al. Evaluation of peritoneal fluid following intestinal resection and anastomosis in horses. *Am J Vet Res*. 1992;53:216–221.
30. Weil MH, Shubin H. Proposed reclassification of shock states with special reference to distributive defects. *Adv Exp Med Biol*. 1971;23:13–23.
31. Ellender TJ, Skinner JC. The use of vasopressors and inotropes in the emergency medical treatment of shock. *Emerg Med Clin North Am*. 2008;26:759–786.
32. Rodgers KG. Cardiovascular shock. *Emerg Med Clin North Am*. 1995;13:793–810.
33. Spaniol JR, Knight AR, Zebley JL, et al. Fluid resuscitation therapy for hemorrhagic shock. *J Trauma Nurs*. 2007;14:152–160.
34. Lefer AM, Lefer DJ. Pharmacology of the endothelium in ischemia-reperfusion and circulatory shock. *Annu Rev Pharmacol Toxicol*. 1993;33:71–90.
35. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. *N Engl J Med*. 2001;345:588–595.
36. Tuite PK. Recognition and management of shock in the pediatric patient. *Crit Care Nurs Q*. 1997;20:52–61.
37. Holmes CL. Vasoactive drugs in the intensive care unit. *Curr Opin Crit Care*. 2005;11:413–417.
38. Ruffolo Jr RR, Nichols AJ, Stadel JM, et al. Pharmacologic and therapeutic applications of alpha 2-adrenoceptor subtypes. *Annu Rev Pharmacol Toxicol*. 1993;33:243–279.
39. Nagashima M, Hattori Y, Akaishi Y, et al. Alpha 1-adrenoceptor subtypes mediating inotropic and electrophysiological effects in mammalian myocardium. *Am J Physiol*. 1996;271:H1423–H1432.
40. Huang L, Tang W. Vasopressor agents: old and new components. *Curr Opin Crit Care*. 2004;10:183–187.
41. Steel A, Bihari D. Choice of catecholamine: does it matter? *Curr Opin Crit Care*. 2000;6:347–353.
42. Girault JA, Greengard P. The neurobiology of dopamine signaling. *Arch Neurol*. 2004;61:641–644.
43. Holmes CL, Landry DW, Granton JT. Science review: vasopressin and the cardiovascular system part 1: receptor physiology. *Crit Care*. 2003;7:427–434.
44. Vincent JL. Vasopressin in hypotensive and shock states. *Crit Care Clin*. 2006;22:187–197.
45. Holmes CL, Landry DW, Granton JT. Science review: vasopressin and the cardiovascular system part 2: clinical physiology. *Crit Care*. 2004;8:15–23.
46. Valverde A, Giguere S, Sanchez LC, et al. Effects of dobutamine, norepinephrine, and vasopressin on cardiovascular function in anesthetized neonatal foals with induced hypotension. *Am J Vet Res*. 2006;67(10):1730–1737.
47. Corley KT, McKenzie HC, Amoroso LM, et al. Initial experience with norepinephrine infusion in critically ill foals. *J Vet Emerg Crit Care*. 2000;10(4):267–276.
48. Martin C, Viviand X, Leone M, et al. Effect of norepinephrine on the outcome of septic shock. *Crit Care Med*. 2000;28(8):2758–2765.
49. Woolsey CA, Coopersmith CM. Vasoactive drugs and the gut: is there anything new? *Curr Opin Crit Care*. 2006;12:155–159.
50. Zhong JQ, Dorian P. Epinephrine and vasopressin during cardiopulmonary resuscitation. *Resuscitation*. 2005;66:263–269.
51. Kellum JA, Pinsky MR. Use of vasopressor agents in critically ill patients. *Curr Opin Crit Care*. 2002;8:236–241.
52. Smit AJ. Dopamine in heart failure and critical care. *Clin Exp Hypertens*. 2000;22:269–276.
53. Corley KTT. Inotropes and vasopressors in adults and foals. *Vet Clin North Amer*. 2004;20:77–106.
54. Patel BM, Chittock DR, Russell JA, et al. Beneficial effects of short-term vasopressin infusion during severe septic shock. *Anesthesiology*. 2002;96:576–582.
55. Malay MB, Ashton Jr RC, Landry DW, et al. Low-dose vasopressin in the treatment of vasodilatory septic shock. *J Trauma*. 1999;47:699–703.
56. Asfar P, De Backer D, Meier-Hellmann A, et al. Clinical review: influence of vasoactive and other therapies on intestinal and hepatic circulations in patients with septic shock. *Crit Care*. 2004;8:170–179.
57. Wong DM, Vo DT, Alcott CJ, et al. Plasma vasopressin concentrations in healthy foals from birth to 3 months of age. *J Vet Intern Med*. 2008;22:1259–1261.
58. Hurcombe SD, Toribio RE, Slovis N, et al. Blood arginine vasopressin, adrenocorticotropin hormone, and cortisol concentrations at admission in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2008;22:639–647.
59. Hollis AR, Boston RC, Corley KT. Plasma aldosterone, vasopressin and atrial natriuretic peptide in hypovolaemia: a preliminary comparative study of neonatal and mature horses. *Equine Vet J*. 2008;40:64–69.
60. Dembek KA, Hurcombe SD, Stewart AJ, et al. Association of aldosterone and arginine vasopressin concentrations and clinical markers of hypoperfusion in neonatal foals. *Equine Vet J*. 2016;48:176–181.
61. Borchers A, Magdesian KG, Schenck PA, Kass PH. Serial plasma vasopressin concentration in healthy and hospitalized neonatal foals. *Equine Vet J*. 2014;46:306–310.
62. Ludders JW, Palos HM, Erb HN, et al. Plasma arginine vasopressin concentration in horses undergoing surgery for colic. *J Vet Emerg Crit Care*. 2009;19:528–535.
63. Krausz MM. Controversies in shock research: hypertonic resuscitation-pros and cons. *Shock*. 1995;3:69–72.
64. Fielding CL, Magdesian KG. A comparison of hypertonic (7.2%) and isotonic (0.9%) saline for fluid resuscitation in horses: a randomized, double-blinded, clinical trial. *J Vet Intern Med*. 2011;25:1138–1143.
65. Corley KTT. Monitoring and treating hemodynamic disturbances in critically ill foals. *Equine Vet Educ*. 2002;14:270–279.
66. Hollis AR, Ousey JC, Palmer L, et al. Effects of norepinephrine and a combined norepinephrine and dobutamine infusion on systemic hemodynamics and indices of renal function in normotensive neonatal thoroughbred foals. *J Vet Intern Med*. 2006;20:1437–1442.
67. Landry DW, Levin HR, Gallant EM, et al. Vasopressin deficiency contributes to the vasodilation of septic shock. *Circulation*. 1997;95:1122–1125.
68. Dickey EJ, McKenzie H, Johnson A, Furr MO. Use of pressor therapy in 24 hypotensive critically ill neonatal foals. *Aust Vet J*. 2010;88:472–477.
69. Rivers MD, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med*. 2001;345:1368–1377.

70. ARISE Investigators. Goal-directed resuscitation for patients with early septic shock. *N Engl J Med*. 2014;371:1496–1506.
71. ProCESS Investigators. A randomized trial of protocol-based care for early septic shock. *N Engl J Med*. 2014;370:1683–1693.
72. Corley KTT, Marr CM. Cardiac monitoring in the ICU patient. *Clin Tech Equine Pract*. 2003;2:145–155.
73. Magdesian KG. Monitoring the critically ill equine patient. *Vet Clin North Am Equine Pract*. 2004;20:11–39.
74. Magdesian KG, Fielding CL, Rhodes DM, et al. Changes in central venous pressure and blood lactate concentration in response to acute blood loss in horses. *J Am Vet Med Assoc*. 2006;229:1458–1462.
75. Allen SE, Holm JL. Lactate: physiology and clinical utility. *J Vet Emer Crit Care*. 2008;18:123–132.
76. Corley KT, Donaldson LL, Durando MM, et al. Cardiac output technologies with special reference to the horse. *J Vet Intern Med*. 2003;17:262–272.
77. McConachie E, Barton MH, Rapoport G, et al. Doppler and volumetric echocardiographic methods for cardiac output measurement in standing adult horses. *J Vet Intern Med*. 2013;27:324–330.
78. West JB. *Respiratory physiology: the essentials*. 8th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2008.
79. Wingfield WE, Raffe MR, eds. *The veterinary ICU book*. Jackson Hole, WY: Teton New Media; 2002.
80. Palmer JE. Ventilatory support of the critically ill foal. *Vet Clin North Am Equine Pract*. 2005;21:457–486.
81. Wong DM, Alcott CJ, Wang C, et al. Physiologic effects of nasopharyngeal administration of supplemental oxygen at various flow rates in neonatal foals. *Am J Vet Res*. 2010;71:1081–1088.
82. Wilson DV, Schott HC, Robinson NE, et al. Response to nasopharyngeal oxygen administration in horses with lung disease. *Equine Vet J*. 2006;38:219–223.
83. McKenzie HC. Characterization of antimicrobial aerosols for administration to horses. *Vet Ther*. 2003;4:110–119.
84. McKenzie HC, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration to horses. *Am J Vet Res*. 2000;61:1185.
85. Duvivier DH, Votion D, Roberts CA, et al. Inhalation therapy of equine respiratory disorders. *Equine Vet Educ*. 1999;11:124.
86. Coutiel LL, Cardwell JM, Gerber V, et al. Inflammatory airway disease of horses–revised consensus statement. *J Vet Intern Med*. 2016;30(2):503–515.
87. Giguere S, Slade JK, Sanchez LC. Retrospective comparison of caffeine and doxapram for the treatment of hypercapnia in foals with hypoxic ischemic encephalopathy. *J Vet Intern Med*. 2008;22:401–405.
88. Taylor SD, Toth B, Townsend WM, et al. Mechanical ventilation and management of an adult horse with presumptive botulism. *J Vet Emerg Crit Care*. 2014;24:594–601.
89. Aguilera-Tejero E, Estepa JC, Lopez I, et al. Arterial blood gases and acid-base balance in healthy young and old horses. *Equine Vet J*. 1998;30:352.
90. Picandet V, Jeanneret S, Jean-Pierre L. Effects of syringe type and storage temperature on results of blood gas analysis in arterial blood of horses. *J Vet Intern Med*. 2007;21:476.
91. Viu J, Armengou L, Rios J, Cesarini Latorre Carlota, Jose Cunilleras Eduardo. Acid base imbalances in ill neonatal foals and their association with survival. *Equine Vet J*. 2015;49(1):51–57.
92. Stampfli HR, Schoster A, Constable PD. Clinical utility of serum biochemical variables for predicting acid-base balance in critically ill horses. *Vet Clin Pathol*. 2014;43:547–556.
93. Matthews NS, Hartke S, Allen Jr JC. An evaluation of pulse oximeters in dogs, cats and horses. *Vet Anaesth Anal*. 2003;30:3.
94. Souba WW. Nutritional support. *N Engl J Med*. 1997;336:41.
95. Weissman C. Nutrition in the intensive care unit. *Crit Care*. 1999;3:R67.
96. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentages in mares. *Equine Vet J*. 1983;15:371.
97. Heidegger CP, Darmon P, Pichard C. Enteral vs parenteral nutrition for the critically ill patient: a combined support should be preferred. *Curr Opin Crit Care*. 2008;14:408.
98. National Research Council. *Nutrient requirements of horses*. Washington, DC: National Academies Press; 2007.
99. Robinson NE, ed. *Current therapy in equine medicine*. 6th ed. St. Louis, MO: Saunders; 2009.
100. Carr EA, Holcombe SJ. Nutrition of critically ill horses. *Vet Clin North Am Equine Pract*. 2009;25:93–108.
101. Jose-Cunilleras E, Viu J, Corradini I, et al. Energy expenditure of critically ill neonatal foals. *Equine Vet J Supp*. 2012;41:48–51.
102. Hansen TO, White NA, Kemp DT. Total parenteral nutrition in four healthy adult horses. *Am J Vet Res*. 1988;49:122.
103. Spurlock SL, Ward MV. Parenteral nutrition in equine patients: principles and theory. *Compend Cont Educ Pract Vet*. 1991;13:461.
104. Furr M. Intravenous nutrition in horses: clinical applications. *Proc Am Coll Vet Intern Med*. 2002;20:186.
105. Myers CJ, Magdesian KG, Kass PH, et al. Parenteral nutrition in neonatal foals: clinical description, complications, and outcome in 53 foals (1995-2005). *Vet J*. 2009;181:137–144.
106. Krause JB, McKenzie HC. Parenteral nutrition in foals: a retrospective study of 45 cases (2000-2004). *Equine Vet J*. 2007;39:74–78.
107. Dunkel B, Palmer JE, Olson KN, et al. Uroperitoneum in 32 foals: influence of intravenous fluid therapy, infection, and sepsis. *J Vet Intern Med*. 2005;19:889–893.
108. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91–97.
109. Brashier MK, Geor RJ, Ames TR, et al. Effect of intravenous calcium administration on gentamicin-induced nephrotoxicosis in ponies. *Am J Vet Res*. 1988;59:1055–1062.
110. Divers TJ. Urine production, renal function, and drug monitoring in the equine intensive care unit. *Clin Tech Equine Pract*. 2003;2:188–192.
111. Johansson AM, Gardner SY, Levine JF, et al. Furosemide continuous rate infusion in the horse: evaluation of enhanced efficacy and reduced side effects. *J Vet Intern Med*. 2003;17:887–895.
112. Wong DM, Witty D, Alcott CJ, et al. Renal replacement therapy in healthy adult horses. *J Vet Intern Med*. 2013;27:308–316.
113. Gallatin LL, Couteil LL, Ash SR. Use of continuous-flow peritoneal dialysis for the treatment of acute renal failure in an adult horse. *J Am Vet Med Assoc*. 2005;226:756–759.
114. De Lahunta A, Glass EN. *Veterinary neuroanatomy and clinical neurology*. 3rd ed. St. Louis, MO: Saunders; 2009.
115. Matthews HK, Andrews FM. Performing a neurologic examination in a standing or recumbent horse. *Vet Med*. 1990;85:1229–1240.
116. Olby N. Current concepts in the management of acute spinal cord injury. *J Vet Intern Med*. 1999;13:399–407.
117. Zurita M, Vaquero J, Oya S, Morales C. Effects of dexamethasone on apoptosis-related cell death after spinal cord injury. *J Neurosurg*. 2002;96:83–89.
118. Bracken MB. Methylprednisolone in the management of acute spinal cord injuries. *Med J Austr*. 1990;153:368.
119. Bracken MB. Treatment of acute spinal cord injury with methylprednisolone: results of a multicenter, randomized clinical trial. *J Neurotrauma*. 1991;1(suppl 8):S47–S50, discussion S51–S42.
120. Hugenholtz H. Methylprednisolone for acute spinal cord injury: not a standard of care. *CMAJ*. 2003;168:1145–1146.
121. Hugenholtz H, Cass DE, Dvorak MF, et al. High-dose methylprednisolone for acute closed spinal cord injury—only a treatment option. *Can J Neurol Sci*. 2002;29:227–235.
122. Arai T, Tsukahara I, Nitta K, Watanabe T. Effects of mannitol on cerebral circulation after transient complete cerebral ischemia in dogs. *Crit Care Med*. 1986;14:634–637.
123. Polderman KH, van de Kraats G, Dixon JM, et al. Increases in spinal fluid osmolarity induced by mannitol. *Crit Care Med*. 2003;31:584–590.

124. Qureshi AI, Suarez JI. Use of hypertonic saline solutions in treatment of cerebral edema and intracranial hypertension. *Crit Care Med*. 2000;28:3301–3313.
125. Schwarz S, Schwab S, Bertram M, et al. Effects of hypertonic saline hydroxyethyl starch solution and mannitol in patients with increased intracranial pressure after stroke. *Stroke*. 1998;29:1550–1555.
126. Peterson B, Khanna S, Fisher B, Marshall L. Prolonged hypernatremia controls elevated intracranial pressure in head-injured pediatric patients. *Crit Care Med*. 2000;28:1136–1143.
127. Hoerlein BF, Redding RW, Hoff EJ, et al. Evaluation of dexamethasone, DMSO, mannitol and solcoseryl in acute spinal cord trauma. *J Am Anim Hosp Assoc*. 1983;19:216.
128. McConnico RS, Clem MF, DeBowes RM. Supportive medical care of recumbent horses. *Compend Cont Educ Pract Vet*. 1991;13:1287–1295.
129. Nout YS, Reed SM. Management and treatment of the recumbent horse. *Equine Vet Educ*. 2005;7:416–432.
130. Thomas DR. Issues and dilemmas in the prevention and treatment of pressure ulcers: a review. *J Gerontol A Biol Sci Med Sci*. 2001;56:M328–M340.
131. Thomas DR. Prevention and treatment of pressure ulcers: what works? what doesn't? *Cleve Clin J Med*. 2001;68:704–722.
132. Rayner SG. Traumatic cerebral partial lobotomy in a Thoroughbred stallion. *Austr Vet J*. 2005;83:674–677.

CHAPTER 5

Internal Medicine and Clinical Nutrition

Raymond J. Geor*

Veterinarians are a primary source of nutritional information and advice for horse owners. Therefore it is reasonable to expect equine practitioners to have some expertise in the clinical assessment of the nutritional status and feeding programs so that they may assist horse owners in the selection of rations for an individual horse or group of horses. Additionally, because diet composition can contribute to the pathophysiology and clinical manifestations of certain chronic diseases (e.g., some forms of chronic exertional rhabdomyolysis), the veterinarian is often consulted to make recommendations for special diets. Special dietary considerations are also required for sick neonatal foals or adult horses. This chapter provides an overview of the principles of clinical assessment of nutritional status and feeding programs, reviews carbohydrate nutrition for horses (including the types of carbohydrates in horse feeds, terminology and methods for analysis of carbohydrates in feeds, and strategies for mitigation of gastrointestinal disturbances associated with carbohydrate nutrition), and summarizes current recommendations for the nutritional support of neonatal or adult horses with acute illness. Other topics are feeding management of thin and starved horses and dietary recommendations for the management of obesity, which is becoming a significant problem in equine medicine. The reader is referred to the most recent edition of the National Research Council's *Nutrient Requirements of Horses* for a complete discussion of equine nutrition.[1]

EVALUATION OF NUTRITIONAL STATUS AND FEEDING PROGRAMS

Clinical assessment of a feeding program for an individual or group of horses involves three basic elements: assessment of general health and dietary history, clinical examination, and evaluation of current diet and feeding method (i.e., types and amounts of feeds and how they are fed). The physiologic state and intended use of the horse (e.g., pregnant, lactating, in athletic work) affect its nutritional requirements; therefore, they are vital to the evaluation of the feeding program and when making any necessary adjustments. It also is useful to inspect housing and feeding facilities, including feed bins, hay storage, and the watering system. Commercial software programs are available to assist with ration evaluation in relation to nutrient requirements.[2] Fig. 5.1 shows an example of a simple form that can be used to collect clinical data, including signalment, body weight (BW) and condition, and details regarding the horse's current and recommended ration. Box 5.1 provides explanations for a number of nutritional terms used to describe feed nutrients and fractions, the knowledge of which is relevant to the interpretation of feed analysis data.

Clinical Examination

Body condition scoring (BCS) and measurement of BW are the cornerstones of the clinical assessment of nutritional status. In some situations laboratory analysis of blood or other tissue samples may be indicated as part of the nutritional evaluation (e.g., measurement of whole-blood selenium concentration). It must be recognized that no single laboratory value is a reliable indicator of nutritional status in an individual animal. Nonetheless, blood or tissue measurements may be valuable for the evaluation of herd problems, in which samples should be obtained from a representative number of animals.

BW and BCS, which assesses subcutaneous fat deposition, are indicators of long-term energy balance, or energy (calorie) intake relative to the horse's needs. Generally, horses that receive inadequate dietary energy will lose BW and condition, whereas weight gain and the development of overconditioning (high BCS) signify energy intake in excess of requirements. Although a number of systems have been used to determine BCS, the most widely applied method is that developed by Henneke.[3] The Henneke system uses a 1- to 9-point scale and requires the assessment of subcutaneous fat deposition in six areas: over the crest of the neck, withers, behind the shoulder, over the ribs, along the back, and around the tailhead. Considerable variation may exist in the pattern of fat deposition among horses; for example, some horses have little fat deposited over the ribs even when other areas of the body are well covered. In addition, fat deposits are sometimes asymmetrically distributed. Therefore it is important to evaluate all six areas of the body on both sides. A score between 1 and 9 is assigned, in which 1 indicates severe emaciation and 9 indicates extreme fatness (Table 5.1). Body condition scores of 4 to 6 are regarded as ideal depending on use of the horse. Studies of weight gain and loss in moderately conditioned (BCS = 4–7) Thoroughbred, Quarter, and Arabian horses indicate that one BCS unit represents approximately 25 to 35 kg of BW.[4,5] The BW associated with each unit of body condition may be higher in fat or thin horses.

The Henneke BCS system, originally developed for use in Quarter Horse broodmares,[3] is most appropriate for use in light breeds such as Thoroughbreds, Arabians, and Standardbreds. This system may not be suitable for ponies and larger

*The editors and authors acknowledge and appreciate the contributions of Debra K. Rooney as a previous contributor to this chapter. Some of her original work has been incorporated into this edition.

NUTRITIONAL STATUS AND FEEDING PROGRAM EVALUATION FORM

HORSE INFORMATION Date _____

Owner _____ Horse Name _____

Description _____
(e.g., age, gender, breed)
Use/physiological state _____

Housing/environment _____

Medical history_____
_____

PHYSICAL ASSESSMENT

Dentition _____ Normal _____ Poor
Hooves _____ Normal _____ Founder lines (laminitic)
Hair Coat _____ Normal _____ Long _____Other
Current Body Condition Score _____
(1 = very thin; 5 = moderate; 9 = very obese)

Notes:_____
(e.g., abnormal fat deposits)

Optional: Neck Circumference (cm or inches)_____

ESTIMATED ENERGY INTAKE ON CURRENT DIET

Forage Consumption

Excellent hay (1.1 Mcal/lb) × _____ lb/d = _____ Mcal digestible energy (DE)
Very good hay (0.9 Mcal/lb) × _____ lb/d = _____ Mcal
Average hay (0.8 Mcal/lb) × _____ lb/d = _____ Mcal
Below-average hay (0.7 Mcal/lb) × _____ lb/d = _____ Mcal

Concentrate Consumption

Oat grain (1.3 Mcal/lb) × _____ lb/d = _____ Mcal
Sweet feed (1.4 Mcal/lb) × _____ lb/d = _____ Mcal
High-fat sweet feed (1.5 Mcal/lb) × _____ lb/d = _____ Mcal

Other Feeds (e.g., protein supplements, beet pulp)

_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal
_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal
_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal

Current Total Energy Intake _____ **Mcal/d**

(NOTE: Other nutrient intakes should also be checked for adequacy)

FIG. **5.1** Worksheet for evaluation of nutritional status and feeding program. (Adapted from Dr. Laurie Lawrence, University of Kentucky, Lexington, Kentucky.)

ESTIMATING THE APPROPRIATE DAILY ENERGY INTAKE

Target Body Condition Score (BCS) _____

Recommended Change in BCS _____

(+1, +2, –1, –2, etc.)

Current Horse Weight _____ **Target Horse Weight** _____

Guide: For an 1100- to 1200-lb horse, change in 1 BCS unit = 45 to 70 lb, but greater weight change may be necessary to alter BCS in very thin or very obese horses. Also, note that altering feed intake can have a rapid effect on body weight by increasing or decreasing gut fill. Thus, a change in gut fill can affect body weight by 10 to 20 lb, without a concomitant change in body condition score.

Recommended Energy Intake for Target Weight _____ **Mcal/d**
(see Box 2)

Suggested Adjustments for Weight Change (estimates)

Increase BCS 1 unit in 60 days (add 6 to 7 Mcal/d)
Increase BCS 2 units in 90 days (add 9 to 10 Mcal/d)
Decrease BCS 1 unit in 60 days (subtract 6 to 7 Mcal/d)
Decrease BCS 2 units in 120 days (subtract 6 to 7 Mcal/d)

Adjusted Recommended Daily Energy Intake _____ **Mcal/d**

RECOMMENDED DIET

Current Level of Dietary Energy

Adequate _____ Insufficient _____ Excessive _____

Current Diet Could Include Minor Modifications: _____

Current Diet Should Be Adjusted as Follows:

Suggestions:

For weight loss: concentrate amounts should be reduced first
For weight gain: hay intake and quality should be adjusted first

ENERGY INTAKE WITH SUGGESTED DIET

Forage Consumption

Excellent hay (1.1 Mcal/lb) × _____ lb/d = _____ Mcal DE
Very good hay (0.9 Mcal/lb) × _____ lb/d = _____ Mcal
Average hay (0.8 Mcal/lb) × _____ lb/d = _____ Mcal
Below-average hay (0.7 Mcal/lb) × _____ lb/d = _____ Mcal

Concentrate Consumption

Oat grain (1.3 Mcal/lb) × _____ lb/d = _____ Mcal
Sweet feed (1.4 Mcal/lb) × _____ lb/d = _____ Mcal

FIG. **5.1, cont'd**

Continued

High-fat, sweet feed (1.5 Mcal/lb) × _____ lb/d = _____ Mcal

Other Feeds (e.g., protein supplements, beet pulp)

_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal

_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal

_____ (_____ Mcal/lb) × _____ lb/d = _____ Mcal

Total Energy Intake _____ **Mcal/d**

FIG. **5.1, cont'd**

BOX 5.1 Nutritional Terminology Relevant to the Interpretation of Feed Composition Data

Moisture: Percentage of feed that is water

Dry matter (DM): 100% minus the water in feed
Most hays and concentrates are approximately 90% water. Fresh pasture can contain 60% to 80% water.
Feed compositions are usually compared on a *DM basis* (100% DM), but feeds with similar concentrations of DM can be compared on an *as-fed basis.*

Crude protein (CP): Also called *total protein;* this value is calculated by measuring total nitrogen.

Acid detergent fiber (ADF): A chemically determined fraction that contains cellulose and lignin. ADF is inversely related to digestibility and is used to estimate the digestible energy content of horse feeds.

Neutral detergent fiber (NDF): A chemically determined fraction that contains cellulose, lignin, and hemicellulose. NDF contains most of the structural carbohydrates in plants. As plants mature, they contain more stem (more structure); therefore, DF increases with maturity. The NDF fraction includes the ADF fraction. There is a general inverse relationship between NDF in forages and voluntary forage intake by horses; in other words, when two hays of similar variety are compared, the forage with lower NDF will be consumed in higher amounts by horses.

Nonfiber carbohydrates (NFCs): This is not a measured fraction. NFC is determined by calculating the difference between total DM and the sum of NDF, crude fat, ash, and CP. Types of carbohydrates that are included in the NFC fraction are true nonfiber carbohydrates, such as monosaccharides and starch, but it also includes some carbohydrates that are resistant to mammalian enzymatic digestion such as pectin (found in beet pulp and alfalfa hay) and fructan (found in some grasses).

Ethanol-soluble carbohydrates (ESCs): Part of the nonstructural carbohydrate fraction. The ESC fraction contains mostly simple sugars (disaccharides). Some laboratories categorize this fraction as *sugars.*

Starch: Contains amylose and amylopectin. The starch analysis does not separate easily digested starch and starch resistant to small intestinal digestion.

Nonstructural carbohydrate (NSC): Previously, the NSC fraction included all carbohydrates not included in NDF, but today it is commonly defined as starch plus ESC. Therefore the NSC in a feed represents the carbohydrates that are expected to be digested and absorbed from the small intestine as glucose or other simple sugars.

Water-soluble carbohydrates (WSCs): This fraction includes the simple carbohydrates that appear in the ESC fraction as well as *some* longer chain carbohydrates, including fructans. Fructans are storage carbohydrates synthesized by some plants, especially cool season grasses. Some nutritionists calculate fructan as the difference between WSC and ESC, but this estimate has not been validated.

Digestible energy (DE): This is not a measured value. DE is calculated from other analyzed fractions, including ADF and CP. The amount of fat in the feed affects the true DE value of a feed, but if crude fat was not a requested item on the feed analysis, the DE might be calculated using an average value for crude fat. For common forages (e.g., hay and pasture), the DE value calculated by a laboratory is a relatively accurate assessment of the true DE value. For concentrate feeds the calculated DE value may not represent the true DE value.

Adjusted CP, % total digestible nutrients (TDN), net energy of lactation (NEL), net energy of maintenance (NEM), net energy of gain (NEG), relative feed value: Not relevant to horse diet analyses.

breed horses (e.g., drafts) that have a different pattern of fat distribution. A 9-point BCS system for Warmblood horses was developed to account for differences in conformation and patterns of fat deposition in this breed compared with Quarter Horses.[6] For example, fat begins to cover the hip bones of Quarter Horses at a BCS of 4, whereas the hip bones of Warmblood horses remain prominent at a BCS of 6 (using the system developed for Warmbloods).

It also should be noted that the BCS system does not register differences in regional adiposity that may signify increased risk of disease. In humans visceral (abdominal) adiposity is more closely linked than generalized obesity to the risk for diabetes and cardiovascular disease, and measurement of waist circumference is a better indicator of abdominal fat accumulation than is body mass index.[7] In horses and ponies there may be a similar association between regional adiposity and disease risk. In studies of horses and ponies with a predisposition to pasture-associated laminitis, some affected animals are not obese on the basis of BCS (i.e., BCS <7) and have no external evidence of regional adiposity, but others have enlarged fat deposits on the neck ("cresty neck") and thoracic and tailhead regions; these fat deposits

TABLE **5.1 Description of Body Condition Scores in Horses**

Condition Score	General Condition	Neck	Shoulder	Withers	Ribs	Loin	Tailhead
1	Very poor	Individual bone structure visible; feels very bony	Bone structure very visible and sharp to touch	Bones easily visible; no fat; razor-like	Ribs very visible and skin furrows between ribs	Spine bones visible; ends feel pointed	Tailhead and hips very visible
Animal extremely emaciated; no fatty tissue can be felt							
2	Very thin	Bones just visible; animal emaciated	Possible to outline bone structure	Withers obvious, very minimal fat covering	Ribs prominent, slight depression between ribs	Slight fat covering other vertical and flat spin projections; ends feel rounded	Tailhead and hip bones obvious to the eye
3	Thin	Thin, flat muscle covering, no raised muscle or fat	Shoulder accentuated; some fat cover but thinner than desirable	Withers thin and accentuated with some, although little, fat cover	Slight fat cover over ribs; rib outline obvious to the eye	Fat buildup halfway on vertical spines, but easily visible; flat spinal bones not felt	Tailhead prominent; hip bones rounded but easily visible; pin bones covered
4	Moderately thin	Neck with some fat; horse not obviously thin	Shoulder not obviously thin with some fat cover	Withers not obviously thin, smooth edges but prominent	Faint outline visible to the eye	Slight outward ridge along back	Fat palpable
5	Moderate	Neck blends smoothly into body with some fat cover	Shoulder blends smoothly into body	Withers smoothly rounded over top	Ribs cannot be seen but can be easily felt	Back level	Fat around tailhead beginning to feel spongy
6	Moderately fleshy	Fat easily palpable	Fat layer palpable	Fat palpable	Fat over ribs feels spongy	May have slight inward crease	Fat around tailhead soft and palpable
7	Fleshy	Visible fat deposits or lumps along neck	Fat buildup behind shoulder	Fat covering withers is firm	Individual ribs still palpable	May have slight inward crease down back	Fat around tailhead soft and rounded off
8	Fat	Noticeable thickening of neck	Area behind shoulder filled in flush with body	Area along withers filled with fat	Difficult to feel ribs	Crease down back evident	Tailhead fat very soft and flabby
Fat deposited along inner buttocks							
9	Extremely fat	Bulging fat	Bulging fat	Bulging fat	Patchy fat over ribs	Obvious deep crease down back	Building fat around tailhead
Fat along inner buttocks may rub together; flank filled in flush							

are sometimes asymmetrically distributed.[8,9] Neck crest adiposity, assessed by the ratio of mean neck circumference to height at the withers (NCHR), is negatively associated with insulin sensitivity in horses and ponies.[9,10] Although cutoff values for NCHR in relation to obesity and disease risk have not been defined, the repeated measurement of mean neck circumference is useful for monitoring the effectiveness of a weight loss program.

Knowledge of a horse's BW is needed for accurate calculation of nutrient and feed requirements. It is important to note that the BW of an individual horse can vary between 5% and 15%, depending on hydration and feeding status and on gastrointestinal fill. Accordingly, it is important to standardize the time of feeding relative to measurement of BW. BW may be measured directly by use of a platform scale or, in mature horses, estimated from body measurements such as heart girth circumference and body length.[11,12] Heart girth is measured immediately behind the elbows, and body length is measured from the point of the shoulder to the tuber ischii:

$$\text{BW (kg)} = \left[\text{Heart girth (cm)}^2 \times \text{length (cm)}\right]/11,800$$

BW also can be estimated from measurement of heart girth circumference by use of a weight tape calibrated for horses. Measurements should be taken in a consistent manner, with the horse standing on a flat surface, and care must be taken not to pull the tape too tightly. Clinicians are advised to take the average of several (e.g., three or four) measurements. Accuracy of these measurements is affected by a number of factors, including breed, conformation, level of fitness, thickness of hair coat, gastrointestinal fill, and pregnancy status.[12] BW based on heart girth is most accurate when this method is applied to mature horses with an average BCS (i.e., 5–7). Underestimation or overestimation of BW may occur when weight tapes are used to estimate the BW of horses with low or high condition scores.

Diet and Ration Evaluation

It is useful to first examine the physical characteristics of feeds (i.e., visual appearance [color, presence of foreign material such as mold and dust] and odor). Preserved forages (e.g., hay) should be evaluated for type (grass vs. legume), leaf-to-stem ratio, and presence of seed heads. Hays with good nutritional value are characterized by a large proportion of leaf; compared with stems, leaves have higher nonstructural carbohydrates (NSCs; starches, sugars, and fructans), lower structural carbohydrates (SCs; hemicellulose, cellulose, and lignin), and higher protein.[1] Therefore second- and third-cutting hays, which often have a higher stem-to-leaf ratio, tend to provide higher nutritional value than first-cutting hay. The latter is usually harvested at a more mature stage. The presence of seed heads and a stemmy, coarse appearance are also indications of advanced maturity at cutting. Some studies have reported an inverse relationship between the neutral detergent fiber (NDF; the fraction that contains cellulose, hemicellulose, and lignin) and voluntary hay intake (i.e., the voluntary intake of stemmy hays with high NDF content will be lower compared with that of hays with high leaf-to-stem ratio).[1,13]

The next step in the evaluation is to estimate daily feed intake. Owners or stable managers should be questioned to determine the amounts of hay, concentrates, and supplements provided (and whether the horse regularly refuses any of these feedstuffs). All feeds, including several flakes or biscuits of hay, should be weighed to estimate the actual amounts of feed provided to the horse. Significant errors may occur when volume (e.g., "a coffee can–full") rather than weight is used to estimate feed provision because grains, concentrates, and hays vary markedly in weight per unit volume. A handheld fish scale or similar scale works well for this task. It is difficult to measure actual feed intake, particularly when forage is fed on the ground, where wastage can be as high as 25%, or fed to groups of horses. The other challenge is estimation of pasture forage intake, which is generally taken as the difference between the mean intake of digestible energy (DE) for horses, with consideration given to their weight, age, and physiologic condition, minus actual daily intakes of hay and grains. As previously mentioned, regular assessment of BW and BCS over time will provide the best guide to the adequacy of energy intake. Reported values for daily dry matter intake (DMI) by horses, ponies, and donkeys range between 0.8% and 5.2% of BW.[1] Young and mature horses appear to have a maximal daily DMI of 3% to 3.2% of BW, but intakes in the range of 2.0% to 2.5% of BW are more typical (this includes horses maintained on an all-hay diet). Some evidence suggests that ponies may have higher daily DMI than horses.[1]

The clinician also should evaluate feeding management, particularly when a problem under investigation may be due to poor feeding practices (e.g., weight loss, colic). For example, what are the relative proportions of forage and grain/concentrate? Does the ration contain adequate high-quality forage? How many times a day is the horse fed? How frequently is the diet changed, and are these changes made gradually (ideally over 7–10 days) or suddenly? Several epidemiologic studies have reported that changes in diet (grain/concentrate or hay, even when switching to hay of the same type) significantly increase the risk of colic.[14-16] The risk of gastrointestinal problems also increases when horses are fed large quantities of starch-rich grain and concentrate feeds or inadequate long-stem roughage.[14,16] As a general guide, all horses should receive a *minimum* of 1.0% of BW per day as hay or the equivalent; forage at 1.5% of BW per day is preferred. For a 450- to 500-kg horse, grain or concentrate meals should be no more than 2.5 kg (approximately 5 lb) to reduce risk of hindgut disturbances associated with the cecal delivery of undigested starch. If the daily ration includes more than 5.0 kg of grain or concentrate, it should be provided in more than two feedings per day (see the section Carbohydrates in Equine Nutrition).[16]

Information on the nutrient content of the diet is needed to determine the adequacy of the ration. Several approaches can be used to estimate the nutrient contents of feeds. The feed tags on commercial feeds provide some nutritional information, including a list of ingredients and data on some nutrients. However, feed manufacturers are required to guarantee only that minimums were met and maximums were not exceeded; in other words, the actual content of listed nutrients is not provided. An alternative source of data on the nutrient content of feeds is published databases (e.g., National Research Council [NRC] publications, feed company websites). Although the nutrient profiles available from these databases provide a useful guide, these data may not correspond exactly to the feedstuff provided to the horse under evaluation because of differences in geography, growing and harvesting conditions, and other factors. The ideal, although not always practical, approach is to submit samples of feed and forages (core hay samples and pasture clippings) for laboratory analysis of nutrient content. At least two samples (approximately 250 g each) of each grain or concentrate should be taken; these should be mixed thoroughly with the composite sample submitted for analysis. Approximately 10% of the hay store should be sampled by use of a hay probe. Again, these samples should be well mixed, with the composite sample shipped to the laboratory in a sealable plastic bag. Garden shears can be used to obtain pasture clippings, which should be sampled in a Z-pattern and cut to grazing height. It may be necessary to sample clippings taken from several pastures to determine the range of nutrients on the farm. The basic nutrient profile from analysis includes dry matter (DM), DE, crude protein (CP), acid detergent fiber, NDF, fat (ether extract), ash, calcium, magnesium, phosphorus, sodium, and potassium (see Box 5.1). Trace element and vitamin analyses are available depending on need and expense. Some laboratories will perform additional analyses and calculations to provide estimates of some of the carbohydrate fractions. For example, Equi-Analytical Laboratories (Ithaca, NY; www.equianalytical.com) analyzes starch and ethanol-soluble carbohydrates (ESCs; which are mostly simple sugars). More information on dietary carbohydrates is presented later in this chapter.

With data on feed composition (and estimated intake of feeds), it is possible to formally evaluate the ration against published feeding standards (e.g., NRC 2007[1]). The NRC developed a simple Web-based program that calculates nutrient requirements derived from the 2007 *Nutrient Requirements of Horses* (http://nrc88.nas.edu/nrh/); this program can also be downloaded to a personal computer. The user is first required to enter basic information such as age, BW, and physiologic state (e.g., stallion, lactating mare, working horse) and then data on the horse's ration, specifically, the amount and nutrient profile of each feed and supplement fed to the horse. The program uses these data to calculate nutrient requirements, dietary supply, and the difference between the two amounts. Unfortunately, only a limited number of nutrients are evaluated: DE, CP, lysine, Ca, P, Na, Cl, and K. The NRC recommendations are also based on minimum nutrient requirements, whereas many nutritionists favor feeding standards based on optimal ranges. Several nutrition companies have developed more comprehensive computer software programs. For example, MicroSteed, developed by Kentucky Equine Research, Inc. (Versailles, Kentucky), can evaluate the ration against both the NRC minimums and a set of optimums recommended by company nutritionists.[17]

The process outlined in the preceding paragraphs will facilitate decisions regarding the adequacy of the current ration. If gross deficiencies or excesses are identified, adjustments can be recommended and implemented. Another important aspect is the need for follow-up monitoring, particularly if a goal has been set for weight loss or gain. Fig. 5.1 outlines a simple way to estimate energy needs and intake and calculate ration adjustments necessary to facilitate weight loss or gain. A more detailed discussion of dietary management of thin or obese horses is presented later in this chapter.

CARBOHYDRATES IN EQUINE NUTRITION

Carbohydrates are the primary source of energy in the diet of horses. As nonruminant herbivores, horses evolved to utilize forages high in SCs, with bacterial fermentation and production of volatile fatty acids (VFAs) in a highly developed large intestine. However, modern horses, particularly those in athletic training, are often fed cereal grains or other starch-rich feeds to meet energy requirements. For example, some survey studies indicate that racehorses weighing 450 to 550 kg typically receive 3 to 6 kg of feed per day, with some horses receiving more than 8 kg per day.[18,19] Such high grain intakes by horses (or a low forage-to-grain ratio such as 30:70) have been implicated in the development of gastrointestinal problems, particularly colic associated with disturbances to hindgut function[20,21] and gastric ulcer disease.[22,23] Additionally, diets rich in starch and sugar contribute to the clinical expression of chronic muscle diseases (polysaccharide storage myopathy and recurrent exertional rhabdomyolysis) in genetically susceptible horses[24] and likely exacerbate insulin resistance and susceptibility to laminitis in horses and ponies with the metabolic syndrome phenotype.[25] Therefore equine veterinarians should have working knowledge of carbohydrate digestion and metabolism and methods for assessment of the carbohydrate fractions in feeds. This information can be used in the formulation of diets and feeding programs that control intake of certain carbohydrates and potentially mitigate risk of these conditions.

Classification and Nomenclature

Plant carbohydrates in equine feeds can be subdivided into the SCs, which largely make up the fibrous portion of the diet and originate from the plant cell wall, and the NSCs, which originate from the cell content. Diets for horses, whether based on pasture, conserved forage, concentrates, or a combination of all three, contain both SCs and NSCs.[26]

All dietary carbohydrates contain similar amounts of gross energy. However, when used by the horse, they provide variable amounts of DE, metabolizable energy, and net energy.[27] Carbohydrates digested and absorbed as monosaccharides in the small intestine yield more energy than carbohydrates digested by microbial action (predominantly fermentation), and a glycemic response to the ingestion of such carbohydrates tends to occur.[28] The type of linkage between the monosaccharide residues in the carbohydrate also affects the site of digestion of these compounds and thus their nutritional value. Hydrolysis of the α1-6 and the α1-4 linkages of starch and maltose, for example, can occur in the equine small intestine, but horses do not produce the enzymes necessary to digest the β1-4 linkages found in cellulose or the mixed linkages found in hemicellulose.[28] Therefore digestion of cellulose and hemicellulose must occur as a result of microbial fermentation, which does not result in a pronounced glycemic response. Stachyose, raffinose, β-glucans, fructooligosaccharides (or fructans), and pectin are also thought to be resistant to enzymatic hydrolysis. Thus an understanding of the various carbohydrate fractions in plants (including means of digestion) is needed to determine the potential for a feed to result in a glycemic–insulinemic response.

Simple Sugars

This fraction comprises monosaccharides and disaccharides (i.e., glucose, fructose, sucrose). The simple sugar content of these plants is low; the sugars produced by way of photosynthesis can be used immediately by the plant to supply energy for metabolism, protein synthesis, and growth, or they can be elaborated into more complex *oligosaccharides* (e.g., raffinose and stachyose) or the *structural polysaccharides* of the plant cell wall (e.g., cellulose, hemicellulose, pectin). When sugar production exceeds the immediate requirements for metabolism, the excess sugars are polymerized to form "storage," or "reserve," carbohydrates. Storage carbohydrates mostly are in the form of starch or fructan. Starches and fructans, together with the simple sugars and the oligosaccharides, make up the NSC fraction of the diet.[26]

Oligosaccharides

The raffinose family of oligosaccharides are α-galactosyl derivatives of sucrose. The most common are the trisaccharide raffinose (composed of galactose, fructose, and glucose) and the tetrasaccharide stachyose. These oligosaccharides are found in sugar beet molasses and whole grains. Soybean oligosaccharides make up approximately 5% of DM in whole beans and up to 8% of DM in soybean meal. Together raffinose and stachyose rank second only to sucrose in abundance as water-soluble carbohydrates (WSCs). However, raffinose and stachyose cannot be directly digested by the horse because of the absence of the enzyme α-galactosidase.

Structural Polysaccharides

This fraction includes dietary fiber that is composed of cellulose, pectin, and hemicelluloses, along with mannans,

galactans, and xyloglucans.[26] Total nonstarch polysaccharides (NSPs) are the sum of the water-soluble and water-insoluble NSPs and include cellulosic and noncellulosic polysaccharides. Although some of the NSPs are water soluble, these are not digestible by mammalian enzymes; therefore, digestion can occur only through fermentation, which may occur proximal to the cecum.

Starch

Starch is the main storage polysaccharide in a number of higher plants, including the forage legumes (e.g., clover, alfalfa). Starch is stored in both the vegetative tissues (i.e., nonreproductive tissues such as leaves and stems) and the reproductive tissues (i.e., flowers, seeds). The starch content of grass seed ranges from approximately 300 to 400 g starch/kg seed DM, whereas oats, barley, and corn contain approximately 400, 550, and 700 g starch/kg DM, respectively.[26] The amount of starch stored in the leaves of legumes rarely exceeds 75 g starch/kg DM.

Starch consists of polymers of glucose, which occur in two forms: amylose and amylopectin. Amylose is a linear α-(1–4) linked molecule, and amylopectin is a larger, highly branched molecule containing both α-(1–4) and α-(1–6) linkages. The ratio of amylose to amylopectin largely depends on the botanical origin of the starch. For example, in wheat flour amylose is around 30% of the total starch, whereas corn can contain up to 70% amylose. The extent to which starch is digested prececally depends on many factors, including the availability of the starch to mammalian enzymes (e.g., the extent to which any outer husk or hull has been broken down), the ratio of amylose to amylopectin within the starch granule, the effect of processing (thermal treatment, for example, improves the digestibility of corn and barley starch), and intestinal passage rate.[29] Glucose availability in the small intestine tends to be higher for starches with high amylopectin content. As discussed in more detail later, the ingestion of excessive levels of starch may exceed the relatively limited amylolytic capacity of the equine foregut. Any undigested starch (including resistant starch) that has not been fermented in the stomach and small intestine by resident microbes will pass into the large intestine, where it will be fermented and yield less net energy than when it is absorbed as glucose.[29]

Fructan

Fructan is the major storage carbohydrate of the vegetative tissues of temperate grasses. Depending on the number of fructose molecules, fructans can be described as oligosaccharides (<10 monosaccharide units) or polysaccharides (>10 units). High levels of fructan can accumulate in the vegetative tissues of pasture grasses, with implications for the development of pasture-associated laminitis.[26] In a 3-year northern European study of the WSC (sugar plus fructan) components in the vegetative structures of temperate grasses, fructan contents of up to 279 g/kg DM were recorded.[26] Fructan accumulation (unlike that for starch) can occur below the temperature threshold for plant growth (approximately 6°C).[30] Therefore cold, bright days that result in high rates of photosynthesis but minimal plant growth may lead to the production of large quantities of excess sucrose and, in turn, substantial fructan accumulation.

Evaluation of the Carbohydrate Content in Feeds

A number of analytical techniques and terms have been used to describe the carbohydrate fractions in feeds. It is important to understand what the different terms mean and to recognize the variation among laboratories with respect to analytical methods and definitions (see Box 5.1).

Water-Soluble Carbohydrates

The WSC fraction includes the simple (glucose, sucrose, and fructose) and more complex (oligosaccharides and fructans) sugars. Although not all of these components can be digested by mammalian enzymes, most can be rapidly fermented by gram-positive bacteria, resulting in the production of lactic acid. However, it is important to note that starch, which also can be fermented in this way, is not included in this category. Some commercial laboratories measure WSC (i.e., free sugars plus oligosaccharides and fructans) but report it simply as sugar, whereas others use the term *sugar* to describe the free sugar fraction only.

Ethanol-Soluble Carbohydrates

The term *ethanol-soluble carbohydrates* (ESCs) refers to the component of the WSC that is digestible by mammalian enzymes and elicits a glycemic response (i.e., simple sugars). The difference between the WSC and ESC fractions has been used to approximate the amount of fructan in a particular feedstuff, although the accuracy of this calculation has not been verified.

Nonstructural Carbohydrates

The NSC fraction includes the monosaccharides and disaccharides, oligosaccharides (including fructan), fructan polysaccharides, and starch. Several methods have been used to estimate the NSC content of feeds. The system developed by Van Soest[31] estimates NSC *by difference,* in which the feed is partitioned into neutral detergent solubles and NDF. The NDF fraction contains cellulose, most of the hemicellulose, and lignin. Until recently, the NSC content of a feed was determined on the basis of feed analysis and the "by-difference method," according to the following equation:

$$\text{NSC} = 100 - (\text{CP \%} + \text{NDF \%} + \text{moisture \%} + \text{fat \%} + \text{ash \%})$$

This estimate was taken to represent the combined sugar, starch, and fructan content of the feed. However, it also includes pectins, gums, and mucilages that, unlike starch, sugar, and fructan, are not subject to very rapid fermentation; therefore, they do not induce the marked changes in lactic acid concentrations and pH within the hindgut that can occur with excess intake of sugars, starch, or fructan. Therefore the NSC by-difference fraction is now referred to as *nonfiber carbohydrate,* or *NFC.* The quantitative difference between measured NSC and NFC is small for some feeds (e.g., cereal grains) but can be quite large for other feeds (e.g., feeds with substantial pectin, such as sugar beet pulp; Table 5.2).

Most commercial feed analysis laboratories do not completely fractionate the carbohydrates that make up the NSC, but in most feeds the amount of NSC can be approximated by summing the amount of starch and WSC. The extent to which the sum of starch and WSC accounts for all NSC depends on the analytic procedures used to measure these fractions. This determination probably more accurately reflects the potential for a feed to be rapidly fermented to produce lactic acid.

TABLE **5.2 Neutral Detergent Fiber, Nonfiber Carbohydrate, and Nonstructural Carbohydrate Composition of Selected Feedstuffs on a Dry Matter Basis**[a]

Feedstuff	% NDF	% NFC[b]	% NSC[c]
Alfalfa hay	43.1	22.0	12.5
Beet pulp	47.3	36.2	19.5
Corn gluten meal	7.0	17.3	12.0
Mixed, mostly grass hay	60.9	16.6	13.6
Soybean meal (48% CP)	9.6	34.4	17.2
Soyhulls	66.6	14.1	5.3

[a]The values shown here may vary from those shown elsewhere in this chapter. They are provided to illustrate differences among feeds and carbohydrate categories. Actual values for individual feeds may vary by stage of maturity, variety, and source.
[b]%NFC = (100% – %CP – %EE – %ash – %NDF).
[c]%NSC determined by direct measurement.
CP, Crude protein; *EE,* ether extract; *NDF,* neutral detergent fiber; *NFC,* nonfiber carbohydrate; *NSC,* nonstructural carbohydrate.

Carbohydrate Nutrition and Colic

Colic is caused by many conditions, each of which may be related to specific risk factors, such as changes in diet, feeding practices, exercise patterns, and housing or inappropriate parasite control programs. An association between feeding practices and disturbances in gastrointestinal function has long been hypothesized,[32,33] but the mechanisms linking diet with the development of intestinal dysfunction are poorly understood. Indeed, the exact relationship between diet and colic is difficult to determine because of the variety of feeds and feeding practices used throughout the world as well as differences in study populations. Furthermore, it is often difficult to separate the effects of diet and feeding schedule from other management practices, which often depend on the horse's breed and use. Nonetheless, the results of recent epidemiologic studies support the proposition that diet composition and recent changes in diet are important risk factors for the development of colic.[34-39] Tinker et al.[37] prospectively examined the risk for colic at 31 horse farms over a 1-year period. Both a change in concentrate feeding (odds ratio [OR] = 3.6 relative to no colic) and the feeding of high levels of concentrate (>2.5 kg/day DM, OR = 4.8, >5 kg/day DM, OR = 6.3, relative to feeding no concentrate) were identified as risk factors for colic. In addition, colic risk increased when processed feeds such as pellets were fed. Hudson et al.[36] reported that a recent (within 2 weeks) change in type of grain or concentrate fed (OR = 2.6), the feeding of more than 2.7 kg of oats per day (OR = 5.9), and a change in the batch of hay fed (OR = 4.9) were significant risk factors for an episode of colic. In another prospective case-control study, neither the amount nor type of concentrate fed was associated with the colic risk, although the researchers did conclude that horses at pasture may have a decreased risk of colic.[38] On the other hand, a recent (within 2 weeks) change in diet, in particular the type of hay fed (including hay from a different source or cutting of the same type of hay) was a significant risk factor for colic.[38] In this study feeding hay other than coastal/Bermuda or alfalfa significantly increased the colic risk, but this finding may have reflected hay quality and digestibility rather than type of hay per se. Changing to a poorer quality, less digestible hay or feeding wheat straw or cornstalks may predispose horses to large colon impaction.[38] In a practitioner-based colic study in the United Kingdom, a recent change in management was associated with at least 43% of the cases of spasmodic or mild undiagnosed colic. The most common management change was turnout onto lush pasture in the spring.[39] In reviewing the results of available epidemiologic studies, Cohen estimated that approximately one third of colic cases had a history of a recent change in diet.[40] The ingestion of high-concentrate and low-forage diets has also been implicated in the development of gastric ulcers, which in turn may result in signs of colic.[23]

These observations raise several questions regarding the effects of diet composition and dietary change on gastrointestinal function, including the capacity of the equine digestive tract for grain (starch) digestion, possible reasons for increased colic risk with high levels of grain feeding, and the effect of a sudden change in diet (grain or forage) on gastrointestinal function.

Carbohydrate Digestion and Hindgut Function

From a digestive viewpoint carbohydrates in horse feedstuffs can be divided into three main fractions: (1) hydrolyzable carbohydrates (CHO-H), which can be digested in the small intestine by mammalian enzymes (or they can be fermented, both in the foregut and hindgut); (2) rapidly fermented carbohydrates ($CHO\text{-}F_R$), which cannot be broken down by mammalian digestive enzymes but are readily available for microbial fermentation; and (3) slowly fermentable carbohydrates ($CHO\text{-}F_S$). The hydrolyzable fraction includes hexoses, disaccharides, some oligosaccharides, and the nonresistant starches. Although some fermentation of these compounds may occur in the stomach, the primary products of digestion of these compounds are monosaccharides that can be absorbed in the small intestine, with a relatively high energy yield. The rapidly fermentable fraction included pectin, fructan, and some oligosaccharides not digested in the small intestine. Resistant starch and neutral detergent hemicellulose could also be included in the rapidly fermentable fraction. The slowly fermentable carbohydrate fraction includes cellulose, hemicellulose, and lignocellulose that result primarily in the production of acetate in the large intestine.

Foregut Digestion

Carbohydrate digestion begins in the stomach, which in the horse is relatively small and inelastic (capacity 9–15 L for a 500-kg horse). Bacterial fermentation of ingested feed is initiated in the cranial (squamous) portion of the stomach, with conversion of some of the available simple sugars or starches to lactic acid.[41] This microbial activity and starch/sugar degradation slows when the gastric contents pass to the fundic

gland region and are mixed with gastric secretions containing pepsinogen. Horse saliva contains minimal amylase activity, and little enzymatic carbohydrate digestion occurs in the stomach.

The small and large intestines are the primary sites of carbohydrate digestion. Starch digestion in the small intestine first involves degradation by α-amylase into disaccharide (maltose), trisaccharide (maltotriose), and α-dextrin units. Subsequently, there is hydrolysis of maltose, maltotriose, and α-dextrin units by small intestine brush border glycanases, primarily amyloglucosidase, to form free glucose.[42] The disaccharidases sucrase, lactase, and maltase are expressed along the length of the small intestine.[43] D-Glucose and D-galactose are transported across the equine intestinal brush border membrane by a Na^+/glucose cotransporter type 1 isoform (SGLT1),[43] and fructose is absorbed by way of an equine-specific GLUT-5 transporter.[15] The activity of both transport proteins is highest in the duodenum and lowest in the ileum.[15] Sugars taken up by enterocytes are transported down concentration gradients into the circulation by way of the GLUT-2 transporter. Preliminary studies have demonstrated upregulation of small intestinal SGLT1 expression with an increase in dietary starch content.[44]

It has been proposed that starch digestion in the small intestine is limited by amylolytic activity (e.g., the availability and activity of α-amylase). The activity of α-amylase in the pancreatic tissue of horses is low relative to that of other species,[45] although the activities of brush border glycanases appear to be comparable to those observed in humans, pigs, and dogs.[46,47] The activity of α-amylase in the pancreatic tissue of horses fed either hay or hay and concentrate for at least 8 weeks was unaffected by diet.[46] However, in a related study, the amylase activity of jejunal chyme was modestly higher in horses that received a diet with added corn, oats, or barley compared with only hay.[47]

The extent of starch digestion in the small intestine is affected by the type and amount of starch digested (see the section Recommendations for Minimizing Digestive Disturbances).[48] At low levels of starch intake (<100 g/100 kg BW from oats, barley, or corn as a single meal), approximately 80% of the starch was digested in the small intestine. When starch feeding was doubled (250–270 g/100 kg BW), prececal starch digestibility decreased to between 50% and 55%.[49] Therefore most nutritionists recommend that an individual grain or concentrate meal should contain no more than 2 g starch/kg BW. For example, if a grain concentrate is 50% starch, no more than 4 g/kg BW or approximately 2 kg for a 500-kg horse should be fed. At higher intakes in a single meal, particularly when unprocessed corn or barley is fed, there is the risk of substantial starch overflow into the hindgut, where it will undergo rapid fermentation.

Hindgut Digestion

The equine large intestine (cecum and colon) is an enlarged fermentative chamber that contains an extremely abundant and highly complex community of microorganisms. Although some fermentation of feedstuffs occurs in the stomach and small intestine, most fermentation occurs in the hindgut. The microbial hydrolysis of dietary plant fiber within the large intestine leads to the release of soluble sugars that are subsequently fermented to the VFAs acetate, propionate, and butyrate, which are an important source of energy. In addition, the VFAs (particularly butyrate) regulate the expression of genes controlling proliferation, apoptosis, and differentiation of gut epithelial cells.[50]

The rate of fermentation and the microbial and biochemical contents of the large intestine are affected by diet composition and feeding pattern (i.e., continuous grazing, or small, frequent meals, versus large meals administered twice daily). A change from forage only to a forage and concentrate diet will result in an increased rate of fermentation and marked changes in the microbial population, luminal pH, and the contents of VFA and lactate.[51] The extent of these changes is likely dependent on the nature and abruptness of the dietary change. With a sudden increase in grain (i.e., starch) feeding, a portion of the ingested starch passes into the cecum undigested, in which it undergoes rapid fermentation with increased production of lactate and gas and a decrease in cecal–colonic pH. Increasing proportions of grain result in decreased acetate and increased propionate and lactate contents of the cecum and colon.[51] Other biochemical changes with the rapid fermentation of starch (or other CHO-F_R, such as fructan) in the hindgut may include an increase in the production of vasoactive monoamines (e.g., tyramine, tryptamine), endotoxins, and exotoxins, all of which have been implicated in the pathogenesis of laminitis.[52]

After an increase in starch feeding, the numbers of lactobacilli and total anaerobic bacteria increase, whereas the numbers of xylanolytic and pectinolytic bacteria decrease.[53] Overall, there is an increase in amylolytic, lactic acid–producing bacteria and decreases in the proportions of acid-utilizing (particularly lactate) and cellulolytic (i.e., fiber-degrading) bacteria. Reduced efficiency of fiber utilization and decreased energy yield may result from the decline in cellulolytic bacteria with high grain feeding. In horses fed forage only, pH within the cecum and colon is in the range of 6.7 to 7.0. The feeding of increasing amounts of corn or barley starch is associated with proportionate decreases in cecal pH, with values approaching 6.0 when 3 to 4 g/kg BW is fed as a single meal.[51,54,55] A similar dose of oat starch was not associated with a significant decrease in pH, consistent with other data demonstrating higher prececal digestibility of oat starch versus barley and corn starch.[55] Some nutritionists have suggested that a cecal pH of 6.0 represents subclinical acidosis and that the risk of clinically apparent intestinal dysfunction (e.g., increased permeability) is substantially increased when hindgut pH is less than 6.0.[2,55] Similar disruptions to the hindgut environment probably occur with the delivery of other rapidly fermentable substrate, such as fructan, which can compose 5% to 40% of grass DM,[26] particularly temperate species such as perennial ryegrass and Timothy grass. In vitro experiments have demonstrated that fructan induces a more rapid decrease in the pH of cecal contents compared with corn starch,[56] and one type of fructan (Raftilose) has been used to induce carbohydrate overload and laminitis.[57]

These disturbances to the hindgut environment put the horse at greater risk for digestive disturbances such as colic, osmotic diarrhea, and laminitis.[32,33] Scenarios favoring the presentation of large loads of rapidly fermentable substrate to the hindgut include (1) a sudden introduction to grain feeding or an abrupt increase in the amount of grain concentrate; (2) the feeding of large grain meals that, even in horses adapted to such feeds, overwhelm the hydrolytic or absorptive capacity of the small intestine; and (3) the grazing of lush pasture or forage with high contents of rapidly fermentable substrate such as

fructan and simple sugars. It is therefore apparent that feeding strategies designed to minimize hindgut disturbances must focus on reducing the flow of rapidly fermented substrate to the cecum and large colon.

Recommendations for Minimizing Digestive Disturbances

Feeding Frequency

Ideally, feeding strategies for horses kept under intensive conditions would mimic the pattern of a grazing animal; that is, an almost continuous feeding pattern that minimizes fluctuations in the rate of delivery of substrate to the large intestine and, when forage makes up the bulk of the diet, ensures some stability of the hindgut ecosystem. A more continuous feeding pattern also may minimize fluctuations in gastric acidity and therefore be of benefit in horses at risk for squamous mucosal ulcer disease. For stabled horses fed two large meals daily, foraging behavior should be encouraged by increasing the availability of hay (or even a variety of different forages) and pasture or dry lot turnout (with forage available). Provision of more frequent (e.g., thrice daily rather than twice daily), smaller concentrate meals throughout the day is also recommended to minimize delivery of undigested hydrolyzable carbohydrate to the hindgut. Extending eating time by diluting the energy density of the meal (e.g., chopped hay mixed with concentrates) or feeding forage before grain or concentrate may be helpful. For some greedy eaters placement of several large stones in the feeder trough may slow the rate of intake.

Adequate Forage and Fiber

For hard-working horses with high DE requirements, the provision of roughage is often restricted in favor of grain concentrates to ensure adequate DE intake within limits of typical DM consumption. However, there is considerable circumstantial evidence associating low-roughage diets with digestive disturbances (e.g., hindgut acidosis, colic, gastric ulcers) and behavioral problems. There also is evidence that the adverse effects of high starch intake on hindgut function are mitigated when the ration is at least 50% NDF.[58] Accordingly, there is rationale for feeding programs that promote higher roughage intake. An absolute fiber requirement has not been defined, but a minimum of 1.0 kg long-stem forage per 100 kg BW (i.e., 5.0 kg for a 500-kg horse, as-fed basis) has been recommended. Some nutritionists have suggested that a rate of 1.5 kg per 100 kg BW is preferable. Alternatively, fiber intake can be increased by feeding other sources such as sugar beet pulp or soya hulls, both of which are highly digestible (i.e., the DE yield is higher compared with that of hay) and are now commonly added to energy supplements for horses. This approach also facilitates a decrease in reliance on grain or sweet feed for energy, decreasing the risk of digestive disturbances associated with high starch intake.

Forage quality is another important consideration. The feeding of highly lignified fiber sources (e.g., straw), which are poorly degraded in the large intestine, may increase the risk of impaction colic. High intake of straw is possible when it is used for bedding, particularly when an inadequate amount of hay (or roughage with low palatability) is offered. An increase in the provision of palatable forage or a change in bedding can be helpful in these situations. Moldy forage should not be fed to equids.

Limiting Delivery of Rapidly Fermented Substrate to the Hindgut

Size of Grain-Concentrate Meals. The feeding of large meals rich in starch and sugar can overwhelm the digestive capacity of the small intestine and destabilize the hindgut because of rapid fermentation of these substrates. No more than 2 g starch/kg BW should be fed in a single meal. For a grain or sweet feed mix that is 40% to 50% starch, this upper limit equates to approximately 2.0 kg per meal for a 500-kg horse.

Feed Starch Sources with High Prececal Digestibility. Prececal starch digestibility varies with the type of grain and the nature of any mechanical or thermal processing. Although oat starch has a prececal digestibility of around 80% to 90%, approximately 35% of equivalent doses of barley or corn starch (from unprocessed grains) reaches the cecum undigested. The higher prececal digestibility of oat starch may relate to the small size of the starch granules compared with other grains, providing a large surface area for exposure to intestinal amylase. Milling, grinding, and various heat treatments (e.g., steam flaking, micronization, extrusion) improves the prececal starch digestibility of oats, barley, and corn. In one study the preileal digestibility of ground oats was 97% compared with 83% for whole oats. Rolling or breaking did not improve the preileal digestibility of oats. For corn and barley preileal starch digestibility is substantially increased after heat (e.g., steam-flaked corn, micronized barley) but not mechanical treatment. Overall, oats appear to be the safest source of starch for horses, although barley and corn are acceptable *if* they are subjected to some form of heat treatment.

Use Alternative Sources of Energy. The energy demands of growth, lactation, and performance can be readily met by provision of alternative energy sources such as vegetable oil (fat) and nonstarch carbohydrates (e.g., sugar beet pulp, soya hulls). Commercial concentrates made with these ingredients contain varying amounts of starch and sugar, but generally amounts are substantially lower compared with straight cereals or sweet feed mixes. Compared with more traditional fiber sources such as hay, soya hulls and beet pulp contain lower indigestible material (e.g., lignin) and higher amounts of NSPs, pectins, and gums, which can be digested to a large extent within the time period that they remain within the gastrointestinal tract. This translates to a higher energy yield. A variety of vegetable oils (e.g., corn, soy, safflower, or flaxseed) and other sources of fat (e.g., stabilized rice bran; approximately 20% fat) are suitable for inclusion in equine diets. Corn oil tends to be the most palatable oil, but most types have acceptable palatability provided that they are fresh and not rancid. One recommendation is to feed up to 100 g oil/100 kg BW daily. For reference purposes, 450 mL of oil (approximately 420 g) provides about 3.4 megacalories (Mcal) of DE. This daily amount should be divided into two or three meals and introduced gradually (e.g., starting at 50 mL/day). Vitamin E (100–200 IU/100 mL of oil) should be added to the ration if supplemental vegetable oil is provided.

Pasture Grazing. An unresolved problem is management of the intake of rapidly fermentable substrate (e.g., fructan) by horses on pasture, particularly animals with a history of pasture-associated laminitis or those with recognized risk factors for this disease (i.e., obesity, insulin resistance). The most obvious avoidance strategy is to prevent access to pasture and feed-preserved forage with a low NSC content (e.g., <10% NSC). Alternative approaches are to restrict access to pasture at certain times of the day, avoiding peaks in forage

NSC content that may increase risk of laminitis, or to apply a grazing muzzle that limits forage intake (but allows water intake). Several factors affect the accumulation of fructans and other forms of NSC in pasture plants, including plant growth rate, temperature, and light intensity. There also is marked diurnal variation, with peak concentrations in the afternoon and a nadir during the night and early morning, and it has been suggested that horses grazing in the afternoon may ingest twofold to fourfold quantities of NSC compared with that ingested during night or early morning grazing. These observations are the basis for the recommendation to restrict grazing to late night and early morning, with removal of the horse or pony from pasture by midmorning.

Gradual Dietary Changes

The increased risk of colic in the 2-week period after a change in hay or grain feeding suggests that all changes in diet and pattern of feeding should be gradual. Horses should receive a blend of old and new hays during the transition between hay batches (e.g., over a 7- to 10-day period, with a gradual increase in the proportion of the new forage) and a conservative introduction to concentrate feeding or changes in type of grain or concentrate. One suggestion is to start at about 0.5 kg/day (split into two feedings) for a 450- to 500-kg horse, with increasing increments of no more than 0.5 kg/day until attainment of the target feeding rate.

Efficacy of Probiotic Supplements and Feed Additives Purported to Stabilize the Hindgut Environment

There is considerable interest in the use of feed additives such as live yeast culture, probiotics (bacterial species), and buffers (e.g., sodium bicarbonate) as a strategy to minimize the negative effects of cereal-based diets. Yeast cultures might be beneficial for stabilization of the hindgut environment in the face of high cereal feeding. In horses fed high-starch meals (3.4 g/kg BW per meal, as barley), daily supplementation with 10 g of a live yeast *(Saccharomyces cerevisiae)* culture preparation attenuated postfeeding decreases in cecal and colonic pH and alterations in hindgut microbial populations.[59] Thus supplementation with live yeast culture may be beneficial in horses fed a high grain ration. From a practical standpoint it may be more important to ensure adequate fiber in the diet, decrease the quantity of grain, and emphasize use of nonstarch energy sources that do not adversely affect the hindgut environment.

Probiotics have been defined as live microorganisms that, when ingested or administered orally, provide a beneficial effect beyond that of their nutritional value.[60] Many probiotics are marketed for use in horses; the primary rationale is the treatment or prevention of gastrointestinal diseases (e.g., as an adjunct therapy in the management of acute or chronic diarrhea) or prophylactic administration for prevention of colic associated with disturbances to gut microflora (e.g., for horses fed a high-cereal ration). Anecdotal data suggest that the use of probiotics in horses is widespread; however, as with many nutritional supplements, there is a dearth of scientific data regarding safety and efficacy. An effective probiotic organism must be resistant to destruction by gastric acid, pancreatic secretions, and bile salts and be able to colonize the intestinal tract. Weese and Rousseau[60] have screened equine intestinal microflora for organisms meeting these criteria and initially identified *Lactobacillus pentosus* (WE7) as a potential equine probiotic. However, in a randomized controlled clinical trial of 153 neonatal foals (24–48 hours old), the administration of freeze-dried *L. pentosus* WE7 for 7 days was significantly associated with the development of signs of depression, anorexia, and colic and more days with diarrhea compared with the placebo treatment.[60] On the other hand, supplementation with *S. boulardii* reduced significantly the duration of diarrhea in horses with enterocolitis.[61]

The feeding of a protected sodium bicarbonate product has been reported to moderate the decrease in fecal pH associated with grain feeding in horses,[62] perhaps as a result of the buffering of lactic acid produced in the cecum and colon. This approach may be useful for mitigation of hindgut disturbances associated with grain feeding or the grazing of lush pasture.

NUTRITIONAL SUPPORT OF SICK HORSES

There are few data on the effects of different feeding practices on short- and long-term outcomes of sick horses, and many recommendations regarding nutritional management are based on anecdotal evidence or clinician experience rather than the results of controlled studies. In human medicine nutritional status is an independent determinant of morbidity in hospital patients.[63,64] Nutrient deprivation is associated with immunosuppression and alterations in gastrointestinal function, including decreased motility, villous atrophy, and a decrease in gut barrier function resulting from an increase in intestinal permeability, particularly in patients with preexisting malnutrition.[65] Consequently, nutritional support is standard practice in the care of sick people.

Healthy adult horses can tolerate 2 to 3 days of feed withdrawal without ill effect. Starvation invokes neuroendocrine responses that lower metabolic rate, conserve lean tissues (i.e., skeletal muscle), and promote use of fat stores to meet energy demands.[66] This strategy prolongs life in the face of nutrient deprivation. The metabolic response to critical illness (e.g., sepsis) contrasts with that of simple starvation. Increased sympathetic nervous system activity, inflammatory cytokines (e.g., interleukin-1[IL-1], IL-2, IL-6, tumor necrosis factor-α [TNF-α]), and catabolic hormones (catecholamines, cortisol, and glucagon) combine to increase metabolic rate and induce a state of hypercatabolism.[67] Stimulation of proteolytic pathways in skeletal muscle provides amino acids for hepatic gluconeogenesis and synthesis of acute phase proteins. Amino acids (from catabolism of lean tissues) rather than fatty acids are the primary source of energy substrate. There also is disruption of glucose regulation with the development of marked insulin resistance. Unabated, this hypercatabolic state results in large nitrogen losses, severe muscle wasting, and compromise of immune function and tissue healing.[66-68]

Severe hyperlipidemia has been described in horses with colic, colitis, or both that had clinical and laboratory evidence of systemic inflammatory response syndrome (SIRS).[69] The increase in circulating lipids likely reflects both an increase in the mobilization of fat reserves (lipolysis) and a decrease in lipid clearance from blood, with both processes potentially modulated by SIRS, endotoxemia, or both. Studies in other species have demonstrated that the activity of endothelial lipoprotein lipase, the enzyme responsible for tissue uptake of circulating lipids, is decreased by TNF-α, whereas the activity of hormone-sensitive lipase is increased during

endotoxemia.[68,70,71] In horses with severe hypertriglyceridemia, treatment with intravenous dextrose solution or partial parenteral nutrition (PN) resulted in a decrease in serum triglyceride concentrations to reference limits, and appetite improved coincident with the decrease in circulating lipids.[69]

Candidates for Nutritional Support

Several factors affect the decision to provide nutritional support to a sick horse, including the duration of inappetence or anorexia, the nutritional state before illness (e.g., poor versus good body condition), physiologic state (e.g., growing animals, last trimester of pregnancy, lactation), and the presence of clinical indicators of a hypercatabolic state (e.g., hyperlipidemia or lipemia).[72,73] As a general guide, horses that have not eaten for 48 to 72 hours are candidates for nutritional support. However, earlier intervention should be considered when there is evidence of compromised nutritional status, such as horses in thin body condition (BCS <3); those with a history of weight loss, poor feed intake, or both for more than 48 hours before examination; and those with evidence of sepsis, SIRS, or severe hyperlipidemia (e.g., triglycerides >500 mg/dL). Aggressive nutritional support also is indicated for obese (BCS >7) animals, particularly pony breeds, Miniature Horses, donkeys, and lactating mares, all of which are at high risk for development of hyperlipemia and hepatic lipidosis during periods of negative energy balance. Old horses or those with evidence of endocrine (pituitary pars intermedia dysfunction, equine Cushing's disease) and metabolic disease (equine metabolic syndrome) also are candidates for early nutritional intervention.

Estimating Nutrient and Feed Requirements

The nutritional requirements of sick horses have not been determined, so recommendations are largely based on data from healthy horses, with some extrapolation from data in other species. As for healthy animals, however, the first consideration is energy (calories). As discussed, negative energy balance caused by starvation or underfeeding can compromise immune function, delay wound healing, and result in a marked decrease in lean mass (e.g., skeletal muscle), the latter resulting from the breakdown of endogenous protein for use in energy-requiring processes. On the other hand, studies in humans and other species have shown that an oversupply of energy *(hyperalimentation)* is also detrimental, with complications such as hyperglycemia, hyperinsulinemia, hypertriglyceridemia, insulin resistance, and increased risk of septic complications.[74] A proposed mechanism of increased complications induced by hyperalimentation is an increase in the expression of TNF receptors associated with an increase in nuclear factor κB binding to the nucleus.[75] Furthermore, in septic animals high caloric intake results in an increase in mortality.[76,77] For these reasons, the current thinking in human medicine is to underfeed nonprotein calories (15–25 kilocalories [kcal]/kg daily or no more than 66% of calculated energy requirements) to minimize risk of septic complications.[74]

The sixth edition of *Nutrient Requirements of Horses*[1] reported that the DE needs of mature horses at maintenance range between 30.3 and 36.3 kcal/kg BW daily. The lower end of this range is suitable for minimally active animals with a tendency to gain weight ("easy keepers"), and the high value should be applied to animals with a nervous temperament ("hard keepers"). For healthy horses confined to a stall, daily energy requirements are probably 25% to 40% lower than in horses kept at pasture or in similar circumstances that require them to engage in some voluntary activity. Pagan and Hintz[78] reported that the DE requirement of healthy horses kept in stalls was approximately 22 to 23 kcal/kg BW per day, or 30% lower than the DE required by horses kept at pasture. This *stall maintenance* caloric requirement can be estimated by the following equation:

$$\text{RER} = [21\ \text{kcal} \times \text{BW}\ (\text{kg})] + 975\ \text{kcal}$$

where RER is resting energy requirement. Energy requirements are also affected by the level of feed intake. Thermogenesis associated with the digestion, fermentation, and metabolism of feed can account for 15% to 25% of daily energy expenditure. Because horses recovering from illness generally consume less feed, some reduction in the energy losses associated with digestion and nutrient processing is to be expected.

Another consideration in the estimation of energy requirements is the effect of disease or surgical intervention on metabolic rate. There is conflicting evidence from human studies regarding the effects of surgery, injury, or illness (e.g., sepsis) on metabolic rate and energy requirements. Some studies have demonstrated that abdominal surgery increases energy needs by as much as 30%, whereas others have reported minimal change in energy requirements after gastrointestinal surgery, perhaps a reflection of the decrease in physical activity during hospitalization.[79,80] On balance, it appears that energy needs are minimally altered by surgery or injury unless there are major complications, such as generalized burns or sepsis, in which case energy requirements may increase by 40% to 100%.[80] The potential effects of underlying disease (e.g., endotoxemia or SIRS) on the energy requirements of horses are unknown. However, in sick neonatal foals the resting metabolic rate and energy requirements were considerably lower compared with those of healthy foals of the same age,[81,82] and horses that underwent resection of the small intestine gained weight when fed at true maintenance (i.e., 32–33 kcal/kg/day) during the postoperative period.[83] This author recommends that the caloric requirements of sick horses initially be based on the RER or stall maintenance equation (i.e., 22–23 kcal/kg/day). Thereafter there should be a gradual increase in the ration, although true maintenance DE may not be required until the horse returns to normal management (e.g., field turnout). Practitioners should keep in mind that regular measurement of BW or assessment of BCS should be undertaken during convalescence to judge the adequacy of energy provision and provide a basis for adjustments in feeding.

Protein serves an important role in tissue maintenance, immune function, wound healing, and the slowing of endogenous protein catabolism. Protein requirements must be considered in light of caloric intake and underlying disease process. When energy supply from carbohydrate and fat is limited, endogenous protein will be used for energy, contributing to a loss of lean body mass. Therefore the practitioner should, when developing a nutritional plan, first ensure that minimal energy needs are met and then calculate protein requirements. For humans suggested protein requirements range between 1.2 and 2.0 g protein/kg per day, with the higher end of this range recommended for patients undergoing major intestinal surgery.[84] The CP requirement of healthy adult horses at maintenance is approximately 1.25 g CP/kg BW per day, and this value is an appropriate starting point in the development of a nutritional plan for sick horses: As the efficiency of digestion for most dietary proteins in horse feeds is about 70%, this

level of CP will provide approximately 0.9 g available protein per kilogram BW. For parenteral feeding a slight lowering of protein feeding is reasonable given the higher metabolic availability of amino acids administered by the intravenous route. In recent reports of parenteral feeding of horses, 0.6 to 0.8 g protein/kg per day (1 g per 40–50 kcal) was administered (as a balanced amino acid solution).[83,85] There may be justification for higher levels of dietary protein (e.g., 2 g CP/kg/day), particularly for horses in poor body condition (BCS <3) or those with SIRS, hypoproteinemia, or hypoalbuminemia.

Modes of Nutritional Therapy

The mode of nutritional therapy will depend on the underlying disease, the horse's appetite, and complications that arise during convalescence. The popular phrase in human clinical nutrition "if the gut works, use it" is used when discussing the pros and cons of enteral nutrition (EN) versus PN support. Historically, PN has been associated with several complications, including intestinal atrophy, failure of gut barrier function, bacterial translocation, increased incidence of sepsis, and hyperglycemia. In rodent models intestinal villous atrophy develops within a few days of the start of PN, and withdrawal of enteral feeding has been associated with bacterial translocation, systemic inflammation, and sepsis.[86-88] Early human studies also suggested that complication rates (particularly sepsis) and mortality were higher in patients receiving PN compared with those who received EN. However, critical review of human studies has indicated that PN (i.e., complete bowel rest) is not associated with intestinal atrophy, whereas the incidence of bacterial translocation is the same in patients receiving PN and those receiving EN.[74] Although EN is preferable when the gastrointestinal tract is functional, the weight of evidence from human studies indicates that PN is an important alternative to EN when a risk of malnutrition is present and EN is not tolerated or not possible because of poor gastrointestinal function. These same principles can be applied when developing a plan for the nutritional management of sick horses. PN should be considered for horses with ileus and other intestinal conditions that prevent voluntary or enteral feeding, particularly when the withholding of oral feeding is expected to exceed 48 hours.

Assisted Enteral Feeding

Assisted enteral feeding (AEF) is accomplished by the infusion of a liquid diet through a nasogastric tube. Diet options for AEF include human-marketed enteral products, commercial pelleted horse feeds, and homemade recipes.[89,90] Formulations marketed for humans that have been administered to adult horses include Vital HN and Osmolite HN (Ross Laboratories, Columbus, Ohio). Both formulations are devoid of fiber, which is an advantage because it facilitates administration through a small-diameter nasogastric tube, but there is also a disadvantage: it may lead to the development of diarrhea when these products are fed to horses. There also have been anecdotal reports of laminitis in horses fed these diets. Risk of these complications may be mitigated by gradually introducing the liquid diet over a 3- to 4-day period, but diarrhea remains common, perhaps indicating the importance of dietary fiber for maintenance of normal hindgut function. It also should be recognized that the mix of energy substrates in these fiber-free enteral formulas for human use differs from that in typical equine rations. Osmolite contains approximately 29% of calories from lipid and 54% of calories from hydrolyzable carbohydrate (mostly sugars), and Vital HN provides about 10% of calories from lipids and 74% from carbohydrates. The high lipid content of Osmolite may contribute to digestive disturbances in horses not adapted to fat-supplemented rations, and use of a high-carbohydrate diet such as Vital HN may be contraindicated for horses with abnormal glucose metabolism (insulin resistance). Collectively, these considerations argue against using fiber-free enteral products intended for human use in horses. Diets containing a moderate amount of fiber (10%–20% crude fiber [CF], DM basis) appear to be a more suitable choice for AEF in horses.

A simple approach is to use a commercially available pelleted feed that contains a source of fiber, such as Equine Senior (Land O' Lakes-Purina Feed, St. Louis, MO) or similar "complete feeds" that contain added fiber and can be fed without hay. These products contain about 14% to 25% CF and provide 2.6 to 3.1 Mcal DE/kg diet (as fed). Therefore 3.5 to 4.0 kg of diet would be needed to meet the stall energy requirements of a 500-kg horse. Vegetable oils (75–375 mL per day) can be added to increase the caloric density of the diet (Table 5.3). One standard cup (approximately 225 mL [210 g]) of oil provides about 1.7 Mcal of DE. Vitamin E (100–200 IU per 100 mL of oil) should be added to the ration if supplemental vegetable oil is provided. When providing supplemental fat to a sick horse (450–500 kg BW), 75 to 125 mL/day (¼ to ½ cup) should be given initially and then gradually increased if no adverse response is seen (e.g., diarrhea, steatorrhea, lipemia). Feeding vegetable oil may be contraindicated in horses and ponies with hypertriglyceridemia (triglyceride concentration >40–500 mg/dL) or hepatic lipidosis.

Variations on the alfalfa/dextrose/casein enteral formulation first described by Naylor et al.[90] also can be used for AEF (Table 5.4). The recipe designed by Naylor et al.[90] provides about 3 Mcal DE/kg diet and is 33% CP and 12% CF. The higher protein content, compared with that of typical equine diets, may be beneficial for debilitated or hypoproteinemic horses. In healthy horses this diet reportedly maintained BW and

TABLE **5.3 Enteral Formulation Based on a Complete Pelleted Ration and Recommended Feeding Schedule for a 500-kg Horse**[a]

Ingredient	Day 1 (¼ Ration)	Day 2 (½ Ration)	Day 3 (¾ Ration)	Day 4 (Full Ration)
Complete pelleted horse feed (g)[b]	885	1770	2650	3530
Vegetable oil (mL)	100	177	265	354
Water (L)	8	16	24	24
Digestible energy (Mcal)	3	6	9	12

[a]Energy requirements are at *stall maintenance* for a 500-kg horse (~12 Mcal DE/day). These allowances should be divided and administered into a minimum of four feedings daily.
[b]Equine Senior (Land O'Lakes-Purina Feed, St. Louis, Missouri), 2.6 Mcal DE/kg (as fed).

serum biochemical parameters within reference limits. However, diarrhea and laminitis were occasional complications.

Suggested feeding protocols for a complete pelleted feed with supplemental vegetable oil and for the alfalfa/dextrose/casein formulation are presented in Tables 5.3 and 5.4, respectively. The rate of diet administration should be gradually increased over a 3- to 5-day period. A suggested rate of introduction is to administer ¼ of the final target volume of feed on day 1, ½ total volume on day 2, ¾ total volume on day 3, and the total volume on day 4 or 5. Clinical signs of intolerance to enteral feeding will dictate a slower rate of introduction. In hospital settings the enteral diet should be administered in a minimum of four and preferably six feedings per day, with no more than 6 to 8 L per feeding for a 450- to 500-kg horse (including volume of water used to flush the tube). This volume should be administered over a 10- to 15-minute period. In field settings a more practical approach is to administer two treatments daily, although it will not be possible to meet stall maintenance nutritional requirements with this treatment regimen.

Pelleted feeds should be soaked in warm water to soften before mixing in a blender (ratio of 1 kg pelleted feed to 6 L of water). A fresh batch of diet should be made before each feeding. A tube with a ½-inch (12-mm) inner diameter is suitable for most enteral diets that contain fiber. The end of the tube should be open ended, rather than fenestrated, to prevent the tube from becoming clogged. Intermittent nasogastric intubation or placement of an indwelling nasogastric tube can be used to facilitate feeding. In hospitalized horses feeding tubes can be left in place for up to 8 days, although nasopharyngeal irritation and mucoid nasal discharge are expected outcomes (when longer term AEF is anticipated, placement of the tube through cervical esophagostomy is recommended). Softer silicon tubes are less irritating compared with tubes made of polyvinylchloride, do not tend to harden when left in place, and are generally recommended for horses requiring AEF for a number of days. Before placing the tube, the clinician should establish that the diet solution flows adequately through the tube. It may be necessary to add more water or to mix the feed in a blender a second time. The tube should be positioned in the stomach rather than the distal esophagus to minimize risk of reflux of feed around the tube. The tube should be secured to the halter; between feedings application of a muzzle may be necessary to prevent the horse from dislodging the tube. A marine bilge pump is recommended for infusion of fiber-containing diets. After administration of the diet, the tube should be flushed with approximately 1 L of water followed by a small volume of air to ensure that no feed material remains in the tube. The end of the tube should be capped with a syringe case between feedings.

Close clinical monitoring, particularly of gastrointestinal function, is imperative for horses receiving AEF. Repeated ultrasonographic examinations can be useful for the evaluation of gastric distention and intestinal motility. The presence of residual gastric fluid should be assessed (i.e., by siphoning) before each feeding. Substantial gastric reflux (>1–2 L) is an indication to withhold enteral feeding for at least 1 to 2 hours, with reevaluation before recommencement of diet administration. Persistent gastric reflux indicates intolerance to enteral feeding and the need for parenteral feeding. Similarly, signs of colic, ileus, abdominal distention, and increased digital pulses suggest intolerance to enteral feeding and are an indication to discontinue therapy or decrease the volume and frequency of feedings. The passage of loose feces is not uncommon in horses receiving AEF and of minimal concern if not accompanied by clinical signs of depression, dehydration, ileus, or colic. It is important to measure the total volume of water administered through the nasogastric tube. Daily water requirements (approximately 50 mL/kg/day) can generally be met during AEF if the horse is fed four to five times daily. Frequent measurements of hematocrit and plasma total protein concentration also are useful for monitoring hydration status and the adequacy of water administration. Hypokalemia, ionized hypomagnesemia, and ionized hypocalcemia can occur in horses with gastrointestinal disease. Accordingly, frequent measurements of serum electrolytes and ionized calcium and magnesium are recommended during AEF in these patients. Supplementation with potassium, calcium, magnesium, or both may be necessary. Horses also should be monitored for development of complications associated with repeated or indwelling nasogastric intubation, including rhinitis, pharyngitis, and esophageal ulceration. BW should be measured daily to assess the adequacy of nutritional support, although changes in BW may reflect alterations in fluid balance rather than the effect of feeding.

Parenteral Nutritional Support

PN is indicated for horses with gastrointestinal tract dysfunction (e.g., ileus, gastric reflux) or conditions that mandate complete bowel rest (e.g., small intestinal resection,

TABLE **5.4** **Alfalfa/Dextrose/Casein Enteral Formulation and Recommended Feeding Schedule for a 500-kg Horse**[a]

	Day						
Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Electrolyte mixture (g)[b]	230	230	230	230	230	230	230
Water (L)	21	21	21	21	21	21	21
Dextrose (g)	300	400	500	600	800	800	900
Dehydrated cottage cheese or casein (g)	300	450	600	750	900	900	900
Dehydrated alfalfa meal (g)	2000	2000	2000	2000	2000	2000	2000
Digestible energy (Mcal)	7.4	8.4	9.4	10.4	11.8	11.8	12.2

[a]These allowances should be divided and administered into three or four feedings daily. Stall maintenance requirements for a 500-kg horse are 12 Mcal DE/day.

[b]Composition of electrolyte mixture: sodium chloride (NaCl) 10 g; sodium bicarbonate ($NaHCO_3$) 15 g; potassium chloride (KCl) 75 g; potassium phosphate (dibasic anhydrous, K_2HPO_4) 60 g; calcium chloride ($CaCl_2 \cdot 2H_2O$) 45 g; magnesium oxide (MgO) 25 g.

Adapted from Naylor JM, Freeman DE, Kronfeld DS. Alimentation of hypophagic horses. *Compend Cont Educ Pract Vet*. 1984;6:S93-S99.

duodenitis-proximal jejunitis). Durham et al.[83,85] examined the effects of postoperative PN in 15 horses (vs. 15 control horses) recovering from resection and anastomosis of strangulated small intestine and reported no beneficial effect of PN on time to first oral feeding, duration of hospitalization, costs of treatment, or short-term survival (up until 5 months after discharge). The PN protocol did confer improved nutritional status, as reflected by lower serum concentrations of triglycerides and total bilirubin and higher concentrations of glucose. However, the duration and volume of postoperative gastric reflux were longer in the PN group than in the control horses, perhaps as a result of alterations in gastric or small intestinal motility, and there was an insignificant trend for catheter-site complications in the PN group.[83] The authors concluded that further study of a larger number of horses is necessary to determine the clinical benefits and possible harmful side effects of PN in horses recovering from small intestinal surgery. Studies of the effects of PN in human patients also have yielded equivocal findings. Several studies have demonstrated that perioperative PN is associated with reduced morbidity and mortality in malnourished patients.[91-93] In contrast, perioperative PN in well-nourished human patients has been associated with increased morbidity, particularly septic complications.[94,95] Nonetheless, as previously discussed, the current consensus in human clinical nutrition is that PN is an important component of overall case management, particularly in patients with evidence of malnourishment, gut failure, and increased nutritional requirements (e.g., pregnancy, lactation, growth).

The following paragraphs provide a brief overview of the composition of PN solutions, methods for delivery, and potential complications. As with EN support, the goal of PN is to administer calories and amino acids such that loss of body protein (and lean body mass) is minimized.[73] Carbohydrates, in the form of a 50% dextrose solution (3.4 kcal/g or 1.7 kcal/mL; osmolarity 2525 mOsm/L), and lipid, as a 10% to 20% emulsion (20% emulsion: 9 kcal/g or 2 kcal/mL; osmolarity 260 mOsm/L), are the primary sources of energy used in PN solutions, whereas an amino acid solution (e.g., Travasol 8.5% or 10%; Baxter Health Care Corporation, Deerfield, IL) is used to meet protein requirements (e.g., protein synthesis, immune function). Commercial lipid emulsions (e.g., Intralipid 20%; Baxter Health Care Corporation, Deerfield, IL) consist of soybean oil, egg yolk phospholipid, and glycerin. These emulsions provide mainly unsaturated fatty acids (linoleic, 44%–62%; oleic, 19%–30%; linolenic, 4%–11%; palmitic, 7%–14%). PN solutions with and without lipid can be used (i.e., dextrose/amino acid or dextrose/lipid/amino acid mixtures). The addition of lipids to the PN formula results in a solution with lower osmolarity compared with a dextrose/amino acid mixture of similar caloric density. Hence the lipid-containing solution should be less irritating to peripheral veins. Lipid solutions must be included in the formula if target calorie provision approaches true maintenance (32–33 kcal/kg/day) because this level of calorie delivery from a dextrose/amino acid PN solution often results in marked hyperglycemia and glucosuria. However, when the target daily energy provision is 20 to 22 kcal/kg per day, dextrose/amino acid mixtures can be used. In human medicine this approach is referred to as a *partial PN* and is often used in postoperative patients who require only a few days of intravenous nutritional support;[74] similarly, partial PN is recommended for horses requiring short-term (3–7 days) intravenous feeding. Lipid administration is not recommended for patients at high risk for severe hypertriglyceridemia or hyperlipemia (e.g., ponies, Miniature Horses, donkeys). Serum triglyceride concentrations should be monitored on a regular basis if lipid solutions are administered to these patients. As discussed previously, the provision of amino acids (protein) at a rate of 0.6 to 0.8 g/kg BW daily is one guideline for meeting protein requirements in adult horses, although some authors have recommended 1 to 1.5 g/kg per day, and provision of amino acids at 0.6 to 2 g/kg per day has been used in sick horses without apparent complications.

A suggested PN formula (Table 5.5) comprises 1 L of 50% dextrose (0.5 g/mL dextrose × 3.4 kcal/g × 1000 mL = 1700 kcal), 1 L of a 10% amino acid solution (0.1 g/mL of amino acids × 4 kcal/g × 1000 mL = 400 kcal), and 500 mL of 20% lipid emulsion (0.2 g/mL lipid × 9 kcal/g × 500 mL = 900 kcal). These components are diluted with 4 L of isotonic fluid, yielding a final volume of 6.5 L and a caloric density of approximately 0.45 kcal/mL. A multivitamin supplement may be added to this mixture. This solution can be prepared up to 24 hours before administration, with storage at 4°C until use. Administration of PN solutions should be through a dedicated intravenous catheter (i.e., do not administer

TABLE **5.5 Parenteral Nutrition Formula and Recommended Administration Rate for a 500-kg Horse**[a]

Formula Variable	First 12 hours	Second 12 hours	Day 2
Dextrose 50%	1000 mL	1000 mL	1000 mL
Lipid 20%	500 mL	500 mL	500 mL
Amino acids 10%	1000 mL	1000 mL	1000 mL
Isotonic fluids	4000 mL	4000 mL	4000 mL
Total volume	6500 mL	6500 mL	6500 mL
Kcal per bag	3000	3000	3000
Kcal/h	210	333	480
Rate (mL/h)	—	470	740
Bags required	0.90 per 12 h	1.4 per 12 h	4.0 per 24 h
Kcals per day	—	—	11,500

[a]For parenteral nutrition, daily energy needs are estimated at 23 kcal/kg per day (11.5 Mcal per day for a 500-kg horse).
Adapted from Robinson NE, Sprayberry K, eds. *Current Therapy in Equine Medicine*. 6th ed. St. Louis, MO: Saunders; 2009.

other medications with this catheter), preferably one inserted into a large vein such as the jugular, to minimize the risk of complications associated with the infusion of hyperosmotic solutions. Alternatively, a double-lumen catheter can be used, allowing the PN solution to be given through one port and medications and other fluids through the other port. To minimize the risk of thrombophlebitis, nonthrombogenic catheters such as those made from polyurethane are recommended. Meticulous attention to sterile technique is needed during catheter placement to further minimize the risk of thrombophlebitis and other septic complications. The fluid lines used for delivery of the PN solution should be changed every 24 hours. An infusion pump is required to ensure accurate delivery of the PN solution. The bag containing the PN solution should be covered with a brown bag during administration to protect it from light, which can degrade the amino acids within the solution.

Table 5.5 provides a recommended rate of parenteral feeding for a 500-kg horse. The initial rate of PN solution administration should be approximately 35% of target calorie provision, increasing to 60% to 65% after 12 hours and 100% (23 kcal/kg/day) at 24 hours, provided that there are no complications, such as the development of marked hyperglycemia, glucosuria, or hyperlipemia. Hyperglycemia and hyperlipemia were the most common complications of postoperative PN in horses after intestinal surgery. In one report hyperglycemia was observed in 52 of 79 horses receiving PN,[96] perhaps because of insulin resistance, an excessive rate of administration, or both. Blood glucose concentrations should be measured every 4 to 8 hours in horses receiving PN, and the rate of dextrose administration should be decreased if glucose concentrations exceed renal threshold (approximately 180–200 mg/dL). A constant-rate insulin infusion (e.g., regular insulin at a starting dose of 0.05–0.1 IU/kg/h) can be instituted if the reduction in dextrose administration rate fails to correct the hyperglycemia. Blood glucose concentrations must be closely monitored (e.g., every 2–6 hours). Adjustments in insulin dose may be required to achieve glycemic control. Serum blood urea nitrogen (BUN), triglycerides, and electrolytes should be monitored at least daily. Hypokalemia, hypocalcemia, and hypomagnesemia have been reported in horses receiving PN, and it may be necessary to supplement these nutrients if parenteral feeding is used for more than 48 hours. Finally, BW should be recorded daily or every other day.

Transition to Voluntary Feeding

A decrease in AEF or PN is indicated when appetite returns (or when voluntary oral feeding is no longer contraindicated). Initially, small amounts of palatable (e.g., fresh grass or leafy hay) feed should be offered. If these feedings are tolerated, the level of tube or parenteral feeding can be gradually reduced as the provision of feed for voluntary consumption is increased. Nutritional support can be withdrawn when voluntary feed intake provides at least 75% of stall maintenance DE and protein requirements. As with any feeding program, all changes in diet should be gradual. Hay should be the primary, if not sole, component of the convalescent diet, preferably leafy hay with a fresh aroma. Grain or commercial grain-concentrate feeds should be offered only if hay alone does not meet requirements. An alternative to grain is to feed 1 to 2 lb/day of a high-protein product (approximately 20%–25% CP, usually with added minerals and vitamins) as a supplement to hay.

NUTRITIONAL MANAGEMENT OF THE ORPHANED OR SICK FOAL

Foal Metabolism and Nutrient Requirements

The foal's nutritional requirements and dietary composition change substantially during the gradual transition from neonate to weanling. At birth the foal must transition from a continuous supply of nutrients provided by the dam by way of the placenta to intermittent absorption of ingested nutrients. At the same time, the metabolism of the neonate is no longer able to depend on the maternal glucose concentration to maintain normoglycemia, and the pancreas assumes responsibility for regulating glucose homeostasis.[97] These dramatic alterations in energy metabolism may not always occur smoothly, and the neonatal foal possesses limited energy reserves in the form of glycogen and fat. The result is that hypoglycemia occurs frequently in even the normal neonatal foal, and the sick foal is at risk for profound hypoglycemia if deprived of energy intake for even a few hours.[97]

The neonatal foal has a high metabolic rate and requires frequent ingestion of high volumes of milk to meet its energy requirements for maintenance and growth. In light breeds the average rate of daily gain over the first month of life is 1 to 1.5 kg/day. During the first week of life, calorie needs are as follows: approximately 150 kcal/kg per day, with a gradual decrease to approximately 120 kcal/kg per day at 3 weeks of age, and then to 80 to 100 kcal/kg per day by 1 to 2 months of age.[81,82] Healthy neonatal foals (<7 days of age) have up to seven nursing bouts per hour, most lasting for 1 to 2 minutes. Subsequently, there is a gradual decrease in the frequency of nursing but an increase in the duration of each nursing bout. During the first 24 hours of life, foals consume approximately 15% BW as milk (i.e., 8 L in a 50-kg foal), increasing to between 23% and 25% BW (15 L) by day 3 or day 4.[98] On a DM basis, mare's milk averages about 64, 22, and 13% sugar (as lactose), protein, and fat, respectively, compared with cow's milk, which contains 38, 26, and 30% sugar, protein, and fat, respectively. As a result, glucose (from lactose) is the primary source of energy for the foal.

Starting as early as the second day of life, foals begin ingesting small amounts of hay, grass, and grain while also ingesting maternal feces, which likely provides the initial microbial flora required to support digestion of these feedstuffs. It is unlikely that grain and roughage are well digested until at least several weeks of age, at which point the foal begins the gradual transition from a milk-based diet to a forage-based diet. The amount of milk produced by the mare peaks after approximately 2 months of lactation and then begins a steady decline, which continues until the time of weaning, when the foal must begin relying on solid feed for an increasing proportion of its nutritional requirements. Full maturation of hindgut function may occur by 3 to 4 months of age.

Nutritional Support of Orphan Foals

The primary options for management of orphan foals are to raise by hand or foster to a nurse mare.[98,99] Fostering is the best option for a foal younger than 6 to 8 weeks of age. Draft or Draft-cross mares that are mild in temperament make good nurse mares. Any disparity between the stage of lactation and age of the foal should be noted. A mare that is 4 to 6 weeks into lactation may not have the quality of milk needed to sustain a neonate; it may be necessary to provide the neonatal foal with a mineral supplement when fostered to a mid- to late-lactation mare.

Hand rearing has several disadvantages, including the need for high labor input, the expense of milk substitute, and a high incidence of behavioral problems. The risk of behavioral problems can be mitigated by ensuring that the foal is raised with an equine companion (e.g., a quiet horse or pony or even another orphan foal). Teaching a foal to drink from a bucket is preferable to bottle feeding because it is less labor intensive for the owner and allows the foal to drink milk on demand. Feeding from a bucket also helps prevent some of the behavioral problems that develop in foals that have bonded to people. Bottle feeding may be the only option in newborn foals, but a quick transition to bucket feeding is recommended. It is also important to ensure adequate colostral intake (and acquisition of passive immunity) in foals that are orphaned at birth.

Fortified cow's milk (by the addition of 20 g/L dextrose to 2% part skimmed milk); goat milk; and calf, lamb, and kid milk substitutes have been used to rear foals, but a high-quality mare-milk formula is preferred. On a DM basis foal milk replacers should contain approximately 15% fat and 22% CP, with a fiber content of less than 0.5%.[99] Milk replacers should be fed as a 10% to 15% solution when diluted with water and generally provide about 500 kcal/L when mixed according to the manufacturer's instructions. The volume of milk should be fed over a 7- to 10-day period. One recommendation is to start at 5% to 10% of BW, increasing to 20% to 25% BW by day 10. Intake of 20% to 25% BW/day as milk replacer will meet energy and nutrient requirements for growth. As a general guide, most light-breed foals need 14 to 17.5 L (4–5 gallons) of milk substitute each day during the first 2 to 3 weeks of life, and draft foals require 21 to 28 L (6–8 gallons). Foals may be fed as infrequently as two to four times daily, although more frequent feedings (six to nine daily) are recommended for young foals and during the initial phase of hand rearing to keep from overwhelming the digestive tract. Feeding buckets should be thoroughly cleaned on a daily basis. Similarly, strict hygiene is required during the preparation of the milk substitute. Fresh water should be available at all times.

BW or linear measures that predict weight and height gain[100] should be monitored on a regular (e.g., weekly) basis. Expected rates of growth and weight gain are available for some horse breeds. Studies of hand-reared foals have demonstrated growth rates similar to those observed in foals reared with their dams.[101] Solid feed should be introduced as early as 2 to 3 weeks of age (e.g., small amounts of high-quality hay, milk-based pellets, or both). Up to 1 kg of milk pellets per day can be fed with a concomitant reduction in liquid milk feeding. Orphan foals can be weaned from milk at 10 to 12 weeks of age, with a transition to a diet of forage and a balanced foal feed (16%–18% CP).

Nutrition of the Sick Neonatal Foal

The energy (calorie) requirements of sick, recumbent foals are less than those of healthy foals, in part because of their lower physical activity and a temporary decrease in growth rate compared with healthy neonates. In one study the metabolic rate of healthy foals was 130 to 140 kcal/kg BW per day, whereas metabolic rate in immature foals and foals with perinatal asphyxia syndrome was approximately 62 to 69 kcal/kg BW per day.[81] It is possible that sepsis and endotoxemia increase metabolic rate, although studies in neonatal humans indicate that critical illness is not accompanied by a hypermetabolic state. Currently providing critically ill, recumbent foals approximately 45 to 50 kcal/kg BW per day is recommended.[98,102] This "hypocaloric" approach to nutritional support may prevent complications associated with overfeeding, such as hyperglycemia and glucosuria, particularly in foals with sepsis in which the systemic inflammatory response contributes to insulin resistance and carbohydrate intolerance. Neonatal foals require approximately 1.5 g protein/kg BW per day.

The adage, "if the gut works, use it", also applies to the nutritional support of sick neonatal foals. Milk contains trophic substances that promote growth and development of the gastrointestinal tract. Additionally, enteral feeding is believed to lower the risk of sepsis associated with bacterial translocation. However, parenteral feeding is required in foals with poor gastrointestinal tract function.

Enteral Nutrition

Thorough assessment of gastrointestinal function is needed before institution of EN support, including abdominal auscultation and evaluation of abdominal distention and the presence of gastric reflux. Abdominal radiographs and ultrasonographic examination for evaluation of bowel dimensions and motility also may be indicated. Foals with evidence of gastrointestinal dysfunction such as gastric reflux, bowel distention, increased bowel wall thickness, and ileus are unlikely to tolerate enteral feeding. A conservative approach to enteral feeding is also indicated for premature or immature foals in which the gastrointestinal tract may not be completely developed. Foals with perinatal asphyxia syndrome or severe sepsis may be intolerant of enteral feeding as a result of intestinal ischemic injury.

Mare's milk is the preferred substrate for enteral feeding; it is highly digestible and obviously provides the correct balance of nutrients for normal growth and development. Commercial mare milk replacers can be used, but practitioners should bear in mind that these products are bovine in origin and have lower digestibility compared with mare's milk. This may increase the risk of intestinal dysfunction associated with enteral feeding. Part skimmed (2% fat) cow's milk to which 20 g/L dextrose (corn sugar) is added can be used if mare's milk (or a mare's milk replacer) is unavailable. Foals that are unable to nurse the mare (or when no mare is available) may be fed through a bottle, bowl, or nasogastric feeding tube. Bottle feeding is recommended when the foal is expected to be transitioned to a mare within a short period of time. The practitioner should remember, however, that bottle feeding is very labor intensive and does carry some risk of milk aspiration if the milk flow rate from the bottle exceeds the foal's capacity to nurse properly. The risk of aspiration is lower with bowl feeding because the head is positioned with the nose down during drinking.

Many sick, recumbent foals have a weak or uncoordinated suck reflex and are at risk of milk aspiration and pneumonia. Milk should be administered to these patients through a feeding tube. Use of small-bore, indwelling nasogastric feeding tubes (e.g., NG1243–12Fr × 108 cm [43 inches], MILA International, Erlanger, KY) and feeding of small volumes at frequent intervals (e.g., every 20 minutes) are preferred over repeated passage of a nasogastric tube at 1- to 2-hour intervals. Large-bolus feedings may overwhelm digestive capacity, and repeated passage of a stomach tube is an unnecessary stress on the foal. Another advantage of the small-bore indwelling tubes is that they do not interfere with the suckle response. Therefore the tube may be left in place as the foal is transitioned to feeding from the mare. The feeding tube should be inserted with the foal in sternal recumbency, and correct placement

TABLE **5.6 Feeding Recommendations for Neonatal and Growing Foals**

Foal Age (days)	Energy Requirement	Volume of Mare's Milk or Milk Replacer	Percentage of Body Weight Fed
0–1	150 kcal/kg/day	2–3 mL/kg/h	5%–7%
2–3	150 kcal/kg/day	4–5 mL/kg/h	10%–12%
4–7	150 kcal/kg/day	6–8 mL/kg/h	14%–20%
8–30	120 kcal/kg/day	9–10 mL/kg/h	22%
30–weaning	80–100 kcal/kg/day	Gradually decreasing and replaced with solid feed	—

within the esophagus should be confirmed by radiography or endoscopy. The tube should be fastened to the external nares by sutures or may be retained using a circumferential elastic bandage around the muzzle. It is important to check at each feeding that the tube is still in place and there is no gastric reflux. The foal should be in sternal recumbency or standing when it is fed. Milk should be administered by gravity flow and followed by a small amount of clean water to flush the tube. The tube should be capped between feedings to prevent aspiration of air. Feeding tubes should be replaced every 1 or 2 days to reduce the risk of gastrointestinal tract infection.

A suggested initial rate of milk delivery is 2 to 3 mL/kg BW per hour or 100 to 150 mL/h for a 50-kg foal, which will provide 2.4 to 3.6 L of milk to a 50-kg foal during the first 24 hours of enteral feeding (Table 5.6). Dextrose-containing fluids can be administered intravenously to provide additional calories during the transition to an adequate level of enteral feeding. The feeding rate can be gradually increased over the next 2 to 3 days (e.g., increase to 4–5 mL/kg/h on day 2 and then to 6–8 mL/kg/h on day 3), which represents a total daily intake of 10% to 15% BW. As the rate of enteral feeding increases, intravenous caloric support (dextrose) can be gradually withdrawn. This feeding level will likely meet the maintenance energy requirements of hospitalized foals. Depending on the rate of clinical improvement and the length of hospitalization, it may be possible to increase the volume of feeding to 22% to 23% BW per day, which approximates the milk intake of healthy neonatal foals. Clinical monitoring should include frequent assessments of gastrointestinal function, including gastric reflux, intestinal sounds, abdominal distention, and quantity and quality of feces. Gastric reflux, bloating, colic, diarrhea, or constipation can indicate intolerance to enteral feeding and the need for adjustments to the feeding program. Adjustments may involve a decrease in the volume or frequency of enteral feedings. Foals fed milk replacers appear to be more likely to develop loose feces than foals fed mare's milk. In some cases clinical improvement is observed after supplementation with 6000 units of lactase (Lactaid; McNeil Nutritionals, LLC, Ft. Washington, PA) per 50-kg foal every 3 to 8 hours, mixed into the milk replacer before feeding.[103]

Parenteral Nutrition

PN support is indicated for foals with poor gastrointestinal function and intolerance to enteral feeding. Short-term parenteral supplementation (less than 24 hours) does not require the use of a balanced PN solution that contains sources of carbohydrate, amino acids, and lipids. However, if PN is expected to be administered for a longer period, then a more complete formula should be used.

Short-term caloric supplementation can be accomplished by administrations of an intravenous fluid containing 5% dextrose (e.g., 5% dextrose in water [D_5W], lactated Ringer's solution with 5% dextrose, 0.45% saline with 5% dextrose), and hypotonic maintenance electrolyte solutions containing 5% dextrose.[97] Fluids containing dextrose should not be used for initial large-volume fluid resuscitation because this often results in profound hyperglycemia. After initial fluid resuscitation the solutions containing electrolytes as well as dextrose may be used as the primary fluids for maintenance therapy in foals with minimal ongoing fluid losses. As a maintenance solution, D_5W is not a good choice because it lacks electrolytes; it is primarily useful in providing free water to patients suffering from hyperosmolar conditions. The caloric content of a 5% dextrose solution is 0.17 kcal/mL, so an infusion rate of 10 mL/kg per hour would be required to deliver approximately 40 kcal/kg per day (0.17 kcal/kg/h × 24 h/day = 41 kcal/kg/day). However, this rate of infusion is more than twice the maintenance fluid requirements of neonatal foals (4–5 mL/kg/h). Therefore an infusion rate of 5 mL/kg per hour (providing approximately 20 kcal/kg/day) is a more appropriate target for partial caloric support using 5% dextrose solutions.[97] Caution is required when adjusting the infusion rate in response to changes in hydration state to prevent excessive dextrose administration, especially in premature or very sick foals that may be glucose intolerant.

An alternative approach involves the administration of a 50% dextrose solution by use of an infusion pump, provided that isotonic fluids are administered concurrently to minimize the risk of vascular endothelial injury caused by the hyperosmolar 50% dextrose solution. Use of 50% dextrose solution is not recommended unless an infusion pump is available. The caloric content of 50% dextrose solution is 1.7 kcal/mL; therefore, an infusion rate of 1 mL/kg per hour will provide about 40 kcal/kg per day (1.7 kcal/kg/h × 24 h/day = 41 kcal/kg/day). At this low rate of infusion, the primary fluid needs of the patient can be met with a dextrose-free isotonic electrolyte-containing fluid, the infusion rate of which can be altered in response to changes in patient fluid status without concerns related to the requirements of the nutritional plan.[97]

Two approaches have been used for formulation of PN solutions for foals.[97,98,104] The first involves the exact determination of the anticipated metabolic needs of the patient, followed by the development of a formulation that will meet all of these needs using a mixture of dextrose, amino acids, and lipids. The second approach is simpler and more practical and uses one of two basic PN formulas (Table 5.7):

1. Solution I: A mix of equal volumes of 50% dextrose and 8.5% amino acid solutions, intended for short-term use (caloric density = 1.02 kcal/mL)
2. Solution II: A mix of three parts 50% dextrose, four parts 8.5% amino acid, and one part 20% lipid solutions; this solution is preferred for foals that are poorly tolerant of infused dextrose (caloric density = 1.08 kcal/mL)

TABLE **5.7 Formulation of Parenteral Nutrition Solutions for Neonatal Foals**

Formulation	Composition	Caloric Density (kcal/mL)	Nonprotein Calories/g Nitrogen
Formula I	1500 mL 50% dextrose, 1500 mL 8.5% amino acids	1.02	125
Formula II	1500 mL 50% dextrose, 500 mL 20% lipids, 2000 mL 8.5% amino acids	1.08	131

As previously discussed, protein degradation in muscle tissue occurs during critical illnesses such as sepsis. Thus provision of adequate energy and amino acids to counter this catabolic response is an important goal of PN support. The recommended ratio for nonprotein calories to nitrogen is 100 to 200 nonprotein calories per gram of nitrogen.[105] In one report the amount of protein provided affected weight gain of neonatal foals, with a negative association between the nonprotein nitrogen calories: grams nitrogen ratio (NPC/g N) and the rate of weight gain.[106] Solution I provides 125 NPC/g N, and Solution II contains 131 NPC/g N.

The inclusion of lipids in the PN formulation allows for the provision of a larger number of calories per unit volume compared with solutions containing only dextrose. Another advantage of lipid emulsions is that they are isotonic, moderating the hypertonicity of the PN formulation and potentially decreasing the risk of thrombophlebitis. In a recent report the use of lipid-containing PN solutions allowed for the provision of 40 to 92 kcal/kg per day (mean = 63 kcal/kg/day) to foals, compared with 25 to 66 kcal/kg per day (mean = 41 kcal/kg/day) with a dextrose-based solution.[104]

Methods for delivery of PN solutions have been discussed (see the previous section Nutritional Support of Sick Horses). The catheter and catheter site should be monitored at least twice daily for heat, swelling, or exudation. Increased resistance to fluid flow in the catheter may be an indication of thrombosis deeper within the vasculature and will often necessitate the placement of a catheter in an alternative site, such as the opposite jugular vein, a cephalic vein, or a lateral thoracic vein. The rate of infusion (in mL/h) is calculated on the basis of the desired kcal/kg per day to be administered. A reasonable initial goal is 40 to 50 kcal/kg per day; higher rates of energy administration often result in hyperglycemia and hyperlipidemia. The initial infusion rate of PN solutions should be 50% of the calculated final rate, and the rate should be gradually increased every 1 to 3 hours after monitoring of the blood glucose concentration to ensure that hyperglycemia (blood glucose >180 mg/dL) is not present.

Rectal temperature should be closely monitored during PN support because fever is a common early manifestation of systemic infection. Blood glucose concentrations should be closely monitored, the frequency of which depends on the stability of blood glucose concentrations. Blood glucose should be maintained between 90 and 180 mg/dL. Although the renal threshold for glucose is not well described in foals, glucosuria and diuresis will be observed in many when blood glucose levels exceed 180 mg/dL. Urine output should be monitored continuously, in combination with intermittent monitoring of urine glucose concentration, because of the risk of hyperglycemia-induced diuresis and glucosuria. Additional clinicopathologic monitoring should consist of daily complete blood counts and serum chemistry profiles in the critical case; these can be performed every 48 to 72 hours in more stable patients. BW should be assessed on a daily basis to ensure that the foal is at least maintaining BW while on PN support.

Some critically ill foals are intolerant of even a conservative rate of dextrose administration because of insulin resistance. The administration of insulin is needed to control hyperglycemia and allow attainment of the goal level of PN support.[97] Use of continuous rate infusion (CRI) for the administration of insulin is preferred to intermittent bolus dosing. An initial insulin infusion rate of 0.07 IU/kg per hour is generally well tolerated. If possible, simultaneous alterations in both the insulin and PN infusion rate should be avoided, because this can result in marked fluctuations in blood glucose concentrations. Blood glucose monitoring should be performed at least hourly for the first 2 to 3 hours after initiation of the insulin CRI, and if hyperglycemia (blood glucose >150 mg/dL) is persistent beyond the first 2 hours of insulin therapy, then the insulin infusion rate may be increased by 50%, followed by hourly blood glucose monitoring for an additional 2 or 3 hours. This procedure for increasing the insulin infusion rate may be repeated if hyperglycemia persists. Conversely, if hypoglycemia (blood glucose <60 mg/dL) is noted, then a bolus of 0.25 to 0.5 mL/kg of 50% dextrose solution should be administered intravenously over 3 to 5 minutes. The blood glucose level should then be reassessed every 30 minutes for at least 90 minutes. If hypoglycemia recurs, a second bolus of dextrose should be administered and the insulin infusion rate decreased by 50%. Close monitoring will then be required for an additional 60 to 90 minutes to assess the stability of blood glucose concentration. Further changes to the insulin infusion rate are not usually necessary once a steady state has been achieved, in which the blood glucose level is stable and the desired rate of PN administration has been achieved.

When PN support is to be discontinued, it is recommended that the infusion rate be gradually reduced by 25% to 50% increments every 4 to 6 hours while gradually introducing enteral feeding. The monitoring of blood glucose must continue during this weaning process to prevent severe hypoglycemia.

MANAGEMENT OF OBESITY

Obesity is an emergent problem in companion animal equid populations. It has been associated with insulin resistance in horses and ponies, and both obesity and insulin resistance have been associated with increased risk of laminitis, particularly the pasture-associated form of this disease.[8,9,107] There is no universal definition of obesity in horses and ponies. According to the Henneke system, horses with a BCS of 8 (fat) or 9 (extremely fat) can be defined as obese, and animals with a BCS of 7 might be considered overweight, if not obese. Few studies have examined the prevalence of obesity in horse and pony populations. The 1998 National Animal Health Monitoring System (NAHMS) study estimated that 4.5% of the horse

TABLE **5.8 Recommended Digestible Energy Intake by Mature Horses (Mcal/day)**[a]

	Target Body Weight		
Type of Horse	**400 kg (880 lb)**	**500 kg (1100 lb)**	**600 kg (1320 lb)**
Adult sedentary[b]			
Minimum voluntary activity	12.1	15.2	18.2
Average voluntary activity	13.3	16.7	20.0
Elevated voluntary activity	14.5	18.2	21.8
Adult light exercise	16.0	20.3	24.0
Adult moderate exercise	18.6	23.3	28.0
Adult heavy exercise	21.3	26.6	32.0
Adult very heavy exercise	27.6	35.6	41.4
Pregnant: 0–4 months	13.3	16.7	20.0
5 months	13.7	17.1	20.5
6 months	13.9	17.4	20.9
7 months	14.3	17.9	21.5
8 months	14.8	18.5	22.2
9 months	15.4	19.2	23.1
10 months	16.2	20.2	24.2
11 months	17.1	21.4	25.7
Lactating: 1st month	25.4	31.7	38.1
2nd month	25.3	31.7	38.0
3rd month	24.5	30.6	36.7
4th month	23.6	29.4	35.3
5th month	22.7	28.3	34.0
6th month	21.8	27.2	32.7

[a]Recommended intakes for stallions and growing horses can be found in the NRC publication.
[b]Sedentary adults: Minimum = very inactive in stall/paddock, easy keeper; elevated = very active in stall/paddock, hard keeper. Light exercise: 1 to 3 h/week; walking, trotting. Moderate exercise: 3 to 5 h/week; walking, trotting, some canter; easy skills. Heavy exercise: 4 to 5 h/week; trotting, cantering; hard skills. Very heavy: racing; elite 3-day; endurance racing.
From Committee on Nutrient Requirements of Horses, National Research Council. *Nutrient Requirements of Horses*. 6th ed. rev. Washington, DC: National Academies Press; 2007.

population in the United States was overweight or obese.[108] However, the accuracy of this estimate may be questioned because it was based on owner reports, not the results of physical examination. Anecdotal observations by equine veterinarians suggest that the prevalence of obesity is far higher than the NAHMS estimate. In support of this contention, a recent cross-sectional, prospective study of 300 mature horses (ponies were excluded) in southwestern Virginia reported obesity (BCS of 8 or 9) in 56 animals (i.e., a prevalence of 19%).[109] In a study of 319 pleasure riding horses in Scotland, 32% were obese and a further 35% were considered fat.[110]

The cause of obesity in horses and ponies is likely multifactorial. However, overfeeding and lack of physical activity are likely contributing factors. Many horses are kept in confinement (e.g., stall, small pen) for much of the day, and even if used for riding activities 2 or 3 days per week, they may not require any more than maintenance energy intakes. Nevertheless, many of these horses are fed much more than the maintenance energy requirement, which is a problem that is compounded by the provision of grains, sweet feeds, and other feeds with high caloric density. Obesity also can be a problem in some horses and ponies given unrestricted access to pasture, particularly during the spring and fall, when pasture forage is actively growing, plentiful, and energy rich. In a study by Thatcher et al., more than 60% of the surveyed horses were maintained at pasture year round and were not used for any type of riding activity.[109]

Maintenance DE requirements for horses range between 30 and 36 kcal/kg BW (Table 5.8), with variation among horses resulting from differences in dietary composition, age, breed, environment, and body composition, among other factors.[1] Thus for a 500-kg (1100-lb) horse, minimum maintenance energy needs are 15.2 Mcal DE/day. This requirement can be met by about 7 kg (15 lb) of high-quality hay (i.e., no supplemental grain is required). To illustrate the impact of even moderate overfeeding on BW, consider the effect of adding 1 kg of grain or sweet feed per day to this horse's ration for a 1-year period (i.e., an additional 3 Mcal/day or >1000 Mcal over the 1-year period); if it is assumed that 20 to 25 Mcal of DE *over maintenance* is required for 1 kg of weight gain, the horse in this example will gain upward of 40 kg BW during this period (with a 1- to 1½-unit increase in BCS).

Genetics may be another factor in the predisposition to obesity. Horse owners and veterinarians often use the term *easy keeper* to describe a horse or pony that has a tendency to be overweight and appears to require fewer calories than most horses to maintain condition. Ponies and certain horse breeds (e.g., Morgans, Arabians, Paso Finos) appear to fit this description. One hypothesis is that certain lines of horses and ponies have inherited genetic traits that have facilitated survival on

poor-quality forages or in the face of limited feed availability: the so-called thrifty genotype.[8,111] When these animals are supplied with abundant feed, particularly grains or pasture forage rich in NSC, weight gain and obesity result.

Obesity in horses and ponies is a risk factor for laminitis. Mechanical trauma caused by increased load on the feet is one possible reason that obesity is linked with laminitis. However, the increased risk of laminitis in obese equids is more likely related to the attendant insulin resistance. In one recent study of ponies, a phenotype characterized by generalized or regional adiposity (especially a cresty neck), hyperinsulinemia, and hypertriglyceridemia was associated with 10-fold higher risk for development of pasture-associated laminitis. The clustering of these risk factors for laminitis is termed *prelaminitic syndrome*.[8] Similar associations between obesity, insulin resistance, and laminitis have been observed in horses, leading to the use of the term *equine metabolic syndrome*.[112] Obese ponies, donkeys, and Miniature Horses are prone to the development of hyperlipemia during times of stress or negative energy balance (e.g., concurrent disease, lactation). Other proposed effects of obesity include impaired thermoregulation in hot weather, reduced athletic performance, and increased risk of joint injuries, but evidence is lacking. In broodmares obesity and insulin resistance have been associated with prolonged luteal phase and lengthened interovulatory intervals,[112] but the impact of these alterations in the estrous cycle on reproductive performance has not been extensively studied.

Weight Management Programs

In horses, as in humans, eating less and exercising more are the key strategies for improving BW and condition. Important steps in the development of a weight management program include the following:

- Owner/trainer recognition that the horse or pony is overweight or obese: As the old adage states, "beauty is in the eye of the beholder," and different equestrian disciplines and breeds have adopted different standards to evaluate body condition. Nonetheless, the effectiveness of any weight loss program is critically dependent on the willingness of the owner or caregiver to comply with the plan.
- Evaluation of the current feeding program and housing: This includes a thorough evaluation of the type of feed that is being provided (including supplementary feed, hay, pasture quality, and time allowed for grazing) and in what quantities.
- Assessment of the weekly workload and soundness for exercise: Is the horse or pony engaged in structured physical activity (e.g., being ridden)? If so, how much? Many obese equids receive little structured exercise. Information on current activity level and soundness for exercise forms the basis for recommendations regarding physical activity.
- Set realistic goals for weight loss and regularly monitor progress: In this author's experience, there is wide variation in the response of obese horses and ponies to weight loss treatment programs. Some undergo a substantial loss of BW and adiposity after 2 to 3 months of diet restriction and increased physical activity. In others, progress can be frustratingly slow, and further adjustments to diet and the level of physical activity may be needed for satisfactory improvement. As a guide, an effective weight loss regimen should result in the loss of approximately 25 to 30 kg over a 4- to 6-week period. This decrease in BW may be accompanied by the loss of approximately 1 unit of BCS. However, initial weight loss may result from a decrease in abdominal fat or abdominal fat mass, and further weight loss may be required before noticeable changes in BCS occur. BW and body condition should be assessed regularly (e.g., every 2–4 weeks) during the *weight reduction* program so that progress can be monitored and the program adjusted accordingly.
- Make all dietary changes gradually and avoid prolonged periods of feed withholding. Abrupt starvation in obese ponies, donkeys, and Miniature Horses carries the risk of hyperlipemia and hepatic and renal lipidosis.
- Develop an appropriate *weight maintenance* program once the target weight and body condition have been achieved. This includes monthly assessment of BW and condition to ensure that the feeding program is appropriate to the current level of physical activity and other environmental influences on energy requirements (e.g., ambient conditions).

In obese people the combination of caloric restriction and regular physical activity can result in more substantial weight loss than either strategy alone. However, studies in humans also have demonstrated that physical activity is beneficial even when weight loss does not occur, as demonstrated by improvements in insulin resistance, blood lipid profile, and markers of inflammation, all of which are risk factors for cardiovascular disease. Similarly, a study in a small number of obese mares demonstrated improvements in insulin sensitivity without a change in BW after 7 days of round pen exercise (15–20 min/day).[113] Accordingly, a program of regular exercise is likely to be beneficial in the management of obese (but sound) horses and ponies. In the author's experience, weight reduction and subsequent control are improved when dietary restriction is combined with a program of riding or longeing. A suggested exercise regimen is to start with two or three exercise sessions per week (20–30 min per session), subsequently building to 4 or 5 times per week, with a gradual increase in the intensity and duration of exercise.

Feeding Obese Horses

Caloric restriction is of paramount importance in the management of obese equids; creation of a state of negative energy balance is needed to achieve loss of BW. Several different dietary strategies can be applied depending on the horse's present and desired body condition and other individual circumstances. A certain amount of trial and reassessment is invariably required to achieve the goal weight and condition in an individual animal. Key considerations are the quantity and composition of the ration. Removal from pasture (e.g., to a large dry lot) is necessary for adequate control of dietary intake. Some nutritionists and veterinarians have recommended restrictive grazing as a means to decrease caloric intake in overweight equids. However, a study in obese pony mares reported no change in BW when ponies were allowed access to pasture for 12 hours daily (either during day or night),[114] perhaps because of increased forage consumption during the restricted grazing period. In another study it was estimated that ponies could consume 40% of their daily DM intake during 3 hours of pasture turnout.[115] Strategies that allow turnout while minimizing forage intake include application of grazing muzzles, strip grazing behind other horses, mowing the pasture and removing clippings before providing access, putting a deep layer of wood chips over a small paddock, and using dry lots or indoor arenas. It is important to ensure that horses wearing grazing muzzles are able to consume water. In some obese horses and ponies, a return to less restricted pasture access is possible after attainment of goal BW

and condition. Even then, however, reduced grazing may be justified during periods of rapid growth (i.e., spring and fall) given the likelihood for weight gain and exacerbation of insulin resistance, with attendant increased risk of laminitis.

Generally, rations for overweight and obese horses should be high in fiber and low in NSC. Horses at maintenance require approximately 2% BW as forage or forage plus supplement to meet daily nutrient requirements. As a first step toward calorie restriction and weight loss, grain and other concentrated sources of calories (e.g., commercial sweet feeds, feeds containing added fats) should be reduced (for overweight animals) or totally removed (for obese animals) from the diet. Excessive feeding of other treats, such as carrots and apples, also should be curtailed. Forage (as hay or hay substitute such as chop, chaff, or haylage) should be the primary, if not sole, energy-providing component of the ration. In some areas forage-based, low-calorie feeds complete with vitamins and minerals are available commercially; this type of feed is convenient and may be used as a substitute for hay or fed as a component of the ration along with hay. In a study of obese ponies provided a free-choice forage (chaff) diet during winter and summer, voluntary intake (DM basis) was about 2% of BW, and BCS was unchanged during the study period.[116] As a general guide, therefore, hay or hay substitute should initially be provided at no more than 1.5% of current BW per day (clients should be instructed to weigh the ration), with subsequent further reductions in feed depending on the extent of weight loss (e.g., 1% of *target* BW). It is preferable not to decrease forage provision below 1% of BW; feeding smaller amounts of forage can increase the risk of hindgut dysfunction, stereotypical behaviors (e.g., wood chewing), ingestion of bedding, and coprophagy. The ration should be divided into three to four feedings per day.

Mature grass hay (i.e., with visible seedheads and a high stem-to-leaf ratio) has higher fiber and lower energy and NSC than immature hay and is suitable forage for the obese horse or pony. Alfalfa hay or other legumes, such as clover, are less preferred because, on average, these forages have higher energy and NSC content than grass hay. Ensiled forages generally have lower NSC contents than hay made from the same crop. However, despite the generally lower NSC content of haylage compared with hay, the high palatability of some haylages may result in higher total NSC intake. Ideally, the results of proximate nutrient analysis, including direct measurement of starch and ESC, should be reviewed before selection of the hay. An NSC content of less than 10% is recommended. Poorly digestible, highly silicated forages should be used with caution; according to anecdotal reports, this practice increases the risk of impaction colic in some animals.

Forage-only diets do not provide adequate protein, minerals, or vitamins. It is possible that the lack of protein over the long term could lead to the loss of muscle mass rather than fat. Therefore the forage diet should be supplemented with a low-calorie commercial ration balancer product that contains sources of high-quality protein and a mixture of vitamins and minerals to balance the low vitamin E, vitamin, copper, zinc, selenium, and other minerals typically found in mature grass hays. These products are often designed to be fed in small quantities (e.g., 0.5–1.0 kg/day); they can be mixed with chaff (hay chop) to increase the size of the meal and extend feeding time, which may alleviate boredom in animals provided a restricted diet.

Ponies, donkeys, and Miniature Horses must not be abruptly starved to reduce their BW or prevented from eating for prolonged periods; these strategies have been associated with the development of hyperlipemia. This risk is increased at times of stress, such as during transportation, lactation, pregnancy, and management changes. As mentioned previously, the target duration for weight loss should be weeks to months rather than days.

Additional factors should be considered when planning a weight reduction program, including strategies to extend feeding time and relieve boredom in the face of limited feed provision, the need for individual rather than group feeding, the potential need for a change in the type of bedding, and the potential use of pharmacologic agents (e.g., levothyroxine) purported to enhance weight loss or mitigate comorbidities such as insulin resistance. Recent studies have demonstrated that levothyroxine sodium (48 mg/day for an adult horse) can enhance weight loss in healthy horses.[117]

Use of hay nets with small openings or double hay nets can extend feeding time in some horses. The size of the meal and duration of feeding can be increased by mixing the feed with chaff or chopped straw. In group housing situations it may be necessary to separate the obese horse or pony to allow strict control of feed intake. For animals housed in stalls, dietary restriction can promote intake of bedding. Because some straws retain some of the cereal heads, ingestion of bedding can substantially increase daily caloric intake (and possibly the risk of colic). In this situation use of shavings, paper, or other nonstraw alternatives for bedding is recommended.

FEEDING THIN AND STARVED HORSES

Failure to meet a horse's energy (DE) requirements will result in weight loss. Prolonged nutritional restriction (energy and protein) will result in emaciation and, in severe cases, death. Chronically starved horses have low body condition (BCS of 2 or lower), with minimal subcutaneous fat and reduced muscle mass. The hair coat is often unthrifty in appearance, and muscle weakness may result in recumbency. Healthy horses starved of feed take approximately 60 to 90 days to become recumbent. After 36 to 48 hours of recumbency, horses are often in lateral recumbency, may not be able to raise their heads, and can display seizure-like activity. The prognosis for survival is very poor in horses recumbent for more than 72 hours, even when appropriate nutritional support and nursing care are instituted.[118]

Laboratory findings in chronically starved horses may include anemia, hypertriglyceridemia, hyperbilirubinemia (especially unconjugated bilirubin), high nonesterified fatty acid concentrations, lymphopenia, hypophosphatemia, and hypomagnesemia. Protein deficiency may result in hypoalbuminemia and low BUN concentration. When presented with a thin or emaciated horse, the clinician first must determine the cause (e.g., feed restriction versus medical problems such as intestinal parasites, malabsorption syndromes, poor dentition, old age). Thorough clinical evaluation is therefore required to identify the cause of weight loss and emaciation. If the horse has been neglected, obtaining an accurate history of its diet and feeding program may be difficult. However, observation of the environment and other horses on the farm may indicate feed restriction as the cause of thin body condition or starvation. Herdmates also may be in poor body condition, pastures overgrazed, and hay quality poor or fed in quantities insufficient to meet the requirements of the individual or herd. Harsh environmental conditions (e.g., dry summer, cold winter) can contribute to feed restriction in horses kept at pasture.

Recommendations for feeding management of thin and emaciated horses vary depending on the severity and chronicity of starvation and appetite. Severely debilitated horses with poor appetite may require AEF with a slurry made from commercial complete feeds or PN support (as described earlier in this chapter). For horses in moderately poor body condition (BCS 3 or 4) with good appetite, a simple increase in energy intake will result in weight gain. The first step is to thoroughly evaluate the current ration, with particular reference to the adequacy of DE and protein intake (see Fig. 5.1 and Table 5.8). Requirements for weight gain can then be estimated. The relationships among energy intake, change in BW, and change in BCS have not been well described. However, in light-breed horses it appears that each unit of body condition increase requires at least 20 kg of weight gain. Some experts suggest that 16 to 24 Mcal of DE are required per kilogram of gain in mature horses of about 500-kg (1100-lb) BW.[1] Using an average value of 20 Mcal DE/kg weight gain, a 1-unit increase in BCS (20-kg gain) will require 400 Mcal of DE over maintenance requirements, or an additional 6 to 7 Mcal per day for the 500-kg horse over a 60-day period. Using similar assumptions, a 2-unit increase in BCS (approximately 40 kg) may be achieved over a 90-day period by feeding an additional 9 to 10 Mcal of DE per day (see Fig. 5.1). Note that these estimates have been derived from limited data, and individual responses are likely to vary. Also bear in mind that the efficiency of conversion of DE to usable energy for tissue deposition (net energy) varies among energy sources; less DE may be needed per unit of gain if a high-fat feed is provided compared with a high-fiber feed such as grass hay. The addition of 3 kg of good-quality hay (e.g., alfalfa, DE 2.4 Mcal/kg on DM basis) or sugar beet pulp (2.8 Mcal DE/kg DM) to the ration will provide the extra DE required for a 1-unit increase in BCS over a 60-day period. Alternatively, a smaller amount of additional hay or beet pulp may be combined with vegetable oil (1.7 Mcal DE per standard cup [225 mL]). The provision of 2 to 2.5 kg/day of a commercial fat-supplemented feed (8%–10% fat; typical DE of 3–3.5 Mcal/kg) is another option.

A more conservative approach is indicated for the initial nutritional support of starved horses. In malnourished human patients aggressive refeeding can result in potentially fatal shifts in fluids and electrolytes as a result of hormonal and metabolic responses to rapid refeeding, whether enteral or parenteral; this problem has been named *refeeding syndrome.* The hallmark biochemical feature of refeeding syndrome is hypophosphatemia, but other electrolyte abnormalities may occur, including hypokalemia and hypomagnesemia.[119] Thiamine deficiency is another possible feature of refeeding syndrome. During prolonged starvation the intracellular concentrations of these substances become severely depleted, although serum concentrations may remain within reference limits. With refeeding, glycemia leads to an increase in circulating insulin, which stimulates glycogen, and fat and protein synthesis. These processes require cofactors such as phosphate, magnesium, and thiamine. Insulin stimulates cellular uptake of potassium, and magnesium and phosphate also move into the cells. Water follows by osmosis. Consequently, there can be marked decreases in serum phosphate, magnesium, and potassium, with potential development of cardiac dysfunction (e.g., arrhythmias, cardiac arrest) and neuromuscular complications.

The incidence of refeeding syndrome in chronically starved horses is unknown. Anecdotally, however, metabolic and electrolyte derangements similar to those observed in humans with refeeding syndrome have been observed in starved horses provided a diet rich in starches and sugars (i.e., high NSC). Therefore some experts recommend restricting dietary NSC in the ration provided to chronically starved horses, specifically less than 20% NSC in the total diet.[73] One study evaluated the metabolic responses of chronically starved horses refed one of three diets: alfalfa hay, oat hay, or a diet of half oat hay and half an extruded (commercial) feed.[120] The diets were initially offered at 50% of estimated daily DE requirements and, over the subsequent 10-day period, gradually increased to 100% of daily needs. There were minimal differences among treatments regarding metabolic responses (e.g., blood glucose, nonesterified fatty acid, mineral concentrations), but serum insulin concentrations were higher in horses fed hay in addition to extruded feed. Weight gain over the 10-day period did not differ among treatments. Another study compared starved horses fed a diet of alfalfa alone and those fed alfalfa and corn oil.[121] Phosphorus intake was lower in horses fed the alfalfa and corn oil diet, which is associated with lower serum phosphorus concentrations.

Generally, a diet consisting mostly of forage (e.g., hay) is recommended during the rehabilitation of chronically starved horses. Grass or legume hay (or a mixture of the two) should be fed. The NSC content of these forages is generally less than 15% DM. Alfalfa hay is a good choice for initial refeeding because it has a higher mineral content than grass hay. Grains (e.g., oats, corn, barley) and sweet feeds are not recommended because of their high NSC content. It is advisable to supplement the hay ration with a vitamin and mineral supplement or balancer pellet. The energy density of the ration may be increased by adding vegetable oil (e.g., ¼ to 1 cup per day, starting at the low end of this range) or providing a commercial fat-supplemented feed (8%–12% fat, as fed with NSC <20%). As previously mentioned, thiamine deficiency is thought to contribute to the pathophysiology of refeeding syndrome in humans. Accordingly, the administration of a B-vitamin preparation may be justified.

The DE (caloric) requirements should be calculated on the basis of *RERs* at the current BW (22–23 kcal/kg/day) and the true maintenance requirements at the ideal BW (30–36 kcal/kg/day; see Table 5.8). Starved, emaciated horses may have lost 25% to 30% of BW; therefore, maintenance DE needs for ideal BW will be based on 125% to 130% of BW measured at the time of first examination. A gradual increase in daily DE intake has been recommended,[73] starting at 25% to 50% of resting requirements at current BW, building to 100% of resting requirements over the next 2 to 3 days, and followed by a gradual (over 7–10 days) transition to maintenance energy requirements for the ideal BW. Energy provision should not exceed 100% of resting requirements if AEF is used for provision of the ration, with transition to true maintenance after the start of voluntary feed consumption. Regardless of feeding method (voluntary vs. AEF), the ration should be divided into four to six feedings daily during the first 10 to 14 days of rehabilitation. Subsequently, the ration can be provided into two to three meals per day.

Regular (every 1–2 days) measurement of blood glucose, serum magnesium, phosphorus, and potassium is advised during the first 7 to 10 days of refeeding. Frequent assessment of hydration status and gastrointestinal function (e.g., borborygmi, quantity and character of feces) is also recommended. Administration of intravenous fluids, oral electrolyte-mineral preparations, or both may be necessary to correct electrolyte imbalances.

REFERENCES

1. National Research Council. *Nutrient requirements of horses*. 6th ed. Washington, DC: National Academies Press; 2007.
2. Pagan JD, Geor RJ, eds. *Advances in equine nutrition II*. Nottingham, UK: Nottingham University Press; 2001.
3. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15:371–372.
4. Pratt SE, Geor RJ, McCutcheon LJ. Effect of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in standardbred horses. *Equine Vet J*. 2006;(suppl 36):579–584.
5. Suagee JK, Burk AO, Quinn JK, et al. Effects of diet and weight gain on body condition scoring in Thoroughbred geldings. *J Equine Vet Sci*. 2008;28:156–166.
6. Kienzle E, Schramme S. Body condition scoring and prediction of bodyweight in adult warm-blooded horses. *Pferdeheilkunde*. 2004;20:517–524.
7. Lee S, Bacha F, Gungor N, et al. Waist circumference is an independent predictor of insulin resistance in black and white youths. *J Pediatr*. 2006;148:188–194.
8. Treiber KH, Kronfeld DS, Hess TM, et al. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J Am Vet Med Assoc*. 2006;228:1538–1545.
9. Carter RA, Treiber KH, Geor RJ, et al. Prediction of incipient pasture-associated laminitis from hyperinsulinemia, hyperleptinemia, and generalized and localized obesity in a cohort of ponies. *Equine Vet J*. 2009;41:171–178.
10. Frank N, Elliott SB, Brandt LE, et al. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J Am Vet Med Assoc*. 2006;228:1383–1390.
11. Caroll C, Huntington P. Body condition scoring and weight estimation of horses. *Equine Vet J*. 1988;20:41–45.
12. Ellis JM, Hollands T. Accuracy of different methods of estimating the weight of horses. *Vet Rec*. 1998;143:335–336.
13. St. Lawrence AC, Lawrence LM, Coleman R. Using an empirical equation to predict the voluntary intake of grass hays by mature equids. In: *Proceedings of the 17th Equine Nutrition and Physiological Society Symposium*. 2001:99–100.
14. Archer DC, Proudman CJ. Epidemiological clues to preventing colic. *Vet J*. 2006;172:29–39.
15. Shirazi-Beechey SP. Molecular insights into dietary induced colic. *Equine Vet J*. 2008;40:414–421.
16. Geor RJ, Harris PA. How to minimize gastrointestinal disease associated with carbohydrate nutrition in horses. In: *Proceedings of the 53rd Annual Convention of the American Association of Equine Practitioners*. 2007:178–185.
17. Pagan JD, Jackson S, Duren S. Computing horse nutrition: how to properly conduct an equine nutrition evaluation using MicroSteed equine ration evaluation software. *World Equine Vet Rev*. 1996;1:11–17.
18. Gallagher K, Leech J, Stowe H. Protein, energy and dry matter consumption by racing Thoroughbreds: a field study. *J Equine Vet Sci*. 1988;12:43–47.
19. Southwood LL, Evans DL, Bryden WL, et al. Nutrient intake of horses in Thoroughbred and Standardbred stables. *Aust Vet J*. 1993;70:164–168.
20. White NA. Equine colic. II. Causes and risk factors for colic. In: *Proceedings of the 52nd Annual Convention of the American Association of Equine Practitioners*. 2006:115–119.
21. Richards N, Hinch GN, Rowe JB. The effect of current grain feeding practices on hindgut starch fermentation and acidosis in the Australian racing Thoroughbred. *Aust Vet J*. 2006;84:402–407.
22. Nadeau JA, Andrews FM, Mathew AG, et al. Evaluation of diet as a cause of gastric ulcers in horses. *Am J Vet Res*. 2000;61:784–790.
23. Reese RE, Andrews FM. Nutrition and dietary management of equine gastric ulcer syndrome. *Vet Clin Nth Am Equine Pract*. 2009;25:79–92.
24. McKenzie EC, Valberg SJ, Godden SM, et al. Effect of dietary starch, fat and bicarbonate content on exercise responses and serum creatine kinase activity in equine recurrent exertional rhabdomyolysis. *J Vet Intern Med*. 2003;17:693–701.
25. Geor RJ. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *J Equine Vet Sci*. 2008;28:753–759.
26. Longland AC. Starch, sugar and fructans, what are they and how important are they in diets for horses. In: *Proceedings of the 1st WALTHAM-RVC Laminitis Conference*. 2007:7–14.
27. Harris PA. Energy requirements of the exercising horse. *Ann Rev Nutr*. 1997;17:185–210.
28. Harris PA, Geor RJ. Primer on dietary carbohydrates and utility of the glycemic index in equine nutrition. *Vet Clin Nth Am Equine Pract*. 2009;25:23–37.
29. de Fombelle A, Veiga L, Drogoul C, et al. Effect of diet composition and feeding pattern on the prececal digestibility of starches from diverse botanical origins measured with the mobile nylon bag technique. *J Anim Sci*. 2004;82:3625–3634.
30. Pollock CJ, Lloyd EJ. The effect of low temperature upon starch, sucrose and fructan synthesis in leaves. *Ann Bot*. 1987;60:231–235.
31. Van Soest PJ. *Nutritional ecology of the ruminant*. 2nd ed. Ithaca, NY: Comstock Publishing; 1995.
32. Argenzio RA. Functions of the equine large intestine and their interrelationship in disease. *Cornell Vet*. 1979;65:303–330.
33. Clarke LL, Roberts MC, Argenzio RA. Feeding and digestive problems in horses. Physiologic responses to a concentrated meal. *Vet Clin North Am Equine Pract*. 1990;6:433–450.
34. Cohen ND, Gibbs PG. Dietary and other management factors associated with colic in horses. *J Am Vet Med Assoc*. 1999;215:53–60.
35. Hillyer MH, Taylor FGR, Proudman CJ, et al. Case control study to identify risk factors for simple colonic obstruction and distension colic in horses. *Equine Vet J*. 2002;34:55–463.
36. Hudson JM, Cohen ND, Gibbs PG, et al. Feeding practices associated with colic in horses. *J Am Vet Med Assoc*. 2001;219:1419–1425.
37. Tinker MK, White NA, Lessard P, et al. Retrospective study of equine colic risk factors. *Equine Vet J*. 1997;29:454–458.
38. Cohen ND, Peloso JG. Risk factors for history of previous colic and for chronic, intermittent colic in a population of horses. *J Am Vet Med Assoc*. 1995;208:607–703.
39. Proudman CJ. A two year, prospective survey of equine colic in general practice. *Equine Vet J*. 1992;24:90–93.
40. Cohen ND. The John Hickman Memorial Lecture: colic by numbers. *Equine Vet J*. 2003;35:343–349.
41. Varloud M, Goachet AG, de Fombelle A, et al. Effect of the diet on prececal digestibility of dietary starch measured in horses with acid insoluble ash as an internal marker. In: *Proceedings of the 18th Equine Nutrition and Physiological Society Symposium*. 2003:117–118.
42. Cummings JH, Englyst HN. Gastrointestinal effects of food carbohydrate. *Am J Clin Nutr*. 1995;(suppl 61):S938–S945.
43. Dyer J, Fernandez-Castano E, Salmon KS, et al. Molecular characterisation of carbohydrate digestion and absorption in equine small intestine. *Equine Vet J*. 2002;34:349–358.
44. Dyer J, Al Rammahi M, Waterfall L, et al. Adaptive response of equine intestinal Na(+)/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflugers Arch*. 2009;458:419–430.
45. Lorenzo-Figueras M, Morisset SM, Morriset J, et al. Digestive enzyme concentrations and activities in healthy pancreatic tissue. *Am J Vet Res*. 2007;68:1070–1072.
46. Kienzle E. Small intestinal digestion of starch in the horse. *Revue Med Vet*. 1993;145:199–204.

47. Kienzle E, Radicke S, Wilke W, et al. Activity of amylase in the gastrointestinal tract of the horse. *J Anim Physiol Am Nutr*. 1994;72:234–241.
48. Meyer H, Radicke S, Kienzle E, et al. Investigations on preileal digestion of oats, corn and barley starch in relation to grain processing. In: *Proceedings of the 13th Equine Nutrition and Physiological Society Symposium*. 1993:92–97.
49. Potter GD, Arnold FF, Householder DD, et al. Digestion of starch in the small or large intestine of the equine. *Pferdeheilkunde*. 1992;1:107–111.
50. Bugaut M, Bentejec M. Biological effects of short chain fatty acids in nonruminant mammals. *Annu Rev Nutr*. 1993;13:217–241.
51. De Fombelle A, Julliand V, Drogoul C, et al. Feeding and microbial disorders in horses: part 1—effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. *J Equine Vet Sci*. 2001;21:439–445.
52. Bailey SR, Marr CM, Elliott J. Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet J*. 2004;167:129–142.
53. Goodson J, Tyznik WJ, Cline JH, et al. Effects of an abrupt diet change from hay to concentrate on microbial numbers and physical environment in the cecum of the pony. *Appl Environ Microbiol*. 1988;54:1946–1950.
54. Willard JG, Willard JC, Wolfram SA, et al. Effect of diet on cecal pH and feeding behavior of horses. *J Anim Sci*. 1977;45:87–93.
55. Radicke S, Kienzle E, Meyer H. Preileal apparent digestibility of oat and corn starch and consequences for cecal metabolism. In: *Proceedings of the 13th Equine Nutrition and Physiological Society Symposium*. 1991:43–48.
56. Bailey SR, Rycroft A, Elliott J. Production of amines in equine cecal contents in an *in vitro* model of carbohydrate overload. *J Anim Sci*. 2002;51:1930–1934.
57. French KR, Pollitt CC. Equine laminitis: loss of hemidesmosomes in hoof secondary epidermal lamellae correlates to dose in an oligofructose induction model: an ultrastructural study. *Equine Vet J*. 2004;(suppl 36):230–235.
58. Drogoul C, de Fombelle A, Julliand V. Feeding and microbial disorders in horses. 2: effect of three hay:grain ratios on digesta passage rate and digestibility in ponies. *J Equine Vet Sci*. 2001;21:487–490.
59. Medina B, Girard ID, Jacotot E, et al. Effect of preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. *J Anim Sci*. 2002;80:2600–2609.
60. Weese JS, Rousseau J. Evaluation of *Lactobacillus pentosus* WE7 for prevention of diarrhea in neonatal foals. *J Am Vet Med Assoc*. 2005;226:2031–2034.
61. Desrochers AM, Dolente BA, Roy MF, et al. Efficacy of *Saccharomyces boulardii* for treatment of horses with acute enterocolitis. *J Am Vet Med Assoc*. 2005;227:954–959.
62. Pagan JD, Lawrence TJ. LA: Feeding protected sodium bicarbonate attenuates hindgut acidosis in horses fed a high-grain ration. In: *Proceedings of the 54th Annual Convention of the American Association of Equine Practitioners*. 2007:530–533.
63. Heyland DK. Nutritional support in the critically ill patient: a critical review of the evidence. *Critical Care Clinics*. 1998;14:423–440.
64. Silk DBA, Gow NM. Postoperative starvation after gastrointestinal surgery: early feeding is beneficial. *Br Med J*. 2001;323:761–766.
65. Shukla VK, Roy SK, Kumar J, Vaida MP. Correlation of immune and nutritional status with wound complications in patients undergoing abdominal surgery. *Ann Surg*. 1985;51:442–445.
66. Hasselgren PO, Fischer JE. Counter-regulatory hormones and mechanisms in amino acid metabolism with special reference to the catabolic response in skeletal muscle. *Curr Opin Clin Nutr Metab Care*. 1999;2:9–14.
67. Romijn JA. Substrate metabolism in the metabolic response to injury. *Proc Nutr Soc*. 2000;59:447–449.
68. Leverve X. Inter-organ substrate exchanges in the critically ill. *Curr Opin Clin Nutr Metab Care*. 2001;4:137–142.
69. Dunkel B, McKenzie HC. Severe hypertriglyceridemia in clinically ill horses: diagnosis, treatment and outcome. *Equine Vet J*. 2003;35:590–595.
70. Gelfand RA, Mathews DE, Bier D, et al. Role of counter-regulatory hormones in the catabolic response to stress. *J Clin invest*. 1984;74:2238–2248.
71. Langhans W. Peripheral mechanisms involved with catabolism. *Curr Opin Clin Nutr Metab Care*. 2002;5:419–426.
72. Magdesian KG. Nutrition for critical gastrointestinal illness: feeding horses with diarrhea or colic. *Vet Clin Equine*. 2003;19:617–644.
73. Robinson NE, Sprayberry K, eds. *Current therapy in equine medicine*. 6th ed. St. Louis, MO: Saunders; 2009.
74. Jeejeebhoy KN. Enteral and parenteral nutrition: evidence-based approach. *Proc Nutr Soc*. 2001;60:399–402.
75. Raina N, LaMarre J, Liew C-C, et al. Effect of nutrition on the expression of plasma soluble tumor necrosis factor (TNF) receptors, membrane TNF receptors and MRNA of TNF receptors in rats receiving oral and parenteral nutrition. *Am J Physiol*. 1999;277:E464–E473.
76. Yamazaki K, Maiz A, Moldaver LL, et al. Complications associated with overfeeding of infected animals. *J Surg Res*. 1986;40:152–158.
77. Matsui J, Cameron RG, Kurian R, et al. Nutritional, hepatic, and metabolic effects of cachetin/tumor necrosis factor in rats receiving parenteral nutrition. *Gastroenterology*. 1993;104:235–243.
78. Pagan JD, Hintz HF. Equine energetics. I. Relationship between body weight and energy requirements in horses. *J Anim Sci*. 1986;63:815–821.
79. Stapleton RD, Jones N, Heyland KD, et al. Feeding critically ill patients: what is the optimal amount of energy? *Crit Care Med*. 2007;35:S535–S540.
80. Jeejeebhoy KN. Permissive underfeeding of the critically ill patient. *Nutr Clin Pract*. 2004;19:477–480.
81. Ousey JC, Holdstock NB, Rossdale PD, et al. How much energy do sick neonatal foals require compared to healthy foals? *Pferdeheilkunde*. 1996;12:231–237.
82. Ousey JC, Prandi S, Zimmer J, et al. Effects of various feeding regimens on the energy balance of equine neonates. *Am J Vet Res*. 1997;58:1243–1251.
83. Durham AE, Phillips TJ, Walmsley JP, et al. Study of the clinical effects of postoperative parenteral nutrition in 15 horses. *Vet Rec*. 2003;153:493–498.
84. Adam S, Forrest S. ABC of intensive care. *Br Med J*. 1999;319:175–178.
85. Durham AE, Phillips TJ, Walmsley JP, et al. Nutritional and clinicopathological effects of post operative parenteral nutrition following small intestinal resection and anastomosis in the mature horse. *Equine Vet J*. 2004;36:390–396.
86. Frost P, Bihari D. The role of nutritional support in the critically ill: physiological and economic considerations. *Nutrition*. 1997;(suppl 13):58–63.
87. Miura S, Tanaka S, Yoshioka M, et al. Changes in intestinal absorption of nutrients and brush border glycoproteins after total parenteral nutrition in rats. *Gut*. 1992;33:484–489.
88. Mosenthal AC, Xu D, Deitch EA. Elemental and intravenous total parenteral nutrition diet-induced gut barrier failure is intestinal site specific and can be prevented by feeding nonfermentable fiber. *Crit Care Med*. 2002;30:396–402.
89. Buechner-Maxwell VA, Elvinger F, Thatcher CD, et al. Physiologic response of normal adult horses to a low residue liquid diet. *J Equine Vet Sci*. 2003;23:310–317.
90. Naylor JM, Freeman DE, Kronfeld DS. Alimentation of hypophagic horses. *Comp Cont Educ Pract Vet*. 1984;6:S03–S99.
91. Detsky AS, Baker JP, O'Rouke K, Goel V. Perioperative parenteral nutrition: a meta-analysis. *Ann Intern Med*. 1987;107:195–203.

92. Silk DB, Green CJ. Perioperative nutrition: parenteral versus enteral. *Curr Opin Clin Nutr Metab Care*. 1998;1:21–27.
93. Bozzetti F, Gavazzi C, Miceli R, et al. Perioperative total parenteral nutrition in malnourished gastrointestinal cancer patients: a randomized, clinical trial. *J Parenter Enteral Nutr*. 2000;24: 7–14.
94. Klein S, Kinney J, Jeejeebhoy K, et al. Nutrition support in clinical practice: review of published data and recommendations for future research directions. *J Parenter Enteral Nutr*. 1997;21:133–156.
95. Farinas-Alvarez C, Farinas MC, Fernandez-Mazarrasa C, et al. Analysis of risk factors for nosocomial sepsis in surgical patients. *Br J Surg*. 2000;87:1076–1081.
96. Lopes MA, White NA. Parenteral nutrition for horses with gastrointestinal disease: a retrospective study of 79 cases. *Equine Vet J*. 2002;34:250–257.
97. McKenzie III HC, Geor RJ. Feeding management of sick neonatal foals. *Vet Clin North Am Equine Pract*. 2009;25:109–119.
98. Stoneham S. How to feed the sick neonatal foal. In: *Proceedings of the 1st British Equine Veterinary Association and Waltham Nutrition Symposia*. Suffolk, UK: Equine Veterinary Journal Limited; 2005:33–37.
99. Naylor JM, Bell R. Raising the orphan foal. *Vet Clin N Am Equine Pract*. 1985;1:169–178.
100. Staniar WB, Kronfeld DS, Hoffman RM, et al. Weight prediction from linear measures of growing thoroughbreds. *Equine Vet J*. 2004;36:149–154.
101. Cymbaluk NF, Smart ME, Bristol F, et al. Importance of milk replacer intake and composition in rearing orphan foals. *Can Vet J*. 1993;34:479–486.
102. Ousey JC. Feeding the newborn foal in health and disease. *Equine Vet Educ*. 2003;6:50–54.
103. Magdesian KG. Neonatal foal diarrhea. *Vet Clin N Am Equine Pract*. 2005;21:295–312.
104. Krause JB, McKenzie III HC. Parenteral nutrition in foals: a retrospective study of 45 cases. *Equine Vet J*. 2000-2004;(39): 74–78.
105. Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Saunders; 1990.
106. Spurlock SL, Donaghue S. Weight gains in foals on parenteral nutrition. In: *Proceedings of the 2nd Conference of the International Society of Perinatology*. 1990:61.
107. Geor R, Frank N. Metabolic syndrome: from human organ disease to laminar failure in equids. *Vet Immunol Immunopathol*. 2009;129:151–154.
108. United States Department of Agriculture. NAHMS Equine '98. Part III. Management and health of horses. https://www.aphis.usda.gov/animal_health/nahms/equine/downloads/equine98/Equine98_dr_PartIII.pdf; accessed 28 March 2017.
109. Thatcher C, Pleasant RS, Geor RJ, et al. Prevalence of overconditioning in mature horses in southwest Virginia during the summer. *J Vet Intern Med*. 2012;26:1413–1418.
110. Wyse CA, McNie KA, Tannahil VJ, et al. Prevalence of obesity in riding horses in Scotland. *Vet Rec*. 2008;162:590–591.
111. Treiber KH, Kronfeld DS, Geor RJ. Insulin resistance in equids: possible role in laminitis. *J Nutr*. 2006;136:S2094–S2098.
112. Johnson PJ. The equine metabolic syndrome: peripheral Cushing's syndrome. *Vet Clin N Am Equine Pract*. 2002; 18:271–293.
113. Powell DM, Reedy SE, Sessions DR, et al. Effect of short-term exercise training on insulin sensitivity in obese and lean mares. *Equine Vet J*. 2002;(suppl 34):81–84.
114. Buff PR, Johnson PJ, Wiedmeyer CE, et al. Modulation of leptin, insulin and growth hormone in obese pony mares under chronic nutritional restriction and supplementation with ractopamine hydrochloride. *Vet Ther*. 2007;7:64–72.
115. Ince JC, Longland AC, Moore-Colyer M, et al. A pilot study to estimate the intake of grass by ponies with restricted access to pasture. In: *Proceedings of the British Society of Animal Science*. 2005:109.
116. Dugdale AHA, Curtis GC, Knottenbelt DC, et al. Changes in body condition and fat deposition in ponies offered an *ad libitum* chaff-based diet. In: *Proceedings of the 12th Congress of the European Society of Veterinary Clinical Nutrition*. 2008:39. [abstract].
117. Frank N, Elliott SB, Boston RC. Effects of long-term oral administration of levothyroxine sodium on glucose dynamics in healthy adult horses. *Am J Vet Res*. 2008;69:76–81.
118. Whiting TL, Salmon RH, Wruck GC. Chronically starved horses: predicting survival, economic, and ethical considerations. *Can Vet J*. 2005;46:320–324.
119. Stanga Z, Brunner A, Leuenberger M, et al. Nutrition in clinical practice: the refeeding syndrome: illustrative cases and guidelines for prevention and treatment. *Eur J Clin Nutr*. 2008;62:687–694.
120. Witham CL, Stull CL. Metabolic responses of chronically starved horses to refeeding with three isoenergetic diets. *J Am Vet Med Assoc*. 1998;212:691–696.
121. Stull CL. Nutrition for rehabilitating the starved horse. *J Equine Vet Sci*. 2003;23:456–459.

CHAPTER 6

Clinical Epidemiology and Evidence-Based Medicine

Kenneth W. Hinchcliff

INTRODUCTION

Contemporary veterinary clinical practice is the art and science of providing optimal care for the animal in a context that is consistent with the informed consent of the person responsible (owner) for the care, well-being, and use of that animal. These are two important considerations: the provision of optimal care, which implies knowledge of what is best practice, and provision of this care in a context in which the wishes and resources of the owner are considered. Best practice increasingly relies on an awareness of the scientific and clinical literature on a specific topic and the ability to evaluate the reliability of this information. This process has been called "evidence-based veterinary medicine" (EBVM), and it underlies much of modern veterinary clinical practice. This topic was previously discussed in earlier editions of this textbook.

EBVM can be defined broadly as the dispassionate assessment of the evidence underlying our approaches to diagnosis, treatment, and control of diseases or conditions. This consideration of the evidence is scientifically based and is intended to provide objective assessment of the evidence leading to a finding of the best intervention, diagnostic method, or means of control of a disease. The term *evidence-based medicine (EBM)* was proposed in human medicine in the early 1990s, and the concept was soon extended to veterinary medicine including equine medicine. There is now a substantial body of literature on human EBM and a smaller, but still important, amount of literature on veterinary evidence-based medicine.[1-7]

The basis of EBM and EBVM is scientific in that both the evidence and the method of assessing it are grounded in science. EBVM is an evaluation of the scientific evidence, as available in the published literature, using prescribed and predetermined methodologies and criteria, as discussed further in this chapter. What is often not clear is the role of clinical experience in evaluating this scientific evidence. Early definitions of EBM in human medicine explicitly included consideration of individual clinical expertise, but a definition of veterinary EBM referred to the "use of current best evidence," implying that individual, or collective, clinical experience is not a basis for the application of the evidence.[7] Although perhaps not the intended approach, exclusion of clinical experience would appear to be a limitation of the purely evidence-based approach to practice, and this is recognized in more current definitions.[1]

The emerging practice in human medicine is more holistic and includes a consideration of the evidence and its quality in the context in which the evidence will be used. This approach, which is captured in the Grading of Recommendations Assessment, Development and Evaluation (GRADE) process explicitly acknowledges that good clinical practice is complex and not solely based on the use of high-quality evidence. Good clinical practice includes a consideration of multiple factors such as the seriousness of the outcome, risk of adverse events, and preferences of the owner or trainer (or, in human medicine, the patient; see later). GRADE provides a conceptual framework that involves a systematic and transparent approach to moving from the evidence to a recommendation or decision while recognizing that the high quality of evidence is a fundamental, but not the sole, basis for making strong recommendations for clinical practice.[8,9]

Evidence-Based Veterinary Medicine: Arriving at a Recommendation

There are three key components and five steps to arriving at a recommendation using EBVM.[1] The fundamental components of EBM are identifying and clearly stating the question, assembling and evaluating the evidence through a systematic review, and considering the evidence in making a recommendation.[10] These aims can be divided into five steps.[1]

Ask the Pertinent Question

All EBVM begins with asking the right question. The start of any search for evidence must begin with a clear and concise response to "What is the question?" A common method of formulating the question is the PICO method: patient (animal including species, age, sex, disease, and other demographically or clinically defining characteristics), intervention (or diagnostic method or prophylaxis), comparator (the situation to which the intervention is being applied, such as a placebo, different surgical or diagnostic technique), and the outcome of interest.[10] A relevant question must include a consideration of the setting (primary practice or referral hospital), the population of interest (e.g., foals <5 months of age on a stud farm with endemic *Rhodococcus (Prescottella) equi* pneumonia), the intervention being applied (e.g., administration of azithromycin), the comparator (e.g., administration of a placebo), and well-defined and measurable outcomes (e.g., survival at 300 days of age).[11]

The question should be framed in a very utilitarian context: the population, intervention, comparator, and outcomes should all be practicable and available to the person seeking to apply the recommendations arising from the evidence in their particular setting. In particular, the outcome should be important to the patient, owner, or trainer. An irrelevant or trivial outcome yields only low-grade evidence and at best enables recommendations that are weak or ineffectual.

Acquire the Evidence

There are essentially two ways of sourcing evidence with which to answer the question being considered: find evidence that someone else has generated or generate the evidence (i.e., do research) yourself. The latter approach is usually, but not always, restricted to academic institutions or large practices and often has a time frame of months to years. The former approach involves going to the literature to find evidence that someone else has created. This approach can be very efficient, compared with actually doing a research study yourself, but involves finding the relevant literature, evaluating its applicability to your situation, and making an assessment of its quality.

Appraise the Quality of Evidence

Generally, the strength of a recommendation for a particular intervention is strongly correlated to the quality of the evidence used to develop the recommendation.[12] High-quality evidence (as defined later in this chapter) provides clear indications of the magnitude of the effect of the intervention on the key outcome in the population of interest.[10] It should also include high-quality evidence of adverse effects or outcomes. This then allows formulation of strong recommendations. Weak or equivocal evidence or evidence from a population that is not relevant to the question does not provide the confidence to make strong recommendations.

Apply the Evidence to Practice

Taking evidence and converting it to a practice situation is not quite as easy as one might first think. For any particular question there might be a body of evidence, some of it conflicting, and often the body of evidence will have important gaps. These complexities mean that rather than relying on the results of one study (a much less than ideal situation), one must consider a diversity of evidence in formulating an answer to the question. The author's answer, the recommendation, must be a synthesis of the evidence and ideally considers factors other than just the quality of the evidence. For instance, the recommendation should also include consideration of the cost of the intervention, its availability for use in the population in question, the preferences of owners and trainers, and the risk and severity of adverse effects or outcomes.

Systematic reviews of evidence do not, or should not, make recommendations. A process that is additional to evaluating the quality of evidence is needed to make recommendations applicable to clinical practice. This process has been formalized as the GRADE process in which the quality of evidence is considered along with a number of other factors (see later) in arriving at a recommendation about an intervention.[13]

Assessing the Efficacy of the Intervention

A key component of any evidence-based approach to clinical practice is evaluating if the intervention, diagnostic technique, or prophylaxis was effective. This should ideally be done prospectively with metrics that provide evidence of the effect of the intervention on the outcome of interest.

This chapter will consider what represents evidence in veterinary practice, how the quality of this evidence is assessed, and how this evidence can be used to inform the practice.

What Is Evidence and What Is Quality of Evidence?

Evidence is an observation, fact, or organized body of information offered to support or justify inferences or beliefs in the demonstration of some proposition or matter at issue.[14] Evidence evaluated in EBVM is usually in written format and is commonly in the form of a scientific article or report. However, it could be contained in a database, such as online collations of,[15] or in hospital records or similar clinical databases.

Not all evidence has the same usefulness (evidentiary value) in informing our decision, and an important aspect of EBM is deciding on the evidentiary value of evidence in the context in which you need it. High-quality evidence exists in publications that describe studies of high quality and in which we have higher degrees of confidence that the results of the study accurately reflect our reality. High-quality evidence gives confidence that the results are repeatable, provides estimates of the effect of the intervention that are usefully narrow, and is consistent with other high-quality studies addressing the same question. Most important, information of high evidentiary value is relevant to the practice situation and the question we are seeking to answer. For this reason, even the highest quality evidence of efficacy of an intervention in, for example, humans, dogs, or cats, has limited evidentiary value in equids.

Veterinary Scientific Literature

Perhaps the greatest single advance in veterinary medicine has been the increase in the amount of information and, presumably, the proportionate increase in knowledge. This increase in the amount of information is most clearly demonstrated by the 2.3-fold increase in the number of articles published each year in the veterinary scientific literature (from 8815 in 1996 to 20,549 in 2014).[16,17] Not only did the number of articles increase, but so did the number of veterinary journals indexed by Scopus (from 109 in 1996 to 211 in 2014), although this does not include all journals that publish veterinary material.[16-18] These increases are representative of the increase in information available for equine veterinary science, with 123,146 equine-related articles published between 1996 and 2014, rising from 2534 in 1996 to 7722 in 2014.[19] There is clearly an abundance of scientific literature in which we can seek answers to important clinical questions.

The large increase in the volume of information creates some important challenges for practitioners and veterinarians seeking to use this information. These challenges include the following:

- Identifying that the information exists: how does one find out that the relevant publications exist?
- Sourcing the information: having identified that the information exists, how does one then access these publications?
- Evaluating the information: is the information reliable, is it valid, and does it apply to this particular circumstance?
- Using the information: how does one make sense of and apply the information that is related to a particular problem?

Identifying That the Information Exists

The first challenge facing a veterinarian is to locate current information about the topic. There are various ways of doing

so, including sourcing information from colleagues, continuing education events or notes, scientific or clinical journals, textbooks, and Internet sources.[20]

When presented with a difficult case, 44% of veterinarians in the United Kingdom first sought advice from a colleague (not a specialist), and 23% consulted a textbook.[20] Accessing information in publications was less important to veterinarians, with fewer than 5% seeking information in scientific articles. Equine veterinarians in the United Kingdom most frequently read the journal *In Practice* (81%), which publishes only commissioned, peer-reviewed articles; the peer-reviewed *Veterinary Record* (75%) and *Equine Veterinary Education* (43%); and nonpeer-reviewed material in the *Veterinary Times* (79%) and *UK Vet* (49%). This usage is likely a reflection of the availability of these publications, with the *Veterinary Times* and *UK Vet* being free resources, the *Veterinary Record* and *In Practice* included as part of membership of the British Veterinary Association (BVA), and *Equine Veterinary Education* included as part of membership of the British Equine Veterinary Association.[20] Uncertainty about how to identify suitable information through Internet-based searches, limited access to publications, and esoteric content (for practitioners) of many scientific articles could be reasons for limited uptake of information from scientific journals.

Veterinary Literature Database

Until late in the last century, sourcing information in the scientific or peer-reviewed clinical literature was only achieved by physically visiting scholarly libraries and pouring through printed indices (Index Veterinarius), consulting specialized textbooks or tomes, and scouring the index of scientific journals. Sourcing of information has changed markedly with the availability of specialized veterinary electronic databases and almost universal access to the Internet and search engines. However, access to specialized databases and many scientific journals is limited to academics and those who choose and have the financial resources to subscribe to these resources. Although there is greater availability of open access journals, which make the articles they publish available without payment by readers, most veterinarians rely on nonspecialized sources identified using search engines.[20] For instance, 71% of veterinarians in the United Kingdom rely on Google searches as their primary source of electronic veterinary information, although not surprisingly veterinarians in academia prefer PubMed (which is also available to the public).[20] The concern with accessing information from the Internet is that the source, while ostensibly authoritative, might not be so. This is balanced with the ease with which the information can be accessed. Finding the answer will involve using information sources that provide reliable, contemporary, and current information or guidance.

There are a number of databases that cover topics in veterinary medicine and related fields.[21,22] Many of these databases restrict access by requiring a subscription, such as through a university library or institution, and are not readily accessible to veterinarians in practice. However, a number of professional organizations, such as the American College of Veterinary Internal Medicine,[23] have agreements that provide access to these databases by their members. Access to relevant databases by practicing veterinarians is an impediment to use for EBVM in clinical practice outside of universities.[24]

Databases that index journals that publish articles relevant to veterinary medicine include the following[21,22]:

- Agricola: Indexes the agricultural literature including topics such as animal science, cytology, feed science, microbiology, and parasitology. Agricola is open access.[25]
- Biological Abstracts: As the major index for life sciences literature, Biological Abstracts covers bioengineering, biotechnology, microbiology, and other areas of biology.[26]
- CAB Abstracts: Indexes the veterinary medical literature more completely than any other database.[18] CAB Abstracts contains records from more than 8000 journals, conference proceedings, and books. It can be accessed through the following platforms: CAB Direct (Center for Biosciences and Agriculture International [CABI]'s own platform), Dialog, Dimdi, EBSCO, OvidSP, STN International, and Thomson Web of Knowledge. In 2012 CAB Abstracts indexed almost all the principal veterinary journals (98%) and 90% of journals that include any veterinary content.[18,27]
- CONSULTANT: This is a database of animal diseases indexed and searchable by diagnosis or clinical signs. Outcomes of a search include citations of selected scientific articles. CONSULTANT is open access.[28]
- Embase: This an Elsevier product that offers additional tools for compiling systematic reviews. As of 2012 it indexed 85% of the principal veterinary journals and 38% of journals that publish any veterinary content.[18,29]
- Google Scholar: This is a search engine provided by Google that searches the scholarly literature. It, and Google, are frequently used sources of information for veterinary students and veterinarians in practice.[20,30] Google Scholar searches provide different results from PubMed searches in the human literature, often missing the "gray" literature of abstracts and conference proceedings.[31-35] Use of Google Scholar is free, but there is no information about the proportion of veterinary journals that it indexes or locates. Google Scholar is open access.[36]
- MEDLINE: Indexes roughly 100 veterinary medical journals, but overall coverage of veterinary science is mediocre with the database covering approximately 82% of the principal veterinary journals and only 37% of journals that publish any veterinary content.[18] Notable journals not covered by MEDLINE include *Advances in Veterinary Dermatology, Equine Veterinary Education, Veterinary Economics,* and *Veterinary Medicine.*[37]
- PubMed: The overlap between PubMed and MEDLINE is roughly 98% with PubMed including life science journals that submit full text to PubMed Central and additional articles in journals that are selectively indexed by MEDLINE.[21] PubMed Central is a free online archive of the biomedical and life sciences literature.[38]
- Scopus: This database, owned by Elsevier, in 2012 covered almost all the principal veterinary journals (98%) but only 58% of journals that publish any veterinary content.[18,39]
- SPORTDiscus: This is the major database for sports medicine. It covers horse biomechanics, medicine, and physiology.
- VetMed Resource: Produced by CABI Abstracts, this database and resource center includes all CAB Abstracts of [?] veterinary-related records for journals and conference proceedings. The VetMed Resource is marketed to practicing veterinarians and includes an attractive and useful landing page, continuous alerts to recent articles, ready access to full text sources, information about continuing education, and the capacity to create user profiles (as do some other sites).[40]

- VetSRev: This is a database of systematic reviews of the veterinary literature. It is useful for identifying reviews on particular topics.[41]
- Web of Science: Indexes the major journals in biomedicine, the sciences, and engineering including, as of 2012, 93% of principal veterinary journals and 53% of journals that publish any veterinary content.[18,42]
- Wikipedia: This also contains articles that address veterinary topics. The articles are not primary source research articles, do not have an identified single author or identified groups of authors, and are sometimes unreliable or assertions are not verified. The articles are publicly curated.[43]

Searching the Literature

Regardless of the database used, key to identifying relevant literature is formulating an effective search strategy. Briefly, an effective search strategy involves clearly defining the purpose of the search, which can usually be framed in terms of PICO.[22] For instance, searching for articles on the effectiveness of the use of ponazuril compared with pyrimethamine in treatment of horses with clinical signs consistent with equine protozoal myeloencephalitis can be framed as P, equine; I, ponazuril; C, pyrimethamine; and O, protozoal myeloencephalitis. This search yields four articles dealing with the use of ponazuril to treat equine protozoal myeloencephalitis. Further details on effective search strategies in veterinary medicine are available in publications dealing with this important topic.[1,2,7,22,44] Search techniques useful for identifying evidence in human medical literature are not sufficiently sensitive (detect a sufficient proportion of the articles) for use in veterinary medicine.[45]

Sourcing the Information

Accessing information in scientific publications can be challenging for veterinarians who are not associated with universities or similar institutions that have subscriptions to large numbers of veterinary and other journals. Most scientific journals require a subscription to access articles that they publish or they provide access to individual articles at a cost per article (often $25 or more). Other journals charge authors to publish and then make their articles freely available to readers (so-called open access publishing). The availability of journals publishing open access articles of interest to practicing veterinarians is increasing, and a catalog of journals that provide free access to readers is available at the Directory of Open Access Journals.[46]

Journals that publish veterinary topics and provide open access to articles include *Acta Vet Scandinavica, BMC Veterinary Research, Equine Disease Quarterly, Frontiers in Veterinary Science, Irish Veterinary Journal, Journal of Veterinary Internal Medicine, PLoS One, Veterinary Record Open, and Veterinary Research,* among others. Articles that are available through open access can be accessed through searches of databases, including using VetSRev or CONSULTANT. PubMed Central is a repository of open access articles that contains many veterinary articles.[38]

The International Veterinary Information Service (IVIS) is an online publisher of veterinary books and proceedings. The IVIS website currently includes 1673 book chapters and proceeding articles and access is free.[47] It does not publish articles describing primary scientific research. Similarly, the Veterinary Information Network (VIN) indexes more than 150 core veterinary journals and publishes the proceedings of at least 21 veterinary conferences including the American College of Veterinary Internal Medicine (ACVIM) Forum. VIN is a commercial product marketed to practicing veterinarians.[48]

A list of libraries holding collections of veterinary literature is available from Veterinary Medical and Related Libraries: An International Directory (http://vmls.mlanet.org/libraries).

Evaluating the Quality of Evidence

Confidence in an evidence-based approach to veterinary practice depends on our assessment of the quality (evidentiary value) of the available evidence.[49] The higher the quality of evidence, the greater confidence we have in recommendations derived from this evidence. Assessing the quality of evidence is not simple and must involve the consideration of a combination of factors including the design of the study, with some study designs being considered more likely to provide high-quality evidence (e.g., a randomized, controlled trial [RCT] compared with a case report); the rigor of the design, conduct and reporting of individual studies, with studies that are considered likely to provide high-quality evidence being "rated down" because of flaws in the design of the individual study; and the journal or format in which the study is published, with some scientific journals being of higher reputational value than others and, by implication, publishing articles that are of higher evidentiary value.

Grading Quality of Evidence. There are a number of methodologies, and reporting scales, that are used to categorize the quality of evidence. All scales are ordinal; thus, they rank the quality of evidence. A relatively simple but effective series of definitions of varying quality of evidence is provided by GRADE[13]:

- High: Further research is very unlikely to change our confidence in the estimate of the effect (i.e., the magnitude, precision, and direction of the effect of an intervention).
- Moderate: Further research is likely to have an impact on our confidence in the estimate of the effect (magnitude, precision, and direction) and might change the estimate.
- Low: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
- Very low: Any estimate of the effect is very uncertain.

This methodology for ranking the quality of evidence is particularly important in conducting systematic reviews and forming guidelines for practice.

Journal Reputation and Article-Level Metrics. Veterinary scientific journals do not all have the same reputational value; some are considered to commonly publish articles of higher scientific merit. This reputational value has been based on the "impact factor" developed and reported annually by Thomson Reuters as Journal Citation Reports.[50-52] Scopus provides a similar ranking of journal impact factor (journal analytics) using slightly different methods of calculating impact.[53] Impact factor is calculated by dividing the number of citations to articles that were published in a 2-year, 3-year, or 5-year period by a journal by the number of articles published by that journal during a defined period. For example, if a journal received 800 citations to articles it published during a 2-year period, and during that time the journal published 400 articles, the impact factor would be 800/400 = 2. This is the average number of citations per article published in that journal and is considered to be a metric of the influence of the journal; it is not a measure of the impact of individual articles or of each article's evidentiary value. Other, more

sophisticated indices take into account the quality of journals that cite articles in a particular journal (assessing quality of a journal by the company it keeps), whereas others attempt to normalize or account for variations in citation rates across disciplines. The usefulness of these metrics as surrogates for the quality of individual or particular articles in a journal is quite limited.

There are a number of shortcomings in using the impact factor to assess the quality of evidence of individual articles. Importantly, articles in clinical areas in human medicine, and especially those describing therapeutic interventions, receive fewer citations than do articles describing basic biomedical research or diagnostic testing.[54] Citation counts are not evidence of research impact on clinical applications, public health outcomes, or meaningful health outcomes in human medicine, nor do high citation counts provide evidence of a higher quality of research or greater influence.[50] It is likely that a similar situation exists in veterinary medicine and that articles with high clinical utility and providing high quality of evidence for clinically important topics are not always more frequently cited. Furthermore, for articles in veterinary journals there is no association between the number of citations to an article and the level of evidence of the study design of that article.[55] The impact factor ranking of the journal and the number of citations to an individual article are generally not important factors to consider when determining the evidentiary value of an article.

Article level metrics, such as the number of times an article is downloaded or viewed online, the amount of social media comment it attracts, and the number of links to online referencing services provide other metrics that could indicate the influence of an article.[50] However, the link between these article-level metrics and the quality of evidence is unknown; more frequently downloaded or commented on articles might not provide higher quality of evidence.

Appraising the Evidentiary Value of an Individual Article. Articles are considered to have a certain evidentiary value by virtue of their overall study design (see the later section "Study Design and Evidentiary Value"). This initial rating of evidentiary value is based on the assumption that the individual article conforms with the highest standards in terms of conduct and reporting of the study and that the study patients, intervention, comparator, and outcomes are those of interest to the person seeking the evidence. Unfortunately, this is frequently, indeed mostly, not the case, and the evidentiary value of individual articles must be considered in the context of the features of that study. This usually involves decreasing the evidentiary value of an article, although GRADE provides for a mechanism for increasing the value of some very well-conducted and reported observational studies.

Critical appraisal of an article is a systematic process that aims to identify the strengths and weaknesses of the publication.[56] Critical appraisal is the basis for deciding on the use of the article by clinicians. Evaluation of the quality of an individual article often requires some expertise or understanding of experimental design and statistics and the recognition that different study designs are prone to particular weaknesses

TABLE **6.1** **Summary of Strengths and Limitations of Various Types of Studies Included in the Veterinary Literature**

Study Type	Strengths	Limitations
Systematic review or meta-analysis of randomized, controlled trials	Involves use of the full body of literature to estimate the direction and magnitude of effect of potential risk factors, protective factors, and interventions Provides analytic methods to estimate magnitude of effect and degree of certainty	Restricted to combining studies with a similar design and duration of follow-up (meta-analysis) Findings subject to publication bias
Randomized, controlled trials	Allows good control for bias and confounding Provides the strongest evidence for cause-and-effect relationships	Involves restrictive study population and environment that can limit generalizability of results to real-word settings. Allows testing of few variables Often involves a brief follow-up period between interventions and outcome assessment Involves experimentally induced (not naturally occurring) disease in some situations
Retrospective or prospective cohort study	Involves use of real-world population and environment Able to test a large number of risk factors provided the study population is sufficiently large Able to test for effect of uncommon risk factors Allows prolonged follow-up period between factors or exposures and outcomes	Can be prone to selection bias, information bias, and confounding Can be expensive to conduct prospectively because of the long follow-up period between factors or exposures and outcomes Subjects can be lost to follow-up when conducted prospectively Dependent on quality of case records
Case-control study	Involves use of subjects from real-world populations and environments Can be used to investigate risk factors for uncommon diseases while involving few animals Less expensive to conduct than randomized, controlled trials and prospective cohort studies Can generate questions that can be investigated by use of study types with better control for bias and confounding	Highly prone to selection bias, information bias, and confounding Relies on accuracy and completeness of historical records Does not provide evidence for causation because timing of exposure to risk factors is different from that of the outcome of interest Occurrence of risk factors and outcomes is unclear

TABLE **6.1 Summary of Strengths and Limitations of Various Types of Studies Included in the Veterinary Literature—cont'd**

Study Type	Strengths	Limitations
Cross-sectional study	Involves use of subjects from real-world populations and environments Often inexpensive to perform Results can be used to generate hypotheses or objectives that can be investigated in other studies	Can be prone to selection bias, information bias, and confounding Does not provide evidence of causation because chronology of exposure to the risk factor(s) differs to that of the outcome
Case reports or series	Useful for reporting rare diseases or novel potential treatments or interventions Inexpensive Results can generate questions that can be studied in other formats involving larger populations or more controlled study conditions.	A descriptive account of clinical findings Inconsistent format results in variable quality of reporting Does not control for bias or confounding Do not provide evidence of proof; the intervention might not have had the effect that was observed
Induced disease in species of interest	Demonstrates clinical signs and diagnostic features of the disease Enables testing of accuracy of diagnostic tests or efficacy of therapeutic interventions in controlled conditions	Does not replicate full range of conditions occurring in the naturally occurring disease that might affect efficacy of treatment, disease progression, or accuracy of diagnostic tests Expensive to conduct Concerns about animal welfare
Physiologic studies or in vitro studies	Provide understanding of mechanisms of disease Usually high quality with internal validity (good experimental design) Readily reproduced	Do not provide evidence of risk factors or outcomes for naturally occurring disease Require cautious and considered extrapolation to naturally occurring disease
Consensus statement and guidelines	Useful for providing narrative summary of a topic compiled by a panel of experts Provides guidance on topics that are contentious Provides best assessment of available evidence	Variable quality of reporting of methodology; hence, they provide evidence or recommendations of variable quality Expert panel is often self-appointed Subject to group think bias Often do not differentiate between systematic reviews, which should not provide recommendations, and guidelines
Expert opinion	Provides considered opinion of an expert, or self-appointed expert, in the field	Unreliable or unknown evidence base and subject to bias Not subject to peer review or critique by peers

Modified from Larson RL, White BJ. *Importance of the role of the scientific literature in clinical decision making. J Am Vet Med Assoc*. 2015;247:58–64.

(Table 6.1). A series of "filters" can be applied when deciding if an individual article is likely to be useful and are based on the following questions: (1) Is the study valid?, (2) What are the results?, and (3) Are the results of use to me?[57-59]:

1. Decide on the question, preferably using the PICO format, and conduct the literature search.
2. Review each article's title and abstract to decide if the topic of the article is relevant to your question. If the topic is relevant, proceed to the next step; if not, discard the article.
3. Review the results of the study. If correct, will they change your current practice or inform your decision in a meaningful way? If not, discard the article. If so, proceed to the next step.
4. Review the materials and methods for appropriate study design (controls, randomization, interventions, blinding, etc.) and control of bias and confounding factors. If these are not adequate, discard the article. If they are of acceptable standards, proceed to the next step.
5. Review statistical handling and reporting of data. If they are adequate, proceed to the next step.
6. Interpret the findings of the study in the light of your particular clinical context.

The previous six points provide a rudimentary guide to evaluating a single article. More detailed guidelines for appraising the quality of studies are available including a comprehensive set of tool kits and guides from the Critical Appraisal Skills Programme (CASP).[59]

Quality of Reporting of Studies. Fundamental to the evaluation of the quality of the study described in an article is that the details of the study are reported in sufficient detail that the reader can evaluate the validity of the study. This realization has led to the development and use of reporting guidelines. These guidelines, which are tailored to particular study designs, provide advice and direction on optimal reporting of the study. There is evidence in the veterinary literature that inadequate, or poor, reporting of the details of studies is significantly associated with reports of positive outcomes in the study.[60] This result implies that imperfectly or inadequately reported studies are more likely to report positive effects or estimates that are not reliable. Guidelines for reporting are useful to editors, reviewers, and readers in assessing the completeness of reporting and, therefore, the quality of the article and evidence it contains.[61,62] For example, the CONSORT checklist provides a means of assessing quality and completeness of reporting of randomized clinical trials (Table 6.4),[63] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines provide a structure for conducting and reporting systematic reviews (Fig. 6.1).[64]

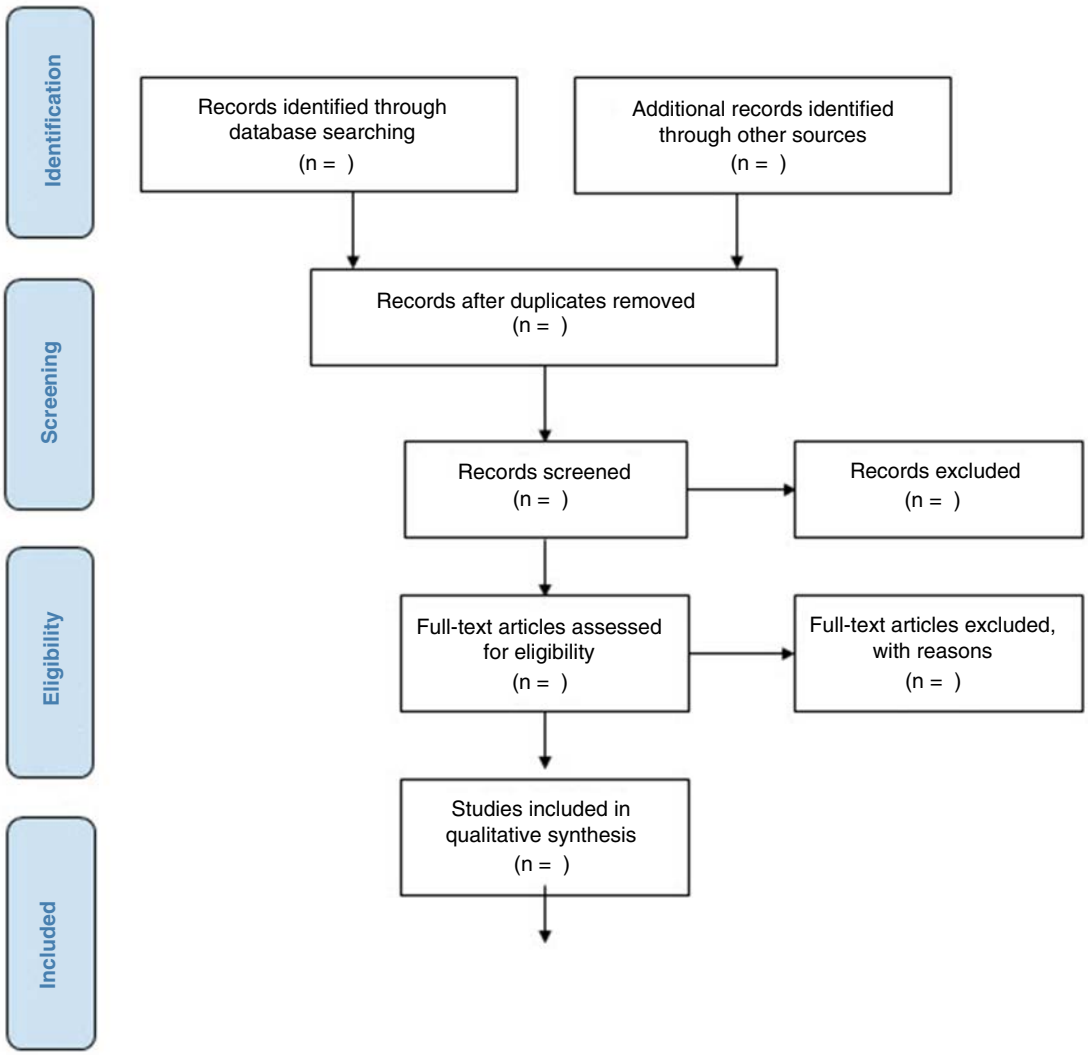


FIG. **6.1** Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram for inclusion of articles in systematic reviews.[74]

There are a large number of reporting guidelines (>317) with a small number tailored for the veterinary literature (REFLECT[65] and STROBE-vet). Guidelines are available at the EQUATOR network site.[66]

Study Design and Evidentiary Value. The design of studies reported in the veterinary literature varies, but they can be grouped broadly into one of nine categories (see Table 6.1). The conventional view is that not all study designs are of equal merit or utility and that the type of study design should be considered when evaluating the quality of the evidence derived from that article. This view, which is held steadfastly by some authorities, provides for a hierarchy of quality of evidence based on overall study design (Table 6.2).[7] Others contend that evidence can be understood not as being ordered by methodology but rather in the context of its use and method of its production.[14] An intermediate view is presented by the GRADE process, which while allocating an *a priori* ranking (high, moderate, low, and very low) based on the design of studies, allows for the quality of evidence of a particular study, or group of studies, to be revised up or down (Table 6.3). For instance, RCTs are initially categorized as "high quality," and observational studies are initially categorized as "low quality."[67] The categorization of an RCT can be decreased by at least one level (to "moderate") if there are particular methodological problems with the RCT and that of an observational study increased by up to two levels (to "high") if the evidence from the observational study is particularly strong. Using the GRADE approach, some observational studies might provide higher quality evidence than do some RCTs.

The Oxford hierarchy of evidence has been modified and revised by numerous authors to fit the veterinary context and can be restated as follows[1,7]:

- Systematic review including meta-analysis
- Systematic review of RCTs
- RCTs performed under field conditions
- Nonrandomized trials, cohort studies, or models of induced disease in the species of interest
- Case-control or cross-sectional studies
- Case reports and case series
- Expert opinion/editorials

TABLE **6.2 Levels of Quality of Evidence**

Level of Evidence	Method
1a	Systematic review (with homogeneity of outcomes and narrow confidence intervals around consistent effects of the intervention) of RCTs
1b	Individual RCT (with narrow confidence interval about the effect of the intervention)
1c	All or none: met when all patients died before the intervention became available, but some now survive on it, or when some patients died before the intervention became available, but none now die on it
2a	Systematic review of cohort studies demonstrating homogeneity of outcomes or effect measures
2b	Individual cohort study or low-quality RCT
2c	"'Outcomes"' research such as audit studies
3a	Systematic review of case-control studies demonstrating homogeneity of outcomes or effect measures
3b	Individual case-control study
4	Case-series or poor quality cohort and case-control studies
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research, or "first principles"

RCT, Randomized, controlled trial.
Modified slightly from the evidence hierarchy of the Oxford Centre For Evidence-Based Medicine. *Anon*. Oxford Centre for Evidence-Based Medicine: levels of evidence (March 2009). http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/; 2016. Accessed 9 May 2017.

TABLE **6.3 Methodology for Assigning the Grade of Quality of Evidence for Individual or Groups of Studies**[13]

A priori evidence grade based on study design:
- Randomized trial = high
- Observational study = low
- All others = very low

Decrease grade of evidence if the following are evident in the study:
- Serious (–1 grade) or very serious (–2) limitations to study quality
- Important inconsistency with other studies (–1)
- Some (–1) or important (–2) uncertainty about directness
- Imprecise or sparse data
- High probability of reporting bias (–1)

Increase evidence grade if
- There is evidence of a strong association–significant relative risk of >2 or <0.5 based on two or more observational studies with no plausible confounding (+1)
- Evidence of very strong association–significant relative risk of >5 or <0.2 based on direct evidence with no major threats to validity (+2)
- An evident dose:response gradient (+1)
- All plausible confounders would have reduced the effect (+1)

Notes: Inconsistency refers to lack of similarity of estimates across a number of studies, which then reduces confidence in estimates of effect for that outcome. Directness refers the extent that patients, interventions, and outcomes are similar to those of interest.[13]

- Animal models of the disease of interest (induced disease in species other than the species in which the disease occurs naturally, e.g., mouse model of a disease in horses)
- In vitro studies with an appropriate control group

The characteristics and evidentiary value of publications in veterinary medicine and veterinary science vary and can be categorized as described in Table 6.1. They can be further differentiated as being of either experimental or observation study designs.[7,68]

Observational Studies

Observational studies are those in which there is no intervention by the investigators. Data are obtained by simply observing and recording events without any attempt to impose interventions that could alter the course of events. There is no experimental aspect to observational studies in that there is no testing of a hypothesis. These studies are solely based on observing details about a case or series of cases, or groups of animals, and summarizing associations among variables. Observational studies can be descriptive or analytic.

Descriptive studies are useful in providing information on the presentation of disease including such information as clinical signs, outcomes, or simply that the disease occurs. They do so by observing events and recording facts, often in a retrospective fashion using medical or other case records or, less frequently, in prospective studies. Observational descriptive studies do not include numeric or statistical analysis and cannot report on formal assessments of risk factors or effects of interventions. Examples of descriptive studies include case reports and case series and some types of cross-sectional studies.

Descriptive Observational Studies (Case Reports and Case Series). *Case reports* provide a detailed description of a small number (usually 1–3) of cases with no attempt to formally identify common characteristics of the cases beyond one factor (such as the diagnosis or presenting signs). Reports of a series of cases (usually >3) can attempt to subjectively identify common characteristics, such as breed, sex, predisposition to the disease, or outcome, in a series of nonrandomly selected cases. These reports can be useful in identifying new diseases or rare adverse effects with treatments but without providing definitive evidence of these associations.

The shortcomings of observational descriptive studies are the high risk of selection bias or confounding caused by the nonrandom availability of cases and case records. Selection bias occurs because the investigators cannot take measures to increase the likelihood that cases included in the study reflect the larger population of cases. For instance, reports of outcomes of cases treated at a referral hospital might have little similarity to outcomes of cases of the same disease treated in ambulatory practice. The quality of observational studies can also be impaired by the quality of record keeping, especially in retrospective studies, and the often high frequency of missing data. Results of case reports or case series are usually not generalizable to a larger, or different, population of animals and have limited external validity. Indeed, the characteristics of the case or cases might not be representative of the more common or important manifestations or outcomes of the disease.

TABLE **6.4 CONSORT Checklist for Reporting of Randomized, Controlled Clinical Trials**[63]

Section/Topic	Number	Checklist Item	Reported on Page Number
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable, background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; and systematic review registration number	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., web address), and, if available, provide registration information including registration number	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level) and how this information is to be used in any data synthesis	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means)	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies)	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, metaregression), if done, indicating which were prespecified	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period), and provide the citations	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see Item 12)	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study (a) simple summary data for each intervention group, (b) effect estimates and confidence intervals, ideally with a forest plot	
Synthesis of results	21	Present the main results of the review If meta-analyses are done, include for each confidence intervals and measures of consistency	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15)	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, metaregression [see Item 16])	

TABLE **6.4 CONSORT Checklist for Reporting of Randomized, Controlled Clinical Trials[63]—cont'd**

Section/Topic	Number	Checklist Item	Reported on Page Number
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias) and at review level (e.g., incomplete retrieval of identified research, reporting bias)	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence and implications for future research	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data) and role of funders for the systematic review	

A particular type of observational study is a cross-sectional description of cases in a population at a single point in time. These studies provide a one point in time estimate of prevalence and can attempt to identify risk factors if both cases and controls are included in the study; however, there is considerable risk of selection bias and of confounding (in which a third, perhaps unidentified, variable influences the effect of another variable on the outcome of interest). Cross-sectional studies can be inexpensive to undertake. Surveys are a form of cross-sectional study.[7]

Analytic Observational Studies. This type of study design is often useful when studying naturally occurring diseases. Common designs in veterinary medicine are cohort and case-control studies.[7]

A *cohort study* is a type of longitudinal study that is commonly used to study associations between naturally occurring exposure to a putative risk factor for disease and subsequent development of disease. Another variation is to look at responses to different therapeutic interventions. In either case, an initial population of animals is identified and then animals in that population are categorized as exposed to the particular risk factor of interest or not and their progression to disease determined. Similarly, a population of animals with a particular disease might be divided in two or more groups, each of which receives a different treatment. The outcomes of each group are then compared. Cohort studies can be retrospective or prospective.

Prospective cohort studies can require long time periods to complete if there is a delay between exposure and appearance of disease. Retrospective cohort studies are relatively inexpensive to conduct because they use already collected data, often in the form of medical records. However, a disadvantage of retrospective cohort studies is that the data were collected for purposes other than research; thus, information relevant to the study may not have been recorded. Cohort studies do not permit blinding, and matching of animals in each group of factors other than the risk factor of interest can be difficult. Prospective cohort study results should be reported as relative risk, and retrospective cohort studies should be reported as odds ratios.

The *case-control study* is retrospective in that cases are identified and then matched with animals that did not develop the disease. The records of each group are then examined to identify risk factors that differ between cases and controls. Case-control studies are relatively inexpensive and easy to perform, but they do not usually allow for control of confounding variables because of the difficulty in closely matching cases and controls. As with other retrospective observational studies, data quality can be limiting.

Experimental Studies

Experimental study designs are those in which the investigator chooses the animals being studied and assigns the intervention to a proportion of the study population. By manipulating one variable, the aim is to establish a causal relationship by measuring the outcome or dependent variable. Typically, experimental studies involve a group of animals to which the new intervention is applied, a control group that might receive an alternative intervention, or no active intervention (placebo) with random assignment of animals to each group. This design attempts to minimize the effect of known or unknown factors, other than that of the interventions, on the outcome. The best justification for placing RCTs at the top of evidence hierarchies for individual studies is that RCTs minimize certain forms of bias, especially selection bias, therefore producing the best evidence of a treatment effect or of causation.

RCTs in equids can be conducted under laboratory conditions, usually with induced disease, or as clinical trials using animals with naturally occurring disease.[7] When evaluating an RCT for quality of evidence, it is important to consider the relevance of the intervention, the population being studied (especially if the study uses induced rather than naturally occurring disease), and the setting (field service versus referral clinic). RCTs using experimental animals are efficient and effective to perform and provide for strict control of potentially confounding factors. Such experimental trials might not mimic or replicate the condition or disease in the field, and care should be taken extrapolating results of these studies to animals with naturally occurring disease. Conversely, RCTs conducted in the field are often highly relevant to animals with naturally occurring disease but are more challenging to perform than laboratory studies.[7]

RCTs should include blinding (masking) of those investigators who determine which treatment an animal receives, of those administering the treatments, and those evaluating the outcomes of the intervention to minimize the likelihood of conscious or unconscious bias.

RCTs are also parsimoniously powered to get answers related to the efficacy of the intervention on the outcome, which is usually treatment of a disease. As a result, RCTs and meta-analyses that use them are often underpowered to detect infrequent or rare adverse effects.[69]

Synthesis of Evidence: Reviews

The wealth and volume of veterinary scientific literature have both prompted and enabled individuals or groups to attempt to provide a considered synthesis of information on a particular topic. The purpose of such reviews is to take the primary literature and create a generalizable understanding of the disease or intervention on the assumption that compiling information from a number of studies will provide more reliable information than that from one study alone. Although there are at least 14 forms of review identified in the library literature, most reviews in the veterinary literature are narrative (integrative) or systematic (aggregative) reviews or meta-analyses.[70,71] The most common form of this information synthesis in veterinary medical literature is the narrative (integrative) review. Detailed descriptions of these types of reviews, including strengths and weaknesses, are available elsewhere.[70]

Narrative Review. Narrative, or otherwise known as integrative, reviews seek to bring together the literature on a particular topic, not with the intention of answering a specific question or questions but rather to provide a commentary on the results of the available primary studies on the topic. These types of reviews, which are typical of textbooks or review articles in journals, are often useful summaries of a body of literature. Such reviews do not typically include an explicit methodology, such as how the literature search was conducted, how evidence was appraised, and how articles were included or excluded. As such, narrative reviews might not consider all the available literature on a topic. Lack of a predetermined methodology also increases the possibility of conscious or unconscious bias in the inclusion of primary studies in the review or the interpretation of results. Authors of narrative reviews are often self-selected and might, or might not, be expert in the topic of the review. The author's reasons for writing the review might not be revealed to readers.

Systematic Reviews. The highest quality evidence is provided by systematic reviews, which might include a meta-analysis. Systematic reviews differ from narrative reviews, which have much lower evidentiary value, in that systematic reviews are approached in a manner and with methodology designed to ensure the validity of the conclusions.[70] Systematic reviews should be based on a clearly defined question and prespecified criteria for inclusion and evaluation of the literature, among other factors. Criteria and methodology for performing systematic reviews are available.[44,72,73] The PRISMA statement provides a widely, although not universally, used methodology for reporting systematic reviews, including a recommended flowchart and reporting checklist (see Fig. 6.1).[64,74,75]

Systematic reviews should be methodologically rigorous assessments of the literature and should not make recommendations on clinical practice.[12] The fundamental characteristics of systematic reviews are as follows[76]:

1. A clearly stated set of objectives with predefined eligibility criteria for inclusion of studies
2. An explicit and reproducible methodology
3. Use of a systematic and thorough search that attempts to identify all reports of studies that meet with eligibility criteria
4. A systematic assessment of the validity of the findings of studies that are included in the review
5. A systematic assessment of the characteristics and findings of the included studies and a systematic presentation of those findings

A systematic review therefore provides a dispassionate assessment of the published evidence on an issue. It makes an assessment about the quality of evidence and, in doing so, enables readers to weigh the use of the evidence as they decide on the answer to their clinical question. Systematic reviews consider only the quality of the evidence and do not provide a methodology for taking the next step, which is that of making a recommendation on use of the evidence.

From Evidence to Recommendation: Practice Guidelines

The approach of using evidence to guide clinical decision making has been formalized in the past two decades in human medicine and is gaining traction in veterinary practice. As veterinary clinicians we have ethical and legal obligations to use methods and practices that are most likely to provide the "best" outcomes for the animals we treat and their owners. A traditional approach to deciding on the "best" treatments, diagnostic tests or methods, and preventive measures has been to identify the highest quality evidence of effectiveness and to adopt the approach with the strongest evidence of efficacy. The Cochrane collaboration and the Cochrane reviews exemplify and lead this approach in human medicine.[76]

This "evidence-based" approach has the implicit assumption that one should rely on the highest quality evidence and that high-quality evidence of efficacy necessarily leads to the adoption of that treatment, diagnostic test, or prophylaxis. However, this approach falls short when formulating recommendations for use in clinical practice. What practitioners need are recommendations that are based on the available evidence but that also take into account the other factors that must be considered when advising an owner or trainer on the "best" approach to dealing with their animal's (and their) problem. Clinical practice guidelines are statements that include recommendations intended to optimize patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options.[77] Importantly, development of practice guidelines (recommendations) includes both a systematic review and consideration of the trade-offs between benefits and harms.[77]

Although an analysis of the quantitative literature, such as RCTs, is important, it is also important to consider the literature describing more qualitative outcomes. This literature can be evaluated according to four criteria, each of which has five grades (Table 6.5). The criteria are feasibility, appropriateness, meaningfulness, and effectiveness.[78]

The recognition that systematic reviews of the literature are an essential but not the sole, or best, means for making recommendations for interventions resulted in the development of the GRADE process.[79] GRADE works by providing a framework to determine a final recommendation on an intervention through use of the following:

1. Quality of evidence (Cochrane and similar evaluations of quality of evidence stop here)
2. Seriousness of the outcome
3. Magnitude of the treatment effect
4. Precision of the treatment effect
5. Risk of the target event (how frequent)
6. Risk of adverse events associated with the intervention
7. Cost of the intervention
8. Values and preferences of the end users

TABLE **6.5 Example of Elements of Qualitative Assessment and Review Instrument Levels of Applicability of Evidence**

Feasibility	Appropriateness	Meaningfulness	Effectiveness
Immediately practicable	Acceptable and justifiable and within ethical guidelines	Provides a rationale for practice development	Effectiveness established to a degree that merits application in clinical practice
Practicable with limited local training or modest additional resources	Acceptable after minor revision	Provides a rationale for local, regional, or national reform	Effectiveness established to a degree that suggests application in some clinical situations
Practicable with extensive additional training or resources	Acceptable after major revision	Provides a rationale for practice-relevant research	Effectiveness established to a degree that warrants consideration of applying the findings
Practicable with significant national or regulatory reforms	Acceptable after development of new ethical guidelines	Provides a rationale for advocating change	Effectiveness established to a limited degree and benefits are unclear
Impracticable to practitioners	Ethically unacceptable	Evidence unlikely to make sense	Effectiveness not established

Modified from Pearson A. Balancing the evidence: incorporating the synthesis of qualitative data into systematic reviews. *JBI Rep*. 2004;2:45–64.

All of these criteria have at least some applicability in veterinary medicine. Briefly, judgments about the quality of recommendations require consideration of the following:

- The quality of evidence on which the recommendation is based: The quality of evidence is assessed on the type of study imprecision of the results over a number of studies, inconsistency of the studies, indirectness, reporting bias, magnitude of the effect, biologic plausibility, and strength of association.[67,80-83] Systematic reviews of the literature are fundamental to the development of appropriate recommendations.
- The balance between benefits and harms: Will the intervention do more good than harm? What is the extent of the benefit and of the potential harm?
- Feasibility of translating the evidence into the circumstance in which the intervention will be made: Can I apply this in my practice? Is it affordable?
- Certainty of the baseline risk (i.e., How important is the problem?)
- Cost (both monetary and in terms of resources)

The balance between benefit and harm (the trade-off) can be categorized as follows:

- Net benefits: The intervention clearly does more good than harm.
- Trade-off: There are important trade-offs between the benefits and harms.
- Uncertain trade-offs: It is unclear whether the intervention does more good than harm.
- No net benefits: The intervention clearly does more harm than benefit.

Finally, these considerations can be distilled down to the following recommendations being "a judgment that most well-informed people would make"[12]:

- "Do it": There is high-quality evidence of net benefits within appropriate resource constraints (costs) for a problem that has significant importance (a judgment that most well-informed people would make).
- "Probably do it": When the strength of evidence is moderate or when the benefit:harm trade-off is unclear or marginal.
- "Probably do not do it": When the strength of evidence is low or very low, when the benefit:harm trade-off is unclear or marginal, or when the baseline risk is low.
- "Do not do it": There is high-quality evidence of harm clearly exceeding benefits, the cost is too great compared with benefits, or the baseline risk is very low (i.e., the problem is not important).

The GRADE guidelines, although not well established for veterinary medicine, have been used and provide the opportunity to make evidence-based recommendations to practitioners.[84]

CONCLUSIONS

The techniques underlying EBVM are useful as we seek answers to problems in equine medicine. The techniques allow us to ask the relevant question, seek out the information, and assess its quality. We can then decide how best to apply this information to the problem. This final step involves more than just an evaluation of the quality of evidence; it requires that we consider a number of other factors in arriving at a recommendation and deciding on the strength of that recommendation. This final step is crucial to the sensible application of the principles of EBVM.

REFERENCES

1. Anon. *Evidence-based veterinary medicine*. Nottingham, UK: University of Nottingham.
2. Cockcroft P, Holmes M. Evidence-based veterinary medicine 2. Identifying information needs and finding the evidence. *In Practice*. 2004;26:96–102.
3. Holmes M, Cockcroft P. Evidence-based veterinary medicine 3. Appraising the evidence. *In Practice*. 2004;26:154–164.
4. Holmes M, Cockcroft P. Evidence-based veterinary medicine 1. Why is it important and what skills are needed? *In Practice*. 2004;26:28–33.
5. Malynicz G. Evidence-based medicine. *Vet Rec*. 1998 Nov 28; 143(22):619.
6. More SJ. Evidence is at the core of scientific method: a challenge for clinicians. *Vet J*. 2012;191:11–12.
7. Cockcroft P, Holmes MA. *Handbook of evidence-based veterinary medicine*. Oxford: Blackwell Publishing; 2003.
8. Guyatt GH, Oxman AD, Schuenemann HJ, et al. GRADE guidelines: a new series of articles in the *Journal of Clinical Epidemiology*. *Journal of Clinical Epidemiology*. 2011;64:380–382.
9. Djulbegovic B, Kumar A, Kaufman RM, et al. Quality of evidence is a key determinant for making a strong GRADE guidelines recommendation. *Journal of Clinical Epidemiology*. 2015;68: 727–732.

10. Larson RL, White BJ. First steps to efficient use of the scientific literature in veterinary practice. *Javma-Journal of the American Veterinary Medical Association*. 2015;247:254–258.
11. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 2. Framing the question and deciding on important outcomes. *Journal of Clinical Epidemiology*. 2011;64:395–400.
12. Guyatt GH, Oxman AD, Kunz R, et al. GRADE: going from evidence to recommendations. *British Medical Journal*. 2008;336:1049–1051.
13. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. *British Medical Journal*. 2004;328:1490–1494.
14. Upshur REG, VanDenKerkhof EG, Goel V. Meaning and measurement: an inclusive model of evidence in health care. *Journal of Evaluation in Clinical Practice*. 2001;7:91–96.
15. Nicholas F, Online Mendelian Inheritance in Animals (OMIA). *Online Mendelian Inheritance in Animals (OMIA)*. Faculty of Veterinary Science, University of Sydney; 2012.
16. Christopher MM, Marusic A. Geographic trends in research output and citations in veterinary medicine: insight into global research capacity, species specialization, and interdisciplinary relationships. *BMC Veterinary Research*. 2013:9.
17. Anon. SJR–SCImago Journal & Country Rank: SCImago. (2007), 2016.
18. Grindlay DJC, Brennan ML, Dean RS. Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. *Journal of Veterinary Medical Education*. 2012;39:404–412.
19. Anon. Web of Science: Thomson Reuters, 2016.
20. Nielsen TD, Dean RS, Massey A, et al. Survey of the UK veterinary profession 2: sources of information used by veterinarians. *Veterinary Record*. 2015 Aug 15;177(7):172.
21. Buchanan RA, Wooldridge AA. Staying current by searching the veterinary literature. *Journal of Veterinary Medical Education*. 2011;38:10–15.
22. Murphy SA. Searching for veterinary evidence: strategies and resources for locating clinical research. *Veterinary Clinics of North America-Small Animal Practice*. 2007;37:433ff.
23. ACVIM. Access to information: American College of Veterinary Internal Medicine. http://www.acvim.org/Diplomates/Membership; 2016.
24. Vandeweerd JM, Kirschvink N, Clegg P, et al. Is evidence-based medicine so evident in veterinary research and practice? History, obstacles and perspectives. *Veterinary Journal*. 2012;191:28–34.
25. AGRICOLA: https://www.ebscohost.com/academic/agricola; 2016.
26. Biological Abstracts: http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/biosis-citation-index.html; 2016.
27. CABI Abstracts: www.cabi.org/publishing-products/online-information-resources/cab-abstracts/; 2016.
28. White ME. CONSULTANT, 2016.
29. Embase: www.elsevier.com/solutions/embase-biomedical-research; 2016.
30. Weiner SA, Stephens G, Nour AYM. Information-seeking behaviors of first-semester veterinary students: a preliminary report. *Journal of Veterinary Medical Education*. 2011;38:21–32.
31. Anders ME, Evans DP. Comparison of PubMed and Google Scholar literature searches. *Respiratory Care*. 2010;55:578–583.
32. Boeker M, Vach W, Motschall E. Google Scholar as replacement for systematic literature searches: good relative recall and precision are not enough. *BMC Medical Research Methodology*. 2013:13.
33. Haddaway NR, Collins AM, Coughlin D, Kirk S, et al. The role of Google Scholar in evidence reviews and its applicability to Grey literature searching. *PLoS One*. 2015 Sep 17;10(9).
34. Nourbakhsh E, Nugent R, Wang HL, et al. Medical literature searches: a comparison of PubMed and Google Scholar. *Health Information and Libraries Journal*. 2012;29:214–222.
35. Shariff SZ, Bejaimal SAD, Sontrop JM, et al. Retrieving clinical evidence: a comparison of PubMed and Google Scholar for quick clinical searches. *Journal of Medical Internet Research*. 2013 Aug 15;15(8).
36. Google Scholar: https://scholar.google.com; 2016.
37. MEDLINE: https://www.nlm.nih.gov/bsd/pmresources.html; 2016.
38. Pubmed Central: https://www.ncbi.nlm.nih.gov/pmc/; 2016.
39. Scopus: https://www.elsevier.com/solutions/scopus; 2016.
40. VetMed Resource: www.cabi.org/vetmedresource/; 2016.
41. VetSRev: http://webapps.nottingham.ac.uk/refbase/index.php; 2016.
42. Web of Science: http://thomsonreuters.com/en/products-services/scholarly-scientific-research/scholarly-search-and-discovery/web-of-science.html; 2016.
43. Wikipedia: https://www.wikipedia.org; 2016.
44. O'Connor AM, Anderson KM, Goodell CK, et al. Conducting systematic reviews of intervention questions I: writing the review protocol, formulating the question and searching the literature. *Zoonoses and Public Health*. 2014;61:28–38.
45. Murphy SA. Research methodology search filters: are they effective for locating research for evidence-based veterinary medicine in PubMed? *Journal of the Medical Library Association*. 2003;91:484–489.
46. Directory of Open Access Journals: https://doaj.org; 2016.
47. International Veterinary Information Service: www.ivis.org/home.asp; 2016.
48. Anon. Veterinary Information Network. http://www.vin.com; 2016.
49. Sargeant JM, Kelton DF, O'Connor AM. Study designs and systematic reviews of interventions: building evidence across study designs. *Zoonoses and Public Health*. 2014;61:10–17.
50. Carpenter CR, Cone DC, Sarli CC. Using publication metrics to highlight academic productivity and research impact. *Academic Emergency Medicine*. 2014;21:1160–1172.
51. Neylon C, Wu S. Article-level metrics and the evolution of scientific impact. *PLoS Biol*. 2009 Nov;7(11).
52. Christopher MM. Weighing the impact (factor) of publishing in veterinary journals. *Journal of Veterinary Cardiology*. 2015;17:77–82.
53. Lab S. Scimago Journal and Country Rank: SCOPUS, 2016.
54. van Eck NJ, Waltman L, van Raan AFJ, et al. Citation analysis may severely underestimate the impact of clinical research as compared to basic research. *PLoS One*. 2013 Apr 24;8(4).
55. Giuffrida MA, Brown DC. Association between article citation rate and level of evidence in the companion animal literature. *journal of veterinary internal medicine*. 2012;26:252–258.
56. Young JM, Solomon MJ. How to critically appraise an article. *Nature Clinical Practice Gastroenterology & Hepatology*. 2009;6:82–91.
57. White BJ, Larson RL. Systematic evaluation of scientific research for appropriateness of data analysis to improve clinical decision making. *Javma-Journal of the American Veterinary Medical Association*. 2015;247:759–762.
58. White BJ, Larson RL. Systematic evaluation of scientific research for clinical relevance and control of bias to improve clinical decision making. *Javma-Journal of the American Veterinary Medical Association*. 2015;247:496–500.
59. CASP. Critical Appraisal Skills Programme. http://www.casp-uk.net/; 2013.
60. Sargeant JM, Thompson A, Valcour J, et al. Quality of reporting of clinical trials of dogs and cats and associations with treatment effects. *Journal of Veterinary Internal Medicine*. 2010;24:44–50.
61. Grindlay D. Reporting guidelines: how can they be implemented by veterinary journals? *Equine Veterinary Journal*. 2015;47:133–134.
62. Grindlay DJ, Dean RS, Christopher MM, Brennan ML. A survey of the awareness, knowledge, policies and views of veterinary journal editors-in-chief on reporting guidelines for publication of research. *BMC Vet Res*. 2014 Jan 10;10:10.
63. Schulz KF, Altman DG, Moher D, et al. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Journal of Clinical Epidemiology*. 2010;63:834–840.

64. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med*. 2009 Jul 21;6(7).
65. O'Connor AM, Sargeant JM, Gardner IA, et al. The REFLECT statement: methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. *Journal of Veterinary Internal Medicine*. 2010;24:57–64.
66. Network E. EQUATOR network: enhancing the quality and transparency of health research, www.equator-network.org; 2016.
67. Guyatt GH, Oxman AD, Sultan S, et al. GRADE guidelines: 9. Rating up the quality of evidence. *Journal of Clinical Epidemiology*. 2011;64:1311–1316.
68. Institute TJB. *Joanna Briggs Institute reviewers' manual: 2014 edition*. The University of Adelaide, South Australia 5005: The Joanna Briggs Institute; 2014.
69. Upshur REG. Looking for rules in a world of exceptions–reflections on evidence-based practice. *Perspectives in Biology and Medicine*. 2005;48:477–489.
70. O'Connor A, Sargeant J. Research synthesis in veterinary science: Narrative reviews, systematic reviews and meta-analysis. *Veterinary Journal*. 2015;206:261–267.
71. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. *Health Information and Libraries Journal*. 2009;26:91–108.
72. O'Connor AM, Sargeant JM, Wang C. Conducting systematic reviews of intervention questions III: synthesizing data from intervention studies using meta-analysis. *Zoonoses and Public Health*. 2014;61:52–63.
73. Sargeant JM, O'Connor AM. Conducting systematic reviews of intervention questions II: relevance screening, data extraction, assessing risk of bias, presenting the results and interpreting the findings. *Zoonoses and Public Health*. 2014;61:39–51.
74. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009 Jul 21;6(7).
75. Tao KM, Li XQ, Zhou QH, Moher D, Ling CQ, Yu WF, et al. From QUOROM to PRISMA: A survey of high-impact medical journals' instructions to authors and a review of systematic reviews in anesthesia literature. *PLoS One*. 2011;6(11).
76. Higgins JPT, Green S. *Cochrane handbook for systematic reviews of interventions*. 1st ed. Hoboken: Wiley; 2008.
77. Sox HC. Do clinical guidelines still make sense? *Yes. The Annals of Family Medicine*. 2014;12:200–201.
78. Pearson A. Balancing the evidence: incorporating the synthesis of qualitative data into systematic reviews. *JBI REPORTS*. 2004;2:45–64.
79. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. *Journal of Clinical Epidemiology*. 2011;64: 383–394.
80. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines 6. Rating the quality of evidence—imprecision. *Journal of Clinical Epidemiology*. 2011;64:1283–1293.
81. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 8. Rating the quality of evidence—indirectness. *Journal of Clinical Epidemiology*. 2011;64:1303–1310.
82. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 7. Rating the quality of evidence—inconsistency. *Journal of Clinical Epidemiology*. 2011;64:1294–1302.
83. Guyatt GH, Oxman AD, Vist G, et al. GRADE guidelines: 4. Rating the quality of evidence—study limitations (risk of bias). *Journal of Clinical Epidemiology*. 2011;64:407–415.
84. Hinchcliff KW, Couetil LL, Knight PK, et al. Exercise induced pulmonary hemorrhage in horses: American College of Veterinary Internal Medicine consensus statement. *Journal of Veterinary Internal Medicine*. 2015;29:743–758.

CHAPTER 7

Clinical Approach to Commonly Encountered Problems

Melissa T. Hines*

CHANGES IN BODY TEMPERATURE

Assessment of body temperature is an essential part of every physical examination. As with all mammalian species, horses normally maintain their core body temperatures within a narrow range despite extremes in environmental conditions.[1,2] The core temperatures may vary by approximately 1°C (2°F) between individuals. In adult horses the normal body temperature ranges from 37.2° to 38.3°C (99.0°–101.0°F), whereas in neonatal foals the temperature tends to be slightly higher, ranging from 37.8° to 38.9°C (100.0°–102.0°F). Diurnal variation of up to 1°C (2°F) may occur, with the low point typically occurring in the morning and the peak in the late afternoon.

Mechanisms Controlling Body Temperature

The set-point, which is the crucial temperature that the body attempts to maintain, is regulated primarily via neuronal control operating through temperature centers in the hypothalamus.[3-5] Both peripheral and central thermoreceptors sense changes in ambient and core body temperatures and activate feedback mechanisms that bring the temperature back to the set-point. Specifically, peripheral thermoreceptors, which are generally most sensitive to low temperatures, are located in the skin and around certain great veins, as well as in some deep tissues, such as the spinal cord and abdominal viscera. Central thermoreceptors include large numbers of heat-sensitive neurons and lower numbers of cold-sensitive neurons in the preoptic area of the anterior hypothalamus (POA). In response to changes in temperature, the peripheral and central thermoreceptors transmit signals into the posterior hypothalamic area, subsequently activating autonomic and behavioral effector responses to regulate body temperature. These responses affect the balance between heat loss and heat production.

There are multiple mechanisms for cooling in response to elevations in body temperature, which include both means of increasing heat loss and decreasing heat production.[3-5] One means of increasing heat loss is by transferring heat from the body core to the surface by increasing blood flow to the skin. Changes in core body temperature and environmental temperature cause the sympathetic nervous system to regulate the degree of vasoconstriction and thus the amount of blood flow, with increases in temperature resulting in cutaneous vasodilation and increased skin blood flow. Heat is lost from body surfaces to the surroundings by several physical mechanisms, including radiation, conduction, and convection. Evaporation is also an important mechanism of heat loss in horses.[5] To some extent, the amount of evaporative heat loss is controlled by the rate of sweating. However, even when the animal is not sweating, water evaporates insensibly from the skin and lungs, resulting in continual heat loss. In horses, evaporative heat loss, primarily through increased sweating but also through increased respiration, becomes more important as the ambient temperature rises and during exercise.[5,6] In addition to increased heat loss when the body temperature rises, the horse also decreases temperature further by inhibiting means of heat production, such as shivering, and by behavioral responses, such as seeking shade and wind currents and wading into water.[6-8]

Mechanisms that increase body temperature are triggered when the body temperature is too low.[2] Heat is conserved by stimulation of the posterior hypothalamic sympathetic centers, leading to cutaneous vasoconstriction and piloerection. Heat production also increases and may occur through increased muscle activity ranging from inapparent contractions to generalized shivering. Shivering may increase heat production by 4 to 5 times baseline. The primary motor center for shivering is in the posterior hypothalamus, which normally is stimulated by cold signals from the peripheral receptors and to some extent central receptors in the POA. Digestion of food also contributes to total body heat. Sympathetic stimulation may increase the rate of cellular metabolism, increasing heat production by chemical thermogenesis. Cooling also increases the production of thyrotropin-releasing hormone, ultimately increasing thyroid hormones and cellular metabolism and further contributing to chemical thermogenesis. In addition to these physiologic adaptations, behavioral responses to conserve heat also occur, such as adopting a huddled stance, aggregating in groups, and seeking shelter.[9-11] It has been demonstrated that horses voluntarily select shelter, especially under wet, windy conditions and that shelter selection is affected by breed, body condition score, and hair coat weight.[9]

Increased Body Temperature: Hyperthermia and Fever

Elevation of the body temperature above normal is one of the most common clinical problems encountered, and although

*The editors and authors acknowledge and appreciate the contributions of Bonnie R. Rush, Kenneth W. Hinchcliff, and Siddra A. Hinesas as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

classically associated with infection, a variety of disorders may cause increased body temperature.[3-5] Veterinarians should distinguish between conditions of hyperthermia, in which the temperature set-point is unaltered, and true fever, in which the set-point actually increases.

Hyperthermia

Mechanisms of Hyperthermia. The body temperature may become elevated without an increase in the set-point when there is a loss of equilibrium in the heat balance equation.[4] Increased heat production or absorption of heat beyond the ability of the body to dissipate heat may occur. In some conditions impaired heat loss also may occur.

Conditions Associated with Hyperthermia. Hyperthermic conditions include problems such as exercise-related hyperthermia, heat stroke, anhidrosis, malignant hyperthermia, central nervous system disorders, and reactions to certain toxins or drugs (Box 7.1). In general, these conditions do not respond to treatment with antipyretic drugs.

Exercise-Related Hyperthermia. During sustained or high-intensity exercise, increased heat production is associated with muscular activity.[5,6,12,13] The heat produced may exceed the ability of the body to lose heat, resulting in an increased core body temperature. Typically, the temperature returns to normal with rest as heat loss mechanisms remain activated. There is some evidence that ageing compromises the ability of horses to thermoregulate during exercise.[14] Elevated temperature also may occur with the intense muscle activity associated with generalized seizures.

BOX 7.1 Causes of Changes in Body Temperature

HYPERTHERMIA

Exercise-related hyperthermia
Heat stroke
Anhidrosis
Malignant hyperthermia
Central nervous system disorders
Toxins or drugs
Macrolide-induced hyperthermia

FEVER

Infection
Neoplasia
Immune-mediated disease
Other
- Toxic hepatopathy
- Inflammatory bowel disease
- Other

HYPOTHERMIA

Accidental Hypothermia

Exposure to harsh environmental conditions
Surgical procedures/anesthesia

Pathologic Hypothermia

Sepsis/inflammation (maladaptive response)
Intracranial disease
Hypothyroidism (neonates)

Heat Stroke. Heat stroke occurs when the body temperature rises above a critical temperature, leading to multisystemic problems. In horses signs of heat stroke may develop when the body temperature is above 41.5°C (107°F), which most often occurs in association with exercise in environmentally stressful conditions.[13,15] Although horses can acclimatize to various weather conditions to some extent, the efficiency of evaporative heat loss may be compromised significantly in hot, humid weather.[6,12,13,15] Susceptibility to heat stroke may increase if sweating leads to dehydration and electrolyte imbalances. Once the body temperature reaches the critical point, the homeostatic mechanisms of thermoregulation fail, resulting in peripheral vasoconstriction, decreased cardiac output, and decreased blood pressure. Affected horses are lethargic with weak, flaccid muscles. Prostration, circulatory shock, disseminated intravascular coagulation, multiple organ failure, and death may occur.

Anhidrosis. Anhidrosis is an inappropriate response to prolonged climatic stress characterized by a partial or total loss of the ability to sweat.[16-18] This condition occurs primarily in horses living in hot, humid environments and has been documented in about 2% to 6% of horses living in Florida.[16,19] The impaired sweating results in diminished heat loss, frequently resulting in hyperthermia. Other clinical signs include tachypnea, poor performance, and poor hair coat.

Malignant Hyperthermia. Malignant hyperthermia (MH) encompasses a group of inherited skeletal muscle disorders in which calcium metabolism is altered. Although the condition is most common in human beings and pigs, it has been reported in several species, including dogs and horses.[20-23] MH is characterized by a hypermetabolic state of muscle that generally is induced by halogenated inhalation anesthetics, depolarizing skeletal muscle relaxants, and occasionally local anesthetics or stressors such as heat or vigorous exercise. Clinical signs include a rapid increase in core body temperature, skeletal muscle rigidity, and tachycardia. Affected animals may develop significant acidosis and muscle necrosis, and the condition can be fatal. MH is most often associated with a defect in the ryanodine receptor.[20,23] In Quarter Horses and American Paint Horses, MH is inherited as an autosomal dominant trait that is linked to a single point mutation in the skeletal muscle ryanodine receptor 1 (RyR1) gene at nucleotide C7360G.[23,24] This mutation can be identified by genetic testing. Horses with polysaccharide storage myopathy tend to have a more severe clinical phenotype if they are concurrently affected with the MH mutation.[24]

Central Nervous System. Alterations in body temperature have been documented with a variety of conditions affecting areas of the hypothalamus involved in thermoregulation, with hyperthermia being more common than hypothermia.[1,3,4,25] Central hyperthermia has been associated with conditions such as hemorrhage, traumatic brain injury, neoplasms or abscesses, infectious/inflammatory changes, and degenerative disorders. It is usually characterized by a lack of diurnal variation, absence of sweating, resistance to antipyretic drugs, and excessive response to external cooling. In some cases, damage to the hypothalamus results in a disruption in the hypothalamic set-point temperature, resulting in neurogenic fever rather than true hyperthermia.[25]

Toxins or Drugs. Hyperthermia has occasionally been associated with some toxins or drugs.[26-28] Exposure to compounds that uncouple oxidative phosphorylation, such as the wood preservative pentachlorophenol, could potentially

cause a significant rise in body temperature.[27] Foals treated with macrolide antibiotics are at risk of developing hyperthermia.[28,29] Erythromycin causes a drug-induced anhidrosis that is the likely cause of the hyperthermia.[29] Because the sweat response of treated foals is impaired, these foals are at particular risk of hyperthermia when exposed to hot, humid environments and direct sunlight.

Fever

Mechanisms of Fever. In true fever the set-point for the desired core body temperature increases and then is maintained by the same mechanisms that maintain the normal body temperature. Fever is a part of the acute phase response to infection or inflammation. The pathogenesis of fever is complex, and multiple integrative signaling pathways come into play[3,4,30,31] (Fig. 7.1). Currently it is thought that both neuronal and humoral mechanisms are involved in the induction of fever. Neuronal mechanisms in part involve stimulation of vagal afferents, whereas humoral mechanisms involve several proinflammatory cytokines that act as endogenous pyrogens.[32,33] Prostaglandin E2 (PGE2), which may be produced peripherally or centrally, is regarded as the principal downstream mediator of fever, acting on thermosensitive hypothalamic neurons to influence the febrile response.[34,35] Other mediators, such as orexin, may also play a role in thermoregulation.[36,37]

The substance most often used in experimental studies of the febrile response is bacterial lipopolysaccharide (LPS).[38-40] Like many microbial products acting as exogenous pyrogens, LPS initiates fever by activation of the innate immune system, specifically via the complement cascade and Toll-like receptors (TLRs). The response is generally biphasic, with early and late phases. In the early phase of fever, LPS taken up by the Kupffer cells of the liver activates the complement cascade, with C5a rapidly stimulating the Kupffer cells to produce PGE2. This peripherally produced PGE2 may then act primarily via two pathways to affect thermoregulation. First, this PGE2 may be transported by the bloodstream to the ventromedial POA where it acts on thermoregulatory neurons to increase the set-point. Second, it may interact with receptors on hepatic vagal afferents, leading to activation of pathways projecting to the medulla oblongata and then to the POA via the ventral noradrenergic bundle. Consequently, norepinephrine is secreted, which then stimulates α_1-adrenoreceptors on thermoregulatory neurons, evoking a rise in core temperature. In the late phase of fever, there is further production of PGE2 following

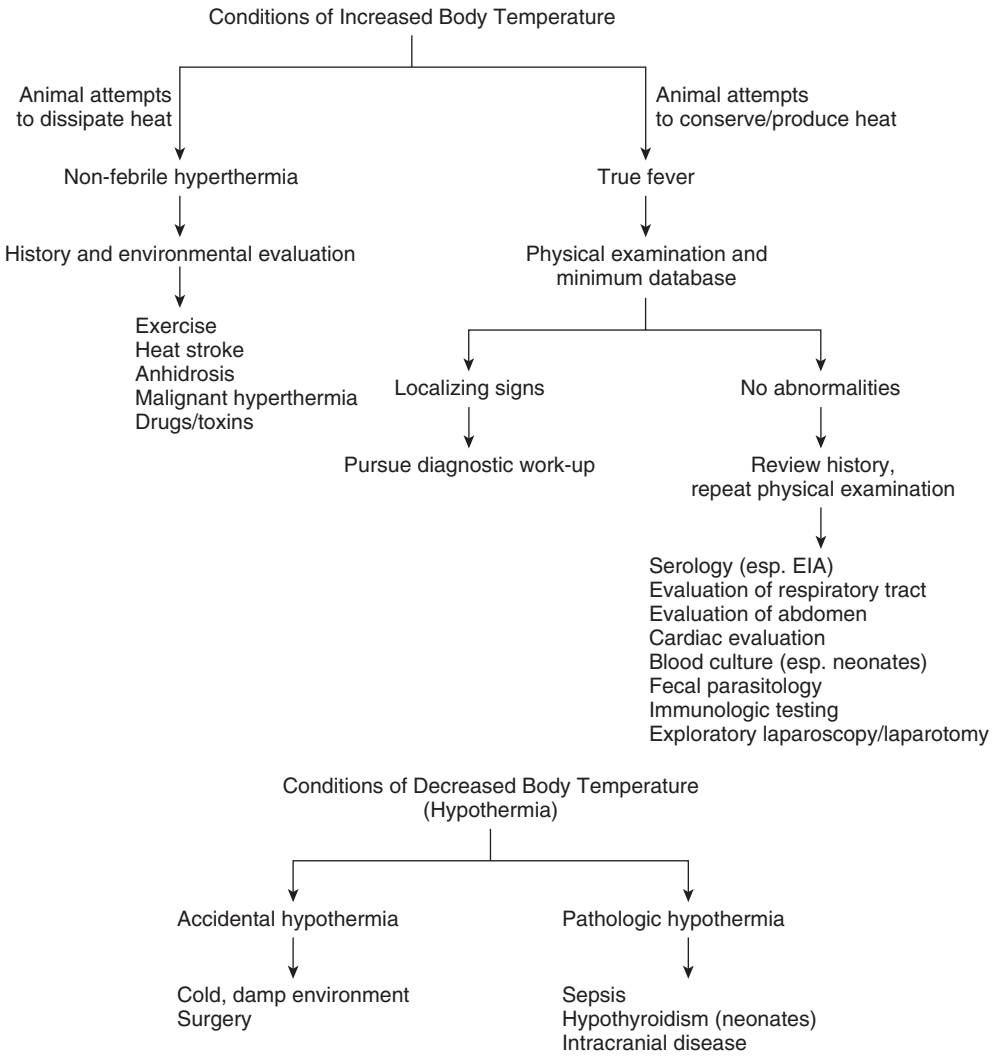


FIG. **7.1** Approach to changes in body temperature.

both direct induction of its synthesizing enzymes via TLR signaling and the production of endogenous pyrogens resulting in the central production of PGE2.

Endogenous pyrogen was initially assumed to be a single molecule produced by leukocytes, thus the name *leukocytic* or *granulocytic pyrogen*. Currently, at least 11 cytokines have been shown to be intrinsically pyrogenic.[30,32,33] These cytokines are produced predominantly by monocytes and macrophages. Among the most potent of the endogenous pyrogens are interleukin-1 (IL-1) α and β and tumor necrosis factor–alpha (TNF-α). Others include TNF-β, IL-6, and interferon-α. The precise means by which these cytokines influence thermoregulation are complex and remain to be fully elucidated. It appears that endogenous pyrogens have both peripheral and central mechanisms of action that include stimulation of afferent neuronal pathways, induction of PGE2 synthesis in endothelial cells in both the periphery and brain, and direct action in the brain via neuronal cytokine receptors.

The organum vasculosum laminae terminalis (OVLT) is a rich vascular network associated with neurons of the POA that plays an important role in the generation of fever.[33,35,41,42] This region is part of the circumventricular organs, which are unique structures of the brain with extensive vasculature and a minimal blood-brain barrier. As such, endothelial cells lining this region may allow direct movement of exogenous or endogenous pyrogens and peripherally produced PGE2 into the brain. Also, exogenous and endogenous pyrogens may interact with specific receptors on endothelial cells of the OVLT to further produce PGE2. Ablation of the OVLT prevents fever after a peripheral injection of endogenous pyrogens but has no effect when endogenous pyrogens are injected directly into the brain tissue.[42]

The production of both peripheral and central PGE2 is key in the pathogenesis of fever. PGE2 is basically synthesized in three steps involving phospholipase A2, cyclooxygenase, and terminal PGE synthetase. These enzymes are upregulated in fever.[34] The cyclooxygenase 2 (COX-2) pathway is clearly important in the pathogenesis of fever because COX inhibitors, and specifically COX-2 inhibitors, effectively reduce the febrile response but have no effect on the normal body temperature.[43,44] At the same time that there is upregulation of proteins involved in prostaglandin production, there is transcriptional downregulation of proteins involved in PGE2 inactivation, such as 15-hydroxy-prostaglandin dehydrogenase. Once produced, PGE2 interacts with E type prostaglandin receptors (EP receptors), of which there are currently four identified subtypes with differential expression in various areas of the hypothalamus and brainstem.[45] It appears that the EP3 receptor in the median preoptic nucleus within the POA of the hypothalamus is particularly important in the febrile response. The interaction of prostaglandins with their receptors initiates neuronal signaling by producing a cascade of changes in cyclic nucleotides, calcium, and monoamines leading to a higher set-point in the hypothalamic thermoregulatory center. For example, interaction with the EP3 receptor mediates decreases in intracellular cyclic adenosine monophosphate (cAMP), which results in decreased firing of warm-sensitive neurons, increased firing of cold-sensitive neurons, and ultimately fever.

Feedback mechanisms exist to prevent an excessive rise in body temperature. A number of antipyretic substances, sometimes referred to as either *endogenous* or *autogenous cryogens*, may be liberated systemically or within the brain during fever.[31,46-50] Some of the antipyretic effects occur via an inhibitory influence on the formation or action or endogenous pyrogens or via effects on neuronal thermoregulatory circuits that are activated during fever. The interactions between pyrogens and cryogens are complex, and some cytokines function as either depending on the circumstance. Some endogenous antipyretic substances include glucocorticoids, neuropeptides/hormones (arginine vasopressin, adrenocorticotropic hormone, α-melanocyte-stimulating hormone MSH, γ-MSH), cytokines (TNF-α, IL-10), lipocortin, nitric oxide, and epoxyeicosanoids.[31,47,49] One of the major antipyretics is IL-10. Following induction by pyrogenic cytokines, IL-10 inhibits further production of IL-1 and TNF. Also, α-MSH has significant antipyretic effects, being more effective in controlling fever than acetaminophen when administered to human beings.[49] Nitric oxide also has an antipyretic role, mediated by cyclic guanosine monophosphate in the POA.[50] Even PGE2, whose role is primarily in the induction of fever, may produce hypothermic responses when interacting with the EP4 receptor.[45]

The cytokines that act as endogenous pyrogens are generally proinflammatory, with a variety of biologic effects in addition to fever.[1,4,30,33,40,51] Thus fever is usually accompanied by additional hematologic, immunologic, and metabolic changes referred to as the *acute phase response*. Among these effects is the synthesis of acute phase proteins by hepatocytes, including fibrinogen, C-reactive protein, haptoglobin, serum amyloid A, and others. Also, hypoferremia, hypozincemia, and hypercupremia are cytokine mediated, as is the activation of lymphocytes, which in turn produce additional cytokines.

Prostaglandins induced by endogenous pyrogens stimulate the muscle catabolism associated with fever and induce collagenase synthesis from synovial cells, contributing to the muscle and joint pain often seen with fever. Local tissue responses to IL-1β and TNF-α may stimulate afferent neural impulses that lead to behavioral responses associated with fever, such as lethargy and anorexia. As expected, treatment with COX inhibitors can diminish many of the signs of fever.

Fever is a host defense mechanism that has been preserved within the animal kingdom. Although much attention is focused on the adverse effects of fever, including patient discomfort, there is evidence that fever can be beneficial.[52-58] Some studies have demonstrated an association between a rise in body temperature and a decrease in mortality and morbidity during infection. Although the elevation in temperature may directly impair organism growth in some cases, the beneficial effects are thought to be primarily associated with enhanced host defenses.[40,52-57] Fever has beneficial effects on multiple aspects of both the innate and adaptive immune response. As part of the acute phase response associated with fever, the concentration of iron, which is required by many bacteria for multiplication, decreases.[59-61] The beneficial effects of fever, however, are not universal, and many of the positive effects of fever are reversed when temperature becomes extremely high.[43,62] In rabbits experimentally infected with *Pasteurella multocida*, the survival rate increased in association with elevations in body temperature up to 2.25°C (4.5°F) above normal but decreased with elevations above this level.[63] The increased catabolism, variable anorexia, and increased metabolic rate can lead to muscle wasting and weakness when fever is prolonged. Although seizures induced by fever are uncommon in horses, they

can be seen in neonates when the temperature is above 42°C (108°F).[2] In debilitated animals, prolonged fever has been associated with cardiovascular failure.

Conditions Associated with Fever. Fever is part of the physiologic response to infection and inflammation (see Box 7.1). It is considered a cardinal sign of infection and has been associated with infections caused by essentially all types of organisms. Although there is considerable variation, viral infections are often associated with high fevers. In addition to infection, fever can be a prominent component of many inflammatory, neoplastic, and immunologic conditions. In intensive care units in human hospitals, the incidence of fever ranges from 23% to 70% and is related to an infectious process in approximately half of the cases.[64,65]

Fever of Unknown Origin. *Fever of unknown origin* in human medicine is defined specifically as a fever that occurs on several occasions over at least 3 weeks in which the diagnosis remains unclear after an initial diagnostic workup. In veterinary medicine, the term *fever of unknown origin* is often used more loosely, referring to any prolonged, unexplained fever. In many cases, the cause of a fever of unknown origin is a common disease with an unusual presentation. In a review of 63 cases of fever of unknown origin in the horse, the specific criteria used to define fever of unknown origin included (1) illness of at least 3 weeks' duration associated with nonspecific signs, (2) body temperature of at least 38.6°C (101.5°F) on several occasions, and (3) no clear diagnosis after an initial complete blood count and serum biochemical profile.[66] The most common cause was found to be infection, which was responsible for 43% of the cases. Other causes included neoplasms in 22% of cases; immune-mediated diseases in 6.5%; and miscellaneous diseases such as toxic hepatopathy, parasitism, and others in 19%. In 9.5% of cases no diagnosis was made. Specifically, the most common conditions identified were abdominal infections (peritonitis/abdominal abscessation) and lymphosarcoma, each accounting for approximately 16% of cases. Pneumonia, pleuropneumonia, or both were found in 11% of cases. Three horses (approximately 5%) were found to have bacterial endocarditis, and in each case a murmur was not identified initially but developed within several weeks of the onset of illness. Thus the diagnosis of fever of unknown origin requires a systematic approach with emphasis on the evaluation of infectious disease.

Diagnostic Approach to Elevations in Body Temperature

Increased body temperature is a common clinical sign with diverse causes. Fortunately, in many cases the cause may be readily apparent on the basis of the signalment, history, and physical examination. In other cases, additional diagnostic testing may be required (see Fig. 7.1).

Hyperthermia. Many causes of hyperthermia, such as exercise-related hyperthermia and malignant hyperthermia, can be distinguished from fever based largely on the signalment and history of the patient. Older horses may be more susceptible to exercise-related hyperthermia.[14] Treatment with macrolide antibiotics is significant due to the potential for macrolides to cause hyperthermia.[28,29] A sweat test, such as the quantitative intradermal terbutaline sweat test, can be useful in confirming anhidrosis.[18,67] Genetic testing is available for detection of the RyR1 mutation in cases of suspect malignant hyperthermia.[23,24]

Fever. Multiple conditions may result in fever. Infectious diseases remain the most common cause, although other inflammatory conditions such as neoplasia and immune-mediated disease may also cause fever. Often localizing clinical signs such as nasal discharge or diarrhea aid in determining the specific etiology. If the underlying condition responsible for the fever is not readily apparent, additional diagnostic tests are warranted.

Documentation of Fever. It may be useful to have the body temperature taken twice daily over a period of time to document fever and identify any pattern. Fevers can be categorized as intermittent, remittent, biphasic, or sustained, although some inconsistencies in the precise definitions of these patterns exist. In general, intermittent fevers are characterized by recurring paroxysms of elevated temperature followed by periods of normal temperature, such as those fevers that demonstrate diurnal variation. In most cases of intermittent fever the temperature tends to peak in the late afternoon or evening. Intermittent fevers most often are associated with infectious causes, particularly viral infections, although they may be seen with a variety of other conditions. Remittent fevers are those in which diurnal variation is exaggerated without a return to normal body temperature or those with a cyclic pattern in which the temperature elevation lasts for several days, such as may be seen with equine infectious anemia virus. Biphasic fevers, in which an initial rise in body temperature precedes a period of normal temperature and then a second rise, are characteristic of certain diseases such as equine neorickettsiosis (Potomac horse fever). Sustained fevers are those in which the elevation of temperature is consistent.

Signalment and History. The signalment and history should be considered when investigating fever. Some causes of fever may be more common in certain age groups. For example, although not a consistent finding, fever is often seen in neonatal foals in association with septicemia, omphalophlebitis, or septic arthritis. *Rhodococcus equi* is predominantly seen in foals 1 to 6 months of age and is often associated with fever. Young horses, especially those that have been recently exposed to new horses, may be particularly at risk for respiratory tract infections. Any exposure to *Streptococcus equi* subspecies *equi* (strangles) may be significant because of the association of this organism with internal abscessation. Geographic location and travel history may be relevant, as some diseases, such as equine neorickettsiosis, piroplasmosis, and coccidioidomycosis, are more common in certain geographic locations.

Physical Examination. A thorough physical examination, including auscultation of the thorax with a rebreathing bag and rectal palpation, is indicated in the evaluation of fever. Repeating the physical examination may yield new information. A neurologic examination can also be useful as disorders of the central nervous system may cause aberrations in temperature through pyrogenic cytokines or in some cases through direct effects on thermoregulatory centers.

Ancillary Diagnostic Aids. Ancillary diagnostic tests are commonly used in the diagnosis of fever, particularly in cases of fever of unknown origin. Localizing signs may help direct selection of the most appropriate tests but are not always present.

CLINICAL PATHOLOGY/LABORATORY TESTING. A database, including complete blood count (CBC), fibrinogen, biochemical profile with bile acids, and urinalysis, should be obtained. Hemoparasites may occasionally be seen on the blood smear,

but the apparent absence of organisms does not rule out a parasitemia that is below detectable limits. Abnormalities consistent with chronic infection or inflammation, including anemia, hyperfibrinogenemia, hyperglobulinemia, and thrombocytosis, are common but nonspecific findings. Although inflammation is the most common cause of hyperglobulinemia, further assessment by serum protein electrophoresis and specific immunoglobulin quantitation may be indicated in some cases. A monoclonal gammopathy is characteristic of plasma cell myeloma but may also be seen with other tumors of the reticuloendothelial system and occasionally other conditions. Immunodeficiencies, which in some cases are associated with low lymphocyte counts or low immunoglobulin concentrations, can predispose affected horses to chronic infections. In cases of hypoalbuminemia, gastrointestinal or renal loss, third space loss, and decreased production associated with significant hepatic disease should be investigated. The presence of hypercalcemia can be helpful in the diagnosis of disease, as it is most often linked with either renal disease or certain neoplasms in horses. Bone marrow aspiration may be useful, particularly in those horses with persistent abnormalities in circulating cell populations.

Infections of the respiratory tract and abdomen frequently are associated with fever in the horse, and therefore these systems should be thoroughly evaluated. In many cases this includes cytologic evaluation and culture and/or polymerase chain reaction (PCR) of samples from the respiratory tract and abdomen. The upper airway is often sampled by nasal swabs, nasal washes, or guttural pouch lavage, and the lower airway can be sampled by bronchoalveolar lavage or transtracheal aspiration. Thoracocentesis may be considered because abnormalities of pleural fluid are occasionally present even without increases in the volume of fluid. Similarly, abnormalities in peritoneal fluid can be found without increases in fluid volume, and abdominocentesis should be considered as part of the diagnostic plan. It should be remembered that neoplastic cells often do not exfoliate into fluid; therefore neoplasia cannot be ruled out based on cytologic evaluation of fluid.

Although only occasionally associated with fever, the presence of gastrointestinal parasites is so common that feces from horses with fever of unknown origin should be examined for parasite ova. In cases of suspected gastrointestinal protein loss, diarrhea, or melena, one should consider diagnostic procedures such as fecal culture and analysis for clostridial toxins, culture and PCR for *Salmonella,* rectal mucosal biopsy, or absorption tests.

Blood cultures are generally most useful in neonates but can yield valuable information in adult horses with fever as well. Ideally, three to five samples should be collected at least 45 minutes apart when the horse is not on a regimen of antibiotic therapy. Sampling just before and during a temperature rise is most likely to yield a positive culture.

Serologic evaluation can be useful in the assessment of fever, and specific tests can be prioritized based on the patient and the geographic area. Due to variability in the clinical presentation and regulatory concerns, equine infectious anemia should be considered as a differential diagnosis for horses with fever of unknown origin, and testing should be performed. A serologic test for detection of antibodies to the M protein of *Streptococcus equi* ssp. *equi* has been developed and may be a useful aid in the diagnosis of metastatic abscessation associated with the strangles organism.[68] Serologic tests for several other infectious diseases are also available, including tests for *Corynebacterium pseudotuberculosis,* equine piroplasmosis, brucellosis, and coccidioidomycosis, among others.

Immune-mediated disorders such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, systemic lupus erythematosis, vasculitides, and rheumatoid arthritis have been implicated as causes of fever of unknown origin but more commonly in human beings and small animals than in horses. Appropriate diagnostic tests, such as the Coombs' test, skin biopsy, and antinuclear antibody testing, may be useful in some patients.

ENDOSCOPY. Endoscopy is commonly used for evaluation of the respiratory tract, especially the upper respiratory tract including the guttural pouches. Pleuroscopy allows direct visual examination of the pleural space and may facilitate biopsy of any masses. Also, endoscopy can be useful in evaluation of the esophagus, stomach, and urinary tract.

DIAGNOSTIC IMAGING. Ultrasound is a practical, noninvasive means of assessing the thorax and parts of the abdomen. It can help identify abnormalities that need further evaluation, such as consolidated lung, abdominal masses, or pathologic liver and kidney conditions. It may also help identify fluid for collection. Echocardiography can aid in the diagnosis of bacterial endocarditis.

Radiographs of the thorax are helpful in the evaluation of pulmonary disease. Although the practicality and utility of abdominal radiographs are limited, especially in adult horses, they may be of value in individual cases, especially in neonates. Nuclear imaging using labeled white blood cells may identify a site of infection or inflammation.

OTHER. Exploratory laparoscopy or laparotomy is indicated when abdominal involvement is suspected or the animal is becoming progressively debilitated. In horses for which a specific diagnosis has not been made, therapeutic trials with antimicrobials may help, and in cases of suspected immune-mediated disease, corticosteroids may help.

Decreased Body Temperature: Hypothermia

Mechanisms of Hypothermia

Hypothermia occurs when the core body temperature drops below accepted normal values.[4] Clinically, hypothermia can be characterized as either accidental or pathologic. In accidental hypothermia the body's ability to produce heat is overwhelmed, often in association with harsh environmental conditions. There is a spontaneous decrease in the core body temperature independent of actual disruption to the thermoregulatory system.

Pathologic causes of hypothermia should be considered when no clear reason for accidental hypothermia is evident. Pathologic hypothermia occurs in association with disorders that decrease metabolic activity or directly affect the thermoregulatory center, such as endocrine disorders, sepsis, and intracranial disease. When hypothermia is seen with systemic inflammation, it is often considered a maladaptive thermoregulatory response. The mechanisms involved in producing hypothermia are not fully understood, but several cytokines (including TNF-α, interleukins, and interferon-gamma [IFN-γ]) are involved.[69] Some of these same cytokines may also act as pyrogens under other conditions.

The ability to generate heat through shivering is impaired or lost once the body temperature becomes too low. The animal experiences a decrease in the metabolic rate of most tissues. Heart rate, cardiac output, glomerular filtration, and blood pressure may decrease.

Conditions Associated with Hypothermia

Accidental Hypothermia. Accidental hypothermia is most often associated with cold or cold, damp, windy environments (see Box 7.1). Occasionally extreme situations occur, such as an animal falling through ice. Mild accidental hypothermia sometimes occurs with surgical procedures. Accidental hypothermia is particularly common in neonates and geriatric or debilitated adult horses. Although central thermoregulation through the hypothalamus is normal in neonates, foals have a large ratio of surface area to body weight, enhancing heat loss.[2] Sick or debilitated animals often have decreases in activity and nutritional intake and alterations in circulation that can contribute to hypothermia. Severe hypothermia may result in significant metabolic changes and death.

Pathologic Hypothermia. Pathologic hypothermia can be associated with sepsis, neurologic disorders, and endocrine disorders (see Box 7.1). Hypothermia has been observed with septicemia and shock, especially in neonates, in which 24% of septic foals were found to have a decreased body temperature.[2]

Hypothyroidism is an uncommon clinical problem in horses; however, impaired thermoregulation has been seen in foals with congenital hypothyroidism.[70,71] Hypothermia may be more common in donkeys than horses. In one study, histologic lesions of the thyroid gland were identified in four of five hypothermic donkeys.[72]

Approach to Hypothermia

Accidental Hypothermia. Accidental hypothermia can generally be identified from the signalment and history (see Fig. 7.1). It is important to assess environmental conditions to determine whether environmental stress, especially cold, wet, and windy conditions, is contributing to the hypothermia. Neonatal, geriatric, and debilitated patients are at high risk.

Pathologic Hypothermia

SIGNALMENT AND HISTORY. The signalment and history should be considered when assessing cases of potential pathologic hypothermia (see Fig. 7.1). It is important to consider environmental conditions, as in some cases both accidental and pathologic causes may contribute to the hypothermia, such as in septic neonates born in cold, damp environments.

PHYSICAL EXAMINATION. A thorough physical examination should be performed in horses with hypothermia. Patients should be assessed for potential underlying conditions such as sepsis or inflammation and neurologic or endocrine disorders. A neurologic examination may be useful.

ANCILLARY DIAGNOSTIC TESTS. A CBC, serum biochemical profile, fibrinogen, and urinalysis can be useful in determining the underlying cause of hypothermia. Blood cultures are indicated in cases of potential sepsis. Hypothyroidism is uncommon, but if suspected, diagnostic testing may include a thyroid-stimulating hormone or thyrotropin-releasing hormone (TRH) response test. Ultrasound and aspiration or biopsy of the thyroid may also be useful, especially in foals with suspected congenital or nutritional hypothyroidism.

CHANGES IN BODY WEIGHT

Changes in body weight and condition are common clinical problems in horses. Both weight loss and obesity have the potential to adversely affect the health of the horse. Weight loss can range from mild physiologic weight loss, such as that seen with increased exercise, to dramatic, life-threatening weight loss.[73,74] Severe malnutrition, regardless of the underlying cause, can result in starvation and the accompanying signs of marked weakness and wasting, known as *inanition*. Ultimately starvation can lead to organ failure and death. Obesity appears to be increasing in prevalence in the horse population and has been linked to serious health concerns, particularly laminitis.[75-78] Some horses that are prone to obesity ("easy keepers") have an endocrinologic disorder known as *equine metabolic syndrome* (EMS).[79-81]

The most accurate means of assessing body weight and monitoring changes in weight is to use a scale, but this has practical limitations. Thus a variety of weight tapes and equations based on morphometric measurements have been developed to estimate body weight.[82,83] To account for differences in body type between breeds, some breed-specific equations are available.[82] The ideal body weight depends on the type and use of the horse, and equations have been developed to calculate the ideal body weight for horses of certain breeds. Several online tools are available to aid in the calculation of actual and ideal body weight.

Weight alone does not necessarily reflect the body condition of the horse. Because muscle weighs more than fat, athletic horses may be heavier than nonathletic horses of similar size. To better evaluate body condition, a number of scoring systems have been developed to estimate the extent of adiposity.[83-87] One commonly used system to determine a body condition score (BCS) is the Henneke system, which uses a scale of 1 to 9, with 1 representing an extremely emaciated horse, 9 representing an extremely fat one, and 5 being close to ideal[86,87] (Table 7.1). A BCS system that ranges from 0 to 5 is also occasionally used, with 0 being emaciated, 3 good, and 5 very fat. In addition to body condition scoring, a number of morphometric measurements can be used to help estimate adiposity, some of which may be particularly useful in evaluating localized adiposity.[83,88,89] The girth-to-height ratio correlates well with the BCS and is a suitable morphometric measure for assessment of overall adiposity.[83] The cresty neck score, which ranges from 0 to 5, can be used to evaluate adiposity in the neck, with a score of 3 or greater being considered a cresty neck (Table 7.2). Some additional measurements used to assess neck adiposity include crest height, neck circumference, and neck circumference–to–height ratio. It has been demonstrated in ponies that measures of generalized and localized obesity, including a BCS of 7 or greater, a cresty neck score of 4 or greater, and a neck circumference–to–height ratio greater than 0.71, are useful in the prediction of laminitis.[88] A more quantitative means of measuring adiposity is by ultrasound.[90] One method is to take a measurement 5 cm lateral from the midline at the midpoint of the pelvic bone and the percent body fat is calculated by the following equation: % body fat = 2.47 + 5.47 (rump fat in cm). Most lean horses are approximately 8% to 14% body fat, whereas overconditioned horses are 16% to 30%. Ultrasound can also be used to assess retroperitoneal fat. A topline score can be used to evaluate epaxial musculature. Body condition scoring is also valuable in evaluating thin horses and is often used to assess horses in animal welfare cases.

Mechanisms Controlling Body Weight

The stability of body weight and body composition is related to the balance of energy intake and energy expenditure.[73] Dietary carbohydrates, fats, and proteins provide energy to support the body's metabolic needs. When the energy intake exceeds the expenditure, the excess energy is stored, primarily as fat, resulting in an increase in body weight. When the

TABLE **7.1 Horse Body Condition Scores on the Henneke Scale**[86]

Score	Description
1. Poor	Extremely emaciated; no fatty tissue; vertebrae, ribs, tail head, and bones of withers, shoulder, and neck are visible
2. Very thin	Emaciated; slight tissue cover over bones; vertebrae, ribs, tail head, and bones of withers, shoulder, and neck are visible
3. Thin	Slight fat cover over body; individual vertebrae and ribs no longer visibly discernible; withers, shoulders, and neck do not appear overly thin
4. Moderately thin	Ridge of spine and outline of ribs are visible; tail head may or may not be visible depending on the breed; withers, shoulders, and neck do not appear overly thin
5. Moderate	Spine and ribs cannot be seen, but ribs can be felt; tail head is spongy; withers, shoulders, and neck are rounded and smooth
6. Moderately fleshy	Slight crease down spine; ribs and tail head feel spongy; fat deposits along withers and neck and behind shoulders
7. Fleshy	Crease down spine; ribs have fat filling between them; tail head spongy; fat deposits along withers and neck and behind shoulders
8. Fat	Apparent crease down spine; ribs difficult to feel; soft fat surrounding tail head; fat deposits along withers, behind shoulders, and on inner thighs; neck is large
9. Extremely fat	Obvious crease down spine; patchy fat on ribs; bulging fat on tail head, withers, behind shoulders, and on neck; fat fills in flank and on inner thighs

TABLE **7.2 Cresty Neck Score Used in the Assessment of Regional Adiposity**[83]

Score	Description
0	No palpable crest
1	No visual appearance of a crest but slight filling felt with palpation
2	Noticeable appearance of a crest Fat deposited fairly evenly from poll to withers Crest easily cupped in one hand and bent from side to side
3	Crest enlarged and thickened Fat deposited most heavily in middle of neck giving a mounded appearance Crest fills cupped hand and begins losing side-to-side flexibility
4	Crest grossly enlarged and thickened May have wrinkles perpendicular to the topline Crest can no longer be cupped in one hand or easily bent from side to side
5	Crest is so large it falls to one side

energy intake is insufficient to meet metabolic needs, energy stores are used and there is a loss of body weight. Both weight loss and obesity can be serious clinical problems.

Weight Gain/Obesity

Obesity is being increasingly recognized in the equine population. In the 1990s, a study by the National Animal Health Monitoring System of the U.S. Department of Agriculture (USDA) estimated that approximately 5% of the horse population was obese. Several more recent studies in the United States and Europe have found that 45% to 50% of horses are overconditioned, with 10% to 35% being obese.[75-78,91-93] Breed has been shown to be strongly associated with the risk of obesity. In many cases, owners do not recognize their horse as obese. Horses that are not ridden or used for pleasure riding rather than competition appear to be at increased risk of obesity.[75,76] In a study of outdoor-living horses in the United Kingdom, seasonal variation in BCS was documented, with the prevalence of obesity increasing from 27% at the end of winter to 35% at the end of summer.[76] In this study, providing supplementary feed was not a strong predictor of obesity, supporting the belief that grass consumption is an important factor influencing obesity in outdoor-living horses.

Obesity has been linked to adverse health effects in many species. In horses, the increased risk of laminitis associated with obesity is a major concern.[81,94] Other potential problems seen with obesity include thermoregulatory inefficiency, exercise intolerance, altered reproductive performance, hyperlipemia syndrome, and the development of lipomas in mesenteric adipose.

Mechanisms of Weight Gain

Weight gain occurs when energy intake exceeds energy expenditure. Although this is a fairly straightforward concept, the actual etiology of obesity can be complex, involving interactions between genetics, hormones, and management. In many cases, management factors including diets high in sugar and starch coupled with a lack of sufficient exercise contribute to obesity. Even when horses are ridden regularly as pleasure horses, the relatively low-intensity exercise provided often does not use significant energy.[75,76]

Some horses are particularly susceptible to obesity. At one time, it was proposed that this was due to hypothyroidism, but evidence did not support this. In 2002 EMS was introduced as a metabolic and endocrine disorder contributing to obesity.[79] EMS is a complex problem that shares some characteristics with metabolic syndrome in people.[79-81,94,95] The major phenotypic features of EMS include obesity and/or regional adiposity, insulin resistance, and a predisposition for laminitis.

The pathophysiologic mechanisms involved in both obesity in general and EMS are complex, involving interactions between multiple factors including genetics. It appears that some individuals may inherit traits that facilitate survival in harsh environments, making them especially thrifty. EMS can be recognized in any breed of horse, but an increased susceptibility has been recognized in certain breeds, including several pony breeds, Morgan Horses, Paso Finos, Arabians, Saddlebreds, Spanish Mustangs, and Warmblood breeds.[81] Studies are ongoing to help better define the role of genetics in EMS.

Insulin dysregulation is a key factor in the diverse physiologic processes that contribute to EMS.[79-81,96,97] Insulin dysregulation refers collectively to abnormalities of insulin metabolism including excessive insulin responses to sugars,

fasting hyperinsulinemia, and insulin resistance (IR). The relationship between IR and obesity might be described as a vicious circle, as each may promote the other. In one study that demonstrated differences in the insulin response to glucose between ponies and horses, all animals were in moderate body condition, suggesting that the breed-related differences in insulin dynamics were independent of obesity.[98] In addition to genetics, EMS, and obesity itself, some factors that can potentially cause IR include physiologic responses to stress or pregnancy, systemic inflammation, and pituitary pars intermedia dysfunction (PPID).

There is increasing awareness of the role fat itself plays in the development of metabolic disease and inflammation.[99-101] Previously, adipose tissue was considered simply an energy storage depot with a fixed number of adipocytes. Any increase in adipose mass was thought to occur due to increased fat storage within existing cells resulting in hypertrophy. However, it is now recognized that adipose tissue is a complex endocrine organ that contains a large pool of stem cells and preadipocytes that can be recruited once existing adipocytes reach a critical level of hypertrophy. Thus in obesity there is generally both hypertrophy and proliferation of adipocytes. The adipocytes are biologically active and influence multiple physiologic processes including energy metabolism, cardiovascular function, reproductive function, inflammation, and immunity. The cells secrete a wide variety of cell-signaling proteins, or cytokines, known as *adipokines*, that function in both an autocrine/paracrine and endocrine fashion.[100] Due to local influences, adipocytes from differing anatomic locations may vary in their specific pattern of expression of these adipokines. Currently at least 100 substances are thought to be secreted by adipocytes. Some important adipose-derived hormones include leptin, adiponectin, resistin, visfatin, and angiotensinogen. Adipokines that act as proinflammatory cytokines and inflammatory mediators include TNF-α, IL-1α and IL-1β, IL-6, and IL-8, as well as monocyte chemotactic protein–1 and plasminogen activator inhibitor. The altered expression of adipokines in obesity can influence a number of metabolic processes and contribute to EMS.[101-107] Adipokines may inhibit insulin signal transduction pathways contributing to insulin resistance. Leptin, which is involved in satiety and regulation of obesity, is elevated in horses with IR, suggesting leptin resistance. Adiponectin, an insulin-sensitizing hormone, can be decreased in obese horses. In humans and rodents, obesity is linked with an upregulation of proinflammatory cytokines secreted by adipocytes. This results in a chronic inflammatory state and contributes to insulin dysregulation. Studies in horses, however, have yielded variable results regarding the expression of inflammatory mediators in obese horses, and further investigation is needed to understand their role in EMS.[95,104-106]

Several other factors may influence the development of obesity in addition to insulin dysregulation and the action of adipokines. The mechanisms of central control of body weight remain somewhat obscure, and it has been proposed that there is a set-point for body weight regulated through a region of the hypothalamus.[108] Myostatin, a myokine and negative regulator of skeletal muscle mass, has been implicated in obesity in other species, and limited data in the horse suggest a possible role for myostatin and its receptor in equine obesity.[109] Recently, the central role of the intestinal microbiota in the progression and prevention of metabolic dysfunction has become apparent, and the role of the gut microbiota in obesity and insulin dysregulation is under investigation in multiple species, including horses.[110-112]

BOX 7.2 Causes of Weight Gain/Obesity

PHYSIOLOGIC WEIGHT GAIN
Growth
Pregnancy

MANAGEMENT
Overfeeding/excessive grass consumption
Lack of exercise

ENDOCRINOLOGIC/METABOLIC DISORDERS
Equine metabolic syndrome
Pituitary pars intermedia dysfunction
Often causes weight loss

An increased risk of laminitis is an important feature of EMS.[79-81,113] The mechanisms involved in the relationship of obesity and insulin dysregulation to laminitis are not fully understood, and this remains an active area of research. The pathogenesis is likely multifactorial, and some proposed mechanisms include impaired glucose uptake by epidermal laminar cells, altered function of epidermal cells, endothelial cell dysfunction within the vasculature of the foot, digital vasoconstriction, and activation of metalloproteinase by glucose deprivation or reactive oxygen species.

Conditions Associated with Weight Gain

An increase in body weight occurs when the energy intake is greater than the energy expenditure. Being overweight should be distinguished from the normal physiologic weight gain associated with pregnancy and from pathologic conditions that cause abdominal distention, such as ascites or peritonitis (Box 7.2).

Overconditioning and obesity are often related to management problems associated with supplemental feed or excessive grass consumption, often combined with a lack of significant exercise. Horses with EMS are particularly susceptible to weight gain, and EMS is an important cause of obesity and regional adiposity. PPID can have variable effects on body weight. Whereas many horses with PPID may experience weight loss and muscle wasting, others may exhibit obesity or regional adiposity, especially if they have concurrent IR.

Diagnostic Approach to Weight Gain

Signalment and History. Any breed may develop EMS, but some appear to be at increased risk, including pony breeds, Morgan Horses, Paso Finos, Arabians, Saddlebreds, Spanish Mustangs, and Warmblood breeds.[81] The condition has also been commonly recognized in American Miniature Horses, draft breeds, and Norwegian Fjords, as well as donkeys and mules. It appears to be less common in Thoroughbreds and Standardbreds. The prevalence of PPID increases with age.

A thorough history is important. Particular attention should be given to the feeding practices as well as the amount and type of exercise that the horse receives. Previous episodes of laminitis may point to an increased likelihood of EMS or PPID. A history of abnormal shedding or polyuria/polydipsia increases the suspicion of PPID. The breeding history should be established. In some cases, the breeding history may be unknown, especially if the horse has changed ownership.

Physical Examination. A complete physical examination should be performed in cases of weight gain. This may help to rule out medical conditions that may cause abdominal distention such as ascites, bloat, uroperitoneum, hemoabdomen, or peritonitis. Palpation per rectum may be indicated to rule out pregnancy, especially in horses that have an unknown breeding history.

The body weight and BCS should be assessed, and the horse should be evaluated for regional adiposity.[82-87] Several morphometric measurements can be useful in the evaluation of body weight and condition. It is important to palpate carefully over the shoulder, elbow, ribs, withers, sternum, loin, and tailhead. The girth:height ratio can be useful in the assessment of overall adiposity.[83] A cresty neck score, as well as the neck crest height and neck circumference–to–height ratio, are useful in the assessment of neck adiposity.[83] In many cases, careful evaluation for lameness, increased digital pulses, and sensitivity to hoof testers is warranted due to the association of obesity with laminitis.

Ancillary Diagnostic Tests. Routine blood work is often unremarkable in cases of weight gain. As normal values may vary among laboratories, it is ideal to use reference ranges specific for the laboratory being used, especially when evaluating IR and PPID. Also, any significant ongoing pain or stress should be taken into account as these conditions can affect glucose and insulin dynamics, as well as adrenocorticotropic hormone (ACTH) and cortisol. Horses with both suspected EMS and PPID should be screened for IR. Current tests for evaluating IR focus primarily on measurements of glucose and insulin.[80,81] It is generally recommended to limit feed before sampling to one flake of grass hay low in nonstructural carbohydrates per 500 kg of body weight given no later than 10 PM the night before. As IR is often well compensated, baseline concentrations of glucose and insulin may be normal or in many cases the glucose is normal whereas the insulin is elevated. Occasionally horses will develop diminished glycemic control and become hyperglycemic (greater than 150 mg/dL) consistent with type 2 diabetes mellitus. There are several mathematical calculations such as the glucose:insulin ratio and reciprocal inverse square of insulin (RISQI) that are occasionally used to help assess insulin sensitivity.[114] Because resting concentrations of both glucose and insulin can be normal in IR, dynamic testing is often indicated. Practical dynamic tests include the oral sugar test (OST) and the combined intravenous glucose-insulin test (CGIT). Other diagnostic tests that may be useful in assessing IR include measurement of triglyceride and leptin concentrations, both of which may be elevated in IR.

Evaluation for PPID should be considered in horses with weight gain or regional adiposity, as well as in those with loss of body condition.[115,116] There are several options for diagnostic testing. Currently, frequently recommended tests include measurement of baseline ACTH, a low-dose dexamethasone suppression test, and a TRH stimulation test with measurement of ACTH.

Weight Loss

Mechanisms of Weight Loss

Weight loss occurs when the energy intake is exceeded by the expenditure. Thus there are basically two general mechanisms of weight loss, decreased energy intake and increased energy demand. In many horses with weight loss, both of these mechanisms come into play. Muscle loss often occurs with weight loss but can also occur as a result of neurogenic or disuse muscle atrophy, in which case the muscle loss may be independent of generalized weight loss.

Causes of Decreased Energy Intake

1. *Limited access to feed or poor quality feed:* The most direct cause of protein-calorie malnutrition and weight loss is an inadequate volume or quality of feed to meet the dietary requirements of the animal. This can result from actual underfeeding or from other factors such as excessive competition between horses or lameness preventing the horse from accessing the feed easily.[117] Also, the nutritional value of feed, particularly forage, can vary widely, and poor quality feed can be a significant factor in malnutrition.
2. *Dysphagia:* Dysphagia, difficulty with prehension, mastication, or swallowing, can occur in horses for many reasons with resultant decreased feed intake and weight loss. One common cause of difficulty eating, especially in older horses, is abnormal dentition.
3. *Malabsorption/malassimilation:* Malabsorption is the inadequate assimilation of dietary substances due to defects in digestion, absorption, or transport.[118] Both macronutrients (carbohydrates, fats, and proteins) and micronutrients (vitamins and minerals) can be affected. Normal absorption is a complex process involving multiple organs, enzymes, and hormones, as well as transport and secretory mechanisms. Impairment of any of the steps in this process can result in malabsorption. Rapid gastrointestinal transit time can also contribute to malabsorption. In horses, malabsorption is often associated with conditions that damage the intestinal wall, such as inflammatory bowel diseases, infiltrative neoplasms, parasitism, and certain gastrointestinal infections.[119] In foals, both primary and secondary lactase deficiency have been identified as causes of malabsorption.[120] Depending on the underlying cause of the malabsorption, weight loss may occur in the face of normal dietary intake.
4. *Anorexia:* Anorexia, which is a loss of appetite, frequently contributes to weight loss, especially when prolonged. Anorexia may be either partial or complete, and occasionally the term *hyporexia* is used to describe partial anorexia. A wide variety of diseases are associated with anorexia, and in many cases, the decreased desire for feed is combined with increased energy expenditure, resulting in significant weight loss. The pathophysiologic mechanisms involved in anorexia are not fully understood, but it appears that there are disturbances of hypothalamic pathways controlling energy homeostasis and that multiple mediators including hormones, neuropeptides, and cytokines such as IL-1 and TNF-α are involved.[121,122]

Causes of Increased Energy Demand. In general, causes of increased energy demand can be categorized as physiologic or pathologic.

1. *Physiologic causes of increased energy demand:* Physiologic weight loss may be seen with conditions requiring increased energy, such as late pregnancy, early lactation, and intense exercise. Environmental stress, especially decreased ambient temperature, can also have a significant impact on caloric requirements.[123-125] In adult horses, the lower critical temperature, which is the temperature below which the horse must increase metabolic heat production to maintain normal body temperature, ranges from 5°C (4°F) to −15°C (5°F) depending on the horse's adaptation to the environment. The digestible energy requirements are estimated to increase by 2.5% for every degree Celsius below the lower critical temperature. Energy requirements are further

increased by wet, windy conditions. It is estimated that in cold temperatures when the hair coat is wet the maintenance digestible energy requirement may increase by as much as 50%.

2. *Pathologic causes of increased energy demand:* Many disease processes will result in weight loss, not only because anorexia is common, but because there is frequently a concomitant increase in energy expenditure.[126] Fever itself appears to increase energy needs. In human patients, the resting energy requirement is estimated to increase by approximately 14% for each degree C increase in body temperature. However, the increase in energy requirement associated with disease is not consistent. For example, it was determined that the resting energy requirement of critically ill neonatal foals (40–50 kcal/kg body weight per day) was less than that of control foals (60–80 kcal/kg body weight per day).[127] The regulation of energy requirements in disease is complex and remains poorly understood.
3. Cachexia is a specific multifactorial syndrome associated with underlying illness that is defined by the loss of skeletal muscle with or without the loss of fat mass.[128-130] Cachexia is characterized by a negative protein and energy balance driven by a variable combination of reduced feed intake and abnormal metabolism, and it cannot be fully reversed by nutritional support. The pathophysiology of this syndrome involves multiple mechanisms.[130-133] It is in part mediated by cytokines, including TNF-α, IL-6, IL-1β, IFN-γ, and proteolysis inducing factor. TNF-α was originally designated as cachectin due to its catabolic effects. In human patients, muscle catabolism has been associated with TNF-α in a variety of conditions such as cancer, congestive heart failure, and chronic obstructive pulmonary disease. Based on studies in cultured muscle cells, it appears the increased muscle catabolism associated with TNF-α is mediated by reactive oxygen species and nuclear factor-κβ, which upregulate ubiquitin/proteasome activity.[134] There are several additional mechanisms of cytokine mediated cachexia, including activation of the hypothalamic melanocortin system and upregulation of the cytokine-activated transcription factor STAT3.[128,129,132-134] The endocrine system also has a role in cachexia. There is often a reduction in circulating anabolic hormones associated with disease. In cancer patients, tumor-derived parathyroid hormone–related protein (PTHrP) appears to play a role in cachexia.[131] In chronic disease states such as congestive heart failure and chronic kidney disease, it appears that the renin-angiotensin system has a major role in producing cachexia.[133] Angiotensin II concentrations are often elevated in chronic disease, and treatment with angiotensin converting enzyme inhibitors can improve weight loss.
4. Several additional mechanisms may contribute to increased energy requirements during systemic disease.[126,128-130] The stress associated with disease may alter metabolism due to increases in sympathetic and hormonal activity. Chronic pain may also result in elevated systemic catecholamine and cortisol concentrations, resulting in a catabolic state. In recurrent airway obstruction, the work of breathing can increase energy requirements. An increase in nutrient demand can be seen in association with protein loss in conditions such as peritonitis, pleuritis, colitis, inflammatory bowel disease, and burns. Also, although thyroid disease is uncommon in horses, weight loss has been recognized in association with hyperthyroidism and the accompanying increase in basal metabolic rate.[135,136]
5. Aging in multiple species has been associated with unintentional weight loss and sarcopenia, which is a loss in muscle mass, quality, and strength associated specifically with aging. The mechanisms behind this structural and functional decline in skeletal muscle are complex.[137] In horses, aging alone has been associated with a loss of weight and muscles.[138,139] Horses affected with PPID may be particularly susceptible to muscle loss due to atrophy of type 2A and 2B muscle fibers and loss of type 2B fibers.[115,140] Proposed mechanisms of steroid-related myopathy in horses with PPID include myocyte apoptosis, negative regulation of nuclear factor kappa B (NF-κB) activation and function, and oxidative stress.
6. It is not uncommon for weight loss to involve multiple mechanisms at the same time. Not only may decreased energy intake and increased energy demand be present concurrently, but there may be multiple mechanisms contributing to the decreased intake and increased demand. For example, in recurrent airway obstruction there may be increased demands from both the work of breathing and the presence of inflammatory cytokines. In parasitism, there is competition for nutrients within the gastrointestinal tract, as well as damage to the gastrointestinal wall resulting in inflammation and malabsorption of both macronutrients and micronutrients.

Conditions Associated with Weight Loss

A wide variety of problems can be associated with weight loss (Box 7.3). An inadequate amount or quality of feed for the age and use of the horse is an important cause that should not be overlooked. In some cases, adequate feed is provided, but the horse is unable to eat a sufficient amount due to factors such as competition with other horses or the presence of a gait deficit that limit's the horse's ability to access feed readily. Also, actual dysphagia can contribute to weight loss if prolonged. Some of the more common problems causing dysphagia include dental disorders, oral or pharyngeal foreign bodies, esophageal disorders, masseter myopathy, and several neurologic conditions such as neuropathy associated with guttural pouch disease, nigropallidal encephalomalacia, equine protozoal myelitis, and botulism.

Almost any disease process, including parasitism, infectious diseases, neoplasms, immunologic disorders, toxicities, chronic organ dysfunction, and endocrinopathies, can result in weight loss. In some instances the underlying cause of weight loss is readily apparent, such as in cases of pleuropneumonia or diarrhea. Weight loss is common with many gastrointestinal diseases, particularly protein losing enteropathies. In a retrospective study of 40 horses presented for weight loss despite a good appetite, a definitive diagnosis was established in 24 of 40 cases (60%), and 16 of 40 cases (40%) were idiopathic.[141] The most common diagnoses were inflammatory bowel disease (13 of 40, 32.5%) and intestinal lymphosarcoma (4 of 40, 10%). Hypoalbuminemia was identified in 58% of the total cases, and the severity of hypoalbuminemia was related with nonsurvival. A review of inflammatory bowel diseases of the horse identified cases of granulomatous enteritis, multisystemic eosinophilic epitheliotropic disease, lymphocytic-plasmacytic enterocolitis, and idiopathic eosinophilic enterocolitis.[142] Some other potential gastrointestinal causes of weight loss and hypoproteinemia in horses include

BOX 7.3 Mechanisms and Selected Differential Diagnoses for Decreased Body Weight

Mechanism	Differential Diagnoses
Lack of access to appropriate food	Unappetizing or inappropriate types of feed Poor-quality feed Insufficient quantity of feed Inability to access the available feed: Lameness, other musculoskeletal abnormality Social factors within the herd ("pecking order")
Lack of ingestion of available nutrients	Lack of appetite Inadequate prehension Nigropallidal encephalomalacia Masseter myodegeneration Abnormal mastication Poor dentition Masseter myodegeneration Abnormal swallowing Dysphagia Abnormal esophageal transit Esophageal abnormalities
Abnormal digestion, absorption, or metabolism of nutrients	Gastrointestinal dysfunction Gastrointestinal ulceration Inflammatory bowel disorder Neoplasia Parasitism Hepatic dysfunction Toxicities
Inadequate delivery of nutrients to peripheral tissues	Cardiovascular disease Heart failure Respiratory disease Recurrent airway obstruction (chronic obstructive pulmonary disease) Hepatic disease
Increased rate of protein and energy use or loss	Unusual levels of physical activity Late gestation, early lactation Adaptation to environmental conditions (e.g., cold) Neoplasia Infection, inflammation Pneumonia Pleuritis Peritonitis Equine infectious anemia Pain Heart failure Endocrine disorder Pituitary pars intermedia dysfunction Gastrointestinal disease Renal disease
Primary muscle wasting disorders	Neurogenic muscle atrophy Primary muscle disease Equine motor neuron disease Immune-mediated myopathy Polysaccharide storage myopathy

parasitism, right dorsal colitis, and proliferative enteropathy due to *Lawsonia intracellularis.*

Other important causes of weight loss include internal abscessation, neoplasia, and chronic organ dysfunction.[141,143-149] Internal abscesses can be difficult to diagnose. Although a multitude of organisms may be involved, *Streptococcus equi* subspecies *equi* (strangles) and *Corynebacterium pseudotuberculosis* (pigeon fever) are particularly important. Weight loss is a common clinical finding in horses with either chronic hepatic or renal disease, and weight loss has also been recognized with heart failure. In some cases, recurrent airway obstruction can also be associated with significant weight loss. Several neoplastic conditions can cause weight loss, and in horses, lymphosarcoma is particularly common.

Endocrine disorders have also been associated with changes in body weight. In horses, PPID is a common disorder that can be associated with either abnormal fat deposition or weight loss and muscle wasting.[115,140] Although uncommon, hyperthyroidism has been documented in horses, with weight loss being a major feature.[135,136,150,151] Rarely, diabetes mellitus has been identified in horses.[152]

Weight loss can be a component of a variety of other conditions. Persistent infection with equine infectious anemia virus can result in weight loss, although many cases are subclinical.[153] Several toxins have been associated with weight loss through a variety of mechanisms. For example, yellow star thistle toxicity causes significant dysphagia, and pyrollizidine alkaloids and alsike clover cause hepatic disease. Some other potential toxic causes of weight loss include selenium, lead, wild jasmine *(Cestrum diurnum),* and hairy vetch *(Vicia villosa),* among others. Chronic pain may also result in weight loss.

Muscle atrophy may occur either as a component of generalized weight loss, as in starvation and cachexia, or as a separate entity. Muscle loss is often prominent in association with aging and PPID. Some direct causes of muscle atrophy include disuse and neurogenic muscle atrophy. Progressive muscle wasting and weight loss are prominent features of equine motor neuron disease, which is a neurodegenerative disorder linked to vitamin E deficiency.[154] Other signs include weakness, muscle fasciculations, shifting weight, and increased recumbency. Also, rapid muscle atrophy affecting primarily the epaxial and gluteal muscles has been associated with immune-mediated myositis, particularly in Quarter Horses.[155]

Diagnostic Approach to Weight Loss

Signalment and History. The signalment and history should be considered when evaluating a horse with weight loss. Some conditions, such as certain dental disorders and PPID, are more common in older horses. *Lawsonia intracellularis* primarily affects weanling-age foals.

An accurate diet history is particularly important. The type, amount, and quality of feed should be documented, and it should be determined whether additional micronutrients or supplements are being fed. It is important to ensure that assessment of the amount is accurate by weight, because many owners feed by volume. The age, use, and condition of the horse should be considered when evaluating the feeding program because these may affect nutritional requirements. The feed should be evaluated for the presence of mold or toxic plants. The potential availability of vitamin E in the diet should be considered. Feed analysis can often be beneficial in assessing feed quality. The particulars of the feeding management should be determined, such as the feeding schedule, the types

of feeders, potential access to sand, and the water source. It should be established if there are other horses on the property and if so whether there is competition among horses for feed and if the weight loss affects more than one individual. Also, the appetite of the horse should be determined.

General historical information is also important when evaluating weight loss. This includes the history of deworming, dental care, and any use of medications, such as nonsteroidal antiinflammatory drugs. Any history of abnormal shedding, as well as any history of previous disease, such as strangles, pigeon fever, or laminitis, can be relevant.

Physical Examination. A complete general physical examination should be performed. The BCS and, if possible, the body weight should be determined. Observing the animal while eating can be important to establish whether it can prehend, masticate, and swallow normally. Neonatal foals should be observed suckling and evaluated for milk coming from the nostrils. The mare's udder and the milk should be examined. The clinical examination should include a thorough oral examination, rebreathing examination, and rectal palpation. A neurologic examination may also yield useful information. The presence of concurrent clinical signs, such as fever, diarrhea, cough, nasal discharge, a heart murmur, polyuria/polydipsia, icterus, and abnormal shedding or hypertrichosis, can point more quickly to a specific cause of weight loss and help direct additional testing.

Ancillary Diagnostic Tests. A number of ancillary tests can be useful in the diagnosis of weight loss, and tests should be prioritized based on the signalment, history, and physical examination findings.

CLINICAL PATHOLOGY/LABORATORY TESTING. A CBC, fibrinogen, and serum chemistry can be useful and is generally part of the minimum database. The white blood cell count and fibrinogen may be elevated with active infection. Several parameters may support a diagnosis of chronic inflammation including anemia, hyperfibrinogenemia, hyperglobulinemia, and thrombocytosis. These changes may support internal abscessation. Hypoproteinemia, and particularly hypoalbuminemia, is seen with protein losing conditions, such as gastrointestinal or renal disease and third space loss, or with decreased protein production, which can be seen with significant liver disease. A common cause of hypoalbuminemia in horses with weight loss is protein losing enteropathy. The serum chemistry may support a diagnosis of renal or hepatic disease, and a urinalysis can help in the evaluation of renal disease. The presence of hypercalcemia may indicate possible renal disease, hyperparathyroidism, or neoplasia. However, it should be remembered that hypercalcemia of malignancy is an inconsistent finding in horses with neoplasia.

Parasitism can contribute to weight loss even if it is not the primary problem. Assessment of parasite status should include fecal flotation with a fecal egg count; a Baermann analysis and tapeworm enzyme-linked immunosorbent assay (ELISA) should be considered. Feces should also be assessed for the consistency and fiber length, as well as the presence of sand.

Sampling of the airway via transtracheal aspirate or bronchoalveolar lavage can be useful in evaluating pulmonary disease. Culture and cytology of pleural or peritoneal fluid can also be useful; however, it should be remembered that many neoplastic conditions do not exfoliate significant numbers of cells, making diagnosis of neoplasia often challenging. Aspiration or biopsy of appropriate tissues may be indicated when possible.

Additional diagnostic testing, such as serum or whole blood trace mineral analysis, determination of vitamin E concentrations, thyroid testing, and testing for PPID, may be indicated for some horses with weight loss, determination of vitamin E concentrations, thyroid testing, and testing for PPID. Baseline concentrations of thyroid hormones often do not accurately reflect thyroid status due to the number of confounding variables, and additional testing is generally recommended.[150,151,156-158]

ENDOSCOPY. Endoscopic evaluation may aid in the evaluation of pharyngeal function and guttural pouch disease. It can also be helpful in assessing the esophagus and examining the stomach for equine gastric ulcer syndrome or gastric squamous cell carcinoma.

DIAGNOSTIC IMAGING. Imaging of the thorax by ultrasound and radiographs can be useful in the evaluation of thoracic disease. Ultrasound can be used to assess the abdomen and can help to assess fluid volume and character, intestinal wall thickness, and abdominal masses. Abdominal radiographs may be useful in foals, but in adult horses they are often impractical, and the diagnostic utility is generally limited to the identification of enteroliths or excessive sand accumulation. Echocardiography may be indicated especially in cases where a murmur or arrhythmia is identified on physical examination.

OTHER. Oral absorption tests using either D(+)-xylose or glucose to identify malabsorption can be useful in the evaluation of unexplained weight loss.[142,159,160] Although xylose is less affected by metabolic factors, glucose absorption is a valid, practical means of assessing malabsorption. Intestinal biopsy is often necessary to identify the specific cause of the malabsorption.

COUGH

Cough is a sudden, forceful expulsion of air through the glottis that generates a characteristic sound. Although cough is a normal reflex mechanism that is important in protection of the airways, it is also a common sign of disease in multiple species. Cough promotes ciliary activity and generates the high air flows necessary to shear mucus from the airway walls, thus acting to remove secretions and foreign particles from the respiratory tract and prevent pulmonary aspiration. Specific data related to the importance of an intact cough mechanism in horses are limited, but in human patients an inefficient cough reflex has been associated with atelectasis and recurrent pneumonia.[161-163] Although cough is an important protective mechanism, excessive cough is a common sign of disease and can have deleterious effects. In human medicine, cough has been estimated to account for up to 40% of outpatient practice.[164-166] Cough is also a common clinical problem in horses. Even when not associated with significant systemic disease, cough can be performance limiting in the equine athlete.

Mechanisms of Cough

Cough is a highly coordinated reflex action that can be modulated by voluntary input. All mammalian species that have been studied either cough or display a similar respiratory reflex.[167] Despite the prevalence and clinical importance of cough, the precise mechanisms involved are not fully understood. Much of the information reported regarding the mechanism of cough in horses has been extrapolated from other species. Some species-related differences, however, are known

to exist.[167] Age and gender are also known to influence cough. The cough reflex can be poorly developed in neonates and can be compromised in association with aging.[168,169] These age-related differences may contribute to an increased susceptibility to aspiration and respiratory infections in these age groups. Interestingly, in humans an increased sensitivity of the cough reflex has been recognized in females following puberty, and women are more likely to develop chronic cough.[170-172]

Cough Cycle

There are three phases of the cough cycle: inspiratory, compressive, and expiratory.[173-175] These phases are generally followed by a period of relaxation. In the inspiratory phase, inhalation essentially generates the volume necessary for an effective cough. A variable amount of air is inhaled and serves to lengthen the expiratory muscles, optimizing the length-tension relationship. This expansion of lung volume allows for an increase in the subsequent velocity of expiratory airflow due to greater force of contraction from the lengthened muscles and greater elastic recoil pressure.

The compressive phase of cough consists of a very brief (200 msec) closure of the glottis to maintain lung volume as isometric contraction of the expiratory muscles occurs. Muscles of the chest wall, diaphragm, and abdominal wall contract against the closed glottis, resulting in a rapid rise in intrathoracic pressure.

The expiratory phase starts with opening of the glottis as the expiratory muscles continue to contract, resulting in the release of air. There is a brief period of supramaximal expiratory flow, sometimes referred to as the *cough spike,* which is then followed by lower expiratory flows. Due to relative changes in pleural and airway pressures there is dynamic compression of the larger airways during expiration, which helps to maximize the velocity of airflow by decreasing the airway diameter. The high-velocity turbulent airflow results in airway vibration, which is largely responsible for the sound recognized as cough. Importantly, the high flows generated in the expiratory phase dislodge mucus and facilitate the removal of mucus and debris from the tracheobronchial tree. At the end of cough, expiratory muscles relax, intrapleural pressure decreases and transient bronchodilation may occur.

The specific pattern of cough can vary and is sometimes broadly categorized into two types.[173] Laryngeal cough, which functions primarily to protect the airway from aspiration, is triggered by mechanical stimulation of laryngeal receptors and is a true reflex, sometimes referred to as the *expiratory reflex*. The inspiratory phase is minimal, and expiration occurs rapidly. Tracheobronchial cough is stimulated primarily by receptors distal to the larynx and has a more prominent inspiratory phase in order to generate the greater airflows necessary to remove secretions and debris from the tracheobronchial tree. It may occur either as a reflex or by voluntary control.

Neurophysiology of Cough

Coughing is a complex visceral reflex that can be both voluntarily and involuntarily regulated.[176-179] The neurologic control of cough is still under investigation, and an improved understanding has therapeutic implications for the control of cough. Basically, cough can be compartmentalized into three components: (1) stimulation of sensory afferent nerves, (2) integration of the information in the brainstem and higher brain centers, and (3) activation of efferent pathways to the muscles involved in the generation of cough[176-179] (Fig. 7.2).

Afferent Pathways Regulating Cough. Cough is initiated by the activation of sensory vagal afferent nerves that primarily innervate the respiratory tract, although they may be found in additional sites such as the ear in some species.[178-182] Though all vagal in origin, this population of afferent nerves is not homogeneous, and several subtypes have been identified. Some of the differences between subtypes include their distribution within the respiratory tract as well as the presence of myelination, the receptor type and sensitivity to various stimuli, and the location of the cell bodies, which can be within either the nodose and/or jugular ganglia. In general, these nerves respond to chemical and/or mechanical stimuli, with different thresholds for the various stimuli. There is no single classification scheme, but the subtypes are often functionally classified broadly as being either *primarily* sensitive to mechanical stimuli (mechanosensors) or to chemical stimuli (chemosensors, sometimes referred to as nociceptors). Although both types can be located throughout the airways, mechanosensors tend to predominate in the larynx and large airways. Among the most studied subtypes of sensory afferents are the rapid-adapting receptors (rapidly adapting stretch receptors), slow-adapting receptors (slowly adapting stretch receptors), and C-fibers. Several other receptors, including a possible specific cough receptor, have been described. Overall, the relative contribution of the various subtypes in the generation of cough is still uncertain and may be species dependent. These nerves often contribute to other functions within the respiratory tract, such as enhancing mucus secretion, initiating bronchoconstriction, and influencing the rate and depth of respiration.

RAPID-ADAPTING RECEPTORS AND SLOW-ADAPTING RECEPTORS. Rapid-adapting receptors (RARs) and slow-adapting receptors (SARs) are myelinated nerves that originate primarily in the nodose ganglion.[178-181] RARs are present in both extrapulmonary and intrapulmonary airways, whereas SARs are predominantly within the intrapulmonary airways and lung parenchyma and are sparse in extrapulmonary airways. Although there are some differences in the exact stimuli they respond to, they are both predominantly low threshold mechanosensors that may respond to a variety of stimuli such as changes in lung volume and changes in airway diameter due to smooth muscle constriction, airway wall edema, or the presence of mucus.

C-FIBERS. The majority of vagal airway afferent nerves are unmyelinated C-fibers that originate primarily in the jugular ganglion.[178-181] They innervate the epithelial layer throughout the extrapulmonary and intrapulmonary respiratory tract. A type of nociceptor, C-fibers are predominantly chemosensors that respond to a wide variety of stimuli including both high and low temperatures, nonisotonicity, low pH, irritants, and inflammatory mediators. They can have multiple receptors, including neuronal transient receptor potential (TRP) channels such as ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1).[183,184] Most C-fibers are known to react with capsaicin, and it appears that this occurs primarily through the TRPV1 receptor. Also, the TRP receptors may be important in disease states as they can be activated by reactive oxygen species generated during inflammation, and TRP channel antagonists are being investigated as potential antitussive agents.[185-187] PGE_2, which is released in association with airway inflammation, acts directly on pulmonary C-fibers to increase their sensitivity and lower the cough threshold.[188]

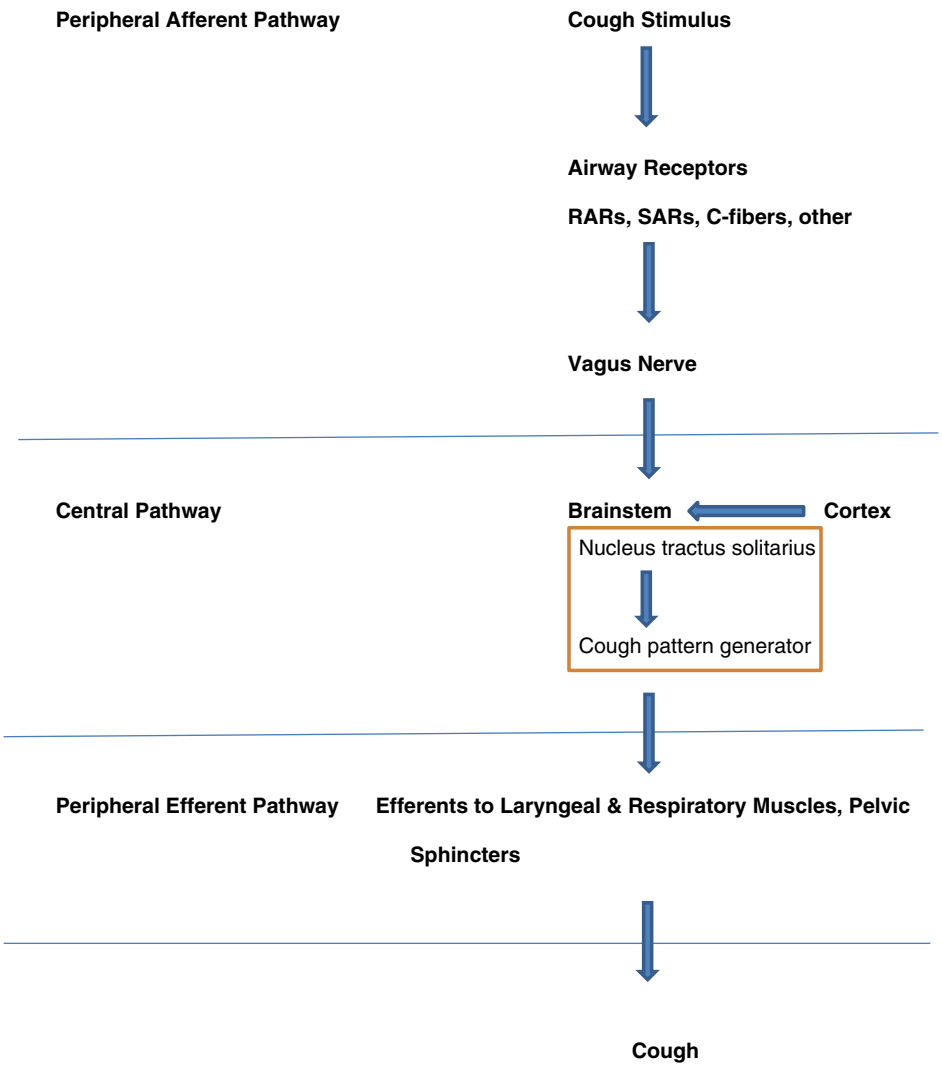


FIG. **7.2** Simplified schematic of the neurophysiology of cough.

The initiation of cough is a complex activity with multiple interactions between the afferent nerve subtypes.[178-181,189] For example, activated C-fibers release neuropeptides including substance P, neurokinin A, and calcitonin gene–related peptide. These neuropeptides can cause bronchospasm, edema formation, and mucus secretion, which in turn can lead to activation of the RARs. Without the activity of the RARs and SARs, C-fibers are limited in their ability to evoke cough.

Bronchoconstriction and cough are closely interrelated but can also have distinct pathways.[190,191] Some, but not all, stimuli for cough will also cause reflex bronchoconstriction, which generally has a slower onset and longer duration than cough. Bronchoconstriction itself can be a stimulus for cough and, up to a point, can improve the efficiency of cough by decreasing airway diameter and thus increasing airflow velocity. Some therapies affect either bronchoconstriction or cough, whereas others affect both. For example, some bronchodilator drugs can also decrease cough by desensitizing airway receptors that initiate cough.

Central Regulation of Cough. The mechanisms controlling the central regulation of cough are complex, involving both the brainstem and higher brain centers.[177,179,192-194] The afferent sensory nerve input arising from the respiratory tract is centrally integrated primarily within the nucleus tractus solitarius (nTS) of the medulla oblongata in the brainstem. Neurons within the nTS communicate with other brainstem neurons as part of a multifunctional, dynamic neural network that functions in both generation of the normal breathing pattern and cough. This neural network has been referred to as the cough pattern generator as well as the respiratory pattern generator or central controller. When cough occurs, there is reshaping of the discharge patterns of respiratory neurons within this neural network resulting in discharges being transmitted to the motor neurons involved in cough. Projections between the brainstem and cortical centers add complexity to the regulation of both breathing and cough. Due to cortical modulation, some cough can be voluntarily initiated and suppressed. Among the receptors involved in the generation of cough in the brainstem are *N*-methyl-D-aspartate (NMDA) receptors, and in several species antagonists of NMDA-type glutamate receptors such as dextromethorphan have been effective antitussives.[195,196]

Activation of Efferent Pathways. Signals from the brainstem and cerebral cortex are transmitted via a number of efferent nerves to the muscles involved in cough.[173,178] Important efferent nerves include the vagus, phrenic, intercostal, and

lumbar nerves. In addition to the vagus, several other cranial nerves play a role, including the trigeminal, facial, hypoglossal, and accessory nerves. The efferent nerves involved in cough supply the muscles of the larynx and tracheobronchial tree, the diaphragm, and the intercostal, abdominal, and pelvic muscles, as well as secretory glands of the respiratory tract. The coordinated activity of these muscles results in cough.

Cough Efficiency/Adverse Effects. The effectiveness of cough is dependent on the ability to generate high flow velocities and the interaction of the flowing air with airway secretions.[173] In general, cough is more effective in clearing large airways as opposed to smaller peripheral airways. Cough efficiency is affected by the integrity of the neurophysiologic pathway of cough as well as physical aspects, such as respiratory muscle strength and mucus quality. Normal mucus acts to improve airway clearance and protect receptors from irritants, and changes in the amount or tenacity of mucus can both mechanically stimulate cough receptors and cause flow limitation.[173,197,198] Cough is often less effective as a clearance mechanism when airway disease is present.

Not only does cough lose its defensive function is some disease conditions, but it may even have deleterious effects.[173,199] Lung expansion during the inspiratory phase may allow for the spread of infectious agents and particulate matter into the smaller airways. Paroxysmal or persistent coughing can cause fatigue and may decrease feed intake, particularly in foals. Chronic cough can result in bronchial muscle hypertrophy. Cough can also have significant cardiovascular effects due to changes in abdominal and intrathoracic pressures.[199,200] During the expiratory phase of cough there is an initial rise in systemic arterial blood pressure and a concurrent rise in cerebral venous and cerebrospinal fluid pressures. This is followed by a period of hypotension, which can reduce the effective perfusion pressure of the brain, especially when there is high cerebral venous pressure. The resulting cerebral hypoperfusion and anoxia have been associated with cough-induced syncope in dogs as well as people.[201,202] Some other potential adverse effects of cough that have been reported in small animals and people include pneumothorax, pneumomediastinum, and lung lobe torsion, as well as rib and vertebral fractures.[199,202]

Conditions Associated with Cough

The potential causes of cough are diverse, as the cough receptors can respond to a wide variety of mechanical and irritant stimuli.[173-181,183,203] Some general stimuli of cough include inhaled particles or irritants, inflammatory mediators, bronchoconstriction, excessive mucus, exposure to cold or hot air, sloughing of airway epithelial cells, and pulmonary edema. Intramural or extramural tension on the airways, such as that seen in association with masses or decreased pulmonary compliance, can result in cough. The sensitivity of cough receptors is influenced by a number of factors, such as genetics, age, and the presence of disease. Most often, the cough reflex becomes hyperresponsive once respiratory disease is established due to both increased exposure of the cough receptors due to a loss of the integrity of the epithelial lining and increased sensitivity of the receptors in response to inflammation. It is important to remember, however, that significant respiratory disease can be present without cough.

The specific causes of cough have been categorized in a number of ways, such as the duration of signs (acute or chronic), the presence or absence of fever, whether the cough is productive or not (wet or dry), and whether it originates from the upper or lower airway. Categorizing cough can be useful in establishing a list of differentials for cough, although there can be considerable overlap between the categories. The presence or absence of fever may be particularly useful when considering the causes of cough (Box 7.4).

BOX 7.4 Causes of Cough with Fever

Common Causes	Major Diagnostic Test(s)
Viral respiratory tract disease Equine influenzavirus Equine herpesviruses Equine rhinitis viruses A and B Equine viral arteritis Adenovirus Other	Nasal or nasopharyngeal swab Polymerase chain reaction (PCR) Viral isolation Viral antigen detection Serology
Bacterial pneumonia/pleuropneumonia (including aspiration pneumonia, pulmonary abscesses)	Radiographs/ultrasound Transtracheal aspirate/thoracocentesis Cytology Culture and sensitivity PCR
Less Common Causes	**Major Diagnostic Test(s)**
Fungal pneumonia	Radiographs/ultrasound Transtracheal aspirate/thoracocentesis Cytology Fungal culture Serology
Interstitial pneumonia Equine multinodular pulmonary fibrosis Syndromes of acute lung injury, acute respiratory distress	Radiographs PCR—EHV 5 Lung biopsy
Neoplasia	Radiographs/ultrasound Endoscopy Transtracheal aspirate/thoracocentesis Cytology (often low yield) Biopsy
Smoke inhalation	History
Other—parasitic pneumonitis, cardiogenic cough	

Causes of Cough Associated with Fever

Cough in conjunction with fever is most often associated with infection of the respiratory tract, although some noninfectious conditions such as neoplasia can also occasionally present with fever. Infection of the respiratory tract may be primary or may be secondary to an underlying condition such as inflammation of the airways. Viral infections of the respiratory tract are a common cause of cough in horses and are particularly important as they can cause outbreaks of respiratory disease.[203,204] Affected horses often present with an acute onset of a dry cough in conjunction with fever, lethargy, and anorexia. Some important equine respiratory

viruses include equine influenzavirus, equine herpesviruses (EHVs), equine rhinitis A and B, and equine viral arteritis. With regard to the equine herpesviruses, EHV-4 and EHV-1 are the major pathogens associated with acute respiratory disease in the domestic horse population.[204,205] Respiratory viruses frequently cause exposure and sensitization of cough receptors, along with injury to epithelial cells and decreased mucociliary clearance, which can predispose the affected horse to secondary bacterial infection. In addition to viruses, both bacterial and fungal agents can cause infection of the respiratory tract, and are often associated with a periodic wet cough due to increased mucus production and neutrophil accumulation. Bacterial infection is particularly common in the lower respiratory tract and can cause bronchitis, pneumonia, or pleuropneumonia.[206,207] A variety of organisms have been isolated and polymicrobial infections may occur. Some common pathogens include *Streptococcus equi* subspecies *zooepidemicus, Pasteurella, Actinobacillus* spp., *Escherichia coli,* and *Klebsiella pneumoniae. Rhodococcus equi* is an important cause of pneumonia in foals. Anaerobic organisms, such as *Bacteroides fragilis* and *Peptostreptococcus anaerobius,* may also infect the lower airways of horses. *Mycobacterium* and *Mycoplasma* spp. are occasionally isolated from horses with respiratory tract disease.[206,208] Fungal respiratory disease in horses is uncommon and is most often recognized in the paranasal sinuses, guttural pouches, and lungs.[209,210] Some primary fungal pathogens that have been identified in association with respiratory disease in the horse include *Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis,* and *Conidiobolus coronatus.* Opportunistic fungi, such as *Aspergillus* spp., *Candida* spp., and *Pneumocystis carinii,* can also cause respiratory tract disease. Following recovery from infectious respiratory tract disease of any cause, horses will often have a persistent wet or dry cough as the cough receptors remain hypersensitive.

Interstitial pneumonia has been reported in horses of all ages in association with both acute and chronic respiratory disease.[211,212] Horses with interstitial pneumonia frequently have marked tachypnea with increased respiratory effort, and cough and fever can be present as well. Some specific types of interstitial pneumonias include equine multinodular pulmonary fibrosis and syndromes of acute lung injury and acute respiratory distress. Although interstitial pneumonia has been linked to a variety of causes, in many cases an underlying cause is not identified. Equine multinodular pulmonary fibrosis has been linked to EHV-5 infection.[212]

Other conditions may occasionally present with cough in association with fever. Thoracic neoplasia is uncommon in horses and presents with variable clinical signs that may include cough with or without fever.[213] Smoke inhalation may cause a significant inflammatory response and edema resulting in tachypnea, cough, and fever.[214] Fever may occasionally be present with parasitic or cardiogenic cough.

Causes of Cough Without Fever

Among the most common causes of cough without fever in horses is a spectrum of chronic inflammatory airway disease that is now referred to as *equine asthma syndrome*.[215-218] Included in this syndrome are inflammatory airway disease (IAD, mild to moderate equine asthma) and recurrent airway obstruction (RAO, severe equine asthma). Summer pasture-associated RAO (SPARAO, summer pasture-associated obstructive pulmonary disease, SPAOPD) is a subset of RAO in which signs occur after exposure to pasture in warm summer months. RAO primarily affects horses over 7 years of age. The prevalence of RAO is approximately 10% to 20%, and a familial predisposition has been identified.[219-222] Affected horses typically have frequent coughing, increased respiratory effort at rest, and exercise intolerance. With IAD, horses may be affected at any age, and the clinical signs are more subtle. Both RAO and IAD are characterized by increased mucus in the airways. With regard to the specific cytologic abnormalities in the bronchoalveolar lavage fluid (BALF), RAO is typically characterized by pronounced neutrophilia (greater than 25%), whereas in IAD the abnormalities tend to be milder and more variable. Other conditions that can resemble equine asthma clinically include silicosis, lungworm infection, and idiopathic eosinophilic pneumonia.[223-226]

It is not uncommon for horses to cough occasionally, especially at the beginning of exercise. These coughs are sometimes referred to as *arena cough, warm-up cough,* or *nuisance cough* depending on the exact circumstances. This type of cough may reflect cough's function as part of the normal defense mechanisms of the respiratory tract, as it is not uncommon for clinically normal horses to have some mucus accumulated behind the larynx waiting to be expelled. Also, horses breathe more deeply during exercise, and the environment in some riding arenas can be dusty. Although this type of cough is generally relatively innocuous, it may indicate poor air quality and may also be a sign of early disease. Thus all horses with cough should be monitored closely.

Parasitic pneumonitis is another cause of cough that is generally afebrile, although occasionally fever may be seen. In foals, the larvae of *Parascaris equorum* migrate through the lungs and can occasionally cause a significant inflammatory response resulting in cough and nasal discharge.[203] Horses infected with the lungworm *Dictyocaulus arnfeldi* can have chronic cough and mucoid nasal discharge.[224,225] Affected horses often have a history of contact with donkeys or mules. In donkeys and mules, infection is generally asymptomatic but is patent, providing a source of eggs in the feces. In horses and ponies, infection often results in an inflammatory response and cough and is typically not patent, with larval development arrested in the lungs.

Exercise-induced pulmonary hemorrhage (EIPH) is defined by the presence of hemorrhage in the airways of a horse after exercise.[227,228] It is common in Thoroughbred and Standardbred racehorses and in other horses performing strenuous exercise, such as barrel racing horses. The clinical abnormalities seen with EIPH are not consistent but may include epistaxis, increased respiratory rate, poor performance, and coughing.

Congestive heart failure, although not common in horses, can result in cough.[229,230] In particular, in left-sided heart failure, blood is not effectively pumped from the left side of the heart, leading to increased blood in the pulmonary circulation and increased pulmonary vascular pressure. Vascular congestion and pulmonary edema follow, and cough receptors are stimulated. In a study of 14 horses with congestive heart failure, all horses presented with a heart murmur and tachycardia.[229] Other common clinical signs included cough, crackles, tachypnea, ventral edema, and either jugular distention or pulsation. The underlying causes for heart failure included congenital defects, traumatic vascular rupture, pericarditis, pulmonary hypertension secondary to RAO, and valvular

dysplasia. Fever is not generally associated with cardiogenic cough but may be present in some horses, especially those with pericarditis or endocarditis.

A number of disorders of the upper airway can result in cough. Pharyngitis, also referred to as *pharyngeal* or *follicular lymphoid hyperplasia,* is a relatively common disorder that is recognized primarily in young horses.[203,231,232] It is most likely the result of the lymphoid response to a variety of irritant or infectious stimuli. Although many cases are subclinical, coughing, nasal discharge, and mild submandibular lymphadenopathy may be present. Dynamic or static compression of the upper airway such as may be seen with dorsal displacement of the soft palate, fourth branchial arch defect (rostral displacement of the palatopharyngeal arch, laryngeal dysplasia), aryepiglottic fold entrapment, subepiglottic cyst, arytenoid chondritis/chondrosis, and laryngeal hemiplegia, may also be associated with cough, which is often dry in nature.[203] Although clinical signs vary, these disorders are often characterized by abnormal breath sounds such as stridor or stertor at rest or during exercise as well as normal lung sounds and absent or minimal mucopurulent discharge. Soft palate problems, including soft palate paresis, dorsal displacement of the soft palate and cleft palate, and other causes of dysphagia, can result in cough that is often associated with eating. In some cases, disorders such as sinusitis and guttural pouch mycosis or empyema will result in cough due to the presence of exudate in the upper airways.

Tracheal abnormalities may also result in cough. These include tracheal stenosis, collapse, and partial obstruction such as may be seen with a foreign body or neoplasia. Tracheal collapse is typically associated with an inspiratory honking sound. It is most often diagnosed in American Miniature Horses but may be seen in other horses as well and has been reported in association with pneumonia.[233-235]

Diagnostic Approach to Cough

Cough is a common clinical problem that can be associated with a number of diverse disorders. The diagnosis of the specific cause of cough is based on consideration of the signalment, history, and clinical signs, as well as the use of appropriate diagnostic aids.

Signalment and History

The signalment can help to prioritize the differential diagnoses in a horse presented for cough. Pneumonia caused by *Rhodococcus equi* is most common in foals 1 to 6 months of age.[207] Although viral respiratory diseases can be seen in any age horse, they are particularly common in weanling and yearling horses.[204] Pharyngitis is also most common in young horses.[231,232] Horses with RAO are typically over 7 years of age, and a familial predisposition has been identified.[215-218] The risk of developing RAO is threefold when one parent is affected and almost fivefold when both parents are affected.[221,222]

Several components of the history are important in the assessment of a horse with cough. Routine management factors such as vaccination, deworming, stabling, and bedding type, as well as the feed type, feed quality, and feeding practices, are of particular importance. Risk factors for infectious respiratory disease include exposure to new horses, either at an event or through new arrivals to the farm, and stress, such as recent transport, surgery, weaning, or strenuous activity. A history of other clinical signs such as fever, lethargy, and anorexia, as well as the involvement of other horses, may increase the suspicion of infectious respiratory disease. The risk of lungworm infection is increased by exposure to donkeys or mules.[224,225] EIPH is associated with strenuous exercise.[227] The geographic area may raise the suspicion of some conditions such as silicosis, which has been reported primarily in California, and certain mycotic infections, such as *Coccidioides immitis,* which is found primarily in the southwestern United States.[209,210,223] Any history of previous disease, as well as a history of signs such as stridor or exercise intolerance, can also be important.

The historical aspects of the cough itself can be helpful in the evaluation of horses with cough. These include whether the cough is acute or chronic, any seasonal pattern, the frequency of the cough, and any association with feeding or exercise. It can be helpful to establish if the cough is nonproductive or productive, although this can be difficult to discern.

Physical Examination

A complete physical examination, including a detailed examination of the respiratory system, should be performed in horses presented for evaluation of cough. The character of any nasal discharge should be noted. The presence of a fever increases the likelihood of infectious respiratory tract disease, although noninfectious conditions may occasionally be associated with fever. It should be remembered that fever can be intermittent and can be suppressed by the administration of nonsteroidal antiinflammatory drugs. Careful auscultation of the thorax should be performed. The presence of a heart murmur and tachycardia may suggest congestive heart failure, especially if there is jugular distention or pulsation.[229] If the horse can tolerate it, a rebreathing examination should be performed, especially if there are no obvious abnormal sounds during quiet breathing. The rebreathing examination, which is performed by loosely placing a plastic bag over the nostrils, will cause the horse to increase tidal volume, accentuating breath sounds. Cough may be induced, especially if there is exudate in the airways. The presence of crackles and/or wheezes on auscultation suggests pulmonary parenchymal disease. With pulmonary consolidation, atelectasis, or pleural fluid, breath sounds are typically decreased or absent ventrally, although occasionally sounds may be accentuated due to referral of sounds from aerated lung. Percussion can be used to identify areas of dullness. In horses with significant RAO, percussion can be used to identify a shift of the caudal lung border.[236]

Ancillary Diagnostic Tests

Clinical Pathology. A CBC and fibrinogen are frequently indicated in the assessment of horses with cough, and in some cases a serum chemistry may also be useful. Acute viral infection can be associated with leukopenia characterized primarily by lymphopenia.[204] A transient anemia may be seen as well. Neutrophilia and hyperfibrinogenemia are seen with many inflammatory conditions, especially bacterial or fungal pneumonia.[206] Inflammatory conditions may also be associated with anemia, hyperglobulinemia, and thrombocytosis, especially if chronic. Hematologic values are usually unremarkable in horses with uncomplicated equine asthma.[216] Although not consistent, peripheral eosinophilia may be seen with idiopathic eosinophilic pneumonia, parasitic pneumonitis, and occasionally eosinophilic IAD.[216,224-226] Hypercalcemia can be seen in some horses with malignancy.

An arterial blood gas evaluates air exchange and helps to monitor the severity of respiratory disease. In some horses

with chronic or recurrent infectious disease, measurement of immunoglobulin concentrations may help identify an underlying immunodeficiency. There has been a growing interest in the use of biomarkers to evaluate disease states. Although findings are often nonspecific, serum amyloid A and fibrinogen are two biomarkers commonly used to evaluate inflammatory disease.[237] In racehorses with IAD, increased concentrations of surfactant protein D (SP-D) have been described, and in horses with RAO, soluble CD14 concentrations are increased compared with controls.[238,239]

Airway Cytology. Several techniques have been described for sampling the respiratory tract of horses.[240-246] The primary means of sampling the lower respiratory tract include bronchoalveolar lavage and tracheal wash. Tracheal wash is often used for the diagnosis of infectious pneumonia. Analysis of BALF is recommended for the diagnosis of equine asthma.[215,216] RAO is characterized by a moderate to severe neutrophilia (greater than 25%), whereas in IAD the changes are more varied and may include a mild to moderate increase in neutrophils (greater than 10%), eosinophils (greater than 5%), and/or mast cells (greater than 5%). BALF fluid cytology is also useful in the diagnosis of EIPH, which is characterized by the presence of erythrocytes and hemosiderophages.

Assessment of Infection. Currently a nasal or nasopharyngeal swab is the preferred sample for diagnosis of most viral respiratory infections.[204] A Dacron or rayon swab is preferred compared with cotton. Detection of genetic material by PCR is a common means of virus identification, although virus isolation can be performed as well. There are also several means of viral antigen detection. For example, direct immunofluorescence can be used to demonstrate EHV antigens and several enzyme-linked immunosorbent assays are available for the detection of influenza-virus nucleoprotein.

Appropriate samples for the identification of bacterial or fungal agents in respiratory tract disease depend on the suspected site of infection and organism. Common techniques for sampling the upper airways include nasal or nasopharyngeal swab, nasal wash, guttural pouch wash, and sinus trephination. Transtracheal aspiration is commonly used for sampling the lower airway in cases of suspected pneumonia. Other techniques include endoscopic tracheal aspiration or brushing and sterilely performed BAL. Pleural fluid can be sampled by thoracocentesis. Cytology and Gram stain of the samples can support the presence of infection. Culture and PCR are used for identification of specific pathogens. Many laboratories now offer diagnostic panels for respiratory disease that test for multiple common pathogens.

Serologic testing is available to detect the host response to several infectious agents. Depending on the organism, however, it may be difficult to distinguish active infection from exposure or vaccination. Serology can be useful in the diagnosis of several fungal infections, such as cryptococcosis, coccidiomycosis, blastomycosis, and conidiobolomycosis.[209,210]

The Baermann fecal flotation technique can be used in horses with suspected lungworm infection. However, as infections in horses are often not patent, a negative Baermann does not rule out the presence of lungworms.[224,225] Routine fecal flotation can be used to detect *Parascaris equorum* in foals with suspected parasitic pneumonitis, but in some cases the infection may not yet be patent.

Endoscopy. Endoscopic examination is particularly valuable in evaluation of the upper airways and trachea. Because sedation can alter airway function, endoscopy is best performed without sedation if possible. Endoscopy can identify exudate, anatomic or functional abnormalities, mass lesions, and airway obstruction. On occasion, lungworm larvae may be seen in the trachea. Pleuroscopy can be used to evaluate the pleural space.

An endoscopic mucus scoring system has been developed to quantify mucus in the trachea (0—no visible mucus, 1—single to multiple small blobs of mucus, 2—larger but nonconfluent blobs, 3—confluent or stream forming mucus, 4—pool forming mucus, 5—profuse amounts of mucus).[247] Normal horses tend to be either grade 0 or 1, whereas those with equine asthma are 2 to 5.[216,248] Studies have shown associations between the amount of mucus present in the airways with coughing and poor performance.[232,249-251]

Diagnostic Imaging. Radiographs and ultrasound are useful in the evaluation of horses with either suspected upper or lower airway cough.[246] Radiographs of the upper airway can demonstrate fluid accumulation, soft tissue masses, and abnormalities of pharyngeal and laryngeal structures. Ultrasound can be used to evaluate laryngeal problems such as left laryngeal hemiplegia, laryngeal dysplasia, arytenoid chondritis, and congenital malformations. Thoracic ultrasonography is a practical means of assessing the thorax and can provide diagnostic information about the pleural cavity, lung, and mediastinum. The presence and character of pleural fluid can be determined, and abnormalities such as consolidation and abscessation in the periphery of the lung can be identified. In addition, the caudal lung borders can be determined. If congestive heart failure is suspected, the heart can be evaluated by echocardiography. Thoracic radiography can identify abnormalities of the pleural space, pulmonary parenchyma, mediastinum, and diaphragm. In the lungs, pulmonary consolidation, abscesses, peribronchial disease, and interstitial disease may be evident.

Other Diagnostic Aids. Several additional diagnostic tests can be helpful in the evaluation of equine asthma, especially pulmonary function testing.[215-218,249,252] Other tests that may be useful include a hay challenge to induce disease, histamine provocation tests to assess airway reactivity, and bronchodilator response testing, typically with atropine, *N*-butylscopolammonium bromide, or albuterol. Although allergen testing has limitations in horses, it may be useful in some cases to identify specific triggers. Endobronchial biopsy specimens have also been used in the evaluation of horses with asthma.[253] Percutaneous lung biopsy, although not commonly performed, can be used to evaluate respiratory disease.[254] Both histologic and microbiologic evaluation of tissue may be useful. Lung biopsy may be particularly helpful in the diagnosis of pulmonary neoplasia, equine multinodular pulmonary fibrosis, and silicosis.

RESPIRATORY DISTRESS

Respiratory distress is defined as labored breathing and is characterized by an inappropriate degree of effort to breathe based on rate, rhythm, and subjective evaluation of respiratory effort.[255] *Dyspnea* refers to the sensation of arduous, uncomfortable, or difficult breathing that occurs when the demand for ventilation exceeds the patient's ability to respond.[256] Because dyspnea describes a subjective feeling, it is technically a symptom rather than a clinical sign and thus not strictly applicable in veterinary medicine although the term is often used. The clinical signs of respiratory distress vary with the severity and

origin of impaired gas exchange. Clinical signs commonly observed in horses with respiratory distress include flared nostrils, exercise intolerance, inactivity, exaggerated abdominal effort, abnormal respiratory noise (stertor or stridor), anxious expression, extended head and neck, and synchronous pumping of the anus with the respiratory cycle.[255,257,217] In severe cases, cyanosis may be seen. Horses with chronic respiratory distress may develop a heave line resulting from hypertrophy of the cutaneous trunci and abdominal muscles, which assist during forced expiration.[257,217] Weight loss may be seen in association with decreased feed intake and a high energy demand for respiration. In fact, it has been shown that in horses with recurrent airway obstruction (RAO), which is part of the syndrome of equine asthma, the work of breathing has the potential to approximate the energy expenditure of a horse trotting for 12 hours a day.[258,216]

Mechanisms of Respiratory Distress

Problems with both ventilation, which is the process of moving air into and out of the lungs, and respiration, which is the process of gas exchange, can impair the efficient exchange of oxygen and carbon dioxide leading to respiratory distress. Common causes of respiratory distress include primary pulmonary disease, airway obstruction, or impairment of the muscles and supporting structures necessary for ventilation.[255,256,259] In some horses, respiratory distress can occur in the absence of impaired gas exchange in response to pain, metabolic acidosis, or high environmental temperature. Familiarity with the mechanics of breathing and control of ventilation in healthy and diseased lungs facilitates the diagnosis and treatment of respiratory distress.[217,257]

Control of Ventilation

The partial pressure of oxygen (Pa_{O_2}) and the partial pressure of carbon dioxide (Pa_{CO_2}) in arterial blood are maintained within a narrow range through rigid control of gas exchange.[256] The control of ventilation is complex but essentially involves three components: (1) stimulation of sensory afferents via chemoreceptors and mechanoreceptors, (2) integration of the information in the brainstem, and (3) efferent signals to the muscles of respiration[256,260-262] (Fig. 7.3). Within the brainstem, ventilation is controlled by a complex neuronal network known as the central controller or respiratory pattern generator, which is the same neuronal network involved in the cough reflex. In response to afferent signals, the central controller alters the rate and depth of respiration via efferent signals to the muscles of respiration. The central controller therefore adjusts alveolar ventilation to the metabolic rate of the individual. Although this process is involuntary, higher brain centers can influence ventilation, and there can be voluntary, conscious control of ventilation.

Neuromuscular Physiology of Ventilation

SENSORY AFFERENTS. Afferent input into the central respiratory centers in the brainstem arises primarily from three groups of neural receptors: (1) peripheral arterial chemoreceptors, (2) central chemoreceptors in the brainstem, and (3) chemoreceptors and mechanoreceptors in the respiratory tract[260-264] (see Fig. 7.3). In addition, mechanoreceptors in the diaphragm and thoracic wall have some influence on the level and timing of ventilatory activity in response to changes in length, tension, or movement. All of these receptors collectively provide information to the central controller, resulting in the modification of ventilation.

CENTRAL AND PERIPHERAL ARTERIAL CHEMORECEPTORS. The major chemoreceptors include the central chemoreceptors in the ventral medulla of the brainstem and the peripheral chemoreceptors in the arterial vasculature.[256] These chemoreceptors identify changes in metabolism and oxygenation, providing feedback to the central controller and thus influencing ventilation.

The central chemoreceptors monitor alterations in the pH of intracerebral interstitial fluid and cerebrospinal fluid. As the blood-brain barrier is impermeable to bicarbonate and hydrogen ions but is freely permeable to carbon dioxide, acidification of the intracerebral interstitial fluid and thus stimulation of the central chemoreceptors occur predominantly in response to hypercapnia and respiratory acidosis. The severity of acidosis in the intracerebral interstitial fluid caused by hypercapnia is amplified by two features of the central nervous system: (1) hypercapnia produces cerebral vasodilation, increasing the delivery of CO_2 to the central nervous system, and (2) cerebrospinal fluid has poor buffering capacity because of low total protein concentrations.[256]

Peripheral chemoreceptors include the carotid bodies, situated at the bifurcation of the common carotid artery, and the aortic bodies, which are located near the aortic arch.[256] Carotid bodies are generally considered the primary peripheral chemoreceptor, but the aortic bodies can also influence ventilation. These receptors relay information to the central controller regarding arterial gas tensions via the glossopharyngeal and vagus nerves. Their responsiveness to alterations in Pa_{CO_2} is less consequential than the central chemoreceptors. They also can have a slight response to metabolic acidosis. The peripheral chemoreceptors are highly sensitive to changes in the partial pressure of oxygen, however, and are solely responsible for the hypoxic ventilatory drive. The peripheral chemoreceptors demonstrate a nonlinear response to low arterial oxygen tension. They are insensitive to alterations in Pa_{O_2} above 100 mm Hg, exhibit moderate response to arterial O_2 tensions between 50 and 100 mm Hg, and demonstrate a dramatic increase in responsiveness when the partial pressure of oxygen falls below 50 mm Hg in the arterial circulation.[256] The respiratory pattern elicited by hypoxia differs from that stimulated by hypercapnia.[265,266] Hypoxia evokes an increase in respiratory frequency, whereas hypercapnia triggers an elevation in tidal volume. In addition, hypoxia stimulates recruitment of the inspiratory muscles, whereas hypercapnia potentiates the activity of inspiratory and expiratory muscles.

The sensitivity of peripheral chemoreceptors should be considered in the treatment of patients with complex acid-base and blood-gas abnormalities. A patient suffering simultaneously from impaired gas exchange caused by pulmonary disease and metabolic acidosis resulting from shock can manifest respiratory distress in response to hypoxemia, hypercapnia, and acidosis. Oxygen supplementation likely will improve the patient's arterial oxygen tension; however, such treatment may abolish the hypoxic ventilatory drive and consequently slow the ventilatory rate. This decreased ventilation could exacerbate respiratory acidosis and result in decompensation of the patient.[259] Although treatment of metabolic acidosis may be indicated, it can also contribute to respiratory acidosis by ultimately increasing CO_2. Thus to prevent life-threatening acidemia, treatment of respiratory acidosis, often by assisted ventilation, may be indicated depending on the specifics of the case.

Respiratory Tract Receptors. Receptors located in the upper and lower respiratory tract respond to mechanical and

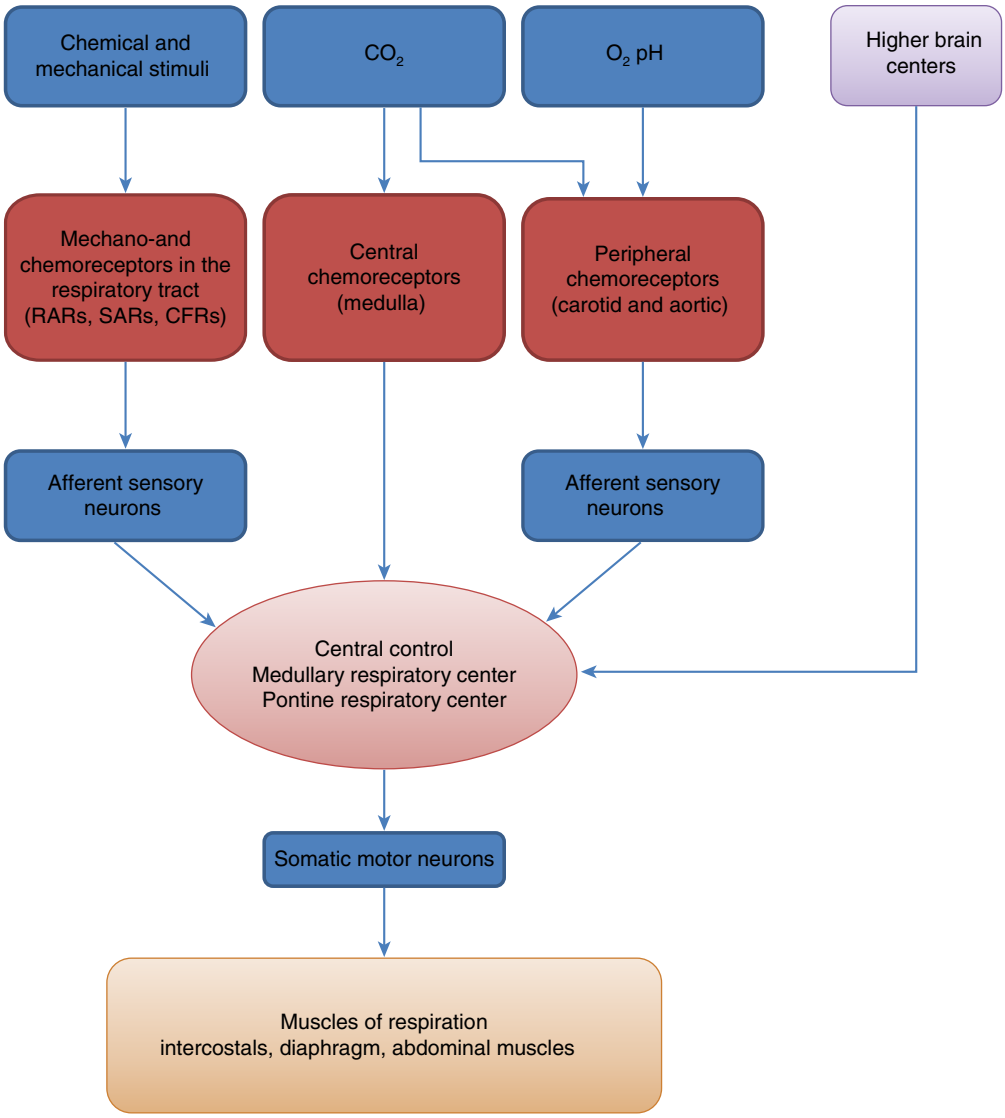


FIG. **7.3** Simplified schematic outlining the control of ventilation. *CFRs,* C-fiber receptors; *RARs,* rapid-adapting receptors; *SARs,* slow-adapting receptors.

chemical stimuli and relay afferent information to the central controller of respiration via the vagus nerve.[255,256,263,264] Vagal blockade abolishes tachypnea in horses with pulmonary disease; therefore these receptors are likely to play an important role in development of respiratory distress associated with primary pulmonary disease.[267-269]

As discussed earlier, some of the major respiratory tract receptors influencing ventilation are the *slow-adapting receptors* (SARs, slowly adapting stretch receptors), *rapid-adapting receptors* (RARs, rapidly adapting stretch receptors), and *C-fiber receptors* (CFRs).[261,263,264] These receptors also function in mucus secretion, bronchoconstriction, and the generation of cough.[180] The SARs, also known as pulmonary stretch receptors, are located primarily within smooth muscle fibers in the walls of the trachea and bronchi and are sparse in extrapulmonary airways.[256,259,263] The SARs are primarily mechanosensors and are stimulated in part by pulmonary inflation, inhibiting further inflation of the lung (Hering-Breuer reflex). Conversely, at end expiration these receptors stimulate inspiratory activity. These receptors are considered to be partially responsible for controlling the depth and rate of respiration.

The RARs are found in and under the epithelium of the respiratory tract from the nasopharynx to the bronchi, and their pattern of response varies with their location.[256,264] Although they are highly sensitive to mechanical stimuli, those in the bronchi are more chemosensitive and are sometimes referred to as *irritant receptors*. The wide variety of stimuli they may respond to include exogenous agents such as smoke, irritant gases, and dust, as well as endogenously produced inflammatory mediators, including histamine and prostaglandins. RARs are not likely to function in regulation of breathing in

a normal resting horse.[259] Stimulation of these receptors by noxious stimuli triggers bronchoconstriction, cough, tachypnea, mucus production, and release of inflammatory mediators.[255,256,180] In horses with RAO (severe equine asthma), the production of histamine, prostaglandins, and other inflammatory mediators increases, and the resulting stimulation of RARs by these inflammatory mediators may be in part responsible for the bronchoconstriction, mucus production, and tachypnea observed in horses with this disorder.[217,270-272] In addition to their role as chemoreceptors, the RARs or irritant receptors also function as mechanoreceptors throughout the airways.[263,264] An abrupt change in end-expiratory lung volume, such as occurs with pneumothorax or pleural effusion, can produce tachypnea in response to stimulation of these receptors. Increased negative pressure (upper airway obstruction) within the airway stimulates the mechanoreceptors of the larynx and produces prolongation of inspiratory time and activation of upper airway dilator muscles.[273,274]

Unmyelinated C-fibers innervate receptors found in the epithelial layer throughout the intrapulmonary and extrapulmonary respiratory tract.[256,263] C-fiber receptors are predominantly chemosensors, although they may also respond to hyperinflation of the lung. These receptors respond to pulmonary edema, congestion, and inflammatory mediators, and stimulation activates tachypnea. In addition, C-fiber receptors may stimulate the release of pulmonary neuropeptides, which produce bronchoconstriction, vasodilation, protein extravasation, and cytokine production. C-fiber receptors located within the alveolar walls in juxtaposition to pulmonary capillaries are sometimes referred to as juxtacapillary receptors. Information from other species suggests that these receptors can respond to increased interstitial fluid volume and may be involved in the sensation of difficult breathing.[256]

CENTRAL CONTROL OF VENTILATION

Brainstem Control. The mechanisms involved in the central control of ventilation are still not completely understood. The central controller of the brainstem integrates signals from the sensory afferent neurons and then initiates phasic activity of the diaphragmatic, intercostal, and abdominal respiratory muscles.[256] Although it is a complex neuronal network, the central controller consists of two basic regions with several subregions: (1) the medullary respiratory center in the reticular formation, which includes the ventral and dorsal respiratory groups, and (2) the pontine respiratory center, which includes the pneumotaxic and apneustic centers. The medullary respiratory center controls the rhythmic pattern of respiration. The dorsal respiratory group helps to coordinate inspiratory activity by assimilating afferent information from the glossopharyngeal and vagus nerves and transmitting efferent signals to the muscles of inspiration and neurons in the ventral respiratory group. The ventral respiratory group consists of inspiratory and expiratory motor neurons. One subregion, the pre-Botzinger complex, appears to have a major role in generating the basic breathing rhythm.[260-262,275] Closely associated neurons in the retrotrapezoid nucleus and parafacial respiratory group are important in regulating expiration.[275,276] The ventral respiratory group also helps to regulate ventilation during exercise. In the pontine respiratory center, the apneustic center provides stimulatory input to inspiratory motor neurons, activating and prolonging inhalation.[260-262] Damage to this region, which can result from problems such as trauma or neonatal encephalopathy, results in prolonged inspiratory gasps interrupted by transient expiratory efforts.[259] The pneumotaxic center, also in the pons, inhibits the inspiratory centers and helps to regulate the volume and rate of respiration. The pneumotaxic center is not required to maintain a normal respiratory rhythm; instead, this center functions to fine-tune the respiratory rhythm, receiving afferent input from the vagus nerve regarding Pao_2, $Paco_2$, and pulmonary inflation.

Input of Higher Brain Centers. Ventilation is primarily under involuntary control via the brainstem, but higher brain centers can also play a role.[260-262] Conditions that influence ventilation via higher brain centers include the emotional state, via input from the limbic system, and temperature, via input from the hypothalamus, as well as conscious control. Conscious or voluntary control of ventilation, which is centered in the cerebral cortex, can be overridden by the chemoreceptor reflex.

EFFECTORS OF VENTILATION. The muscles required for ventilation include the diaphragm, the external and internal intercostal muscles, and the abdominal muscles.[256] Horses have a somewhat unique biphasic inspiratory and expiratory airflow pattern in which there is both a passive and an active phase to inspiration and expiration.[277] The single most important muscle required for the inspiratory phase of the respiratory cycle is the diaphragm.[278] The relaxation volume is the static equilibrium volume of the relaxed respiratory system at which the net elastic recoil pressure is zero. In horses, the first part of inhalation following exhalation is passive until the relaxation volume is reached, at which point the diaphragm and external intercostal muscles complete the inspiratory phase. Contraction of the diaphragm forces the abdominal contents back, increasing the length of the thoracic cavity, and pulls the ribs abaxially, increasing the width of the abdominal cavity. In addition, the external intercostal muscles participate in inspiration by pulling the ribs abaxially to increase the width of the thoracic cavity. The net effect is an increase in the size of the thoracic cavity, producing subatmospheric intrathoracic pressure that drives inspiration and pulmonary inflation. In most species, expiration at rest is a passive process and relies on elastic recoil of the lung to create positive intrathoracic pressure.[256] This is the case during the first portion of expiration in horses, which relies on elastic recoil to the point of relaxation volume, when the tendency for pulmonary collapse equals the tendency for expansion by the thoracic wall. Horses then further decrease lung volume by active compression of the chest wall through contraction of the internal intercostal muscles and muscles of the abdominal wall.[278] The passive phase of inhalation follows. Dysfunction of the diaphragm and intercostal muscles due to either mechanical (abdominal distention, trauma to the thoracic wall) or neuromuscular (botulism, phrenic nerve damage, nutritional myodegeneration) problems prevents expansion of the thoracic wall and can produce hypoventilation, hypoxemia, and respiratory distress.[255,257] Horses with torsion of the large colon can develop significant abdominal distention and respiratory distress, and respiratory failure caused by impaired diaphragmatic function can play an important role in the pathophysiology and mortality associated with this intestinal accident.

Control of Airway Diameter

The diameter of the conducting airways, which is primarily controlled by the autonomic nervous system, is an important determinant of the degree of pulmonary resistance and work of breathing.[256] The control of airway diameter by the autonomic nervous system involves both sympathetic and

parasympathetic innervation with activation of adrenergic and muscarinic receptors, respectively.[256,279-281] The receptors are generally widely expressed in the lung, although the pattern of specific receptor expression can vary among species. The control of normal airway function and airway diameter is complex, requiring interactions or "crosstalk" between receptors. Vagal-mediated parasympathetic stimulation is important in the regulation of airway smooth muscle tone, and stimulation can cause bronchoconstriction as well as mucus secretion and bronchial vasodilation. Specifically, bronchoconstriction can occur due to the action of acetylcholine from parasympathetic fibers on M3 muscarinic receptors located on airway smooth muscle. This mechanism of bronchoconstriction has been associated with allergic airway disease in several species. The administration of anticholinergics such as atropine or *N*-butylscopolammonium bromide can result in rapid relief of bronchoconstriction in some horses with RAO, demonstrating the important role of parasympathetic bronchoconstriction in the pathogenesis of this disorder.[257,259,282,283] With regard to sympathetic effects, both α- and β-adrenergic receptors are found in the lung.[28-30] However, direct sympathetic innervation of airway smooth muscle is minimal to none, and thus stimulation of the adrenergic receptors is primarily via circulating catecholamines.[279-281] β_2-Adrenergic receptors are abundant throughout the lung on multiple cell types, including bronchial smooth muscle, bronchial epithelial cells, and several immune cells, and thus activation can have multiple effects. The stimulation of β_2-receptors on airway smooth muscle can cause smooth muscle relaxation and bronchodilation. Airways must be constricted for β_2-receptor stimulation or the anticholinergic action of atropine to increase airway diameter.[284,285] β_2-Receptors appear to be present in normal or increased numbers on asthmatic airway smooth muscle but appear to be hyporesponsive, thus allowing bronchoconstriction.[281,286] β_1-Adrenergic receptors are less abundant than β_2-receptors in the airways. The effects of both β_1- and α_1-adrenergic receptors in the regulation of airway diameter in horses appear to be minimal.[285-287]

Nonadrenergic-noncholinergic (NANC) innervation, including both excitatory and inhibitory components, also contributes to large airway diameter.[288,289] Stimulation of excitatory NANC nerves initiates a process of neurogenic inflammation that involves bronchoconstriction, mucus secretion, increased vascular permeability, vasodilation, and cough. Inhibitory NANC nerves appear to be important neural pathways for bronchodilation, and activation results in the relaxation of smooth muscles of the trachea and bronchi. Dysfunction of the NANC system may play a role in asthma in both people and horses, although the precise role remains to be elucidated. In RAO-affected horses with clinical signs of airway obstruction, inhibitory NANC function is absent.[290] Failure of the inhibitory NANC system may result from the inflammatory response during acute RAO or may be an inherent autonomic dysfunction of the conducting airways of RAO-affected horses.

Role of Hypoxemia and Hypercapnia in Respiratory Distress

Respiratory distress most often originates from inadequate pulmonary gas exchange to meet the metabolic demands of the individual, resulting in hypoxemia and hypercapnia. Hypoxemia results from one or more of five basic pathophysiologic mechanisms: hypoventilation, ventilation-perfusion mismatch, right-to-left shunting of blood, diffusion impairment, and reduced inspired oxygen concentration.[256] The degree of associated hypercapnia and the response to oxygen supplementation vary depending on the mechanism of impaired gas exchange, and determination of these two parameters is useful in defining the pathophysiologic process predominantly responsible for the development of hypoxemia.[256]

Hypoventilation. *Alveolar hypoventilation* is defined as insufficient ventilation leading to hypercapnia.[256] The elevation in $Paco_2$ is inversely proportional to the reduction in alveolar ventilation; halving alveolar ventilation doubles $Paco_2$.[256] The reduction in arterial oxygen tension is almost directly proportional to the increase in CO_2. For instance, if $Paco_2$ increases from 40 to 80 mm Hg, the Pao_2 decreases from 100 to 60 mm Hg. Therefore hypoxemia resulting from hypoventilation is rarely life threatening. In addition, oxygen supplementation easily abolishes hypoxemia caused by pure hypoventilation. Acidosis caused by hypercapnia is the most clinically significant feature of hypoventilation and may be life threatening.[256] Metabolic alkalosis or central nervous system depression (e.g., from head trauma, encephalitis, narcotic drugs) can produce hypoventilation; however, horses with these disorders may not demonstrate clinical signs of respiratory distress. Disorders that can cause alveolar hypoventilation that are often associated with clinical signs of respiratory distress include dysfunction of the respiratory muscles from mechanical (abdominal distention, trauma to the thoracic wall) or neuromuscular (botulism, phrenic nerve damage, nutritional myodegeneration) conditions, restrictive pulmonary disease (silicosis, pulmonary fibrosis, pneumothorax, pleural effusion), and upper airway obstruction.[255,257,259]

Ventilation-Perfusion Mismatch. Ventilation-perfusion (V-Q) mismatch is the most common cause of hypoxemia and is characterized by unequal distribution of alveolar ventilation and blood flow.[256,259] Either a low or high V-Q ratio can result in hypoxemia. Pulmonary regions that are overperfused in relation to ventilation (low V-Q ratio) contribute disproportionate amounts of blood with low arterial oxygen content to the systemic circulation.[256] Respiratory diseases characterized by low V-Q ratios include RAO, pulmonary atelectasis, and consolidation.[259] If ventilation exceeds perfusion (high V-Q ratio), the ventilated pulmonary units are inefficient for CO_2 elimination and O_2 uptake. Ventilation of poorly or nonperfused units is wasted ventilation, termed *alveolar dead space.*[256] Conditions associated with high V-Q ratios include pulmonary thromboembolism and shock (low pulmonary artery pressure). Patients with V-Q mismatch often have a normal arterial Pco_2. The ventilatory drive to maintain normal $Paco_2$ is powerful, and as the CO_2 dissociation curve is basically a straight line (direct relationship), increased ventilation efficiently decreases $Paco_2$ at high and low V-Q ratios. However, also due to the nearly flat shape of the O_2 dissociation curve, increasing ventilation is inefficient for proportionally increasing the arterial Po_2. Only pulmonary units with moderate to low V-Q ratios benefit from increased ventilation. Therefore the increased ventilatory effort to maintain normal $Paco_2$ is wasted and unnecessarily increases the work of breathing. Oxygen supplementation tends to increase $Paco_2$ in patients with a V-Q mismatch. Also, elevation in arterial O_2 is delayed compared with hypoventilation and in some cases may be incomplete.[256] Compensatory mechanisms are present to minimize unequal distribution of ventilation and perfusion in diseased lungs to prevent the development of hypoxemia

until the pulmonary pathologic condition is severe.[256] Reflex pulmonary arterial constriction (hypoxic vasoconstriction) prevents perfusion of unventilated alveolar units and attempts to redirect blood flow to alveoli that are ventilated adequately. Airway hypocapnia causes bronchoconstriction of airways that conduct to unperfused alveolar units, redirecting air flow to better perfused alveoli.

Shunt. *Shunt* is defined as blood that is not exposed to ventilated areas of the lung and is added to the arteries of the systemic circulation.[256] Shunting can occur as an extreme form of V-Q mismatch or with direct addition of unoxygenated blood to the arterial system. *Physiologic shunting* is defined as perfusion of nonventilated or collapsed regions of the lung and occurs with pulmonary consolidation, atelectasis, and edema. Certain congenital heart diseases, such as tetralogy of Fallot and some cardiac septal defects, are examples of direct right-to-left shunts wherein unoxygenated blood from the right side of the heart is added to oxygenated blood from the left side of the heart. Right-to-left shunting may also contribute to hypoxemia in some cases of persistent pulmonary hypertension. In these conditions hypoxemia cannot be abolished by increasing the oxygen content of inspired air. The shunted blood is never exposed to the higher concentration of inspired oxygen in the alveolus, and the addition of a small amount of shunted blood with its low O_2 content greatly reduces the P_{O_2} of arterial blood. Compared with breathing room air, the decrement in P_{O_2} is much greater at P_{O_2} levels associated with the inhalation of O_2-enriched air because the O_2 dissociation curve is so flat at high P_{O_2} levels. Only hypoxemia caused by right-to-left shunting behaves in this manner when the patient is permitted to inspire high percentages of oxygen (70%–100%). Shunts do not usually cause hypercapnia.[256] Chemoreceptors detect excess arterial CO_2, and ventilation increases to reduce the content of CO_2 in unshunted blood until arterial P_{CO_2} reaches the normal range. In some cases of shunt the arterial P_{CO_2} is below normal because of hyperventilation stimulated by the hypoxemic ventilatory drive.

Diffusion Impairment. Gas exchange between the alveolus and the capillary occurs by passive diffusion, which is driven by the property of molecules to move randomly from an area of high concentration to one of low concentration.[256] Factors that determine the rate of gas exchange include the concentration gradient between the alveolus and capillary blood, solubility of the gas, surface area available for diffusion, and width of the air-blood barrier. Diseases characterized by pure diffusion impairment are rare in veterinary medicine.[259] However, some degree of diffusion impairment can occur with disorders such as pulmonary fibrosis, interstitial pneumonia, silicosis, or pulmonary edema and is most often associated with increased width of the barrier or decreased surface area available for gas exchange. Although the major component of hypoxemia in these conditions is a V-Q mismatch, diffusion impairment can contribute to the severity of hypoxemia. Supplemental oxygen therapy is effective in treating hypoxemia caused by diffusion impairment because it creates a more favorable concentration gradient and increases the driving pressure of oxygen to move from the alveolus into the blood. Transport of CO_2 is less affected by diseases of diffusion impairment because of its greater solubility compared with O_2.[256]

Reduction of Inspired Oxygen. Hypoxemia resulting from decreased inspired oxygen content is uncommon and occurs only under special circumstances. High altitude and iatrogenic ventilation with a low oxygen concentration are the most common circumstances in which hypoxemia is attributed to reduction of inspired oxygen content.[256]

Most pulmonary diseases in horses incorporate more than one of these pathophysiologic mechanisms for the development of hypoxemia. Horses with pleuropneumonia, for example, may develop hypoxemia caused by hypoventilation (extrapulmonary restriction by pleural effusion), V-Q mismatch (accumulation of exudate and edema within alveoli and conducting airways), and diffusion impairment (exudate and edema within the interstitial spaces).

Role of Obstructive Disease in Respiratory Distress

Airway obstruction can limit air flow and contribute to respiratory distress. The location (intrathoracic or extrathoracic) and nature (fixed or dynamic) of airway obstruction determine whether impedance to air flow occurs during inspiration, expiration, or both.[257] The phase of the respiratory cycle that is affected by air flow obstruction is prolonged and may be associated with a respiratory noise (stertor, stridor, or wheeze).[256,257,291]

The horse is an obligate nasal breather and can breathe efficiently only through the nares.[259] Therefore upper airway obstruction within the nasal passages cannot be bypassed by mouth breathing. In addition, approximately 80% of the total airway resistance to air flow is located in the upper airway.[291] A 50% decrease in the radius of an airway increases its resistance by 16 times (Poiseuille's law).[256] Therefore small changes in the upper airway diameter dramatically affect the overall resistance to air flow and work of breathing for the horse. Extrathoracic airway pressures are subatmospheric during inspiration; therefore poorly supported structures in the upper airway narrow or collapse during inspiration (dynamic collapse). There are several causes of upper airway obstruction in horses, the most common being laryngeal hemiplegia.

Of the total airway resistance 20% is attributable to the small airways.[291] Although the radius of individual bronchioles is small, many of them exist and the sum or collective radius is large, with the result that their overall contribution to pulmonary resistance is low.[256] Because the resistance of the bronchioles is low, advanced disease must be present for routine measurements of airway resistance to detect an abnormality, and obstruction of these airways must be extensive before a horse would suffer from respiratory distress. During pulmonary inflation intrathoracic pressures are subatmospheric. Small airways are pulled open by negative intrathoracic pressure and stretched parenchymal attachments at high lung volumes. Thus resistance to air flow in small airways is low during the inspiratory phase of respiration.[256] During exhalation intrathoracic pressure is positive and the diameter of small airways is decreased, and bronchioles may even close at low lung volumes. Therefore resistance to air flow in small airways is greatest during the expiratory phase. In horses with RAO the airway diameter is reduced by inflammatory exudate, edema, and bronchoconstriction.[257,259,290] As lung volume decreases during expiration, the narrowed bronchioles are compressed shut (dynamic airway collapse) and trap air distal to the site of closure.[259] This is an example of severe flow limitation, which may lead ultimately to the development of emphysema. Flow limitation forces horses with RAO to breathe at higher lung volumes and maintain a higher functional residual capacity to reduce or prevent dynamic airway collapse. Affected horses

attempt to reduce the end-expiratory lung volume by recruiting abdominal muscles to increase the intrathoracic pressures during expiration. However, the greater the end-expiratory pressure, the greater is the likelihood of small airway compression and collapse. Hypertrophy of the cutaneous trunci and expiratory abdominal muscles, especially the external abdominal oblique, produces the characteristic "heave line" associated with RAO.[259] Because dynamic airway narrowing and collapse occur during exhalation, wheezes are typically loudest at end expiration in horses with RAO.[257,259]

Role of Restrictive Disease in Respiratory Distress

Restrictive disease is less common than obstructive pulmonary disease in horses.[259] By definition, restrictive disease inhibits pulmonary expansion and leads to inspiratory respiratory distress.[256] The vital capacity and compliance (pulmonary or chest wall) decrease, expiratory flow rates and elastic recoil increase, and airway resistance is normal. The characteristic respiratory pattern in horses with restrictive pulmonary disease is rapid, shallow respiration at low lung volumes.[259] This strategy takes advantage of high pulmonary compliance at low lung volumes and decreases the work of breathing. This respiratory pattern has the disadvantage of increased ventilation of anatomic dead space.[256] Restrictive diseases may be classified as intrapulmonary (pulmonary fibrosis, silicosis, and interstitial pneumonia) and extrapulmonary (pleural effusion, pneumothorax, mediastinal mass, botulism, and nutritional myodegeneration).[255,259] Hypoxemia observed in horses with intrapulmonary restrictive disease is largely attributed to V-Q mismatch and diffusion impairment, although hypoventilation may also have a role. Stimulation of juxtacapillary receptors may contribute to respiratory distress observed in these patients.[256] The pathophysiologic mechanism for hypoxemia in horses with extrapulmonary restriction is hypoventilation.[259] In horses with pleural effusion and pneumothorax, respiratory distress is likely to be exacerbated by thoracic pain.

Nonpulmonary Respiratory Distress

Respiratory distress does not always originate from dysfunction of the pulmonary system and its supporting structures. Nonpulmonary respiratory distress can occur because of inadequate oxygen-carrying capacity of the blood, pain, hyperthermia, or compensation for metabolic acidosis.[255-257]

Impaired oxygen-carrying capacity of the blood may occur because of anemia (blood loss, hemolytic, or aplastic) or dysfunction of red blood cells (methemoglobinemia, carbon monoxide toxicity). In these cases the arterial Po_2 tension (quantity of dissolved oxygen) is normal; however, the total oxygen content of the blood is reduced greatly.[256] Tachypnea and respiratory distress occur in response to impaired oxygen delivery and tissue hypoxia.

The respiratory system can compensate for metabolic acidosis by increasing ventilation to lower $Paco_2$ and attenuate acidemia.[256] The ventilatory drive increases in response to stimulation by peripheral chemoreceptors by circulating hydrogen ions. Hypocarbic compensation for mild to moderate metabolic acidosis is effective in returning blood pH to normal until renal compensatory mechanisms can be established.[256]

Pain and anxiety are physiologic causes of tachypnea and hyperpnea. Horses with musculoskeletal pain are unlikely to demonstrate significant respiratory distress; however, rhabdomyolysis and laminitis are painful musculoskeletal conditions that may produce tachypnea.[257] Marked respiratory distress is observed frequently in horses with abdominal pain; however, the respiratory distress is not caused solely by pain and is exacerbated by abdominal distention, shock, acidosis, and endotoxemia.

Elevations in body temperature caused by fever or hyperthermia associated with exercise, heat stroke, anhidrosis, or macrolide-induced hyperthermia can produce respiratory distress in horses. Hyperpnea is an effective means of heat dissipation in human beings, dogs, and ruminants.[257] Unfortunately, in horses increased ventilation is an inefficient mechanism for heat dissipation.[257,259]

Conditions Associated with Respiratory Distress

A number of conditions, both respiratory and nonrespiratory in origin, can cause some degree of respiratory distress in horses (Box 7.5). Among the more common respiratory causes are infectious conditions. In particular, bacterial infections of the respiratory tract such as bacterial pneumonia, aspiration pneumonia, pleuropneumonia, pleuritis, and pulmonary abscessation can result in respiratory distress.[206,207] Strangles can result in respiratory distress in association with airway obstruction from lymph node abscessation and less frequently due to pneumonia. Viral respiratory tract infections, which are common in horses, may sometimes result in respiratory distress.[204] Fungal pneumonia can result in significant respiratory distress in some patients but is uncommon in horses.[209,210] Respiratory distress is often a prominent feature of syndromes of interstitial pneumonia, including acute lung injury, acute respiratory distress, and equine multinodular pulmonary fibrosis.[211,212,292,293] In neonatal foals, immature lungs and lack of surfactant can contribute to respiratory distress.[294] Idiopathic or transient tachypnea has been reported in neonatal foals, especially in Clydesdales, Thoroughbreds, and Arabians.[294] RAO is a common condition in horses that has the potential to cause significant respiratory distress, both in association with acute exacerbations of the condition and with chronic disease.[215,216,217,218,290] Some other respiratory problems that can be associated with respiratory distress include exercise-induced pulmonary hemorrhage, eosinophilic pulmonary disease, silicosis, smoke inhalation, and neoplasia.[213,214,223,226,227,295-297]

Upper airway obstruction is another important cause of respiratory distress in horses, especially when oxygen demand is increased by exercise.[255,257,259] The most common cause of non–fixed upper airway obstruction in horses is laryngeal hemiplegia, which produces inspiratory stridor during exercise.[257,298-300] Intraluminal masses and arytenoid chondritis cause fixed upper airway obstruction and produce inspiratory and expiratory respiratory distress.[257] Several other upper airway abnormalities have been documented, such as fourth branchial arch defect, subepiglottic cyst, aryepiglottic fold entrapment, and dorsal displacement of the soft palate.[257,301] Laryngeal and pharyngeal dysfunction has been reported in horses that are homozygous for hyperkalemic periodic paralysis.[302,303] Infrequently, laryngeal paralysis has been associated with hepatic dysfunction.[304] A wide variety of other conditions have been associated with respiratory distress such as pneumothorax, diaphragmatic hernia, choanal atresia, nasopharyngeal cicatrix syndrome, tracheal collapse, and others.[233-235,305,306] Although heart disease is relatively uncommon in horses, it may result in respiratory distress via pulmonary

BOX 7.5 Causes of Respiratory Distress

RESPIRATORY CAUSES

- Respiratory tract infection
  - Bacterial pneumonia, pleuropneumonia (including aspiration, pulmonary abscesses)
  - Strangles
  - Viral respiratory tract infection
  - Fungal respiratory tract infection
- Interstitial pneumonia
  - Equine multinodular pulmonary fibrosis
  - Syndromes of acute lung injury, acute respiratory distress
- Recurrent airway obstruction (severe asthma)
- Upper airway obstruction
  - Left laryngeal hemiplegia
  - Arytenoid chondritis
  - Fourth branchial arch defect
  - Subepiglottic cyst
  - Dorsal displacement of the soft palate
  - Aryepiglottic fold entrapment
  - Intraluminal masses, abscessation (strangles, other)
  - Laryngeal and pharyngeal dysfunction associated with HYPP
  - Choanal atresia
  - Nasopharyngeal cicatrix syndrome
- Exercise-induced pulmonary hemorrhage
- Pneumothorax
- Diaphragmatic hernia
- Tracheal abnormalities
  - Tracheal collapse, stenosis
  - Foreign body
  - Intraluminal mass
- Cardiac disease
  - Pulmonary edema
  - Left-to-right shunting
- Other
  - Silicosis
  - Smoke inhalation
  - Neoplasia
  - Eosinophilic pulmonary disease
  - *Crotolaria equorum*—pyrrolizidine alkaloid toxicity
- Additional causes in foals
  - Inadequate surfactant, pulmonary immaturity
  - Idiopathic tachypnea (transient)

NONRESPIRATORY CAUSES

- Systemic inflammatory response syndrome
- Pain
- Elevated body temperature
- Fever
- Hyperthermia
  - Exercise-related hyperthermia
  - Heat stroke
  - Anhidrosis
  - CNS disorders
  - Macrolide-induced hyperthermia
- Anemia
- Monensin
- Snake envenomation
- Neuroborreliosis

edema or right-to-left shunting.[229,230] Respiratory distress is a prominent sign of a rare form of pyrollizidine alkaloid toxicity associated with *Crotolaria equorum*.[307]

Several nonrespiratory conditions can also cause respiratory distress. Respiratory distress is often a feature of the systemic inflammatory response syndrome.[255,257] Pain, fever, or hyperthermia may lead to respiratory distress. Tachypnea and elevation in body temperature are the most prominent clinical signs in horses with anhidrosis.[16] Other nonrespiratory causes of respiratory distress include disorders that cause anemia, such as blood loss, autoimmune hemolytic activity, neonatal isoerythrolysis, and red maple toxicosis. In addition to red maple, other toxins that can affect respiration include monensin and snake envenomization. Episodic respiratory distress was seen in 5 of 16 horses diagnosed with neuroborreliosis.[308]

Approach to Respiratory Distress

Signalment and History

Both breed and age have been linked with specific causes of respiratory distress. For example, American Miniature Horses are at increased risk of tracheal collapse.[233] Idiopathic laryngeal hemiplegia is more common in large, long-necked horses such as draft horses, Thoroughbreds, and Warmbloods.[298,299] In Quarter Horses and related breeds, some foals affected with hyperkalemic periodic paralysis will show signs of respiratory stertor usually within the first week of life as a result of laryngeal dysfunction, especially if homozygous.[302,303] Inadequate lung development, surfactant deficiency, persistent pulmonary hypertension, and meconium aspiration are problems unique to neonatal foals.[294] Congenital problems such as choanal atresia and cardiac defects should also be considered in neonates. Rhodococcal pneumonia is primarily a disease of foals 1 to 6 months of age.[207] Horses affected with RAO are generally over 7 years of age, and a familial predisposition has been identified.[215,216]

Some important historical considerations include environmental conditions such as heat and humidity and relocation to high altitude. A history of recent transport may raise the index of suspicion for pleuropneumonia. Useful information can also be obtained from specifics related to the respiratory distress such as the speed of onset, progression of signs, history of previous episodes, and association with exercise or specific locations/housing. Any history of recent trauma or potential exposure to toxins should be determined. A history of any other problems such as cough, nasal discharge, dysphagia, anorexia, or lethargy can also be important.

In neonatal foals, the circumstances of gestation and parturition can help to establish the likelihood of problems such as immaturity, rib fractures, meconium aspiration, and sepsis.

Physical Examination

A thorough physical examination is essential to determine the origin of respiratory distress, identify concurrent disease, and direct further diagnostic testing. Prolonged inspiration is consistent with restrictive or extrathoracic, nonfixed, obstructive disease, whereas horses with intrathoracic airway obstruction exhibit expiratory difficulty.[256,257] Respiratory distress

associated with inspiration and expiration may indicate an extrathoracic fixed obstruction. Stertor is a low-pitched, "snoring" respiratory noise caused by partial obstruction above the larynx and is inspiratory. In comparison, stridor is a more musical sound that is generated by obstruction of the larynx and less frequently the trachea or bronchi. Stridor can be heard during inspiration and/or expiration but is most often audible during inspiration.[257]

Thoracic auscultation identifies abnormal respiratory sounds (crackles and wheezes) or regions of decreased breath sounds caused by pleural effusion, pneumothorax, or pulmonary consolidation.[259,308] Percussion of the thoracic wall generates a resonant and hollow sound when performed over regions of normal lung. Pleural effusion and pulmonary consolidation sound dull and flat during thoracic percussion, whereas pneumothorax produces a hyperresonant sound.[246,259]

Normal air flow occurs in laminar flow; therefore normal horses at rest do not generate easily audible sounds.[259] If the horse is not in significant distress, auscultation with a rebreathing bag can help enhance sounds by increasing tidal volume. Respiratory sounds are generated from vibration in tissue and sudden changes in pressure of gas moving within the airway lumen. Airway narrowing and exudate generate audible sounds by creating disturbances in laminar flow, turbulence, and sudden changes in pressure of moving gas.[256] Crackles are intermittent or explosive sounds, generated by bubbling of air through secretions or by equilibration of airway pressures after sudden opening of collapsed small airways. The generation of crackles requires an air-fluid interface, and these abnormal lung sounds occur in horses with pneumonia, interstitial fibrosis, RAO, pulmonary edema, and atelectasis.[246,259] Wheezes are continuous, musical sounds that originate from oscillation of small airway walls before complete closing (expiratory wheeze) or opening (inspiratory wheeze).[256] Expiratory wheezes are the hallmark of obstructive pulmonary disease.[256]

Horses with nonpulmonary respiratory distress demonstrate increased rate and/or depth of respiration without producing abnormal respiratory noise. Stertor and stridor are absent, and auscultation of the thorax is normal.

Ancillary Diagnostic Tests

Clinical Pathology

BLOOD WORK. A CBC and fibrinogen can help to evaluate hemoconcentration, anemia, and the presence of inflammation. Serum amyloid A can also be used in the assessment of inflammation.

Arterial blood gas analysis provides a quantitative evaluation of pulmonary function, alveolar ventilation, and acid-base status and may identify the origin of respiratory distress (hypercapnia, hypoxemia, or acidemia).[256] The clinician may determine the pathophysiologic mechanism of hypoxemia by examining the $Paco_2$ level and by investigating the response of Pao_2 to supplemental oxygen therapy. In addition, serial blood gas monitoring can determine response to bronchodilator, parasympathomimetic, or anti-inflammatory therapy.

Airway Cytology/Assessment of Infection. Appropriate samples for cytologic evaluation and culture can be obtained from the respiratory tract depending on the primary differential diagnoses.[240,241,246] The degree of respiratory distress should be considered, as some diagnostic procedures can be stressful in horses with significant respiratory distress, especially foals. Currently, for most viral respiratory tract infections, the most common means of diagnosis is detection of viral genetic material by PCR from a nasal or nasopharyngeal swab. For strangles, PCR and/or culture of a nasal or nasopharyngeal swab, nasal wash, guttural pouch wash, or abscess can be diagnostic. Several techniques have been described for sampling the lower airways of horses including transtracheal aspiration, bronchoalveolar lavage, and various transendoscopic procedures. Thoracocentesis can be useful in cases of suspected pleuropneumonia and in some cases of suspected neoplasia. For the diagnosis of RAO, cytologic analysis of bronchoalveolar lavage fluid is recommended and is typically characterized by a significant increase in neutrophils (greater than 25%). Bronchoalveolar lavage can also be helpful in the evaluation of suspected EIPH.

Endoscopy. An endoscopic examination of the upper airway is indicated in horses with stertor or stridor and suspected upper airway obstruction.[246,259] Endoscopy can be used to evaluate the trachea and can help to identify tracheal collapse, foreign bodies, mucus, or hemorrhage. Horses with extreme respiratory distress may resent endoscopic examination, and forced examination may precipitate a respiratory crisis.

Diagnostic Imaging. The findings during thoracic auscultation and percussion are valuable in determining whether ultrasonography rather than radiography is indicated.[246,259] Pulmonary consolidation, abscessation, fibrosis, interstitial pneumonia, peribronchial infiltration, and mediastinal masses are typically imaged best via thoracic radiography. Thoracic ultrasonography is superior to radiography in detecting and characterizing pleural fluid and peripheral pulmonary abscessation and consolidation in horses.[309] Air reflects the ultrasound beam; therefore ultrasonography does not image deep pulmonary lesions if the overlying lung is aerated.

Other Diagnostic Aids. The administration of atropine or *N*-butylscopolammonium bromide in horses with RAO may provide rapid relief of respiratory distress if the major component of airway obstruction is reversible bronchoconstriction.[282] The bronchodilatory properties of *N*-butylscopolammonium bromide appear to be similar to those of atropine, although of shorter duration and associated with fewer systemic side effects.[283] Horses that respond to an atropine or *N*-butylscopolammonium bromide challenge likely will respond favorably to bronchodilator therapy. Incomplete response to these bronchodilators in horses with RAO indicates that exudate or fibrosis is contributing to airway obstruction, and limited response to bronchodilator therapy is anticipated.[257,259] Other tests that may be of value in the assessment of equine asthma include a hay challenge, histamine provocation test, and pulmonary function testing.

Both endobronchial and percutaneous lung biopsies may be used in the diagnosis of respiratory disease but are not commonly performed.[253,254] In horses with suspected anhidrosis, a terbutaline sweat test can be used to evaluate the patient's ability to sweat.[67]

EDEMA

Edema is defined as the excessive and abnormal accumulation of fluid in the interstitium, which is the intercellular connective tissue that lies between the cellular elements of tissues.[310,311] Interstitial fluid accumulates as a result of imbalances between the rates at which fluid enters and exits the interstitium. Factors that either increase the rate of fluid flux from the capillary or impair reabsorption and lymph drainage to the extent that

normal compensatory mechanisms are overwhelmed result in the accumulation of fluid in the interstitial space and the development of edema.

Edema is often classified according to the anatomic location. The most frequently recognized type of edema in horses is peripheral edema, which is often seen in the limbs or along the ventrum. Most peripheral edema is "pitting," which means that an indentation persists after pressure is applied to an area and released—for example, using the tip of a finger to indent the fluid. Occasionally nonpitting peripheral edema will be recognized, which in horses is most often associated with lymphedema, which is fluid retained in the interstitium as a result of impaired lymphatic drainage. Organ-specific edema, such as cerebral, pulmonary, and corneal edema, also occurs and is often clinically important. Generalized edema occurs when edema is present both peripherally and in multiple organs.

General Physiology of Fluid Movement

The volume of interstitial fluid and lymph fluid in the normal horse is 8% to 10% of body mass,[310] or 36 to 45 L in a 450-kg horse. Interstitial fluid consists largely of water, protein, and electrolytes. Compared with plasma, interstitial fluid has a slightly lower concentration of cationic electrolytes, a slightly higher concentration of chloride, and a much lower concentration of protein (1.2 vs. 0.2 mOsm/L of water).[311] The overall amount and function of the proteins within the interstitial space are not inconsequential. A constant circulation of plasma proteins occurs between the vascular and interstitial spaces, with about half of the protein circulating every 24 hours in human beings. More than half of the plasma protein content of the body is contained within the interstitial space at any one time. Plasma proteins within the interstitial space are important in the transport of water-insoluble substances from the vascular space and in resistance to infection.[312]

The extracellular tissue of the interstitium, except in the case of bone, consists of a three-dimensional collagen fiber network embedded in a proteoglycan gel matrix.[313] Normally most of the interstitial water is contained within the proteoglycan interstitial gel, with a small proportion existing as free water. However, in edematous states, the proportion of fluid as free water within the interstitium increases.[311]

The source of interstitial fluid is the intravascular space. The volume of interstitial fluid is determined by the functional relationships of three major anatomic structures: the capillary, the interstitial space, and the lymphatics.[314] Functionally, the volume of fluid that accumulates in the interstitium is determined by the rate of ingress of fluid from the vascular space, the compliance of the interstitium, and the rate at which fluid is evacuated from the interstitium.

Traditionally the fundamental principle guiding microvascular filtration and transcapillary fluid shifts has been defined by the Starling equation, first introduced in 1896. In this model, fluid movement is passive and dependent on pressure gradients across the endothelium, which are determined by hydrostatic and colloid osmotic pressures.[311,315] However, it is now recognized that the regulation of fluid flux is not simply governed by transcapillary hydrostatic and oncotic pressure differences but is much more complex. The role of non-Starling mechanisms of barrier regulation and fluid movement are an area of active investigation. Important factors that mediate processes central to the development of edema include the endothelial glycocalyx layer, the endothelial basement membrane, and selective water channels known as *aquaporins*.[312,316-319]

The Starling Equation

The net rate of ingress of fluid from capillaries into the interstitium is determined by a number of factors acting across the capillary membrane, the effects of which are related by Starling's equation:

$$J = Kf\left([P_c - P_t] - \sigma\left[\pi_p - \pi_t\right]\right)$$

in which J equals the volume flow across the capillary wall; Kf equals the filtration coefficient of the capillary wall (volume flow per unit time per 100 g of tissue per unit pressure); P_c equals capillary hydrostatic pressure; P_t equals interstitial fluid hydrostatic pressure; σ equals the osmotic reflection coefficient; π_p equals the colloid osmotic (oncotic) pressure of the plasma; and π_t equals the colloid osmotic (oncotic) pressure of the interstitial fluid.[315] Although all these factors act in concert to determine the rate of net fluid efflux from the capillary, considering them individually is conceptually easier.

Filtration *(Kf)* and Reflection (σ) Coefficients. Together the filtration and reflection coefficients describe the properties of the capillary membrane that determine the ease with which water, protein, and other plasma constituents move from the vascular space to the interstitium. The filtration coefficient, which is the product of the hydraulic permeability and surface area of the capillary, is a measure of the ease with which water crosses the capillary membrane. The reflection coefficient is a mathematical expression of the capillary permeability to a particular substance and ranges from 0, indicating the substance crosses the membrane as easily as water, to 1, indicating that the substance does not cross the membrane. The reflection coefficient is dependent on both the substance in question and the tissue bed.[311,320,321] For example, in lung endothelium, the reflection coefficients for urea, glucose and albumin are approximately 0.3, 0.5, and 0.7. Reflection coefficients for albumin vary among tissues, being 0.1 in the hepatic sinusoids, 0.9 in muscle, and 0.99 in brain.

The filtration and reflection coefficients together partially determine the rate of fluid flux across the capillary wall and the composition of the fluid. For a given hydrostatic and oncotic pressure difference, tissues with higher filtration coefficients (whether because of a larger capillary surface area or more porous capillaries) will have a greater fluid flux. Conversely, under the same circumstance, increases in the reflection coefficient will reduce solute movement across the capillary wall, affecting the osmotic pressure gradient and reducing fluid flux. The differential permeability of the capillary membrane to water and protein has important consequences in the maintenance of the oncotic pressure difference between plasma and interstitial fluid. Multiple factors influence the movement of fluid and solutes across the endothelium, including the concentration of the solutes on either side of the membrane, solute charge and interaction with other solutes, and capillary pore configuration.[322]

Hydrostatic and Colloid Osmotic Pressures (Starling's Forces). Transcapillary fluid flow results from an imbalance between the hydraulic forces favoring movement of water from the capillary into the interstitium and the forces favoring movement of water in the reverse direction. The forces contributing to fluid movement out of the capillary are the intracapillary hydrostatic pressure and the interstitial colloid

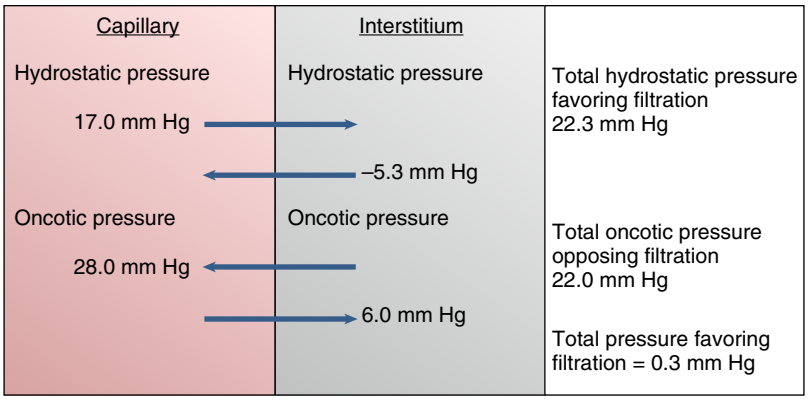


FIG. **7.4** Basic Starling's forces—mean forces influencing fluid movement into or out of the capillary. (From Guyton AC, Hall JE: The microcirculation and the lymphatic system: capillary fluid exchange, interstitial fluid and lymph flow. The body fluid compartments: extracellular and intracellular fluids; interstitial fluid and edema. In Guyton AC, Hall JE, editors: *Textbook of medical physiology,* ed 11, Philadelphia: Elsevier/Saunders; 2006.)

osmotic pressure, whereas those forces favoring movement of fluid from the interstitium to the capillary are the interstitial hydrostatic pressure (if it is positive) and the plasma colloid osmotic pressure[323] (Fig. 7.4).

The principal force favoring fluid efflux from the capillary is the hydrostatic pressure within the capillary. Capillary hydrostatic pressure varies among different tissues and decreases along the length of the capillary. Hydrostatic pressure within a capillary is determined by the arterial and venous pressures and by the precapillary and postcapillary resistances.[324] Specifically, capillary pressure *(Pc)* is determined by the ratio of the postcapillary resistance *(Ra)* to the precapillary resistance *(Rv),* and by the arterial *(Pa)* and venous *(Pv)* pressures:

$$Pc = [(Rv/Ra)\,Pa + Pv] / [1 + (Rv/Ra)]$$

Thus although an increase in either arterial or venous pressure will increase capillary pressure, a small increase in venous pressure has a much greater effect than does an increase in arterial pressure. For this reason the hydrostatic pressure is greater in capillaries below the heart (e.g., legs) than in those above the heart (e.g., head).

The colloid osmotic pressure of the plasma is the principal force minimizing fluid efflux from the capillary. The colloid osmotic pressure is generated both because the plasma and interstitial fluid are separated by a semipermeable membrane, the endothelium, and because they vary slightly, but significantly, in composition. As noted previously, the interstitial fluid has a lower protein concentration than does plasma but has an essentially identical electrolyte concentration. The difference in protein concentration across the semipermeable endothelium generates an osmotic force that tends to draw water from the interstitium into the plasma.

In addition to the capillary hydrostatic pressure, the colloid osmotic pressure and negative hydrostatic pressure of the interstitial fluid favor fluid movement out of the capillary. Fluid flux across the capillary results from the summation of these forces (see Fig. 7.4). These figures should be recognized as representing the forces at the midpoint of an idealized capillary; the forces are dynamic, changing between tissues and even along the length of the capillary. In fact, a large net flux of fluid from the capillary occurs at its arteriolar end, where capillary hydrostatic forces are greatest and the oncotic gradient is least, whereas a net flux of fluid into the capillary occurs toward its venous end, where capillary hydrostatic forces are least and the oncotic pressure gradient favoring reabsorption is greatest following dilution of protein in the interstitium (Fig. 7.5).

The small imbalance in filtration forces results in a net efflux of fluid from the capillary into the interstitial tissue. Normally this fluid does not accumulate in the interstitium, as it is removed by the lymphatics.

Other Factors Influencing Fluid and Solute Movement

Vascular Endothelium. The endothelium forms a dynamic barrier between the blood and the tissue. Although endothelial cells can have distinct morphologies and functions depending on the tissue and even the specific vessel, in general one function is to restrict the extravasation of larger molecules and cells from the vasculature to the interstitium.[325] The endothelial glycocalyx, which is a layer of macromolecules at the luminal surface of the vascular endothelium, plays an important role in fluid homeostasis and solute exchange.[317,325,326] The composition of the endothelial glycocalyx is dynamic, but the major constituents are hyaluronic acid and the negatively charged heparin sulfate proteoglycans. Changes in the endothelial glycocalyx appear to play a key role in endothelial dysfunction and the formation of edema. Also, endothelial permeability is in part regulated by the dynamic opening and closing of cell-cell adherens junctions that are composed primarily of vascular endothelial-cadherin.[318,327,328]

Aquaporins. Aquaporins are a diverse family of membrane proteins that are expressed predominantly in tissues in which edema and fluid imbalances are of major concern.[319,329] Water movement across cell membranes is driven by osmotic and hydrostatic forces, but the speed of this process can be influenced by the presence of specific aquaporin channels. These channels are primarily water channels, although some are also permeable to small solutes. Aquaporin-4 water channels play a central role in brain water regulation in neurologic disorders.[330-332] The pharmacologic modulation of the expression and activity of various aquaporins potentially could provide novel treatments for a variety of disorders, including brain edema.

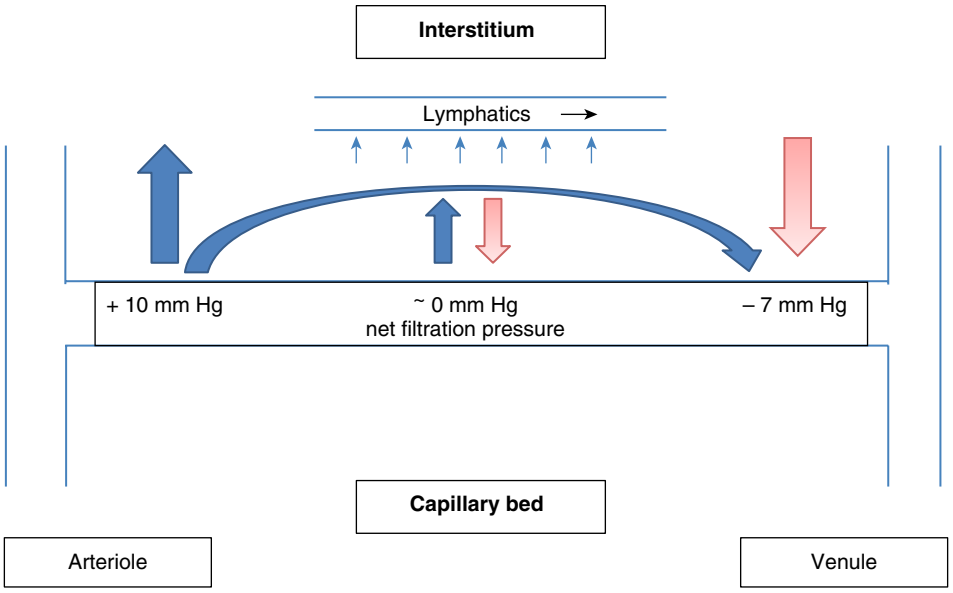


FIG. **7.5** Schematic of normal fluid movement in a capillary bed. Filtration occurs at the arterial end where capillary hydrostatic pressure is highest, and absorption occurs at the venous end where capillary hydrostatic pressure is lower, allowing plasma colloid oncotic pressure to predominate. Excess fluid is removed by the lymphatics.

Lymphatics. The lymphatics drain the interstitium of fluid and substances, notably proteins, that are not absorbed by the capillaries.[311] The lymphatics represent the only means by which interstitial protein is returned to the circulation. Interstitial fluid (and, with it, protein) moves down a pressure gradient into lymphatic capillaries through clefts between the lymphatic endothelial cells. Lymphatic endothelial cells are supported, and the lymphatic capillaries maintained patent, by anchoring filaments that attach the endothelial cells to surrounding connective tissue. Lymphatic fluid progresses centripetally through progressively larger vessels before draining into the great veins of the chest. Lymphatic valves prevent the retrograde flow of fluid from the lymphatics. Lymph is propelled by factors extrinsic to the lymphatics, including muscle activity, active and passive motion, posture, respiration, and blood vessel pulsation. Exercise increases lymph flow, at least in part because of the increase in tissue pressure that is associated with muscle contraction, although passive motion also increases lymph flow. In human beings, standing results in significant diminution or cessation of lymph flow from the lower extremities, which can result in the accumulation of interstitial fluid and edema. In addition to the extrinsic factors affecting lymph flow, coordinated contractions of lymphatic vessels contribute substantially to the centripetal flow of lymph.[313]

Mechanisms of Edema Formation

Simply stated, accumulation of excessive fluid in the interstitial spaces—edema—results from an imbalance of the rates of fluid filtration from the capillaries and drainage by the lymphatics. The exact pathophysiologic mechanisms involved in edema formation are complex and involve some tissue-specific mechanisms. Basic mechanisms of edema include alterations in Starling's forces, changes in vascular permeability, and impaired lymph drainage.

BOX 7.6 Mean Forces (mm Hg) Influencing Fluid Movement into or out of the Capillary

Type of Pressure	Mean Force
HYDROSTATIC PRESSURES	
Mean capillary pressure	17.0
Interstitial pressure	–5.3
Total hydrostatic pressure favoring filtration	22.3
COLLOID ONCOTIC PRESSURES	
Plasma oncotic pressure	28.0
Interstitial oncotic pressure	6.0
Total oncotic pressure opposing filtration	22.0
Total pressure favoring filtration	0.3

Data from Guyton AC: *Textbook of medical physiology,* ed 11, Philadelphia, Saunders, 2005.

Alterations in Starling's Forces

Perturbations of one or more of the forces that affect filtration across the capillary alter the rate at which fluid enters the interstitium.[311,312] Increases in capillary hydrostatic pressure, decreases in plasma oncotic pressure, and increases in interstitial oncotic pressure all favor increased fluid filtration. Conversely, increased interstitial hydrostatic pressure and decreased interstitial oncotic pressure act to inhibit fluid filtration.

There are several fundamental mechanisms by which excessive interstitial fluid can accumulate (Box 7.6). Increases in capillary hydrostatic pressure, which occur with venous obstruction or arteriolar dilation, such as that associated with inflammation, increase net fluid efflux. The edema that occurs with congestive heart failure likely has an increase in capillary hydrostatic pressure as one of its causes, although the mechanism is complex.[313]

Posture also affects capillary hydrostatic pressure; capillaries below the level of the heart have higher hydrostatic pressures than do capillaries above the level of the heart.

A decrease in the oncotic gradient across the capillary endothelium, which occurs with a decreased plasma oncotic pressure or an increased interstitial oncotic pressure, results in an increase in efflux of fluid from the capillary. A decrease in plasma oncotic pressure decreases the oncotic gradient that favors movement of fluid into the capillary. Consequently, the capillary hydrostatic pressure, which favors filtration, predominates and fluid accumulates in the interstitium. Plasma oncotic pressure decreases when plasma protein concentration declines. Albumin is the plasma protein that exerts the preponderance of the oncotic force and edema often is associated with hypoalbuminemia.[311,321]

Changes in Vascular Permeability

An increase in the permeability of the capillary membrane can increase fluid and protein transport into the interstitium and decrease the ability of the membrane to maintain a difference in oncotic pressure between the plasma and the interstitium.[314] Thus changes in vascular permeability can affect fluid flux both directly and via changes in Starling's forces.

The regulation of vascular permeability is complex with multiple pathophysiologic triggers contributing to increased permeability.[325-327] The microvasculature is sensitive to damage via several mechanisms including mechanical forces, ischemia-reperfusion injury, sepsis, and inflammation. Vasodilation and increased blood flow alone can increase vascular permeability. Numerous molecular regulators affect vascular permeability, including hormones, inflammatory cytokines, and other plasma constituents. Vascular endothelial growth factor is a potent angiogenic factor that can affect regulation of the vascular barrier.[325,333,334] Also, a number of inflammatory cytokines contribute to vasculitis. Among these are histamine and bradykinin, both of which can ultimately increase the generation of nitric oxide.[325]

The precise means by which vascular permeability is increased after stress to the endothelium are complicated and still under investigation. Inflammatory mediators and hormones can affect the integrity and function of the endothelial glycocalyx layer, and the changes in this layer frequently result in increased permeability and edema.[317,325,326] The organization and function of vascular endothelial-cadherin and other proteins at the adherens junctions can also be affected.[318,325,327,328] In addition, changes in the transcriptional, translational, and posttranslational regulation of transporters and ion and water channels, including aquaporins, occur.[325,329-332] The complexity of the regulation of vascular permeability and other factors contributing to edema can in part explain some of the limitations of colloid therapy. Improved understanding of the mechanisms controlling vascular permeability might lead to the formulation of pharmacologic strategies to control the barrier function of the endothelium.

Impaired Lymph Drainage

Lymphatic obstruction prevents the removal of interstitial fluid and protein.[311] Filtration of fluid and passage of small amounts of protein into the interstitial space continues in the presence of lymphatic obstruction. The interstitial fluid is reabsorbed by the capillaries, but the protein is not. Consequently, the protein content of the interstitial fluid gradually increases, with a resultant increase in interstitial oncotic pressure that favors filtration of fluid. The increased interstitial oncotic pressure causes fluid to accumulate in the interstitium, thus exacerbating the edema.

Compensatory Mechanisms Limiting Edema Formation

Alterations in the magnitude of one or more of Starling's forces can be offset by compensatory changes in lymph flow and other of Starling's forces.[311] In concert Starling's forces and lymph flow act as "edema safety factors" to prevent the excess accumulation of interstitial fluid and development of frank edema. For example, lymph flow increases with the increased filtration associated with increased capillary hydrostatic pressure. Thus a larger volume of fluid enters and is removed from the interstitial space. The interstitial protein concentration decreases as increased fluid flow washes protein out of the interstitial space. Reduced interstitial space protein concentration minimizes oncotic forces drawing fluid from the capillary to the interstitial space.[315]

Conditions Associated with Edema

Edema is not in itself a disease; rather, it is a sign of a disease process. Therefore the identification of conditions leading to the accumulation of edema is based on an understanding of the pathogenesis of edema. The most common form of edema recognized in horses is peripheral edema, and multiple causes have been identified (Table 7.3). Some specific causes of peripheral edema are discussed later.

A nonpainful edema of the limbs is often seen in horses in association with immobility. This condition, commonly referred to as "stocking up," is apparently associated with venous congestion and decreased lymph flow. It can affect all four limbs but is most common in the hindlimbs.

Hypoproteinemia, particularly hypoalbuminemia, is an important cause of edema.[311] In horses, the most common cause of hypoalbuminemia is protein loss, particularly through the gastrointestinal tract. In addition to acute colitis, some specific gastrointestinal conditions that are frequently associated with protein loss include right dorsal colitis, proliferative enteropathy due to *Lawsonia intracellularis,* and inflammatory bowel disease.[142.335,336] Other potential causes of protein loss include renal disease, especially chronic renal failure, amyloidosis, extensive skin wounds (burns), and third space loss as is seen in some cases of pleuritis or peritonitis. Occasionally hypoalbuminemia can be seen in association with decreased albumin production due to liver disease.[337] Severe burn injuries can induce generalized edema independent of protein loss, likely secondary to endothelial injury.[214,338]

Vasculitis is another important cause of edema in horses.[339-343] Some infectious diseases in which vasculitis is often prominent include equine viral arteritis, equine infectious anemia virus, African horse sickness, and equine granulocytic anaplasmosis *(Anaplasma phagocytophilum).* Purpura hemorrhagica is an immune-mediated vasculitis that is often associated with marked edema.[343] Many, but not all, horses affected with purpura hemorrhagica have been exposed to or infected with *Streptococcus equi* subspecies *equi.*

Several other causes of edema have been recognized in horses. Congestive heart failure is relatively uncommon in horses, but when it does occur ventral edema is among the most common clinical signs along with a heart murmur accompanied by either jugular distention or pulsation, tachycardia, and tachypnea.[229] Lymphangitis is an inflammation of the lymphatic vessels that often results in impaired lymphatic drainage and swelling. The condition can occur in any limb

TABLE **7.3 Common Causes of Peripheral or Ventral Edema in Horses**

CONGESTIVE HEART FAILURE
Valvular disease
Myocarditis
Monensin toxicosis
VASCULITIS
Equine viral arteritis
Equine ehrlichiosis
Purpura hemorrhagica
Equine infectious anemia
VENOUS OBSTRUCTION AND CONGESTION
Catheter-related thrombophlebitis
Disseminated intravascular coagulation
Tight bandages
Tumors
Immobility
CELLULITIS
Staphylococcal
Clostridial
Counterirritant application
LYMPHATIC OBSTRUCTION
Ulcerative lymphangitis
Lymphadenitis *(Streptococcus equi, Corynebacterium pseudotuberculosis)*
Lymphosarcoma
Tumors
HYPOALBUMINEMIA
Parasitism
Pleural and peritoneal effusions
Protein loss (gastrointestinal, renal, or wounds)
Inadequate production (starvation)
Hemodilution (subsequent to hemorrhage)
SHOCK
Hemorrhagic
Endotoxic
PLEURITIS
Late-term pregnancy
Prepubic tendon rupture
Starvation (inadequate intake; malabsorption)

but is more common in the hindlimbs. It is usually caused by a bacterial infection, although culture is frequently unrewarding. Some cases of *Corynebacterium pseudotuberculosis* infection manifest as an ulcerative lymphangitis.[344] Chronic progressive lymphedema is a condition recognized in some draft horse breeds including Clydesdales, Shires, and Belgians.[345] The condition is thought to be caused by altered elastin metabolism leading to impaired lymphatic function. Affected horses have chronic lymphedema with swelling of the distal limbs and development of fibrosis. Massive envenomation by bees (bee stings) causes generalized edema in horses.[346] Snake envenomation can also cause edema.[347]

Diagnostic Approach to the Patient with Edema

The diagnostic approach to the patient with edema is dependent on an understanding of the mechanisms of edema formation and a knowledge of the diseases likely to be involved. The diagnostic approach to an animal with edema should not be any different from that for any other sign of disease. A clinical examination, including history and physical examination, permits the development of a list of potential diagnoses and dictates the appropriate subsequent steps in confirming the diagnosis. The reader is referred to those sections of the text that deal with specific diseases for a more in-depth description of the conditions and their diagnosis.

Signalment and History

The signalment can be important when considering specific conditions associated with edema. For example, *Lawsonia intracellularis* is primarily a disease of weanling-age horses.[335] Chronic progressive lymphedema is particularly common in certain draft breeds, including Shires, Clydesdales, and Belgians.[229]

When taking the history of a horse that has edema, the veterinarian should focus on acquiring facts that have the greatest diagnostic use in differentiating among those diseases that have edema as a sign. The veterinarian should consider the following aspects: housing, season, and geographic region; vaccine and parasiticide administration; exposure to other horses and diseases present within the herd; the duration of the edema and its distribution; and the presence of any other clinical signs. The veterinarian should investigate the remainder of the history depending on the responses to initial questions.

Physical Examination

The physical examination should begin with a visual evaluation of the attitude and physical condition of the horse. The temperature, pulse rate, and respiratory rate should be recorded. Although the physical examination should be complete, particular attention should be paid to those body systems that the preliminary examination indicates might be involved in the disease process. The physical examination reveals the distribution and severity of edema. Edema that is localized to one extremity or is not bilaterally symmetric is more likely to be caused by local factors (e.g., lymphangitis, venous obstruction) than by systemic disease. Conversely, edema that involves several areas of the body and has a symmetric distribution is likely to be associated with systemic disease, such as the ventral edema of congestive heart failure. It is important to note that distribution of edema associated with hypoproteinemia can vary and can be widespread or limited to the ventrum, limbs, or submandibular area.

Ancillary Diagnostic Tests

The clinician will have developed an ordered list of potential diagnoses after the initial clinical examination, and this list will help direct the most appropriate ancillary diagnostic tests. Confirmation or elimination of the potential diagnoses may depend on the subsequent diagnostic procedures, including the response to therapy. A CBC and serum biochemical profile are often useful as part of a minimum database and will establish whether hypoproteinemia is present. Sections of this text deal with the specific disease processes and appropriate diagnostic procedures.

COLLAPSE IN HORSES: SYNCOPE, SEIZURES, AND SLEEP DISORDERS

Collapse is characterized by a loss of postural tone with or without progression to recumbency and with or without loss

of consciousness. Collapse in horses is uncommon, but it has serious implications for both horse welfare and human safety. In horses, it appears that collapse most often occurs at rest, but episodes can occur during exercise.[348]

Collapse can be categorized as syncopal or nonsyncopal. Syncopal collapse, which is sometimes referred to as *fainting,* is a self-limiting loss of consciousness and postural tone resulting from cerebral hypoperfusion.[349,350] Nonsyncopal collapse includes a number of disorders that can be further subdivided into those that are associated with some degree of loss of consciousness, including seizures, sleep disorders, and hypoglycemia, and those with no loss of consciousness, including musculoskeletal and metabolic problems, such as hyperkalemic periodic paralysis.

Mechanisms of Collapse

Syncope

Syncope is defined as a temporary interruption of cerebral perfusion resulting in a sudden and transient loss of consciousness and postural tone.[349,350] The brain has a high metabolic demand and is highly sensitive to changes in perfusion. Normally cerebral blood flow is tightly regulated by a complex system of cerebral autoregulation involving metabolic, myogenic, and neurologic components. This system maintains cerebral blood flow despite changes in blood pressure, and syncope can occur when this system fails. As syncope is uncommon in horses, much of the information regarding syncope is extrapolated from other species.

The causes of syncope can be broadly classified as cardiovascular, noncardiovascular, and unknown.[350,351] Cardiovascular syncope is due to reduced cardiac output and has been associated with structural heart disease, arrhythmias, and, in people, coronary heart disease. Causes of noncardiovascular syncope include primarily neurologic and metabolic disorders. One cause of noncardiogenic syncope in people is orthostatic or postural hypotension, which is defined as a decrease of 20 mm Hg in systolic blood pressure or 10 mm Hg in diastolic blood pressure within 3 minutes of changing position from sitting or supine to standing.[350-352] It results from an inadequate physiologic response to postural changes and has been associated with an impairment of peripheral vasoconstriction or reduction of intravascular volume. Specific causes of syncope related to orthostatic hypotension include dehydration or blood loss, as well as a variety of neurologic, cardiovascular, and endocrine disorders. Another type of noncardiogenic syncope is neurally mediated syncope, which is associated with disturbances of reflex cardiovascular control.[350,351,353,354] Some cases of neurally mediated syncope are associated with pain, stresss, or specific triggers. Although the pathogenesis of this phenomenon is not fully understood, it is characterized by peripheral vasodilation and/or transient bradycardia that is associated with inappropriate triggering of neural reflexes. In some but not all cases, neurally mediated syncope is associated with concurrent cardiac disease. The most common form of neurally mediated syncope is neurocardiogenic or vasovagal syncope, in which there is an increase in vagal parasympathetic tone and a decrease in sympathetic tone that results in bradycardia and hypotension associated with peripheral vasodilation.[353,354] In two cases of presumptive neurocardiogenic syncope in horses, no bradycardia was noted before collapse, suggesting a purely vasodepressor response in those cases.[348] Some other forms of neurally mediated syncope that have been recognized in people include carotid sinus syndrome, postmicturition syncope, and swallow syncope.

Syncope is common in people, with approximately one third of the population experiencing syncope at least once during their lifetime.[350,351] In humans, presyncopal signs such as weakness, blurred vision, and nausea frequently precede the collapse. Recovery is spontaneous, and following the episode people can experience persistent drowsiness, dizziness, headache, or nausea but typically not confusion. In horses, signs such as stumbling, head elevation, or anxiousness can precede collapse, but in most cases there are little or no premonitory warning signs.[355] The collapse is associated with a transient comatose state followed by recovery. Horses may struggle during recovery but are normal thereafter unless they sustain significant trauma during the episode. A syndrome of convulsive syncope that is difficult to differentiate from seizure activity has been described in people, but it is uncertain if this occurs in horses.[356,357]

Seizures

Seizures are the manifestation of the excessive and/or hypersynchronous discharge of neurons in the cerebral cortex that leads to voluntary alterations of motor activity, consciousness, and autonomic or sensory functions.[349,355,358-360] Normal neuronal function reflects a balance between excitatory and inhibitory activity. When intracranial neurons experience either excessive excitation or loss of inhibition (disinhibition) the resulting excessive depolarization can result in seizure activity. The seizure threshold reflects the level of neuronal inhibition that must be exceeded for uncontrolled discharge of a population of neurons to occur.

Normally there are sporadic low-frequency spontaneous depolarizations within the brain that are contained by a process known as *surround inhibition*. However, in some cases, groups of cells will depolarize with recurrent high-frequency bursts of action potentials that can manifest as focal seizure activity. Depolarizations of sufficient magnitude can overcome the surrounding inhibitory zone and be conducted to a more widespread area via normal anatomic connections between neurons, thus propagating seizure activity and potentially involving the entire cortex. Although occasionally depolarizations in the brainstem and thalamus can result in seizures, these depolarizations are projected to the cortex. The mechanisms of seizure termination are not fully understood, but factors that may be involved include metabolic exhaustion of neurons and the activity of extracortical inhibitory centers within the cerebellum. Other regions of the brain, such as the caudate and reticular formation, may also affect seizure activity.

Multiple factors may trigger the uncontrolled synchronous neuronal activity involved in the generation of seizures, reflecting the complexity of the neuronal environment.[358-361] Among the factors influencing this environment are the neuronal lipoprotein cell membrane with its associated ion channels and enzymes. Also, neuronal function is influenced by the ionic environment, including concentrations of sodium, chloride, calcium, and potassium. A variety of neurotransmitters are present, including both excitatory neurotransmitters such as glutamate, aspartate, and acetylcholine, and also inhibitory neurotransmitters such as γ-aminobutyric acid, glycine, taurine, and norepinephrine. Several hormones and neurosteroids can influence epileptogenesis, having variable effects depending on the substance.[361] Any alterations in the complex neuronal environment can potentiate seizures. For

example, hypoglycemia can result in a loss of energy substrate for the Na^+-K^+ ATPase pump, causing cells to move toward lower positivity and allowing excessive excitation. In hepatoencephalopathy, there may be a decrease in the function of inhibitory neurotransmitters, resulting in a lack of inhibition and unregulated depolarization. Multiple factors contribute to the development of seizures associated with traumatic brain injury, infection, or neoplasia including neuronal loss, altered cellular metabolism and blood flow, and neuroinflammation, among others.[358-362]

Seizures can present with a variety of forms and severities, and the classification and terminology can be confusing. In humans, the Commission on Classification and Terminology of the International League Against Epilepsy has developed a comprehensive scheme for the classification of seizures to more accurately describe seizures and develop a systematic approach to their diagnosis and treatment.[363] Efforts have been made to similarly classify seizures in horses.[364,365] The term *epilepsy* is defined as two or more seizures that occur in more than 24 hours regardless of the cause. Seizures can be described as either focal or generalized, both of which have been described in horses. Focal seizures, which have previously been referred to as partial, represent a focal area of abnormal discharge within the brain and result in localized motor signs or sensations. Focal seizures can be further categorized as simple, if alertness and normal mentation are maintained, and complex, if impairment of consciousness is present. Generalized seizures involve the entire cerebral cortex and typically result in generalized bilateral motor activity and loss of consciousness. Generalized seizures can be primary, meaning they are generalized from the outset, or secondary, meaning they progress from a focal seizure. Seizures can also be classified by the cause. Reactive seizures are those in which the seizure is a result of a temporary systemic disease with normal brain function such as seizures seen in association with acute hemorrhage or hypoglycemia. Other causes of seizures have been grouped as follows: (1) symptomatic, in which there is a documented structural brain disorder such as brain damage from trauma or hypoxia, congenital anomalies, neoplasia, and infection, including meningitis, encephalitis, and abscessation; (2) cryptogenic, in which symptomatic causes are suspected but cannot be documented; and (3) idiopathic, in which there are no underlying structural or metabolic abnormalities and there is a suspected genetic predisposition.

The clinical presentation of seizures in horses is similar to that seen in other species.[355,364] In a study of 104 horses over 3 weeks of age with seizures, precipitating factors were identified in approximately 11% of cases and included xylazine, noise, trimming the mane, touching the neck, and estrus.[364] Prodromal or preictal signs are not consistently recognized but can be seen in some horses with either focal or generalized seizures and include anxious behavior, isolation from other horses, standing in a corner, swishing of the tail, and changes in personality. Focal seizures may involve the face or body and some of the more common signs include ear or lip twitching, tongue prolapse, opening and closing of jaw, compulsive kicking, head turning to one side, and tremors.[364] In some cases, impaired consciousness and behavior changes have also been reported. Generalized seizures are typically characterized by tonic-clonic motor activity. Eye globe deviation and nystagmus are common. Vocalization and autonomic disturbances such as mydriasis, urination, defecation, and profuse sweating have also been reported. Affected horses may have generalized rigidity or a loss of muscle tone. Postictal signs have also been reported in horses. In the study of 104 horses with seizures, 31% of horses affected by complex focal or generalized seizures had postictal signs typically lasting from minutes to hours.[364] These signs included ataxia, disorientation, lethargy, agitation, blindness, hypersensitivity, and shallow breathing.

Sleep Disorders

Sleep is defined as unconsciousness from which one can be aroused by sensory or other stimuli.[349,355] Sleep has been investigated in domestic horses, and four stages of vigilance have been identified: wakefulness, drowsiness, slow wave sleep (SWS), and rapid eye movement (REM) sleep, also known as *paradoxic* or *desynchronized sleep*.[366-369] REM sleep appears to be an important regenerative part of the sleep cycle. Adult horses typically require 3 to 5 hours per day of sleep with approximately 0.5 hour per day of that being REM sleep. The majority of REM sleep in horses appears to occur during recumbency, although occasionally REM sleep has been observed in standing horses that are not habituated to their environment.[368]

One cause of excessive drowsiness and possible collapse in horses is recumbent sleep deprivation. The level of tolerance for sleep deprivation among individual horses appears to vary widely, with some horses being able to tolerate weeks of being unable to lie down to sleep whereas others will show signs of sleep deprivation after just several days.[355,368,370]

Narcolepsy is a sleep disorder that is primarily characterized by excessive drowsiness and sleep attacks with or without cataplexy, which is a sudden loss of muscle tone and somatic areflexia.[355,371,372] In people, disturbed nocturnal sleep, hypnagogic hallucinations, and sleep paralysis can also be seen in association with narcolepsy. The sleep cycle is disrupted, and people with narcolepsy frequently enter REM sleep at the onset of sleep rather than later in the sleep cycle. Both sporadic and familial forms of narcolepsy have been identified in humans, dogs, and horses.[370-379] The pathophysiology of narcolepsy remains unclear and may vary among species and individuals. It appears to be a complex biochemical disorder affecting several regions of the brain (brainstem, hypothalamus, limbic system, and possibly the striatum and cortex) with dysfunction of dopaminergic, cholinergic, and noradrenergic neurotransmitters.[372,381] Defective hypocretin signaling has been described in both familial and sporadic narcolepsy in some species.[371-373,382] Hypocretin 1 and 2, also known as *orexins,* are neuropeptides that appear to be specifically expressed in certain hypothalamic neurons. They appear to have an important role in the regulation of sleep and arousal states, as well as appetite. In some dog breeds, a familial form of narcolepsy has been identified in association with a mutation in hypocretin-2 receptor gene.[374] In these dogs, hypocretin concentration in the cerebrospinal fluid (CSF) is normal. In humans, narcolepsy is only rarely associated with mutations in the hypocretin receptor gene. In both humans and dogs, the sporadic form of narcolepsy has been associated with decreased hypocretin concentration in the CSF. Many cases of narcolepsy in people are linked to the human leukocyte antigen, and an autoimmune component may play a role in the development of disease. It has been postulated that there is an immune-mediated destruction of hypothalamic neurons producing hypocretin, resulting in a progressive decline in the concentration of hypocretin. The role of hypocretin in equine narcolepsy is currently uncertain. The concentration of hypocretin in the CSF of an

Icelandic foal with narcolepsy without cataplexy was similar to controls, although it was speculated that concentrations could decrease over time.[377] Occasionally secondary narcolepsy may develop after disease or organic insult to the brain, such as following traumatic brain injury.[383,384]

Most cases of narcolepsy in horses have been diagnosed in foals, but sporadic cases have been seen in adults.[355,374-380] Given the difficulties in diagnosing narcolepsy, some of these cases may represent recumbent sleep deprivation or idiopathic hypersomnia, which is another disorder of the central nervous system characterized by excessive sleepiness. Better characterization of sleep disorders in horses is needed.

Sleep disorders in horses are often characterized by drowsiness, with gradual lowering of head and buckling, which most often occurs in the forward direction. In some cases there is complete collapse. In cases of narcolepsy with cataplexy there will be limb atonia and areflexia. Occasionally swaying and stumbling when walking have been described in narcoleptic horses. In cases of narcolepsy in other species, cataplexy can sometimes be induced by play or feeding, and similar triggers have been described occasionally in equine cases of narcolepsy. Many normal newborn foals can be induced into a sleeplike cataplectic state by firm, whole-body restraint or squeezing.[355]

Conditions Causing Collapse

Several conditions have been reported in association with collapse in horses, but information is somewhat limited (Box 7.7). It can be challenging to make a definitive diagnosis for the cause of collapse. In a retrospective study of 25 horses with episodic collapse, a final diagnosis was established in 11 cases and a presumptive diagnosis was made in 8, with 6 being undiagnosed.[348] Overall, the most common cause of collapse in this series of cases was syncope, which accounted for 63% of the cases in which a definitive or presumptive diagnosis was made. In the 11 horses with a specific final diagnosis, causes included cardiac disease (5 of 11), generalized seizures (2 of 11), hypoglycemia secondary to neoplasia (2 of 11), and sleep disorders (2 of 11). In the 8 horses with a presumptive diagnosis, causes included neurocardiogenic syncope (5 of 8), syncope associated with severe EIPH (2 of 8), and seizures (1 of 8).

Syncope

Syncope in horses has been associated with cardiovascular, noncardiovascular, and unexplained causes, with cardiovascular and presumed neurocardiogenic syncope being the most common. Specific cardiovascular problems that have been reported in association with syncope in horses include cardiac arrhythmias, including third-degree atrioventricular (AV) block, atrial tachycardia with advanced second-degree AV block, and atrial fibrillation.[348,355,385,386] Right-sided heart failure, ruptured chordae tendineae, myocardial infarction, myocardial fibrosis, aortic endocarditis, and pericarditis have also been associated with syncope.[355,385,386] In some cases, a definitive cardiac disease can be hard to confirm. Depending on the underlying problem some horses with cardiovascular syncope may have other signs of cardiac failure, but this is not consistent. Presumptive neurocardiogenic syncope has been diagnosed in horses in which there are no abnormal findings on clinical, neurologic, or cardiac examinations.[348,355] In general, collapse was reported to occur without warning at rest, and horses were described to sink abruptly or to raise their heads and fall backward to the ground. Confirmation of neurocardiogenic syncope would require ECG and blood pressure monitoring. Polycythemia and upper airway obstruction have also been reported to cause syncopal episodes.[387,388]

Seizures

A number of disorders have been associated with seizures in horses. The clinical presentation of the seizure appears to be

BOX 7.7 Causes of Collapse

SYNCOPE

Cardiovascular
- Arrhythmia
  - 3° atrioventricular (AV) block
  - Atrial tachycardia with 2° AV block
  - Atrial fibrillation
  - Other
- Right-sided heart failure
- Ruptured chordae tendinae
- Myocardial infarction, myocardial fibrosis
- Endocarditis
- Pericarditis

Noncardiovascular
- Neurocardiogenic (vasovagal) syncope
- Polycythemia
- Airway obstruction

Undetermined

SEIZURES

Reactive (no structural brain lesion)
- Hepatoencephalopathy
- Hemorrhage
- Electrolyte abnormalities
- Hypoglycemia

Symptomatic
- Traumatic brain injury (skull fracture, cerebral hemorrhage)
- Cerebral edema
- Neonatal encephalopathy
- Neoplasia
- Cholesterol granuloma (can be incidental)
- Vasculitis
- Viral, bacterial, or verminous meningoencephalitis
- Abscessation
- Equine protozoal myeloencephalitis
- Intracranial vascular events
- Leukoencephalomalacia
- Congenital anomalies
- Ear ticks

Cryptogenic
- Lesion suspected but cannot be identified

Idiopathic
- Juvenile idiopathic epilepsy of Arabian foals

SLEEP DISORDERS

Recumbent sleep deprivation
Narcolepsy

OTHER

Hyperkalemic periodic paralysis
Hypoglycemia
Hyperthermia
Undetermined

independent of the underlying cause, reflecting the area and extent of the cerebral cortex involved.[364] In the study evaluating 104 horses with seizures, 2 horses were found to have reactive seizures not associated with abnormal brain function. These horses were diagnosed with liver failure and severe acute systemic hemorrhage. The remaining 102 horses were felt to have underlying brain disease, although a specific diagnosis was not always possible. Of these, 28% had a single seizure episode and 70% had two or more seizures and were diagnosed with epilepsy. Of those horses with a single seizure episode, an underlying structural brain lesion was confirmed or suspected in 16 of 29 horses, with acute head trauma being the most common (6 of 16). In those horses with epilepsy, the condition was categorized as symptomatic in 35.6% of cases and cryptogenic in 54.8%, and 2 foals were diagnosed with idiopathic epilepsy. Overall there was no breed, age, or gender association in horses with epilepsy, although juvenile idiopathic epilepsy is seen in Arabian foals. Specific causes of symptomatic seizures that were identified included skull fracture, cerebral hemorrhage, cerebral edema, neoplasia, cholesterol granuloma, vasculitis, meningoencephalitis, abscessation, intracranial vascular events, leukoencephalomalacia, congenital abnormalities, and equine protozoal myeloencephalitis (EPM). The presence of ear ticks has rarely been associated with seizures.[355] Meningoencephalitis due to infection with the nematode *Halicephalobus gingivalis* has been associated with multiple neurologic deficits, including seizures.[389] Neonatal foals have a lower seizure threshold than adults, and seizures have been reported in association with sepsis, neonatal encephalopathy, and electrolyte abnormalities, including hyponatremia.[355,390-392] Hypoglycemia may cause seizures but is more often associated with weakness and lethargy. Hyperbilirubinemic encephalopathy (kernicterus) may rarely occur in foals.

Sleep Disorders

Sleep deprivation can be induced by preventing horses from being able to lie down, such as when horses are kept on a tie-line to prevent them from exacerbating a musculoskeletal injury by getting up and down.[355,370] Other conditions in which horses do not lie down to sleep include musculoskeletal, abdominal, thoracic, or neurologic problems that either create pain or mechanical difficulties on attempt at recumbency or rising to stand. Environmental insecurity, including such things as herd dynamics, stall size, and excessive noise, may prevent horses from feeling comfortable enough to lie down. It has also been proposed that monotony, such as being in crossties for an extended time, may cause horses to exhibit drowsiness, with the head lowering to the point of near collapse as the horse begins to transition from SWS to REM sleep.

Familial narcolepsy has been identified in American Miniature Horses, Shetland Ponies, and Lipizzaners.[355,375,376,378] At this time the precise genetics have not been determined. Sporadic narcolepsy, generally without cataplexy, has been identified in several breeds including Warmbloods, Icelandic Horses, and Thoroughbreds.[355,378]

Other

A number of other disorders can occasionally result in collapse. Hyperkalemic periodic paralysis can cause weakness, muscle fasciculations, and collapse without alterations in consciousness.[302] Other electrolyte abnormalities in addition to hyperkalemia may result in collapse including hypocalcemia, hyponatremia, and hypokalemia.[355] Hypoglycemia can be associated with weakness and collapse, and in some cases seizures may occur. Some other disorders that can be result in collapse include trauma to the motor pathways, botulism, and hyperthermia.[355]

Diagnostic Approach to Collapse

The diagnosis of the cause of collapse can be challenging, and in many cases a definitive cause is not determined. Often the clinician is unable to witness the episode, and although accounts by the owner can be helpful, they can also be inaccurate and may omit key information if the owner is unaware of the significance. A final diagnosis was found to be more likely in horses that experienced multiple episodes of collapse than in horses that were observed to collapse only once.[348] This may be because they may be more likely to have ongoing signs of disease and also because the sensitivity of diagnostic procedures may be improved by carrying them out either during or immediately after a collapsing episode. For example, some cardiac arrhythmias are paroxysmal and may not be present between episodes.

Signalment and History

There are some age, breed, and sex associations with some causes of collapse. In general, neonatal foals are more susceptible to seizures than adult horses and may seize in response to a number of metabolic and systemic diseases.[355,390-392] Juvenile idiopathic epilepsy is a disease of Egyptian Arabian foals with an age of onset ranging from 2 days to 6 months.[355,393] Familial forms of narcolepsy have been identified in American Miniature Horses, Shetland Ponies, and Lipizzaners.[355,375,376,378] Hyperkalemic periodic paralysis should be considered as a possible cause of collapse in Quarter Horses and related breeds.[302] In the study of 104 horses with seizures, seizure type was significantly associated with gender, with females being more prone to generalized seizures and one mare demonstrating generalized seizures during estrus.[364] Estrogen and progesterone concentrations are related to alterations in seizure threshold in women and female dogs, and a similar risk factor may be present in horses.[361,394]

A detailed history should be obtained, including a performance history and the occurrence of any other problems, such as EIPH or lameness. With regard to the episodes of collapse, the onset and duration as well as any specific triggers and the features of the episode should be determined. Possible reasons for recumbent sleep deprivation should be investigated, including underlying medical problems and management changes such as moving the horse to a new environment with different horses, excessive noise, or limited space.

Physical Examination

A thorough physical examination should be performed. In some cases, additional clinical signs may be present depending on the underlying disease. For example, fever and lethargy may be present in horses with viral or bacterial encephalitis and icterus, and weight loss may be seen in horses with hepatoencephalopathy. Horses with sleep disorders may have abrasions on the fetlocks from repeatedly buckling forward. Particular attention should be focused on the cardiovascular and neurologic systems. In some cases of potential cardiac disease, evaluating the horse after exercise may be useful.

Video surveillance may be needed to allow observation of the actual episode of collapse. Although differentiation of the conditions can be difficult, clinical features can vary among syncope, seizures, and sleep disorders. Generalized seizures generally differ from syncope in that they involve tonic-clonic

convulsive activity and may be associated with opisthotonos or nystagmus, as well as urination and defecation. Seizures are also often followed by a postictal phase that is not seen in syncope or narcolepsy. In horses with sleep disorders, the collapse is often preceded by a gradual lowering of the head, and the horse most often falls forward. Eye movement may be seen in association with REM sleep during a sleep attack, but this appears to be uncommon in horses.

Ancillary Diagnostic Aids

Clinical Pathology. A CBC, fibrinogen, and serum chemistry can be helpful in identifying metabolic or systemic abnormalities, such as sepsis, liver disease, or electrolyte abnormalities. In some horses, such as those with hyperkalemic periodic paralysis (HYPP), the abnormality may only be present during the episode. Measurement of cardiac troponin can be useful to identify cardiac disease.[395,396] Analysis of CSF and testing for EPM can provide useful information.

Electrodiagnostic Testing. An electrocardiogram (ECG) is valuable in evaluating the cardiac rhythm in horses with episodes of collapse. As some arrhythmias are intermittent, continuous ECG monitoring may be necessary to identify the arrhythmia. This is most often done with a 24-hour Holter monitor. An insertable cardiac monitor, also known as *insertable* or *implantable loop recorder,* has been used in horses to record an ECG during the collapse episodes and can improve the diagnostic yield.[348] Blood pressure monitoring during an episode could be useful but has not been done in horses.

An electroencephalogram (EEG) assesses electrical brain activity and can be helpful in the identification of seizures. However, due to the paroxysmal nature of epileptiform activity, a normal EEG does not rule out the presence of seizures, especially if performed during the interictal period. In Arabian foals with juvenile idiopathic epilepsy, epileptiform activity was found on EEG in 9 of 13 foals.[393] Continuous EEG monitoring improves the chance of detecting abnormal brain activity. A device for ambulatory EEG has been used in horses for long-duration EEG recording and has been shown to identify abnormal brain acitivity.[397,398] EEG can also evaluate electrical activity in the sleep cycle and may be useful in the assessment of narcolepsy.

Diagnostic Imaging. Echocardiography can be useful in the evaluation of cardiac disease. It can also be used to evaluate the liver in horses with suspected hepatic disease and hepatoencephalopathy.

Radiographs, computed tomography (CT), and magnetic resonance imaging (MRI) can all be useful in the diagnostic evaluation of horses with collapse, especially with suspected seizure activity.[348,364,399,400] Skull radiographs and CT are particularly useful in horses with skull trauma.[400] CT can be used to identify other lesions as well but has some limitations in identifying inflammatory disease and small or diffuse parenchymal lesions. MRI has been shown to be a valuable diagnostic tool, especially in the evaluation of symptomatic epilepsy.[364,399] There has been a good correlation between MRI findings and intraoperative or postmortem results. It is important to remember, however, that it is not uncommon for seizures to occur in the absence of a structural brain lesion.

Additional Testing. Challenge testing has been used in the assessment of possible narcolepsy in horses, but results have not been consistent.[374-377] Physostigmine may induce narcoleptic episodes, and atropine may eliminate signs. In humans and dogs, hypocretin concentration in CSF can be used in the evaluation of narcolepsy, but at this time the value of hypocretin testing in horses is uncertain.[377,382]

DYSPHAGIA

Dysphagia is defined as difficulty swallowing. Normal swallowing, or deglutition, occurs as a complex sequence of events with three phases: oral, pharyngeal, and esophageal.[401] In equine medicine, dysphagia is often used in the broad sense to include any difficulty in the prehension and uptake of feed, as well as in actual swallowing. The clinical signs of dysphagia vary depending on the cause but frequently include dropping feed or packing feed between the cheek teeth and buccal mucosa, ptyalism, extension of the neck, gagging, coughing, and the presence of feed-tinged nasal discharge. Dysphagia can be a significant problem for the patient due to the risk of aspiration and the potential compromise in nutritional and hydration status.

Mechanisms of Dysphagia

Normal eating and drinking are complex neuromuscular activities that are initiated with the uptake of material into the oral cavity.[401-403] The process of swallowing allows the transport of this material from the oral cavity to the stomach while protecting the airway. The initial stage of swallowing is the oral phase, which encompasses the formation and positioning of a feed bolus, which is defined as feed that is of suitable size and consistency to be swallowed. Mastication and moistening by saliva lead to formation of the bolus and also initiate digestion by mechanical breakdown of the feed and addition of digestive enzymes from saliva. The bolus is moved to the base of the tongue, where coordinated movements of the tongue and pharynx initiate the pharyngeal phase of swallowing. The oropharynx relaxes and the soft palate elevates to seal the palatopharyngeal arch and nasopharynx. As the bolus enters the oropharynx, the hyoid apparatus moves rostrodorsally, drawing the larynx and common pharynx forward while at the same time the epiglottis tips caudally to prevent the bolus from entering the larynx. The bolus is moved through the pharynx by sequential contraction of the pharyngeal constrictor muscles along with some driving force from the tongue. The lower portion of the inferior pharyngeal constrictor muscle, known as the *cricopharyngeus,* is the major muscle in the upper esophageal sphincter. At the end of the pharyngeal stage of the swallow, this muscle relaxes, allowing the bolus to enter the esophagus, and then contracts to prevent oroesophageal reflux and aerophagia. As the bolus enters the esophagus, the esophagus, including the lower esophageal sphincter, relaxes and the bolus is propelled toward the stomach by primary peristaltic waves. Gravity can assist in moving a liquid bolus toward the stomach. After the bolus enters the stomach, the lower esophageal sphincter contracts to prevent gastroesophageal reflux. Any minor reflux that does occur is typically cleared by secondary peristaltic waves, which are waves that occur in the thoracic esophagus without an associated pharyngeal contraction. Active antiperistalsis does not normally occur in horses, and thus horses generally do not actively vomit. All of the activity involved in swallowing occurs relatively rapidly. In herbivores, breathing can continue uninterrupted during swallowing.

Several anatomic structures and at least 30 muscles, including both striated and smooth muscles, are involved in the process of eating and drinking.[401,402] Among the muscles used

are the masseter, temporalis, and medial and lateral pterygoid muscles, often referred to as the *muscles of mastication*. Also muscles within the lips, oral cavity, pharynx, larynx, and esophagus are involved. Sophisticated integration among these muscles and the peripheral and central nervous systems is required for normal swallowing to occur.

Each phase of swallowing is under complex neurologic control, with both voluntary (somatic) and involuntary (autonomic) input into the process.[401-403] Cranial nerves (CNs) that play a major role in prehension and swallowing include the trigeminal (CN V), facial (CN VII), glossopharyngeal (CN IX), vagus (CN X), spinal accessory (CN XI), and hypoglossal (CN XII). The activity of these six CNs is mediated centrally via the medulla oblongata of the brainstem, where a network of sensory and motor nuclei and interneurons form the swallowing center. In addition to the brainstem, the cortical and subcortical regions of the brain play an integral part in mediating the swallow, especially the oral phase.

Both sensory and motor function play a role in the uptake of feed, as well as throughout swallowing.[403] With regard to the uptake of feed, sensory input from the olfactory and optic nerves is important in providing smell and sight while the trigeminal nerve provides sensation to the rostral oral mucosa and lips. Once feed is in the oral cavity and oropharynx, peripheral receptors stimulate the trigeminal, facial, and glossopharyngeal nerves providing sensory input to the brainstem, which leads to activation of the swallowing reflex. Patterned discharges of excitation and inhibition are sent from neurons in the swallowing center to motor nuclei of the cranial nerves, resulting in swallowing. Swallowing can also be initiated by stimulation of cortical neurons, and often cortical and peripheral inputs act together in eliciting swallowing. Once swallowing is initiated, continuing sensory input modulates motor activity.

A variety of problems, either congenital or acquired, can disrupt swallowing.[404-406] Problems causing dysphagia may be grouped by the anatomic location as oral, pharyngeal, or esophageal. Also, general mechanisms of dysphagia may be classified as either morphologic or functional. Morphologic causes include pain and inflammatory conditions, as well as mechanical problems, such as anatomic abnormalities and obstruction. Functional causes include a variety of neurologic, neuromuscular, and muscular disorders that can interfere with the highly regulated activity of swallowing. Functional disorders may affect any phase in the process of eating and drinking, but most often affect the oral and pharyngeal phases of swallowing. Some disorders, such as guttural pouch empyema, have the potential to cause both physical obstruction and neurologic impairment.

BOX 7.8 Morphologic Causes of Dysphagia

- Anatomic abnormalities
  - Cleft palate
  - Wry nose, severe malocclusions
  - Branchial remnant cysts
  - Subepiglottic cysts
- Dental conditions
  - Points, hooks
  - Retained deciduous premolars
  - Wave, step, or shear mouth
  - Periodontitis
  - Fractured teeth
  - Equine odontoclastic tooth resorption and hypercementosis
  - Other
- Temporomandibular joint disease
- Oral ulceration
  - Dental problems
  - Vesicular stomatitis virus
  - Plant awns
  - Caustic substances, medications
  - Cantharidin toxicosis
- Trauma
  - Fractures of skull, mandible, or maxilla
  - Tongue lacerations
- Foreign bodies
- Neoplasia
  - Oral, esophageal—squamous cell carcinoma, dental
- Epiglottitis
- Retropharyngeal masses causing compression
  - Abscesses
  - Guttural pouch enlargement
  - Empyema, mycosis
  - Tympany
  - Hemorrhage associated with rupture of the longus capitus
  - Neoplasia
  - Septic sialoadenitis
- Esophageal disorders
  - Obstruction (choke)
  - Esophagitis
  - Perforation, fistula
  - Stricture
  - Diverticula, megaesophagus
  - Congenital abnormalities—cysts
  - Neoplasia
- Snakebite

Conditions Associated with Dysphagia

Morphologic Causes

Several morphologic causes of dysphagia have been identified in horses (Box 7.8). Anatomic abnormalities that have been recognized in association with dysphagia include a cleft palate, wry nose, branchial remnant cysts, and subepiglottic cysts.[405-408] Pain and inflammation from a number of underlying causes often contribute to dysphagia. Dental problems can cause both pain and mechanical problems with mastication.[409,410] Some painful dental conditions include ulcerations from points or hooks, periodontitis, infundibular caries, fractured teeth, and equine odontoclastic tooth resorption and hypercementosis syndrome (EOTRH). Problems such as retained deciduous premolars, as well as a wave, step, or shear mouth, and occasionally severe malocclusions may make it mechanically difficult to chew effectively. Oral ulceration causing discomfort may be seen with plant awns, vesicular stomatitis virus, cantharidin toxicosis, and exposure to caustic substances or some medications, such as phenylbutazone or enrofloxacin.[404-406] Dysphagia has been reported in a foal with equine herpesvirus 2–associated oral and esophageal ulceration.[411] Other painful conditions include epiglottitis, foreign bodies, temporomandibular joint disease, mandibular or maxillary fractures, and other traumatic injuries, such as hyoid bone injury or tongue

lacerations.[405,406,412,413] Although uncommon, septic sialoadenitis involving either the parotid or mandibular salivary gland can be a significant cause of pain, inappetance, and dysphagia.[414]

The most common obstructive problem causing dysphagia is esophageal obstruction, or choke.[404-406,415] This most often results from a feed impaction, although esophageal foreign bodies may occur as well. In addition to obstruction, other esophageal conditions that can result in dysphagia include esophagitis, esophageal rupture, fistula, strictures, diverticula, megaesophagus, and congenital disorders, such as esophageal cysts.[415,416] Esophageal tumors are uncommon, but primary esophageal squamous cell carcinoma has been reported.[415,417-419] Both squamous cell carcinoma and leiomyosarcoma have been seen to extend from the stomach into the esophagus. Idiopathic muscular hypertrophy of the distal esophagus has been recognized but in many cases is of no clinical significance.[420]

External compression of the pharynx or esophagus may also result in obstruction. Pharyngeal collapse may be seen with enlargement of the retropharyngeal lymph nodes, most often due to abscessation associated with *Streptococcus equi* subspecies *equi,* as well as with severe guttural pouch disease, including empyema, mycosis, or tympany, and occasionally neoplasia.[405,406,421-424] Pharyngeal collapse is often associated with dyspnea, as well as dysphagia. Oral neoplasms of either dental or nondental origin are relatively uncommon but can cause pain or act as a space-occupying mass resulting in dysphagia.[405,406,425,426] A number of dental tumors, as well as osteogenic tumors of nondental origin, such as osteomas, ossifying fibromas, and osteochondromas, have been described. Squamous cell carcinoma is the most common soft tissue neoplasm reported in the oral cavity, but others have been identified, including melanoma and lymphosarcoma. Adenocarcinoma of the tongue and epiglottis has been reported as a cause of severe dysphagia in a horse.[427] Snakebite may cause severe edema of the head and dysphagia.[347,428]

Functional Causes

Guttural pouch diseases affecting the cranial nerves within the pouch are one of the more common functional problems causing dysphagia[404-406,424,429-431] (Box 7.9). The dorsolateral wall of the medial compartment of the guttural pouch contains several nerves, including the glossopharyngeal (IX); branches of the vagus (X), spinal accessory (XI), and hypoglossal nerves (XII); and the cranial cervical ganglion. In addition, a small portion of the facial nerve (VII) is located in the dorsal wall of the lateral compartment. Thus guttural pouch disease can result in pharyngeal dysfunction, dysphagia, and occasionally other neurologic signs such as laryngeal paralysis, Horner's syndrome, and facial paralysis.[404-406,424,429-431] Important diseases of the guttural pouch causing dysphagia include guttural pouch empyema and guttural pouch mycosis. Skull trauma resulting in rupture of the longus capitus muscle at the site of attachment to the basisphenoid bone may result in significant hemorrhage into the pouch, potentially affecting the cranial nerves.[429] Occasionally flushing of the guttural pouch and surgical correction of guttural pouch tympany results in damage to the cranial nerves coursing through the pouch with consequent neurologic dysfunction.[405,406,431] Although temporohyoid osteoarthropathy most often results in deficits of the facial (CN VII) and vestibulocochlear (CN VIII) nerves, occasionally deficits of the glossopharyngeal (CN IX) and vagus (X) nerves occur, resulting in dysphagia.[432,433] Lead toxicity may result in a peripheral neuropathy with dysphagia.[434] Some horses with polyneuritis equi, an inflammatory condition affecting primarily the cauda equina, will have involvement of the cranial nerves and present with cranial nerve signs including dysphagia, occasionally even before the development of signs related to cauda equina dysfunction.[435,436]

Central neurologic diseases and neuromuscular or muscle disorders that can result in dysphagia often have other concurrent signs in addition to problems eating. Some important central neurologic diseases that can be associated with dysphagia include viral encephalitis (rabies, eastern and western encephalitis, West Nile virus, equine herpesvirus), equine protozoal myeloencephalitis, toxic neuropathies (leukoencephalomalacia and nigropallidal encephalomalacia), tetanus, cerebral trauma, and rarely migrating parasites.[404-406,437-441] Dysphagia is a relatively common finding in horses affected with rabies.[404] Horses with nigropallidal encephalomalacia have lesions of the basal ganglia and can swallow but are unable to prehend

BOX 7.9 Functional Causes of Dysphagia

PERIPHERAL NERVE DISEASE
- Guttural pouch disease affecting the cranial nerves
  - Empyema, mycosis
  - Tympany
  - Hemorrhage associated with rupture of the longus capitus
  - Neoplasia
- Temporohyoid osteoarthropathy
- Lead poisoning
- Polyneuritis equi

CENTRAL NEUROLOGIC DISEASE
- Viral encephalitis/encephalomyelitis
  - Rabies
  - Eastern, western, and Venezuelan encephalitis (EEE, WEE, VEE)
  - West Nile virus (WNV)
  - Equine herpes myeloencephalopathy
- Equine protozoal myeloencephalitis
- Toxicity
  - Nigropallidal encephalomalacia
  - Leukoencephalomalacia
- Trauma
- Tetanus
- Migrating parasites
- Equine grass sickness
  - Affects central, peripheral, and enteric nervous systems
- Pharyngeal dysfunction in neonatal foals

NEUROMUSCULAR DISEASE
- Botulism
- Tetanus
- Organophosphate toxicity
  - Primarily acute cholinergic signs, dysphagia rare
- Tick paralysis

MUSCLE DISEASE
- Nutritional myodegeneration (white muscle disease)
- Hyperkalemic periodic paralysis

feed as they lack coordination of the lips and tongue. Tetanus toxin acts at several sites within the nervous system, including the inhibitory interneurons in the central nervous system and the neuromuscular junction, causing muscle paralysis.[442] It typically results in increased tonus of the masticatory muscles, known as *trismus,* and dysphagia. The mouth may be difficult to open, thus the term *lockjaw.* Some cases of marked hypocalcemia may result in tetany and dysphagia. A syndrome of dysphagia related to presumed pharyngeal dysfunction has been reported in neonatal foals.[443] Although the pathogenesis is not fully understood, some affected foals were premature and/or diagnosed with neonatal encephalopathy. Other central neurologic problems that may potentially cause dysphagia include bacterial or fungal meningitis and intracranial masses.

Neuromuscular problems that can result in dysphagia include organophosphate toxicity, tick paralysis, botulism, and to some extent tetanus, which also has a central component.[404-406,442,444,445] One of the early signs of botulism can be that the horse eats slowly. Equine grass sickness (EGS), also referred to as *equine dysautonomia,* is a largely fatal neurodegenerative disease affecting the peripheral, central, and enteric nervous systems of grazing equids.[446-448] The gastrointestinal tract is the most severely affected body system, with the predominant clinical signs being colic, weight loss, and dysphagia. Although the disease primarily occurs within Great Britain, cases have been identified in regions of mainland Europe and rarely in other locations including the United States. A possible link to *Clostridium botulinum* types C and D has been proposed, but the cause of EGS remains unclear.[446]

The primary muscle disease resulting in dysphagia is nutritional myodegeneration (nutritional muscular dystrophy, white muscle disease) associated with selenium deficiency.[449-451] Although reported more frequently in foals, nutritional myodegeneration can affect horses of any age. Although multiple muscles can be affected, myodegeneration may be limited to the masseter muscle and/or tongue in some cases, making dysphagia the predominant sign. Foals affected with HYPP may have dysphagia, particularly when they are homozygous.[302]

Diagnostic Approach to Dysphagia

The evaluation of dysphagia focuses on determining whether morphologic and/or functional abnormalities are present. The initial assessment should include consideration of the signalment and history and a thorough physical examination, including observation of the horse eating. Additional tests such as a neurologic examination and ancillary diagnostic aids are often indicated.

Signalment and History

Some age and breed associations have been associated with dysphagia. Anatomic abnormalities such as a cleft palate or wry nose are generally apparent at an early age. Some dental disorders can be age related.[409,410] The deciduous premolars are lost between 2 and 5 years of age and may be retained as caps. Older horses are more are more likely to have a severe wave mouth, missing teeth, or EOTRH and can be at increased risk of esophageal obstruction. Dysphagia associated with pharyngeal dysfunction may be seen in neonatal foals, sometimes in association with prematurity or neonatal encephalopathy.[443] In foals with Quarter Horse breeding, HYPP can be a cause of dysphagia.[302] Friesian horses have an increased prevalence of esophageal disorders, specifically megaesophagus.[452]

A thorough general history is important in the evaluation of dysphagia. The history may help to differentiate dysphagia from a decrease in appetite. The diet, dental history, and any recent disease and treatment should be determined. A history of dropping feed during mastication, or *quidding,* may suggest dental disease. The potential for exposure to toxins such as lead, organophosphates, or yellow star thistle and Russian knapweed should be assessed. The vaccination status of the horse should be established, especially with regard to tetanus, West Nile virus, eastern equine encephalitis virus (EEE), western equine encephalitis virus (WEE), equine herpesvirus, and rabies. It should be determined whether other animals on the farm are having problems as certain conditions, such as strangles and botulism, may affect more than one individual. Trauma is often associated with a history of an acute onset of dysphagia. In some cases there is a known history of a traumatic event such as a fall or kick, but in other cases the cause of the trauma in unknown. Occasionally trauma may occur during the administration of medication or passage of a nasogastric tube. In addition, a history of flushing the guttural pouch can be of significance as the procedure may rarely cause inflammation and tissue damage.

Physical Examination

Biosecurity procedures should be followed during the initial assessment of horses with dysphagia as rabies is a potential differential. Ideally, veterinarians and assistants should have an adequate rabies antibody titer.

A complete physical examination should be performed. The presence of a fever suggests an infectious or inflammatory process. Observing the horse while eating, and if possible drinking, can be valuable. Some horses can continue to drink despite difficulty swallowing feed whereas others cannot. Offering feed may help distinguish dysphagia from anorexia. Depending on the underlying cause, many dysphagic horses are hungry and will attempt to eat. Some horses, such as those with retropharyngeal abscessation due to strangles, may have both dysphagia and a decreased appetite. Observation of eating can also help determine the phase of eating affected, which can help in prioritizing differentials. In cases where prehension is primarily affected, nigropallidal encephalomalacia should be considered in areas where yellow star thistle or Russian knapweed is present. Dropping feed while chewing is consistent with abnormal mastication, which is often associated with dental disease. The regurgitation of feed through the nares while eating is generally associated with pharyngeal or esophageal disorders rather than oral cavity problems. Those horses with feed in the nares are at increased risk of aspiration of feed material, and coughing or increased respiratory rate and effort may be seen. Careful auscultation of the thorax, including a rebreathing evaluation, should be performed.

A thorough oral examination using a mouth speculum is usually indicated. The teeth should be examined for the presence of problems such as retained deciduous caps, sharp points or hooks, wave mouth or step mouth, missing teeth, diastema, periodontitis, infundibular caries, dental fractures, and EOTRH.[409,410] The oral cavity and tongue should be examined for the presence of ulcers or other abnormalities, such as lacerations, foreign bodies, and masses. Foreign bodies may become wedged between the molars or under the tongue, and wire foreign bodies or awns may become embedded in the tissue. The submandibular and throat latch area should

be examined for evidence of lymphadenopathy or guttural pouch enlargement. Occasionally an esophageal obstruction can be palpated in the neck. An esophageal perforation should be considered if there is heat or swelling in the neck. If ptyalism is observed without dysphagia, ingestion of clover that is contaminated with *Rhizoctonia leguminicola,* a fungus that produces the mycotoxin *slaframine,* should be considered.[453] Excess salivation stops once the horse is no longer exposed to the toxin.

Nasogastric Intubation

A complete esophageal obstruction can be ruled out if a nasogastric tube can be passed into the stomach. Common sites for esophageal obstruction to occur are the proximal esophagus just distal to the larynx, at the thoracic inlet, and at the base of the heart.

Neurologic Examination

A complete neurologic examination is often indicated in the evaluation of dysphagia, especially when morphologic causes have been ruled out. The neurologic examination helps establish a neuroanatomic localization. In general, horses with dysphagia due to peripheral neurologic disease may exhibit only dysphagia, whereas those with central neurologic or neuromuscular disease tend to have additional deficits. For example, the presence of generalized weakness in association with dysphagia often suggests a neuromuscular problem, such as botulism or organophosphate toxicity.[404-406,444,445] Equine motor neuron disease results in generalized weakness and muscle atrophy; however, affected horses do not exhibit cranial nerve deficits and are not dysphagic.[454] Tetanus causes spastic paralysis and can present with a variety of signs, including dysphagia, hyperesthesia, and prolapse of third eyelid, in addition to a stiff gait, which may progress to recumbency.[442] Spinal ataxia associated with dysphagia suggests a diffuse or multifocal disease affecting the spinal cord and brainstem, such as equine protozoal myelitis, rabies, equine herpes myeloencephalopathy, or a migrating parasite.[437-440] Viral encephalitis should be considered in dysphagic horses with mentation changes, especially if the horse is febrile. Horses with polyneuritis equi occasionally have cranial nerve involvement in addition to the classic signs of cauda equina involvement, which include slowly progressive paralysis of the tail, rectum, anus, and bladder.[435,436] Affected horses may also have ataxia and weakness of the hindlimbs.

Ancillary Diagnostic Aids

Clinical Pathology/Laboratory Testing. A CBC, fibrinogen, and serum chemistry may be helpful in documenting an inflammatory process. In cases of suspected nutritional myodegeneration, measurement of selenium and glutathione peroxidase can help in evaluating the selenium status of the patient.

Further diagnostic tests are often indicated in cases of suspected neurologic disease. These may include an evaluation of CSF for cytologic and biochemical abnormalities, as well as specific testing for antibodies to *Sarcocystis neurona* or *Neospora hughsei.*[440] In cases of suspected West Nile virus infection, the immunoglobulin M (IgM) capture ELISA can be used. Cranial nerve involvement is infrequent with equine herpesvirus myeloencephalopathy, but testing by PCR or virus isolation on nasal swabs and/or blood should be considered if appropriate.[438,439] Botulism can be a difficult disease to diagnose, and diagnosis is often based on the history and clinical signs along with the exclusion of other diseases.[444,445] Several tests are available for definitive diagnosis, including testing for toxin by mouse bioassay or PCR in feed, manure, gastrointestinal contents, serum, or wound exudate. The antemortem diagnosis of polyneuritis equi can be difficult, but a biopsy of skeletal muscle innervated by nerves arising from the cauda equina may demonstrate extensive cellular infiltrates in intramuscular nerve fibers.[435,436]

Endoscopy. Endoscopic examination allows visualization of the nasal passageways, nasopharynx, pharynx, larynx, and guttural pouches. Pharyngeal and laryngeal function can be assessed, although the use of sedation should be taken into account as it may affect function. Endoscopic examination of the guttural pouch can identify the presence of empyema or guttural pouch mycosis, as well as other less common conditions such as trauma-related hemorrhage and neoplasia. The stylohyoid bone should be carefully evaluated. In temporohyoid osteoarthropathy there is often osseous proliferation primarily affecting the proximal portion of the stylohyoid.[432,433] Depending on the length of the endoscope, the esophagus may also be evaluated for evidence of esophagitis, obstruction, strictures, diverticula, perforations, or other abnormalities.

Diagnostic Imaging. Diagnostic imaging can provide useful information in the evaluation of dysphagia. Skull radiographs, CT, or MRI can help in the diagnosis of fractures, dental disease, temporohyoid osteoarthropathy, temporomandibular joint disease, guttural pouch disease, and retropharyngeal masses.[405,406,409,410,412,421] Radiopaque foreign bodies, such as a wire in the oral cavity, pharynx, or esophagus, can be identified on radiographs.[413] In cases of esophageal perforation, extraluminal radiolucencies consistent with subcutaneous air may be visualized. Contrast radiography with the use of barium sulfate can be useful in the diagnosis of certain esophageal disorders such as strictures, diverticula, or megaesophagus.[415] Due to the risk of aspiration in horses with dysphagia, radiographs and ultrasound of the thorax are often indicated, especially in horses with abnormal thoracic auscultation, nasal discharge, cough, increased respiratory rate, or increased respiratory effort as evidenced by increased abdominal effort or nasal flare. Ultrasound may also be useful in the detection of foreign bodies and in evaluation of swelling in the throatlatch region.

COLIC

Colic is defined as the manifestation of abdominal pain. In horses colic is a serious medical and economic problem worldwide. In the United States the annual incidence of equine colic has been reported to be anywhere between 3.5% and 26%.[455-458] A study performed by the National Animal Health Monitoring System in 1998 assessed the annual incidence of colic to be 4.2% with an estimated total cost of approximately $115 million.[455] In this study only 1.4% of colic episodes resulted in surgery, but the overall fatality rate for all colic was 11%. When evaluating general causes of mortality in horses over 6 months of age, including euthanasia, colic was found to be responsible for 22.2% of equine deaths in 1998 and 15.2% in 2005.[459] This makes colic among the most common causes of death in horses along with old age (24.8% and 30.4% of deaths) and injury (12.7% and 16.0% of deaths). Even in less serious cases, colic can be associated with a significant loss of use. In a study of working horses in Egypt, the prevalence of colic was found to be 54.6%.[460] These numbers illustrate the enormous impact that colic has on the equine population.

Horses express clinical signs of colic in a variety of ways.[461,462] Often the first sign recognized by owners is inappetence, but other early signs may include general restlessness and extended periods of lying down. More overt signs, such as kicking or biting at the abdomen, pawing, and stretching out as if to urinate, may also be observed. Bruxism is occasionally seen. As the level of discomfort increases, horses may repeatedly get up and down and try to roll, sometimes violently.[461] In some cases, the horse may acutely have much pain. The level of stoicism varies widely among individual horses, and the severity of signs does not always correlate to the severity of the lesion.

Mechanisms of Colic

The precise pathophysiologic mechanisms involved in cases of colic can vary because of the myriad causes for colic in the horse. Most cases of colic, sometimes referred to as "true" colics, are gastrointestinal in origin. However, disorders of other body systems, such as cholelithiasis and uterine torsion, may also manifest as colic. True colic can be classified on the basis of small intestinal versus large intestinal disorders, physical versus functional disorders, obstructive versus nonobstructive lesions, and strangulating versus nonstrangulating lesions. In all of these colic classifications, the simplest basic etiologies for damage to the gastrointestinal tract are inflammation and ischemia.[462-464] Gastrointestinal distention, ileus, mesenteric tension, and endotoxemia also play a role in the development of disease in many cases.

Ischemia of the intestine can result from a strangulating lesion or even from a simple obstruction. Strangulating lesions cause acute direct occlusion of vessels leading to rapid tissue hypoxia, ischemia, and ultimately necrosis. With obstructions significant pressure within the distended intestine may lead to venous collapse, which over time may result in ischemia as the intestinal vasculature becomes increasingly compromised.[462-464] The mucosa is the layer most sensitive to hypoxia because of its high metabolic activity. Damage to the mucosa can be assessed on a scale of Grade I through Grade V, with Grade V being the most severe.[462] The villous tips are especially sensitive to ischemia, and damage typically begins there. Crypt cells are affected later as the ischemia becomes more complete and longer in duration. In the equine colon complete ischemia leads to necrosis and detachment of surface epithelial cells and most likely to capillary thrombosis and occlusion.[462] Smooth muscle is less sensitive to hypoxia, and therefore it is destruction of the mucosa that is the main factor leading to the pathologic changes associated with colic.

Prolonged intestinal distention proximal to an obstructive lesion can eventually lead to tissue ischemia. Distention also results in edema and additional secretion of fluid into the lumen of the gut as venous collapse occurs.[462-464] As veins are occluded, hydrostatic pressure within the capillaries increases, causing increased filtration. Lymphatic drainage is also often impaired, and the resultant excess fluid becomes edema and secreted intestinal fluid. Distention is one of the main causes of pain associated with colic, as stretch pain receptors are triggered within the wall of the intestine.[462]

Inflammation plays a major role in almost every type of colic and often occurs secondary to ischemia. The inflammatory response is generally meant to protect the intestine against long-term damage. The pathophysiology of inflammation is complex and beyond the scope of this section, but the basic process in the gastrointestinal tract is similar to that of other body systems. Almost all cells in the intestine can play a role in the development of inflammation by either cytokine production or cell activation. Some specific cell types involved in the initiation of intestinal inflammation include mucosal cells, endothelial cells, fibroblasts, neutrophils, macrophages, neurons, eosinophils, and mast cells.[463] Cytokines, growth factors, and adhesion molecules produced by these cells are important in the initiation of inflammation. Some important cytokines include IL-1, TNF-α, platelet activating factor, complement, interferon, and histamine.[463]

Endothelial cells are stimulated by ischemia or macrophage cytokines to attract neutrophils, which subsequently migrate to the affected tissue facilitated by adhesion molecules. Vascular permeability is also increased, resulting in the formation of edema and facilitating the migration of inflammatory cells. Neurons, fibroblasts, and muscle cells detect and release other cytokines, leading to the activation of many effector cell types.[463,464]

Eventually the intestinal barrier is compromised to the extent that endotoxin begins to leak into systemic circulation. Endotoxemia (discussed in detail in Chapter 12) is most common with colitis and to a lesser extent with strangulating lesions. The response to endotoxin is complex, involving a massive release of cytokines and mediators that results in systemic inflammation and fever. Classical endotoxemia has more recently been described as *systemic inflammatory response syndrome (SIRS),* because it likely involves more than just endotoxin as an initiating factor.[463] The result is hemodynamic responses that include both vasodilation and vasoconstriction, platelet aggregation, and eventual development of a hypercoagulable state and consumptive coagulopathy.[462,463]

Finally, ileus should be addressed as an important component of the pathophysiology of colic. Ischemic bowel has decreased motility, although bowel more proximal to the lesion tends to have an increase in motility until it becomes so distended that it can no longer contract normally. Postoperative ileus appears to involve both dopaminergic and adrenergic stimulation. It has also been suggested that prostaglandins E_1 and E_2, as well as nitric oxide, play a role in disrupting intestinal motility patterns.[462]

Owners often question why horses have colic, and several studies have attempted to identify risk factors for colic with varying results.[465-473] Some factors that may increase the risk of colic in horses include stall confinement, feeding of excessive concentrate, infrequent large meals, recent feed changes, decreased water consumption or lack of access to water even during turnout, feeding of Coastal Bermuda grass hay, and off-the-ground feeding. Other risk factors include inadequate deworming or dentistry, exposure to sand, transport, changes in activity, previous colic episodes, and the use of nonsteroidal antiinflammatory drugs (NSAIDs). Colic has also been linked to stereotypic behaviors such as crib-biting, which has been shown in some studies to be associated with an increased risk of both colic in general and epiploic foramen entrapment.[470,474-476] It is important to remember that colic can occur regardless of management practices.

Conditions Associated with Colic

Colic encompasses a wide variety of conditions, both gastrointestinal and nongastrointestinal in origin (Table 7.4). The actual incidence of specific disorders causing colic in the general

TABLE **7.4 Gastrointestinal Causes of Colic**

Gas and spasmodic	
Impactions	Pelvic flexure
Feed	Large colon
Sand (primarily pelvic flexure, large colon)	Cecum
	Small colon
Other intraluminal obstructions	Ileal
	Gastric
	Meconium impaction (foals)
	Enteroliths
	Fecaliths
	Foreign body
Large colon displacements	Right dorsal displacement
	Left dorsal displacement (nephrosplenic entrapment)
	Other
Large colon torsion and volvulus	
Small intestinal volvulus	
Strangulating lipoma	
Entrapment of small intestine	Epiploic foramen
	Mesenteric rent
Intussusception	
Ulceration	Gastric
	Right dorsal colitis
Enteritis/colitis	Duodenitis/proximal jejunitis (anterior enteritis)
Inflammatory bowel disease	Colitis
Parasites	Ascarid impactions
	Tapeworms
	Strongylus vulgaris
	Cyathostomes
Herniation	Inguinal
	Umbilical
	Diaphragmatic
Peritonitis	
Abdominal abscessation	
Hemoperitoneum	
Toxins	Cantharidin
	Monensin
	Other
Other	Ileus
	Overo lethal white syndrome
	Congenital anomalies
	Neoplasia

equine population is not known, partially because a definitive diagnosis is not always established. In general, most colics are gastrointestinal in origin, with simple gas or spasmodic colics being most common.[456,477-479] Other common causes include large colon impaction and large colon displacement. In one study of 604 horses that were *referred* for evaluation of colic, 327 cases were medical (54.1%) and 277 cases were surgical (45.9%).[479] Of the medical cases, the most common problems were large colon impaction (39.6%) and spasmodic colic (20.8%). Of the surgical cases, the most common problems were large colon displacement (24.5%), followed by large colon torsion (14.3%) and strangulating lipoma (13.5%).[479]

Disorders of several body systems other than the gastrointestinal tract can present with signs of colic.[480-484] Causes of colic involving the reproductive tract include uterine torsion or tears in mares and testicular torsion in stallions. Occasionally ovarian activity may cause transient colic in mares. Some other potential nongastrointestinal causes of colic include cholelithiasis, liver lobe torsion, urolithiasis, and pheochromocytoma.

Diagnostic Approach to Colic

Colic can be caused by a number of different conditions. Several other clinical problems may be confused with colic, such as pleuritis, exertional rhabdomyolysis, laminitis, hyperkalemic periodic paralysis, renal disease, and even neurologic abnormalities.[485] When a horse presents with colic, it is important to determine that the horse is exhibiting signs of abdominal pain and then arrive at a specific diagnosis if possible. In some cases the evaluation of a horse with colic may be straightforward, but other cases may be more challenging. Fortunately, although a specific diagnosis may not be made, many cases of colic either resolve spontaneously or with minimal intervention. However, in some horses, the condition can be life threatening, and a delay in surgical exploration can result in increased mortality. Thus the initial goal in the assessment of a horse with colic is often to determine whether the case is an uncomplicated one, such as a gas or spasmodic colic, rather than one requiring either extensive medical management or surgical exploration. Signalment, history, physical examination, clinicopathologic data, imaging findings, and endoscopy may all contribute to the evaluation of a patient with colic.[485,486] A number of studies have focused on identifying means to aid in the early classification of colic cases as medical or surgical and to determine a prognosis. Most of these studies have assessed a number of physical examination and laboratory variables.[486-492]

Signalment and History

The signalment of the horse can provide information that may increase the degree of suspicion for a specific cause of colic. Weanling-age foals are more prone to ascarid impactions, whereas younger foals are at higher risk for small intestinal volvulus or intussusception.[485,486,493,494] Older horses have a far greater likelihood of developing strangulating lipomas.[462,477]

Colic may affect horses of any breed, but some breed predispositions have been recognized. An increased incidence of colic has been recognized in Arabians and Thoroughbreds.[455,457,485,495] Arabians are specifically predisposed to ileal impaction, small colon impaction, and enterolith formation. Miniature horses, especially when young, are prone to small colon impactions and fecaliths.[462,485] Standardbred, Tennessee Walking Horse, and Warmblood stallions have a higher risk of developing inguinal hernias than stallions of other breeds, which is felt to be related to increased size of the inguinal rings.[462,485] Overo lethal white syndrome is a fatal, recessive genetic condition that causes ileocolonic aganglionosis in neonatal foals.[496] Affected foals are white or predominantly white with small dark markings and generally develop signs of colic shortly after birth. The defective gene is found predominantly in American Paint Horses but has also been found in horses of other breeds with overo coloring, such as American Miniature Horses and half-Arabians.

Gender is also important to consider in cases of colic. In addition to inguinal hernias, testicular torsion can be a cause

of colic in stallions.[462] Postpartum mares are predisposed to torsion of the large colon, whereas colic in a late-gestation mare may be associated with normal parturition or dystocia.[462,485] Pregnant mares exhibit signs of colic with uterine torsion.[480,485] Nonpregnant mares can have mild transient signs of colic associated with ovarian activity.

The history of the patient can provide vital clues as to the severity and possible etiology of the colic.[485,486] With regard to the current episode, it is important to determine the duration and severity of pain, recent defecation and character of feces, appetite, previous treatment, and response to treatment. Previous history of colic, including colic surgery, history of other surgeries, recent management changes, geographic area, breeding history, and pregnancy status, are all important components in the medical history of a colicky horse. Additionally, specific details as to feed, access to sand, water source, deworming, dentistry, history of medications (e.g., NSAIDs, antibiotics), activity level, and stereotypic behavior such as crib-biting/windsucking may also provide valuable diagnostic information.

Physical Examination

A thorough physical examination is essential to the evaluation of colic. It is important to assess the degree of pain, and in some cases the horse may be so uncomfortable initially that sedation will be necessary for safe completion of the examination. If possible, heart rate, respiratory rate, and auscultation of gut sounds should be evaluated before sedation because these can change significantly in response to sedation.[497,498] Because rectal temperature can also be significantly decreased after rectal palpation, it is advisable to obtain the body temperature before palpation.[461] A finding of pyrexia may increase suspicion of colitis, enteritis, peritonitis, or intraabdominal abscessation in the patient, although previous administration of an NSAID may mask this clinical finding.[462,485,486] Tachycardia can be an indicator of pain, hypovolemia, tachyarrhythmia, or endotoxemia, and rates of greater than 80 beats per minute generally indicate serious disease.[462,485,486] During auscultation the patient should be evaluated for the presence of any cardiac abnormalities that would put the horse at increased risk under sedation. Respiratory rate may be increased in horses with colic as a response to some combination of pain, fever, and/or metabolic acidosis. Mucous membranes and capillary refill time (CRT) give a rough assessment of cardiovascular status and peripheral perfusion. Normal mucous membranes should be pink and moist with a CRT of less than 2 seconds. Tacky mucous membranes are generally observed with dehydration of at least 5% to 7%.[485,486] Prolonged CRT and either grayish or dark red mucous membranes indicate impaired cardiovascular status and poor perfusion. With poor perfusion, there may also be cool extremities and reduced jugular fill. Significant changes in mucous membrane color, often with a dark "toxic" line adjacent to the teeth, may accompany endotoxemia, which is common in gastrointestinal disease, particularly in horses with colitis, proximal enteritis, and strangulating lesions. Icterus is relatively common in horses that have been off feed for more than 48 hours due to equine fasting hyperbilirubinemia. However, icterus may also be seen with hepatobiliary disease or hemolysis. Other important considerations during the physical examination include assessment of hydration status, attitude, abdominal distention, and the presence of injuries that indicate self-trauma, which often reflects the degree of pain. As previously mentioned, in stallions careful palpation of the inguinal region must be performed to identify an inguinal hernia or testicular torsion. Severe abdominal distention may markedly increase the respiratory effort and eventually lead to respiratory distress. Auscultation of abdominal quadrants (left dorsal, left ventral, right dorsal, right ventral) can give an estimation of gastrointestinal motility.[485,486] Assessing the abdomen for pings can help to localize gas. Auscultation of the most ventral part of the abdomen should also be performed to assess for the presence of sand.[499] Although not always present, a characteristic "waves on the beach" sound can sometimes be heard, indicating the presence of sand in the colon. If feces can be collected from the horse, it can be useful to evaluate for the presence of sand. A simple way to do this is to mix a few fecal balls with warm water in a rectal sleeve and hang the sleeve with the fingers down. After about 5 minutes any sand in the feces should sediment into the ends of the glove fingers.[485]

Additional diagnostics for horses with colic include rectal palpation, nasogastric intubation, and, in many cases, abdominocentesis. Adequate restraint is important for these procedures because not only is the veterinarian at increased risk of injury with an uncooperative horse, but the horse also is at increased risk of rectal tears, significant epistaxis, and abdominocentesis complications such as enterocentesis or contamination of the collection site. The most appropriate means of restraint is dependent on the individual patient and may include sedation and/or the application of a nose twitch. When selecting the method of restraint the safety of the examiner and the horse must be considered, while at the same time it is important to not further compromise an unstable patient or dramatically affect assessment of the horse's level of pain. Use of xylazine hydrochloride intravenously at 0.2 to 0.5 mg/kg (typically 100–250 mg for a 500-kg horse) is often adequate, but butorphanol tartrate at 0.01 to 0.08 mg/kg intravenously can be added when necessary.[461] Use of detomidine is usually discouraged in the initial management of colic because of its long duration of action and heightened ability to mask more significant pain that may affect determination of the horse's treatment.[465,466] However, if indicated, detomidine can be administered at 0.01 to 0.04 mg/kg intravenously or intramuscularly.[461] It is most commonly used when a longer duration of sedation is required to keep a horse quiet during transport to a referral center.

Nasogastric intubation should be performed as a part of a complete colic examination. If a horse is exhibiting significant pain or has a heart rate greater than 60 beats per minute on initial evaluation, nasogastric intubation should be performed before other diagnostic procedures because it may have both diagnostic and therapeutic value. If a horse has significant gastric distention, gastric decompression may help prevent gastric rupture and decrease pain, allowing for a more thorough physical examination. The nasogastric tube can be left in place if a significant amount of net reflux is obtained to allow for repeated decompression. If no reflux is obtained and a simple colic is suspected, oral fluids with or without electrolytes or magnesium sulfate (Epsom salt) can be administered via the tube. Although mineral oil has long been considered a part of standard colic treatment, its use is controversial. Mineral oil may be valuable as a diagnostic tool, providing an estimate of gastrointestinal transit time. In a normal horse mineral oil can be observed in the feces and on the perineum and tail in 12 to 24 hours.[500] The absence of mineral oil after this period may indicate delayed gastrointestinal transit. However, mineral

oil can sometimes pass around an impaction that is present, thereby falsely suggesting adequate gastrointestinal transit when in fact digesta is not moving normally.[500] In addition, if a horse ultimately requires abdominal surgery, the presence of mineral oil can complicate an enterotomy.[501]

Palpation per rectum can be important in the assessment of colic. Even with the use of appropriate sedation, some horses remain resistant to rectal examination, placing them at increased risk for rectal tears. To potentially improve the quality and safety of palpation per rectum, intravenous administration of *N*-butylscopolammonium can help to decrease rectal pressure and peristalsis.[502] Application of lidocaine within the rectal lumen has also been used to facilitate palpation. Although rectal palpation can provide valuable diagnostic information, the presence or absence of a particular lesion cannot always be definitively determined. For instance, although a pelvic flexure or cecal impaction may be palpable, if no impaction is palpated, it cannot be ruled out. Additionally, gas distention might be palpable in gas colic, but it may also reflect a more serious problem, such as a displacement or torsion. Rectal palpation therefore can provide evidence to support a diagnosis but is often not diagnostic in itself. Nephrosplenic entrapment may be suspected if the spleen is displaced medially or the gut is palpated between the spleen and kidney.[485,486] Another useful finding is the identification of distended small intestine. Although small intestinal distention may occasionally occur secondary to a large intestinal problem such as a displacement, it is most often an indication of small intestinal disease. Although rectal palpation cannot differentiate a surgical small intestinal problem such as a strangulating lesion from a medical problem such as proximal enteritis, small intestinal disease is almost always an indication for referral.[485,486,503] Therefore palpable loops of distended small intestine are a significant finding on rectal examination. Rectal palpation can also be useful for identifying masses in the abdomen, which may be a cause of chronic colic.

Ancillary Diagnostic Tests

Clinical Pathology. A packed cell volume (PCV) and total protein should ideally be evaluated to assist with assessment of hydration status and possible protein loss. These values can be affected by stress, which can increase the PCV, anemia, protein loss, or hyperproteinemia, confounding the effects of dehydration.[462,504] Therefore these values must be evaluated in the context of the history and physical examination of the patient, as well as their relationship to each other. In general, the greatest negative indicator is a significantly increased PCV (i.e., greater than 65%) coupled with a significantly low plasma protein concentration (i.e., less than 4 g/dL), especially if serial values are trending toward these extremes.[462,485,486,504]

Although further blood work usually is not performed on a standard simple colic, a CBC and serum chemistry can provide valuable information if a colic goes beyond simple treatment and requires more advanced care.[504,505] In acute colic CBC values will typically be normal or reflect stress, but in more chronic conditions nonspecific signs of inflammation such as leukocytosis and hyperfibrinogenemia can develop, as well as a possible normochromic normocytic anemia of inflammation and chronic disease. If significant colon wall compromise occurs, leukopenia with neutropenia and a left shift with toxic changes can occur as a result of endotoxemia.[461,485,504] Thrombocytopenia may also be present with endotoxemia.

Electrolyte abnormalities, especially hypokalemia and hypocalcemia, are relatively common abnormalities observed on a chemistry panel in horses with colic.[505] Anorexia, dehydration, diarrhea, and excessive nasogastric reflux can all contribute to derangement of electrolytes. In addition to calcium and potassium, sodium, chloride, magnesium, and bicarbonate can be lost with diarrhea or significant gastric reflux.[472] Leakage from damaged cells may result in elevated serum phosphate. Metabolic acidosis caused by elevation of lactate, loss of bicarbonate, or both may also be observed. Hyperglycemia is relatively common in horses with acute gastrointestinal disease in association with dysregulation of glucose homeostasis. Significant elevations in glucose have been associated with a worse prognosis for hospital discharge.[506] Occasionally, hepatic enzyme activities are increased, especially γ-glutamyltransferase (GGT). This most commonly occurs with right dorsal displacement of the large colon or proximal enteritis.[507,508] In a study of horses with large colon displacements, 49% of horses with right dorsal displacements had concentrations of GGT above the reference range compared with 1% of horses with left dorsal displacements.[508] Dehydration often results in elevations in creatinine and serum urea nitrogen, and these parameters should be monitored, especially when NSAIDs or aminoglycoside antibiotics are used. Elevations in muscle enzymes are common in horses with colic. In a study of horses undergoing celiotomy for acute gastrointestinal pain, elevations in creatine kinase (CK) and aspartate aminotransferase (AST) activity were significantly associated with the presence of lesions resulting in intestinal ischemia.[509]

Abdominocentesis can be performed both in the field and in a referral setting. Most often the procedure is performed with either an 18-g, 1.5-inch needle or a teat cannula for the collection of fluid. The typical location in which the procedure is performed is to the right of midline (to avoid splenocentesis) on the most ventral point of the abdomen at least several inches caudal to the xiphoid.[462,485] However, occasionally other locations, such as on the ventral midline, will be used. Also, ultrasound of the abdomen can be used to identify pockets of fluid, but it should be remembered that fluid can often still be obtained even when a significant volume of fluid is not apparent on ultrasound. If severe gas distention or small intestinal distention is present, or if a sand enterocolitis is suspected, it may be advisable to forego abdominocentesis to avoid puncture of a distended compromised viscus or a heavy sand-filled viscus that is sitting flush with the ventral body wall. Although a practitioner may not have access to a full fluid analysis and cytology, a basic assessment of abdominal fluid can be made by visual evaluation for color and clarity, and total protein can be evaluated with a refractometer. Normal fluid should be odorless, nonturbid, and a clear to pale yellow color.[462,510,511] Increased turbidity can indicate increased protein, increased total nucleated cell count, or both. Total protein should be less than 2.5 g/dL. A normal cell count is typically less than 5000 cells/μL, although it may be normal up to 10,000/μL.[458,475] Serosanguineous fluid usually reflects a strangulating obstruction or significant compromise to the bowel wall. With a ruptured gastrointestinal structure, the fluid is generally dark and smells of ingesta. These results should be confirmed, however, because an accidental enterocentesis will appear the same.[462] If the spleen is accidentally punctured, the fluid will look like frank blood and will usually have a PCV equal to or greater than the peripheral PCV.[511]

Measurement of lactate concentrations in both the circulation and peritoneal fluid is useful in the assessment of colic.[512-516] Several point-of-care analyzers are available, making measurement of lactate practical.[516,517] In general, in horses with colic, an elevation in lactate concentration in either the circulation or peritoneal fluid suggests intestinal hypoperfusion and correlates with more severe disease. Elevations in peritoneal fluid lactate, especially if higher than the simultaneously measured blood lactate, as well as increases in peritoneal fluid lactate over time, are predictors of intestinal ischemia secondary to a strangulating obstruction.[512,515,516] There is some evidence that pony and miniature breeds presenting for gastrointestinal disease have higher blood lactate concentrations compared with large-breed horses, suggesting that different cutoff values should be used when determining the necessity of surgery and the prognosis in these breeds.[518]

Diagnostic Imaging. Ultrasound examination of the abdomen is often useful in the evaluation of horses with colic.[519-525] Adequate transabdominal ultrasound examination is most easily performed with a 2.5- to 5-MHz curved linear transducer; however, an examination can also be performed using a transrectal probe.[519,520,525] A protocol for fast localized abdominal sonography of horses admitted for colic (FLASH) has been developed.[524] This protocol allows for rapid evaluation of specific abdominal regions of particular importance in the assessment of colic. On the left side of the horse the regions evaluated include the ventral abdomen, gastric window, splenorenal window, and left middle third of the abdomen, and on the right side the regions evaluated include the duodenal window, right middle third of the abdomen, and cranial ventral thorax.

Ultrasonography is particularly valuable in the evaluation of small intestinal distention and motility.[519,520,522,524] Small intestine can most reliably be found in the inguinal region or on the ventral abdomen, and it should normally be collapsed or have a very small diameter with frequent peristalsis. Small intestine can be evaluated for dilation, motility, and thickening of the intestinal wall. The presence of dilated turgid small intestinal loops is highly sensitive and specific (80% and 96.15%) for small intestinal obstruction, although enteritis cannot entirely be ruled out. An intussusception may be identified as a target-shaped lesion on cross-sectional view.[519,520,526] Ultrasound can also be used to identify a left dorsal displacement of the large colon, also referred to as a nephrosplenic entrapment.[519,520,527] With this condition, the spleen cannot be visualized against the body wall, or the kidney cannot be visualized on the left side because of the interference of large colon. Visualization of colonic mesenteric vasculature on the right side of the abdomen can be a predictor of right dorsal displacement of the large colon, a 180° large colon volvulus, or both, although this change is not present in all cases.[528] Ultrasonographic assessment of peritoneal fluid volume and character may be useful in assessing the patient and in identifying pockets of representative fluid for abdominocentesis.[519,520] The thickness of intestinal wall should be evaluated at numerous locations, especially the right dorsal colon, which may be extraordinarily thickened with right dorsal colitis.[519,520] Investigation for abdominal masses also is often undertaken using ultrasound, coupled with rectal palpation.

A study comparing the findings of rectal examination and ultrasonographic findings in horses with colic demonstrated that ultrasonography was a more sensitive diagnostic technique than rectal examination in horses with small intestinal obstruction, left dorsal displacement of the large colon (nephrosplenic entrapment), and large colon torsion in the long axis, but was less sensitive than rectal examination in horses with impactions and most large colon displacements.[521] It was concluded that although ultrasonography is advisable in evaluation of the equine acute abdomen, it should not replace traditional rectal palpation.

Radiographs of the abdomen are of limited usefulness but may be diagnostic for two problems associated with colic in the adult horse: sand accumulation and enterolithiasis.[529,530] Sand is usually visualized best in the ventral large colon. It is important to keep in mind that some sand can be visualized in many normal horses, so the presence of sand in and of itself has no diagnostic relevance. Radiographs may, however, provide some information as to the volume of sand present.[504] Enteroliths represent the other main lesion identifiable by radiographs, although, depending on the location, they may be difficult to visualize. In a study of 141 horses evaluated for enterolithiasis, abdominal radiography had a sensitivity of 76.9% and a specificity of 94.4%.[530] The abdomen can be more thoroughly assessed via radiography in a neonate as opposed to an adult horse, although results still cannot be considered conclusively diagnostic. Radiographs of a foal's abdomen can identify gas distention of the stomach, small intestine, or large colon, which are nonspecific but generally pathologic findings.[494,531] Radiographs can aid in the diagnosis of meconium impaction. A radiographic pattern known as *pneumatosis intestinalis,* characterized by linear radiolucencies within the bowel wall, is typical for necrotizing enterocolitis.[494] Excessive peritoneal fluid and pneumoperitoneum can also be identified.

Endoscopy/Other. Gastroscopy is important in cases of colic where equine gastric ulcer syndrome is suspected. Ideally the squamous and glandular regions of the stomach, as well as the pyloric antrum, should be examined.

Evaluation for parasites may be indicated for some patients. Ideally both fecal flotation and a fecal egg count should be performed. Because standard flotation and fecal egg count methods are unreliable in the diagnosis of tapeworm infestation, however, a tapeworm ELISA, which detects antibody to tapeworms, should be considered.

An underlying cause is not found in some horses with chronic colic after routine diagnostic evaluation. In these cases, an exploratory laparotomy or laparoscopy may be indicated.

Referral Indications

Referral to a secondary care center is indicated when there is a strong likelihood that the horse will require surgery or more extensive medical care is necessary. The most common basis for referral is severe or persistent pain with a lack of response to analgesics.[485,486,503] If a horse demonstrates intractable pain or requires repeated doses of analgesics to remain comfortable, referral is warranted. Referral is also indicated when a horse has not resolved the signs of colic in 24 to 48 hours after onset, or if a 1.1-mg/kg dose of flunixin meglumine does not maintain comfort for at least 8 to 12 hours.[503] A persistently elevated heart rate can be a good indicator of pain or endotoxemia and necessitates referral. Other indications of endotoxemia or impaired cardiovascular status include weak pulses, abnormal mucous membrane color (pale, hyperemic, injected, purple, or cyanotic), and delayed capillary refill time. Rectal palpation of a potentially surgical lesion or small intestinal distention, an abnormal abdominocentesis, net nasogastric reflux of greater than 2 to 3 L, or significant depression are

all findings that would also support referral.[485,486,503] In addition, significant dehydration may require extensive treatment with intravenous or oral fluids or both because dehydration has the potential to exacerbate even apparently simple colic problems and affects the patient's systemic health. Dehydration can result in worsening of an impaction that is already present or contribute to development of an impaction when coupled with ileus and an inciting primary colic problem such as colonic displacement.

DIARRHEA

Diarrhea, defined as an increase in the fluidity, frequency, or volume of feces, is a commonly encountered clinical problem in the horse. It is often the result of a primary gastrointestinal disease but may also occur as a secondary response to another disease process, such as sepsis, endotoxemia, or hepatic disease. In some cases, diarrhea may rapidly develop into a life-threatening condition.

The function of the equine gastrointestinal tract is complex and involves maintenance of normal fluid balance and digestion and absorption.[532-534] As a result of dietary intake and endogenous secretions, a large volume of fluid normally enters the gastrointestinal tract, most of which is reabsorbed. In the adult horse absorption occurs predominantly in the large bowel, where a volume of water approximately equal to the total extracellular fluid volume of the animal, or about 100 L, is recovered during the course of the day. Because the large colon is the primary site of water resorption, most significant diarrheal disease in the adult horse involves the colon. In young foals, however, small intestinal disorders such as rotaviral infection also may result in diarrhea.[535]

A second critical function of the large bowel is that of microbial digestion of carbohydrates and, to some extent, protein or nonprotein nitrogen.[532-534] Microbial fermentation of carbohydrates in the cecum and colon results primarily in the production of volatile fatty acids (VFAs), which are absorbed readily, providing up to 75% of the energy requirement of the horse. The intestinal microbiome is a complex polymicrobial ecosystem that appears to play an important role in the maintenance of health.[534,536,537] Therefore maintaining a stable environment for the microbial population is important. In general, efficient function of the large bowel requires mechanisms that limit the rate of digesta passage, provide optimal conditions for microbial digestion, and allow for efficient transport of solutes and water.

The characteristics of normal equine feces and the frequency of defecation can vary somewhat with diet, age, and sex.[538] On average, adult horses defecate approximately 8 to 10 times daily. The frequency of defecation tends to be slightly higher in stallions and foals. Generally, equine feces are tan, brown, or greenish, and, although approximately 75% water, they are well formed. An average size adult horse on a typical diet of grass hay and grain produces about 20 to 28 g of feces per kilogram of body weight per day, or about 11 to 13 kg of feces per day.[538] In cases of diarrhea the amount of feces may increase up to tenfold, with some horses producing more than 200 g/kg/day, which can equate to more than 90 L of diarrhea. As a result, diarrhea can cause significant losses of electrolytes and water. Especially in cases of acute diarrhea, these losses may result in severe dehydration, electrolyte imbalances, and acid-base abnormalities. However, horses with chronic diarrhea seldom develop these abnormalities as they are often able to compensate for increased fecal losses. Protein loss may be seen with both acute and chronic diarrhea.

Mechanisms of Diarrhea

Several basic mechanisms of diarrhea have been described, and in many diarrheal diseases more than one mechanism is involved. Inflammation within the bowel often plays a central role in the pathogenesis of diarrhea and may contribute to diarrhea via multiple mechanisms. Basic mechanisms of diarrhea include the following:

1. *Malabsorption:* Malabsorption results from a decrease in the functional absorptive surface area of the gastrointestinal tract. In the small intestine, villus atrophy, such as that seen with rotaviral enteritis, equine proliferative enteropathy *(Lawsonia intracellularis),* and infiltrative bowel disease, can result in malabsorption because of the loss of functional epithelium and maldigestion caused by decreased production of digestive enzymes.[142,535,539-577] In the colon, a number of insults can result in inflammation and disruption of absorptive cells and tight junctions, leading to decreased absorptive capacity and decreased ability to retain absorbed fluid (i.e., increased loss). Several inflammatory mediators, such as TNF-α, histamines, and prostaglandins, can contribute to the colonic inflammation. These mediators are produced primarily by inflammatory cells in the lamina propria and inhibit absorption through a variety of mechanisms.[542-546]
2. *Increased secretion:* The increased secretion of solutes and water by the inflamed colon can contribute significantly to the development of diarrhea. Although the precise mechanisms of secretion in the equine colon are not understood fully, active secretion and passive fluid loss occur.[542-549] Control of active secretion is complex, involving two primary pathways: first, the activation of adenyl cyclase, resulting in an increase of intracellular cyclic adenosine monophosphate concentrations, and second, the activation of calcium channels, leading to increased intracellular calcium concentrations.[547,548] Cyclic adenosine monophosphate and calcium stimulate specific secretory activities, primarily through chloride channels. In some cases of diarrhea, bacterial enterotoxins such as those produced by certain strains of *Escherichia coli* and *Salmonella* spp. stimulate adenyl cyclase activity, thus increasing active secretion. This is true hypersecretory diarrhea. Also, a number of inflammatory mediators produced by the inflamed colon, particularly prostaglandin E_2, increase intracellular concentrations of cyclic adenosine monophosphate and to some extent calcium, thereby increasing active secretion by mucosal cells.[547-549] Inflammation also enhances passive fluid loss through a number of factors, such as changes in hydrostatic pressure in the colonic capillaries, mucosal damage, and loss of tight junctions. In horses with severe mucosal injury, the loss of protein can decrease vascular oncotic pressure and further potentiate fluid exchange across the endothelium.
3. *Decreased transit time (abnormal motility):* Sufficient retention time and thorough mixing are required for digestion and absorption of nutrients and fluid to occur, and thus decreased intestinal transit time can contribute to diarrhea.[532-534,550] Primary motility disorders causing diarrhea are not well recognized, although diarrhea associated with stress or excitement may represent this phenomenon. However, it appears that secondary motility disorders are

common in response to a variety of gastrointestinal problems, although the exact role that these changes in motility play in the pathogenesis of diarrhea is not entirely clear. Both inflammatory and infectious conditions may influence motility in addition to potentially altering absorption and secretion. Absorption of endotoxin and the release of inflammatory mediators, including prostaglandins, disrupts normal motility patterns.[551] In general, these changes result in hypermotility and decreased intestinal transit time, although hypomotility has been recognized as well. Some progressive motility must be present for diarrhea to occur. In some cases of acute colitis, a period of ileus may occur without diarrhea. This is usually transient and diarrhea develops, although occasionally ileus may persist. With diarrheal diseases, the elimination of gastrointestinal contents is part of the normal host defense mechanism, and thus treatments specifically aimed at decreasing motility are not indicated in most cases.

4. *Osmotic overload:* Any increase in osmotically active particles within the intestinal lumen can result in diarrhea. The increase can be associated with the administration or ingestion of osmotically active substances such as magnesium sulfate. The increase also may be associated with overloading of the intestine with carbohydrates or occasionally lipids beyond the amount that can be digested and absorbed. Therefore sudden dietary changes that result in significant shifts in gut flora and changes in fermentation or gastrointestinal diseases that result in malabsorption or maldigestion also may result in an osmotic diarrhea. In foals the loss of villous epithelial cells in the small intestine associated with disorders such as rotavirus infection and clostridiosis may lead not only to malabsorption but also to maldigestion caused by the decreased production of lactase and secondary lactose intolerance.[535,539,552] Although uncommon, primary lactose intolerance has also been reported in foals.[553] Lactose intolerance allows excess lactose to enter the large intestine, increasing the osmotic load.
5. *Increased hydraulic pressure from the blood to the lumen:* The pressure change may result in increased fluid movement to the lumen and decreased net fluid absorption. This mechanism of diarrhea is more common in chronic conditions, such as congestive heart failure or inflammatory bowel disease. The condition may result from decreased oncotic pressure associated with hypoproteinemia, increased capillary hydrostatic pressure (as in heart failure), or decreased lymphatic drainage associated with inflammation of lymphatics and lymph nodes.

The normal equine fecal microbiota is very complex. In horses with diarrhea, the bacterial diversity of the gut microbiota may be lower than in horses with normal feces.[537] This microbial imbalance, referred to as *microbiome dysbiosis,* may be both a result and a cause of intestinal disease and diarrhea. Characterization of the microbiome and its role in health and disease is a very active area of research.

Understanding the mechanisms of diarrhea can be helpful in directing therapy. It is important to remember that most disorders that cause diarrhea, whether infectious or noninfectious, do so through inflammatory mechanisms resulting in multiple functional alterations.

Conditions Associated with Diarrhea

Diarrhea is a common, and sometimes fatal, clinical problem of adult horses and foals. A number of specific causes for acute and chronic diarrhea have been identified (Tables 7.5, 7.6, and 7.7). In some cases, rapid dietary changes or stress can result in diarrhea that is generally mild and transient. Also, although sand ingestion is frequently associated with impactions, it may also result in diarrhea due to mechanical irritation to the gastrointestinal tract.

TABLE **7.5 Differential Diagnoses for Acute Diarrhea in Adult Horses**

Common Causes	Major Diagnostic Test(s)
Salmonellosis	Fecal culture or polymerase chain reaction (PCR), culture of rectal mucosal biopsy
Potomac horse fever *(Neorickettsia risticii)*	PCR (feces, peripheral blood), paired serologic tests
Clostridiosis *(Clostridium difficile, C. perfringens)*	Fecal culture, toxin analysis
Antibiotic-associated diarrhea	History
Nonsteroidal antiinflammatory toxicity (primarily right dorsal colitis)	History and supportive clinicopathologic findings, ultrasonography, exploratory surgery with biopsy
Undiagnosed	Other conditions ruled out
Less Common	
Cantharidin toxicity	
Parasitism (strongylosis, cyathostomiasis, other)	
Aeromonas, Campylobacter spp.	
Sand	
Carbohydrate overload	
Arsenic toxicity, other toxicities	
Thromboembolic disease	
Anaphylaxis	

Several infectious agents are associated with diarrhea in horses. *Salmonella* and *Clostridium* species are among the most common causes of infectious diarrhea in horses of any age.[554,555] *Salmonella* is of particular importance because under certain circumstances it can be highly contagious, leading to significant outbreaks of disease, especially in hospitalized patients.[556] *Clostridium difficile* and *Clostridium perfringens* are the most common clostridia associated with colitis in horses, although occasionally other species may be involved.[552,557-561] In certain geographic areas equine neorickettsiosis (Potomac horse fever) caused by *Neorickettsia risticii* is also common.[562] *Lawsonia intracellularis* can cause equine proliferative enteropathy resulting in diarrhea, colic, edema, and weight loss.[335,601-565] *Lawsonia intracellularis* can potentially affect horses of any age, but it is most common in weanling-age foals. Although primarily a respiratory pathogen, *Rhodococcus equi* also can cause diarrhea, particularly in foals 2 to 4 months of age.[566] *Escherichia coli* is an uncommon cause of diarrhea in foals, unlike in calves and piglets. However, enterotoxigenic strains, characterized by the presence of virulence factors, have been identified in foals.[567] Other, less common bacterial agents potentially associated with diarrhea include *Campylobacter* spp. and *Aeromonas* spp.[568,569]

Both equine rotavirus and coronavirus can be important enteropathogens. Rotaviral infection is a common cause of

TABLE **7.6 Differential Diagnoses for Chronic Diarrhea in Adult Horses**

Cause of Diarrhea	Major Diagnostic Test(s)
Chronic salmonellosis	Fecal culture or polymerase chain reaction, culture
Sand	Fecal sedimentation
Parasitism (strongylosis, cyathostomiasis)	Fecal egg count, empirical deworming
Nonsteroidal antiinflammatory toxicity (primarily right dorsal colitis)	History and supportive clinicopathologic findings, ultrasonography, exploratory surgery with biopsy
Inflammatory or infiltrative disorders	Histopathologic examination, absorption tests (supportive but nonspecific)
Inflammatory bowel disease (granulomatous, lymphocytic-plasmacytic, or eosinophilic enterocolitis)	
Mucosal lymphosarcoma	
Amyloidosis	
Dietary: abnormal fermentation	History
Neoplasms: lymphosarcoma, squamous cell carcinoma	Histopathologic examination
Peritonitis, abdominal abscessation	Peritoneal fluid analysis, ultrasound, exploratory surgery
Nongastrointestinal causes (chronic liver disease, congestive heart failure, renal disease)	Physical examination, clinicopathologic findings

TABLE **7.7 Differential Diagnoses for Diarrhea in Foals**

Cause of Diarrhea	Major Diagnostic Test(s)
Salmonellosis	Fecal culture or PCR
Clostridiosis *(Clostridium difficile, C. perfringens)*	Fecal culture, toxin analysis
Endotoxemia, gram-negative septicemia	Blood culture, physical examination, complete blood count, sepsis score
Antibiotic-associated diarrhea	History
Foal heat diarrhea	History, physical examination
Viral: rotavirus; rarely coronavirus or adenovirus	Electron microscopy, enzyme immunoassay
Protozoan: cryptosporidiosis	Fecal analysis
Secondary lactose intolerance therapy	Oral lactose tolerance test, response to
Rhodococcus equi	Culture, PCR
Lawsonia intracellulare	Fecal PCR, serologic testing
Gastric ulcer disease syndrome	Gastric endoscopy
Strongyloides westeri	Fecal egg count
Sand	Fecal sedimentation

PCR, Polymerase chain reaction.

diarrhea in foals and is most often recognized in foals from 1 to 4 weeks of age.[535,539,570,571] Equine coronavirus, also sometimes referred to as equine enteric coronavirus, has been recognized in both foals and adult horses.[571-575] Since 2011 several outbreaks of equine coronavirus have been identified in adult horses, causing it to be named as an emerging pathogen. The most common signs include anorexia, lethargy, and fever; diarrhea and colic are less common.

The signs associated with internal parasites can vary depending on the type and severity of infestation, but diarrhea may occur. Often it is a chronic diarrhea, but acute diarrhea may be seen. Encysted cyathosomin larvae are ubiquitous in grazing horses, but large numbers of encysted larvae place the horse at risk for developing larval cyathostominosis.[576,577] This condition occurs when there is a mass emergence of the larvae, resulting in acute typhlocolitis. The inflammatory responses trigger diarrhea, especially in individuals with high parasite burdens. The case fatality rate may be as high as 50%. The majority of infections with *Strongyloides westeri* are subclinical, but occasionally diarrhea can occur in foals with high worm burdens.[578,579] A 2014 study in Thoroughbred foals in central Kentucky found a mean prevalence of *S. westeri* infection of 30%, which was greater than previous reports from the area.[579] *Cryptosporidium* spp. are coccidian parasites that have been linked to diarrhea in foals and occasionally adult horses.[580-582]

Medications may be involved in the pathogenesis of diarrhea, with the two classes of drugs most commonly incriminated being antibiotics and NSAIDs. Potentially any systemic antibiotic may cause diarrhea, but the risk appears to be higher with certain antibiotics such as trimethoprim-potentiated sulfonamides and erythromycin.[28,583,584] Although the mechanisms of antibiotic-associated diarrhea may be multifactorial, an important factor is that the use of systemic antimicrobials leads to changes in the intestinal microbiota.[585,586] The use of NSAIDs has been linked to adverse gastrointestinal effects including gastric ulcers, right dorsal colitis, and inhibition of mucosal barrier healing.[336,587,588,589,626] Phenylbutazone causes changes in right dorsal colon arterial blood flow and changes in VFA production.[589]

Diarrhea is a component of the clinical syndrome associated with several toxins. Cantharidin (blister beetle toxin) can cause severe colitis in horses.[590] Some plants associated with diarrhea include alfalfa dodder *(Cuscuta campestris),* acorns, and oleander.[591-593] Other toxins that may result in diarrhea include lead, selenium, and arsenic.[594,595]

Cellular infiltrative disorders such as inflammatory bowel disease and neoplasia can cause diarrhea, which is generally chronic in nature. Alimentary lymphosarcoma is the most common intestinal neoplasia of horses, and it can cause significant cellular infiltration of the intestine and associated lymph nodes.[596] It has been recognized across a broad age range, including relatively young horses. Several inflammatory bowel diseases have been recognized in horses, including granulomatous enteritis, lymphocytic-plasmacytic enterocolitis, eosinophilic enterocolitis, and multisystemic eosinophilic epitheliotropic disease.[142]

Some additional causes of diarrhea occur in neonatal foals. Foal heat diarrhea is a normal physiologic diarrhea that occurs typically between 5 and 15 days of age.[597] Foals are otherwise healthy and the diarrhea is generally mild and self-limiting. With respect to infectious agents, coinfections appear to be

BOX 7.10 Outline of Diagnostic Approach to Diarrhea

I. SIGNALMENT, HISTORY, AND PHYSICAL EXAMINATION

II. CLINICAL PATHOLOGY

1. Minimum database: complete blood count, fibrinogen, and serum chemistry profile
   a. Assess hydration, acid-base status, electrolyte abnormalities, and protein status
   b. Assess renal and hepatic function
   c. Assess endotoxemia
2. Serum protein electrophoresis and immunoglobulin quantitation
3. Serologic testing: *Neorickettsia risticii* and *Lawsonia intracellulare*
4. Peritoneal fluid analysis

III. EVALUATION OF FECES

1. Gross appearance: severity, hemorrhage, odor, and presence of sand
2. Direct smear: evaluation of protozoan populations and presence of leukocytes and epithelial cells
3. Parasite evaluation: including evaluation for *Cryptosporidium parvum,* especially in foals
4. Evaluation of bacterial pathogens
   a. Gram stain and spore stain
   b. Aerobic and anaerobic culture (culture of multiple samples or rectal mucosal biopsy for *Salmonella* spp.)
   c. Clostridial toxin analysis
   d. Polymerase chain reaction: *Salmonella* spp., *N. risticii,* and *L. intracellulare*
5. Foals: evaluation of viral pathogens, primarily rotavirus (electron microscopy and enzyme immunoassay)

IV. DIAGNOSTIC IMAGING: RADIOGRAPHY AND ULTRASONOGRAPHY

V. ENDOSCOPIC EXAMINATION: STOMACH, RECTUM, AND DESCENDING COLON

VI. ABSORPTION TESTS (GLUCOSE OR XYLOSE ABSORPTION): PRIMARILY FOR CHRONIC PROTEIN-LOSING ENTEROPATHY

VII. HISTOPATHOLOGIC EXAMINATION

VIII. TOXIN EVALUATION: CANTHARIDIN IN URINE OR GASTROINTESTINAL CONTENTS, ARSENIC IN LIVER, OR OTHER

IX. RESPONSE TO THERAPY

common in neonatal foals.[571] Secondary lactose intolerance has been documented in foals with gastrointestinal infections, and primary lactose intolerance has been reported as well.[539,552,553] Diarrhea has been seen in neonatal foals in association with hypoxic-ischemic gastrointestinal damage, sepsis, and rarely acute pancreatitis.[598]

Diagnostic Approach to Diarrhea

A comprehensive evaluation may help in establishing a diagnosis and developing a treatment plan in cases of diarrhea (Box 7.10). However, even in severe cases a definitive diagnosis often is not made, making the problem particularly frustrating.[554,555,568,571,599]

Signalment and History

The veterinarian should consider the signalment and history carefully when evaluating a patient with diarrhea. Age is particularly important because several disorders, such as foal heat diarrhea, rotaviral diarrhea, and equine proliferative enteropathy, are age related. The genetic background also may be significant because diarrhea has been associated with certain heritable immunodeficiencies, and granulomatous bowel disease has been identified in three sibling horses.[600-602] Establishing whether the diarrhea is acute or chronic is important. Other historical questions of particular relevance include the type and source of feed, as well as any dietary changes; deworming program; involvement of single versus multiple animals; exposure to sand; and the use of medications, especially antibiotics and NSAIDs. Other concurrent diseases, stress, possible exposure to toxins, weight loss, water consumption, and salt availability also may be significant. The information obtained helps the veterinarian prioritize differential diagnoses and direct further testing.

Physical Examination

The clinician should perform a complete physical examination. Attention should be given to good biosecurity measures, especially as some causes of equine diarrhea are potentially zoonotic in addition to being transmissible among horses. The body condition of the horse and the presence of any edema should be noted. The presence of fever, dehydration, or signs of endotoxemia may help in assessing the severity of the disease and differentiating the cause because some causes of diarrhea are not typically associated with systemic signs of illness. The presence of oral ulcers and synchronous diaphragmatic flutter increase the likelihood of cantharidin toxicity especially in horses being fed alfalfa.[590] In horses presented for diarrhea with a concurrent arrhythmia, oleander toxicity should be considered if exposure is possible.[593] Careful evaluation of the abdomen should be performed. Visible abdominal distention is often an indication of large intestinal distention, which may occur in association with acute colitis. However, distention also may be visible with extreme dilation of multiple loops of small intestine. Careful auscultation of the abdomen can be useful in assessing motility. Although the frequency of borborygmi in horses is variable, horses with normal motility generally have 1 to 3 borborygmi in a 60-second time period. Auscultation behind the xiphoid process may help identify the presence of sand or gravel if particles can be heard grinding together during contractions of the colon.[499] Percussion of the abdomen while ausculting may help to identify high-pitched resonant sounds associated with gas-distended bowel. Particularly in foals, transabdominal palpation and ballottement may be useful to identify increased abdominal fluid or large masses near the body wall. Transrectal palpation can be helpful in assessing the size of intestinal segments, consistency of contents, and wall thickness, as well as in identifying masses, enlarged lymph nodes, or mesenteric arteritis.

Ancillary Diagnostic Tests

Clinical Pathology. Routine analysis of blood work typically does not identify a specific cause of diarrhea, although occasionally *N. risticii* can be found in circulating monocytes. However, blood work is often important in directing appropriate supportive care and may help to establish whether

diarrhea is caused by another condition, such as hepatic or renal disease. Some important parameters to evaluate include the presence of leukopenia, particularly neutropenia with a left shift, and toxic changes in the white blood cells. These abnormalities suggest a systemic inflammatory response, often associated with endotoxemia. Thrombocytopenia and coagulopathies may also be present. The clinician also should evaluate the concentration of protein, as well as the albumin-to-globulin ratio. Both acute and chronic diarrhea can cause significant protein loss, resulting in hypoproteinemia and particularly hypoalbuminemia. Hyperglobulinemia may indicate a chronic inflammatory condition. Disturbances in acid-base balance, especially metabolic acidosis, and electrolyte abnormalities frequently occur in horses with acute diarrhea but are uncommon with chronic diarrhea. Although hypocalcemia is relatively common in horses with colitis, marked hypocalcemia may be suggestive of possible cantharidin toxicity.[63] Because of the dehydration frequently seen with acute diarrhea, prerenal azotemia is common and is important to recognize because some therapies, especially NSAIDs, may worsen the condition. In a study of 122 horses with acute diarrhea, horses with azotemia and clinicopathologic findings consistent with hemoconcentration and hypoproteinemia were less likely to survive.[599]

The diagnostic and prognostic value of serum protein electrophoresis has been evaluated in horses with chronic diarrhea.[603] Significantly higher levels of β_1-globulin were found in horses with larval cyathostominosis than in other horses, and such values in conjunction with a decreased albumin were helpful in diagnosing intestinal parasitism. However, a normal β_1-globulin concentration was not a reliable indicator of the absence of the disease. Significantly lower albumin concentrations and significantly higher α_2-globulin concentrations were found in horses that did not survive, suggesting that these parameters are nonspecific indicators of the severity of inflammatory changes within the intestinal wall. Parasitic infections, particularly strongylosis, also may be associated with elevated serum concentrations of the immunoglobulin G (IgG) isotype IgG(T).[604]

Because immunodeficiencies are infrequently associated with diarrhea,[600,601] further evaluation of immune status may be indicated in some horses. This evaluation may include specific immunoglobulin quantitation, evaluation of specific lymphocyte subsets, or functional assays. The clinician should consider genetic testing for severe combined immunodeficiency in sick foals of Arabian breeding.

Analysis of peritoneal fluid may be useful in some horses with diarrhea. Abnormalities in the peritoneal fluid may reflect the severity of inflammation and in some cases may help to establish a specific diagnosis. Increases in protein and sometimes nucleated cell count may be seen in association with ulcerative colitis. In horses with bacterial peritonitis, the veterinarian may find organisms on cytologic examination or culture. Occasionally, the veterinarian may identify neoplastic cells in the peritoneal fluid, although their absence does not rule out the presence of neoplasia.

Evaluation of Feces. Evaluation of the feces may yield important information in horses with diarrhea. Even the gross appearance of the feces can be helpful. For example, profuse, watery diarrhea is not generally consistent with a diagnosis of right dorsal colitis, which is most often associated with soft, poorly formed feces. Frank blood in the feces suggests bleeding into the distal colon from mucosal damage. Hemorrhagic, foul-smelling feces often are seen in association with clostridial diarrhea. The clinician also can assess the feces for the presence of occult blood, which indicates bleeding from any source. Although excess sand in the feces is readily apparent in some cases, other cases require mixing the feces in a rectal sleeve with water and allowing the sand to settle. Acorn husks may be present in the feces of horses with acorn toxicity. Less commonly used tests include evaluation of fecal osmolality and electrolyte concentrations (sodium and potassium). If the concentration of sodium plus potassium is much less than the osmolality, the result indicates the presence of osmotically active nonelectrolytes, confirming an osmotic diarrhea.

Microscopic examination of the feces for evidence of parasitism and evaluation of viable protozoal populations also may be useful. A direct smear of fresh feces allows for observation of the motility of ciliates and can be used as a screen for the presence of ova and oocysts. Ideally evaluation of parasitism should include fecal flotation and a quantitative method that allows for estimation of the number of eggs per gram of feces, such as McMaster's or Stolley's. Cryptosporidial oocysts are typically difficult to identify on routine fecal flotation, but detection can be improved by acid-fast or acridine orange staining as well as immunofluorescent staining or flow cytometry.[579-582] It is important to remember that fecal examination for parasites sometimes can be misleading, giving false-negative results.

Fecal samples also can be examined microscopically for leukocytes and epithelial cells. In general, the cellularity increases with the severity of diarrhea. Fecal leukocytes and epithelial cells are increased in salmonellosis but are not specific for this disorder.[605] The presence of more than 10 leukocytes per high-power field has been associated with salmonellosis.

Evaluation of the feces for infectious agents is essential in the diagnostic evaluation of horses with diarrhea. Gram stain and spore stain of fecal smears can help to identify and quantitate the bacterial populations present, particularly clostridial species. However, although large numbers of gram-positive rods or spores have been identified in horses with clostridial enterocolitis, the results of direct staining may be misleading.[557-561] *Clostridium perfringens* has been cultured from 59% of samples in which no gram-positive rods were visible. Some clostridial strains also are likely parts of the normal microflora.[561,606] Large numbers of yeast in the feces should alert the clinician to the possibility of candidiasis, especially in compromised neonatal foals.

Fecal culture is used commonly to establish a diagnosis in horses with bacterial diarrhea. When culturing feces, especially if an outside laboratory is used, the clinician must consider proper sample handling, particularly for anaerobic clostridia.[607] *Salmonella* spp. are one of the most significant bacterial pathogens in equine feces.[556] Although the number of *Salmonella* organisms isolated from the feces of horses with clinical salmonellosis is generally greater than from horses with asymptomatic infections, the volume of feces in horses with profuse diarrhea may decrease the likelihood of positive culture. Culture of multiple fecal samples, typically five, is recommended to increase the sensitivity.[608] Culture of a rectal mucosal biopsy or rectal scraping is an alternative to fecal cultures and may increase sensitivity because *Salmonella* spp. are intracellular organisms. Identifying clostridial species requires anaerobic culture. However, evaluating the presence of toxin in cases of suspected clostridial diarrhea also is critical because *Clostridium* spp., particularly *C. perfringens* type A, may be present in normal equine feces.[557-561,606] Depending on the clostridial species and the laboratory, toxin can be assessed by detecting preformed toxin in the feces, toxin being produced by the isolate in culture, or the toxin gene in the isolate.

An increasing number of polymerase chain reaction (PCR) assays are available for detecting causative agents of equine diarrhea. In comparing a PCR with microbial culture for detection of salmonellae in equine feces and environmental samples, the PCR method was found to be more sensitive and more rapid and required submission of fewer samples.[609,610] For diagnosis of equine neorickettsiosis (Potomac horse fever), PCR is currently recommended for detection of *Neorickettsia risticii* in feces and peripheral blood.[562,611,612] Fecal PCR analysis is also useful in documenting *Clostridium, Lawsonia intracellularis,* and *Cryptosporidium* spp., as well as both equine rotavirus and equine coronavirus.[335,539,563,572,613,614] Both rotavirus and coronavirus can also be identified in the feces by electron microscopy.[539,572,615] A number of commercial assays are also available for the detection of rotavirus antigen.[539,616]

Serologic methods, evaluating the presence of antibodies, have been used in the diagnosis of *Neorickettsia risticii* and *Lawsonia intracellularis.*[562-564,612,614] In the case of *N. risticii,* the serologic tests are of limited value in the diagnosis of disease.[562] For *L. intracellularis,* it is recommended to use both PCR and serologic diagnostic testing as these assays have high analytic specificity but variable sensitivity depending on the circumstances.[563,614]

Coinfections have been documented in both adult horses and foals with diarrhea. In a study of neonatal foals, coinfections were significantly associated with the risk of gastrointestinal disease.[571] The use of diagnostic panels rather than individual tests in combination with quantitative toxin gene analysis may be helpful in the diagnosis of coinfections.

Diagnostic Imaging. Diagnostic imaging, although particularly useful in foals, also can be valuable in adult horses. In foals radiographs can detect gas distention in the lumen of the gastrointestinal tract, and the gas pattern may help to differentiate ileus from mechanical obstruction. Occasionally, gas may be seen within the bowel wall in severe cases of clostridial necrotizing enterocolitis. In adult horses abdominal radiography is limited somewhat by having the proper facilities and equipment to perform the procedure safely. However, radiographs can be effective in identifying radiodense material, such as enteroliths and sand. Ultrasonography can be used in horses of all ages to evaluate the amount and character of the peritoneal fluid, masses, small intestinal motility, intestinal distention, and wall thickness. In horses with right dorsal colitis the diagnosis has been supported by ultrasonographic evidence of an increase in mural thickness of the right dorsal colon.[617] The right dorsal colon may have a prominent hypoechoic layer most likely associated with submucosal edema and inflammatory infiltrates. Although isotope-labeled white blood cell scintigraphic scans also may help identify colonic ulcerations, the availability and sensitivity of the procedure are limited.[618] In cases of *L. intracellularis,* ultrasonography may reveal thickened small intestinal loops and possibly excess free abdominal fluid.[335,563]

Endoscopy. Endoscopic examination of the stomach and proximal duodenum may reveal the presence of neoplasms or ulceration. Diarrhea and inappetence can be seen in symptomatic foals with ulceration of the squamous gastric mucosa. Endoscopy also can be used for inspection of the mucosa of the rectum and descending colon, allowing for evaluation of mural masses or mucosal inflammation.

Additional Diagnostic Tests. Absorption tests are used primarily in horses with chronic diarrhea or weight loss to evaluate the small intestinal absorptive capacity. Oral glucose and oral xylose absorption tests have been used.[160,619] Although the plasma concentration of glucose may reflect glucose metabolism as well as absorption from the gastrointestinal tract, the assay is reliable in the diagnosis of significant malabsorptive conditions. Xylose is influenced less by the metabolic status of the horse, but the compound is more expensive than glucose, and the assay is not available in many laboratories. Results of both assays are nonspecific, but abnormal results support malabsorption and may indicate the necessity of biopsy.

Diagnosing neoplasms and chronic inflammatory or infiltrative disorders often requires histopathologic examination. A rectal mucosal biopsy is easy to collect and can be cultured, but the area that can be reached for biopsy is limited. Laparoscopy allows for visualization of the abdomen and certain biopsies. The veterinarian can obtain a full-thickness intestinal biopsy during exploratory celiotomy.

The diagnosis of cantharidin toxicity can be made in suspect cases by the identification of blister beetles in the feed source and detection of cantharidin in urine or gastrointestinal contents.[590] If a heavy metal or trace mineral toxicity is suspected, lead and selenium can be measured in the blood and liver or arsenic can be measured in the blood, urine, liver, or kidney as appropriate.[594,595] Oleandrin is detectable in urine and gastrointestinal contents.[593]

Evaluation of Response to Therapy

Evaluating the response to empirical therapy may be helpful in some horses with undiagnosed diarrhea, especially in chronic cases. Dietary changes may decrease diarrhea in some cases, and often a diet of grass hay alone is recommended. In cases in which right dorsal colitis is suspected but cannot be confirmed, using pelleted feed and the addition of psyllium mucilloid and corn oil to the diet may be beneficial. Psyllium mucilloid also has been used for treatment of horses in which sand was suspected as contributing to the diarrhea. Any medications that the horse has been receiving, especially NSAIDs or antibiotics, should be discontinued in case they are contributing to the diarrhea.

Fecal microbiota transplant (FMT), also known as *transfaunation,* can be used in an attempt to restore normal flora.[620] Although not a new concept, there has been renewed interest in FMT due in part to increased awareness of the importance of the microbiota and the success of FMT in the management of *Clostridium difficile* infection in humans. Currently, data regarding FMT in horses are limited. Some guidelines for the use of FMT in horses, including case selection and FMT procedure, have been proposed.[620] Several commercial prebiotics and probiotics are available, and their efficacy is under investigation.[621] The yeast *Saccharomyces boulardii* may decrease the severity and duration of clinical signs in horses with acute enterocolitis.[622]

A course of corticosteroids can be tried in cases of chronic diarrhea in which infectious causes have been ruled out. Treatment with a larvicidal anthelmintic may be beneficial in some cases and sometimes is used with corticosteroids. Some horses with chronic diarrhea have responded to iodochlorhydroxyquin (10 g/450 kg/day for 2 weeks). This drug sometimes has been used concurrently with trimethoprim-sulfa. Occasionally, transfusion with plasma seems to suppress diarrhea in young horses.

POLYURIA AND POLYDIPSIA

Polyuria and polydipsia are infrequently reported clinical problems in the horse that can be a source of inconvenience to owners, may indicate a major abnormality in the mechanisms controlling water balance, and can be associated with significant disease. As water intake and urine output are closely

linked, polydipsia and polyuria generally occur together. Establishing whether the primary problem is polyuria or polydipsia can be helpful in developing a management strategy and providing an accurate prognosis.

Normal fluid intake and urine production in horses are influenced by a number of factors, including age, diet, physiologic demands such as exercise or lactation, environmental temperature and humidity, and gastrointestinal water absorption.[623-630] In adult horses, water requirements range from 25 to 70 mL/kg per day. These requirements are proportional to metabolic body size rather than body mass, with larger horses requiring less water per kilogram than smaller horses or ponies. Because fat is low in water content compared with lean body tissue, horses with significant body fat require less water than lean horses. Fluid requirements are higher in neonatal foals than adult horses due to their high total body water.

Water is obtained from three sources: drinking water, water in feed, and metabolic water from the catabolism of fats, carbohydrates, and protein. Water is lost in urine and feces as well as through evaporation across the skin and respiratory tract. In adult horses, normal water intake through drinking is approximately 50 to 60 mL/kg per day (20–30 L/day for an average adult horse) and normal urine production is approximately 15 to 30 mL/kg per day (5–15 L/day for an average adult horse).[623-627] Diet is a major influence on drinking and urine output. In neonatal foals on a milk diet, fluid intake is approximately 250 mL/kg per day to meet their caloric needs.[631-633] As a result, the kidneys actively excrete water and the urine output approaches 148 mL/kg per day, which is almost 5 times that of adults. In adult horses, water intake correlates with dry matter intake.[628,629] Depending on weather conditions, water consumption may be minimal in horses on good quality pasture due to the high moisture content of grass. Water requirements are higher for horses consuming hay compared with pasture or grain-based rations and vary with the type of hay. Horses on legume hay have significantly higher urine production than those on grass hay. Voluntary water intake is also affected by ambient temperature, increasing when temperatures are high and water losses in sweat are increased[623,625,634,635] (Table 7.8). Water consumption may increase significantly in heavily exercised horses such as endurance horses to replace the additional fluid lost in sweat.[634,635] Horses with diarrhea may also have significantly increased water consumption.

Polyuria and polydipsia may be clearly evident in some affected horses but in others may be more subtle. The conditions are sometimes hard for owners to recognize, particularly in horses housed at pasture and especially if they are with other horses. Some owners may misinterpret frequent urination, or pollakiuria, as polyuria. Objective documentation of polyuria and polydipsia requires measurement of water consumption and 12- to 24-hour urine production. In adult horses, polydipsia has been defined as water intake greater than 100 mL/kg per day and polyuria as urine output greater than 50 mL/kg per day. Consideration of factors that can influence water consumption and urine production, such as diet, climatic conditions, and level of exercise, should be taken into consideration when documenting polyuria and polydipsia.

TABLE **7.8 Causes of Polyuria and Polydipsia**

SOLUTE DIURESIS
Primary renal insufficiency or failure
Glucosuria (PPID)
Psychogenic salt consumption
Diabetes mellitus
Postobstructive diuresis
WATER DIURESIS
Insufficient antidiuretic hormone (central diabetes insipidus)
PPID
Head trauma
(Potassium depletion)[a]
Insufficient response of collecting ducts to antidiuretic hormone
Acquired nephrogenic diabetes insipidus
Hyperadrenocorticism (glucocorticoid excess with PPID)
Endotoxemia
(Drugs: gentamicin, lithium, methoxyflurane, amphotericin B, propoxyphene, etc.)
(Congenital nephrogenic diabetes insipidus)
Renal medullary solute washout
Chronic diuresis of any cause
Inappropriate renal tubular sodium handling
Apparent psychogenic polydipsia
(Chronic liver disease)
(Polycythemia)
(Pyometra)
(Hypercalcemia)
(Potassium depletion)
IATROGENIC
Intravenous fluid therapy
Excess dietary salt
Drugs
Diuretics
Glucocorticoids
(Drugs causing acquired diabetes insipidus)

[a]Not reported in horses.
Modified from Fenner WR: *Quick reference to veterinary medicine,* ed 3, Philadelphia, Wiley-Blackwell, 2001.
PPID, Pituitary pars intermedia dysfunction.

Mechanisms of Polyuria and Polydipsia

Maintenance of Normal Water Balance

The maintenance of adequate body water that has a relatively constant concentration of electrolytes and other solutes is essential for normal cellular function. Under normal conditions, plasma osmolality remains relatively constant, varying only by approximately 2%.[636] Body water is controlled by two general mechanisms: (1) fluid intake, which is regulated by factors that influence thirst, and (2) renal excretion of water, which is regulated by factors that affect glomerular filtration and tubular reabsorption. In normal horses, glomerular filtration exceeds 1000 L/day, but 99% is reabsorbed in the renal tubules and collecting ducts. Normal urine is 3 to 4 times more concentrated than plasma with an osmolality of 900 to 1200 mOsm/kg and a specific gravity of 1.025 to 1.050. The ability to concentrate urine is dependent primarily on three factors: (1) the production of arginine vasopressin (AVP), also known as *antidiuretic hormone (ADH);* (2) the presence of a sufficient number of nephrons that are sensitive to AVP; and (3) the presence of a hyperosmotic renal medullary interstitium.[636,637]

Role of Arginine Vasopressin. Arginine vasopressin is an important peptide hormone that is highly conserved among species.[636-639] Initially, AVP was recognized as having two functions, antidiuresis and vasoconstriction. It is

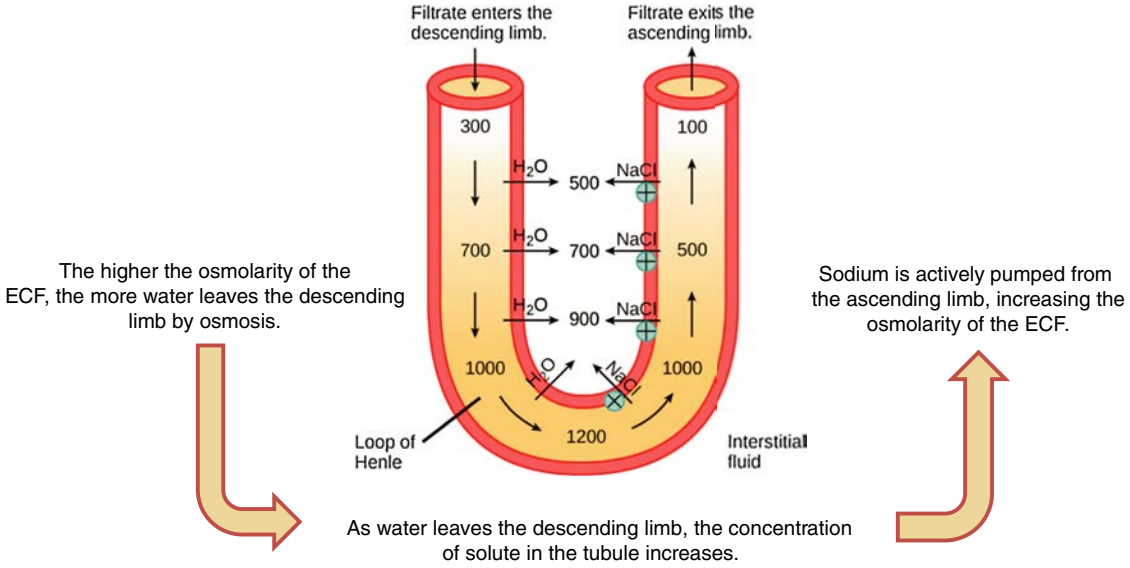


FIG. **7.6** Simplified schematic of the countercurrent multiplier in the loop of Henle. *ECF,* Extracellular fluid, concentrations in mOsm/L.

now known to have diverse effects, some of which include modulation of pituitary functions, as well as immune responses and behavior. A small peptide of nine amino acids, AVP is synthesized primarily in the supraoptic and paraventricular nuclei of the hypothalamus, with smaller quantities produced by various tissues outside the hypothalamus. It is produced as a precursor, prepro-vasopressin, which is packaged into neurosecretory granules and transported axonally via the pituitary stalk (infundibulum) to the posterior pituitary (neurohypophysis). During transport, prepro-vasopressin is processed into the active hormone, which is stored for secretion in the posterior pituitary.

AVP is primarily released in response to signals from osmoreceptors located in the anterior hypothalamus near the supraoptic nuclei and in the anteroventral region of the third ventricle.[636-639] These osmoreceptors rapidly respond to small increases in the osmolarity of the extracellular fluid, which is primarily determined by the sodium concentration. Once stimulated, osmoreceptors send signals to nerve cells in the supraoptic nuclei, which relay the signals down the pituitary stalk to the posterior pituitary stimulating the release of AVP from secretory granules in the nerve endings. Although the exact threshold for AVP release in the horse is unknown, in healthy ponies, an increase of approximately 8 mOsm/kg in plasma osmolality (about 3%) following water deprivation was associated with an increase in plasma AVP concentration from 1.53 ± 0.36 pg/mL to 4.32 ± 1.12 pg/mL.[640] When the extracellular fluid becomes hypo-osmolar, less AVP is released, causing less water to be reabsorbed. Although changes in osmolarity are the major stimulus for AVP release, the stimulation of arterial and cardiopulmonary baroreceptors by a reduction in circulating volume and hypotension can also result in AVP release.[636,638] Other stimuli for AVP release that have been reported in some species include stress, pain, nausea, hypoglycemia, and certain drugs such as morphine.

There are three different receptors for AVP, V1a, V1b, and V2, with V2 being the receptor found on the epithelial cells of the distal convoluted tubule and collecting duct of the kidney.[638] Extrarenal V2 receptors are present in the endothelium. Within the kidney, one mechanism of action of AVP is to influence aquaporins, which are integral membrane proteins that can form pores in the cell membrane that act primarily as water channels. Binding of AVP to the renal V2 receptor increases aquaporin-2 synthesis and leads to aquaporin-2 water channel insertion into the apical membrane. This aquaporin channel then allows water to move down the osmotic gradient and out of the nephron, increasing water reabsorption by the kidney. AVP also acts to increase urea permeability in the terminal inner medullary collecting duct by regulating urea transporters, increasing the concentration of urea in the medullary interstitium.[641] Also, the V2 receptors are active in the loop of Henle, where activation stimulates the reabsorption of sodium.

Role of the Renal Hyperosmotic Medullary Interstitium. The osmolarity of the interstitial fluid in the medulla of the kidney is approximately 1200 to 1400 mOsm/L compared with about 300 mOsm/L for interstitial fluid in other locations. This high osmolarity of the renal medullary interstitium surrounding the collecting ducts provides the osmotic gradient necessary for water reabsorption to occur when AVP concentrations are high. The primary mechanism maintaining the hyperosmolar interstitium is countercurrent multiplication, which depends on the structure and function of the loop of Henle[636] (Fig. 7.6). Essentially, the ongoing active reabsorption of ions from the thick ascending loop of Henle combined with the continued flow of new ions into the loop of Henle ultimately multiplies the medullary solute concentration. Due to the presence of aquaporin channels, the descending loop of Henle is permeable to water, but it is relatively impermeable to solute. Thus as tubular fluid enters the descending loop and moves toward the inner medulla, it gradually becomes more hypertonic as water moves out into the interstitium. In the thin ascending loop of Henle, ions begin to move into the interstitium by passive diffusion. Once fluid enters the thick ascending loop of Henle, sodium, potassium, and chloride are actively reabsorbed via the apical $Na^+/K^+/2Cl^-$ cotransporter. This active transport of ions, combined with the

impermeability of this section to water, results in an increased osmolarity in the renal medullary interstitium. This creates an osmotic pressure gradient, drawing water from the descending limb and increasing the osmolarity of the tubular fluid. This process is repeated as fluid continues to flow through the tubules, with the net effect of continuing to add sodium to the medullary interstitium until the osmolarity reaches 1200 to 1400 mOsm/L. The collecting ducts also contribute to the hyperosmolar interstitium through the active transport of ions and the facilitated diffusion of urea into the interstitium. The relatively low blood flow in the renal medulla and the unique characteristics of the vasa recta help to maintain the hyperosmolar interstitium.

Countercurrent multiplication and maintenance of the hyperosmolar interstitium play important roles in the concentration of urine. When luminal fluid enters the thick ascending loop of Henle, approximately 80% of the glomerular filtrate has been reabsorbed. The active resorption of NaCl in the thick ascending loop of Henle, which is impermeable to water, causes the fluid entering the distal tubule in the renal cortex to be hypotonic. Water is then reabsorbed from the distal tubules and cortical portions of the collecting ducts under the action of AVP. Reabsorbed water is transported rapidly out of the interstitium by the extensive cortical capillary network, and interstitial hypertonicity is preserved.

Thirst. Adequate fluid intake, which is regulated by thirst, contributes to the maintenance of water balance along with the renal excretion of water.[630,634,636,642] The thirst center in the hypothalamus consists of osmoreceptors in the anteroventral wall of the third ventricle and anterolateral preoptic nucleus that are in close proximity to those that regulate AVP secretion. A major stimulus for thirst is increased osmolarity of the extracellular fluid, which causes intracellular dehydration in the thirst centers, stimulating the sensation of thirst. In human beings the threshold for stimulation of thirst is approximately 2 to 5 mOsm/kg greater than that for stimulation of AVP release. Also, decreases in extracellular fluid volume and arterial pressure can stimulate thirst via mechanisms that are distinct from osmolarity. These mechanisms include neural input from cardiopulmonary and systemic baroreceptors and the release of angiotensin II, which can both stimulate thirst and decrease renal fluid excretion. Peripherally, mechanoreceptors in the oropharynx can sense dryness of the mouth, stimulating thirst. Following experimental water deprivation, ponies drank when their plasma osmolalities increased by 3%, when plasma Na concentrations increased by approximately 5%, and after induction of a plasma volume deficit of 6%.[8]

General Mechanisms of Polyuria and Polydipsia

General mechanisms of polyuria and polydipsia include water or solute diuresis, and in some cases both mechanisms may contribute.[625,630,634,636] Water diuresis is caused by either decreased water absorption in the collecting tubules or excessive voluntary water intake. In water diuresis, either polyuria or polydipsia may be primary. Solute diuresis is caused by excessive renal excretion of a nonreabsorbed solute such as glucose or sodium, resulting in primary polyuria.

Polydipsia as the Primary Problem. Excessive water intake results in water diuresis.[636] By expanding the extracellular fluid volume and decreasing plasma osmolarity, excessive fluid intake causes a suppression of AVP secretion. This then makes the collecting ducts less permeable to water, increasing urine output.

Polyuria as the Primary Problem. Polyuria can result from renal disease or several systemic disorders. Although polyuria is commonly associated with renal failure, the precise mechanisms are not fully understood and are most likely multiple.[643] With primary renal insufficiency or failure, there is a decrease in functional nephrons. This can result in solute diuresis, as increasing amounts of glomerular filtrate are presented to the remaining tubules, often exceeding their reasborptive capacity. The resultant increase in urinary solute is accompanied by an increase in water loss. Also, the increased tubular flow rate in the remaining tubules may simply allow less time for the reabsorption of water. In addition, diuresis may result from decreased hypertonicity of the medullary interstitium associated with decreased transport of sodium and chloride out of the loop of Henle when there is renal compromise. Another factor that may contribute to polyuria in renal failure is acquired nephrogenic diabetes insipidus due to an inability of the diseased collecting ducts to respond to AVP.

The syndrome of diabetes insipidus, which is characterized by the excretion of large volumes of dilute urine associated with abnormalities related to AVP, has several fundamental causes.[644,645] In primary polydipsia, AVP secretion is suppressed by excessive fluid intake. Also, in human patients, gestational diabetes insipidus can occur due to degradation of AVP by a placental enzyme. Central or neurogenic diabetes insipidus occurs when there is inadequate production or secretion of AVP. This form of diabetes, or inflammatory disease involves the hypothalamus or posterior pituitary. In nephrogenic diabetes insipidus there is insensitivity of the renal collecting duct epithelial cells to AVP, and the condition may be primary or acquired. Primary nephrogenic diabetes insipidus implies isolated dysfunction of the response to AVP not associated with actual structural or metabolic problems of the kidney. In people this occurs as a familial disorder with an X-linked autosomal semirecessive mode of inheritance, and there is some evidence this may occur as a familial problem in horses as well. Acquired nephrogenic diabetes insipidus can be associated with renal disease or altered sensitivity of nephrons to AVP due to endotoxemia or cortisol excess. Also, in some species, hypercalcemia, potassium depletion, and the administration of certain drugs such as gentamicin have been reported to cause insensitivity of the collecting duct receptors to AVP.

Renal medullary washout is a syndrome in which the relative hypertonicity of the renal medulla is reduced, typically from prolonged diuresis of any cause.[636,643] The increased tubular flow rates during diuresis may result in an inability to resorb sufficient sodium and urea from the tubular lumen. The resulting decrease in medullary hypertonicity leads to impairment of renal concentrating ability and polyuria with secondary polydipsia. Enhanced medullary blood flow may deplete medullary solute further.

Polyuria as a result of solute diuresis can occur in association with excessive salt consumption and hyperglycemia in addition to renal failure.[643,646-649] When blood glucose concentrations increase above the renal threshold of approximately 150 to 200 mg/dL in the horse, the resultant glucosuria results in increased urine flow. Solute diuresis may also occur following obstructive urinary tract disease, but this is not commonly reported in horses.

Conditions Associated with Polyuria and Polydipsia

There are several potential specific causes of polyuria and polydipsia in horses.[650,651] The most common causes include renal failure, PPID, and primary psychogenic polydipsia. Other less common causes include excessive salt consumption, central and nephrogenic diabetes insipidus, and diabetes mellitus. Polyuria and polydipsia may also occur secondary to sepsis and endotoxemia or may be iatrogenic following the use of α_2-agonists, corticosteroids, or diuretics.[650-652]

Polydipsia

The most common reason for excessive water intake in horses is psychogenic water drinking. The extent of polydipsia and therefore polyuria seen with psychogenic water drinking can be substantial. Psychogenic water drinking can be a stable vice associated with confinement and boredom.[642,653] The behavior has also been associated with changes in environmental conditions, stabling, and diet. It has been suggested that primary polydipsia is particularly common in horses residing in the southern United States when ambient temperature and humidity are high.

Excessive salt consumption may also result in increased thirst, although a significant amount of salt needs to be consumed to stimulate thirst in horses.[648] In human patients, polydipsia has been reported in association with conditions affecting the osmoreceptors involved in the regulation of thirst, such as neurologic disease affecting the hypothalamic thirst center.[654,655] This could potentially occur in horses. For example, the enlarged pituitary gland in horses with PPID could compress the hypothalamus.

The ability to concentrate urine is retained in cases of primary polydipsia. However, occasionally the long-standing diuresis can result in medullary washout and a decrease in concentrating ability.

Polyuria

Important causes of primary polyuria in horses include renal failure and pituitary pars intermedia dysfunction.[650,651] Diabetes insipidus and diabetes mellitus are less common causes.

Renal Failure. In the acute stage of renal failure there is frequently a period of oliguria or anuria, which is then followed by polyuria.[643,646] The repair of tubules following an acute insult may take several weeks, and in some cases the repair may not be complete, resulting in chronic renal failure and persistently diminished concentrating ability.[643,647] Some potential causes of renal failure in horses include failure to recover from ischemic renal damage, pigmenturia, chronic infection, urinary obstruction, and exposure to nephrotoxins such as nonsteroidal antiinflammory drugs, gentamicin, and polymixin.[646,647,656,657] Other possible causes include immune-mediated disease, glomerulonephritis, congenital abnormalities, renal helminthiasis, and amyloidosis. In some horses, the inciting cause of chronic renal failure may be hard to identify.

Pituitary Pars Intermedia Dysfunction. Polyuria and polydipsia are recognized in approximately 30% of horses with *pituitary pars intermedia dysfunction* (PPID), although numbers vary among studies.[658-660] Other clinical signs that are commonly associated with PPID include hypertrichosis, laminitis, muscle atrophy, abnormal fat deposition, and recurrent infections. The pathogenesis of polyuria and polydipsia in PPID is not completely understood and most likely involves multiple factors. As some horses with PPID are hyperglycemic, osmotic diuresis associated with glucosuria may play a role. Although it has not been documented, the enlarged pars intermedia may impinge on the posterior pituitary and/or hypothalamus, potentially causing secondary neurogenic diabetes insipidus by interfering either with the production or release of AVP or possibly with osmoreceptors involved in thirst. Also, an increase in plasma cortisol associated with PPID may result in antagonism of the action of AVP at the renal tubule.

Diabetes Insipidus. Neurogenic diabetes insipidus appears to be rare in the horse, with two cases reported in the literature.[661,662] In both cases, the horses were unable to concentrate urine in response to water deprivation but responded to the administration of exogenous vasopressin with a decrease in urine volume and increase in urine concentration. In one case the condition was felt to be idiopathic, and in the other it was felt to be acquired secondary to encephalitis.

Primary nephrogenic diabetes insipidus also appears to be rare in horses, having been reported in three colts and a 14-year-old gelding.[663-665] Two of the colts were sibling Thoroughbreds, suggesting that an inherited form of nephrogenic diabetes insipidus similar to what is seen in people may occur in horses.[664] In addition to signs of polyuria and polydipsia, two of the three colts were underweight. Following water deprivation, affected colts could not increase urine concentration although plasma AVP concentrations increased. In addition, affected horses had minimal response to the administration of exogenous vasopressin, confirming resistance of the collecting ducts to the antidiuretic action of vasopressin.

Nephrogenic diabetes insidipus has been recognized in horses with some frequency as a secondary problem in which there is an acquired decrease in renal responsiveness to AVP.[645] This acquired nephrogenic diabetes insipidus has been associated with a variety of conditions, including renal disease. It has also been described following drug therapy, as well as with a variety of endocrine, metabolic, infectious, neoplastic, and postobstructive disorders.

Diabetes Mellitus. Diabetes mellitus is characterized by chronic hyperglycemia. In type 1 or insulin-dependent diabetes mellitus, there is a lack of insulin, whereas in type 2 or non–insulin-dependent diabetes mellitus, insulin is adequate but tissue sensitivity to insulin is impaired. Although it is a relatively common cause of hyperglycemia and glucosuria in other species, type 1 diabetes mellitus has been infrequently diagnosed in the horse.[152, 666-671] Type 2 diabetes mellitus has been recognized in horses in association with several conditions, especially PPID and equine metabolic syndrome.[80, 672-676] Diabetes mellitus has also been reported in a horse with bilateral granulosa cell tumors.[672]

Other. Medullary washout may result from chronic diuresis of any cause and may be a more common complication of primary diseases and their therapy in horses than has been reported to date. Polyuria and polydipsia have occasionally been reported in association with sepsis and endotoxemia in multiple species, including horses. The mechanisms are unclear but may involve endotoxin-induced production of PGE_2, which can cause renal vasodilation and inhibit the actions of AVP. Although infrequently reported in horses, polyuria and polydipsia have been recognized in association with severe liver disease. Some conditions that have been associated with polyuria and polydipsia in other

species include primary polycythemia, hypercalcemia, and potassium depletion.

Diagnostic Approach to Polyuria and Polydipsia

Signalment and History

The signalment and history can be valuable in the assessment of polyuria and polydipsia. Congenital problems such as renal anomalies are generally recognized in young animals, whereas PPID increases in prevalence with age. Primary nephrogenic diabetes insipidus has been described in two related Thoroughbred colts. A detailed history should be obtained with particular emphasis on the duration of signs, any management changes, diet including any supplements, and any current or previous medication use. In some horses, iatrogenic causes of polyuria and polydipsia can be ruled out by assessment of the history and return to normal urine output and water intake after discontinuing medications, intravenous fluids, or excess dietary salt.

Physical Examination

A complete physical examination should be performed with careful attention to hydration status. Palpation per rectum can be useful in evaluation of the urinary tract. Horses with psychogenic polydipsia are generally normal on physical examination. Chronic renal insufficiency is often associated with a poor body condition, and in some cases, appetite may be decreased. The clinical signs of PPID can be quite variable depending on the individual and the stage of disease, but commonly recognized signs include hypertrichosis, muscle atrophy, abnormal fat deposition, chronic infections, and laminitis.

Measurement of Water Intake and Urine Volume

The measurement of 24-hour water intake and urine production can be helpful in confirming the problem, especially in those cases in which polyuria and polydipsia are less obvious.[677-680] It can also establish the severity, which can help to prioritize differentials. In general, the degree of polydipsia and polyuria tends to be more pronounced with psychogenic polydipsia and diabetes insipidus than with renal failure or PPID. Several means of urine collection have been devised. Generally close observation and cross-tying are required to ensure the device stays in place and measurements are accurate. In general water intake over 100 mL/kg per day and urine output over 50 mL/kg per day are consistent with polydipsia and polyuria in adult horses. Factors that can influence water intake and urine production, such as diet and environmental conditions, should be taken into consideration. When measuring water consumption, behavioral tendencies to spill or play in the water should be taken into account.

Ancillary Diagnostic Aids

Clinical Pathology. A hemogram, serum biochemistries, and urinalysis should be assessed in horses presented for polyuria and polydipsia. Urine specific gravity (USG) is decreased in horses with polyuria and polydipsia. The extent of the decrease in USG, along with the presence or absence of other abnormalities on laboratory tests, can help prioritize differential diagnoses and may direct additional testing. Horses with psychogenic polydipsia tend to present with hyposthenuria (USG less than 1.008 and osmolality less than 260 mOsm/kg) without other laboratory abnormalities. As hyposthenuria indicates that renal diluting ability is intact, horses with renal failure or complete medullary washout are unlikely to have hyposthenuria, although it may occasionally be present in horses recovering from acute renal failure. Chronic renal failure is suggested by the presence of isothenuria (USG 1.008–1.014 and osmolality 260–300 mOsm/kg) with concurrent azotemia.[681-683] Other laboratory abnormalities that may be seen with chronic renal failure include mild anemia, hypoalbuminemia, hyponatremia, hypochloridemia, hyperkalemia, hypercalcemia, and hypophosphatemia. Some additional laboratory tests that may be used to assess renal function include fractional excretion of electrolytes, urinary protein–to–urinary creatinine ratio, creatinine clearance, and measurement of urinary enzymes. Measurement of glomerular filtration rate is a sensitive indicator of renal dysfunction but is infrequently performed primarily for practical reasons. Measurement of symmetric dimethylarginine, a methylated form of arginine that is eliminated primarily by renal excretion, is useful in the evaluation of renal function in small animals and is under investigation in horses.[684]

There are no consistent laboratory abnormalities in horses with PPID, although neutrophilia, lymphopenia, hyperglycemia, and glucosuria may be present.[658-660] Additional endocrine testing is required to confirm the presence of PPID. Some recommended tests include measurement of baseline ACTH concentration, measurement of ACTH concentration following the administration of thyrotropin-releasing hormone, or a low-dose dexamethasone suppression test. Hyperglycemia and glucosuria may also be seen with equine metabolic syndrome, underlying systemic disease and diabetes mellitus.

Water Deprivation Testing. Water deprivation evaluates the ability to conserve water and is most often used to differentiate psychogenic polydipsia from diabetes insipidus.[624,685,686] It is most clearly indicated in horses that are hyposthenuric rather than isosssthenuric. Water deprivation testing is contraindicated in horses that are dehydrated or azotemic. In normal animals, the increase in plasma osmolality that occurs with water deprivation results in the release of AVP, which acts on the renal tubules and collecting ducts to conserve water, increasing USG. Before initiating the test, baseline values for USG, serum urea nitrogen, creatinine, and body weight should be obtained and the bladder emptied by catheterization. After water is removed, USG and body weight should be monitored every 6 to 12 hours. Serum urea nitrogen and creatinine may be monitored as well. In response to 24 to 72 hours of water deprivation, normal horses will increase their USG over 1.045 with an osmolality over 1500 mOsm/kg. For practical purposes, if USG increases above 1.025 within 24 hours or by the time that 5% of body weight is lost, concentrating ability is adequate and the test can be discontinued. The test should be terminated if there is a loss of more than 5% of the body weight or if clinical signs of dehydration or azotemia develop. Typically, horses with psychogenic polydipsia can concentrate their urine in response to water deprivation whereas those with diabetes insipidus cannot. Occasionally horses with psychogenic polydipsia will have incomplete concentrating ability due to medullary washout and loss of the medullary interstitial osmotic gradient. These horses may respond to a modified water deprivation test where water intake is gradually restricted for several

days allowing for restoration of the medullary interstitial osmotic gradient.[687] Horses with either central or nephrogenic diabetes insipidus cannot effectively concentrate their urine in response to water deprivation, and to distinguish these conditions additional testing such as measurement of endogenous AVP concentrations and evaluation of the response to exogenous AVP is indicated.

Measurement of Arginine Vasopressin and Response to Exogenous Arginine Vasopressin or Analogs. The plasma concentration of AVP can be measured during water deprivation testing or following the administration of 7.5% sodium chloride solution to increase plasma osmolality. In normal ponies, AVP concentrations increased from baseline values of 1.53 ± 0.36 pg/mL to 4.32 ± 1.12 pg/mL after 24 hours of water deprivation.[13] Plasma concentrations of AVP will increase in horses with psychogenic polydipsia or nephrogenic diabetes insipidus, but any increase will be minimal with central diabetes insipidus. In nephrogenic diabetes insipidus, the elevation in AVP is not accompanied by an increase in USG.

The ability of renal tubules to respond to AVP can be assessed by a challenge test.[688] USG is monitored following the administration of either exogenous synthetic vasopressin or desmopressin acetate, a synthetic vasopressin analog. USG is expected to increase over 1.020, and failure to do so is consistent with a diagnosis of nephrogenic diabetes insipidus if medullary washout is not present.

Diagnostic Imaging

Transabdominal and transrectal ultrasound examination can be useful in evaluation of the urinary tract. Although not consistent, changes in the size and/or architecture of the kidneys may be present in both acute and chronic renal failure. Ultrasound can also be helpful in the diagnosis of urolithiasis.

POOR PERFORMANCE

Poor performance is the inability of an individual to perform at a level that can be reasonably expected based on the individual's physical characteristics, level of training, and/or previous performance. Any decrease in performance may be critical to the equine athlete. In some cases, poor performance may be easily documented, such as when the problem is severe or when there is a drop in performance that can be objectively measured (e.g., in a discipline such as racing). However, in other cases, poor performance may be subtle and difficult to document.

Mechanisms Affecting Performance

Numerous factors influence performance, including genetics, training, desire, body composition, and overall health. Peak athletic performance requires optimal function of all body systems, particularly those involved in locomotion and oxygen transport.

Genetic Factors

Genetic factors play a role in several aspects of performance. In multiple species, genetics are known to influence athletic potential. As the knowledge of equine genomics expands, understanding of the heritability of specific traits associated with elite athletic performance will increase.[689-691] For example, variation at the myostatin gene locus has been found to be associated with a horse's best racing distance.[690] In addition to influencing inherent athletic ability, genetic traits can potentially impair performance either by direct effects on the individual's health or by increasing the likelihood of the individual developing secondary problems. For example, heritable muscle disorders can directly affect performance, and poor structural conformation can increase the risk of injury.[3,4] Similarly, it has been suggested that Warmblood horses that must work in top gear to achieve the speeds required for upper levels of 3-day eventing are more prone to injury.[692]

Locomotion

Movement is a key component of athletic performance, and therefore musculoskeletal, neurologic, and neuromuscular disorders can all have a negative impact on performance.[691-695] The mechanisms by which they affect performance are multiple and include pain, mechanical restrictions, incoordination, and weakness. Pain can make the horse reluctant to exercise and can also limit the amount of training so that the horse is unable to achieve the level of fitness required for optimal performance. Any gait deficit can potentially make efficient movement mechanically difficult. Also, lameness at one site can contribute to the development of secondary problems such as back pain or chronic myopathy that can further impair performance. Neurologic disease can cause ataxia and weakness, adversely affecting performance.

Physical activity requires muscles to generate and sustain the power for movement. Horses are generally considered to be a highly athletic species, and there are several adaptations in equine skeletal muscle that contribute to this athletic ability, including an increased muscle mass relative to body weight, high locomotor efficiency based on muscle-tendon architecture, high intrinsic shortening velocities, and efficient muscle energetics.[696,697] In horses, muscle tissue comprises about 45% of body weight in most equine breeds and up to 55% of body weight in Thoroughbreds, compared with 30% to 40% in many other species. Horses that have the ability to run fast tend to have a large overall skeletal muscle mass, longer muscle fascicles, a high percentage of fast-twitch muscle fibers, and a low percentage of body fat.[90] Any change in muscle mass, architecture, or function can have a profound impact on performance. If muscle fatigues prematurely or is painful, such as with rhabdomyolysis, the horse may not be able to generate the necessary power for efficient movement.

Oxygen Uptake and Transport

Any process that impairs the uptake or transport of oxygen can limit the ability to produce the energy required for optimal performance. Thus diseases of both the upper and lower respiratory tract, and less frequently the cardiovascular system, are important causes of poor performance. A number of upper airway obstructive disorders have been recognized that restrict the movement of air into and/or out of the lung.[301,698-,699-702,739] Disease of the lower respiratory tract may increase the work of breathing and impede normal gas exchange in the lung. For example, inflammatory airway disease (IAD) has been shown to alter pulmonary mechanics through changes in lung compliance, greater viscous lung resistance, and rate of dynamic work of breathing.[703] Due to the increase in ventilatory load, the energy required for breathing is significantly higher in affected horses, which may limit their performance. Also, a higher neutrophil percentage in the bronchoalveolar lavage fluid of horses with poor racing performance has been correlated with significantly lower relative gas flows during inspiration and expiration.[704]

Although the effects of exercise-induced pulmonary hemorrhage (EIPH) on performance are not entirely clear, in a study of 132 horses with a history of poor performance, exercise-induced hypoxemia was seen in horses with IAD and EIPH.[705] Gas exchange was most significantly impaired in those horses with concurrent EIPH and upper airway obstruction. Once oxygen is taken up into the circulation from the lung, it must be delivered to the tissue, especially working muscle, for optimal performance. Thus cardiovascular disease can impair performance by decreasing the effectiveness of oxygen delivery to the tissues.

Other

Almost any systemic disease or source of pain has the potential to make the horse reluctant to work. For example, poor performance has been reported in association with equine gastric ulcer syndrome and possibly dental disease.[700,706,707] Also, in horses with anhidrosis, their decreased ability to maintain normal body temperature can limit performance in warm environmental conditions.[708]

Conditions Associated with Poor Performance

It is not possible to list all potential causes of poor performance as so many factors influence performance (Box 7.11). Certainly almost any systemic disease, as well as desire and training, can affect performance. When evaluating horses presented specifically for the complaint of poor performance, the conditions most often identified have been abnormalities of the respiratory and musculoskeletal systems.[695,699,700,709,710] It is not uncommon for multiple problems to occur concurrently. In a study of 275 racehorses with a history of poor racing performance, 84% were found to have more than one abnormality.[699] Similarly, in a study of 27 endurance horses with poor performance, 66.7% had multiple concomitant disorders.[710]

Locomotor Disorders

A surprising number of horses presented for poor performance are found to be lame even when lameness has not been reported by the owner or trainer.[692,695,699,700,709,710] Although some horses may compete successfully with low-grade lameness, any degree of lameness has the potential to impair performance. A wide variety of musculoskeletal conditions have been identified in association with poor performance.[692,695,699,700,709-712] In addition to those horses with forelimb or hindlimb lameness, horses with other sites of musculoskeletal pain, such as neck, back, or sacroiliac joint pain, may also have poor performance.[693,694,712] Neurologic deficits also have the potential to adversely affect performance.

Several muscle disorders have been linked to poor performance, including both sporadic and recurrent rhabdomyolysis (tying-up).[698,700,713,714] In many cases, affected horses do not exhibit the classic clinical signs of rhabdomyolysis but have increased muscle enzymes in response to exercise. In one study, 53 of 348 horses examined for poor performance had this form of subclinical myopathy identified only by increased creatine kinase concentrations after exercise, and 10 of 348 had clinical exertional rhabdomyolysis.[700]

Polysaccharide storage myopathy (PSSM) is an inherited glycogen storage disorder that is an important cause of muscle disease in a number of breeds, including Quarter Horses and related breeds, many draft and Warmblood breeds, and others.[714] Clinical signs vary depending on the individual and breed, and the clinical presentation of affected horses ranges from being asymptomatic to exhibiting muscle atrophy and progressive weakness, muscle soreness, and gait abnormalities, or acute rhabdomyolysis. In Quarter Horses and related breeds it appears there is a second mutation, malignant hyperthermia, that potentially worsens the signs of PSSM in affected horses.[24]

BOX 7.11 Causes of Poor Performance

SYSTEMIC DISEASE

BODY CONDITION

Overconditioned
Underconditioned

TRAINING

Inadequate
Overtraining

MUSCULOSKELETAL

Lameness
Back pain
Neck pain
Muscle disorders
- Sporadic exertional rhabdomyolysis
- Subclinical exertional rhabdomyolysis
- Polysaccharide storage myopathy
- Recurrent exertional rhabdomyolysis
- Vitamin E myopathy
- Muscle injury/strain
- Mitochondrial myopathy
- Myofibrillar myopathy

NEUROLOGIC DISEASE

RESPIRATORY TRACT DISEASE

Upper airway obstruction
- Dorsal displacement of the soft palate
- Recurrent laryngeal neuropathy
- Axial deviation of the aryepiglottic folds
- Dynamic pharyngeal collapse
- Epiglottic entrapment
- Subepiglottic cyst
- Rostral displacement of the palatopharyngeal arch
- Redundant alar folds

Lower airway disease
- Inflammatory airway disease; recurrent airway obstruction
- Exercise-induced pulmonary hemorrhage

CARDIOVASCULAR DISEASE

Cardiac arrhythmia
Myocardial dysfunction
Murmurs (often not clinically significant)

OTHER

Dental disorders
Equine gastric ulcer syndrome
Anhidrosis
Saddle fit; horse-saddle-rider interaction
Disease in early neonatal period

Several other muscle disorders have been identified in horses. Recurrent exertional rhabdomyolysis (RER) is a heritable abnormality of intramuscular calcium regulation seen primarily in Thoroughbreds and to a lesser extent in Standardbreds and possibly Arabians.[715] A skeletal muscle mitochondrial myopathy has been described in an Arabian in association with impaired oxidative energy metabolism; muscle stiffness and exercise intolerance due to a suspected myofibrillar myopathy have also been reported in Arabian horses.[716,717] Vitamin E deficiency has been associated with several clinical problems, one of which is a myopathy.[718] Horses affected with myopathy related to vitamin E deficiency may present with signs of muscle atrophy, weakness, toe dragging, and muscle fasciculations, as well as poor performance.

Muscle strain or soreness can also be a cause of decreased performance.[698] Equine activities can often be athletically challenging and may require either high intensity or prolonged effort. Some disciplines require a combination of activities and may require sudden moves or maintaining a precise body position. In some cases, the horse is not adequately conditioned, making the risk of muscle injury greater. Any muscle group may be affected, and clinical signs vary depending on the muscle involved and the severity of the injury.

Respiratory Disorders

Respiratory disease has been the most common cause of poor performance in several studies. Among the most common respiratory problems associated with poor performance in the equine athlete is dynamic airway obstruction, affecting both racing and nonracing performance horses.[300,699,700,702,709,719] In the study of 275 racehorses evaluated for poor performance, 110 (40%) were found to have dynamic airway obstruction.[699] Similarly, in the study of 348 racehorses and show horses with poor performance, 148 (42.6%) had dynamic airway obstruction.[700] Of these 148 affected horses, 39 were found to have multiple airway abnormalities, and an additional 22 were found to have a concurrent cardiac arrhythmia. The most common conditions causing airway obstruction include dorsal displacement of the soft palate and idiopathic left laryngeal hemiplegia with arytenoid collapse, also referred to as recurrent laryngeal neuropathy. Other conditions diagnosed have included axial deviation of the aryepiglottic folds, dynamic pharyngeal collapse, epiglottic entrapment, subepiglottic cyst, fourth branchial arch defect (rostral displacement of the palatopharyngeal arch, laryngeal dysplasia), and redundant alar folds. Horses of Quarter Horse breeding with hyperkalemic periodic paralysis (HYPP) may suffer dynamic collapse of the airway when exercising.[720]

Disease of the lower respiratory tract is also a common cause of decreased performance.[227,251,755,710,721,722] In the study of 27 endurance horses with poor performance, respiratory tract abnormalities, specifically tracheal fluid neutrophilia and IAD, were the most commonly identified problems.[710] IAD was identified in 70% of national hunt horses presented for poor athletic performance.[721] Excess mucus accumulation in the airway has been associated with poor willingness to perform in sport horses.[251,721] In addition to IAD, other subtypes of equine asthma, including recurrent airway obstruction (RAO) and summer-pasture–associated obstructive pulmonary disease, can limit athletic performance. The effects of EIPH on performance have been difficult to measure because there are so many confounding factors, but current evidence suggests that moderate to severe EIPH adversely affects athletic capacity.[227,722] Clinical bacterial or viral respiratory tract infection significantly affects performance, but the role of subclinical infections is less clear. Studies in Standardbred trotters have not found an association between subclinical viral infection and poor performance.[723,724]

Cardiovascular Disorders

Cardiovascular disease can also cause poor performance, although it is less common than either musculoskeletal or respiratory disease.[699,700,709,710] The prevalence and clinical relevance of cardiac arrhythmias have varied between studies.[699,700,709,710,725-727] In the study of 348 horses evaluated for poor performance, a clinically significant cardiac arrhythmia was the sole abnormality found in 33 horses, and an arrhythmia in conjunction with dynamic airway obstruction was found in 22 horses.[700] The most frequent arrhythmias observed were atrial and ventricular premature depolarizations. However, in the study of 275 racehorses, arrhythmias were noted in just two horses.[699] Isolated premature depolarizations were common in a study of 88 Thoroughbreds with poor performance, with 55 horses having at least one premature ventricular or supraventricular premature depolarization.[727] The clinical significance of these depolarizations was uncertain. Ventricular tachycardia and paroxysmal atrial fibrillation have also been identified in horses with poor performance. Changes in the T wave, once thought to be related to poor performance, and second-degree atrioventricular block have been found to have no effect on exercise capacity.[728]

Many horses have murmurs that are of little clinical significance.[699,700,709,710,729,730] In the study of 348 horses with poor performance, 102 were found to have murmurs, the most common being mitral regurgitation.[700] In all cases the murmur was determined to be clinically unimportant. Similarly, in the study of endurance horses, neither the prevalence nor the grade of valvular regurgitation was significantly different between well-performing horses and those with a decreased performance level.[710] Although many murmurs appear to be clinically unimportant, in some individual cases a murmur may be associated with more significant cardiac disease and poor performance.

Echocardiography has been used to evaluate cardiac function in cases of poor performance. In the study of 348 horses, decreased fractional shortening indicating left ventricular myocardial dysfunction after exercise was found in 19 horses, only 8 of which had echocardiographic changes at rest.[700] Six of the 19 horses had what was felt to be a clinically significant ventricular arrhythmia.

Other

A wide variety of other disorders have been linked to poor performance. Body condition plays a role in performance, and horses that are both overconditioned and underconditioned may have suboptimal performance.[90,697,731,732] A study in endurance horses demonstrated that body condition score at the start of the ride had a significant effect on performance, with horses with lower scores being less likely to complete the ride.[732] Some other problems potentially associated with poor performance include dental disease, gastric ulcers, anhidrosis, and both undertraining and overtraining.[706-708,733] Poor saddle fit may cause focal areas of increased pressure resulting in an unwillingness to work. The dynamic interaction between the horse, saddle, and rider may not be optimal.[734] Some foals that were sick within the first 18 hours of birth may have decreased performance as an adult.[735]

Diagnostic Approach to Poor Performance

Determining the cause of poor performance in those horses without overt clinical disease often is challenging.[695,698,699-701] In the study of 348 cases of poor performance, a definitive diagnosis was established in 73.5% of cases after in-depth examination, which included the use of a high-speed treadmill.[700] Multiple factors can contribute to the difficulty in establishing a definitive diagnosis. First, it can even be hard to determine whether the horse has actual poor performance or if it is simply not performing up to expectations that are unrealistic for the individual's inherent ability or current level of training. Medical conditions can certainly influence the willingness to work, but there may also be a behavioral component that can be difficult to assess. The abnormalities that are sufficient to impair performance can be quite subtle and may be evident only during exercise. Multiple problems may be present concurrently, which can make it hard to determine the actual clinical significance of any given problem.

Equine athletes presented for poor performance should undergo a comprehensive evaluation, the basic components of which include a history, detailed physical examination, and laboratory screening. The clinician should emphasize examination of the respiratory, musculoskeletal, and cardiovascular systems because these systems most often are linked to performance problems. In many cases standardized exercise testing, generally on a high-speed treadmill, is critical in identifying the problem. Endoscopic examination of the upper airways during exercise has proven particularly useful. It may also be helpful to evaluate the horse while it's being ridden.

Signalment and History

The signalment may be helpful in the assessment of some cases of poor performance. Horses that present at a young age may be more likely to have a congenital or heritable condition or may have limited genetic potential. Diseases with a heritable basis are more often seen in certain breeds. PSSM is found in a wide variety of breeds but is common in Quarter Horses and related breeds as well as some draft horse and Warmblood breeds, whereas RER is primarily seen in Thoroughbreds and to a lesser extent Standardbreds and possibly Arabians.[713-715] RER is more common in females and in horses with a nervous temperament. Recurrent laryngeal neuropathy appears to be more common in large, long-necked horses especially of Thoroughbred or draft horse breeding.[736]

Obtaining a complete history is a fundamental part of evaluating poor performance. The clinician should establish the use of the horse, the time in training, and the specifics of the training program. Determining whether the horse has never performed as expected or has experienced a decline in the level of performance is crucial. If the horse has never performed as expected, the veterinarian should consider a lack of ability, congenital abnormalities, and training problems. A change in performance, either sudden or insidious, often is associated with an acquired problem. The clinician should characterize specifically the decline in performance, including the intensity of exercise at which signs are observed and whether performance is abnormal from the onset of exercise or declines during an exercise bout. In those cases in which performance drops off during exercise, the clinician should determine whether the decline is acute or gradual and whether any other signs, such as stridor, are associated with it.

Other elements of the history with particular relevance to athletic performance include any previous respiratory disease, respiratory noise, cough, or respiratory distress associated with exercise. Any change in gait also may be significant. Establishing the housing environment, feeding practices, changes in appetite or body condition, the type of tack used, and whether sweating is appropriate is important. The clinician should determine the response to any medications that have been used, such as phenylbutazone or furosemide. The information obtained in the history may help direct the investigation.

Physical Examination

The clinician should perform a complete physical examination in all cases. The body condition score should be determined. In-depth assessment of the gait, as well as the respiratory and cardiovascular systems, is generally indicated.

Respiratory Examination. The examination should include evaluation of air flow from the nares and percussion of the sinuses, as well as assessment of any cough or nasal discharge. Careful palpation of the larynx may reveal an increase in prominence of the muscular process of the left arytenoid cartilage resulting from a loss of mass of the left dorsal cricoarytenoid muscle associated with idiopathic hemiplegia. The clinician can use the laryngeal adductor response test, or slap test, to evaluate adduction of the arytenoid cartilages by slapping the withers during expiration and evaluating movement of the contralateral arytenoid by endoscopy or palpation. It is important to note that many of the horses with airway obstruction did not have a history of abnormal respiratory noise and did not have abnormalities at rest.[300,699,700,702,709,719] At the same time, not all abnormalities observed at rest caused obstruction. These findings emphasize the importance of videoendoscopy during exercise as a component of a performance evaluation.

Thorough auscultation of the trachea and lungs should be performed. Having the horse rebreathe from a plastic bag placed over the nostrils increases the respiratory rate and tidal volume, thus accentuating sounds. In addition to auscultation, the clinician should note the character and pattern of respiration, including the presence of any abdominal component, and the recovery time. Percussion of the thorax may be useful in establishing the lung border and any dull or hyperresonant areas, as well as in detecting pleural pain.

Cardiovascular Examination. Any decrease in cardiac output potentially can limit performance, making thorough evaluation of the cardiovascular system essential. On basic physical examination the clinician should evaluate the mucous membrane color, capillary refill time, and arterial and venous peripheral pulses, although finding abnormalities in these parameters in horses presented for decreased performance is uncommon. The clinician should perform careful auscultation of the heart on both sides of the thorax to evaluate the cardiac rhythm and murmurs. Electrocardiography can be used to further evaluate the cardiac rhythm and ideally should be performed before, during, and after exercise using radiotelemetry.

Musculoskeletal and Neurologic Examination. A lameness examination should be performed in all horses presented for evaluation of poor performance both because lameness is a common cause of poor performance and because it is important to establish that the horse is sound enough to safely perform an exercise test if indicated. In some horses presented for poor performance, the gait asymmetry may be subtle and only discernible at high speed, making diagnosis by traditional methods difficult. In these cases use of a sensor-based motion

analysis system, gait analysis on the treadmill, and advanced diagnostic techniques such as nuclear scintigraphy, thermography, and CT or MRI may be useful. In some cases it can be helpful to perform a ridden evaluation. Careful palpation of the muscles should be performed to identify any heat, swelling, or reactivity. In many cases a neurologic examination is indicated to identify any neurologic deficits that could contribute to poor performance.

Ancillary Diagnostic Aids

Clinical Pathology/Selected Laboratory Tests. Hematologic testing and a biochemical profile are indicated, although in most horses presented for poor performance without obvious clinical abnormalities, routine evaluation of a single sample is within normal limits. Because exercise can induce some changes in laboratory parameters, such as an increase in the packed cell volume and neutrophil count, considering the time of sample collection relative to exercise is important.[737-739] Potentially significant findings include changes consistent with chronic inflammation, such as anemia, hyperglobulinemia, and possibly hyperfibrinogenemia or thrombocytosis. Subclinical infections may have only slight alterations in the leukocyte count and differential. Viral infections, especially in the early stages, may be associated with a leukopenia and neutropenia. A decrease in the neutrophil-to-lymphocyte ratio has been associated with overtraining, although this is not a reliable correlation.[739] Changes in the hormonal response to exercise have been reported with overtraining, primarily a decrease in cortisol.[740]

Horses at rest normally maintain a significant proportion of red blood cells and hemoglobin in the splenic reserve.[737,738,741] Although total body hemoglobin increases in response to training and may correlate with performance, this cannot be determined from a resting sample. Special techniques must be used to document total red cell mass or hemoglobin.[741,792] Anemia can decrease the oxygen-carrying capacity during exercise, resulting in suboptimal performance.

Signs of organ dysfunction in horses presented for poor performance are not common findings but are occasionally present. Much attention has been paid to the importance of electrolytes and exercise; however, abnormalities seldom are found. In general, circulating electrolyte concentrations are regulated tightly and may not reflect closely the total body electrolyte status.[628] However, a concentration of potassium consistently below 3 mEq/L may suggest a potassium deficit. Performing renal fractional excretion of electrolytes can help in detecting chronic electrolyte deficiencies.

Myopathy can lead to decreased performance, and muscle enzymes may be elevated, although many cases of myopathy are subclinical and require evaluation of muscle enzymes after exercise.[700,713] Creatine kinase is measured before exercise and ideally 4 to 6 hours after an exercise bout consisting of 15 to 30 minutes at the trot. In normal horses this light exercise rarely causes more than a threefold increase in creatine kinase. An increase of fivefold or more indicates exertional rhabdomyolysis. Genetic testing, muscle biopsies, and measurement of vitamin E and selenium can also be helpful in defining myopathies.

Myocardial disease may contribute to left ventricular dysfunction and arrhythmias. Elevations in myocardial fractions of creatine kinase, lactate dehydrogenase, and troponin support myocardial disease but are not present in all cases.

Analysis of fluid from the lower airways is important in the diagnosis of lower airway disease. Although exact sampling techniques can vary, in most cases cytologic evaluation of bronchoalveolar lavage fluid is used to aid in the diagnosis of EIPH and forms of equine asthma such as IAD and RAO.

Endoscopy. Endoscopic examination of the upper airway is an important part of the evaluation of poor performance. As many of the abnormalities associated with poor performance are related to dynamic airway obstruction, either treadmill or overground videoendoscopy is generally indicated regardless of the history and physical examination findings.[300,719,743-745] Endoscopy also can be useful in identifying respiratory problems other than dynamic airway collapse. For example, the clinician can identify narrowing of the ventral nasal meatus associated with sinusitis, nasal masses, and pharyngitis. In some cases evidence of inflammation and retropharyngeal lymphadenopathy on endoscopic examination of the guttural pouches has been associated with dorsal displacement of the soft palate, which may result from neuropathy of the pharyngeal branch of the vagus nerve.[746]

Tracheal injury and secretions in the lower respiratory tract can be visualized if the endoscope is sufficiently long. Airway secretions may be sampled via the endoscope or via a bronchoalveolar lavage catheter. Endoscopy can also be useful in diagnosing equine gastric ulcer syndrome.

Diagnostic Imaging. Diagnostic imaging can be useful in the evaluation of horses with poor performance. Radiographs and ultrasound are routinely used in the evaluation of musculoskeletal problems. In some cases, additional imaging techniques may be indicated such as nuclear scintigraphy, thermography, CT, or MRI.

Radiographs and ultrasound can also be helpful in evaluation of the respiratory tract. Thoracic ultrasound and radiographs are often used in the evaluation of lower respiratory tract disease but may be normal in conditions commonly associated with poor performance such as IAD. Radiographs of the upper airways can allow for the evaluation of soft tissue masses or fluid accumulations. In addition, abnormalities of the pharyngeal and laryngeal structures such as thickening of the soft palate or hypoplasia of the epiglottis may be seen. Upper airway ultrasonography can also be helpful, particularly in the assessment of recurrent laryngeal neuropathy.[747] The denervated laryngeal muscles are hyperechoic and more homogeneous than normal muscle. Other conditions that can be diagnosed by ultrasound include arytenoid chondritis, laryngeal dysplasia, and congenital malformations of the larynx. CT and MRI have also been used to evaluate the upper airway.

Echocardiography can be used in addition to electrocardiography for further assessment of the heart.[698,748] In assessing cardiac function, it is ideal to measure indices such as percentage fractional shortening and wall motion indices, both before and after exercise. It should be taken into account that there is considerable normal variation in postexercise indices.[749]

Exercise Testing. Exercise testing provides a mechanism for evaluating a range of body systems under standard exercise conditions.[695,698,750,751] In particular, measurements of cardiorespiratory and metabolic function taken during an exercise test provide information about the capacity and efficiency of key body systems involved in energy production. From a clinical standpoint exercise testing is generally most useful in assessing the significance of abnormalities found on a physical examination; however, testing may also help to establish the reason for reduced athletic capacity in horses that have no abnormalities on basic examinations. Exercise testing can be

done in the field, which mimics the condition in which the horse actually performs. However, most testing is currently done on a treadmill, which provides more consistent conditions and an opportunity to perform a greater range of measurements. The specific protocol used for exercise testing may vary somewhat.[695,698,750-752] Occasionally a high-speed test is performed in which the horse is accelerated rapidly to maximal speed and run to fatigue. However, the most common type of test is an incremental test in which the speed increases every 1 to 2 minutes until the horse reaches fatigue, allowing for the generation of data during submaximal and maximal exercise. In most cases the test is performed with the treadmill at a slope of 10%. This slope is not so steep as to be completely unrepresentative of normal exercise, yet it ensures that maximum-intensity exercise can be performed without reaching speeds that may be too fast for horse safety. Some parameters that can be assessed in an exercise test include heart rate, blood lactate level, arterial blood gases, total red cell volume, stride length, and oxygen uptake. Various spirometers, which are masks for measuring pulmonary ventilation, can be used to measure parameters such as air flow rates, tidal volume, and the durations of phases of the respiratory cycle.[698] As previously discussed, treadmill videoendoscopy is often valuable.

Heart Rate during Exercise. Evaluation of the heart rate during exercise provides an indirect index of cardiovascular capacity and function. Several heart rate monitors are available.[753] Radiotelemetry also can be used to evaluate the heart rate and rhythm, particularly at the end of exercise. Because the stroke volume does not change greatly with increasing exercise speed, the heart rate provides a guide to changes in cardiac output. In general, a linear increase in heart rate occurs with increasing exercise speed up to the point at which the maximal heart rate is reached.[752,754-756] The maximal heart rate (HRmax) is identified when no further increase in heart rate occurs despite an increase in exercise speed. The HRmax does not change with training state, although the speed at which it is reached increases with increasing fitness.

One reference point for comparison of cardiovascular capacity is the treadmill speed at a heart rate of 200 beats per minute (V_{200}). At a heart rate of 200 beats per minute, most horses are close to the point of onset of blood lactate accumulation. The V_{200} can be calculated by linear regression analysis or plotted using measurements taken at three to four submaximal exercise speeds, without the horse reaching maximal exercise. The clinician should take care when using the V_{200} to assess exercise capacity because at a heart rate of 200 beats per minute, horses may be exercising at different proportions of their HRmax and therefore their maximal oxygen uptake (VO_{2max}). In general, however, horses with the highest cardiovascular and metabolic capacities have the highest V_{200} values; that is, the better horses reach a heart rate of 200 beats per minute at higher speeds than those with a lower exercise capacity. The V_{200} increases with training and can be useful for monitoring changes in fitness. The better quality Thoroughbreds have a V_{200} of 8 to 9 m/sec in an exercise test with the treadmill set at a 10% slope. Values less than 7 m/sec are abnormal and if found in a fit horse indicate decreased cardiac capacity.

Another measurement of cardiovascular capacity is the treadmill speed at which the horse reaches HRmax, known as V_{HRmax}. This value correlates with VO_{2max} and exercise capacity but requires the horse to exercise up to maximal speeds so that a plateau in heart rate can be identified.

Heart rate measurements are helpful in determining the actual significance of cardiac abnormalities such as murmurs and arrhythmias. In horses with functional cardiac disease, the reduced stroke volume necessitates higher heart rates to maintain adequate cardiac output. It should be taken into account that a high heart rate does not always indicate a cardiac problem. For example, studies in Standardbred racehorses have suggested that horses with musculoskeletal problems have an increased V_{200} and that monitoring the V_{200} may help to identify subclinical lameness.

Blood or Plasma Lactate Measurement. Exercising muscles produce lactate to some extent during all intensities of exercise, but production increases exponentially with the intensity of exercise.[756-758] As exercise becomes more intense, the aerobic energy contribution becomes insufficient to meet total energy requirements, and increased anaerobic metabolism results in increased lactate production. Lactate diffuses from muscle to blood, and therefore blood or plasma concentrations of lactate reflect muscle lactate. Some evidence suggests that whole blood concentrations most accurately measure lactate accumulation because red blood cells actively take up lactate.[758-761]

The rate of increase of lactate in the blood may be used as an indirect indicator of cardiovascular and metabolic capacity. Horses with the highest aerobic capacities because of a high maximal cardiac output tend to have lower lactate values at submaximal exercise intensities than those with lower aerobic capacities. Lactate values can be used to compare horses or to evaluate training in the same horse. The treadmill speed at which a plasma lactate of 4 mmol/L (V_{LA4}) is reached is one measure of lactate production, and a high value reflects good aerobic capacity. The V_{LA4} has been used to monitor changes in fitness. In fit Thoroughbred horses 3 years of age and older, values for V_{LA4} range from 8.0 to 9.5 m/sec. Horses that are not fit or that have respiratory disease have lower values. Another useful reference is the blood or plasma lactate at conclusion of the 10-m/sec exercise step of the incremental test, and highly fit, athletic horses usually have values below 5 mmol/L. High-quality sprint horses, which perform largely under anaerobic conditions and have a high anaerobic capacity, may have high peak lactate values.

Oxygen Uptake. The measurement of oxygen uptake (VO_2) is critical to assessing athletic performance.[754,755] The VO_{2max} has been used as a key indicator of exercise capacity in human athletes since the 1950s. As the VO_2 increases linearly with increasing treadmill speed, VO_{2max} can be identified when VO_2 reaches a plateau despite an increase in speed. The Thoroughbred horse has VO_{2max} values that are higher than those of many other mammalian species when expressed on a mass-specific basis. The major factor responsible for the high VO_{2max} in athletic horses is their high oxygen-carrying capacity, which arises from a high maximum stroke volume and to some extent a large arteriovenous oxygen content difference. The VO_{2max} is a good index of changes in fitness and a measurement of exercise capacity in performance horses.

Maximum Oxygen Pulse. The oxygen pulse is defined as the VO_2/heart rate and is expressed as mL/kg/beat. This value provides an indication of the maximum stroke volume, and in high-quality horses values range from 0.66 to 0.76 mL/kg/beat. Those horses with cardiac problems resulting in low cardiac outputs and individuals with low VO_{2max} values usually have values in the range of 0.5 to 0.56 mL/kg/beat. The maximum oxygen pulse also has been shown to correlate with treadmill total run time.

Arterial Blood Gas Analysis during Exercise. Arterial blood gas analysis during exercise may be indicated, especially in horses in which respiratory disorders are the suspected cause of poor performance. For an accurate blood gas analysis, the clinician should take into account the temperature of the blood because it may reach 42°C during maximal exercise. At exercise intensities above 65% VO_{2max}, athletic horses become hypoxemic, although the extent varies among individuals.[762-765] Horses with low VO_{2max} values do not necessarily have a significant decrease in arterial oxygen tension.

Additional Pulmonary Function Evaluation. Assessment of pulmonary ventilation can be facilitated by measurements of respiratory resistance, air flow rates, tidal volume, and the durations of phases of the respiratory cycle. Tests can be performed during and after exercise. This testing generally requires specialized equipment such as spirometers and ergospirometers, which may become more widely available for use.

Hematocrit and Total Red Cell Volume during Exercise. The total volume of red cells is a major determinant of oxygen-carrying capacity, and therefore measurement of red cell volume can give some index of exercise capacity. A postexercise packed cell volume test is not a reliable indicator of total red cell volume primarily because of plasma volume variations, but it does provide a rough estimate of total circulating red cells.

The clinician can make an accurate determination of red cell volume by techniques that use dye dilution after mobilization of the splenic erythrocyte pool to measure the plasma volume. Although total red cell volume increases with training, some evidence indicates that Standardbred racehorses with overtraining syndrome may develop an abnormal red cell hypervolemia that contributes to poor performance.[792]

Peak Running Speed and Total Run Time. The peak treadmill running speed and the total run time may indicate exercise capacity. In some studies of human athletes, the peak treadmill running speed during an exercise test was shown to be a predictor of performance. Athletic Thoroughbred racehorses can complete 60 seconds at 13 m/sec during an incremental exercise test at a 10% slope.

Stride Length. Athletic horses are thought to have better stride characteristics.[701,766] Some studies have shown a correlation between maximum stride length and the treadmill run time. An accelerometric device has been used to provide quantitative information about locomotor variables that may be useful in evaluating performance.[766]

It is important to remember that although exercise testing can be useful in the evaluation of poor performance, a diagnosis usually cannot be made based on a single measurement. An integrative approach taking into account the history, clinical findings, and all ancillary diagnostic testing is generally required.

REFERENCES

1. Klein BG. Thermoregulation. In: Klein BG, ed. *Cunningham's textbook of veterinary physiology*. 5th ed. St. Louis: Elsevier; 2012.
2. Koterba AM. Physical examination, neonatal infection. In: Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Lea and Febiger; 1990.
3. Dinarello CA. Thermoregulation and the pathogenesis of fever. *Infect Dis Clin North Am*. 1996;10:433.
4. Guyton AC, Hall JE. Body temperature, temperature regulation, and fever. In: Guyton AC, Hall JE, eds. *Textbook of medical physiology*. 11th ed. Philadelphia: Elsevier/Saunders; 2006.
5. Guthrie AJ, Lund RJ. Thermoregulation: base mechanisms and hyperthermia. *Vet Clin North Am Equine Pract*. 1998;14:45.
6. Marlin DJ, Schroter RC, White SL, et al. Recovery from transport and acclimatisation of competition horses in a hot humid environment. *Equine Vet J*. 2001;33:371.
7. Holcomb KE, Tucker CB, Stull CL. Preference of domestic horses for shade in a hot, sunny environment. *J Anim Sci*. 2014;92:1708.
8. Holcomb KE, Stull CL. Effect of time and weather on preference, frequency and duration of shade use by horses. *J Anim Sci*. 2016;94:1653.
9. Jorgensen GH, Aanensen L, Mejdell CM, et al. Preference for shelter and additional heat in horses exposed to Nordic winter conditions. *Equine Vet J*. 2015. http://dx.doi.org/10.1111/evj.12522.
10. Stachurska A, Robovsky J, Bocian K, et al. Changes of coat cover in primitive horses living on a reserve. *J Anim Sci*. 2015;93:1411.
11. Brinkmann L, Gerken M, Hambly C, et al. Saving energy during hard times: energetic adaptations of Shetland pony mares. *Exp Biol*. 2014;217(Pt 24):4320.
12. Geor RJ, McCutcheon LJ, Ecker GL, et al. Heat storage in horses during submaximal exercise before and after humid heat acclimation. *J Appl Physiol*. 2000;89:2283.
13. Geor RJ, McCutcheon LJ. Thermoregulatory adaptations associated with training and heat acclimation. *Vet Clin North Am Equine Pract*. 1998;14:97.
14. McKeever KH, Eaton TL, Geiser S, et al. Age related decreases in thermoregulation and cardiovascular function in horses. *Equine Vet J Suppl*. 2010;38:220.
15. Lindinger MI. Exercise in the heat: thermoregulatory limitations to performance in humans and horses. *Can J Appl Physiol*. 1999;24:152.
16. Mayhew IG, Ferguson HO. Clinical, clinicopathological, and epidemiological features of anhidrosis in central Florida Thoroughbred horses. *J Vet Intern Med*. 1987;1:136.
17. McEwan Jenkinson D, Elder HY, Bovell DL. Equine sweating and anhidrosis part 1—equine sweating. *Vet Dermatol*. 2006;17:361.
18. McEwan Jenkinson D, Elder HY, Bovell DL. Equine sweating and anhidrosis part 2: anhidrosis. *Vet Dermatol*. 2007;18:2.
19. Johnson EB, MacKay RJ, Hernandez JA. An epidemiologic study of anhidrosis in horses in Florida. *J Am Vet Med Assoc*. 2010;236:1091.
20. Fujii J, Otsu K, Zorzato F, et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*. 1991;253:448.
21. Manley SV, Kelly AB, Hodgson D. Malignant hyperthermia-like reactions in three anesthetized horses. *J Am Vet Med Assoc*. 1983;183:85.
22. Smyth GB. Spinal cord decompression and stabilization of a comminuted axis fracture complicated by intraoperative malignant hyperthermia-like reaction in a filly. *Aust Equine Vet*. 1992;10:133.
23. Aleman M, Nieto JE, Magdesian KG. Malignant hyperthermia associated with ryanodine receptor 1 (C7360G) mutation in Quarter Horses. *J Vet Intern Med*. 2009;23:329.
24. McCue ME, Valberg SJ, Jackson M, et al. Polysaccharide storage myopathy phenotype in quarter horse-related breeds is modified by the presence of an RYR1 mutation. *Neuromuscul Disord*. 2009;19:37.
25. Agrawal A, Timothy J, Thapa A. Neurogenic fever. *Singapore Med*. 2007;48:492.
26. Cuddy ML. The effects of drugs on thermoregulation. *AACN Clin Issues*. 2004;15:238.
27. Exon JH. A review of chlorinated phenols. *Vet Hum Toxicol*. 1984;26:508.
28. Stratton-Phelps M, Wilson WD, Gardner IA. Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases (1986–1996). *J Am Vet Med Assoc*. 2000;68:217.

29. Stieler AL, Sanchez LC, Mallicote MF, et al. Macrolide-induced hyperthermia in foals: role of impaired sweat responses. *Equine Vet J*. 2015. http://dx.doi.org/10.1111/evj.12481.
30. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res*. 2004;10:201.
31. Kozak W, Kluger MJ, Tesfaigzi J, et al. Molecular mechanisms of fever and endogenous antipyresis. *Ann N Y Acad Sci*. 2000;917:121.
32. Dinarello CA. Cytokines as endogenous pyrogens. *J Infect Dis*. 1999;179(suppl 2):S294.
33. Luheshi GN. Cytokines and fever: mechanisms and sites of action. *Ann N Y Acad Sci*. 1998;856:83.
34. Ivanov AI, Romanovsky AA. Prostaglandin E2 as a mediator of fever: synthesis and catabolism, Front Biosci. 1977;9:2004.
35. Simm B, Ott D, Pollatzek E, et al. Effects of prostaglandin E2 on cells cultured from the rat organum vasculosum laminae terminalis and median preoptic nucleus. *Neuroscience*. 2016;28:313.
36. Rusyniak DE, Zaretsky DV, Zaretskaia, et al. The role of Orexin-1 receptors in physiologic responses evoked by microinjection of PgE2 or muscimol into the medial preoptic area. *Neurosci Lett*. 2011;498:162.
37. Kozak W, Fraifeld V. Non-prostaglandin eicosanoids in fever and anapyrexia. *Front Biosci*. 2004;9:3339.
38. Roth J, Blatteis CM. Mechanisms of fever production and lysis: lessons learned from experimental LPS fever. *Compr Physiol*. 2014;4:1563.
39. Blatteis CM, Li S, Feleder C, et al. Cytokines, PGE2 and endotoxic fever: a re-assessment. *Prostglandins Other Lipid Mediat*. 2005;76:1.
40. Evans SS, Repasky EA, Fisher DT. Fever and the thermal regulation of immunity: the immune system feels the heat. *Nature Reviews Immunol*. 2015;15:335.
41. Still JT. Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile response of rabbits and rats. *J Physiol*. 1985;368:501.
42. Blatteis CM, Bealer SL, Hunter WS, et al. Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res Bull*. 1983;11:519.
43. Plaisacne KI, Mackowiak PA. Antipyretic therapy: physiologic rationale, diagnostic implications and clinical consequences. *Arch Intern Med*. 2000;160:449.
44. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med*. 2001;111:304.
45. Lazarus M. The differential role of prostaglandin E2 receptors EP3 and EP4 in regulation of fever. *Mol Nutr Food Res*. 2006;50:451.
46. Tatro JB. Endogenous antipyretics. *Clin Infect Dis*. 2000;5 (suppl):S190.
47. Roth J. Endogenous antipyretics. *Clinica Chimica Acta*. 2006;371:13.
48. Catania A, Lipton JM. Peptide modulation of fever and inflammation within the brain. *Ann N Y Acad Sci*. 1998;856:62.
49. Lipton JM, Catania A. Anti-inflammatory actions of the neuroimmunomodulator alpha-MSH. *Immunol Today*. 1997;18:140.
50. Steiner AA, Antunes-Rodrigues J, McCann SM, et al. Antipyretic role of the NP-cGMP pathway in the anteroventral preoptic region of the rat brain. *Am J Physiol Regul Integr Comp Physiol*. 2002;282:R584.
51. Morimota A, Sakata Y, Watanabe N, et al. Charactistics of fever and acute-phase response induced in rabbits by IL-1 and TNF. *Am J Physiol*. 1989;256:R35.
52. Soszynski D. The pathogenesis and adaptive value of fever. *Postepy Hig Med Dosw*. 2003;57:531.
53. Repasky EA, Evans SS, Dewhirst MW. Temperature matters! And why it should matter to tumor immunologists. *Cancer Immunol Res*. 2013;1:210.
54. Lee CT, Zhong L, Mace TA, et al. Elevation in body temperature to fever range enhances and prolongs subsequent responsiveness of macrophages to endotoxin challenge. *PLoS One*. 2012;7:e30077.
55. Jiang Q, Cross AS, Singh IS, et al. Febrile core temperature is essential for optimal host defense in bacterial peritonitis. *Infect Immun*. 2000;68:1265.
56. Weinstein MP, Iannini PB, Stratton CW, Eickhoff TC. Spontaneous bacterial peritonitis: a review of 28 cases with emphasis on improved survival and factors influencing prognosis. *Am J Med*. 1978;64:592.
57. Banet M. Fever and survival in the rat: the effect of enhancing fever. *Pflugers Arch*. 1979;381:35.
58. Launey Y, Nesseler N, Malledant, et al. Clinical review: fever in septic ICU patients—friend or foe? *Critical Care*. 2011;15:222.
59. Grieger TA, Kluger MJ. Fever and survival: the role of serum iron. *J Physiol*. 1978;279:187.
60. Kluger MJ, Rothenburg BA. Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science*. 1979;203:374.
61. Ballantyne GH. Rapid drop in serum iron concentrations as a host defense mechanism: a review of experimental and clinical evidence. *Am Surg*. 1984;50:405.
62. Mackowiak PA, Plaisacne KI. Benefits and risks of antipyretic therapy. *Ann N Y Acad Sci*. 1998;856:214.
63. Kluger MJ, Vaughn LK. Fever and survival in rabbits infected with *Pasteurella multocida*. *J Physiol*. 1978;282:243.
64. Laupland KB. Fever in the critically ill medical patient. *Crit Care Med*. 2009;37:S273.
65. Circiumara B, Baldock G, Cohen J. A prospective study of fever in the intensive care unit. *Intensive Care Med*. 1999;25:668.
66. Mair TS, Taylor FG, Pinsent PJ. Fever of unknown origin in the horse: a review of 63 cases. *Equine Vet J*. 1989;21:260.
67. MacKay RJ. Quantitative intradermal terbutaline sweat test in horses. *Equine Vet J*. 2008;40:518.
68. Sheoran AS, Sponseller BT, Holmes N, et al. Serum and mucosal antibody isotype responses to M-like protein (SeM) of *Streptococcus equi* in convalescent and vaccinated horses. *Vet Immunol Immunopathol*. 1997;59:239.
69. Leon LR. Hypothermia in systemic inflammation: role of cytokines. *Front Biosci*. 1877;9:2004.
70. Irvine CH. Hypothyroidism in the foal. *Equine Vet J*. 1984;16:302.
71. Murray MJ. Hypothyroidism and respiratory insufficiency in a neonatal foal. *J Am Vet Med Assoc*. 1990;197:1635.
72. Stephen JO, Baptiste KE, Townsend HG. Clinical and pathologic findings in donkeys with hypothermia: 10 cases (1988–1998). *J Am Vet Med Assoc*. 2000;216(725).
73. Guyton AC, Hall JE. Dietary balances; regulation of feeding; obesity and starvation; vitamins and minerals. In: Guyton AC, Hall JE, eds. *Textbook of medical physiology*. 11th ed. Philadelphia: Elsevier/Saunders; 2006.
74. Kronfeld DS. Starvation and malnutrition of horses: recognition and treatment. *J Equine Vet Sci*. 1993;13:298.
75. Robin CA, Ireland JL, Wylie CE, et al. Prevalence of and risk factors for equine obesity in Great Britain based on owner-reported body condition scores. *Equine Vet J*. 2015;47:196.
76. Giles SL, Rands SA, Nicol CJ, et al. Obesity prevalence and associated risk factors in outdoor living domestic horses and ponies. *PeerJ*. 2014;2:e299.
77. Ireland JL, Wylie CE, Collins SN, et al. Preventive health care and owner-reported disease prevalence of horses and ponies in Great Britain. *Res Vet Sci*. 2013;95:418.
78. Thatcher CD, Pleasant RS, Geor RJ, et al. Prevalence of overconditioning in mature horses in southwest Virginia during the summer. *J Vet Intern Med*. 2012;26:1413.
79. Johnson PJ. The equine metabolic syndrome peripheral Cushing's syndrome. *Vet Clin North Am Equine Pract*. 2002;18:271.
80. Johnson PJ, Wiedmeyer CE, LaCarrubba A, et al. Diabetes, insulin resistance and metabolic syndrome in horses. *J Diabetes Sci Technol*. 2012;6:534.
81. Frank N, Geor RJ, Bailey SR, et al. Equine metabolic syndrome. *J Vet Intern Med*. 2010;24:467.

82. Martinson KL, Coleman RC, Rendahl AK, et al. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. *J Anim Sci*. 2014;92:2230.
83. Carter RA, Geor RJ, Burton Staniar W, et al. Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. *Vet J*. 2009;179:204.
84. Hallebeek JM. The body condition score in horses. *Tijdschr Diergeneeskd*. 2014;139:30.
85. Dugdale AH, Grove-White D, Curtis GC, et al. Body condition scoring as a predictor of body fat in horses and ponies. *Vet J*. 2012;194.
86. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15:371.
87. Mottet R, Onan G, Hiney K. Revisiting the Henneke body condition scoring system: 25 years later. *J Equine Vet Sci*. 1983;29:417.
88. Carter RA, Treiber KH, Geor RJ, et al. Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalized and localized obesity in a cohort of ponies. *Equine Vet J*. 2009;41:171.
89. Giles SL, Nicol CJ, Rands SA, et al. Assessing the seasonal prevalence and risk factors for nuchal crest adiposity in domestic horses and ponies using the cresty neck score. *BMC Vet Res*. 2015;11:13.
90. Kearns CF, McKeever KH, Kumagai K, et al. Fat-free mass is related to one-mile race performance in elite Standardbred horses. *Vet J*. 2002;163:260.
91. Wyse CA, McNie KA, Tannahill VJ, et al: Prevalence of obesity in riding horses in Scotland. 162:590, 2008.
92. Thatcher CD, Pleasant RS, Geor RJ, et al. Prevalence of obesity in mature horses: an equine body condition study. *J Anim Physiol Anim Nutr*. 2008;92:222.
93. Stephenson HM, Green MJ, Freeman SL. Prevalence of obesity in a population of horses in the UK. *Vet Rec*. 2011;168:131.
94. Johnson PJ, Wiedmeyer CE, Messer NT, et al. Medical implications of obesity in horses—lessons for human obesity. *J Diabetes Sci Technol*. 2009;3:163.
95. Ertelt A, Barton AK, Schmitz RR, et al. Metabolic syndrome: is equine disease comparable to what we know in humans? *Endocr Connect*. 2014;3:R81.
96. de Graaf-Roelfsema E. Glucose homeostasis and the enteroinsular axis in the horse: a possible role in equine metabolic syndrome. *Vet J*. 2014;199:11.
97. Frank N, Tadros EM. Insulin dysregulation. *Equine Vet J*. 2014;46:103.
98. Bamford NJ, Potter SJ, Harris PA, et al. Breed differences in insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of moderate body condition score. *Domest Anim Endocrinol*. 2014;47:101.
99. Fischer-Posovszky P, Wabitsch M, Hochberg Z. Endocrinology of adipose tissue—an update. *Horm Metab Res*. 2007;39:314.
100. Radin MJ, Sharkey LC, Holycross BJ. Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. *Vet Clin Pathol*. 2009;38:136.
101. Bamford NJ, Potter SJ, Harris PA, et al. Effect of increased adiposity on insulin sensitivity and adipokine concentrations in horses and ponies fed a high fat diet, with or without a once daily high glycaemic meal. *Equine Vet J*. 2015. doi: 10:111/evj.12434.
102. Kearns CF, McKeever KH, Roegner V, et al. Adiponectin and leptin are related to fat mass in horses. *Vet J*. 2006;172:460.
103. Pleasant RS, Suagee JK, Thatcher CD, et al. Adiposity, plasma insulin, leptin, lipids, and oxidative stress in mature light breed horses. *J Vet Intern Med*. 2013;27:576.
104. Suagee JK, Corl BA, Crisman MV, et al. Relationships between body condition score and plasma inflammatory cytokines, insulin, and lipids in a mixed population of light-breed horses. *J Vet Intern Med*. 2013;27:157.
105. Suagee JK, Corl BA, Geor RJ. A potential role for pro-inflammatory cytokines in the development of insulin resistance in horses. *Animals (Basel)*. 2012;2:243.
106. Holbrook TC, Tipton T, McFarlane D. Neutrophil and cytokine dysregulation in hyperinsulinemic obese horses. *Vet Immunol Immunopathol*. 2012;145:283.
107. Tadros EM, Frank N, Donnell RL. Effects of equine metabolic syndrome on inflammatory responses of horses to intravenous lipopolysaccharide infusion. *Am J Vet Res*. 2013;74:1010.
108. Farr OM, Li CS, Mantzoros CS. Central nervous system regulation of eating: insight from human brain imaging. *Metabolism*. 2016;65:699.
109. Morrison PK, Bing C, Harris PA, et al. Preliminary investigation into a potential role for myostatin and its receptor (ActRIIB) in lean and obese horses and ponies. *PLos One*. 2014;9:e112621.
110. Raoult D. Microbiota, obesity and malnutrition. *Microb Pathog*. 2016. doi: 10.1016.
111. Shepherd ML, Ponder MA, Burk AO, et al. Fibre digestibility, abundance of faecal bacteria and plasma acetate concentrations in overweight adult mares. *J Nutr Sci*. 2014;3:e10.
112. Patterson E, Ryan PM, Cryan JF, et al. Gut microbiota, obesity and diabetes. *Postgrad Med J*. 2015. http://dx.doi.org/10.1136/ppostgrad medj.
113. Treiber KH, Kronfeld DS, Hess TM, et al. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J Am Vet Med Assoc*. 2006;228:1538.
114. Treiber KH, Kronfeld DS, Hess TM, et al. Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. *Am J Vet Res*. 2005;66:2114.
115. Schott HC. Pituitary pars intermedia dysfunction: challenges of diagnosis and treatment. *Proc Am Assoc Equine Pract*. 2006;52:60.
116. Beech J, Boston R, Lindborg S. Comparison of cortisol and ACTH responses after administration of thyrotropin releasing hormone in normal horses and those with pituitary pars intermedia dysfunction. *J Vet Intern Med*. 2011;25:1431.
117. Giles SL, Nicol CJ, Harris PA, et al. Dominance rank is associated with body condition in outdoor-living domestic horses *(Equus caballus)*. *Appl Anim Behav Sci*. 2015;166:71.
118. Kiela PR, Ghishan FK. Physiology of intestinal absorption and secretion. *Best Pract Res Clin Gastroenterol*. 2016;30:145.
119. Roberts MC. Malabsorption syndromes in the horse. *Compend Cont Educ Pract Vet*. 1985;7:S637.
120. Sloet van Oldruitenborgh-Oosterbaan MM. Lactose intolerance in foals. *Equine Vet Educ*. 2008;20:252.
121. Laviano A, Inui A, Marks DL, et al. Neural control of the anorexia-cachexia syndrome. *Am J Physiol Endocrinol Metab*. 2008;295:E1000–E1008.
122. Ramos EJ, Suzuki S, Marks D, et al. Cancer anorexia-cachexia syndrome: cytokines and neuropeptides. *Curr Opin Clin Nutr Metab Care*. 2004;7:427.
123. Cymbaluk NE, Christison GI. Environmental effects on thermoregulation and nutrition of horses. *Vet Clin North Am Equine Pract*. 1990;6:355.
124. Morgan K. Thermoneutral zone and critical temperatures of horses. *J Thermal Biol*. 1998;23:59.
125. Ott EA. Influence of temperature stress on the energy and protein metabolism and requirements of the working horse. *Livestock Proc Sci*. 2005;92:123.
126. Plank LD, Hill GL. Energy balance in critical illness. *Proc Nutr Soc*. 2003;62:545.
127. Jose-Cunilleras E, Viu J, Corradini I, et al. Energy expenditure of critically ill neonatal foals. *Equine Vet J Suppl*. 2012;41:48.
128. Evans WJ, Morley JE, Argiles J, et al. Cachexia: a new definition. *Clin Nutr*. 2008;27:793.
129. Fearon K, Strasser F, Anker SD, et al. Definition and classification of cancer cachexia. *The Lancet*. 2011;12:489.

130. Anker SD, Morleoy JE. Cachexia: a nutritional syndrome? *J Cachexia Sarcopenia Muscle*. 2015;6:269.
131. Kir S, Komaba H, Garcia AP, et al. PTH/PTHrP receptor mediates cachexia in models of kidney failure and cancer. *Cell Metab*. 2015. http://dx.doi.org/10.1016/jcmet.
132. Burfeind KG, Michaelis KA, Marks DL. The central role of hypothalamic inflammation in the acute illness response and cachexia. *Semin Cell Dev Biol*. 2015. doi: 10:1016/jsemcbd.
133. Yoshida T, Delafontaine P. Mechanisms of cachexia in chronic disease states. *Am J Med Sci*. 2015;350:250.
134. Reid MB, Yi-Ping L. Tumor necrosis factor-α and muscle wasting: a cellular perspective. *Respir Rec*. 2001;2:269.
135. Ramirez S, McClure JJ, Moore RM, et al. Hyperthyroidism associated with a thyroid adenocarcinoma in a 21-year-old gelding. *J Vet Intern Med*. 1998;12:475.
136. Frank N, Sojka J, Messer NT. Equine thyroid dysfunction. *Vet Clin North Am Equine Pract*. 2002;18:305.
137. Rygiel KA, Piccard M, Turnbull DM. The ageing neuromuscular system and sarcopenia—a mitochondrial perspective. *J Physiol*. 2016. doi: 10.1113.
138. Lehnhard KA, McKeever KH, Kearns CF, et al. Myosin heavy chain profiles and body composition are different in old versus young Standardbred mares. *Vet J*. 2004;167:59.
139. Kim J, Hinchcliff KW, Yamaguchi M, et al. Age-related changes in metabolic properties of equine skeletal muscle associated with muscle plasticity. *Vet J*. 2005;169:397.
140. Aleman M, Watson JK, Williams DC, et al. Myopathy in horses with pituitary pars intermedia dysfunction (Cushing's disease). *Neuromusc Disord*. 2006;16:737.
141. Metcalfe LVA, More SJ, Duggan V, et al. A retrospective study of horses investigated for weight loss despite a good appetite (2002–2011). *Equine Vet J*. 2013;45:340.
142. Schumacher J, Edwards JF, Cohen ND. Chronic inflammatory bowel diseases of the horse. *J Vet Intern Med*. 2000;14:258.
143. Traub JL, Bayly WM, Reed SM, et al. Intra-abdominal neoplasia as a cause of chronic weight loss in the horse. *Compend Cont Educ Pract Vet*. 1983;5:S526.
144. Rumbaugh GE, Smith BP, Carlson GP. Internal abdominal abscesses in the horse: a study of 25 cases. *J Am Vet Med Assoc*. 1978;172:304.
145. Foreman JH, Weidner JP, Parry BA, et al. Pleural effusion secondary to thoracic metastatic mammary adenocarcinoma in a mare. *J Am Vet Med Assoc*. 1990;197:1193.
146. McGorum BC, Murphy D, Love S, et al. Clinicopathological features of equine primary hepatic disease: a review of 50 cases. *Vet Rec*. 1999;145:134.
147. Moore BR, Abood AS, Hinchcliff KW. Hyperlipemia in 9 miniature horses and miniature donkeys. *J Vet Intern Med*. 1994;8:376.
148. West HJ. Clinical and pathological studies in horses with hepatic disease. *Equine Vet J*. 1996;28:146.
149. McLeland S. Diseases of the equine urinary system. *Vet Clin North Am Equine Pract*. 2015;31:377.
150. Tan RH, Davies SE, Crisman MV, et al. Propylthiouracil for treatment of hyperthyroidism in a horse. *J Vet Intern Med*. 2008;22:1253.
151. Alberts MK, McCann JP, Woods PR. Hemithyroidectomy in a horse with confirmed hyperthyroidism. *J Am Vet Med Assoc*. 2000;217:1051.
152. Johnson PJ, Scotty NC, Wiedmeyer C, et al. Diabetes mellitus in a domesticated Spanish Mustang. *J Am Vet Med Assoc*. 2005;226:584.
153. Clabough DL. Equine infectious anemia: the clinical signs, transmission, and diagnostic procedures. *Vet Med*. 1990;85:1007.
154. Divers TJ, Mohammed HO, Hintz HF, et al. Equine motor neuron disease: a review of clinical and experimental studies. *Clin Techniques Equine Pract*. 2006;5:24.
155. Lewis SS, Valberg SJ, Nielsen IL. Suspected immune-mediated myositis in horses. *J Vet Intern Med*. 2007;21:495.
156. Meredith TB, Dobrinski I. Thyroid function and pregnancy status in broodmares. *J Am Vet Med Assoc*. 2004;224:892.
157. Breuhaus BA. Thyroid-stimulating hormone in adult euthyroid and hypothyroid horses. *J Vet Intern Med*. 2002;16:109.
158. Morris DD, Garcia M. Thyroid-stimulating hormone response test in healthy horses, and effect of phenylbutazone on equine thyroid hormones. *Am J Vet Res*. 1983;44:503.
159. Jacobs KA, Bolton JR. Effect of diet on the oral D-xylose absorption test in the horse. *Am J Vet Res*. 1856;43:1982.
160. Mair TS, Hillyer MH, Taylor FG, et al. Small intestinal malabsorption in the horse: an assessment of the specificity of the oral glucose tolerance test. *Equine Vet J*. 1991;23:334.
161. Perrin C, Unterborn JN, Ambrosio CD, Hill NS. Pulmonary complications of chronic neuromuscular diseases and their management. *Muscle Nerve*. 2004;29:5.
162. Schramm CM. Current concepts of respiratory complications of neuromuscular disease in children. *Curr Opin Pediatr*. 2000;12:203.
163. Niimi A, Matsumoto H, Ueda T, et al. Impaired cough reflex in patients with recurrent pneumonia. *Thorax*. 2003;58:152.
164. Irwin RS, Curley FJ, French CL. Chronic cough. The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy. *Am Rev Respir Dis*. 1990;141:640.
165. Irwin RS, Baumann MH, Bolser DC, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest*. 2006;129:1S.
166. Schappert SM, Burt CW. Ambulatory care visits to physicians' offices, hospital outpatient departments, and emergency departments: United States, 2001–02. *Vital Health Stat*. 2006;13:1.
167. Canning BJ. The cough reflex in animals: relevance to human cough research. *Lung*. 2008;186:S23.
168. Chang AB, Widdicombe JG. Cough throughout life: children, adults and the senile. *Pulm Pharmacol Ther*. 2007;20:371.
169. Chang AB. Pediatric cough: children are not miniature adults. *Lung*. 2010;1:S33.
170. Fujjimura M, Sakamoto S, Kamio Y, et al. Female gender as a determinant of cough threshold to inhaled capsaicin. *Eur Resp J*. 1996;9:1624.
171. Kavlcikova-Bogdanova N, Buday T, Plevkova J, Song WJ. Chronic cough as a female gender issue. *Adv Exp Med Biol*. 2016;905:69.
172. Morice AH. Chronic cough hypersensitivity syndrome. *Cough*. 2013;9:14.
173. Chang AB. The physiology of cough. *Paed Resp Rev*. 2006;7:2.
174. McCool FD. Global physiology and pathophysiology of cough. *Chest*. 2006;129:48S.
175. Robinson NE. Pathophysiology of coughing. *Proceedings of the thirty-second convention of the American Association of Equine Practitioners*. Nashville. 1986;291.
176. Canning BJ, Chang AB, Bolser DC, et al. Anatomy and neurophysiology of cough: CHEST guideline and expert panel report. *Chest*. 2014;146:1633.
177. Canning BJ. Encoding of the cough reflex. *Pulm Pharmacol Ther*. 2007;20:396.
178. Polverino M, Polverino F, Fasolino, et al. Anatomy and neuropathophysiology of the cough reflex arc. *Multidisciplinary Resp Med*. 2012;7:5.
179. Canning BJ. Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines. *Chest*. 2006;129:33S.
180. Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol*. 2006;152:223.
181. Mazzone SB. An overview of the sensory receptors regulating cough. *Cough*. 2005;1:2.
182. Bloustine S, Langston L, Miller T. Ear-cough (Arnold's) reflex. *Ann Otol Rhinol Laryngol*. 1976;85:496.
183. Zholos AV. TRP channels in respiratory pathophysiology: the role of oxidative, chemical irritant and temperature stimuli. *Curr Neuropharmacol*. 2015;13:279.

184. Geppetti P, Patacchini R, Nassini R, et al. Cough: the emerging role of the TRPA1 channel. *Lung*. 2010;1:S63.
185. Taylor-Clark TE. Role of reactive oxygen species and TRP channels in the cough reflex. *Cell Calcium*. 2016. doi.org/10.1016.
186. Bonvini SJ, Birrell MA, Smith JA, Belvisi MG. Targeting TRP channels for chronic cough: from bench to bedside. *Naunyn Schmiedebergs Arch Pharmacol*. 2015;388:401.
187. Grace MS, Dubuis E, Birrell MA, Belvisi MG. TRP channel antagonists as potential antitussives. *Lung*. 2012;190:11.
188. Lee LY, Kwong K, Lin YS, et al. Hypersensitivity of bronchopulmonary C-fibers induced by airway mucosal inflammation: cellular mechanisms. *Pulm Pharmacol Ther*. 2002;15:199.
189. Canning BJ. Interactions between vagal afferent nerve subtypes mediating cough. *Pulm Pharmacol Ther*. 2002;15:187.
190. Karlsson JA, Sant'Ambrogio G, Widdicombe J. Afferent neuronal pathways in cough and reflex bronchoconstriction. *J Appl Physiol*. 1988;65:1007.
191. Pounsford J. Cough and bronchoconstriction. *Bull Eur Physiopathol Respir*. 1987;23(S10):37s.
192. Pantaleo T, Bongianni F, Mutolo D. Central nervous mechanisms of cough. *Pulm Pharmacol Ther*. 2002;15:227.
193. Haji A, Kimura S, Ohi Y. A model of the central regulatory system for cough reflex. *Biol Pharm Bull*. 2013;36:501.
194. Shannon R, Baekey DM, Morris KF, et al. Production of reflex cough by brainstem respiratory networks. *Pulm Pharmacol Ther*. 2004;17:369.
195. Canning BJ. Central regulation of the cough reflex: therapeutic implications. *Pulm Pharmacol Ther*. 2009;22:75.
196. Allain H, Bentue-Ferrer D, Daval G, et al. Mechanisms of chronic cough pathophysiology. *Rev Mal Respir*. 2004;21:763.
197. Foster WM. Mucociliary transport and cough in humans. *Pulm Pharmacol Ther*. 2002;15:277.
198. Rubin BK. Secretion properties, clearance and therapy in airway disease. *Transl Respir Med*. 2014;2:6.
199. Irwin RS. Complications of cough: Evidence-based clinical practice guidelines. *Chest*. 2006;129:55S.
200. Johansson AM, Gardner SY, Atkins CE, et al. Cardiovascular effects of acute pulmonary obstruction in horses with recurrent airway obstruction. *J Vet Intern Med*. 2007;21:302.
201. Dicpinigaitis PV, Lim L, Farmakidis C. Cough syncope. *Respir Med*. 2014;108:244.
202. Nelson RW, Couto CG, eds. *Essentials of small animal internal medicine*. 2nd ed. St. Louis: Mosby; 1992.
203. Clayton H, Murphy J. The coughing horse. *In Practice*. 1980;2:25.
204. Gilkerson JR, Bailey KE, Diaz-Mendez A, Hartley CA. Update on viral diseases of the equine respiratory tract. *Vet Clin North Am Equine Pract*. 2015;31:91.
205. Ma G, Azab W, Osterrieder N. Equine herpes viruses type 1 (EHV-1) and 4 (EHV-4)—masters of co-evolution and a constant threat to equids and beyond. *Vet Microbiol*. 2013;167:123.
206. Reuss SM, Giguere S. Update on bacterial pneumonia and pleuropneumonia in the adult horse. *Vet Clin North Am Equine Pract*. 2015;31:105.
207. Reuss SM, Cohen ND. Update on bacterial pneumonia in the foal and weanling. *Vet Clin North Am Equine Pract*. 2015;31:121.
208. Ogilvie TH, Rosendal S, Blackwell TE, et al. *Mycoplasma felis* as a cause of pleuritis in horses. *J Am Vet Med Assoc*. 1983;192:1374.
209. Stewart AJ, Cuming RS. Update on fungal respiratory disease in horses. *Vet Clin North Am Equine Pract*. 2015;31:43.
210. Cafarchia C, Figueredo LA, Otranto D. Fungal diseases of horses. *Vet Microbiol*. 2013;167:215.
211. Wilkins PA, Lascola KM. Update on interstitial pneumonia. *Vet Clin North Am Equine Pract*. 2015;31:137.
212. Williams KJ, Maes R, Del Piero F, et al. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. *Vet Pathol*. 2007;44:849.
213. Scarrett WK, Crisman MV. Neoplasia of the respiratory tract. *Vet Clin North Am Equine Pract*. 1998;14:451.
214. Marsh PS. Fire and smoke inhalation injury in horses. *Vet Clin North Am Equine Pract*. 2007;23:19.
215. Couetil LL, Hoffman AM, Hodgson J, et al. Inflammatory airway disease of horses. *J Am Vet Intern Med*. 2007;21:356.
216. Couetil LL, Cardwell JM, Gerber V, et al. Inflammatory airway disease of horses—revised consensus statement. *J Vet Intern Med*. 2016;30:503.
217. Mazan MR. Update on noninfectious inflammatory diseases of the lower airway. *Vet Clin North Am Equine Pract*. 2015;31:159.
218. Pirie RS. Recurrent airway obstruction: a review. *Equine Vet J*. 2014;46:276.
219. Ireland JL, Christley RM, McGowan CM, et al. Prevalence of and risk factors for recurrent airway obstruction in geriatric horses and ponies. *Equine Vet J*. 2015;47:25.
220. Hotchkiss JW, Reid SW, Christley RM. A survey of horse owners in Great Britain regarding horses in their care. Part 2: risk factors for recurrent airway obstruction. *Equine Vet J*. 2007;39:301.
221. Gerber V, Swinburne JE, Blott SE, et al. Genetics of recurrent airway obstruction (RAO). *Dtsch Tierarztl Wochenschr*. 2008;115:271.
222. Gerber V, Tessier C, Marti E. Genetics of upper and lower airway diseases in the horse. *Equine Vet J*. 2015;47:390.
223. Schwartz LW, Knight HD, Whittig LD, et al. Silicate pneumoconiosis and pulmonary fibrosis in horses from the Monterey-Carmel peninsula. *Chest*. 1981;80:82.
224. Lyons ET, Tolliver SC, Drudge JH, et al. Lungworms *(Dictyocaulus arnfeldi):* prevalence in live equids in Kentucky. *Am J Vet Res*. 1985;46:921.
225. Goetz TE. *Dictyocaulus arnfeldi* as a possible cause of chronic cough in 14 horses. *Equine Pract*. 1984;6:33.
226. Bell SA, Drew CP, Wilson WD, Pusterla N. Idiopathic chronic eosinophilic pneumonia in 7 horses. *J Vet Intern Med*. 2008;22:648.
227. Hinchcliff KW, Couetil LL, Knight PK, et al. Exercise induced pulmonary hemorrhage in horses: American College of Veterinary Internal Medicine consensus statement. *J Am Vet Intern Med*. 2015;29:743.
228. Leguillette R, Steinmann M, Bond SL, Stanton B. Tracheobronchoscopic assessment of exercise-induced pulmonary hemorrhage and airway inflammation in barrel racing horses. *J Vet Intern Med.* doi:10.1111/jvim. 2016;13959.
229. Davis JL, Gardner SY, Schwabenton B, et al. Congestive heart failure in horses: 14 cases (1984–2001). *J Am Vet Med Assoc*. 2002;220:1512.
230. Leroux AA, Detilleux J, Sandersen CF, et al. Prevalence and risk factors for cardiac diseases in a hospital-based population of 3,434 horses (1994–2011). *J Vet Intern Med*. 2013;27:1563.
231. Auer DE, Wilson RG, Groenendyk S. Pharyngeal lymphoid hyperplasia in Thoroughbred racehorses in training. *Aust Vet J*. 1985;62:124.
232. Christley RM, Hodgson DR, Rose RJ, et al. Coughing in Thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. *Vet Rec*. 2001;148:99.
233. Aleman M, Nieto JE, Benak J, et al: Tracheal collapse in American Miniature Horses: 13 cases (1985–2007). 2008;233:1302.
234. Tetens J, Hubert JD, Eddy AL, et al. Dynamic tracheal collapse as a cause of exercise intolerance in a Thoroughbred. *J Am Vet Med Assoc*. 2000;216:722.
235. Fenger CK, Kohn CW. Tracheal obstruction from tracheal collapse associated with pneumonia in a horse. *J Am Vet Med Assoc*. 1992;200:1698.
236. Bakos Z, Voros K, Kellokoski H, Reiczigel J. Comparison of the caudal lung borders determined by percussion and ultrasonography in horses with recurrent airway obstruction. *Acta Vet Hung*. 2003;51:249.
237. Kusano K, Hobo S, Ode H, Ishikawa Y. Tracheal endoscopic and cytologic findings and blood examination results in Thoroughbred racehorses suspected to have lower respiratory tract disease. *J Equine Sci*. 2008;19:97.

238. Richard EA, Pitel PH, Christmann U, et al. Serum concentration of surfactant protein D in horses with lower airway inflammation. *Equine Vet J*. 2012;44:277.
239. Wagner B, Ainsworth DM, Freer H. Analysis of soluble CD14 and its use as a biomarker in neonatal foals with septicemia and horses with recurrent airway obstruction. *Vet Immunol Immunopathol*. 2013;155:124.
240. Hoffman AM, Viel L. Techniques for sampling the respiratory tract of horses. *Vet Clin North Am Equine Pract*. 1997;13:463.
241. Hoffman AM. Bronchoalveolar lavage: sampling technique and guidelines for cytologic preparation and interpretation. *Vet Clin North Am Equine Pract*. 2008;24:423.
242. Wysocka B, Klusinski W. Cytological evaluation of tracheal aspirate and broncho-alveolar lavage fluid in comparison to endoscopic assessment of lower airways in horses with recurrent airways obstruction or inflammatory airway disease. *Pol J Vet Sci*. 2015;18:587.
243. Sweeney CR, Sweeney RW, Benson CE. Comparison of bacteria isolated from specimens obtained by use of endoscopic guarded tracheal swabbing and percutaneous tracheal aspiration in horses. *J Am Vet Med Assoc*. 1989;195:1225.
244. Moore BR, Dradowka S, Robertson JT, et al. Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. *Am J Vet Res*. 1995;56:562.
245. Rossier Y, Sweeney CR, Ziemer EL. Bronchoalveolar lavage fluid cytologic findings in horses with pneumonia or pleuropneumonia. *J Am Vet Med Assoc*. 1991;198:1001.
246. Hewson J, Arroyo LG. Respiratory disease: diagnostic approaches in the horse. *Vet Clin North Am Equine Pract*. 2015; 31:307.
247. Gerber V, Straub R, Marti E, et al. Endoscopic scoring of mucus quantity and quality: observer and horse variance and relationship to inflammation, mucus viscoelasticity and volume. *Equine Vet J*. 2004;36:576.
248. Koblinger K, Nicol J, McDonald K, et al. Endoscopic assessment of airway inflammation in horses. *J Vet Intern Med*. 2011;25:1118.
249. Rettmer H, Hoffman AM, Lanz S, et al. Owner-reported coughing and nasal discharge are associated with clinical findings, arterial oxygen tension, mucus score and bronchoprovocation in horses with recurrent airway obstruction in a field setting. *Equine Vet J*. 2015;47:291.
250. Holcombe SJ, Robinson NE, Derksen FJ, et al. Effect of tracheal mucus and tracheal cytology on racing performance in Thoroughbred racehorses. *Equine Vet J*. 2006;38:300.
251. Widmer A, Doherr MG, Tessier C, et al. Association of increased tracheal mucus accumulation with poor willingness to perform in show-jumpers and dressage horses. *Vet J*. 2009;182:430.
252. Bedenice D, Mazan MR, Hoffman AM. Association between cough and cytology of bronchoalveolar lavage fluid and pulmonary function in horses diagnosed with inflammatory airway disease. *J Vet Intern Med*. 2008;22:1022.
253. Bullone M, Helie P, Joubert P, Lavoie JP. Development of a semiquantitative histological score for the diagnosis of heaves using endobronchial biopsy specimens of horses. *J Vet Intern Med*. 2016;14556. http://dx.doi.org/10.111/jvim.
254. Venner M, Schmidbauer S, Drommer W, Deegen E. Percutaneous lung biopsy in the horse: comparsion of two instruments and repeated biopsy in horses with induced acute interstitial pneumopathy. *J Vet Intern Med*. 2006;20:968.
255. Ainsworth DM, Davidow E. Respiratory distress in large animals. *Proc Forum ACVIM*. 1994;12:589.
256. West JB. *Respiratory physiology: the essentials*. 9th ed. Baltimore: Lippincott, Williams & Wilkins; 2012.
257. Wilson WD, Lakritz J. Alterations in respiratory function. In: Smith BP, ed. *Large animal internal medicine*. 5th ed. St. Louis: Elsevier Mosby; 2015.
258. Mazan MR, Deveney EF, DeWitt S, et al. Energetic cost of breathing, body composition, and pulmonary function in horses with recurrent airway obstruction. *J Appl Physiol*. 2004;97:91.
259. Beech J, ed. *Equine respiratory disorders*. Philadelphia: Lea & Febiger; 1991.
260. Garcia AJ, Zanella S, Koch H, et al. Chapter 3—networks within networks: the neuronal control of breathing. *Prog Brain Res*. 2011;188:31.
261. Dempsey JA, Smith CA. Pathophysiology of human ventilatory control. *Eur Respir J*. 2014;44:495.
262. McCrimmon DR, Ramirez JM, Alford S, Zuperku EJ. Unraveling the mechanism for respiratory rhythm generation. *Bioessays*. 2000;22:6.
263. Widdicombe J. Reflexes from the lungs and airways: historical perspective. *J Appl Physiol*. 2006;102:268.
264. Widdicombe J. Functional morphology and physiology of pulmonary rapidly reacting receptors (RARs). *Anat Rec A Discov Mol Cell Evol Biol*. 2003;270:2.
265. Ainsworth DM, Ducharme NG, Hackett RP. Regulation of equine respiratory muscles during acute hypoxia and hypercapnia. *Am Rev Respir Dis*. 1993;147:A700.
266. Muir WW, Moore CA, Hamlin RL. Ventilatory alterations in normal horses in response to changes in inspired oxygen and carbon dioxide. *Am J Vet Res*. 1975;36:155.
267. Derksen FJ, Robinson NE, Slocombe RF. Ovalbumin induced allergic lung disease in the pony: role of vagal mechanisms. *J Appl Physiol*. 1982;53:719.
268. Derksen F, Robinson N, Slocombe R. 3-Methylindole-induced pulmonary toxicosis in ponies. *Am J Vet Res*. 1982;43:603.
269. Derksen FJ, Robinson NE, Stick JA. Technique for reversible vagal blockade in the standing conscious pony. *Am J Vet Res*. 1981;42:523.
270. McGorum BC. Quantification of histamine in plasma and pulmonary fluids from horses with chronic obstructive pulmonary disease, before and after "natural" (hay and straw) challenges. *Vet Immunol Immunopathol*. 1993;36:223.
271. Watson E, Sweeney C, Steensma K. Arachidonate metabolites in bronchoalveolar lavage fluid from horses with and without COPD. *Equine Vet J*. 1992;24:379.
272. Grunig G, Hermann M, Winder C, et al. Procoagulant activity in respiratory tract secretions from horses with chronic pulmonary disease. *Am J Vet Res*. 1988;49:705.
273. Sant'Ambrogio G, Mathew OP, Fisher JT. Laryngeal receptors responding to transmural pressure, airflow, and local muscle activity. *J Appl Physiol*. 1983;65:317.
274. Sant'Ambrogio G, Mathew OP. Laryngeal receptors and their reflex responses. *Clin Chest Med*. 1986;7:211.
275. Feldman JL. Chapter 14—Looking forward to breathing. *Prog Brain Res*. 2011;188:213.
276. Silva JN, Tanabe FM, Moreira TS, Takura AC. Neuroanatomical and physiological evidence that the retrotrapezoid nucleus/parafacial region regulates expiration in adult rats. *Respir Physiol Neurobiol*. 2016;227:9.
277. Koterba AM, Kosch PC, Beech J. The breathing strategy of the adult horse *(Equus caballus)* at rest. *J Appl Physiol*. 1988;64:337.
278. Lessa TB, de Abreu DK, Bertasoli BM, Ambrosio CE. Diaphragm: a vital respiratory muscle in mammals. *Ann Anat*. 2016;205:122.
279. Nijkampf FP. β-adrenergic receptors in the lung: an introduction. *Life Sci*. 1993;52:2073.
280. Proskocil BJ, Fryer AD. Beta2-agonist and anticholinergic drugs in the treatment of lung disease. *Proc Am Thorac Soc*. 2005;3:395.
281. Bai TR. Beta 2 adrenergic receptors in asthma: a current perspective. *Lung*. 1992;170:125.
282. Couetil L, Hammer J, Miscovis Feutz M, et al. Effects of N-butylscopolammonium bromide on lung function in horses with recurrent airway obstruction. *J Vet Intern Med*. 2012;26:1433.

283. De Lagarde M, Rodrigues N, Chevigny M, et al. N-butylscopolammonium bromide causes fewer side effects than atropine when assessing bronchoconstriction reversibility in horses with heaves. *Eq Vet J*. 2014;46:474.
284. Derksen FJ, Scott JS, Slocombe RF, et al. Effect of clenbuterol on histamine-induced airway obstruction in ponies. *Am J Vet Res*. 1987;48:423.
285. Scott J, Broadstone R, Derksen F, et al. Beta-adrenergic blockade in ponies with recurrent obstructive pulmonary disease. *J Appl Physiol*. 1988;64:2324.
286. Scott JS, Berney CE, Derksen FJ, Robinson NE. Beta-adrenergic receptor activity in ponies with recurrent obstructive pulmonary disease. *Am J Vet Res*. 1991;52:1416.
287. Scott JS, Garon HE, Broadstone RV, et al. Alpha 1 adrenergic induced airway obstruction in ponies with recurrent pulmonary disease. *J Appl Physiol*. 1988;65:686.
288. van der Veldenr VH, Hulsmann AR. Autonomic innervation of human airways: structure, function, and pathophysiology in asthma. *Neuroimmunomodulation*. 1999;6:145.
289. Andersson RG, Grundstrom N. Innervation of airway smooth muscle. Efferent mechanisms. *Pharmacol Ther*. 1987;32:107.
290. Robinson NE, Derksen FJ, Olszewski MA, et al. The pathogenesis of chronic obstructive pulmonary disease of horses. *Br Vet J*. 1996;152:283.
291. Macklem PT, Mead J. Resistance of central and peripheral airways measured by retrograde catheter. *J Appl Physiol*. 1967;22:395.
292. Buergelt CD, Hines SA, Cantor G, et al. A retrospective study of proliferative interstitial lung disease of horses in Florida. *Vet Pathol*. 1986;23:750.
293. Lakritz J, Wilson WD, Berry CR, et al. Bronchointerstitial pneumonia and respiratory distress in young horses: clinical, clinicopathologic, radiographic, and pathological findings in 23 cases. *J Vet Intern Med*. 1984–1989;7(277):1993.
294. Wilkins PA. Lower respiratory problems of the neonate. *Vet Clin North Am Equine Pract*. 2003;19:19.
295. Berry CR, O'Brien TR, Madigan JE, et al. Thoracic radiographic features of silicosis in 19 horses. *J Vet Intern Med*. 1991;5:248.
296. Marques FJ, Hehenberger E, Dickinson R, et al. Respiratory distress due to retropharyngeal and neck swelling in a horse with mediastinal lymphosarcoma. *Compend Contin Educ Vet*. 2012;34:E5.
297. Singh K, Holbrook TC, Gilliam LL, et al. Severe pulmonary disease due to multisystemic eosinophilic epitheliotropic disease in a horse. *Vet Pathol*. 2006;43:189.
298. Brakenhoff JE, Holcombe SJ, Hauptman JG, et al. The prevalence of laryngeal disease in a large population of competition draft horses. *Vet Surg*. 2006;35:579.
299. Dixon PM, McGorum BC, Railton DI, et al. Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses. *Equine Vet J*. 2001;33:452.
300. Davidson EJ, Martin BB, Boston RC, et al. Exercising upper respiratory videoendoscopic evaluation of 100 nonracing performance horses with abnormal respiratory noise and/or poor performance. *Equine Vet J*. 2011;43:3.
301. Franklin SH, Naylor JR, Lane JG. Effect of dorsal displacement of the soft palate on ventilation and airflow during high-intensity exercise. *Equine Vet J Suppl*. 2002;34:379.
302. Naylor JM, Nickel DD, Trimino G, et al. Hyperkalemic periodic paralysis in homozygous and heterozygous horses: a co-dominant genetic condition. *Equine Vet J*. 1999;31:153.
303. Carr EA, Spier SJ, Kortz GD, Hoffman EP. Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. *J Am Vet Med Assoc*. 1996;209:798.
304. Hughes KJ, McGorum BC, Love S, Dixon PM. Bilateral laryngeal paralysis associated with hepatic dysfunction and hepatic encephalopathy in six ponies and four horses. *Vet Rec*. 2009;164:142.
305. Norman TE, Chaffin MK, Bisset WT, Thompson JA. Association of clinical signs with endoscopic findings in horses with nasopharyngeal cicatrix syndrome: 118 cases (2003–2008). *J Am Vet Med Assoc*. 2012;240:734.
306. James FM, Parente EJ, Palmer JE. Management of bilateral choanal atresia in a foal. *J Am Vet Med Assoc*. 2006;229:1784.
307. Botha CJ, Lewis A, du Plessis EC, et al. Crotalariosis equorum ("jaagsiekte") in horses in southern Mozambique, a rare form of pyrrolizidine alkaloid poisoning. *J Vet Diagn Invest*. 2012;24:1099.
308. Johnstone LK, Engiles JB, Aceto H, et al. Retrospective evaluation of horses diagnosed with neuroborreliosis on postmortem examination: 16 cases (2004–2015). *J Vet Intern Med*. 2016;30:1350.
309. Reimer JM. Diagnostic ultrasonography of the equine thorax. *Compend Cont Educ Pract Vet*. 1990;12:1321.
310. Carlson GP. Blood chemistry, body fluids, and hematology. In: Gillespie JR, Robinson NE, eds. *Equine exercise physiology*. 2nd ed. Davis, CA: ICEEP Publications; 1987.
311. Guyton AC, Hall JE. The microcirculation and the lymphatic system: capillary fluid exchange, interstitial fluid and lymph flow. The body fluid compartments: extracellular and intracellular fluids; interstitial fluid and edema. In: Guyton AC, Hall JE, eds. *Textbook of medical physiology*. 11th ed. Philadelphia: Elsevier/Saunders; 2006.
312. Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis revisited. *Am J Physiol*. 1986;250:H706.
313. Staub NC, Taylor AE, eds. *Edema*. New York: Raven Press; 1984.
314. Demling RH. Effect of plasma and interstitial protein content on tissue edema formation. *Curr Stud Hematol Blood Transfus*. 1986;53:36.
315. Taylor AE. Capillary fluid filtration: Starling forces and lymph flow. *Circ Res*. 1981;49:557.
316. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res*. 2010;87:198.
317. Collins SR, Blank RS, Deatherage LS, et al. The endothelial glycocalyx: emerging concepts in pulmonary edema and acute lung injury. *Anesth Analg*. 2013;117:664.
318. Dejana E, Orsenigo F, Lampugnani MG. The role of adherens junctions and VE-cadherin in the control of vascular permeability. *J Cell Sci*. 2008;121:2115.
319. Frigeri A, Nicchia GP, Svelto M. Aquaporins as targets for drug discovery. *Curr Pharm Des*. 2007;13:2421.
320. Renkin EM, Michel CC, eds. *Handbook of physiology*. New York: Oxford University Press; 1984.
321. Raj JU, Anderson J. Regional differences in interstitial fluid albumin concentration in edematous lamb lungs. *J Appl Physiol*. 1992;72:699.
322. Costanzo L, ed. *Physiology*. 3rd ed. St. Louis: Saunders; 2006.
323. Michel CC. Microvascular permeability, venous stasis and oedema. *Inter Angiol*. 1984;8:9–13.
324. Green JF. *Fundamental cardiovascular and pulmonary physiology*. Philadelphia: Lea & Febiger; 1987.
325. Claesson-Welsh L. Vascular permeability—the essentials. *Ups J Med Sci*. 2015;120:135.
326. Golden MH. Nutritional and other types of oedema, albumin, complex carbohydrates and the interstitium—a response to Malcom Coulthard's hypothesis: oedema in kwashiorkor is caused by hypoalbuminemia. *Paediatr Int Child Health*. 2015;35:90.
327. Trani M, Dejana E. New insights in the control of vascular permeability: vascular endothelial-cadherin and other players. *Curr Opin Hematol*. 2015;22:267.
328. Vestweber D. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arterioscler Thromb Vasc Biol*. 2008;28:223.
329. Verkman AS. Mammalian aquaporins: diverse physiological roles and potential clinical significance. *Expert Rev Mol Med*. 2008;10:e13.

330. Stokum JA, Kurland DB, Gerzanich V, et al. Mechanisms of astrocyte-mediated cerebral edema. *Neurochem Res*. 2015;40:317.
331. Stokum JA, Gerzanich V, Simard JM. Molecular pathophysiology of cerebral edema. *J Cereb Blood Flow Metab*. 2016;36:513.
332. Hsu Y, Tran M, Linninger AA. Dynamic regulation of aquaporin-4 water channels in neurological disorders. *Croat Med J*. 2015;56:401.
333. Sun Z, Li X, Massena S, et al. VEGFR2 induces c-Src signaling and vascular permeability in vivo via the adaptor protein TSAd. *J Exp Med*. 2012;209:1363.
334. Eliceiri BP, Paul R, Schwartzberg PL, et al. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol Cell*. 1999;4:915.
335. Page AE, Slovis NM, Horohov DW. *Lawsonia intracellularis* and equine proliferative enteropathy. *Vet Clin North Am Equine Pract*. 2014;30:641.
336. Galvin N, Dillon H, McGovern F. Right dorsal colitis in the horse: minireview and reports on three cases in Ireland. *Ir Vet J*. 2004;57:467.
337. Parraga ME, Carlson GP, Thurmon M. Serum protein concentrations in horses with severe liver disease: a retrospective study and review of the literature. *J Vet Intern Med*. 1995;9:154.
338. Demling RH. The burn edema process: current concepts. *J Burn Care Rehabil*. 2005;26:207.
339. Morris DD. Cutaneous vasculitis in horses: 19 cases (1978–1985). *J Am Vet Med Assoc*. 1987;191:460.
340. White SD, Affolter VK, Dewey J, et al. Cutaneous vasculitis in equines: a retrospective study of 72 cases. *Vet Dermatol*. 2009;20:600.
341. Balasuriya UB. Equine viral arteritis. *Vet Clin North Am Equine Pract*. 2014;30:543.
342. Cook RF, Leroux C, Issel CJ. Equine infectious anemia and equine infectious anemia virus in 2013: a review. *Vet Microbiol*. 2013;29:167.
343. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118.
344. Kilcoyne I, Spier SJ, Carter CN, et al. Frequency of *Corynebacterium pseudotuberculosis* infection in horses across the United States during a 10-year period. *J Am Vet Med Assoc*. 2014;245:309.
345. Affolter VK. Chronic progressive lymphedema in draft horses. *Vet Clin North Am Equine Pract*. 2013;29:589.
346. Lewis N, Racklyeft DJ. Mass envenomation of a mare and foal by bees. *Aust Vet J*. 2014;92:141.
347. Fielding CL, Pusterla N, Magdesian KG, et al. Rattlesnake envenomation in horses: 58 cases (1992–2009). *J Am Vet Med Assoc*. 2011;238:631.
348. Lyle CH, Turley G, Blissitt KJ, et al. Retrospective evaluation of episodic collapse in the horse in a referred population: 25 cases (1995–2009). *J Vet Intern Med*. 2010;24:1498.
349. Guyton AC, Hall JE. *Textbook of medical physiology*. 11th ed. Philadelphia: Elsevier; 2006.
350. Gauer RL. Evaluation of syncope. *Am Fam Physician*. 2011;84:640.
351. Hilz MJ, Marthol H, Neundorfer B. Syncope—a systematic overview of classification, pathogenesis, diagnosis and management. *Fortschr Neurol Psychiatr*. 2002;70:95.
352. Lanier JB, Mote MB, Clay EC. Evaluation and management of orthostatic hypotension. *Am Fam Physician*. 2011;84:527.
353. Benditt DG. Neurally mediated syncopal syndromes: pathophysiological concepts and clinical evaluation. *Pacing Clin Electrophysiol*. 1997;20:572.
354. Gatzoulis KA, Toutouzas PK. Neurocardiogenic syncope: aetiology and management. *Drugs*. 2001;61:1415.
355. Mayhew J. *Large animal neurology*. 2nd ed. Sussex, UK: Wiley Blackwell; 2009.
356. Sabu J, Regeti K, Mallappallil M, et al. Convulsive syncope induced by ventricular arrhythmia masquerading as epileptic seizures: case report and review of the literature. *J Clin Med Res*. 2016;8:610.
357. Kanjwal K, Karabin B, Kanjwal Y, et al. Differentiation of convulsive syncope from epilepsy with an implantable loop recorder. *Int J Med Sci*. 2009;6:296.
358. Jefferys JGR. Advances in understanding basic mechanisms of epilepsy and seizures. *Seizure*. 2010;19:638.
359. McNamara JO. Cellular and molecular basis of epilepsy. *J Neurosci*. 1994;14:3413.
360. Staley K. Molecular mechanisms of epilepsy. *Nature*. 2015;18:367.
361. Reddy DS. Role of hormones and neurosteroids in epileptogenesis. *Front Cell Neurosci*. 2013;7:115.
362. Lucke-Wold BP, Nguyen L, Turner RC, et al. Traumatic brain injury and epilepsy: underlying mechanisms leading to seizure. *Seizure*. 2015;33:13.
363. Engel J. A proposal diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on classification and terminology, ILAE Commission Report. *Epilepsia*. 2001;42:796.
364. Lacombe VA, Mayes M, Mosseri S, et al. Distribution and predictive factors of seizure types in 104 cases. *Equine Vet J*. 2013;46:441.
365. Lacombe VA, Mayes M, Mosseri S, et al. Epilepsy in horses: aetiological classification and predictive factors. *Equine Vet J*. 2012;44:646.
366. Zepelin H. Mammalian sleep. In: Kryger MH, Roth T, Dement WC, eds. *Principles and practice of sleep medicine*. 3rd ed. Philadelphia: WB Saunders Co; 2000.
367. Ruckebusch Y. The relevance of drowsiness in the circadian cycle of farm animals. *Anim Behav*. 1972;20:637.
368. Williams DC, Aleman M, Holliday TA, et al. Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *J Vet Intern Med*. 2008;22:630.
369. Hendricks JC, Morrison AR. Normal and abnormal sleep in mammals. *J Am Vet Med Assoc*. 1981;178:121.
370. Bertone JJ. Excessive drowsiness secondary to recumbent sleep deprivation in two horses. *Vet Clin North Am Equine Pract*. 2006;22:157.
371. Mignot EJ, Dement WC. Narcolepsy in animals and man. *Equine Vet*. 1993;25:476.
372. Scammell TE. Narcolepsy. *N Engl J Med*. 2015;373:2654.
373. Ripley B, Fujiki N, Okura M, et al. Hypocretin levels in sporadic and familial cases of canine narcolepsy. *Neurobiol Dis*. 2001;8:525.
374. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999;98:365.
375. Lunn DP, Cuddon PA, Shaftoe S, et al. Familial occurrence of narcolepsy in miniature horses. *Equine Vet J*. 1993;25:483.
376. Ludvikova E, Nishino S, Sakai N, et al. Familial narcolepsy in the Lipizzaner horse: a report of three fillies born to the same sire. *Vet Q*. 2012;32:99.
377. Bathen-Nothen A, Heider C, Fernandez AJ, et al. Hypocretin measurement in an Icelandic foal with narcolepsy. *J Vet Intern Med*. 2009;23:1299.
378. van Nieuwstadt RA, van der Want CJ, Binkhorst GJ. Narcolepsy in horses. *Tijdschr Diergeneeskd*. 1993;118:765.
379. Dreifuss FE, Flynn DV. Narcolepsy in a horse. *J Am Vet Med Assoc*. 1984;184:131.
380. Sweeney CR, Hendricks JC, Beech J, et al. Narcolepsy in a horse. *J Am Vet Med Assoc*. 1983;183:126.
381. Faull KF, Guillemainault C, Berger PA, et al. Cerebrospinal fluid monoamine metabolites in narcolepsy and hypersomnia. *Ann Neurol*. 1983;13:258.
382. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol*. 2002;59:1553.
383. Viola-Saltzman M, Watson MD. Traumatic brain injury and sleep disorders. *Neurol Clin*. 2012;30:1299.

384. Baumann CR, Bassetti CL, Scammell TE. Loss of hypocretin (orexin) neurons with traumatic brain injury. *Ann Neurol*. 2010;66:555.
385. Jesty SA, Reef VB. Evaluation of the horse with acute cardiac crisis. *Clin Tech Equine Pract*. 2006;5:93.
386. Reef VB, Clark ES, Oliver JA, et al. Implantation of a permanent transvenous pacing catheter in a horse with a complete heart block and syncope. *J Am Vet Med Assoc*. 1986;189:449.
387. Hay WP, Baskett A, Abdy MJ. Complete upper airway obstruction and syncope caused by a subepiglottic cyst in a horse. *Equine Vet J*. 1997;29:75.
388. Steiger R, Feige K. Case report: polycythemia in a horse. *Schweiz Arch Tierheilkd*. 1995;137:306.
389. Bryant UK, Lyons ET, Bain FT, et al. *Halicephalobus gingivalis*-associated meningoencephalitis in a Thoroughbred foal. *J Vet Diagn Invest*. 2006;18:612.
390. Hardefeldt LY. Hyponatremic encephalopathy in azotaemic neonatal foals: four cases. *Aust Vet J*. 2014;92:488.
391. Viu J, Monreal L, Jose-Cunilleras E, et al. Clinical findings in 10 foals with bacterial meningoencephalitis. *Equine Vet J Suppl*. 2012;41:100.
392. Wong D, Wilkins PA, Bain FT, et al. Neonatal encephalopathy in foals. *Compend Contin Educ Vet*. 2011;33:E5.
393. Aleman M, Gray LC, Williams DC, et al. Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985–2005). *J Vet Intern Med*. 2006;20:1443.
394. Berendnt M, Gredal H, Pedersen LG, et al. A cross-sectional study in Danish Labrador Retrievers: prevalence and selected risk factors. *J Vet Intern Med*. 2002;16:262.
395. Cornelisse CJ, Schott HC, Olivier NB, et al. Concentration of cardiac troponin I in horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. *J Am Vet Med Assoc*. 2000;217:231.
396. Nath LC, Anderson GA, Hinchcliff KW, et al. Serum cardiac troponin I concentrations in horses with cardiac disease. *Aust Vet J*. 2012;90:351.
397. Wijnberg ID, van der Ree M, van Someren P. The applicability of ambulatory electroencephalography (AEEG) in healthy horses and horses with abnormal behavior or clinical signs of epilepsy. *Vet Q*. 2013;33:121.
398. van der Ree M, Wijnberg I. A review on epilepsy in the horse and the potential of ambulatory EEG as a diagnostic tool, 332:159, 2012.
399. Manso-Diaz G, Dyson SJ, Dennis R, et al. Magnetic resonance imaging characteristics of equine head disorders: 84 cases (2000–2013). *Vet Radiol Ultrasound*. 2015;56:176.
400. Sogaro-Robinson C, Lacombe VA, Reed SM, et al. Factors predictive of abnormal results for computed tomography of the head in horses affected by neurologic disorders. *J Am Vet Med Assoc*. 2009;235:176.
401. Goyal RK, Mashimo H. Physiology of oral, pharyngeal, and esophageal motility. *GI Motility online*. 2006. http://dx.doi.org/10.1038/gimo1.
402. Shaw S, Martino R. The normal swallow: muscular and neurophysiological control. *Otolaryngol Clin N Am*. 2013;46:937.
403. Steele CM, Miller AJ. Sensory input pathways and mechanisms in swallowing: a review. *Dysphagia*. 2010;25:323.
404. Aleman M. Dysphagia of neurogenic origin. In: Robinson NE, Sprayberry KA, eds. *Current therapy in equine medicine 6*. St. Louis: Saunders/Elsevier; 2009.
405. Baum KH, Modransky PD, Halpern NE, et al. Dysphagia in horses: the differential diagnosis, part I. *Compend Cont Educ Pract Vet*. 1988;10:1301.
406. Baum GH, Halpern NE, Banish LD, et al. Dysphagia in horses: the differential diagnosis, part II. *Compend Cont Educ Pract Vet*. 1988;10:1405.
407. Stick JA, Boles C. Subepiglottic cyst in three foals. *J Am Vet Med Assoc*. 1980;177:62.
408. Nolen-Walston RD, Parente EJ, Madigan JE, et al. Branchial remnant cysts of mature and juvenile horses. *Equine Vet J*. 2009;41:918.
409. Dixon PM, Dacre I. A review of equine dental disorders. *Vet J*. 2005;169:165.
410. Staszyk C, Bienert A, Kreutzer R. Equine odontoclastic tooth resorption and hypercementosis. *Vet J*. 2008;178:372.
411. Vengust M, Baird JD, van Dreumel T, et al. Equid herpesvirus 2-associated oral and esophageal ulceration in a foal. *J Vet Diag Invest*. 2008;20:811.
412. Baker GJ. Equine temporomandibular joints (TMJ): morphology, function, and clinical disease. *Proc Am Assoc Equine Pract*. 2002;48:442.
413. Pusterla N, Latson KM, Wilson WD, et al. Metallic foreign bodies in the tongues of 16 horses. *Vet Rec*. 2006;159:485.
414. Kilcoyne I, Watson JL, Spier SJ, et al. Septic sialoadenitis in equids: a retrospective study of 18 cases (1998–2010). *Equine Vet J*. 2010;47:54.
415. Bezdekova B. Esophageal disorders in horses—a review of the literature. *Pferdeheilkunde*. 2012;28:187.
416. Broekamn LE, Kuiper D. Megaesophagus in the horse. A short review of the literature and 18 own cases. *Vet Q*. 2002;24:199.
417. Booth TM, Marmion WJ, Cullimore AM, et al. Esophageal obstruction in an aged pony associated with squamous cell carcinoma. *Equine Vet Educ*. 2008;20:627.
418. Brazil TJ. Recurrent esophageal obstruction caused by gastroesophageal squamous cell carcinoma: a diagnostic challenge? *Equine Vet Educ*. 2008;20:633.
419. Green S, Green EM, Aronson E. Squamous cell carcinoma: an unusual case of esophageal choke in a horse. *Mod Vet Pract*. 1986;65:870.
420. Benders NA, Velduis Kroez EJ, van der Kolk JH. Idiopathic muscular hypertrophy of the esophagus in the horse: a retrospective study of 31 cases. *Equine Vet J*. 2004;36:46.
421. Todhunter RJ, Brown CM, Stickle R. Retropharyngeal infections in five horses. *J Vet Med Assoc*. 1985;187:600.
422. Sweeney CR, Timoney JF, Newton JR, et al. *Streptococcus equi* infections in horses: guidelines for treatment, control and prevention of strangles. *J Vet Intern Med*. 2005;19:123.
423. McCue PM, Freeman DE, Donawick WJ. Guttural pouch tympany: 15 cases (1977–1986). *J Am Vet Med Assoc*. 1989;12:1761.
424. Greet TRC. Outcome of treatment in 35 cases of guttural pouch mycosis. *Equine Vet J*. 1987;19:483.
425. Tremaine WH: Oral cavity neoplasia, ivis.org. AAEP Focus Meeting, Indianapolis, 2006.
426. Casey M. A new understanding of the oral and dental pathology of the equine cheek teeth. *Vet Clin North Am Equine Pract*. 2013;29:301.
427. Laus F, Rossi G, Paggi E, et al. Adenocarcinoma involving the tongue and epiglottis in a horse. *J Vet Med Sci*. 2014;76:467.
428. Anlen KG. Effects of bites by the European adder *(Vipera berus)* in seven Swedish horses. *Vet Rec*. 2008;162:652.
429. Sweeny CR, Freeman DE, Sweeny RW, et al. Hemorrhage into the guttural pouch (auditory tube diverticulum) associated with rupture of the longus capitis muscle in three horses. *J Am Vet Med Assoc*. 1993;202:1129.
430. Dobesova O, Schwarz B, Velde K, et al. Guttural pouch mycosis in horses: a retrospective study of 28 cases. *Vet Rec*. 2012;171:561.
431. Bell C. Pharyngeal neuromuscular dysfunction associated with bilateral guttural pouch tympany in a foal. *Can Vet J*. 2007;48:192.
432. Koch C, Witte T. Temporohyoid osteoarthropathy in the horse. *Equine Vet Ed*. 2014;26:121.
433. Walker AM, Sellon DC, Cornelisse CJ, et al. Temporohyoid osteoarthropathy in 33 horses. *J Vet Intern Med*. 2002;16:697.
434. Burrows GE, Borchard RE. Experimental lead toxicosis in ponies: comparison of the effects of smelter effluent-contaminated hay and lead acetate. *Am J Vet Res*. 1982;43:2129.

435. Yvorchuk-St. Jean K. Neuritis of the cauda equina. *Vet Clin North Am Equine Pract*. 1987;3:421–426.
436. Hahn CN. Polyneuritis equi: the role of T-lymphocytes and importance of differential clinical signs. *Equine Vet J*. 2008;40:100.
437. Long MT. West Nile virus and equine encephalitis viruses: new perspectives. *Vet Clin North Am Equine Pract*. 2014;30:523.
438. Pusterla N, Hussey GS. Equine herpesvirus 1 myeloencephalopathy. *Vet Clin North Am Equine Pract*. 2014;30:489.
439. Kohn CW, Fenner WR. Equine herpes myeloencephalopathy. *Vet Clin North Am Equine Pract*. 1987;3:405.
440. Howe DK, MacKay RJ, Reed SM. Equine protozoal myeloencephalitis. *Vet Clin North Am Equine Pract*. 2014;30:659.
441. Uhlinger C. Clinical and epidemiologic features of an epizootic of equine leukoencephalomalacia. *J Am Vet Med Assoc*. 1991;198:126.
442. van Galen G, Delguste C, Sandersen C, et al. Tetanus in the equine species: a retrospective study of 31 cases. *Tijdschr Diergeneeskd*. 2008;133:512.
443. Holcombe SJ, Hurcombe SD, Barr BS, et al. Dysphagia associated with presumed pharyngeal dysfunction in 16 neonatal foals. *Equine Vet J Suppl*. 2012;41:105.
444. Swerczek TW. Toxicoinfectious botulism in foals and adult horses. *J Am Vet Med Assoc*. 1980;176:217.
445. Wilkins PA, Palmer JE. Botulism in foals less than 6 months of age: 30 cases (1989–2002). *J Vet Intern Med*. 2003;17:702.
446. Doxey DL, Milne EM, Gilmour JS, et al. Clinical and biochemical features of grass sickness (equine dysautonomia). *Equine Vet J*. 1991;23:360.
447. Wylei CE, Proudman CJ. Equine grass sickness: epidemiology, diagnosis, and global distribution. *Vet Clin North Am Equine Pract*. 2009;25:381.
448. McGorum BC, Scholes S, Milne EM, et al. Equine grass sickness, but not botulism, causes autonomic and enteric neurodegeneration and increases soluble N-ethylmaleimide-sensitive factor attachment receptor protein expression within neuronal perikarya. *Equine Vet J*. 2016. http://dx.doi.org/10.1111/evj.13543.
449. Moore RM, Kohn CW. Nutritional muscular dystrophy in foals. *Compend Cont Educ Pract Vet*. 1991;13:476.
450. Step DL, Divers TJ, Cooper B, et al. Severe masseter myonecrosis in a horse. *J Am Vet Med Assoc*. 1991;198:117.
451. Pearson EG, Snyder SP, Saulez MN. Masseter myodegeneration as a cause of trismus or dysphagia in adult horses. *Vet Rec*. 2005;156:642.
452. Komine M, Langohr IM, Kiupel M. Megaesophagus in Friesian horses associated with muscular hypertrophy of the caudal esophagus. *Vet Pathol*. 2014;51:979.
453. Sockett DC, Baker JC, Stowe CM. Slaframine *(Rhizoctonia leguminicola)* intoxication in horses. *J Am Vet Med Assoc*. 1982;181:606.
454. Divers TJ, Mohammed HO, Cummings JR, et al. Equine lower motor neuron disease: findings in 28 horses and proposal of a pathophysiological mechanism. *Equine Vet J*. 1994;26:409.
455. USDA-APHIS: Incidence of colic in U.S. horses. Available at http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/equine/equine98/colic.PDF. Accessed Mar 4, 2009.
456. Tinker MK, White NA, Lessard P, et al. Prospective study of equine colic incidence and mortality. *Equine Vet J*. 1997;29:448.
457. Traub-Dargatz JL, Kopral CA, Seitzinger AH, et al. Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, spring 1998 to spring 1999. *J Am Vet Med Assoc*. 2001;219:67.
458. Uhlinger C. Investigations into the incidence of field colic. *Equine Vet J*. 1992;13:11.
459. USDA-APHIS: Trends in equine mortality, 1998–2005. Available at http://www.aphis.usda.gov/animal_health/nahms/equine/download/equine05/Equine05_ is_Mortality.PDF
460. Salem SE, Scantlebury CE, Ezzat E, et al. Colic in a working horse population in Egypt: prevalence and risk factors. *Equine Vet J*. 2016. http://dx.doi.org/10.1111/wvj.12573.
461. Orsini JA, Divers TJ, eds. *Equine emergencies: treatment and procedures*. St. Louis: Saunders; 2008.
462. Mair T, Divers T, Ducharme N, eds. *Manual of equine gastroenterology*. St. Louis: Saunders; 2002.
463. White NA. II: Intestinal response to injury. *Proc Ann Conv AAEP*. 2006;52:115.
464. Snyder JR. The pathophysiology of intestinal damage: effects of luminal distention and ischemia. *Vet Clin North Am Equine Pract*. 1989;5:247.
465. Kaya G, Sommerfeld-Stur I, Iben C. Risk factors of colic in horses in Austria. *J Anim Physiol Anim Nutr (Berl)*. 2009;93:339.
466. Cohen ND, Gibbs PG, Woods AM. Dietary and other management factors associated with colic in horses. *J Am Vet Med Assoc*. 1999;215:53.
467. Tinker MK, White NA, Lessard P. Prospective study of equine colic risk factors. *Equine Vet J*. 1997;29:454.
468. Hudson JM, Cohen ND, Gibbs PG, et al. Feeding practices associated with colic in horses. *J Am Vet Med Assoc*. 2001;219:1419.
469. Archer DC, Proudman CJ. Epidemiological clues to preventing colic. *Vet J*. 2006;172:29.
470. Scantlebury CE, Archer DC, Proudman CJ, et al. Management and horse-level risk factors for recurrent colic in the UK general equine practice population. *Equine Vet J*. 2015;47:202.
471. Suthers JM, Pinchbeck GL, Proudman CJ, et al. Risk factors for large colon volvulus in the UK. *Equine Vet J*. 2013;45:558.
472. Scantlebury CE, Archer DC, Proudman CJ, et al. Recurrent colic in the horse: incidence and risk factors for recurrence in the general practice population. *Equine Vet J Suppl*. 2011;39:81.
473. White NA. II: Causes and risks for colic. *Proc Ann Conv AAEP*. 2006;52:115.
474. Malamed R, Berger J, Bain MJ, et al. Retrospective evaluation of crib-biting and windsucking behaviours and owner-perceived behavioural traits as risk factors for colic in horses. *Equine Vet J*. 2010;42:686.
475. Escalona EE, Okell CN, Archer DC. Prevalence of and risk factors for colic in horses that display crib-biting behavior. *BMC Vet Res*. 2014;1:S3.
476. Archer DC, Pinchbeck GK, French NP, et al. Risk factors for epiploic foramen entrapment colic: an international study. *Equine Vet J*. 2008;40:224.
477. Proudman CJ. A two year, prospective study of equine colic in general practice. *Equine Vet J*. 1992;24:90.
478. Voigt A, Saulex MN, Donnellan CM, et al. Causes of gastrointestinal colic at an equine referral hospital in South Africa (1998–2007). *J S Afr Vet Assoc*. 2009;80:92.
479. Abutarbush SM, Carmalt JL, Shoemaker JL. Causes of gastrointestinal colic in horses in western Canada: 604 cases (1992–2002). *Can Vet J*. 2005;46:800.
480. Yorke EH, Caldwell FJ, Johnson AK. Uterine torsion in mares. *Compend Contin Educ Vet*. 2012;34:E2.
481. Duesterdieck-Zellmer KF. Equine urolithiasis. *Vet Clin North Am Equine Pract*. 2007;23:613.
482. Johnston JK, Divers TJ, Reef VB, et al. Cholelithiasis in horses: ten cases (1982–1986). *J Am Vet Med Assoc*. 1989;194:405.
483. Tennent-Brown BS, Mudge MC, Hardy J, et al. Liver lobe torsion in six horses. *J Am Vet Med Assoc*. 2012;241:615.
484. Luethy D, Habecker P, Murphy B, et al. Clinical and pathological features of pheochromocytoma in the horse: a multi-center retrospective study of 37 cases (2007–2014). *J Vet Intern Med*. 2016;30:309.
485. Moore BR, Moore RM. Examination of the equine patient with gastrointestinal emergency. *Vet Clin North Am Equine Pract*. 1994;10:549.
486. Cook VL, Hassel DM. Evaluation of the colic in horses: decision for referral. *Vet Clin North Am Equine Pract*. 2014;30:383.
487. Furr MO, Lessard P, White 2nd NA. Development of a colic severity score for predicting the outcome of equine colic. *Vet Surg*. 1995;24:97.

488. Ebert R. Prognostic parameters in equine colic. *Tiearztl Prax*. 1994;22:256.
489. Orsini JA, Elser AH, Galligan DT, et al. Prognostic index for acute abdominal crisis (colic) in horses. *Am J Vet Res*. 1969;49:1988.
490. Parry BW, Anderson GA, Gay CC. Prognosis in equine colic: a study of individual variables used in case assessment. *Equine Vet J*. 1983;15:337.
491. Parry BW, Anderson GA, Gay CC. Prognosis in equine colic: a comparative study of variables used to assess individual cases. *Equine Vet J*. 1983;15:211.
492. Parry BW, Gay CC, Anderson GA. Assessment of the necessity for surgical intervention in cases of equine colic: a retrospective study. *Equine Vet J*. 1983;15:216.
493. Cribb NC, Cote NM, Boure LP, et al. Acute small intestinal obstruction associated with *Parascaris equorum* infection in young horses: 25 cases (1985–2004). *N Z Vet J*. 2006;54:338.
494. Chaffin MK, Cohen ND. Diagnostic assessment of foals with colic. *Proc Ann Conv AAEP*. 1999;45:235.
495. Cohen ND, Matejka PL, Honnas CM, et al. Case-control study of the association between various management factors and the development of colic in horses. Texas Equine Colic Study Group. *J Vet Med Assoc*. 1995;206:667.
496. Santschi EM, Purdy AK, Valberg SJ, et al. Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. *Mamm Genome*. 1998;9:306.
497. Wagner AE, Muir 3rd WW, Hinchcliff KW. Cardiovascular effects of xylazine and detomidine in horses. *Am J Vet Res*. 1991;52:651.
498. Daunt DA, Steffey EP. Alpha-2 adrenergic agonists as analgesics in horses. *Vet Clin North Am Equine Pract*. 2002;18:39.
499. Ragle CA, Meagher DM, Schrader JL, et al. Abdominal auscultation in the detection of experimentally induced gastrointestinal sand accumulation. *J Vet Intern Med*. 1989;3:12.
500. Tillotson K, Traub-Dargatz JL. Gastrointestinal protectants and cathartics. *Vet Clin North Am Equine Pract*. 2003;19:599.
501. White NA, ed. *The equine acute abdomen*. Malvern: Lea & Febiger; 1990.
502. Luo T, Bertone JJ, Greene HM, et al. A comparison of N-butylscopolammonium and lidocaine for control of rectal pressure in horses. *Vet Ther*. 2006;7:243.
503. White NA. Equine colic: how to make the decision for surgery. In: *AAEP Focus Proceedings*. Philadelphia; 2005.
504. Parry BW. Use of clinical pathology in evaluation of horses with colic. *Vet Clin North Am Equine Pract*. 1987;3:529.
505. Navarro M, Monreal L, Segura D, et al. A comparison of traditional and quantitative analysis of acid-base and electrolyte imbalances in horses with gastrointestinal disorders. *J Vet Intern Med*. 2005;19:871.
506. Hassel DM, Hill AE, Rorabeck RA. Association between hyperglycemia and survival in 228 horses with acute gastrointestinal disease. *J Vet Intern Med*. 2009;23:1261.
507. Davis JL, Blikslager AT, Catto K, et al. A retrospective analysis of hepatic injury in horses with proximal enteritis (1984–2002). *J Vet Intern Med*. 2003;17:896.
508. Gardner RB, Nydam DV, Mohammed HO, et al. Serum gamma glutamyl transferase activity in horses with right or left dorsal displacements of the large colon. *J Vet Intern Med*. 2005;19:761.
509. Krueger CR, Ruple-Czerniak A, Hackett ES. Evaluation of plasma muscle enzyme activity as an indicator of lesion characteristics and prognosis in horses undergoing celiotomy for acute gastrointestinal pain. *BMC Vet Res*. 2014;1:S7.
510. Fischer Jr AT. Advances in diagnostic techniques for horses with colic. *Vet Clin North Am Equine Pract*. 1997;13:203.
511. Cowell RL, Tyler RD, eds. *Diagnostic cytology and hematology of the horse*. 2nd ed. St. Louis: Mosby; 2007.
512. Peloso JG, Cohen ND. Use of serial measurements of peritoneal fluid lactate concentration to identify strangulating intestinal lesions in referred horse with signs of colic. *J Am Vet Med Assoc*. 2012;240:1208.
513. Radcliffe RM, Divers TJ, Fletcher DJ, et al. Evaluation of L-lactate and cardiac troponin I in horses undergoing emergency abdominal surgery. *J Vet Emerg Crit Care (San Antonio)*. 2012;22:313.
514. Yamout SZ, Nieto JE, Beldomenico PM, et al. Peritoneal and plasma D-lactate concentrations in horses with colic. *Vet Surg*. 2011;40:817.
515. Latson KM, Nieto JE, Beldomenico PM, et al. Evaluation of peritoneal fluid lactate as a marker of intestinal ischemia in equine colic. *Equine Vet J*. 2005;37:342.
516. Tennent-Brown BS. Interpreting lactate measurement in critically ill horses: diagnosis, treatment, and prognosis. *Compend Contin Educ Vet*. 2012;34:E2.
517. Nieto JE, Dechant JE, le Jeune SS, et al. Evaluation of 3 handheld portable analyzers for measurement of L-lactate concentrations in blood and peritoneal fluid of horses with colic. *Vet Surg*. 2015;44:366.
518. Dunkel B, Kapff JE, Naylor RJ, et al. Blood lactate concentrations in ponies and Miniature Horses with gastrointestinal disease. *Equine Vet J*. 2013;45:666.
519. Freeman SL. Diagnostic ultrasonography of the mature equine abdomen. *Equine Vet Educ*. 2003;15:319.
520. le Jeune S, Whitcomb MB: Ultrasound of the equine acute abdomen. *Vet Clin North Am Equine Pract*. 2014;30:353.
521. Scharner D, Bankert J, Brehm W. Comparison of the findings of rectal examination and ultrasonographic findings in horses with colic. *Tieraztl Prax Ausg G Grosstiere Nutztiere*. 2015;43:278.
522. Cavalleri JM, Biernert-Zeit A, Feige K. Examination of horses with acute colic—clinical pathology and diagnostic imaging. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2013;41:124.
523. Beccati F, Pepe M, Gialletti R, et al. Is there a statistical correlation between ultrasonographic findings and definitive diagnosis in horses with acute abdominal pain? *Equine Vet J Suppl*. 2011;39:98.
524. Busoni V, De Busscher V, Lopez D, et al. Evaluation of a protocol for fast localized abdominal sonography of horses (FLASH) admitted for colic. *Vet J*. 2011;188:77.
525. Bradecamp EA. How to image the adult equine abdomen and thorax in ambulatory practice using a 5-mHz rectal probe. *Proc Ann Conv AAEP*. 2007;53:537.
526. Bernard WV, Reef VB, Reimer JM, et al. Ultrasonographic diagnosis of small-intestinal intussusception in three foals. *J Am Vet Med Assoc*. 1989;194:395.
527. Santschi EM, Slone DE, Frank WM. Use of ultrasound in horses for diagnosis of left dorsal displacement of the large colon and monitoring its nonsurgical correction. *Vet Surg*. 1993;22:281.
528. Ness SL, Bain FT, Zantingh AJ, et al. Ultrasonographic visualization of colonic mesenteric vasculature as an indicator of large colon right dorsal displacement or 180° volvulus (or both) in horses. *Can Vet J*. 2012;53:378.
529. Keppie NJ, Rosensein DS, Holcombe SJ, et al. Objective radiographic assessment of abdominal sand accumulation in horses. *Vet Radiol Ultrasound*. 2008;49:122.
530. Yarbrough TB, Langer DL, Snyder JL, et al. Abdominal radiography for diagnosis of enterolithiasis in horses: 141 cases (1990–1992). *J Am Vet Med Assoc*. 1994;205:592.
531. Gerhards H, Klein HJ, Offeney F. Radiographic diagnosis of abdominal diseases in foals and ponys. II. Pathologic findings in 60 cases. *Tierarztl Prax*. 1990;18:383.
532. Argenzio RA, Lowe JE, Pickard DW, et al. Digesta passage and water exchange in the equine large intestine. *Am J Physiol*. 1974;226:1035.
533. Argenzio RA, Stevens CE. Cyclic changes in ionic composition of digesta in the equine intestinal tract. *Am J Physiol*. 1975;228:1224.
534. Argenzio RA. Functions of the large intestine and their interrelationship with disease. *Cornell Vet*. 1975;65:303.

535. Conner ME, Darlington RW. Rotavirus infection in foals. *Am J Vet Res*. 1980;41:1699.
536. Costa MC, Stampfli HR, Allen-Vercoe E, et al. Development of the faecal microbiota in foals. *Equine Vet J*. 2015. doi: 10.1111.
537. Rodriguez C, Taminiau B, Brevers B, et al. Faecal microbiota characterization of horses using rdna barcoded pyrosequencing, and carriage rate of *Clostridium difficile* at hospital admission. *BMC Microbiol*. 2015;15:181.
538. Holland JL, Kronfeld DS, Sklan D, et al. Calculation of fecal kinetics in horses fed hay or hay and concentrate. *J Anim Sci*. 1934;76:1998.
539. Bailey KE, Gilkerson JR, Browning GF, et al. Equine rotaviruses—current understanding and continuing challenges. *Vet Microbiol*. 2013;167:135.
540. Vannucci FA, Gebhart CJ. Recent advances in understanding the pathogenesis of *Lawsonia intracellularis* infections. *Vet Pathol*. 2014;51:465.
541. Wong DM, Alcott CJ, Sponseller BA, et al. Impaired intestinal absorption of glucose in 4 foals with *Lawsonis intracellularis* infection. *J Vet Intern Med*. 2009;23:940.
542. O'Louglin EV, Scott RB, Gall DG. Pathophysiology of infectious diarrhea: changes in intestinal structure and function. *J Pediatr Gastroenterol Nutr*. 1991;12:5.
543. Rachmilewitz D. Prostaglandins and diarrhea. *Dig Dis Sci*. 1980;25:897.
544. Hardcastle J, Hardcastle PT. Involvement of prostaglandin in histamine-induced fluid and electrolyte secretion by rat colon. *J Pharm Pharmacol*. 1988;40:106.
545. Wang YZ, Su HC, Cooke, et al. Histamine augments colonic secretion in guinea pig distal colon. *Am J Physiol*. 1990;258: G432.
546. Clarke LL, Argenzio RA. NaCl transport across equine proximal colon and the effect of endogenous prostaglandins. *Am J Physiol*. 1990;259:G62.
547. Halm DR, Rechkemmer GR, Schoumache RA, et al. Apical membrane chloride channels in a colonic cell line activated by secretory agonists. *Am J Physiol*. 1988;254:C505.
548. Cliff WH, Frizzell RA. Separate Cl^- conductances activated by cAMP and Ca2 in Cl(−)-secreting epithelial cells. *Proc Natl Acad Sci USA*. 1990;87:4956.
549. Ling BN, Kokko KE, Eaton DC. Prostaglandin E2 activates clusters of apical Cl^- channels in principal cells via a cyclic adenosine monophosphate-dependent pathway. *J Clin Invest*. 1994;93:829.
550. Guirl MJ, Hogenauer C, Santa Ana CA, et al. Rapid intestinal transit as a primary cause of severe chronic diarrhea in patients with amyloidosis. *Am J Gastroenterol*. 2003;98:2219.
551. King JN, Gerring EL. The action of low dose endotoxin on equine bowel motility. *Equine Vet J*. 1991;23:11.
552. Weese JS, Parsons DA, Staempfli HR. Association of *Clostridium difficile* with enterocolitis and lactose intolerance in a foal. *J Am Vet Med Assoc*. 1999;214:229.
553. Roberts VLH, Knottenbelt DC, Williams A, et al. Suspected primary lactose intolerance in neonatal foals. *Equine Vet Educ*. 2008;20:249.
554. Larsen J. Acute colitis in adult horses. A review with emphasis on aetiology and pathogenesis. *Vet Q*. 1997;19:72.
555. Stewart MC, Hodgson JL, Kim H, et al. Acute febrile diarrhoea in horses: 86 cases (1986–1991). *Aust Vet J*. 1995;72:41.
556. Smith BP. *Salmonella* infection in horses. *Compend Cont Educ Pract Vet*. 1981;3:S4.
557. Jones RL. Clostridial enterocolitis. *Vet Clin North Am Eq Pract16*. 2000;471.
558. Diab SS, Songer G, Uzal FA. *Clostridium difficile* infection in horses: a review. *Vet Microbiol*. 2013;167:42.
559. East LM, Savage CJ, Traub-Dargatz JL, et al. Enterocolitis associated with *Clostridium perfringens* infection in neonatal foals: 54 cases (1988–1997). *J Am Vet Med Assoc*. 1998;212:1751.
560. Magdesian KG, Hirsh DC, Jang SS, et al. Characterization of *Clostridium difficile* isolates from foals with diarrhea: 28 cases (1993–1997). *J Am Vet Med Assoc*. 2002;220:67.
561. Tillotson K, Traub-Dargatz JL, Dickinson CE, et al. Population-based study of fecal shedding of *Clostridium perfringens* in broodmares and foals. *J Am Vet Med Assoc*. 2002;220:342.
562. Berlin FR, Reising A, Slovis NM, et al. Clinical and clinicopathological findings associated with survival in 44 horses with equine neorickettsiosis (Potomac horse fever). *J Vet Intern Med*. 2013;27:1528.
563. Pusterla N, Gebhart CJ. Equine proliferative enteropathy—a review of recent developments. *Equine Vet J*. 2013;45:403.
564. Platell JL, Gibson JS. Proliferative enteropathy in foals: disease, diagnostics and transmission. *Vet J*. 2013;195:135.
565. Lavoie JP, Drolet R, Parsons D, et al. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinemia in foals on three breeding farms in Canada. *Equine Vet J*. 2000;32:418.
566. Cimprich RE, Rooney JR. *Corynebacterium equi* enteritis in foals. *Vet Pathol*. 1977;14:95.
567. Holland RE, Sriranganathan N, Dupont L. Isolation of enterotoxigenic *Escherichia coli* from a foal with diarrhea. *J Am Vet Med Assoc*. 1989;194:389.
568. Hurcombe SD, Fox JG, Kohn CW. Isolation of *Campylobacter fetus* subspecies *fetus* in a 2-year-old quarter horse with chronic diarrhea of an undetermined etiology. *J Vet Diagn Invest*. 2009;21:266.
569. Hathcock TL, Schumacher J, Wright JC, et al. The prevalence of *Aeromonas* species in feces of horses with diarrhea. *J Vet Intern Med*. 1999;13:357.
570. Browning GF, Chalmers RM, Snodgrass DR, et al. The prevalence of enteric pathogens in diarrhoeic Thoroughbred foals in Britain and Ireland. *Equine Vet J*. 1991;23:397.
571. Slovis NM, Elam J, Estrada M, et al. Infectious agents associated with diarrhoea in neonatal foals in central Kentucky: a comprehensive molecular study. *Equine Vet J*. 2014;46:311.
572. Pusterla N, Mapes S, Wademan C, et al. Emerging outbreaks associated with equine coronavirus in adult horses. *Vet Microbiol* 2013;162:228.
573. Fielding CL, Higgins JK, Higgins JC, et al. Disease associated with equine coronavirus infection and high case fatality rate. *J Vet Intern Med*. 2015;29:307.
574. Oue Y, Morita Y, Kondo T, et al. Epidemic of equine coronavirus at Obihiro Racecourse, Hokkaido, Japan in 2012. *J Vet Med Sci*. 2013;75:1261.
575. Guy JS, Breslin JJ, Breuhaus B, et al. Characterization of a coronavirus isolated from a diarrheic foal. *J Clin Microbiol*. 2000;38:4523.
576. Corning S. Equine cyathostomins: a review of biology, clinical significance and therapy. *Parasit Vectors Suppl*. 2009;2:S1.
577. Nielsen MK, Loynachan AT, Jacobsen S, et al. Local and systemic inflammatory and immunologic reactions to cyathostomin larvicidal therapy in horses. *Vet Immunol and Immunopathol*. 2015;168:203.
578. Netherwood T, Wood JL, Townsend HG, et al. Foal diarrhoea between 1991 and 1994 in the United Kingdom associated with *Clostridium perfringens*, rotavirus, *Strongyloides westeri* and *Cryptosporidium* spp. *Epidemiol Infect*. 1996;117:375.
579. Lyons ET, Tolliver SC. Prevalence of patent *Strongyloides westeri* infections in Thoroughbred foals in 2014. *Parasitol Res*. 2014;113:4163.
580. Galuppi R, Piva S, Castagnetti C, et al. *Cryptosporidium parvum*: from foal to veterinary students. *Vet Parasitol*. 2016;219:53.
581. Liu A, Zhang J, Zhao, et al. The first report of *Cryptosporidium andersoni* in horses with diarrhea and multilocus subtype analysis. *Parasit Vectors*. 2015;8:483.
582. Cole DJ, Cohen ND, Snowden K, et al. Prevalence of and risk factors for fecal shedding of *Cryptosporidium parvum* oocysts in horses. *J Am Vet Med Assoc*. 1998;213:1296.

583. Wilson DA, MacFadden KE, Green EM, et al. Case control and historical cohort study of diarrhea associated with administration of trimethoprim-potentiated sulphonamides to horses and ponies. *J Vet Intern Med*. 1996;10:258.
584. Baverud V, Franklin A, Gunnarson A, et al. *Clostridium difficile* associated with acute colitis in mares when their foals are treated with erythromycin and rifampin for *Rhodococcus equi* pneumonia. *Equine Vet J*. 1998;30:482.
585. Costa MC, Stampfli HR, Arroyo LG, et al. Changes in the equine fecal microbiota associated with the use of systemic antimicrobial drugs. *BMC Vet Res*. 2015;11:19.
586. Harlow BE, Lawrence LM, Flythe MD. Diarrhea-associated pathogens, lactobacilli and cellulolytic bacteria in equine feces: responses to antibiotic challenge. *Vet Microbiol*. 2013;166:225.
587. Karcher LF, Dill SG, Anderson WI, et al. Right dorsal colitis. *J Vet Intern Med*. 1990;4:247.
588. Marshall JF, Blikslager AT. The effect of nonsteroidal anti-inflammatory drugs on the equine intestine. *Equine Vet J Suppl*. 2011;39:140.
589. McConnico RS, Morgan TW, Williams CC, et al. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *Am J Vet Res*. 2008;69:1496.
590. Schmitz DG. Cantharidin toxicosis in horses. *J Vet Intern Med*. 1989;3:208.
591. Abutarbush SM. Alfalfa dodder *(Cuscuta campestris)* toxicity in horses: clinical, haematological and serum biochemical findings. *Vet Rec*. 2013;173:95.
592. Smith S, Naylor RJ, Knowles EJ, et al. Suspected acorn toxicity in nine horses. *Equine Vet J*. 2015;47:568.
593. Renier AC, Kass PH, Magdesian KG, et al. Oleander toxicosis in equids: 30 cases (1995–2010). *J Am Vet Med Assoc*. 2013;242:540.
594. Pace LW, Turnquist SE, Casteel SW, et al. Acute arsenic toxicosis in five horses. *Vet Pathol*. 1997;34:160.
595. Casteel SW. Metal toxicosis in horses. *Vet Clin North Am Equine Pract*. 2001;17:517.
596. Taylor SD, Pusterla N, Vaugan B, et al. Intestinal neoplasia in horses. *J Vet Intern Med*. 2006;20:1429.
597. Gianini M, Sutter O, Burger D, et al. Gastrointestinal and endocrine function during "foal heat diarrhea" in healthy foals. *J Reprod Fertil Suppl*. 2000;56:717.
598. Olivett TL, Divers TJ, Cushing T, et al. Acute pancreatitis in two 5-day-old Appaloosa foals. *Equine Vet J Suppl*. 2012;41:96.
599. Cohen ND, Woods AM. Characteristics and risk factors for failure of horses with acute diarrhea to survive: 122 cases (1990–1996). *J Am Vet Med Assoc*. 1999;214:382.
600. Mair TS, Taylor FG, Harbour DA, Pearson GR. Concurrent cryptosporidium and coronavirus infections in an Arabian foal with combined immunodeficiency syndrome. *Vet Rec*. 1990;126:127.
601. Richards AF, Kelly DF, Knottenbelt DC, et al. Anaemia, diarrhoea and opportunistic infections in Fell ponies. *Equine Vet J*. 2000;32:386.
602. Sweeney RW, Sweeney CR, Saik J, et al. Chronic granulomatous bowel disease in three sibling horses. *J Am Vet Med Assoc*. 1986;188:1192.
603. Mair TS, Cripps PJ, Ricketts SW. Diagnostic and prognostic value of serum protein electrophoresis in horses with chronic diarrhoea. *Equine Vet J*. 1993;25:324.
604. Patton S, Mock RE, Drudge JH, et al. Increase of immunoglobulin T concentrations in ponies as a response to experimental infection with the nematode *Strongylus vulgaris*. *Am J Vet Res*. 1978;39:19.
605. Morris DD, Whitlock RH, Palmer JE. Fecal leukocytes and epithelial cells in horses with diarrhea. *Cornell Vet*. 1983;73:265.
606. Schoster A, Arroyo LG, Staempfli HR, et al. Presence and molecular characterization of *Clostridium difficile* and *Clostridium perfringens* in intestinal compartments of healthy horses. *BMC Vet Res*. 2012;8:94.
607. Weese JS, Staemplfi HR, Prescott JF. Test selections and interpretation in the diagnosis of *Clostridium difficile*–associated colitis. *Proc Ann AAEP Conv*. 1999;45:502.
608. Hyatt DR, Weese JS. *Salmonella* culture: sampling procedures and laboratory techniques. *Vet Clin North Am Equine Pract*. 2004;20:577.
609. Cohen ND, Neibergs HL, Wallis DE, et al. Genus-specific detection of salmonellae in equine feces by use of the polymerase chain reaction. *Am J Vet Res*. 1994;55:1049.
610. Cohen ND, Martin LJ, Simpson RB, et al. Comparison of polymerase chain reaction and microbiological culture for detection of salmonellae in equine feces and environmental samples. *Am J Vet Res*. 1996;57:780.
611. Barlough JE, Rikihisa Y, Madigan JE. Nested polymerase chain reaction for detection of *Ehrlichia risticii* genomic DNA in infected horses. *Vet Parasitol*. 1997;68:367.
612. Mott F, Rikihisa T, Zhang Y, et al. Comparison of PCR and culture to the indirect fluorescent-antibody test for diagnosis of Potomac horse fever. *J Clin Microbiol*. 1997;35:2215.
613. Magdesian KG, Leutenegger CM. Real-time PCR and typing of *Clostridium difficile* isolates colonizing mare foal pairs. *Vet J*. 2011;190:119.
614. Page AE, Slovis NM, Gebhart CJ, et al. Serial use of serologic assays and fecal PCR assays to aid in identification of subclinical *Lawsonia intracellularis* infection for targeted treatment of Thoroughbred foals and weanlings. *J Am Vet Med Assoc*. 2011;238:1482.
615. Ellis GR, Daniels E. Comparison of direct electron microscopy and enzyme immunoassay for the detection of rotaviruses in calves, lambs, piglets and foals. *Aust Vet J*. 1988;65:133.
616. Mino S, Kern A, Barrandeguy M, et al. Comparison of two commercial kits and an in-house ELISA for the detection of equine rotavirus in foal feces. *J Virol Methods*. 2015;222:1.
617. Jones SL, Davis J, Rowlingson K. Ultrasonographic findings in horses with right dorsal colitis: five cases (2000–2001). *J Am Vet Med Assoc*. 2003;222:1248.
618. East LM, Trumble TN, Steyn PF, et al. The application of technetium-99m hexamethylpropyleneamine oxime (99mTc-HMPAO) labeled white blood cells for the diagnosis of right dorsal ulcerative colitis in two horses. *Vet Radiol Ultrasound*. 2000;41:360.
619. Roberts MC, Norman P. A re-evaluation of the D (+) xylose absorption test in the horse. *Equine Vet J*. 1979;11:239.
620. Mullen KR, Yasuda K, Divers TJ, et al. Equine faecal microbiota transplant: current knowledge, proposed guidelines and future directions. *Equine Vet Educ*. 2016. http://dx.doi.org/10.1111/eve. 12559.
621. Schoster A, Weese JS, Guardabassi L. Probiotic use in horses—what is the evidence for their clinical efficacy? *J Vet Intern Med*. 2014;28:1640.
622. Desrochers AM, Dolente BA, Roy MF, et al. Efficacy of *Saccharomyces boulardii* for treatment of horses with acute enterocolitis. *J Am Vet Med Assoc*. 2005;227:954.
623. Groenendyk S, English PB, Abetz I. External balance of water and electrolytes in the horse. *Equine Vet J*. 1988;20:189.
624. Rumbaugh GE, Carlson GP, Harrold D. Urinary production in the healthy horse and in horses deprived of feed and water. *Am J Vet Res*. 1982;43:735.
625. Tasker JB. Fluid and electrolyte studies in the horses. III. Intake and output of water, sodium and potassium in normal horses. *Cornell Vet*. 1967;57:649.
626. Morris DD, Divers TJ, Whitlock RH. Renal clearance and fractional excretion of electrolytes over a 24-hour period in horses. *Am J Vet Res*. 1984;45:2431.
627. Kohn CW, Strasser SL. 24-hour renal clearance and excretion of endogenous substances in the mare. *Am J Vet Res*. 1986;47:1332.
628. Rose RJ. Electrolytes: clinical application. *Vet Clin North Am Equine Pract*. 1990;6:281.

629. Cymbaluk NF. Water balance of horses fed various diets. *Equine Pract*. 1989;11:19.
630. Sufit E, Houpt KA, Sweeting M. Physiological stimuli of thirst and drinking patterns in ponies. *Equine Vet J*. 1985;17:12.
631. Brewer BD, Clement SF, Lotz WS, et al. Renal clearance, urinary excretion of endogenous substances and urinary diagnostic indices in healthy neonatal foals. *J Vet Intern Med*. 1991;5:28.
632. Edwards DJ, Brownlow MA, Hutchins DR. Indices of renal function; values in eight normal foals from birth to 56 days. *Aust Vet J*. 1990;67:251.
633. Martin RG, McMeniman NP, Dowsett KF. Milk and water intakes of foals sucking grazing mares. *Equine Vet J*. 1992;24:295.
634. Rose BD. *Clinical physiology of acid-base and electrolyte disorders*. New York: McGraw-Hill Information Services; 1989.
635. McCutcheon LJ, Geor RJ. Sweat fluid and ion losses in horses during training and competition in cool vs. hot ambient conditions: implications for ion supplementation. *Equine Vet J*. 1996;22(S):54.
636. Guyton AC, Hall J. Regulation of extracellular fluid osmolarity and sodium concentration. In: *Textbook of medical physiology*. Philadelphia: Elsevier Saunders; 2006.
637. Jamison RL, Maffly RH. The urinary concentrating mechanism. *N Engl J Med*. 1976;295:1059.
638. Rotondo F, Butz H, Syro LV, et al. Arginine vasopressin (AVP): a review of its historical perspectives, current research and multifunctional role in the hypothalmo-hypophysial system. *Pituitary*. 2016;19:345.
639. Boone M, Deen PM. Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. *Pflugers Arch*. 2008;456:1005.
640. Houpt KA, Thornton SN, Allen WR. Vasopressin in dehydrated and rehydrated ponies. *Physiol Behav*. 1989;45:659.
641. Sands JM, Blount MA, Klein JD. Regulation of renal urea transport by vasopressin. *Trans Am Clin Climatol Assoc*. 2011;122:82.
642. Houpt KA. Thirst in horses: the physiological and psychological causes. *Equine Pract*. 1987;9:28.
643. Guyton AC, Hall J. Kidney diseases and diuretics. In: *Textbook of Medical Physiology*. Philadelphia: Elsevier Saunders; 2006.
644. Robertson GL. Diabetes insipidus: differential diagnosis and management. *Best Pract Red Clin Endocrinol Metab*. 2016;30:205.
645. Schott HC. Water homeostasis and diabetes insipidus in horses. *Vet Clin North Am Equine Pract*. 2011;27:175.
646. Geor RJ. Acute renal failure in horses. *Vet Clin North Am Equine Pract*. 2007;23:577.
647. Schott HC. Chronic renal failure in horses. *Vet Clin North Am Equine Pract*. 2007;23:593.
648. Buntain BJ, Coffman JR. Polyuria and polydipsia in a horse induced by psychogenic salt consumption. *Equine Vet J*. 1981;13:266.
649. Taylor FGR, Hillyer MH. The differential diagnosis of hyperglycaemia in horses. *Equine Vet Educ*. 1992;4:135.
650. Knottenbelt DC. Polyuria-polydipsia in the horse. *Equine Vet Educ*. 2000;12:179.
651. McKenzie EC. Polyuria and polydipsia in horses. *Vet Clin North Am Equine Pract*. 2007;23:641.
652. Rye SH, Kim BS, Lee CW, et al. Glucocorticoid-induced laminitis with hepatopathy in a Thoroughbred filly. *J Vet Sci*. 2004;5:271.
653. Browning AP. Polydipsia and polyuria in two horses caused by psychogenic polydipsia. *Equine Vet Educ*. 2000;12:175.
654. Andersson B, Rundgren M. Thirst and its disorders. *Ann Rev Med*. 1982;33:231.
655. Robertson GL. Abnormalities of thirst regulation. *Kidney International*. 1984;25:460.
656. Sturgeon BP, Bassett H. Polydipsia in a foal with renal helminthiasis. *Vet Rec*. 2000;147:23.
657. Wooldridge AA, Seahorn TL, Williams J, et al. Chronic renal failure associated with nephrolithiasis, ureterolithiasis, and renal dysplasia in a 2-year-old Quarter Horse gelding. *Vet Radiol Ultrasound*. 1999;40:361.
658. McGowan TW, Pinchbeck GP, McGowan CM. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. *Equine Vet J*. 2013;45:74.
659. McFarlane D. Equine pituitary pars intermedia dysfunction. *Vet Clin North Am Equine Pract*. 2011;27:93.
660. Schott HC. Pituitary pars intermedia dysfunction: equine Cushing's disease. *Vet Clin North Am Equine Pract*. 2002;18:237.
661. Breukink HJ, Van Wegen P, Schotman AJH. Idiopathic diabetes insipidus in a Welsh pony. *Equine Vet J*. 1983;15:284.
662. Filar J, Ziolo T, Szalecki J. Diabetes insipidus in the course of encephalitis in the horse. *Med Weter*. 1971;27:205.
663. Morgan RA, Malalana F, McGowan CM. Nephrogenic diabetes insipidus in a 14-year-old gelding. *N Z Vet J*. 2012;60:254.
664. Schott HC, Bayly WM, Reed SM, et al. Nephrogenic diabetes insipidus in sibling colts. *J Vet Intern Med*. 1993;7:68.
665. Brashier M. Polydipsia and polyuria in a weanling colt caused by nephrogenic diabetes insipidus. *Vet Clin North Am Equine Pract*. 2006;22:219.
666. Corke MJ. Diabetes mellitus: the tip of the iceberg. *Equine Vet J*. 1986;18:87.
667. Menzies-Gow N. Diabetes in the horse: a condition of increasing clinical awareness for differential diagnosis and interpretation of tests. *Equine Vet J*. 2009;41:841.
668. Collobert C, Gillet JP, Sorel P, Minnelo J. Chronic pancreatitis associated with diabetes mellitus in a Standardbred racehorse: a case report. *J Eq Vet Sci*. 1990;10:58.
669. Giri JK, Magdesian KG, Gaffney PM. Insulin-dependent diabetes mellitus associated with presumed autoimmune polyendocrine syndrome in a mare. *Can Vet J*. 2011;52:506.
670. Navas de Solis C. Foreman JH: Transient diabetes mellitus in a neonatal Thoroughbred foal. *J Vet Emerg Critical Care*. 2010;20:611.
671. Jeffrey JR. Diabetes mellitus secondary to chronic pancreatitis in a pony. *J Am Vet Med Assoc*. 1968;153:1168.
672. McCoy DJ. Diabetes mellitus associated with bilateral granulosa cell tumors in a mare. *J Am Vet Med Assoc*. 1986;188:733.
673. Ruoff WW, Baker DC, Morgan SJ. Type II diabetes mellitus in a horse. *Equine Vet J*. 1986;18:143.
674. Baker JR, Ritchie HE. Diabetes mellitus in the horse: a case report and review of the literature. *Equine Vet J*. 1974;6:7.
675. Durham AE, Hughes KJ, Cottle HJ, et al. Type 2 diabetes mellitus with pancreatic beta cell dysfunction in 3 horses confirmed with minimal model analysis. *Equine Vet J*. 2009;41:924.
676. Muylle E, van den Hende C, et al. Non–insulin dependent diabetes mellitus in a horse. *Equine Vet J*. 1986;18:145.
677. Sneddon JC, Colyn P. A practical system for measuring water intake in stabled horses. *Equine Vet Sci*. 1991;11:141.
678. Harris P. Collection of urine. *Equine Vet J*. 1988;20:86.
679. van den Berg IS. Modified apparatus for collection of free-flow urine from mares. *J S Afr Vet Assoc*. 1996;67:214.
680. Tasker JB. Fluid and electrolyte studies in the horse. II. An apparatus for the collection of total daily urine and feces from horses. *Cornell Vet*. 1966;56:77.
681. Kohn CW, Chew DJ. Laboratory diagnosis and characterization of renal disease in horses. *Vet Clin North Am Equine Pract*. 1987;3:585.
682. Bickhardt K, Deegen E, Espelage W. Kidney function tests in horses—methods and reference values in healthy animals. *Dtsch Tierarztl Wochenschr*. 1996;103:117.
683. Satoh H, Abe S, Kato M, et al. Optimum conditions for serum clearance of iodixanol, applicable to the estimation of glomerular filtration rate in horses. *Vet Res Commun*. 2011;35:463.
684. Nabity MB, Lees GE, Boggess MM, et al. Symmetric dimethylarginine assay validation, stability and evaluation as a marker for the early detection of chronic kidney disease in dogs. *J Vet Intern Med*. 2015;29:1036.
685. Brobst DF, Bayly WM. Responses of horses to a water deprivation test. *J Equine Vet Sci*. 1982;2:51.

686. Genetzky RM, Loparco FV, Ledet AE. Clinical pathologic alterations in horses during a water deprivation test. *Am J Vet Res*. 1987;48:1007.
687. Brown CM. *Problems in equine medicine*. Philadelphia: Lea & Febiger; 1989.
688. Zimmer EL. Water deprivation test and vasopressin challenge. In: *Equine medicine and surgery*. Goleta, CA: American Veterinary Publications; 1991.
689. Velie BD, Hamilton NA, Wade CM. Heritability of racing performance in the Australian Thoroughbred racing population. *Anim Genet*. 2015;46:23.
690. Hill EW, McGivney BA, Gu J, et al. A genome-wide SNP-association study confirms a sequence variant (g.66493737C>T) in the equine myostatin (MSTN) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses. *BMC Genomics*. 2010;11:552.
691. Jonsson L, Egenvall A, Roepstorff L, et al. Associations of health status and conformation with longevity and lifetime competition performance in young Swedish Warmblood riding horses. *J Am Vet Med Assoc*. 2014;244:1449.
692. Dyson S. Lameness and poor performance in the sports horse: dressage, show jumping and horse trials (eventing). *AAEP Proceedings*. 2000;46:308.
693. Dyson SJ. Lesions of the equine neck resulting in lameness or poor performance. *Vet Clin North Am Equine Pract*. 2011;27:417.
694. Girodroux M, Dyson S, Murray R. Osteoarthritis of the thoracolumbar synovial intervertebral articulations: clinical and radiographic features in 77 horses with poor performance and back pain. *Equine Vet J*. 2009;41:130.
695. Rose RJ. Poor performance: a clinical and physiological perspective. *Proceedings of the 19th American College of Veterinary Internal Medicine Forum*. 2001;224.
696. Rivero JL, Hill EW. Skeletal muscle adaptations and muscle genomics of performance horses. *Vet J*. 2016;209:5.
697. Kearns CF, McKeever KH, Abe T. Overview of horse body composition and muscle architecture: implications for performance. *Vet J*. 2002;164:224.
698. Evans DL. Physiology of equine performance and associated tests of function. *Equine Vet J*. 2007;39:373.
699. Morris EA, Seeherman HJ. Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. *Equine Vet J*. 1991;23:169.
700. Martin BB, Reef VB, Parente EJ, et al. Causes of poor performance of horses during training, racing or showing: 348 cases (1992–1996). *J Am Vet Med Assoc*. 2000;216:554.
701. Seeherman HJ, Morris E, O'Callaghan MW. The use of sports medicine techniques in evaluating the problem equine athlete. *Vet Clin North Am Equine Pract*. 1991;7:259.
702. Brown JA, Hinchcliff KW, Jackson MA, et al. Prevalence of pharyngeal and laryngeal abnormalities in Thoroughbreds racing in Australia, and their association with performance. *Equine Vet J*. 2005;37:397.
703. Pirrone F, Albertini M, Clement MG, et al. Respiratory mechanics in Standardbred horses with sub-clinical inflammatory airway disease and poor athletic performance. *Vet J*. 2007;173:144.
704. Evans DL, Kiddell L, Smith CL. Pulmonary function measurements immediately after exercise are correlated with neutrophil percentage in tracheal aspirates in horses with poor racing performance. *Res Vet Sci*. 2011;90:510.
705. Sanchez A, Couetil LL, Ward MP, et al. Effect of airway disease on blood gas exchange in racehorses. *J Vet Intern Med*. 2005;19:87.
706. Tamzali Y, Marguet C, Priymenko N, et al. Prevalence of gastric ulcer syndrome in high-level endurance horses. *Equine Vet J*. 2011;43:141.
707. Leahy ER, Burk AO, Greene EA, et al. Nutrition-associated problems facing elite level three-day eventing horses. *Equine Vet J Suppl*. 2010;38:370.
708. Hubert JD, Beadle RE, Norwood G. Equine anhidrosis. *Vet Clin North Am Equine Pract*. 2002;18:355.
709. Richard EA, Fortier GD, Pitel PH, et al. Sub-clinical diseases affecting performance in Standardbred trotters: diagnostic methods and predictive parameters. *Vet J*. 2010;184:282.
710. Fraipont A, Van Erck E, Ramery E, et al. Subclinical diseases underlying poor performance in endurance horses: diagnostic methods and predictive tests. *Vet Rec*. 2011;169:154.
711. Dabareiner RM, Cohen ND, Carter GK, et al. Lameness and poor performance in horses used for team roping: 118 cases (2000–2003). *J Am Vet Med Assoc*. 2005;226:1694.
712. Gorgas D, Luder P, Lang J, et al. Scintigraphic and radiographic appearance of the sacroiliac region in horses with gait abnormalities or poor performance. *Vet Radiol Ultrasound*. 2009;50:208.
713. Aleman M. A review of equine muscle disorders. *Neuromuscul Disord*. 2008;18:277.
714. McCue ME, Valberg SJ, Lucio M, et al. Glycogen synthase (GYS1) mutation in diverse breeds with polysaccharide storage myopathy. *J Vet Intern Med*. 2008;22:1228.
715. Fritz KL, McCue ME, Valberg SJ, et al. Genetic mapping of recurrent exertional rhabdomyolysis in a population of North American Thoroughbreds. *Anim Genet*. 2012;43:730.
716. Valberg SJ, Carlson GP, Cardinet GH, et al. Skeletal muscle myopathy as a cause of exercise intolerance in a horse. *Muscle Nerve*. 1994;17:305.
717. Valberg SJ, McKenzie EC, Eyrich LV, et al. Suspected myofibrillar myopathy in Arabian horses with a history of exertional rhabdomyolysis. *Equine Vet J*. 2015. http://dx.doi.org/10.1111/evj.12493.
718. Bedford HE, Valberg SJ, Firshman AM, et al. Histopathologic findings in the sacrocaudalis dorsalis medialis muscle of horses with vitamin E–responsive muscle atrophy and weakness. *J Am Vet Med Assoc*. 2013;242:1127.
719. Tan RH, Dowling BA, Dart AJ. High-speed treadmill videoendoscopic examination of the upper respiratory tract in the horse: the results of 291 clinical cases. *Vet J*. 2005;170:243.
720. Maxson-Sage A, Parente EJ, Beech J, et al. Effect of high-intensity exercise on arterial blood gas tensions and upper airway and cardiac function in clinically normal Quarter Horses and horses heterozygous and homozygous for hyperkalemic periodic paralysis. *Am J Vet Res*. 1998;59:615.
721. Allen KJ, Tremaine WH, Franklin SH. Prevalence of inflammatory airway disease in national hunt horses referred for investigation of poor athletic performance. *Equine Vet J Suppl*. 2006;36:529.
722. Salz RO, Ahern BJ, Boston R, et al. Association of tracheal mucus or blood and airway neutrophilia with racing performance in Thoroughbred horses in an Australian racing yard. *Aust Vet J*. 2016;94:96.
723. Back H, Penell J, Pringle J, et al. A longitudinal study of poor performance and subclinical respiratory viral activity in Standardbred trotters. *Vet Rec Open*. 2015;2:e000107.
724. Back H, Ullman K, Treiberg Berndtsson L, et al. Viral load of equine herpesviruses 2 and 5 in nasal swabs of actively racing Standardbred trotters: temporal relationship of shedding to clinical findings and poor performance. *Vet Microbiol*. 2015;179:142.
725. Barbesgaard L, Buhl R, Meldgaard C. Prevalence of exercise-associated arrhythmias in normal performing dressage horses. *Equine Vet J Suppl*. 2010;38:202.
726. Buhl R, Meldgaard C, Barbesgaard L. Cardiac arrhythmias in clinically healthy showjumping horses. *Equine Vet J Suppl*. 2010;38:196.
727. Jose-Cunilleras E, Young LE, Newton JR, et al. Cardiac arrhythmias during and after treadmill exercise in poorly performing Thoroughbred racehorses. *Equine Vet J Suppl*. 2006;36:163.

728. King CM, Evans DL, Rose RJ. Significance for exercise capacity of some electrocardiographic findings in racehorses. *Aust Vet J*. 1994;71:200.
729. Kriz NG, Hodgson DR, Rose RJ. Prevalence and clinical importance of heart murmurs in racehorses. *J Am Vet Med Assoc*. 2000;216:1441.
730. Zucca E, Ferrucci F, Stancari G, et al. The prevalence of cardiac murmurs among Standardbred racehorses presented with poor performance. *J Vet Med Sci*. 2010;72:781.
731. Leleu C, Cotrel C. Body composition in young Standardbreds in training: relationships to body condition score, physiologic and locomotor variables during exercise. *Equine Vet J Suppl*. 2006;36:98.
732. Garlinghouse SE, Burrill MJ. Relationship of body condition score to completion rate during 160 km endurance races. *Equine Vet J Suppl*. 1999;30:591.
733. McGowan CM, Golland LC, Evans DL, et al. Effects of prolonged training, overtraining and detraining on skeletal muscle metabolites and enzymes. *Equine Vet J Suppl*. 2002;34:257.
734. Greve L, Dyson S. The horse-saddle-rider interaction. *Vet J*. 2013;195:275.
735. Hemberg E, Kindahl H, Lundeheim N, et al. Relationships between early foal health, future performance and their dams reproductive health. *Reprod Domest Anim*. 2010;45:817.
736. Boyko AR, Brooks SA, Behan-Braman A, et al. Genomic association establishes correlation between growth and laryngeal neuropathy in Thoroughbreds. *BMC Genomics*. 2014;15:259.
737. Rose RJ, Allen JR, Hodgson DR, et al. Response to submaximal treadmill exercise and training in the horse: changes in haematology, arterial blood gas and acid base measurements, plasma biochemical values and heart rate. *Vet Rec*. 1983;113:612.
738. Rose RJ, Allen JR. Hematologic responses to exercise and training. *Vet Clin North Am Equine Pract*. 1985;1:461.
739. Tyler-McGowan CM, Golland LS, Evans DL, et al. Haematological and biochemical responses to training and overtraining. *Equine Exerc Physiol Suppl*. 1999;30:621.
740. Hamlin MJ, Shearman JP, Hopkins WG. Changes in physiologic parameters in overtrained Standardbred racehorses. *Equine Vet J*. 2002;34:383.
741. McKeever KH, Hinchcliff KW, Reed SM, et al. Role of decreased plasma volume in hematocrit alterations during incremental treadmill exercise in horses. *Am J Physiol*. 1993;265:R404.
742. Persson SGB, Osterberg I. Racing performance in red blood cell hypervolaemic Standardbred trotters. *Equine Vet J Suppl*. 1999;30:617.
743. Dart AJ, Dowling BA, Hodgson DR, et al. Evaluation of high-speed treadmill videoscopy for diagnosis of upper respiratory tract dysfunction in horses. *Aust Vet J*. 2001;79:109.
744. Christley RM, Hodgson DR, Evans DL, et al. Cardiorespiratory responses to exercise in horses with different grades of idiopathic laryngeal hemiplegia. *Equine Vet J*. 1997;29:6.
745. King CM, Evans DL, Rose RJ. Cardiorespiratory and metabolic responses to exercise in horses with various abnormalities of the upper respiratory tract. *Equine Vet J*. 1994;71:200.
746. Holcombe SJ, Derksen FJ, Stick JA, et al. Pathophysiology of dorsal displacement of the soft palate in horses. *Equine Vet J Suppl*. 1999;30:45.
747. Chalmers HJ, Cheetham J, Yeager AE, et al. Ultrasonography of the equine larynx. *Vet Radiol Ultrasound*. 2006;47:476.
748. Meyer C, Gerber R, Guthrie AJ. The use of the standard exercise test to establish the clinical significance of mild echocardiographic changes in a Thoroughbred poor performer. *J S Afr Vet Assoc*. 2004;75:100.
749. Durando MM, Reef VB, Birks EK. Right ventricular pressure dynamics during exercise: relationship to stress echocardiography. *Equine Vet J Suppl*. 2002;34:472.
750. Seeherman HJ, Morris EA. Methodology and repeatability of a standardized treadmill exercise test for clinical evaluation of fitness in horses. *Equine Vet J Suppl*. 1990;9:20.
751. Seeherman HJ. Treadmill exercise testing: treadmill installation and training protocols used for clinical evaluations of equine athletes. *Vet Clin North Am Equine Pract*. 1991;7:259.
752. Leleu C, Cotrel C, Courouce-Malblanc A. Relationships between physiological variables and race performance in French Standardbred trotters. *Vet Rec*. 2005;156:339.
753. Evans DL, Rose RJ. Method of investigation of the accuracy of four digital-display heart rate meters suitable for use in the exercising horse. *Equine Vet J*. 1986;18:129.
754. Evans DL, Rose RJ. Cardiovascular and respiratory responses in Thoroughbred horses during treadmill exercise. *J Exp Biol*. 1988;134:397.
755. Evans DL, Rose RJ. Determination and repeatability of maximum oxygen uptake and other cardiorespiratory measurements in the exercising horse. *Equine Vet J*. 1988;20:94.
756. Rose RJ, Hendrickson DK, Knight PK. Clinical exercise testing in the normal Thoroughbred racehorse. *Aust Vet J*. 1990;67:345.
757. Evans DL, Harris RC, Snow DH. Correlation of racing performance with blood lactate and heart rate after exercise in Thoroughbred horses. *Equine Vet J*. 1993;25:441.
758. Rasanen, Lampinen KF, Poso AR. Responses of blood and plasma lactate and plasma purine concentrations to maximal exercise and their relation to performance in Standardbred trotters. *Am J Vet Res*. 1995;56:1651.
759. Vaihkonen LK. Hyyppa, Poso AR. Factors affecting accumulation of lactate in red blood cells. *Equine Vet J Suppl*. 1999;30:443.
760. Rainger JE, Evans DL, Hodgson DR, et al. Distribution of lactate in plasma and erythrocytes during and after exercise in horses. *Br Vet J*. 1995;151:299.
761. Poso AR, Lampinen KJ, Rasanen LA. Distribution of lactate between red blood cells and plasma after exercise. *Equine Vet J Suppl*. 1995;18:231.
762. Bayly WM, Shultz DA, Hodgson DR, et al. Ventilatory responses of the horse to exercise: effect of gas collection systems. *J Appl Physiol*. 1987;63:1210.
763. Christley RM, Evans DL, Hodgson DR, et al. Blood gas changes during incremental and sprint exercise. *Equine Vet J Suppl*. 1999;30:24.
764. Bayly WM, Hodgson DR, Schulz DA, et al. Exercise-induced hypercapnia in the horse. *J Appl Physiol*. 1989;67:958.
765. Christley RM, Hodgson DR, Evans DL, et al. Effects of training on the development of exercise-induced arterial hypoxemia in horses. *Am J Vet Res*. 1997;58:653.
766. Barrey E, Evans SE, Evans DL, et al. Locomotion evaluation for racing in Thoroughbreds. *Equine Vet J Suppl*. 2001;33:99.

PART 2

Disorders of Specific Body Systems

This page intentionally left blank

CHAPTER 8

Disorders of the Respiratory System

Elizabeth Davis*

Equine respiratory disease is the second most common disorder that limits performance in horses, ranked only behind musculoskeletal disease in importance.[1,2] Specifically, the metabolic requirements of the pulmonary system include the transfer of oxygen from inspired air to the arterial system for delivery to the tissues. Concurrently, the carbon dioxide generated during cellular metabolism must be delivered to the lungs by the venous system and exhaled. Pulmonary functional requirements increase dramatically during exercise, which is why horses are recognized as elite athletes, as evidenced by wide fluctuations in gas exchange capabilities during exercise. Even though the respiratory system is highly integrated with other body systems such as the musculoskeletal, central nervous, and endocrine systems, the respiratory system has been well recognized as the limiting factor in determining athletic performance. For this reason, even early, mild, or subclinical respiratory disease will reduce the performance capacity of horses. Therefore, it is ideal to identify horses that suffer from respiratory disease early in the course of events so that the clinician can provide specific and effective care that will enable a favorable prognosis to be given. This is facilitated by the fact that, from a diagnostic standpoint, many aspects of the equine respiratory tract are highly accessible and easily evaluated, and, when pathology is present, these conditions are often responsive to a variety of therapeutic modalities.

The primary defense of the respiratory tract includes a combination of nonspecific, generalized, and highly specialized protective mechanisms. Nonspecific protection is offered by anatomic organization, microbial, and nonspecific immunologic mechanisms. Anatomic protection is provided by the organization of the upper respiratory tract, nasal passages, lymphoid tissue, and pulmonary clearance mechanisms. For instance, when particulate material is inhaled, anatomic upper respiratory tract barriers provide a valuable mechanism for removal via local trapping at the level of the nasal respiratory mucosa. Particles that are suspended in the inhaled air are largely removed by turbulent air flow that redirects their movement toward the mucus-covered epithelial surface. Turbulence created in the airstream results from the conformation of the turbinates, trachea, and bronchi. The combination of turbulence and anatomic organization effectively removes particles as small as 5 μm, so that only particles less than this dimension reach the alveoli.

Once the material has passed this level of protection and has gained entrance to the proximal trachea the mucociliary escalator mechanism will offer protection through trapping, the coughing reflex, and expulsion of material from the airways. Respiratory system mucous secretion is a continual process that aids with the removal of airway debris and particles. Mucous flow moves from the bronchioles in a rostral direction toward the bronchi and trachea due to the wavelike motion of the ciliated respiratory epithelium, and a similar but opposite movement of material passes from the nasal passages toward the pharynx. The mucociliary escalator is highly effective based on the composition of the double layer of mucus that extends from the pharynx to the bronchioles. The particle-laden mucus is swallowed and presumably digested in the intestinal tract.

Respiratory mucous secretions are produced by airway goblet cells that line the airway mucosa. The mucous gel is highly effective at adsorption of soluble host molecules, which include host defensin peptides, cathelicidins, lactoferrin, lysozyme, and surfactant proteins.[3,4] The majority of microbial organisms that encounter this barrier are rapidly destroyed. Those particles that escape the mucociliary clearance mechanism will reach the alveoli and be cleared via phagocytosis by alveolar macrophages, which will also enter the mucociliary clearance mechanism.

There are four major surfactant proteins produced by alveolar type II cells in pulmonary fluid secretions: surfactant protein (SP)-A, SP-B, SP-C, and SP-D. SP-B and SP-C are highly hydrophobic, which provides for low surface tension within the alveoli, preventing lung collapse. In contrast, SP-A and SP-D are antimicrobial because of their hydrophilic nature. These proteins belong to the collectin family of antimicrobial peptides and provide an innate defense mechanism against pathogen challenge.[5] Their hydrophilic nature enables them to bind to the surface of pathogens via their carbohydrate moieties and act as opsonins. Additional immunologic properties provided by SP-A and SP-D include macrophage activation, facilitation of chemotaxis, enhancement of respiratory burst activity, and inflammatory cytokine expression. SPs facilitate apoptotic cell removal from lower airways, which is particularly important for the resolution of pulmonary inflammation. Failure of effective macrophage phagocytosis leads to the persistence of degraded or apoptotic neutrophils

* The editors and authors acknowledge and appreciate the contributions of Dorothy M. Ainsworth and Jonathan Cheetham as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

and cellular debris, which can serve as a nidus for persistent airway inflammation.

The respiratory tract contains an abundant arrangement of lymphoid structures with lymphocytes diffusely distributed throughout the lung and along the airways. Mucosa in the proximal larynx contains a large repertoire of T lymphocytes. Microfold (M) cells are located along lymphoid nodules as well as in association with nasal lymphoid tissues. The predominant immunoglobulin (Ig) molecule in this location is secretory IgA, with the greatest concentrations in the upper respiratory tract. Secretory IgA is bound to the mucous layer through the secretory component, which facilitates the clearance of adherent bacteria. More distal regions of the respiratory tract contain greater concentrations of IgG, particularly in the bronchioles and alveoli. An additional antibody molecule present in the upper respiratory tract includes IgE. Notably, IgA facilitates host protection through the process of immune exclusion, which prevents the entry of potential pathogens through the respiratory epithelium. In contrast, other antibodies typically provide effective immunity through the process of immune elimination, which aims to target the pathogen for destruction and removal through the process of opsonization.

Many resident cells can be found in the lower airways. In healthy horses approximately 50% of the cells in bronchoalveolar lavage fluid (BALF) are macrophages, whereas approximately 40% are lymphocytes and <5% are neutrophils. Based on this cellular composition (i.e., a preponderance of pulmonary T lymphocytes and alveolar macrophages), cell-mediated immune activation may be induced in the lower airways under certain stimulatory conditions. Also, horses are a species that expresses pulmonary intravascular macrophages (PIMs), which provide the equine host with the capacity for clearance of blood-borne pathogens, resulting in the lung potentially serving as a shock organ. For instance, if marked bacteremia or other pathogen challenges occur as blood passes through pulmonary capillary beds, PIM activation can contribute to marked systemic inflammatory response mechanisms.

Although the respiratory tract may be considered as a relatively sterile environment, in reality it contains abundant flora or a microbiome that provides an important endogenous protection barrier. Similar to other surfaces, the mucosa of the respiratory tract is far from a sterile surface because of the presence of the extensive microflora, which provides beneficial microbes aimed at maintaining microbial homeostasis. The symbiotic relationship provided by the diverse microflora or microbiota is well maintained in the healthy host. Disruption of this microbial population, damage to the normal clearance mechanisms, or mechanical epithelial damage may, however, result in altered microbial populations leading to pathogen proliferation or invasion.

The upper respiratory tract contains an abundant microflora of aerobic and anaerobic bacteria, which provides an essential competitive barrier to pathogen colonization.[3] When horses suffer from lower airway infection it is typically caused by colonization with an opportunistic organism from the upper airways. Bacterial isolates associated with the healthy flora of the upper respiratory tract include *Streptococcus* spp., *Pasteurella* spp., *Escherichia coli,* and *Actinomyces* spp. In addition to common aerobic isolates, anaerobic bacteria predominate in the healthy equine oral cavity, including *Bacteroides fragilis, Fusobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Veillonella* spp., and *Megasphaera* spp.[4] Although pathogenetic mechanisms associated with lower airway disease are complex and commonly multifactorial, risk factors that contribute to colonization of the lower airways with commensals from the upper respiratory tract and oral cavity include stress, long-distance transport, esophageal obstruction, and prolonged head elevation.[5-7]



FIG. **8.1** A 7-year-old Saddlebred mare presented for 2-year history of upper airway noise. Endoscopy revealed a subepiglottic cyst.

PATIENT ASSESSMENT

Signalment

Specific patient details may provide insightful information regarding the potential for certain diseases. Congenital or developmental disorders are more likely to manifest as clinical disorders in young animals. Examples of such congenital abnormalities include choanal atresia, subepiglottic cysts (Fig. 8.1), hypoplastic lungs, or expression of upper airway disorders associated with hyperkalemic periodic paralysis (HYPP). Acquired disorders such as infections with *Rhodococcus (Prescottia*[8]*) equi* or *S. equi* subsp. *zooepidemicus* are generally evident at 2 to 3 months of age.

Similarly, performance-associated disorders such as epistaxis with short-burst breezing or barrel racing may increase the index of suspicion for exercise-induced pulmonary hemorrhage (EIPH), whereas having historical information that includes awareness of indoor patient housing during adverse weather conditions in association with the development of cough in a riding horse will make allergic airway inflammation more likely.

Diagnostic Considerations

When the initial examination is conducted it is imperative that the clinician collect a complete and pertinent history, although there may be extraneous factors that influence the presenting complaint. Historical information combined with a thorough evaluation at the time of presentation should aid the clinician in determining the source of the problem. Diagnostic examination of the respiratory tract can be achieved with a variety of modalities that include (but are not limited

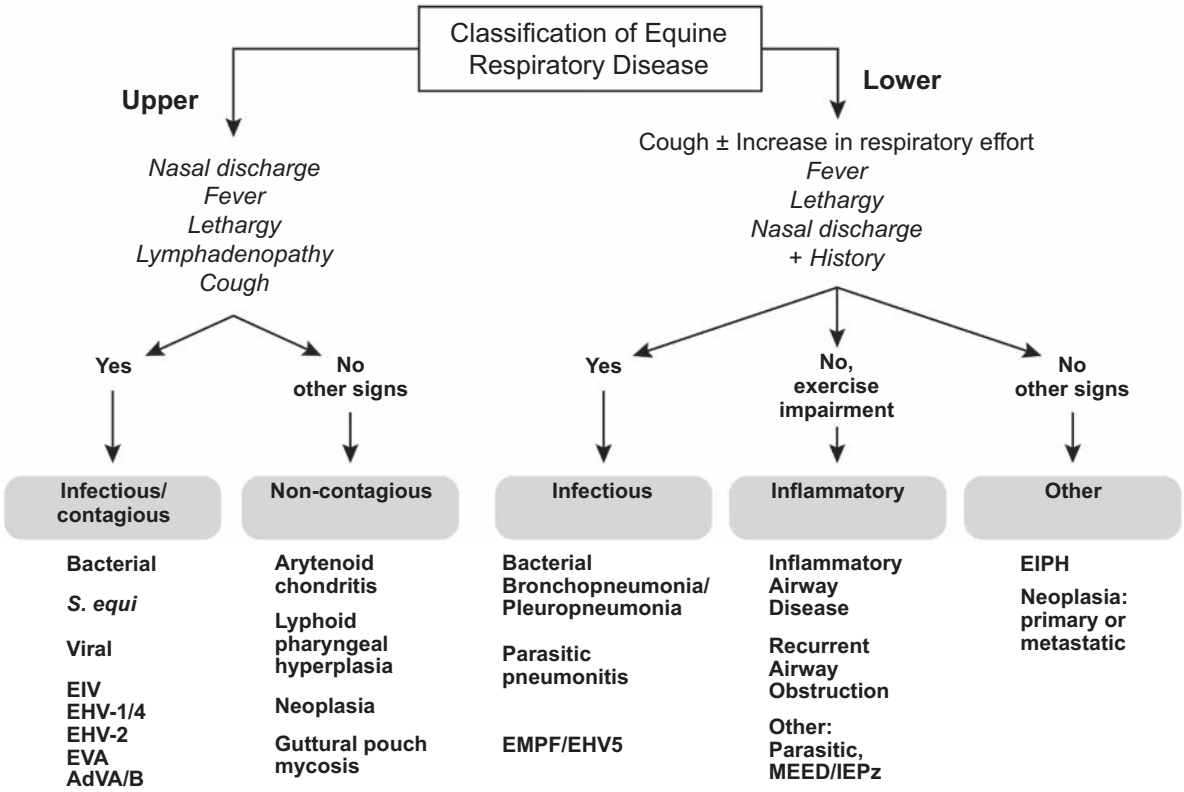


FIG. **8.2** Flowchart diagram differentiating various classifications of equine respiratory diseases. *EHV,* Equine herpesvirus; *EIV,* equine influenza virus; *EMPF,* equine multinodular pulmonary fibrosis; *EVA,* equine virus arteritis.

to) endoscopy, ultrasound, radiography, and respiratory secretion evaluation using transtracheal aspiration and/or bronchoalveolar lavage (BAL) for the collection of samples for culture or cellular analysis, respectively. Advanced imaging techniques include computed tomography (CT) or magnetic resonance imaging (MRI), which may be used for the examination of the upper respiratory tract in adult horses or the entire respiratory tract in small horses/ponies or foals.

Respiratory diseases in horses are typically categorized as upper or lower airway diseases and infectious or noninfectious. Some authors prefer to categorize these conditions according to those that are contagious and noncontagious, as well as infectious and inflammatory or other independent disorders. One way of classifying these disorders is reflected in Fig. 8.2.

Physical Examination

Physical examination should commence with the examination of respiratory rate, effort, and pattern at rest. The clinician should observe the horse in a natural, relaxed environment. It is ideal if the clinician can observe the horse from various angles to specifically observe the inspiratory and expiratory efforts, evidence of nostril flare, abdominal push, or other alteration from normal respiratory parameters. The normal respiratory rate is 8 to 12 breaths per minute. Anxiety, pain, acidosis, hyperthermia, and stress are among the more common reasons that horses can have an increase in respiratory rate. Therefore, the clinician must examine all facets of the patient's status before making the diagnosis of primary respiratory disease.

Clinical signs consistent with respiratory distress in horses include flared nostrils, anxious facial expression, and extended head and neck. The phase of respiration that is abnormal typically aids the clinician with insight into the origin of the respiratory tract disorder. Obstructive and restrictive airway diseases can generally be distinguished by differences in respiratory efforts. Marked inspiratory difficulty, particularly in combination with an audible noise, is consistent with obstructive upper airway disease. The very low airway pressure of the upper respiratory tract is exacerbated during inspiration, which is caused by the movement of soft tissue structures into the lumen in a dynamic manner, exacerbating airway obstruction of the upper respiratory tract. Conversely, a prolonged expiratory phase of respiration, combined with a marked expiratory respiratory effort, provides the clinician with evidence of lower airway obstruction. During expiration, intrathoracic pressure is positive, which narrows the diameter of small airways. If disease includes smooth muscle contraction, mucous production, and increased airway cellularity, these conditions collectively contribute to the development of lower airway obstruction.

Physical examination of the respiratory tract begins with the head. Observation of the character, frequency, and lateralization of nasal discharge may reflect the origin of the disease. Inspiratory noise is characteristic of upper airway obstruction. Maxillary sinusitis may present with unilateral discharge, facial deformity, and/or epiphora. Rostral components of the respiratory tract should be carefully examined for nostril flare, nasal discharge, and appropriate air flow. Nostril flare is not specific for any condition because it may be observed in association with either upper or lower airway disease. However, nostril flare observed in combination with prolonged inspiration and audible noise justifies concern regarding possible

upper airway obstruction. Sounds originating from the upper respiratory tract are classified as stridor, roar, or snore. Stridulous respiratory sounds are characterized by high-pitched inspiratory noise. Diseases characterized by inspiratory stridor include bilateral laryngeal paralysis, a marked pharyngeal or retropharyngeal mass or lymphadenopathy/abscess, arytenoid chondritis, and pharyngeal collapse as observed in cases of Quarter Horses with HYPP. Roaring is a very distinctive noise that occurs when exercising horses suffering from left laryngeal hemiplegia make a low-pitched noise. These horses do not make any audible noise at rest. Unilateral nasal passage obstructions lead to the development of snoring noise. On physical examination, these horses will be noted to have asymmetric airflow through their nostrils and accordingly develop a low-pitched noise that originates from the obstruction in the nasal passages. An exercise-associated low-pitched fluttering sound can be heard with dorsal displacement of the soft palate (DDSP). This might be associated with exercise intolerance, particularly in horses like Thoroughbreds that work at high speed. In the majority of cases, the origin of the airway noise can be successfully identified with standing and/or dynamic endoscopy.

EXAMINATION OF THE LOWER RESPIRATORY TRACT

Examine the horse during eupneic and hyperpneic (by use of a rebreathing bag) breathing. Normal breath sounds are those produced by turbulent air movement through the tracheobronchial tree and vary in intensity and quality depending on the portion of the lung field auscultated. Vesicular sounds, heard over the middle and diaphragmatic lung lobes, are the quietest. Bronchial sounds, which are audible over the trachea and the base of the lung, are the loudest. In the healthy horse, one can hear breath sounds more easily on the right side than on the left. Considerable variation exists among healthy patients in the intensity of the breath sounds. For example, vesicular sounds are often barely audible during normal breathing, whereas in the obese patient they have been described as soft rustling sounds. One may hear breath sounds more easily in a thin or young animal because of less attenuation of lung sounds by the chest wall. The intensity of breath sounds also increases with increased airflow. Thus breath sounds are accentuated in febrile or excited animals or in animals hyperpneic from a variety of causes (exercise, hypoxia, and pain). However, auscultatory findings do not always correlate well with the degree of alveolar ventilation.[9] For example, in horses with pulmonary consolidation, air movement through large airways adjacent to areas of pathology inappropriately gives the examiner the indication that a particular pulmonary region is well ventilated. Breath sounds also may become more difficult to hear in cases of alveolar overinflation in which the aerated tissue of the lung is a poor conduction medium for sound or pneumothorax and pleural effusions in which the sound is reflected at the pleural surface (acoustic impedance).[9] Adventitious pulmonary sounds are abnormal sounds superimposed on the normal breath sounds and are described as crackles or wheezes. Crackles are short, explosive, and discontinuous sounds that are consistent with the sound of cellophane being crumpled. They are usually of low intensity and are audible during the inspiratory phase of respiration. Their production has been attributed to the sudden equalization of pressure in two compartments after airways have reopened. Crackles are often audible in horses with pneumonia, pulmonary edema, or recurrent airway obstruction (RAO). Breathing 100% oxygen also may produce crackles because the nitrogen stent maintaining alveolar distention is eliminated.[10] Crackles are also audible in horses with subcutaneous emphysema. Wheezes are high-pitched musical sounds thought to arise from the vibration of airway walls or tissue masses in close contact with the airway walls and may be audible during inspiration or expiration. Pleuritic friction rubs are sandpaper-like sounds generated by visceral and parietal pleural movement. Percussion of the thorax is accomplished by evaluating the types of sounds produced as one systematically taps the intercostal spaces (ICS) of the thorax using a plexor and pleximeter (foals) or a large spoon and neurologic hammer (adults). Aerated tissues produce a resonant sound, whereas fluid-filled structures (bowel, heart, lung abscesses, and consolidated lung) produce a dull sound. The examiner should identify the transitional site in which the sound quality changes during percussion to establish the boundaries of lung fields. Defining caudal lung borders with thoracic percussion is consistent with limits identified by thoracic ultrasonography.[11] Under normal conditions performing thoracic percussion should not result in pain or discomfort to the patient; if the horse appears uncomfortable or distressed this may indicate pleural pain or the presence of rib fractures. Percussion that reveals ventral dullness suggests pleural effusion, pleural thickening, lung consolidation, masses, or pericardial effusion. Occasionally, caudal borders may be expanded, suggesting alveolar overinflation, which may accompany recurrent airway disease.

Endoscopy

Endoscopic examination is a cost -efficient diagnostic modality that enables the clinician to visualize many critical structures of the upper and lower respiratory tract. Horses are tolerant of endoscopic examination and in some instances do not require sedation, which may be highly desirable when examining laryngeal function. In some clinical settings light to moderate sedation provides the clinician with the ability to effectively complete a thorough endoscopic examination. Endoscopic examination of the respiratory tract provides a diagnostic tool that can be easily adapted to a variety of equine patients, regardless of size or age. The broad utility of endoscopy is extremely helpful to equine clinicians to diagnose DDSP, epiglottic entrapment, rostral displacement of the palatopharyngeal arches, arytenoid chondritis (Fig. 8.3), tracheal collapse or stenosis, and pharyngeal disease that may include narrowing (Fig. 8.4). Developmental or congenital abnormalities may be identified with upper airway endoscopy such as cleft palate, subepiglottic cyst (see Fig. 8.1), or choanal atresia. When exudate or hemorrhage is present, endoscopy can help determine its origin. Examples include guttural pouch mycosis, guttural pouch empyema, progressive ethmoidal hematoma, retropharyngeal lymphadenopathy, pulmonary hemorrhage (Fig. 8.5), or exudate or mucus (Fig. 8.6).

Examination should include tracheal examination and a thorough examination of the lower airways; particular areas of interest will include the mucosa and the presence of exudate or masses. Diagnostic sampling may include a collection of tracheal or bronchial secretions, depending on the purpose of sampling. Tracheal secretion collection is most appropriate for culture purposes, whereas BAL washing of distal airways can be performed for examination of lower airway cellularity.

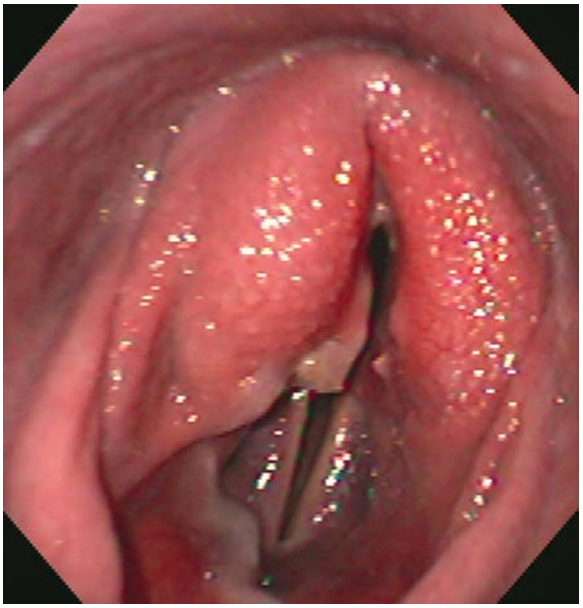


FIG. **8.3** A 24-year-old Paint mare presented for respiratory distress characterized by an increased inspiratory effort. Upper airway endoscopy revealed arytenoid chondritis. Right-sided chondritis is present with right-sided paralysis. Note the exudate exiting from the right arytenoid. Airway distress resulted from severe airway inflammation and near complete airway obstruction. (Video is available in this textbook's website video library.)



FIG. **8.4** Upper airway endoscopy of this 14-year-old Thoroughbred gelding presenting for abnormal whinny and roaring noise with exercise. Marked lymphoid hyperplasia was identified and determined to be caused by neoplastic lymphoid tissue diagnosed as B-cell lymphoma.

In instances where endoscope sterility is in question, transtracheal wash (TTW) is ideally performed using a midcervical, percutaneous approach to avoid the potential for inadvertent bacterial contamination (the TTW procedure is described later).

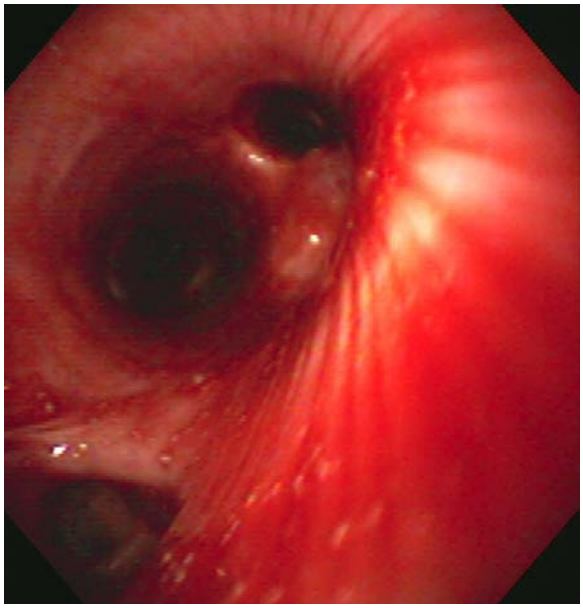


FIG. **8.5** Marked tracheal hemorrhage originating from the lower airway in a horse suffering from exercise-induced pulmonary hemorrhage.

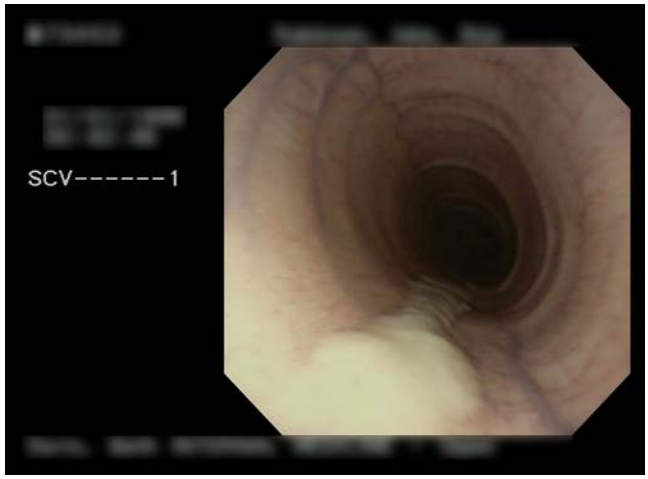


FIG. **8.6** A 5-year-old Holsteiner mare presented for the complaint of cough at the beginning of exercise. Airway endoscopy revealed marked tracheal exudate along the length of the trachea.

Videoendoscopic examination of the upper respiratory tract while on a high-speed treadmill and the use of dynamic endoscopy while the horse is ridden under saddle have become common methods to evaluate a variety of airway disorders. Although many abnormalities associated with the upper respiratory tract can be identified at rest, some conditions are ideally diagnosed with the use of dynamic evaluation while under saddle or on a high-speed treadmill.[12,13]

Radiography

Conventional radiography of the equine skull can be diagnostic under routine conditions of dental disease, the presence of a mass, fluid lines, or fracture. However, equine skull radiography may be challenging to interpret because of the multiple interfaces of radiographic densities and highly complex

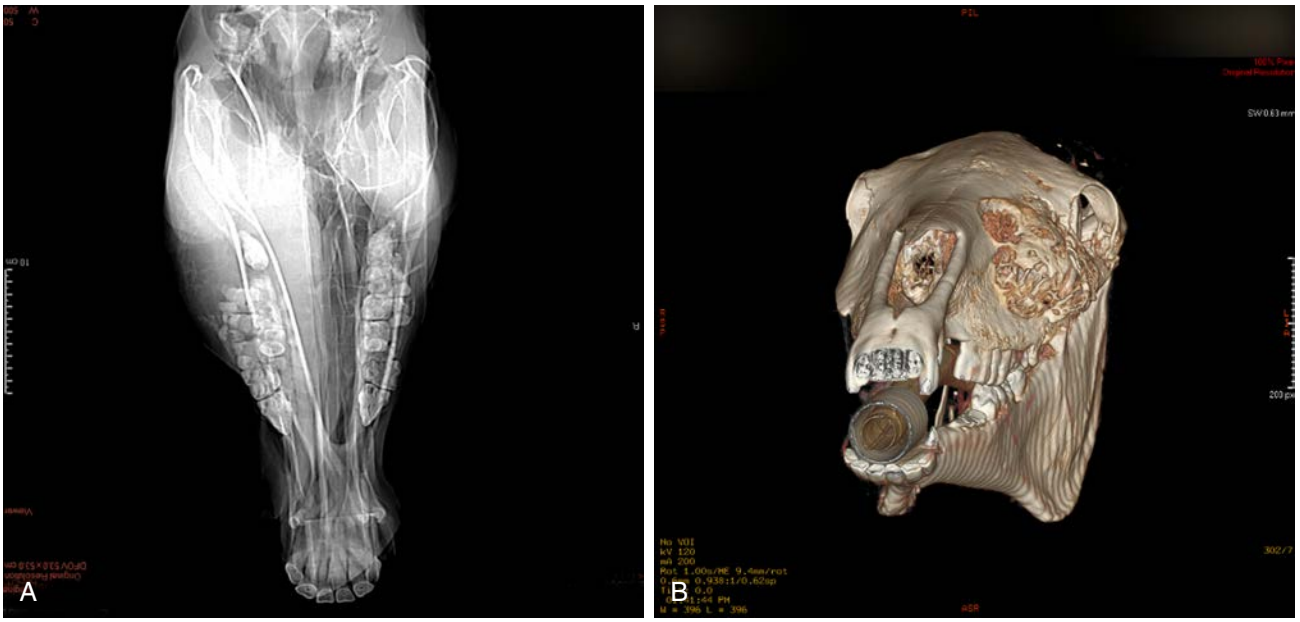


FIG. **8.7** A 31-year-old Arabian gelding presented for a left-sided mass that was rapidly progressive in nature. Although a dorsoventral radiographic imaging provides substantial evidence for a soft tissue mass (A), CT imaging with reconstruction provides remarkable detail regarding the nature and extent of the mass (B).

anatomy of detailed structures. Although examination and conventional radiography may provide some insight into disease origin, additional imaging modalities are often required to fully assess structures in the equine head. CT is a modality that lends itself to detailed imaging of complex structures, particularly when pathology is present.[14] From a methodological standpoint, CT uses a rotated highly collimated radiographic x-ray beam to generate digital cross-sectional images.[15] Collectively, these serial cross-sectional images provide the clinician with greater information and detail compared with conventional radiography alone (Fig. 8.7). (Note: Video is available of this series.) Some limitations of advanced imaging include cost and requirement for general anesthesia. However, CT imagining of the equine head continues to become more common, and in many instances the actual image capture time is shorter than compared with conventional radiographic imaging. Additional evidence to support the use of CT for the equine skull includes complex structures such as nasal turbinates, paranasal sinuses, dentition,[16] nasopharynx, larynx, and guttural pouches.

MRI is an additional imaging modality that can be used to investigate upper respiratory tract disorders. Magnetic resonance images represent a map of tissue protons that, under the influence of a magnetic field, are excited by brief pulses of radiofrequency waves.[15] As the magnetized excited protons relax back to their original magnetized state, a signal is emitted, the intensity of which is converted to a grayscale image. MRI provides excellent cross-sectional evaluations of soft tissue structures, although it also provides useful information about bony structures. Contrast enhancement can be achieved by injecting an intravenous (IV) derivative of gadolinium during the T1-weighted scans.[15] Recent evidence supports the utility of MRI for use in horses. There is a favorable correlation with imaging studies and those identified with surgery or postmortem examination. The increasing availability provides support for the application of this diagnostic modality in equine patients with structural diseases involving the head.[17]

LOWER RESPIRATORY TRACT DIAGNOSTIC SAMPLING

Tracheobronchial Aspiration

Transtracheal aspiration is a routine diagnostic procedure performed in equine respiratory disease workup. The primary indication for performing transtracheal aspiration is to identify pathogenic bacteria colonizing the airway in horses suffering from bronchopneumonia. Following routine sedation and sterile preparation, local subcutaneous anesthetic infiltration is administered to facilitate the procedure. A 3- to 5-mm stab incision is made vertically through the skin and subcutaneous tissue using a no. 15 surgical blade. A TTW trocar is inserted perpendicular to the skin between the tracheal rings, while manually stabilizing the trachea. After penetration of the tracheal lumen, the trocar is removed from the sheath, and the sheath is inserted down the trachea. A lavage catheter is introduced through the sheath into the airway to the level of the thoracic inlet. Approximately 30 to 50 mL of warmed, isotonic crystalline solution is instilled into the tracheal lumen and immediately aspirated. Samples can be used for Gram stains and prepared for aerobic and anaerobic culturing.

Bronchoalveolar Lavage Sampling

BAL can be performed through a 3-m endoscope or using a Bivona (equine BAL) cuffed tube designed specifically to sample the equine lower airway. Routine sedation is administered in combination with appropriate restraint procedures. The BAL tube is initially primed with sterile 0.9% saline containing 2% lidocaine. After entering the nasal passage, the BAL tube is placed in the ventral meatus and moved caudally into the trachea and passed to the lower airways until it is wedged into place at approximately the third- to fourth-generation bronchi. Once wedged into the third- to fourth-generation bronchi, the cuff is fully inflated and the tube is instilled with 200 to

TABLE **8.1 Differential Counts in Bronchoalveolar Lavage Fluid***

Neutrophils	Macrophages	Lymphocytes	Eosinophils	Mast Cells	Epithelial Cells
A 8.9 ± 1.2	45.0 ± 2.8	43.0 ± 2.7	± 1.0	1.2 ± 0.3	3.5 ± 0.7
B 5.0 ± 4.0	72 ± 10	18 ± 3.0	2.0 ± 4.0	1.0 ± 1.4	—
C 6.2 ± 5.0	70.3 ± 15.2	7.6 ± 3.9	1.0 ± 1.4	0.6 ± 1.4	14.3 ± 13.4
D 6.2 ± 2.4	48.5 ± 2.5	35.3 ± 2.5	2.5 ± 0.9	5.2 ± 0.8	2.3 ± 1.4

*Percent of total white blood cell count plus-or-minus standard error or standard deviation.
Data from (A) Derksen FJ, Brown CM, Sonea I, et al.: Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease, *Equine Vet J.* 21:23, 1989; (B) Fogarty U: Evaluation of a bronchoalveolar lavage technique, *Equine Vet J.* 22:174, 1990; (C) Mair TS, Stokes CR, Bourne FJ: Cellular content of secretions obtained by lavage from different levels of the equine respiratory tract, *Equine Vet J.* 19:458, 1987; (D) Deegan E, Beedle RE, editors: *Lung function and respiratory diseases in the horse.* Stuttgart, Germany, 1986, Hippiatrika.

500 mL of sterile, physiologic solution. After instillation, fluid is aspirated with gentle but constant pressure. Marked suction should be avoided to minimize the chance for iatrogenic trauma and associated blood contamination, because blood contamination into the BAL fluid can complicate cytologic interpretation. In addition, horses with marked lower airway inflammation will be more likely to collapse their bronchi; thus care should be taken to avoid exuberant aspiration.[18,19] After completion of the sample collection (approximately 50%–70% recovery of instilled fluid) the cuff is deflated and the tube removed. Sample collection of BALF provides valuable diagnostic information in horses suffering from lower airway inflammation. Representative cytologic findings from bronchoalveolar lavage studies of normal horses are shown in Table 8.1

Thoracocentesis

Pleural fluid accumulation occurs in horses with pleuropneumonia, penetrating wounds that enter into the thoracic cavity, thoracic neoplasia, and hydatid cysts.[20] Percutaneous sampling of pleural fluid is performed for diagnostic and therapeutic purposes. Ideally the anatomic location selected for thoracocentesis is based on ultrasonographic identification of the largest and most ventral location of pleural fluid accumulation. Ventral location is particularly important when an indwelling thoracic drain is being placed, so that the greatest volume of fluid can be evacuated from the pleural space. Care should be taken to identify the location of the diaphragm and heart, noting that large-volume fluid can sometime result in caudal displacement of the heart. Skin preparation generally involves routine clipping and aseptic preparation. The skin and periosteum of the rib are locally blocked with 2% lidocaine. A small stab incision is made for placement of a teat cannula or indwelling thoracic drain. Following placement of the stab incision, a thoracic tube with trocar can be placed to facilitate drainage of pleural fluid. When fluid is drained from the pleural space, large-volume fluid should be removed slowly with concurrent administration of IV, isotonic fluid therapy. Rapid and large-volume pleural fluid drainage may lead to patient decompensation caused by re-expansion hypotension and potential development of pulmonary edema.[21,22] Concurrent IV fluid therapy will aid with maintenance of physiologic status. If the patient appears weak or anxious, it is recommended to periodically clamp off the chest tube while continuing to administer IV fluids to facilitate a stable patient status. When evacuating pleural fluid early in the course of disease or in conditions with low fibrin accumulation, fluid may be removed unilaterally yet effectively from both hemithoraces because horses do not have a complete mediastinum. However, with marked fibrin accumulation, it is unlikely that fluid evacuation from an individual side will successfully remove fluid from the contralateral hemithorax, necessitating bilateral indwelling thoracic tube placement. Ultrasonographic examination will enable the clinician to determine the necessity and frequency of fluid evacuation and thoracic tube placement. Once the indwelling thoracic tube has been placed, a one-way (Heimlich) valve can be placed on the end to facilitate one-way flow of fluid from the pleural space with protection to avoid ascending introduction of microorganisms into the pleural space (Fig. 8.8).



FIG. **8.8** The use of a one-way Heimlich valve placed on the end of an indwelling thoracotomy tube to provide effective elimination of pleural fluid while concurrently avoiding ascending contamination of the pleural space with room air or microbial contaminants.

If ultrasound imaging is not available and a blind approach to thoracocentesis is selected, landmarks should include the sixth or seventh ICS on the right hemithorax and the seventh

or eighth ICS on the left hemithorax. Sampling should be performed approximately 10 cm dorsal to the olecranon, dorsal to the location of the costochondral junction.

Thoracic Ultrasonographic Examination

Ultrasonographic imaging of the equine thorax is a routine diagnostic procedure. Thoracic ultrasonography reveals valuable information about the lung and pleural cavity. Examination will aid the clinician with the assessment for potential pulmonary consolidation, atelectasis, pleuropneumonia, abscess formation, masses, and integrity of the diaphragm.

Patient preparation should include cleaning the surface of the hair coat; ideally the area to be imaged should be shaved with surgical no. 40 clipper blades.[23] In some instances this is not conducive to patient management or the horse may have a very fine hair coat, and application of coupling gel or topical isopropyl alcohol will be sufficient to allow image acquisition.

In a healthy horse, pulmonary fields can be imaged from both sides of the thorax from just below the epaxial musculature to the area in which the lung fields join the diaphragm. The ventral border of the lung field is usually identified as a sloping line that originates at the level of the tuber coxae at the 17th ICS, then at the level of the ischial tuberosity at the 15th ICS, and at approximately the level of the point of the olecranon at the 5th ICS.[24] Lung fields can easily be imaged from the 5th to the 16th to the 17th ICS, bilaterally. Pulmonary fields can also be imaged in the more cranial lung fields at the third and fourth ICS, but in these locations the triceps musculature is superficial to the lung fields, so probe depth must be adjusted to account for the focal point of interest. The initial lung scan should be performed with a 5-mHz probe, with the depth set at approximately 10 to 15 cm.[25] If more superficial imaging is required, then a 7.5- to 10-mHz probe is indicated to improve image clarity. The depth setting for a higher frequency probe will need to be adjusted to approximately 5 to 10 cm. Higher frequency probes are most appropriate for imaging the foal thorax, including young neonates through weanling-aged individuals.[26] Adult full-sized horses with a particularly thick body wall may require a lower frequency setting to obtain a diagnostic quality image, and probe selection settings may be ideal at the 2.5- to 3.5-mHz range. Additionally, horses with severe pulmonary disease, such as a marked pleuropneumonia, may require an increased depth setting at 25 to 35 cm so that the full area of pathology can be effectively examined. Typically sector scanners will provide optimal imaging ability, because the head of the transducer is small and can be placed in the ICS of most horses. Limitations exist when using linear-array transducers because the depth is fixed at 10 cm with some instruments and the entire probe is not in contact with the skin surface, limiting the quality of the examination. The lung fields should be scanned in the 3rd (right side) or 4th (left side) through the 17th ICS, moving in a dorsal to ventral direction and moving cranial to caudal to ensure that all areas of the lung fields have been examined thoroughly.[26] The probe should be carefully placed in the ICS to avoid inadvertent acoustic shadowing from ribs as this will impair image assessment.

Thoracic Radiography

Thoracic radiography is a valuable component of patient evaluation often used in combination with other diagnostic modalities such as thoracic ultrasound. Standard thoracic radiography will require a 1000-mA, 150-kV unit to successfully obtain diagnostic-quality images.[27] A series of three to four overlapping lateral projections are generally required to successfully obtain the entire thorax. The routine focal spot to film distance is approximately 100 cm. The thoracic cavity of foals may be successfully imaged with portable radiographic equipment. Thoracic radiography provides superior diagnostic information compared with ultrasound when the aim of diagnostic testing is to examine pulmonary parenchymal lesions, mediastinal structures, and thoracic wall abnormalities. Specific clinical indications for thoracic radiographic imaging include patients with pulmonary disease that is not responsive to standard therapy; severe respiratory distress; thoracic trauma; respiratory disease in combination with weight loss, aspiration, or other forms of bronchopneumonia; intrathoracic esophageal disease; or suspected diaphragmatic rupture.[27] Pulmonary abscesses, pulmonary consolidation, interstitial pneumonia, pneumothorax, mediastinal lymphadenopathy, and pulmonary fibrosis are conditions in which thoracic radiography is indicated and will provide additive diagnostic information when combined with thoracic ultrasonography.[26,27] Structures located deeper than the superficial pulmonary surface and those that involve the pulmonary parenchyma cannot be examined in complete detail with ultrasound alone.

Four radiographic patterns are described for thoracic radiographic imaging and include interstitial, alveolar, bronchiolar, and vascular. Although classification of radiographic pattern can provide the clinician with insight as to the primary disease etiology, none of the radiographic patterns are pathognomonic for specific disease, and many times overlapping patterns are observed. Finding characteristic radiographic changes will aid in formulating and possibly narrowing the list of differential possibilities. Among the patterns observed in equine patients, similar to other species, the interstitial pattern is the most commonly identified. Changes within the interstitial tissue lead to an increased background opacity identified on radiographs. Although an increased interstitial pattern is characteristic for older horses and for this reason is sometimes considered a normal finding, marked change or interstitial disease in a young horse is abnormal. An interstitial pattern is often identified in the early course of disease and is very non-specific. Interstitial changes may be observed early in the course of infectious pneumonia, pulmonary neoplasia, cardiogenic-associated pulmonary disease, and allergic airway diseases. Nodular interstitial disease represents cellular infiltrative disease, with the most severe form represented by metastatic neoplasia or fungal pneumonia (Fig. 8.9). An alveolar radiographic pattern is seen as patchy and often with soft tissue dense areas that impair the ability to see vascular structures and airways. The characteristic feature of the alveolar pattern is the presence of air bronchogram(s), which characterize small airways. An air bronchogram is seen as a branching lucency in which the airway walls are not visible, yet the pulmonary fields are opaque because of the presence of fluid-filled alveoli. Air bronchograms are observed in patients with pulmonary edema, hemorrhage, consolidation, or atelectasis. Identification of an air bronchogram in a particular distribution will aid in establishing the differential diagnosis with a cranioventral distribution identified in association with bronchopneumonia (Fig. 8.10), whereas a caudodorsal distribution may represent cardiogenic pulmonary edema, hemorrhagic disease, or hematogenous dissemination and

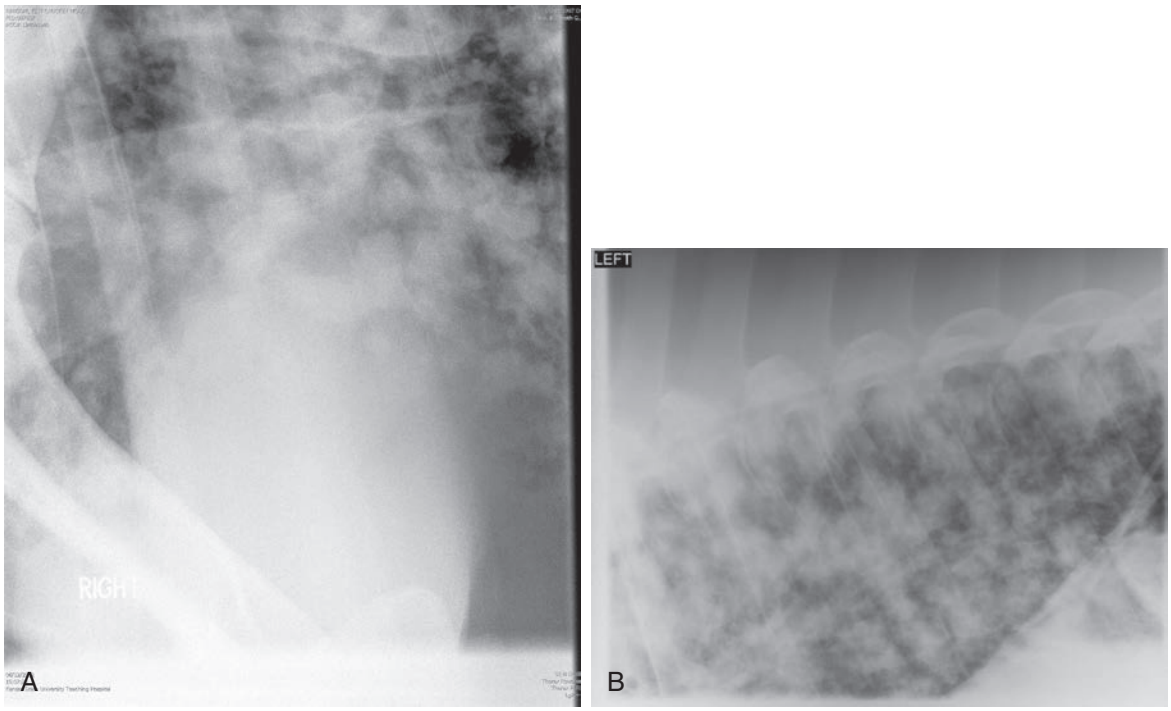


FIG. **8.9** A 15-year-old Thoroughbred gelding with the complaint of weight loss and the diagnosis of equine multinodular pulmonary fibrosis. (A) Nodular interstitial radiographic pattern in a ventral thoracic radiograph centered over the caudal cardiac silhouette. (B) Caudodorsal view of the nodular interstitial radiographic pattern.



FIG. **8.10** A 20-year-old Arabian gelding with the complaint of respiratory distress with acute onset following the administration of mineral oil for colic signs. Note the cranioventral alveolar pattern represented by air bronchograms caused by marked pulmonary fluid accumulation in association with severe mineral oil aspiration pneumonia.

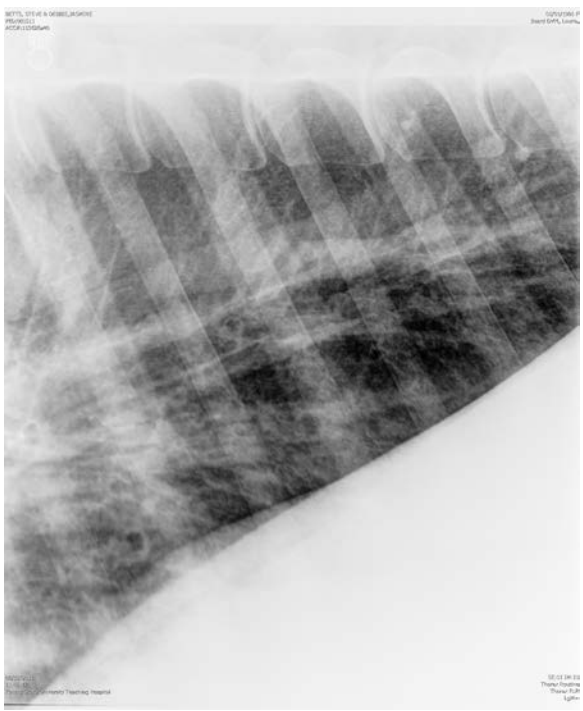


FIG. **8.11** A 15-year-old Arabian mare with the complaint of respiratory distress evidenced by nasal flare and increased expiratory effort. Note the marked bronchointerstitial pattern diffusely present in all lung fields. A marked bronchiolar component is consistent with the presence of chronic peribronchiolar inflammation in association with recurrent airway obstruction.

sepsis. Thickening of the airway results in the identification of a bronchiolar pattern (Fig. 8.11). This represents severe disease of the airways, resulting in thickening of the bronchiolar structures with increased visibility of the bronchi. Increased opacity of the bronchial walls may result from peribronchiolar infiltration or from intraluminal exudate, which is

sometimes referred to as a diphtheritic membrane. Classification of a bronchiolar pattern can be categorized based on sharply defined bronchi or more ill-defined thickened walls termed a *peribronchiolar pattern*. A bronchiolar pattern represents chronic disease with mineralized changes within the bronchiolar wall. In contrast, a peribronchiolar pattern indicates inflammatory disease that surrounds the bronchi, which may develop early in the course of bronchopneumonia or following chronic allergic airway disease such as RAO. The least common pattern is the vascular pattern characterized by a pronounced vascular pattern that mainly occurs following pulmonary overcirculation in association with a left-to-right shunt.

Pulmonary Function Testing

Measurements of mechanical properties of the respiratory system, airway reactivity, and arterial blood gas determinations have been used to determine pulmonary function in horses and are generally available only at select referral centers. Conventional techniques of pulmonary function testing require the horse to wear a breathing apparatus that measures airflow, and pleural pressure changes are measured by an esophageal balloon, which is connected to a pressure transducer. The integration of overall airflow as it relates to time provides a flow rate that aids in the measurement of inspiratory and expiratory volume. Additional parameters obtained using this method of pulmonary function assessment provide inspiratory and expiratory time, respiratory frequency, and peak airflow. From a practical standpoint, the simple measurement of tidal volume, breathing frequency, or minute volume (= tidal volume × breathing frequency) provides limited information regarding the pulmonary function because these values are extremely well maintained until severe respiratory disease leads to marked pulmonary impairment.[28,29]

Measurements of lung movement defined as compliance and airway obstruction characterized by pulmonary resistance provide meaningful information regarding overall pulmonary function. Dynamic compliance (C_{dyn}) is calculated by dividing the tidal volume by the change in pleural pressure occurring at the initiation and completion of inhalation. Pulmonary resistance can be measured by several different techniques, depending on whether the measurement is resistance at peak airflow or at specific ventilatory volumes (i.e., isovolumetric; e.g., 50% tidal volume), which is then divided by the change in pleural (esophageal balloon) pressure over the same interval. Changes in these measurements can provide information regarding the physiologic impact of the pulmonary disease. Horses that suffer from obstructive airway disease of the tracheobronchial tree have reduced dynamic compliance with increased pulmonary resistance. Reduced dynamic compliance without a change in pulmonary resistance indicates that the pulmonary parenchyma is stiffened by alveolar disease or by obstruction of the peripheral bronchioles. Notably, because of the marked surface area of peripheral bronchioles, there will be little change in overall lower airway function until severe, end-stage disease is present. The remarkably large cross-sectional area has minimal impact on the overall resistance of breathing until the disorder is well advanced. Conversely, increased pulmonary resistance without a change in dynamic compliance indicates that the obstruction exists in the respiratory tract, but is does not make a distinction between upper airway, trachea, or bronchus.[28]

An alternative and less invasive technique for measuring pulmonary function uses the forced-oscillation method (or its modification, the impulse-oscillation method). For this method of testing, the horse is fitted with an airtight face mask while an external test signal is imposed on the horse's breathing efforts that generate pressure-flow responses from the respiratory system. The mechanical properties derived from this technique over a wide range of frequencies include total respiratory system impedance (Z_{rs}), respiratory system resistance (R_{rs}), and respiratory system reactance (X_{rs}).[30,31] The impedance represents the *impediment* that the system presents to oscillatory flow and consists of R_{rs} (resistive properties of the respiratory system) and X_{rs} (elastic and inertive properties of the respiratory system).

Measurements of airway hyperresponsiveness are obtained in combination with pulmonary function testing.[32-35] This technique uses a dose of a nebulized bronchoconstrictor agent, such as histamine or methacholine, which causes a 35% increase in baseline R_{rs} or a 35% decrease in baseline lung compliance. The dose to achieve this value in a horse with inflammatory airway disease (IAD) or RAO is considerably less than the dose needed to induce airway reactivity in a healthy horse. Histamine bronchoprovocation testing requires that the horse be sedated and outfitted with an airtight breathing mask. Laboratory assistants must wear protective masks to prevent inadvertent inhalation of the bronchoconstricting agent, which could cause harm to personnel.

Pulmonary function testing of exercising horses and its use in assessing poor athletic performance remains a valuable diagnostic approach offered at select referral centers. With the availability of high-speed treadmills and the ability to measure airflow during exercise,[36] analysis of tidal volume, breathing frequency, dynamic compliance, lung resistance, end-expiratory lung volume, and flow-volume and pressure-volume loops continue to provide additional insights into the cause of the poor performance, the recognition of expiratory flow limitation, and the influence of EIPH on respiratory mechanics.

Lung Biopsy

A histopathologic diagnosis may prove useful in the therapeutic management of certain lung disorders. Percutaneous lung biopsy has been used to investigate disorders characterized radiographically by a pulmonary miliary pattern and disorders for which radiographic or ultrasonographic results are compatible with pulmonary neoplasia or granuloma. A percutaneous approach was originally described,[37] although methods used have been modified because of the routine use of ultrasound during sample collection. Following routine sedation biopsy locations are selected after ultrasonographic confirmation of landmarks (heart and diaphragm) and assessment of tissue accessibility. The biopsy instrument is aseptically advanced through the ICS (cranial to the rib) to the depth of the desired tissues, and samples are obtained for culture and for fixation in 10% formalin. In a study comparing biopsy instruments (manual Tru-Cut versus automated biopsy needle), the investigators found airway bleeding developed in approximately one third of the cases sampled with the manual device and one tenth of the cases biopsied using the automated instrument.[38] This technique is not recommended in patients that are tachypneic, are in respiratory distress, exhibit uncontrollable coughing, or have bleeding disorders. It is not indicated in horses with pulmonary

abscess formation, pleuropneumonia, or pneumonia.[39] In a review of percutaneous lung biopsies performed in 66 horses, this technique yielded a definitive antemortem histologic diagnosis of pulmonary disease in 82% of cases.[40] In the same study, no complications were recognized in 91% of horses sampled. The most common complications observed with lung biopsy are coughing, epistaxis, pulmonary hemorrhage, tachypnea, and respiratory distress. Hemothorax or accidental sampling of abdominal organs (liver and stomach) may occur.[38,40] When horses in respiratory distress are sampled using an automated instrument, epistaxis occurred in 13%, airway bleeding in 39%, and pneumothorax in 4% of patients.[38]

Thoracoscopy

Thoracoscopy or pleuroscopy is a diagnostic technique used in the standing sedated horse to visualize the intrathoracic structures that include the aorta, esophagus, intercostal vessels, sympathetic trunk, vagus nerves, lymph nodes, mainstem bronchi, pulmonary and azygous veins (right hemithorax), diaphragm, and dorsal and lateral surfaces of the lungs. The procedure is used to sample thoracic masses or nodules, to transect pleural adhesions, to place drains for the treatment of pleural abscesses, to repair diaphragmatic hernias, and to resect lung segments either for therapeutic or diagnostic purposes. Thoracoscopy provides a superior method to obtain larger biopsy samples and therefore more accurately assess parenchymal and peripheral airway morphology.

DISEASES OF THE UPPER RESPIRATORY TRACT

Sinusitis

Nasal airways can be infected with a variety of viral, bacterial, fungal, and parasitic agents with resultant sinusitis and/or rhinitis. Rhinitis is defined as infection of the nasal passage independent of the sinus. Infection may include the nasal concha but does not involve the conchal sinuses unless caused by viral agents. Specific viral agents include equine influenza virus (EIV), equine herpesvirus 1 and 4 (EHV-1 and EHV-4), equine rhinoviruses, and adenovirus. Bacterial rhinitis is not common and usually occurs secondary to trauma or foreign body. *Mycoplasma* spp. have been isolated at postmortem examination from horses with rhinitis.[41] A variety of mycotic agents such as *Aspergillus* spp., *Conidiobolus* spp. (usually *C. coronatus*), and *Cryptococcus neoformans* may cause rhinitis in horses.[42] The most common cause of parasitic rhinitis is myiasis caused by *Habronema; Draschia;* and the Russian gadfly, *Rhinoestrus purpureus.* Enzootic lymphangitis or glanders caused by *Burkholderia mallei* causes a specific granuloma within the sinus cavity.[43,44] Horses with sinusitis most commonly have unilateral disease unless the infection is viral or there is extensive involvement of the nasal septa. Most horses present with respiratory stridor and nasal discharge with diminished airflow on the affected side.[41,45-47] Therapy is usually aimed at surgical debridement, debulking of nasal granuloma, and local therapy for the specific agent.[47] Orally administered itraconazole has been described for treatment of recurrent nasal mycoses.[46] Although successful in this case, the pharmacokinetics of this agent are variable, suggesting that an antifungal agent with enhanced bioavailability such as fluconazole may be an appropriate consideration if the susceptibility profile indicates drug efficacy.

Sinonasal disease is very common in horses and likely results from the extensive network of six pairs of sinuses including the conchal sinuses that exchange air with the nasal airway. The frontal sinuses may be affected with granulomatous masses with fungal or parasitic etiologies or bacterial empyema. The most common bacterial isolates are *Streptococcus* spp., which include *S. equi* subsp. *zooepidemicus* and *S. equi* subsp. *equi. Staphylococcus* spp. are the next most frequent isolates.[48] Polymicrobial infection is also identified among sinus disease in horses. *C. neoformans* and *Coccidioides immitis* also may cause granuloma formation within the paranasal sinuses.[49,50] In a study of 277 horses with sinusitis, 24% of the horses had primary sinusitis with no history of predisposing trauma or dental infection.[51] Dental disease of the third to sixth maxillary cheek teeth was the most common predisposing factor for secondary sinusitis (22% of horses) followed by sinus cysts, neoplasia, progressive ethmoidal hematoma, trauma, mycotic infection, sinonasal polyps, and nasal epidermal inclusion cysts. Primary infection of a rostral maxillary cheek root infection was identified in only 4% of cases, although CT evaluation was not used for diagnosis of many of these cases. Nasal discharge (most commonly unilateral but occasionally bilateral) and facial swelling were the most common clinical signs associated with sinusitis in horses. Nasal discharge may be mucopurulent to serosanguineous. A fetid odor is often present and typically attributed to the presence of anaerobic bacteria. Clinical signs commonly persist over several weeks, an initial favorable response to broad-spectrum antibiotics is often only transient. Once antibiotic therapy is discontinued, clinical signs frequently return. Other signs of frontal and maxillary sinus involvement may include lacrimal discharge and exophthalmia. Headshaking syndrome is an uncommon clinical manifestation of fungal sinusitis in horses.[52]

Diagnostic techniques that may facilitate identification of the characterization of sinusitis in horses include endoscopy, radiography, CT, and MRI.[53-55] Endoscopy can detect changes in airway structure (84% of cases) and rule out ethmoidal hematoma.[51,56] Sinuscopy can also be performed through a space created in the skull by trephination in the standing horse.[44,51,56,57]

Radiography is essential for the identification of fluid and masses within sinuses and for preliminary assessment of dentition (Fig. 8.12). Currently available high-detail computed radiography images provide the clinician with a remarkable level of radiographic detail that may provide valuable diagnostic information for the identification of sinus or dental disease.[58] It is not uncommon that the first molar is involved in association with the development of sinusitis. CT and MRI[59] are exceptionally valuable for detection of tooth root involvement and boney changes, which often involve the maxillary bone and facial crest.

Appropriate and effective treatment of sinusitis requires that underlying or predisposing conditions be accurately identified and treated, that debris be removed from the sinus, and that associated infectious agents are properly identified. When fluid is present within a sinus, medical treatment with antibiotics alone is unlikely to be successful. Trephination and flushing or surgical debridement and drainage through a sinus flap are indicated.[60] Establishment of ventral



FIG. **8.12** Skull radiographs can be diagnostic for the identification of dental disease and associated potential sinus disease. This 5-year-old Quarter Horse mare presented with the complaint of facial deformity just rostral to the right facial crest. Radiography demonstrates evidence of periodontal disease, particularly in the region of the right fourth premolar.

drainage of the affected sinuses may be required. Local flushing is likely to be the most important component of therapy, although systemic antimicrobial therapy may be indicated for any horse with signs of osteomyelitis. Prognosis is guarded for complete resolution of clinical signs, especially when apical dental disease is present. Frequently, tooth removal is indicated. Recurrence is most common with ethmoidal hematoma and neoplasia.[61]

PROGRESSIVE ETHMOIDAL HEMATOMA

Definition and Epidemiology

Progressive ethmoidal hematoma (PEH) is characterized by encapsulated, expansive masses that usually arise from the submucosa of the ethmoidal labyrinth.[62,63] The mass is composed of blood and fibrous tissue, is encapsulated by respiratory epithelium, and may extend into the paranasal sinuses.[64]

This condition occurs in 4% to 6% of horses with diseases of the nose and sinus.[50,65] The inciting factor in their development is not known, although they have been hypothesized to be associated with repeated episodes of submucosal hemorrhage. PEH appears bilaterally in 50% of cases and is more prevalent in older horses.[66]

Clinical Signs

The most common clinical sign caused by PEH is intermittent, serosanguineous discharge from the affected nasal passage. Other clinical signs include facial swelling, halitosis, respiratory distress, and coughing.[58,64]

Diagnosis

Diagnosis is based on the clinical signs, endoscopic examination, radiographic evaluation,[51] and, in some cases, CT findings to determine the extent of the lesion.[67] Endoscopy reveals a variably green-colored, smooth glistening mass originating from the ethmoid region. The mass may protrude beyond the nasal septum (and in such cases may cause a bilateral nasal discharge). Radiographs reveal a space-occupying soft tissue density with smooth margins and may demonstrate extension into the paranasal sinuses. Confirmation of the diagnosis is by histopathologic study of the removed tissue,[58] although the clinical appearance is highly suggestive.

Treatment

Treatment options include formalin injection, surgical resection, and laser ablation. Smaller lesions (<10 cm in diameter) can be treated effectively by the intralesional injection of 4% to 10% formalin.[68] Most cases require multiple injections to achieve full resolution.[68a,68b] This procedure can be performed in the standing horse, has a low complication rate, and is associated with 60% resolution after a median of five injections.[64] However, severe neurologic complications can occur if the cribriform plate is fenestrated by the chronic pressure of a large PEH.[69] CT may be indicated to determine the extent of the lesion before treatment.[67]

Surgical removal via a frontal sinus flap is associated with extensive hemorrhage and may require preoperative or postoperative blood transfusions.[64,70] A preoperative crossmatch is warranted, and the horse may also require a postoperative tracheotomy if extensive packing of the nasal cavity is necessary to effect hemostasis. After surgical removal, approximately 20% to 50% of ethmoidal hematomas recur.[70,71] Postoperative complications may include facial incisional dehiscence, suture periostitis, facial bone sequestration, persistent nasal discharges, fungal sinus plaque formation, and encephalitis.[63,64] Laser excision has also been used to treat PEH, with a recurrence rate of 20% described in one report.[72] Recurrence is higher in bilateral PEH than other forms.

GUTTURAL POUCH ANATOMY

The paired guttural pouches are diverticula of the external auditory tubes. Some investigators have suggested that the pouches play a role in cooling the arterial blood supply to the brain.[73] Each pouch has a capacity of about 475 mL and is divided into medial and lateral compartments by the stylohyoid bone.[74] Each pouch fills on inspiration via the plica salpingopharyngeus. The mucosal lining of each pouch is secretory and covered by ciliated pseudostratified epithelium with goblet cells and glands.[74] The medial compartment is three times bigger than the lateral compartment and contains the internal carotid artery and a fold that encloses cranial nerves IX, XI, and XII. The pharyngeal branch of the vagus nerve (cranial nerve X) traverses the floor of the medial pouch, and the cranial sympathetic ganglia are also found within it. The rectus capitis ventralis and longus capitis muscle run just medially to the guttural pouch, and tearing of these muscles after a poll injury can be identified endoscopically as blood in the associated pouch. The lateral compartment of the guttural pouch contains the external carotid and maxillary artery. Branches of the facial nerve (VII), vestibulocochlear (VIII), and mandibular branch of the trigeminal nerve (V) run close to the wall of the lateral compartment.

Guttural Pouch Tympany

Guttural pouch tympany is defined as distention of the guttural pouches with pressurized air. In some instances air accumulation is accompanied by fluid accumulation. Most commonly the condition affects an individual pouch, but it may occur bilaterally. Several reports have described increased frequency of disease in fillies compared with colts.[75-78] Additionally, a breed predilection has been observed for Arabians and Paint foals.[76] Among the affected Arabian foals, many had close genetic relationships. Heritability testing revealed polygenic and mixed monogenic to polygenic models that best described the condition in Arabian foals.[79] Interestingly and consistent with these previous observations, whole genome-wide scanning for guttural pouch tympany in Arabian and German Warmblood horses revealed a gender-specific quantitative trait locus, which is completely consistent with the observation that fillies are more commonly affected than colts.[80]

Clinical Signs

Guttural pouch tympany is most commonly diagnosed in young foals, although it can occur at any time in the first year of life. Characteristic clinical findings include a markedly enlarged area in the throatlatch region. This area can be markedly enlarged and is most commonly unilateral, although bilateral involvement is possible. In cases with severe unilateral involvement, it may be somewhat challenging to determine whether one or both sides are involved because the area of enlargement can be large enough to cross the ventral midline, giving the impression of bilateral disease. The affected area is not painful to palpation, and additional clinical signs may not be apparent. In cases where severe guttural pouch enlargement is present, airway obstruction or dysphagia may be observed. When neurologic deficits are present (e.g., in cases of dysphagia) complete examination should be performed to determine whether evidence of aspiration pneumonia is present.

Pathogenesis

Although the exact etiopathogenesis is incompletely understood, anatomic and physiologic factors must be considered for disease development. It is thought that a mucosal flap (plica salpingopharyngeus) may serve as a one-way valve that prevents the release of air and/or fluid from the affected guttural pouch.[81] Other proposed etiologies include upper airway (viral or bacterial) infection, persistent coughing, or metabolic dysfunction.[82] In most cases there is no obvious anatomic abnormality that can be identified in association with the affected guttural pouch or opening.[76]

Diagnosis

Signalment, history, and clinical findings often provide strong evidence for the diagnosis of guttural pouch tympany in foals. Upper airway endoscopy will provide the clinician with the ability to thoroughly examine the upper airway, nasal passages, guttural pouches, and entire laryngeal region. Although the guttural pouch openings may appear normal, dorsal pharyngeal compression is commonly observed secondary to the trapping of air within the pouch. Additional diagnostic options include skull radiographs, which reveal an enlarged, gas-filled guttural pouch. Although radiographs may be difficult to interpret in foals with bilateral disease, the combination of endoscopy and radiographs will aid the clinician in making an accurate diagnosis.

Treatment

If upper airway inflammation is identified on endoscopy as lymphoid pharyngeal lymphoid hyperplasia, then medical therapy may be initially implemented as a conservative management scheme. Therapy may include nonsteroidal antiinflammatory therapy with flunixin meglumine combined with broad-spectrum antibiotic therapy, trimethoprim-sulfamethoxazole, doxycycline, or minocycline. Therapy should be maintained for 10 to 14 days. If unsuccessful or disease progression is observed, surgical options may be considered.

Surgical approaches include needle decompression and surgical fenestration. Regarding the intricate network of vasculature and nerves in the affected region, an experienced surgeon is best suited to manage affected patients.[81]

Overall, with surgical management, the prognosis remains favorable for recovery and return to function. In some cases, individual surgical decompression is not successful, and repeated surgery is indicated. Nonetheless, when properly applied, even if repeated surgery is required, the prognosis for recovery remains favorable.[76]

Medical conditions that may occur secondarily to the condition of guttural pouch tympany include guttural pouch empyema and aspiration pneumonia. Resolution of the primary condition of guttural pouch tympany is the central focus of disease resolution, but medical management with antibiotics and guttural pouch lavage may be required postoperatively. In some instances, the surgical procedure may result in permanent nerve damage resulting in aspiration caused by persistent dysphagia. In such cases, the prognosis for complete recovery is guarded.[75]

GUTTURAL POUCH MYCOSIS

Guttural pouch mycosis is a life-threatening disease that can lead to fatal hemorrhage in horses. Mycotic plaques are typically located on the roof of the medial compartment associated with the internal carotid artery, and are less commonly on the lateral wall of the lateral compartment of the pouch associated with the external carotid or external maxillary artery. There is no apparent age, gender, breed, or geographic predisposition to this condition. The etiopathogenesis of this disorder remains elusive, but *Aspergillus (Emericella) fumigatus* is the most frequently identified pathogen, which is more readily identified on biopsy versus fungal culture.[83] The fungal plaque is reported to contain a diphtheritic membrane that contains necrotic debris, a multitude of bacterial isolates, and fungal organisms. Although this is typically a condition of more intensely managed horses living in North America, guttural pouch mycosis has also been definitively diagnosed in horses living on pasture in the Southern Hemisphere.[84]

Clinical Signs

Horses most commonly present with the complaint of epistaxis, which may be severe. The hemorrhage originates from the site of fungal plaque localization, which is most commonly over the internal carotid artery, but may involve the maxillary artery in approximately one third of the cases.[73,85-88] Alternate clinical signs many include dysphagia evidenced by feed and saliva containing material exiting from the nostrils, recurrent laryngeal neuropathy (RLN) resulting in turbulent airflow and altered airway mechanics, Horner's syndrome, head extension, parotid enlargement, paralysis of cranial

nerve VII (facial), mycotic encephalitis, and atlantooccipital joint infections.[87,89-92] Even though guttural pouch mycosis is most commonly observed in adult horses, foals have also been reported to develop this disease.[93,94] Epistaxis may be recurrent in nature and can be fatal in approximately 48% of horses; therefore, immediate surgical management is indicated.[87,95]

Pathogenesis

The etiopathogenesis of guttural pouch mycosis remains elusive. It has been postulated that aneurysmal dilations of the vasculature provide a suitable environment for fungal organisms to proliferate.[96] Fungal colonization of the guttural pouch mucosa and underlying vascular and neural structures results in marked inflammation, necrosis, and erosion, which leads to the clinical manifestation of hemorrhage and potential neuropathy. Clinical signs will reflect the severity of disease and specific anatomic structures that are colonized by the fungal organism.

Diagnosis

Diagnostic confirmation of guttural pouch mycosis is based on endoscopic observation of a fungal plaque within the appropriate anatomic location. Notably, in some cases complete examination of the guttural pouch may not be possible because of the severity of hemorrhage and obstruction of view. In such cases, endoscopic examination provides confirmatory diagnostic evidence regarding the area of hemorrhage, even though the exact site of the fungal plaque might not be observed upon initial examination. Based on clinical signs and endoscopic findings the diagnosis of guttural pouch hemorrhage can be established, which will allow a therapeutic plan to be established. An important consideration when performing endoscopy is to avoid dislodgement of an existing clot over a hemorrhagic lesion. For this reason, caution should be used when performing upper airway endoscopy in patients with suspected guttural pouch mycosis.[97]

Neuropathy may result in dysphagia or laryngeal paralysis. Fungal plaque formation involving the pharyngeal branches of the vagus and glossopharyngeal nerves results in dysphagia and potential aspiration pneumonia,[73,98] whereas a mycotic lesion located in the medial compartment of the guttural pouch may cause damage to the cranial cervical ganglion, which can lead to signs of Horner's syndrome on the ipsilateral side. Changes in vocalization or respiratory noise may result from RLN or pharyngeal paresis.[87] The presence of laryngeal paralysis or dysphagia should be documented at the initial examination because both are associated with a more guarded prognosis.[99] A more favorable prognosis is provided in horses that have evidence of dysphagia. Recovery has been observed to occur over a 6- to 18-month period. Horses may also recover from facial nerve paralysis and Horner's syndrome. Radiographs of the guttural pouch may show evidence of fluid accumulation or osteolytic changes in the stylohyoid bone or may suggest mycotic plaque formation.

Treatment

Current options for medical management of fungal disease in horses are hampered by not having a cost-effective, bioavailable drug that can be effectively administered. When hemorrhage is marked, having an efficient therapy is paramount. Regardless of the medical therapy selected, it generally requires weeks of therapy for treatment benefit to be achieved. For these reasons, medical therapy may not be the preferred therapeutic option for guttural pouch mycosis. Potential antifungal therapeutic protocols have been provided for select cases in which this therapy may be considered.[73] Amphotericin has been administered for up to 40 days, but lack of confirmed efficacy combined with safety concerns limit application of this therapy.[100,101] Itraconazole (5 mg/kg PO daily) can reach plasma concentrations greater than the minimum inhibitory concentration in horses and, although itraconazole has a similar spectrum of activity as fluconazole, it is effective against *Aspergillus* spp.[102] In a separate report, a combination of systemically administered itraconazole in combination with topical enilconazole (60 mL of 33.3 mg/mL solution daily flush) provided successful therapy in an individual case of guttural pouch mycosis.[103] Ketoconazole and fluconazole have been considered as potential therapeutic options, but because of a lack of efficacy and absorption, respectively, these drugs are not recommended for the treatment of guttural pouch mycosis. In a separate report of topical therapy, guttural pouch lavage with 0.08% clotrimazole emulsion in 500 mL water for 14 days prevented recurrence of a solitary lesion in an individual report. It has been suggested that a 3-day depot treatment of clotrimazole providing residual antimycotic activity within the pouch may be a superior option.

Additional medical therapy must also include patient needs. Complete assessment for severity of anemia, hydration status, and degree of discomfort must be addressed accordingly. Patients that are dysphagic will require nutritional support. An indwelling feeding tube may be required to provide support until disease resolution is sufficient to result in improved neurologic function.

Surgical Therapy

Commonly, guttural pouch mycosis is only effectively managed with intensive medical therapy to address blood loss and systemic disease manifestation in combination with surgical occlusion of the affected vessel or vessels. Current interventional therapies of choice use a coil or nitinol plug to occlude arterial flow proximal and distal to the fungal lesion.[81] The placement of transarterial coils, via an incision in the common carotid artery under fluoroscopic guidance, is associated with 84% survival and 71% return to performance.[99,99a] Historically, fluoroscopic guidance has been an important procedural component to ensure accurate coil placement. More recent reports describe transarterial coil embolization in standing horses with the use of fluoroscopy.[104] An increased risk of complication is associated with the presence of aberrant vessels, and for this reason fluoroscopic guidance provides the optimal imaging capability when vascular occlusion is performed.[105,106] Correct placement can be difficult in the presence of aberrant vessels.[105,106]

Prognosis

Horses that present with moderate to severe hemorrhage and receive occlusional procedures are likely to have an improved prognosis for survival. Although original reports suggested an approximate 50% survival rate, this was reported before the application of widespread and routine application of interventional therapies.[73] Recovery from guttural pouch mycosis is typically prolonged, and for this reason deficits may persist for extended periods and in some cases may be permanent. Laryngeal paralysis is commonly a residual complication of this disease,[99] but it has been reported to resolve in some cases.[89]

Dysphagia is one of the more serious long-term complicating factors, and, although some horses partially improve, the final outcome may not be apparent for up to 18 months.[87,89] Neuropathy that involves the facial nerve or cranial cervical ganglion leading to facial nerve paralysis or Horner's syndrome, respectively, often resolves completely.[73,107]

GUTTURAL POUCH EMPYEMA

Guttural pouch empyema results from accumulation of purulent material within one or both guttural pouches. Accumulated exudate can accumulate and become inspissated and form chondroids.[108] Most commonly empyema results following upper airway infection with *S. equi* subsp. *equi.* Abscess formation of the retropharyngeal lymph nodes leads to rupture and drainage into one or both guttural pouches. Persistence of purulent material may lead to the development of chondroids, which occurs in approximately 20% of horses that develop guttural pouch empyema.[108] Less commonly, empyema may be a complicating factor resulting from traumatic injury to the stylohyoid bone, potentially resulting in fracture. Iatrogenic infusion of irritating materials or trauma secondary to inadvertent placement of a nasoesophageal tube may result in trauma to the guttural pouch. Rarely, congenital or acquired stenosis of the guttural pouch opening may lead to impaired drainage, resulting in fluid and exudate accumulation.[82,109]

Clinical Signs

Horses with guttural pouch empyema often have intermittent, chronic nasal discharge.[108] Additionally, submandibular lymphadenopathy and parotid enlargement may be observed. Increased respiratory noise is commonly heard resulting from increased upper airway resistance caused by pharyngeal compression. Neuropathy and dysphagia are uncommon but may develop if guttural pouch inflammation is severe. Occasionally hemorrhage or serosanguineous discharge will accompany guttural pouch empyema.[73]

Diagnosis

Upper airway endoscopy will provide diagnostic confirmation for the presence of exudate within the guttural pouches. Examination will enable the clinician to determine the extent of fluid and exudate accumulation and definitively determine whether disease is unilateral or bilateral. Diagnostic testing on samples collected endoscopically generally includes microbiological culture and polymerase chain reaction (PCR) testing for the presence of *S. equi* subsp. *equi.* Radiography will also provide the clinician with diagnostic examination of other structures in the upper airway. Fluid lines within the guttural pouch suggest the presence of fluid and exudate within the guttural pouch. Ultrasonographic examination can provide additional diagnostic evaluation of peripheral soft tissue structures and the extent of lymph node enlargement.

Treatment

Based on the likelihood that *S. equi* subsp. *equi* is associated with the development of guttural pouch empyema, appropriate biosecurity protocols should be adopted to reduce the risk of bacterial dissemination to instruments or personnel. Specific management protocols generally include medical therapy for the physical removal of exudate, preferably while material is liquid rather than when firm mucoid accumulations have developed into chondroids. The horse should be routinely sedated to facilitate a low head position, which will improve drainage of lavage fluid. In acute cases, daily lavage with sterile physiologic or 0.9% saline is generally effective for removal of exudate.[108] Administration of local penicillin may improve treatment success.[110] A gelatin-penicillin mixture can be instilled through a catheter inserted either through an endoscope or using endoscopic guidance into the guttural pouch.[111] A 50-mL mixture is prepared by first heat dissolving 2 g of gelatin in 40 mL of sterile water and then cooling to 45°C to 50°C. Then add 10 million units of sodium benzylpenicillin that have been reconstituted with 10 mL of sterile water. The mixture is ideally stored overnight at 4°C to allow the gel to set. Approximately 25 mL of the mixture is instilled for each pouch that requires treatment.

When chondroids are present, their removal may be attempted with an endoscopic snare, which prevents the risk of surgical complications and minimizes treatment costs. However, complete removal of chondroids can be challenging, particularly if they are numerous.[111,112] When endoscopic removal is unsuccessful or chondroids are numerous, surgical removal may be performed through a modified Whitehouse approach or by using a transendoscopic laser to establish a permanent pharyngeal fistula into the guttural pouch.[81,113,114]

PHARYNGEAL LYMPHOID HYPERPLASIA

Pharyngeal lymphoid hyperplasia is a common condition involving the upper respiratory tract of 2- and 3-year-old racehorses. Most mild cases respond favorably to reduced athletic activity in combination with systemic and topical antiinflammatory therapy. Dexamethasone can be administered at a dose of 0.02 to 0.05 mg/kg orally daily for 1 week, followed by half of the original dose given orally for 1 week, then the same dose administered orally every other day for an additional week. A throat spray comprised of nitrofurazone, dexamethasone, and dimethyl sulfoxide is reported to be of benefit when administered topically.[115] Systemic immune modulation is reported to be effective for the treatment of horses with lower airway inflammation and may also have some benefit in horses suffering from upper airway inflammation.[115-117] Occasionally, chronic disease occurs; reports have suggested that these horses may respond favorably to cautery of the dorsal roof of the pharynx.[118]

Organisms associated with a more prolonged course of pharyngeal hyperplasia include *S. equi* subsp. *equi;* equine influenza; and EHV-1, EHV-2, and EHV-4. The condition is thought to result from chronic inflammation of the localized lymphoid tissues, particularly because these structures have a diffuse distribution within the mucosa in this species. Although some investigators have cultured the oropharynx of affected horses, no consistent etiologic agent has been identified. Normal inhabitants of the equine upper respiratory tract such as *S. equi* subsp. *zooepidemicus, Bordetella bronchiseptica,* and *Moraxella* have been isolated; however, the direct association with the condition has not been determined. A grading system has been established for this condition (Table 8.2). Horses with more severe inflammation have greater numbers of bacterial organisms isolated from their upper respiratory tract.[119]

TABLE **8.2 Grading Scheme for Pharyngeal Lymphoid Hyperplasia**

GRADE 1: Small number of inactive white follicles scattered over the dorsal pharyngeal wall. The follicles are small and inactive; this appearance is normal in horses of all ages.
GRADE 2: Many small inactive white follicles over the dorsal and lateral walls of the pharynx to the level of the guttural pouches. Numerous follicles are larger, pink, edematous and interspersed throughout.
GRADE 3: Many large pink follicles and some shrunken white follicles are distributed over the dorsal and lateral walls of the pharynx. In some individuals the follicles extend onto the dorsal surface of the soft palate and into the dorsal pharyngeal diverticula.
GRADE 4: More numerous pink and edematous follicles packed close together covering the entire pharynx, the dorsal surface of the soft palate, epiglottis, and lining the guttural pouches. Large accumulations appear as polyps.

Adapted from: Raker CW: The nasopharynx. In Mansmann RA, McAllister ES, editors: *Equine medicine and surgery*. Santa Barbara, CA, 1982 Veterinary Publications.

TABLE **8.3 Function and Innervation of Muscles Controlling the Tone of the Equine Soft Palate**

Muscle	Function	Innervation
Tensor veli palatini	Tenses the rostral aspect of the soft palate	Mandibular branch of the trigeminal nerve
Levator veli palatini	Elevates the palate during swallowing and closes the nasopharynx	Pharyngeal branch of the vagus
Palatinus	Shortens and depresses the palate	Pharyngeal branch of the vagus
Palatopharyngeus	Shortens and depresses the palate	Pharyngeal branch of the vagus

DORSAL DISPLACEMENT OF THE SOFT PALATE

DDSP results in an upper airway expiratory obstruction with exercise. It is a common cause of poor performance in racehorses and has a prevalence of 10% to 20%.[120-123] Most affected horses make a loud, vibratory respiratory noise, known as *choking down,*[124,125] although a proportion are silent during displacement.[11,125] Endoscopic examination of the upper airway at rest is a poor predictor of DDSP.[126] A definitive diagnosis is made, during high-speed treadmill examination, if the caudal border of the soft palate is seen dorsal to the epiglottis for more than 8 seconds.[127]

Anatomy

The horse is an obligate intranasal breather, and the epiglottis is usually positioned dorsal to the caudal border of the soft palate. A common analogy is a *button through a buttonhole.*[128] The anatomy of this area is complex. The larynx is suspended from the petrous part of the temporal bone by a chain of paired hyoid bones, the stylohyoid, ceratohyoid, single basihyoid bone, and thyrohyoid, which articulates with the rostral aspect of the thyroid cartilage. The strap muscles (sternohyoid, sternothyroid, and omohyoid) contract during exercise and pull the larynx caudally. Similarly, the genioglossus, geniohyoid, and styloglossus muscles (through their direct or indirect attachments to the hyoid apparatus) pull the larynx rostrally.[129,130] The tone of the soft palate is determined by four pairs of muscles (Table 8.3). The stylopharyngeus muscle is innervated by the glossopharyngeal nerve and tenses the dorsal wall of the nasopharynx.[131]

Etiology

The etiologic factors of DDSP are not completely understood and can be divided into intrinsic and extrinsic causes. Intrinsic dysfunctions are seen with decreased tone in muscles of the palate. Dysfunction of the palatinus and palatopharyngeus muscles results in DDSP.[132] This cause is closely linked to pharyngeal lymphoid hyperplasia in young horses because the pharyngeal branch of the vagus nerve runs through the floor of the guttural pouch, which is also the roof of the pharynx. Transection of the tensor veli palatini tendon alone destabilizes the rostral aspect of the soft palate, leading to inspiratory airway obstruction but not DDSP.[133] Dysfunction of the caudal stylopharyngeus muscle destabilizes the nasopharynx but does not cause DDSP.[131]

Extrinsic causes are related to the musculature that controls the position of the larynx and hyoid apparatus. Transection of the paired thyrohyoid muscles produces DDSP[134] presumably because these muscles, innervated by the hypoglossal nerve, prevent caudal retraction of the larynx at exercise. Partial resection of the sternothyroid and sternohyoid tendons produces an inspiratory airway obstruction but not DDSP.[135]

Investigation targeting the role of the rostral hyoid muscles (geniohyoid, stylohyoid, and styloglossus) in horses is lacking, but in humans these muscles are critical to pharyngeal stability.[136,137] Indirectly, tongue-tie devices, which are designed to prevent caudal retraction of the hyoid apparatus at exercise, have been shown to prevent DDSP in some horses.[138] In addition to these neuromuscular dysfunctions, structural abnormalities such as masses or granulomas associated with the epiglottic cartilage or the soft palate can induce DDSP.[139]

Treatment

Surgical techniques aimed at preventing caudal retraction of the larynx, such as strap muscle resection and the Llewellyn procedure (transection of the tendon of insertion of the sternothyroid muscle), are associated with success rates ranging between 58% and 73%.[125,140,141] Treatments aimed at intrinsic causes include staphylectomy[141] and soft tissue stiffening techniques such as rostral palatoplasty.[142] Conservative management of DDSP, incorporating rest, improvement of fitness, use of a tongue tie, or any combination, has also been reported, with a success rate of up to 61%.[138,143] A case-controlled study demonstrated that composite surgery including staphylectomy, sternothyrohyoideus myectomy, and ventriculocordectomy produced increased race earnings in 60% of horses compared with 40% in controls.[124]

Surgical advancement of the larynx (laryngeal tie-forward procedure) demonstrated a success rate of 80% to 82% in

TABLE **8.4 Grading System for Laryngeal Function Performed in the Standing Unsedated Horse**[152]

Grade	Description
I	All arytenoid cartilage movements are synchronous and symmetrical, and full arytenoid abduction can be achieved and maintained.
II	Arytenoid cartilage movements are asynchronous at times, but full arytenoid abduction can be achieved and maintained.
III	Arytenoid cartilage movements are asynchronous or asymmetrical (or both). Full arytenoid cartilage abduction *cannot* be achieved and maintained.
IV	Immobility of the arytenoid cartilage and vocal fold is complete.

Grades II and III contain several subgrades. Readers should consult the referenced monograph[152] for further information.

TABLE **8.5 Grading System for Laryngeal Function Performed During Treadmill Endoscopy**[153]

Grade	Description
A	Full abduction of the arytenoid cartilages during inspiration
B	Partial abduction of the left arytenoid cartilage (between full abduction and the resting position)
C	Abduction less than resting position, including collapse into the contralateral half of the rima glottidis during inspiration

racehorses.[144] The proposed mechanism of this procedure is that it replaces the action of the thyrohyoid muscles and prevents ventral descent and caudal retraction of the larynx during exercise. This procedure is well described elsewhere.[144] Horses undergoing this procedure are as likely to race postoperatively as matched controls. The procedure restores race earnings to preoperative baseline levels and to those of matched controls.

In addition, an external device (Cornell Collar, Vet-Aire, Inc., Ithaca, NY) that applies forward and upward pressure to the hyoid apparatus and incorporates a figure-eight noseband also prevents experimentally induced DDSP during strenuous exercise.[145]

RECURRENT LARYNGEAL NEUROPATHY

RLN is a major cause of poor performance in racehorses and affects 1.6% to 8% of Thoroughbreds.[146-149] The prevalence in draft horses is nearly 42%,[150] and the risk of RLN increases with increasing height in Belgians and Percherons but not in Clydesdales.[150] Horses as young as 6 months may be affected.[151] The condition produces an inspiratory noise at exercise, which has been described as both *roaring* and *sawing*. The term *recurrent laryngeal neuropathy* is preferred to idiopathic laryngeal hemiplegia.[152]

Diagnosis

A diagnosis can be made in most horses based on a resting endoscopic examination of the arytenoid cartilages. Some confusion has arisen from different grading systems used to diagnose the degree of arytenoid dysfunction present at this examination. A recent meeting of investigators reached a consensus on a four-level grading system (Table 8.4).[152] This resting grading system is most useful in its ability to predict arytenoid function at high-speed exercise. Arytenoid position at exercise is shown in Table 8.5.[153]

All grade 1 horses are normal (grade A) at exercise, and all grade 4 horses show dynamic collapse (grade C).[154] During exercise, 4% of grade 3 horses are normal (grade A), 19% are grade B, and 77% are grade C.[155] For grade 2 horses, 4% are grade B or C at exercise.[154] Recently a technique for examining the lateral cricoarytenoid muscle using ultrasound has been described, and this technique is a good predictor of arytenoid function at exercise.[156] It is especially useful if a high-speed treadmill examination cannot be performed.

Anatomy and Etiology

The left recurrent laryngeal nerve curves medially around the aortic arch during development and is approximately 1 m long.[157] In contrast, the right recurrent laryngeal nerve courses around the right subclavian artery and is approximately 25% shorter.[157] The high prevalence of chronic demyelinating peripheral neuropathy in the left recurrent laryngeal nerve has been attributed to its length.[158,159] The myelin loss is most severe distally[160,161] and is associated with axonal loss.[161a,161b] RLN results in progressive atrophy of the left dorsal cricoarytenoid muscle and associated loss of arytenoid cartilage abduction.[158,160,162] The right recurrent laryngeal nerve is rarely affected, although direct trauma to the nerve can induce dysfunction.

During exercise, arytenoid dysfunction narrows the rima glottidis and increases inspiratory impedance and noise.[163,164] This circumstance, in turn, results in airflow limitation and increased driving pressure during inhalation, leading to severe exercise-induced hypoxemia and decreased performance.[163,165-167]

Treatment

The current gold standard for treatment of RLN in horses is prosthetic laryngoplasty, with or without vocal cordectomy or ventriculectomy.[168-171] This technique involves the placement of a nonabsorbable suture to create arytenoid abduction. One limitation of this technique is the frequent and significant loss of abduction of the arytenoid cartilage seen in the immediate postsurgical period.[171,172] This loss of abduction leads to a reduction in the cross-sectional area of the rima glottidis and the return of exercise intolerance and abnormal respiratory noise.[171-173] Loss of abduction may also contribute to the modest postoperative success rate of 48% to 68% observed in racehorses.[168,169,174-177] A much higher success rate (73%–91%) is reported in horses performing at submaximal exercise.[171]

In draft breeds, the degree of abduction required at laryngoplasty is lower than that for a racehorse, given that the goal is to prevent dynamic collapse of the arytenoid rather than to achieve a normal cross-sectional area of the rima glottidis. Some clinicians recommend vocal cordectomy alone for draft breeds. For racehorses and nonracehorses, the major complications are exercise intolerance, respiratory noise, and coughing.[169,170,177]

Restoration of physiologic function through reinnervation and nerve–muscle pedicle transplant techniques has also produced positive results,[178-180] although a delay of several months occurs before any improvement in arytenoid function is seen.

Partial arytenoidectomy is associated with a fair prognosis for the treatment of RLN in horses[174,181] and is not generally recommended as a first-line surgical option.

EPIGLOTTIC ENTRAPMENT

The aryepiglottic fold is the mucous membrane that extends from the lateral aspect of the arytenoid cartilages to the ventrolateral aspect of the epiglottis, where it blends with the subepiglottic mucosa and the glossoepiglottic fold. In epiglottic entrapment, this membrane envelops the free border of the epiglottis.[182] Billowing of the entrapping membranes during respiration decreases the cross-sectional area of the pharynx and effectively obstructs the airflow, particularly during expiration.

The cause of epiglottic entrapment is not understood completely. In most cases, the epiglottic cartilage and associated soft tissues appear normal. In occasional cases, congenital epiglottic hypoplasia or inflammation of the upper respiratory tract structures appears to contribute to entrapment. Epiglottic entrapment is responsible for 1% to 3% of upper airway obstructive problems in horses.[121,149]

Clinical Signs

Most horses exhibit exercise intolerance and respiratory stertor. Horses may occasionally cough during exercise or while eating.

Diagnosis

The diagnosis is based on endoscopic examination. The membrane obscures the normal serrated margin of the epiglottis and its dorsal vasculature. In contrast to DDSP, the shape of the epiglottis can still be seen. Ulceration of the free margin of the fold and erosion of the entrapped epiglottis may be apparent.[183]

Treatment

Entrapment requires surgical correction. Several different approaches have been used and include transoral[182,184-186] or transnasal[187] axial division with a hooked bistoury and transendoscopic division with a laser.[188-190] The prognosis is good after surgery, although re-entrapment and DDSP may occur after surgery.[182,190,191]

ARYTENOID CHONDRITIS

Arytenoid chondritis is a progressive inflammatory condition of the arytenoid cartilages in adult horses, originating as an infectious condition. Most commonly, upper airway dysfunction is reflected in poor athletic performance and respiratory stridor. Diagnosis is based on upper airway endoscopy. One manifestation of chondritis is the development of granulomas on the axial surface of the arytenoid cartilages. Clinical management of affected patients involves medical or surgical therapy. Although broad-spectrum antibiotic therapy has been attempted in many cases, it is rarely curative. Also of importance in management of some of these cases is placement of a tracheostomy tube. Several techniques are described for placement of a permanent tracheostomy.[192]

ROSTRAL DISPLACEMENT OF THE PALATOPHARYNGEAL ARCH

In the horse, the soft palate terminates caudally at the confluence of the caudal pillars to form the palatopharyngeal arch that covers the esophageal orifice.[193] With rostral displacement of the palatopharyngeal arch, this fold of tissue appears to be displaced forward, overlying the apices of the arytenoid cartilages. The condition is uncommon, with a number of small case series and individual cases being reported.[120,194-197] The displacement of the palatopharyngeal arch may be associated with malformation of the laryngeal cartilages and the cricopharyngeal and cricothyroid muscles.[120] The condition may be present from birth. In most cases, abnormal respiratory noise and poor athletic performance are the presenting complaints. Abnormal pharyngeal conformation prevents normal deglutition, predisposing horses to develop aspiration pneumonia. In severe cases, horses may exhibit dysphagia, nasal discharge of food material, and persistent coughing.[128,196] The diagnosis is based on the clinical signs and history and confirmed with endoscopic examination. The rostrally displaced palatopharyngeal arch obscures the normal view of the apices of the arytenoid cartilages. Rostral displacement of the palatopharyngeal arch represents a major deformation of the laryngeal structures and is associated with a guarded prognosis. Resection of the arch by conventional surgery or laser surgery has not enabled successful athletic performance.[194]

NASOPHARYNGEAL CICATRIX

Nasopharyngeal cicatrix is a condition of upper airway inflammation and subsequent scarring of the pharynx. Additional structures that are commonly affected include the openings to the guttural pouches, the epiglottis, and the arytenoid cartilages. A very specific geographic region is associated with the development of this condition, specifically in the Gulf Coast region of central and southeastern Texas.[198-201] The disease has also been reported in Mississippi, Louisiana, and Florida, but rarely.[183,198] This is an upper airway condition that has been recognized for approximately three decades, but for equine referral clinics this condition has become one of the most commonly diagnosed disorders among horses requiring permanent tracheostomy.[200] Clinical signs commonly include upper respiratory noise, exercise intolerance, and rarely, dysphagia. During acute stages of disease, nasal discharge may be observed.[202] Horses that present with the complaint of respiratory distress are more likely to be in the acute stages of disease. Airway obstruction of >50% leads to marked increase in airway resistance, which increases by 16-fold (or more) depending on the severity of scarring and pharyngeal narrowing. Consistent with the fact that disease is localized to the upper airway and dysphagia is uncommon, coughing is not usually reported in association with nasopharyngeal cicatrix.[202]

Upper airway endoscopy will provide a confirmatory diagnosis. Early diagnosis is important so that environmental factors can be modified to limit disease progression. Removal from pasture and movement to a stall or dry lot is associated with limited disease progression. Although various surgical procedures have been used to remove scar tissue formation, the currently recommended procedure is permanent tracheostomy.[199]

A recent retrospective case-control investigation aimed at determining risk factors for the development of nasopharyngeal cicatrix evaluated 242 horses, 121 with nasopharyngeal cicatrix and 121 control horses. In toto, 1236 horses were examined with the reported affected and control horses meeting inclusion criteria for investigation. Risk factors associated with disease development included horses living on pasture exclusively or access to pasture and age of the horse.[202] There was no breed or gender association with disease development, which was in contrast to a previous investigation that suggested mares may have an increased risk of disease.[198] The age-associated component of disease development is believed to be associated with chronic progression of the disease or that the disease results from repeated bouts of exposure to an allergen or irritant located in the pasture. The repeated bouts of exposure are believed to result in continued bouts of inflammation and fibrous remodeling of the upper airway of affected horses.[202] Based on the available published data reporting this disease, it appears that the proportion of cases have remained steady since the original reports were provided, with the overall proportion being 0.64% of the cases seen in high-risk geographic regions.[198,202] Disease was recognized to have a seasonality, with affected horses presented for examination in summer months. Acute flare-up of disease was more likely to occur in the spring, summer, or fall months, suggesting that exposure to an irritant such as pollen or algal toxin was more likely to occur during warmer months.

Overall, it appears that this condition is more likely to occur in older horses that live predominantly on pasture, particularly during warmer months in southeastern and central Texas. The exact etiology remains elusive, which makes prevention challenging. In horses diagnosed with nasopharyngeal cicatrix resulting in marked airway narrowing and distress, permanent tracheostomy is the treatment of choice, with a favorable prognosis (89%) for outcome and return to function.[200]

STREPTOCOCCUS EQUI SUBSP. *EQUI* INFECTION (STRANGLES)

Bacterial infections involving the upper respiratory tract commonly involve *S. equi* subsp. *equi. S. equi* subsp. *equi* is a gram-positive β-hemolytic coccoid bacterium that is categorized within Lancefield group C and has been responsible for causing primary respiratory disease in horses for centuries.[203] *S. equi* is a highly contagious primary upper respiratory tract bacterial pathogen and is the causative agent of strangles, which is one of the most common infectious diseases of horses. *S. equi* is easily grown on blood agar and produces mucoid, nonpigmented (honeydew) colonies that appear in pairs or chains that are surrounded by a prominent zone of β-hemolysis. Although highly similar genetically, this organism is distinct from *S. equi* subsp. *zooepidemicus,* which is a normal inhabitant of the upper respiratory tract of healthy horses. Although colony morphology may be indistinguishable, *S. equi* is typically differentiated from *S. zooepidemicus* by its inability to ferment carbohydrate sugars, specifically lactose, sorbitol, or trehalose.[204] Typical *S. equi* isolates fail to ferment sugars, whereas atypical *S. equi* isolates may ferment lactose or trehalose but not sorbitol.[205] In addition to biochemical characteristics, DNA-based tests that discriminate between these organisms can provide diagnostic information regarding DNA sequence data that are unique to *S. equi,* which provides the clinician with improved diagnostic sensitivity.[110] Ideally, PCR testing should be performed using primers based on the nucleotide sequence of the gene encoding the *S. equi* M-like (SeM) protein.[205]

Molecular evaluation with multilocus enzyme electrophoresis has confirmed a strong genetic relationship of *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus,* indicating that the former is actually a clone derived from the more genetically diverse *S. equi* subsp. *zooepidemicus.*[206] This finding has led to the recommendation that *S. equi* subsp. *equi* be reclassified as a biovar of *S. equi* subsp. *zooepidemicus.*[207] Even though isolates of these two organisms show greater than 92% DNA homology (similarity), immunity is species specific; immunization with *S. equi* subsp. *zooepidemicus* does not protect against challenge by *S. equi* subsp. *equi.* However, from an immunologic perspective, host response to this pathogen may be complex because of antigen cross-reactivity against these two very similar bacterial organisms.

Because *S. equi* is a primary pathogen of the equine upper respiratory tract, unlike *S. zooepidemicus,* which is pathologic only as a secondary invader, *S. equi* does not require airway epithelial disruption or prior viral infection for effective bacterial colonization and subsequent infection.[208] Three colony types exist for *S. equi,* with morphologic differences directly associated with bacterial virulence. The most widely recognized strain that demonstrates marked pathogenicity in horses produces colonies that are heavily encapsulated and light golden color on blood agar, whereas atypical *S. equi* colonies exhibit a matt appearance within 24 hours of incubation in culture conditions and are nonencapsulated colonies that are dry and small.[209] Morphologic differences are observed among *S. equi* strains as a result of variability in the hyaluronic acid content of the bacterial capsule.

Epidemiology

Infection with *S. equi* is most commonly observed in young horses, typically aged from 1 to 5 years of age, but can occur in horses of any age, particularly if antigen-specific immunity is not present or challenge is marked. Foals up to 3 months of age born from immune mares are known to be resistant to the development of strangles.[210] Therefore in endemic areas foals will have increased susceptibility to infection after waning of maternal antibodies, generally at the time of weaning or later in the first year of life. Immunologically naive individuals are highly susceptible to infection with morbidity rates of approximately 100%, whereas mortality is low (up to 10%).[211] It has been estimated that following infection approximately 75% of horses maintain protective immunity against challenge for greater than 5 years.[212,213] Interestingly, however, the remaining 25% of these horses fail to maintain pathogen-specific immunity; therefore, they are susceptible to reinfection within months of challenge, which may represent a failure to produce or maintain an adequate level of protective mucosal and systemic antibodies.[213] In one study of young horses that had been affected with clinical strangles as foals, researchers found that 83% were resistant to comingling exposure 6 months later.[214] As would be expected, however, immunity is not lifelong. Historical epidemiologic studies report attack rates in horses greater than 3 years of age at 18, 29, and 35%.[211,215,216]



FIG. **8.13** A 12-year-old Quarter Horse gelding with the history of *Streptococcus equi* infection approximately 6 months previously. Intermittent unilateral mucopurulent nasal discharge was observed since the original infection. Upper airway endoscopy revealed marked chondroid development in the left guttural pouch.

S. equi subsp. *equi* can be transmitted by direct contact with an infected horse with purulent discharge associated with disease or via contamination of fomites such as horse handlers, grooming equipment, tack, feed areas, water sources, or other items that may be contaminated with bacterial-laden mucous secretions from an infected horse. Not uncommonly, introduction of a horse that is recovering from infection into a group of naive horses can lead to an outbreak. Although horses can resolve the clinical manifestation of disease in approximately 14 days, bacterial shedding typically continues for 4 to 6 weeks. In some instances, disease outbreak can occur as a result of an apparently healthy horse serving as an asymptomatic chronic carrier animal. Horses can maintain the organism within the guttural pouches for extended periods within inspissated purulent material in the form of chondroids (Fig. 8.13). In a recent report it was recommended that guttural pouch endoscopy may provide the clinician with the most efficient and accurate method of diagnosis in an inapparent carrier horse. This case-control study included 8308 horses over an 8-year period that were examined by the ambulatory service of a major teaching hospital. Overall there were 108 (1.3%) strangles cases, and 215 (2.6%) of the horses presented with pyrexia but did not suffer from *S. equi* infection and therefore served as control animals. The study confirmed previous observations[217-219] that horses with bilateral mucopurulent nasal discharge (Fig. 8.14A–B) and external abscess formation in the pharyngeal region were likely suffering from *S. equi* infection.[220] Additionally it was determined that not all horses demonstrated clinical evidence to support *S. equi* infection and that on examination of suspect horses upper airway endoscopy may provide a more efficient and accurate method for examination of the upper airway and sample collection for microbial culture and molecular diagnostic testing. Previous reports have provided evidence that horses may harbor the organism in the guttural pouches for extended periods, with one report indicating 39 months in the absence of clinical signs.[216,221] The etiopathogenesis of the carrier state for horses with chondroids is incompletely understood but is believed to develop as a result of incomplete drainage of mucoid material from the guttural pouches. It has been proposed that incomplete emptying of the guttural pouches leads to retention of material that develops into chondroid material; the persistent presence of this material in the guttural pouch may maintain a perpetual cycle of inflammation in a relatively closed-off anatomic site. Carrier animals maintained in the general population play a critical role in harboring the bacteria between apparent disease outbreaks.[222] Identification of the carrier animal is paramount to abate future disease outbreak.

Although large-scale controlled field studies aimed at documenting survivability of environmental *S. equi* have not been conducted, there has been a long-standing suspicion that *S. equi* may persist in the environment for extended periods of time.[223] Recommendations for the management of contaminated environments were based on original investigations aimed at determining survival of environmental *S. equi* that suggested that long-term bacterial persistence may be a substantial risk for up to 63 days.[224] However, and in contrast to these original observations, more contemporary evidence suggests more limited survival of *S. equi* in an outdoor environment.[225] In this study, sunlight was associated with survival of less than 24 hours, whereas overall survival was less than 72 hours.[225] The implications from this investigation should not be overinterpreted; however, it is noteworthy than in an outdoor environment, particularly in which ample sunlight exists, long-term sequestering of facilities is unlikely to be necessary. When cleansing of nonporous surfaces is implemented, the organism is susceptible to a 1:200 dilution of phenol, and disinfectants such as povidone-iodine, chlorhexidine, and glutaraldehyde kill the organism within 90 minutes.

S. equi has traditionally been defined as a pathogen restricted to the equine host. However, fatal pneumonia attributed to the organism was reported and characterized at the molecular level in a dromedary camel.[226,227] Although *S. equi* infection may occur in humans and descriptions of *S. equi* bacteremia and meningitis exist, infections in humans are rare and may be associated with overwhelming challenge or impaired host immunity.[228]

Pathogenesis

After infective droplets are inhaled or ingested, the organism adheres to the epithelial cells of the buccal and nasal mucosa of the horse, but it does not colonize the mucosal surface of tonsillar tissues of the nasopharynx. The incubation period for bacterial exposure to clinical manifestation of lymph node abscess formation is approximately 10 to 14 days. Interestingly, *S. equi* cannot be detected by bacterial culture or nasopharyngeal swabs obtained 24 hours after infection or challenge. The mechanism of bacterial adherence remains incompletely understood but may involve exposed bacterial surface proteins such as SzPSe, Se73.0, and Se51.9.[223] What makes *S. equi* unique when compared with *S. zooepidemicus* has been suggested to relate to the genetic sequence of *S. equi,* which results in the ability of this bacterial organism to rapidly translocate the mucosal epithelium and create marked lymph node abscess formation. Of particular relevance to the molecular structure of *S. equi* is the fact that the genome encodes 29 surface proteins that are characterized by the presence of a specific LPXTG motif, which under the influence of sortase enzymes results in the attachment of this protein to the peptidoglycan cell wall.[222,229] Similarly, *S. zooepidemicus* also encodes sortase processed proteins with



FIG. **8.14** Yearling Quarter Horse cross filly with evidence of submandibular lymphadenopathy and bilateral mucopurulent nasal discharge consistent with microbiological culture of *Streptococcus equi* subsp. *equi* (A). (B) Subsequent rupture of submandibular lymph nodes.

one strain, termed *H70*, which encodes 39 proteins. These proteins are defined as pili loci; pili are hairlike projections that extend from the bacterial surface and are believed to play an important role regarding epithelial attachment to host tissues.[222] Because *S. equi* expresses a more limited repertoire of these proteins, this may limit the diversity of host attachments by this bacterial strain, leading to more rapid epithelial translocation.

Among the characteristics of *S. equi* is the ability of this bacteria to evade host defense mechanisms. The sortase-processed M protein of streptococci confer antiphagocytic properties. Specifically, acid-resistant fibrillar molecules that project from the bacterial surface are expressed in pairs that twine around one another into a tight surface coil structure. *S. equi* subsp. *equi*–expressed M proteins are approximately 58 kDa in size. The C-terminal region of SeM is predicted to have an α-helical coiled-coil structure with a high degree of sequence similarity to SzM expressed by *S. zooepidemicus.* In contrast, however, the N-terminal portion of SeM is not coiled, and single strands are expressed that are unique to *S. equi.* Regarding evasion of host immunity, antiphagocytic activity is associated with the fibrinogen binding capacity of these individual protein moieties in addition to the binding capacity with Ig isotypes, IgG4 and IgG7. With the presence of fibrinogen and Ig binding on the bacterial surface C3b-binding sites are blocked, which inhibits the activity of the alternate complement cascade of C3 and C5 convertases. There is a large degree of diversity of this region of SeM.

Although it is recognized that there are many differences among *S. equi* and *S. zooepidemicus,* one of the major differences pertains to fermentation of carbohydrates—namely, ribose, lactose, and sorbitol.[204,222] Mucosal colonization is directly related to carbohydrate metabolism, and it is believed that although *S. zooepidemicus* has maintained this capacity, *S. equi* has lost this through the process of evolution.

Similar to many strains of bacteria, *S. equi* produces a hyaluronic acid capsule. This capsule provides the bacteria with an immune evasion mechanism that limits host destruction of bacterial challenge. This capsule mimics the host molecule that is present in mammalian tissues, conferring a protective shield for the bacterial organism. Nonencapsulated *S. equi* has been compared with a medium capsule-producing strain referred to as the matt strain in addition to a heavily encapsulated or mucoid strain. When compared in vitro, the strains were similar with respect to their M-protein expression, cytotoxin activity, and mitogen expression. However, the nonencapsulated strain did not demonstrate any resistance to in vitro phagocytosis by neutrophils, independent of the presence of opsonins. In contrast, strains with matt or mucoid levels of capsule formation demonstrated near-complete resistance to phagocytosis. In addition, when considering the influence of the capsule on pathogenicity in the equine host, the nonencapsulated strain did not result in clinical disease following challenge, yet seroconversion was observed, whereas encapsulated strains of *S. equi* repeatedly resulted in disease consistent with *S. equi* infection.[230]

Within hours of adhesion, the organism translocates below the mucosa to gain access to the local lymphatics and lymph nodes, in which replication occurs extracellulary.[222,231] Complement-derived chemotactic factors generated after interaction of C1 with bacterial peptidoglycan attract large numbers of neutrophils that contribute to the lymphadenitis

and abscess formation.[213] Metastatic bacterial spread to sites other than the upper respiratory tract lymphoid tissue may occur by hematogenous or lymphatic pathways. Although uncommon and reported in an isolated investigation, *S. equi* may be cultured from the blood 6 to 12 days after intranasal inoculation.[232]

Numerous virulence factors of *S. equi* contribute to its pathogenicity, a few of which include the hyaluronic acid capsule, the SeM protein, an antiphagocytic protein (Se18.9), a leukocidal toxin (streptolysin S–like [SLS] toxin), and cell membrane lipoproteins.[205,232a-c,] Synthesis of the hyaluronic acid capsule is controlled by the *has* operon, which consists of *hasA* (encodes hyaluronate synthase), *hasB* (encodes UDP-glucose dehydrogenase), and *hasC* (encodes UDP-glucose pyrophosphorylase). The capsule is antiphagocytic, ultimately reducing the number of bacteria that are subsequently ingested and killed. The capsule, by virtue of its negative charge and hydrophilicity, also produces a localized reducing environment that protects the activity of oxygen-labile proteases and toxins (SLS). Finally, the capsule is required for the activity of SeM. In nonencapsulated strains of *S. equi,* SeM aggregate and fail to achieve the three-dimensional structure required for their function.[213] Although nonencapsulated strains of *S. equi* are able to colonize the surface of the upper respiratory tract and stimulate production of the serum antibody, they are unable to induce detectable pathologic changes in the retropharyngeal and mandibular lymph nodes. The second virulence factor, the SeM protein, is a 58-kDa cell wall antigen that is also antiphagocytic. At its N-terminal end, SeM binds to fibrinogen; at its central region, it binds to IgG. In doing so, it masks the C3b binding surface on the bacterial cell wall and inhibits the alternative C3 and classical C5 convertase.[213] Sequencing of the SeM gene between codons 38 and 143 from 142 *S. equi* strains has identified 43 alleles of SeM, with each allele characteristic of a particular outbreak in a geographic location.[232d] Thus SeM sequence analysis can be used to complement epidemiologic investigations of strangles outbreaks. The loss of SeM expression leads to a loss of virulence but not to a decrease in the infectivity of the organism. Another M-like protein of *S. equi,* SzPSe, exhibits antigenic cross reactivity with the M-like proteins of *S. zooepidemicus,* but its contribution to the virulence of *S. equi* is unknown.[206] Recently, a protein unique to *S. equi,* Se18.9, was found to also possess antiphagocytic properties.[232c] The net effect of Se18.9 is to decrease deposition of C3 (and hence opsonic C3b) on the bacterial surface, reducing phagocytosis. SLS protein is thought to represent a fourth virulence factor by its cytotoxic properties. It damages host macrophages and neutrophils enabling immune evasion, as well as access to released essential nutrients.[205] A fifth virulence factor resides in the lipoprotein components of the *S. equi* cell membrane. When mutants of a specific lipoprotein are synthesized (PrtM) and the organisms are administered to susceptible ponies, clinical signs are markedly attenuated (0 of 5 infected ponies developed signs compared with controls). These data suggest that this lipoprotein is an important virulence factor and might be targeted for vaccine development.[232b] Additional factors that contribute to the pathogenicity of *S. equi* include extracellular proteins or exotoxins that are mitogenic for peripheral blood mononuclear cells.[230] These mitogenic factors (SePE-H, SePE-I, SePE-K, and SePE-L) bind simultaneously to class II major histocompatibility complex (MHC) molecules on antigen-presenting cells and to the T-cell receptor of T cells to induce nonspecific T-cell stimulation, proliferation, and cytokine release. As a result, an acute phase response ensues, characterized by release of interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and IL-6 from mononuclear cells that contribute to the production of fever, malaise, neutrophilia, and hyperfibri-nogenemia.[213,232a]

Humoral immune responses to many of these virulence factors are apparent in infected horses. During the initial stages of infection, serum IgGa to the SeM protein is induced, followed by the appearance of IgA and IgGb to SeM on the mucosal surfaces.[232e] During convalescence, strong IgGb responses appear to SeM, as well as to other surface-exposed proteins (Se44.2, Se46.8, Se45.5, and Se42.0).[213]

Clinical Signs

The incubation period for typical *S. equi* infection ranges between 2 and 12 days. Generally, horses are febrile (103°F or higher), exhibit lethargy, may have reduced appetite, and, although initially have serous nasal discharge, with the disease progressing, the material becomes mucopurulent. Ocular and nasal mucosal membranes are commonly hyperemic, and a mucopurulent ocular discharge may ensue. Mandibular and retropharyngeal lymph nodes are initially firm but become fluctuant before rupturing 7 to 14 days after the onset of signs. Retropharyngeal lymph nodes may rupture into the guttural pouches, causing neuropathy (dysphagia), empyema, and chondroid formation. Lymphadenopathy, one of the major signs of *S. equi* infections, may be asymmetric and may become so severe that dysphagia, stridor, and respiratory distress ensue. Swelling of the throatlatch area or of Viborg's triangle may be apparent, and palpation of this area may elicit pain. The affected horse may stand with its neck stretched out and be reluctant to swallow. A soft moist cough may be heard. The average course of the syndrome is 23 days.[208] In atypical *S. equi* infections, a mild inflammation of the upper respiratory tract occurs and is characterized by a slight nasal discharge, cough, and fever. Lymph node abscess formation occurs only in a small number of the cases.[209,233,234] In the majority of horses, clinical signs resolve, and bacterial shedding ceases by 4 to 6 weeks. In an estimated 10% of affected horses, continued intermittent shedding of *S. equi* may occur for several additional weeks.

Clinical Pathology

Neutrophilic leukocytosis, hyperfibrinogenemia, hyperglobulinemia, and anemia of chronic infection are characteristic findings with typical strangles cases. In cases complicated by concurrent pneumonia (aspiration), a left shift and the presence of neutrophils with toxic signs may be noted. In horses with purpura hemorrhagica, moderate to marked elevations in muscle enzymes occur. Horses that suffer from infarctive purpura hemorrhagica may have marked elevations of muscle enzymes.[235]

Diagnosis

Diagnosis of strangles in an equine patient is based on clinical signs and the isolation (culture) or detection (via PCR) of *S. equi* from a lymph node, nasal passage swab, nasopharyngeal lavage, or lavage fluid from the guttural pouches. Identification of *S. equi* via culture or PCR testing can generally be achieved 2 to 3 days after the onset of fever. Bacterial shedding can typically be detected for several weeks, often even following resolution of clinical signs. Because *S. equi* does

not colonize the upper airway mucosa culture, PCR testing of an acutely affected horse may initially be negative.[110] Nasopharyngeal lavage samples are more likely to yield positive results compared with swab samples. The lavage fluid will contact an increased surface area, increasing the likelihood of detecting bacteria if present. Following routine sedation, the wash is accomplished by instilling approximately 50 mL of warmed, 0.9% sterile saline into the nasal cavity via a 15-cm soft rubber tubing that has been inserted to the level of the medial canthus. Fluid is collected by gravity, placed into a 50-mL conical tube, and centrifuged; the pellet can be used for culture. Guttural pouch lavage is ideally performed endoscopically, allowing inspection of the medial and lateral compartments for evidence of lymphadenopathy, empyema, or (in more chronic infections) chondroid formation. For the lavage, approximately 50 mL of warmed, sterile 0.9% saline is instilled through the biopsy chamber and gently aspirated for culture, PCR, or (commonly) both. When endoscopy is not feasible, a guttural pouch lavage may yet be possible by advancing a Chambers catheter blindly into the guttural pouch of the sedated horse and lavaging the compartments. In unusual instances blood cultures may become positive for *S. equi* on days 6 to 12 after infection.[232] PCR testing is based on the SeM DNA sequence and is used in conjunction with bacterial culture in diagnostic workups. PCR has greater diagnostic sensitivity compared with bacterial culture and may detect SeM DNA for weeks in guttural pouch lavages after the disappearance of live organisms.[236] Upper airway endoscopy has become commonplace in field settings and may provide superior diagnostic capability for examination and sample collection, particularly when the goal is to identify carrier animals.[220] It is useful in detecting asymptomatic carriers, in establishing *S. equi* infection status before or after transport or commingling, and in establishing success of treatment.[223]

Screening of horses suspected to be suffering from complications of *S. equi* infection includes serologic assessment of the SeM-specific antibody. Horses identified to have an SeM titer in excess of 1:12,800 are suspected to suffer from complicating factors of *S. equi* infection, such as purpura hemorrhagica or metastatic dissemination of *S. equi*.[223] Serologic testing can also be helpful in determining the need for vaccination or in identifying horses that may be hyperresponders and predisposed to the development of purpura hemorrhagica (SeM-specific antibodies >1:1600). An enzyme-linked immunosorbent assay (ELISA) based on SeM-specific antibodies is commercially available through two commercial laboratories (Equine Diagnostic Solutions [EDS] Lexington, KY, and IDEXX Laboratories, Westbrook, ME). Serology may not reliably detect subclinical carriers because of the overlap in the breakpoints between normal and convalescent horses.[237,238]

Upper airway endoscopy is an important component of the examination of horses suffering from *S. equi* infection. Visual inspection of the guttural pouches will provide the clinician with the opportunity to identify evidence of lymphadenopathy, empyema, or chondroids, which can be instrumental in the diagnosis of *S. equi* infections. It can also aid in the identification of asymptomatic carriers, given that endoscopic abnormalities are often still present in these horses.[220,221] Notably, the absence of visible pathology in an asymptomatic carrier emphasizes the need to still obtain samples for concurrent culture and standard (direct) PCR analysis.[110,220,239]

Treatment

In most cases of *S. equi* infection supportive care provided by soft palatable feed, NSAID therapy, and maintenance of hydration will be all the medical care that is required. However, there are some differences of opinion regarding the administration of antibiotics in horses with strangles.[223] Treatment is a function of the stage of the disease, and the general recommendations listed here are those included in the American College of Veterinary Internal Medicine (ACVIM) Consensus Statement.[223] The reader is referred to this excellent review. In treating horses with *S. equi,* penicillin is the drug of choice, although the organism is sensitive to oxytetracycline and the potentiated sulfonamides.[211,231]

Clinical Management of *Streptococcus equi*–Infected Horses and Prevention of Disease Progression

Horses exhibiting early clinical signs of infection (initial fever spike and lethargy) in the absence of lymph node abscess formation should receive penicillin G therapy for 5 days, which can arrest the progression of the disease. One should isolate the horses during their treatment protocol. Treated horses are likely to remain susceptible to infection because sufficient bacterial antigen to elicit a protective immune response may not have been produced as a result of treatment. No evidence has been found that the use of antibiotics during this stage will promote the development of metastatic strangles.[240]

For alert and otherwise clinically stable horses exhibiting lymph node abscess formation, administration of penicillin slows the progression of lymph node abscess formation and is generally contraindicated. Hot-packing the area or areas promotes maturation of the abscess. Once achieved, the clinician should lance the abscess and flush it with a 3% to 5% povidone-iodine solution and, if desirable, then institute antibiotic therapy. Horses may benefit from phenylbutazone or flunixin meglumine administration, which will reduce fever, lessen pharyngeal discomfort associated with the lymphadenitis, and improve demeanor. Horses with *S. equi* infection should be maintained in an isolation area with strict adherence to biosecurity.

Horses that are systemically ill or that develop complications such as dysphagia, aspiration pneumonia, or respiratory distress (particularly if tracheostomy is needed), require supportive care in addition to high levels of IV penicillin and broad-spectrum antimicrobials effective against gram-negative organisms (aminoglycosides) and/or anaerobes (metronidazole). Horses may also require IV fluid therapy, nonsteroidal antiinflammatory drugs, and enteral nutritional support.

Sequelae

The overall complication rate in horses with strangles is estimated to be 20%; most frequently, complications result from metastasis of the organism to other organ systems with the formation of purulent foci.[241] Complications are detailed in the following sections.

Internal Abscessation of the Mesentery or of Parenchymatous Organs

The exact pathogenesis for the development of internal abscess formation is not known.[242] Although anecdotal reports suggest that antibiotic therapy early in the course of infection may predispose horses to the development of metastatic disease,

there is no evidence to support this suggestion. Additionally, because the treatment protocols for horses that suffer from internal abscess formation include long-term and in some cases high-dose antibiotic therapy, it is counterintuitive to consider that antibiotics are a risk factor for the development of metastatic disease. The prevalence of metastatic abscess formation is low, with estimates of 28% based on outbreaks on two farms.[243]

Purpura Hemorrhagica

Immune-mediated type III hypersensitivity leading to vasculitis may occur after re-exposure to *S. equi* by natural infection or following vaccination. Most commonly, purpura hemorrhagica develops during the recovery phase of the disease, 2 to 4 weeks following severe disease manifestation. Immune complex deposition of the vascular intima results in marked edema and necrosis. Clinical manifestation of purpura hemorrhagica involves the development of ventral edema that may be asymmetric in origin. The prevalence of this disorder after natural outbreaks is not known. In a retrospective study of 53 horses diagnosed with purpura hemorrhagica at a university teaching hospital,[244] 17 horses had confirmed exposure or infection with *S. equi,* and 5 had been vaccinated with SeM protein. Horses that are hypersensitive to *S. equi* are at greater risk for developing purpura hemorrhagica defined as having an SeM protein titer of >1:1600.[110] Specifically, the deposition of IgA-containing immune complexes is associated with clinical manifestation of purpura hemorrhagica, whereas an increase in *S. equi*–specific IgG was observed in the clinical recovery stage of purpura hemorrhagica in one report.[171,172] Horses with clinical evidence of purpura hemorrhagica demonstrated markedly elevated IgA titers against SeM-like proteins and culture supernatant compared with IgA titers in horses with uncomplicated strangles.[245] The isolation of immune complexes consisting of IgA and SeM-like proteins in the sera of horses with purpura hemorrhagica has led to the suggestion that IgA is directly involved in the development of purpura.[245,246] The immunologic basis for the increase in serum IgA levels is not known, but possible explanations include clonal expansion of *S. equi*–specific IgA secreting plasma cell populations, impaired IgG production, neutralization, or IgG utilization. Equine purpura hemorrhagica has some similarity with pathogenesis and disease manifestation and is therefore sometimes compared with Henoch-Schönlein purpura, an IgA-mediated immune complex–mediated disease of people.[246]

Guttural Pouch Empyema and Chondroids

This has been described earlier (see page 327).

Septicemia and the Development of Infectious Arthritis and Pneumonia

These conditions warrant a poor prognosis.[247]

Retropharyngeal Abscess Formation

Bacterial translocation across the pharyngeal epithelium with *S. equi* results in lymphadenitis and abscess formation of a variety of regional lymph nodes. Involvement of the retropharyngeal lymph nodes can result in marked pharyngeal compression and airway obstruction. Lymphadenopathy and inflammation in the guttural pouches may result in neuropathy and associated dysphagia, potentially with aspiration. Upper airway endoscopy will reveal nasopharyngeal collapse, which is a deviation of the larynx potentially with drainage of purulent material into the nasopharynx when external pressure is applied to the parotid region.[248] Skull radiographs typically demonstrate a soft tissue opacity in the retropharyngeal area; thickening of the roof of the pharynx; reduction in the diameter of the pharyngeal airway; and distortion or compression of the guttural pouches, pharynx, and trachea.[249] Ultrasonographic evaluation of the affected region caudal to the mandible, dorsal to the trachea, and between the linguofacial vein and maxillary vein can be used to identify the position of the abscessed retropharyngeal lymph nodes.[250,251] Abscess maturation and rupture will occur into the pharynx, which may result in secondary pneumonia, or abscess rupture may occur dorsally into the guttural pouches, which when associated with marked inflammation may lead to neuritis and subsequent dysphagia.[241,248,249]

Abscess Formation Resulting in Respiratory and Systemic Complications

Laryngeal hemiplegia has been reported to occur when abscessed lymph nodes impair normal conduction of the recurrent laryngeal nerve resulting in laryngeal paralysis.[252] In the same report an additional complicating factor involved tracheal compression caused by abscess formation in the region of the cranial mediastinal lymph nodes.[252]

Other Complications

Other complications of *S. equi* infection can also include endocarditis, myocarditis, or cardiac conduction abnormalities. These conditions may result from primary colonization or secondary immune-mediated, inflammatory disorders. An additional complication that can occur in periparturient mares is a condition of agalactia. Although not a result of primary bacterial infection, systemic illness and negative energy balance may play a role for mares reducing or eliminating their lactogenic capacity. Brood mares suffering from *S. equi* infection, that are intended to be raising a foal, should be monitored closely for proper lactation so that foals can be appropriately nutritionally supplemented, as indicated.

Central Nervous System Disease Disorder[243,253,254]

Clinical manifestation of CNS disease will depend on the location of *S. equi* abscess formation. Cerebral cortical lesions may present with the clinical complaint of circling, altered behavior, cortical blindness, marked lethargy, and/or reduced feed intake. Patients presenting with clinical signs that suggest CNS involvement may benefit from advanced imaging. In a previous report T2-weighted MRI images provided valuable diagnostic information regarding lesion location and severity. In addition to imaging acquisition in the cranium, imaging also defined lesion severity in the region of retropharyngeal lymph nodes and sinus involvement. The use of MRI in this report in two of four confirmed positive cases revealed high-quality images that involved soft tissue of the CNS. Lesions that were identified with MRI correlated well with lesions identified at postmortem. MRI is an excellent diagnostic tool that may be considered for the characterization of lesions in affected patients.[243] In a case of *S. equi* meningoencephalomyelitis in a 4-month-old foal, cerebrospinal fluid (CSF) analysis revealed marked elevated total nucleated cell count and total protein concentration and was PCR positive for *S. equi.*[254]

***Streptococcus*-Associated Myopathy.** Myositis may develop as a complication of *S. equi* infection. A serious and potentially fatal form of myositis has been called infarctive purpura hemorrhagica. In this disease horses may initially demonstrate clinical signs of purpura hemorrhagica, but in addition to lethargy, fever, and asymmetric edema, clinical signs of pain evidenced by reluctance to move, marked ventral edema, and colic signs often develop. Complete blood count and serum chemistry analysis reveals neutrophilic leukocytosis with left shift, and hyperproteinemia and hypoalbuminemia are characteristic findings. Most notably marked elevation of creatine (phospho)kinase and aspartate aminotransferase are identified on serum chemistry evaluation. When clinical presentation is consistent with infarctive purpura hemorrhagica and marked muscle involvement is identified, these findings should alert the clinician to the likelihood of extensive muscle damage and necrosis. Postmortem examination reveals muscle infarctions, as well as infarcts in the skin, gastrointestinal tract, pancreas, and pulmonary thromboembolism of the lungs; collectively all findings are compatible with infarctive purpura hemorrhagica. The prognosis is guarded, and affected horses require intense medical management: pain management, IV fluids, penicillin, and high-dose corticosteroid therapy.[235]

Immune-mediated myositis has been reported to occur in horses.[255] Among horses diagnosed with this disease the most common breed included Quarter Horses or closely related breeds.[255] The presenting complaint for affected horses is rapid with marked muscle atrophy (usually symmetric), particularly over the epaxial and gluteal muscles. Additional clinical signs include lethargy, reluctance to move, weakness, and fever. Muscle enzymes creatine kinase and aspartate aminotransferase are typically elevated. Histopathologic evaluation of affected muscle groups reveals macrophages and lymphocytes within myofibers. Phenotypic classification of lymphocytes revealed them to predominantly be CD4+ cells with few B cells, plasma cells, and CD8+ lymphocytes. Although 39% of the horses in the original report had recent exposure to *Streptococcus* spp. organisms, a specific trigger was not determined in all cases.[255] The diagnosis of immune-mediated myositis is based on characteristic clinical findings of marked muscle atrophy in combination with supportive histopathologic findings of lymphocytic myositis obtained from affected muscle tissue. Treatment involves a combination therapy of penicillin and corticosteroid therapy. Response to therapy is rapid and marked in most cases, but a tapering dose of corticosteroids may be needed for several weeks to achieve complete resolution in lymphocytic infiltrates within muscle tissue. Rarely, a poor or incomplete response to therapy is observed.[255]

Outbreak Management

The primary goal when managing an outbreak of *S. equi* is to effectively manage affected horses and minimize disease spread to naive individuals in an effort to minimize the risk of further disease outbreak and potential complicating factors.[223,256] The reader is referred to the ACVIM[223] consensus statement for complete management guidelines, but a summary of outbreak management is provided here:

1. Immediate quarantine of premises that halts equine transport on or off the affected premises to eliminate further disease spread.
   - Horses that are identified to have confirmed *S. equi* disease must be located in an isolation area that is properly designated as such ("dirty" or "strangles cases"). This will allow caregivers to have major areas for horse handling and "clean" and "affected" areas.
2. Determine whether recovering horses are infectious.
   - Three nasopharyngeal lavages (preferably) collected at weekly intervals for 3 weeks; samples should be tested using microbiological culture and PCR testing.
   - Test-negative horses are "clean" of infection.
3. Determine whether apparently healthy horses are *S. equi* positive.
   - Upper airway endoscopy to determine whether there is the presence of disease.
4. Eliminate *S. equi* from guttural pouches of affected horses.
   - Endoscopically guided guttural pouch lavage with isotonic, sterile fluid to remove mucous or chondroid accumulation; manual removal of chondroids may be required if lavage alone is unsuccessful.
   - Systemic and local antibiotic therapy to eliminate *S. equi* infection.
5. Establishment of an isolation area and protocols to prevent indirect cross-infection of *S. equi* to clean areas.
   - Caretakers should have dedicated barrier clothing and instruments. Avoid simultaneous contact with horses. Manage affected horses after clean horses and clean areas.
   - After removal of organic debris, all areas should be cleaned and disinfected.
   - Manure and general waste should be composted in an isolated area.
   - Water troughs should be cleaned daily.
   - Horse transportation vehicles should be cleaned after every use.

Disease Prevention

Among the currently manufactured vaccines there is an inactivated M-protein subunit vaccine and an attenuated live *S. equi* vaccine. The subunit vaccine is a protein-rich acid- and enzyme-extract product that is administered parenterally (e.g., Strepvax II, BIVI). The attenuated live *S. equi* vaccine is administered intranasally (Pinnacle I.N., Zoetis Inc.). In Europe, deletion mutant vaccine is approved as a submucosally administered vaccine. Injection is recommended in the upper lip (Equilis StrepE, Intervet). Among the currently available vaccines, there is no guarantee for the prevention of strangles in vaccinated horses.

Parenterally administered inactivated vaccines induce serum bactericidal activity, but circulating antibodies are not necessarily protective because local mucosal immunity plays a significant role in the resistance to infection.[256] Naive adult horses and foals should receive two to three vaccine doses at 2- to 3-week intervals, followed by annual boosters while they remain at risk for *S. equi* exposure. Vaccine administration is not recommended in horses with high SeM titers (>1:1600) because of the potential for an adverse event such as the development of purpura hemorrhagica.[223]

The attenuated live intranasally administered *S. equi* vaccine is aimed at mimicking the natural exposure to *S. equi,* inducing endogenous local immunity with systemic and locally produced immunoglobulins, notably IgA, aimed at this problematic pathogen. Vaccine manufacturer claims report a significant reduction in the number of horses developing clinical signs of disease following challenge when vaccinates are compared with control animals. Approximately

40% of vaccinees develop clinical signs of strangles, whereas approximately 60% of nonvaccinated control animals develop disease. Although intranasal vaccines are expected to produce fewer adverse reactions than parenteral vaccines, lethargy, inappetence, fever, lymphadenopathy, lymph node abscess formation, purpura hemorrhagica, and intramuscular abscesses have occurred after vaccination.[213] Additionally, clinicians may consider whether to administer the attenuated live vaccine to young horses (<1 year of age). In one report it was suggested that in this age group the potential for disease manifestation exists following vaccination and was observed, yet it should be noted that the sample size was small in this investigation.[257]

Submucosal vaccines have also been associated with the production of adverse reactions, including neck and mandibular lymph node abscess formation.[258]

Attenuated live vaccines should be administered to healthy afebrile animals free of evidence of disease. For the intranasal vaccine, it is recommended that two doses at 2-week intervals followed by an annual booster be administered. Foal vaccination should be considered in light of the mare's vaccine status at the time of parturition. In foals born to unvaccinated mares that are in high-risk situations vaccination may be initiated at 1 to 2 months of age, but the risk of vaccination is that disease-like manifestations may occur.

For the submucosal vaccine available in Europe (Equilis StrepE), two doses (0.2 mL) of vaccine are injected at 4-week intervals in healthy horses older than 4 months of age. Because the duration of immunity by the submucosal vaccine is limited, it is recommended that high-risk horses receive a booster every 3 months, although boosting of horses vaccinated up to 6 months previously in the setting of an outbreak was suggested in one report.[237]

When administering any form of an attenuated live vaccine (intranasal or submucosal vaccines) caution should be used regarding health status and any invasive procedures implemented at the time of *S. equi* vaccination. Specifically, care should be implemented if other vaccines are concurrently administered because potential abscess formation at the site of concurrent intramuscular vaccines may occur. Handling the vaccine should be followed by thorough hygiene measures that include careful handwashing to minimize the transfer of bacteria to other sites or instruments.

Additional areas of concern regarding *S. equi* vaccination include the risk of complications such as purpura hemorrhagica. For this reason, vaccination at the time of disease outbreak is controversial and should be carefully considered. The only horses that should be vaccinated are those that have not had a chance for bacterial exposure and those with an SeM titer of <1:1600. An appropriate amnestic immune response requires approximately 10 days to occur, so exposure to at-risk individuals should not occur before this immunity is expected to be present. No *S. equi* vaccines have any claim to provide protection against *S. zooepidemicus*–associated respiratory (or otherwise) disease.

General Control Measures

Introduction of new arrivals to an equine establishment should include careful biosecurity protocols that include a quarantine period of 3 to 4 weeks. Quarantine areas should be designed so that there is no direct contact of new arrivals with resident horses and that personnel use proper hygiene after working with new arrivals. During this period of quarantine new arrivals should be monitored for any evidence of contagious infectious disease, which includes overall health status, presence of nasal discharge, lymphadenopathy, and rectal temperature monitoring one to two times daily. Comprehensive monitoring of potential *S. equi* carriers should include upper airway endoscopy with guttural pouch lavage. Collection of fluid for *S. equi* PCR testing will aid with the determination of *S. equi* carrier horses. Measures should be implemented to prevent animal or personnel contact between the new additions and resident horses.

Although previous reports have provided suggestions that *S. equi* is a pathogen that may be eradicated, this has not been successfully achieved. Factors that favor eradication include an individual host for infection, poor survival in the environment, minimal antigenic variability, PCR diagnostic availability, and low level of carrier individuals.[259] The primary caveat is that although *S. equi* vaccines are available they are not universally protective. It is anticipated that with the advent of more efficacious vaccine(s), eradication of the serious bacterial pathogen may be achievable. If successful, strangles would become a reportable disease with stringent regulatory control measures implemented to prevent the reintroduction of this pathogen.

EQUINE INFLUENZA (EIV)

EIV is classified as an orthomyxovirus with a single-stranded segmented RNA genome.[260] Influenza viruses are classified on the basis of surface and internal protein antigens into three types (A, B, and C); only type A influenza is reported to infect horses. Major viral antigens include neuraminidase and hemagglutinin (HA). Two type A viral subtypes are known to cause disease in horses: H7N7 and H3N8.[260] The strain H7N7 was initially isolated in 1956 in Prague and designated A/equine/Prague/56. This H7N7 variant, termed equine-1 influenza, has not been isolated since 1980 and is believed to have disappeared from the equine population.[119] The H3N8 EIV, called equine-2, is a strain that was initially isolated in Miami in 1963 and designated A/equine/Miami/63.[261-263] Antigenic drift has subsequently resulted in many subtypes of variant equine-2 among horses, including A/equine/Fontainebleau/79, A/equine/Kentucky/81, A/equine/Saskatoon/90, and A/equine/Newmarket 2/93. Although antigenic drift has been observed for many years, a larger scale change in the antigenic nature of the EIVs has not been documented.

Equine influenza is endemic in Europe, North and South America, North Africa, the Middle East, and Asia. Because of the highly contagious nature of this virus, susceptible equine populations are at risk for major outbreaks if virus is introduced. This scenario occurred in 2007 when breeding stallions from Japan were introduced into Australia and were infected with a Florida Clade I lineage H3N8 equine influenza strain of the virus.[264,265] Australia was influenza free at the time of introduction, and disease outbreak resulted in 76,000 horses developing the viral infection. The outbreak occurred over an 18-week period. Rapid control measures were implemented, including vaccination; the outbreak was successfully managed; and Australia is once again influenza negative.

Influenza is most common in horses commingled under stressful conditions such as race training. Viral spread occurs through aerosolization of viral particles from a contaminated horse to susceptible individuals. Fomites can play a role as well; contamination of personnel can also contribute to viral

spread.[266] Infection occurs via inhalation of viral particles. The virus infects respiratory ciliated epithelium, leading to loss of the mucociliary escalator for pathogen and particle clearance. Therefore, viral infection predisposes affected individuals to secondary bacterial infection.

Clinical Signs

Clinical characteristics of equine influenza include a short incubation period of 1 to 3 days, high fever, depression, and paroxysmal coughing, which can be severe. Nasal discharge may begin as serous fluid and change to a more mucopurulent character with disease progression and bacterial contamination. Submandibular lymphadenopathy is commonly associated with viral disease. Myositis, anorexia, and persistent cough are not uncommon signs observed in association with influenza infection. Immune status and previous vaccination will directly influence disease course; unvaccinated horses or those not previously exposed to the virus typically demonstrate the most severe clinical evidence of disease. Horses with influenza are at risk of secondary bacterial infection that may progress to bronchopneumonia and/or pleuropneumonia.

Diagnosis and Treatment

The recognition of a rapidly spreading respiratory infection in a group of horses is characterized by rapid onset, high fever, depression, and particularly cough, which is strongly presumptive of the equine influenza infection.[267] Vaccinated horses may not demonstrate clinical manifestation of disease, which was illustrated with an outbreak of racehorses in Hong Kong that displayed a variety of clinical signs that delayed definitive diagnosis in this group of horses.[268,269] Additionally, based on experimental challenge data, there is an inverse relationship regarding the level of challenge and time course for clinical manifestation of disease.[270] Collectively, a variety of factors that include host immunity and health, pathogen-specific immunity, and pathogen dose can impact the presence and severity of clinical manifestation of the disease. For these reasons, all factors should be considered when evaluating a potential outbreak with appropriate diagnostic testing performed to determine the specific etiologic agent responsible for disease outbreak.

A variety of diagnostic tests have been developed to determine the presence of this virus in the equine host and include virus isolation, influenza A antigen detection, HA inhibition (paired baseline and convalescent), and real-time quantitative PCR (qPCR). Definitive diagnosis in an outbreak is based on viral detection with nasopharyngeal secretions or by an increasing virus neutralization (VN) titer. Notably, evidence supports that optimal sample collection with improved test sensitivity should be based on nasopharyngeal lavage sampling rather than just nasal swab sampling, particularly in surveillance situations that will lead to quarantine of test-positive individuals.[271] Sample collection with a synthetic swab submitted for PCR testing provides a cost-effective and efficient method of diagnostic testing under most routine clinical settings.[268,272]

Even though specific antiviral treatment for the management of influenza-positive humans has become commonplace, the same cannot be said for equine patients. The primary goal of prevention through application of effective biosecurity measures and vaccine strategies remains the focus for disease avoidance in horses. Among the American Association of Equine Practitioners risk-based vaccine guidelines, vaccination for the prevention of influenza is an achievable goal in many settings. Commercially available vaccines include live attenuated, cold-adapted, inactivated, and recombinant vectored vaccines. The majority of investigation into vaccine efficacy is based on relatively small-scale investigations that consistently identify a correlation between protection from disease and antibody levels, particularly when there is strong similarity between vaccine strain and challenge isolate. In most performance horse populations, regardless of discipline, vaccination practices are applied to aid with enhanced host immunity. In some instances, racing authorities have mandated vaccine protocols aimed at providing support for vaccination to enhance immunity. A recent investigation aimed at determining the optimal vaccine administration interval compared different intervals aimed at stimulating protocols established by various racing authorities. It was concluded that longer vaccine intervals may result in periods of susceptibility, in which vaccine-induced immunity may not be protective, but when needed shortened intervals may be protective when risk of exposure is high.[273] At the time of this writing, the United States Equine Federation has implemented a requirement for all horses competing in sanctioned events or shows to demonstrate proof of vaccination for EIV and EHV in the previous 6 months. Accelerated vaccine protocols with the recombinant canary pox–vectored influenza vaccine have been demonstrated to increase serum antibody levels using the single radial hemolysis assay. In this investigation, time from initial vaccine to second dose was 14 days, followed by a booster vaccine at 105 days.[274] In situations where an accelerated vaccine protocol is needed to enhance viral-specific immunity, accelerated protocols should be considered. Implementation of a protocol with the canary pox–vectored vaccine during the Australian outbreak not only provided enhanced host protection, but this strategy used a vaccine with differentiation of infected and vaccinated individual capacity, which is an added advantage to this protocol in this setting.[268]

EQUINE HERPESVIRUS INFECTION

Herpesviruses are enveloped, double-stranded DNA viruses that are ubiquitous in nature and are well recognized for infecting a variety of hosts. Equids have been reported to have the potential to be infected with nine different herpesviruses.[275] Among the viruses with clinical relevance to the equine host are alphaherpesviruses (EHV-1, EHV-3, and EHV-4) and gammaherpesviruses (EHV-2 and -5).

Among alphaherpesviruses EHV-1 and EHV-4 are most widely recognized for morbidity in horses.[276] Both EHV-1 and EHV-4 are well recognized for induction of upper respiratory disease in horses, whereas EHV-1 is also associated with abortion and in rare instances may induce severe neurologic disease. EHV-1 viral infection is associated with cell-associated viremia, in which infected host leukocytes effectively disseminate virus throughout the infected horse. Differences in these somewhat similar viruses are believed to influence clinical manifestation of the disease. Although both are capable of viral replication in the upper respiratory tract, EHV-1 is well adapted to translocate across the respiratory epithelium, whereas EHV-4 appears to be restricted to the upper airways supported by an airway explant model system,[277] which is believed to have a direct influence on this virus being restricted in anatomic location resulting in rhinopharyngitis and/or tracheobronchitis.[268] However, as a result of viral

dissemination, EHV-1 is recognized for not only upper respiratory disease but also abortion, neonatal illness, and neurologic disease.[278,279] Abortion and myelitis are a direct result of vasculitis that is induced by systemic spread of the virus. Because EHV is neurotropic, latency is likely and associated with the trigeminal (cranial nerve V) ganglion or lymphoid tissue.[280] During times of severe stress or immune suppression disease viral recrudescence and shedding are possible when latent virus is reactivated. Although EHV-1–associated disease has been reported in a variety of situations that include western performance events and a racetrack setting, horses evaluated in a veterinary hospital setting for the potential of EHV-1 viral shedding were not found to be shedding virus.[281]

Pathogenesis

Herpesviruses are well recognized for viral maintenance within the host as a latent virus, and this holds true for EHV-1/4.[282] A continued cycle of viral latency and recrudescence likely plays an important role in the viral maintenance within groups of horses. Immunosuppression is associated with attenuated cell-mediated immunity, which leads to viral shedding among latently infected individuals.[283,284] Horses that are latently infected are viral carriers for life.[268] In a small-scale investigation aimed at viral detection in a group of experimentally infected horses, it was determined that horses challenged 5 years previously were later determined to have maintained the same viral strain as the primary challenge strain.[285]

Clinical Signs

Clinical signs of disease associated with EHV-1/4 typically include pyrexia, marked serous nasal discharge, and occasionally cough. Secondary bacterial colonization will result in nasal secretions changing from mucoid to mucopurulent in nature. Lymphadenopathy and serous ocular discharge may also be observed in association with EHV-1/4 infection. Disease spread is typically not as rapid as with equine influenza, but morbidity among susceptible horses can be very high.[286,287] Clinical manifestation of respiratory disease tends to be worse in younger horses compared with mature adults, with reports commonly including disease outbreak in recently weaned foals. This may in part be influenced by the level of immunity in young horse populations. Diagnosis in affected horses more commonly reveals EHV-4 as the primary pathogen associated with disease.[276,288] EHV-1/4 is well recognized to cause respiratory disease in adult horses. One report described an EHV-1 outbreak among a group of racehorses in training during the winter months, whereas EHV-4 did not have an apparent age or season association with disease outbreak.[289]

When pregnant mares are infected, abortion occurs approximately 2 to 12 weeks following infection, typically during the final months of gestation. Aborted fetuses are typically fresh or demonstrate minimal autolysis while the placenta is passed shortly after abortion. Mares that are infected with EHV-1 rarely demonstrate clinical evidence of illness, particularly before expulsion of the fetus. There is no evidence of damage to the reproductive tract of the mare and no problems with subsequent fertility. Abortions may be individual cases or observed as an abortion storm within a herd. Mares that are virally infected during late gestation might successfully deliver a live foal, yet the foal will have marked disease caused by viral infection. Clinical signs observed in EHV–infected foals may include severe pneumonitis, icterus in association with hepatic involvement, and commonly marked neutropenia as a reflection of severe bone marrow necrosis and destruction.

Among the viral strains of EHV-1 that have been identified, in some instances there is a mutation in the open reading frame for the DNA polymerase (DNApol) gene. Specifically, the mutation has been identified as a single nucleotide polymorphism (SNP) at the nucleotide level (A2254 > G2254),[290] which is associated with increased virulence and the potential for neurologic disease manifestation.[291] Following signs of upper respiratory disease and fever, affected horses may progress with signs of mild incoordination, posterior paresis, posterior paralysis, and recumbency. Commonly clinical signs will include urinary emptying defects that result in marked urine dribbling. Attenuated sensation in the perineal region may also be present. Prognosis is influenced by disease severity and progression to recumbency.

Postmortem examination on foals from aborted mares reveals interlobar pulmonary edema, pleural effusion, and necrosis of lymphoreticular tissues that includes liver, bone marrow, and thymus. Petechiation of the myocardium, adrenal glands, and spleen are commonly observed lesions. Histopathologic examination will reveal intranuclear inclusions, particularly from the liver, lung, and adrenal glands.

Adult horses that succumb to EHV-1–associated myelitis may have no apparent gross lesions, or they may have mild hemorrhage in the meninges, brain, and spinal cord. Histopathology will reveal lymphocytic vasculitis with endothelial damage and perivascular cuffing, thrombus formation, hemorrhage, and possibly malacia. Lesions are most commonly observed in the spinal cord but may be observed in the brain and brainstem.

Diagnosis and Treatment

Horses suffering from contagious infectious respiratory disease should be maintained in an isolation-type environment. In most cases diagnostic confirmation requires days or longer. Because of the inherent nature of respiratory pathogens, horses should be restricted from contact with other horses, particularly in a hospital environment or farm with high-risk individuals such as breeding animals and animals that are traveling frequently for performance-related activities. Because of the similarity in clinical signs, viral respiratory disease requires diagnostic confirmation for pathogen identification. Serologic testing for virus-neutralizing antibodies collected during the acute and convalescent (4 weeks) sampling with a fourfold rise in antibody supports the diagnosis of recent EHV infection. However, when considering the frequency of latently infected adult horses, serologic testing can be challenging to interpret. Additionally, mares that abort their fetus have had a serologic response to viral infection long before delivery of the fetus, further complicating this diagnostic method. Definitive diagnosis (gold standard) is based on virus isolation from samples obtained from upper respiratory nasopharyngeal lavage sampling or leukocyte buffy coat isolation early in the course of infection. PCR testing has become the diagnostic test of choice because of its high analytic sensitivity and specificity. Positive PCR test results may be obtained when culture techniques are not successful because of low-level viral shedding. Samples can be collected from that respiratory tract and include nasal swab sampling or nasopharyngeal lavage collection. Additionally, because of the leukocyte-associated nature of the virus,

whole blood (anticoagulated) buffy coat sampling will provide an additional sample for viral PCR testing. In index cases, nasal swab and whole blood samples should be tested simultaneously (in parallel) to further enhance diagnostic sensitivity.[292] Nasal swab sampling is recommended over pharyngeal lavage sampling because of greater diagnostic sensitivity.[293] Contemporary molecular diagnostic tests have been designed to test for the presence of EHV-1 and the presence of the DNApol SNP at the amino acid level (D752/N752), with D752 more commonly associated with neurologic disease manifestation.[290] Diagnostic testing requires careful interpretation as outlined in the 2009 ACVIM consensus statement on this pathogen and disease process, and the interested reader is referred to this particularly valuable reference for a more detailed diagnostic outline.[292] Careful patient examination is the initial step to determine the course of diagnostic evaluation. In patients suspected to suffer from EHV-1–associated disease, appropriate diagnostic samples should be collected for PCR analysis. In samples that test positive for the EHV-1 sequence, an additional step in molecular characterization will be determining the presence of the mutated EHV-1 biovar. If present in the appropriate clinical setting, proper biosecurity and quarantine measures should be implemented immediately. In contrast, identification of this EHV-1 D752 biovar in an asymptomatic individual (healthy horse with no signs of respiratory or other disease) should not be overinterpreted. Recall that horses are often latently infected with EHV-1, and simply identifying this virus in an otherwise healthy horse does not confirm the presence of future disease manifestation, particularly neurologic disease. There is insufficient evidence to determine the subsequent implications of an individual being infected with this virus. Additionally, the absence of EHV-1 D752 does not preclude the development of equine herpesvirus myeloencephalopathy (EHM) in a horse that has clinical signs of respiratory or neurologic disease. Recommendations regarding diagnostic testing for horses that demonstrate clinical signs suggesting EHV-1 and provided in the EHV-1 ACVIM consensus statement include[292]:

- Uncoagulated (ethylenediaminetetraacetic acid) whole blood sample and nasal swab (synthetic material) for quantitative real-time PCR testing.
- Uncoagulated blood and nasal swab for virus isolation of EHV-1 when strongly suspect disease-positive horse based on clinical signs and PCR testing.
- Paired serum samples collected 12 to 21 days apart for VN and ELISA for specific virus antigen when possible.
- *Avoid* diagnostic testing in healthy horses that have no risk for disease manifestation; screening in healthy horses is not recommended.

There is no specific antiviral treatment recommended for the treatment of horses with respiratory disease associated with EHV-1/4 infection. Rest and proper nursing supportive care are indicated to facilitate disease recovery. High-quality, palatable feeds are recommended to facilitate proper nutritional intake. Horses should be maintained in a comfortable, sheltered environment to minimize the risk of secondary disease manifestation such as bacterial colonization. Horses that demonstrate marked pyrexia, fever >103°F will benefit from the administration of nonsteroidal antiinflammatory therapy in the euhydrated individual. Antibiotic therapy is indicated if clinical signs are consistent with bacterial colonization such as mucopurulent oculonasal discharge or pulmonary disease is evident.

Foals infected in utero with EHV-1 are unlikely to survive despite intensive care therapy. Antiviral therapy with acyclovir or valacyclovir may be considered for virally infected foals. Adult horses that demonstrate clinical manifestation of neurologic disease consistent with EHM are appropriate candidates for antiviral therapy with valacyclovir. Additional nursing care for EHM-affected horses often requires intensive nursing care that may include indwelling urinary catheter placement to avoid urinary bladder rupture. Satisfactory recovery can be achieved although mild persistent neurologic deficits might still be discernible 6 to 12 months later. Even so, these horses are often capable of competitive activity. Horses that remain recumbent for extended periods of time have a more guarded prognosis for survival.

Gammaherpesviruses

EHV-2 is ubiquitous among the equine population even though the exact significance of this virus as a primary respiratory pathogen remains to be fully elucidated. Select clinical signs and conditions that have been implicated with EHV-2 infection include pulmonary inflammation, keratoconjunctivitis, fever, pharyngitis, inappetence, immunosuppression, lymphoma. and lymphadenopathy.[294,295] EHV-2 has been identified within circulating leukocytes, nasal secretions and nasopharyngeal swabs, kidney, bone marrow, spleen, and reproductive and ocular tissues.[296-298] In addition, EHV-2 has been positively identified in lymphoid and neural tissue collected from naturally infected horses. In one investigation of equine respiratory disease tracheal aspirates were infrequently positive for EHV-2 (1/20 foals; 5%) when collected from healthy horses compared with those with clinical respiratory disease (20/30; 67%).[299] Notably, in the same investigation EHV-2 was identified within circulating leukocytes collected from both groups of foals. A subsequent investigation aimed at demonstrating a causal relationship of this viral agent and disease used respiratory secretions collected from a clinically affected EHV-2–infected horse. These were then administered to two immunosuppressed ponies that subsequently developed respiratory disease (coughing and nasal secretions), conjunctivitis, viral shedding, and a demonstrable humoral antibody response to EHV-2.[296] In addition to primary respiratory disease, it has been suggested that EHV-2 may play an important role regarding the disease pathogenesis of other respiratory pathogens, such as EHV-1. It is plausible that altered immune function infection induced by EHV-2 infection may promote recrudescence of EHV-1, resulting in notable clinical respiratory disease.[298]

Equine Herpesvirus-5 and Equine Multinodular Pulmonary Fibrosis

Initial investigations into the relevance of EHV-5 in association with interstitial pneumonia in adult horses resulted from the identification of asinine herpesvirus-5 (AHV-5) and AHV-2 in cases of interstitial pneumonia in donkeys.[300] In addition to the identification of AHV-2/5 in donkeys suffering from pulmonary inflammation and fibrosis, adult horses suffering from poor exercise tolerance and multinodular pulmonary fibrosis have been identified to harbor EHV-5.[301] Subsequent to initial observations, numerous reports support the finding that in nearly all cases of equine multinodular pulmonary fibrosis (EMPF) EHV-5 has been identified. In addition to the identification of EHV-5 in EMPF-affected horses,

Williams et al. administered EHV-5 via bronchial instillation to six healthy horses that subsequently demonstrated an increase in total lung collagen and the appearance of myofibroblasts, which supported the suggestion that EHV-5 is the causative agent for EMPF.[302] It is possible that infection with EHV-5 independently results in the development of pulmonary fibrosis, although it is also reasonable to consider that a synergistic effect may occur in horses that are coinfected with EHV-5 and EHV-2 or AHV-5. Further support regarding the synergism of EHV-2/5 comes from the observation that both gammaherpesviruses have been identified in approximately 30% of the cases of EMPF.[298]

Equine patients that suffer from EMPF are typically adult-aged horses (Fig. 8.15A). Clinical signs often include weight loss, poor overall condition, and increased respiratory rate and effort. Physical examination will localize disease to the respiratory tract characterized by fever, abnormal pulmonary sounds including tracheal rattle, and increased bronchovesicular sounds with wheezes. Additional clinical signs include lymphadenopathy, reluctance to ambulate normally (pain), oral cavity ulcerations, and keratoconjunctivitis.[303-305] Hematologic examination will often reveal leukocytosis, neutrophilia, lymphopenia, anemia, hyperfibrinogenemia, hypoxemia, and hypoalbuminemia.[302] Consistent with the pronounced inflammatory response in affected individuals, elevation of serum amyloid A can also be expected in EMPF patients. A number of investigators suspect that in some cases of infection there is an immune-mediated destruction of mature cells in the peripheral circulation and possible destruction of hematopoietic precursors located within the bone marrow, which can result in notable cytopenia.[306] Although this mechanism remains to be fully elucidated, this author has most commonly observed elevation of leukocyte cell counts in EMPF cases, although in rare individuals marked cytopenia has been observed. EHV-5 has been conclusively identified in the bone marrow of one affected individual that had pancytopenia.[306]

Diagnosis

Diagnostic evaluation of suspect EHV-5 cases should include a complete evaluation of the respiratory tract. Physical examination followed by thoracic ultrasound will provide the clinician with evidence of primary pulmonary disease. Severity of ultrasonographic abnormalities will relate to chronicity of pulmonary disease demonstrated by pleural irregularity and thickening, peripheral nodules, and abscess that can often be visualized in non-acute cases (Fig. 8.15B). Subsequent respiratory system examination will often include thoracic radiographs, which reveal moderate to severe interstitial pulmonary nodules (Fig. 8.15C). Radiographic appearance of pulmonary nodules may resemble those resulting from metastatic neoplasia or fungal granulomas; therefore, additional diagnostic testing will be needed to definitively diagnose EMPF. Additional diagnostic testing includes BAL, which will allow the clinician to examine pulmonary cellularity of the lower airways. Characteristic findings include neutrophilic inflammation with marked mucous accumulation. In some instances, the cytologic characteristics may resemble RAO (heaves). Clinical distinction of the conditions is important. EMPF should be suspected in an equine patient diagnosed with RAO that does not respond to appropriate therapy and environmental management. In addition, EMPF-affected horses have chronic progressive disease, systemic inflammation, and a poor or incomplete response to corticosteroid therapy.

Postmortem examination of EMPF-affected horses reveals evidence of primary pulmonary disease characterized by enlarged lungs that fail to collapse, diffuse pleural thickening, and the presence of numerous fibrous nodules of variable size, in some cases up to 5 cm (or more) in diameter. Bronchial and mediastinal lymphadenopathy is commonly associated with the presence of pulmonary granulomas (Fig. 8.15D–E). There have been two types of pulmonary granulomas described in EMPF patients based on lesion size and distribution. The more common form is referred to as a disseminated nodular form in which multifocal, coalescing fibrotic nodules are present throughout all lung fields with lesions ranging from 1 to 5 cm. In this form of EMPF there is often minimal healthy pulmonary parenchyma present, and approximately 80% of the lungs are composed of fibrous granulomas. Alternatively, a discrete nodular form has been described in which large discreet nodules are separated by normal-appearing pulmonary parenchyma. The nodules in this form of disease are much larger, sometimes measuring 8 to 10 cm in diameter. Regardless of the form of disease on histologic examination, the lesions are characterized by marked interstitial fibrosis with type 2 pneumocyte hyperplasia. Alveoli are often filled with neutrophils, fewer alveolar macrophages, multinucleated giant cells, and eosinophilic proteinaceous material. Intranuclear inclusions, consistent with herpes viral infection, may be observed within macrophages and/or epithelial cells.[302,307]

Definitive diagnosis of EMPF is based on histopathologic evidence of pulmonary tissue obtained by lung biopsy or sample collection at postmortem. Diagnosis is often strongly suspected based on clinical history, ultrasonographic evaluation, thoracic radiographic findings, and pulmonary airway cytology, particularly if cells with intranuclear inclusions are identified. Additional supportive evidence for the diagnosis of EMPF is based on the presence of EHV-5 detected with molecular testing such as PCR and preferably with the qPCR method. Ideal samples for testing include BAL fluid and/or lung tissue. Additional samples that should be tested in parallel include peripheral blood (leukocytes) and nasal swab secretions (synthetic, nylon or Dacron swab). Testing should also include evaluation for additional viruses such as EHV-2 and AHV-5. Although they may not serve a primary role, their presence may potentiate disease development in affected horses.

Treatment

Therapeutic management of EMPF patients is targeted at clearance of pathogen challenge and provision of appropriate supportive care. Specifically, therapy should include broad-spectrum antimicrobials, antiinflammatories that include dexamethasone, and intranasal oxygen therapy in hypoxic individuals. Antiviral therapy is recommended as a component of therapy; although acyclovir[308] has poor bioavailability, valacyclovir has been demonstrated to have improved plasma levels following oral administration.[309] The dosing protocol should be at the higher end of the dosing range, based on limited evidence for successful management of such cases. Valacyclovir should be administered at 30 to 40 mg/kg PO three times daily.[310] Although this treatment protocol remains

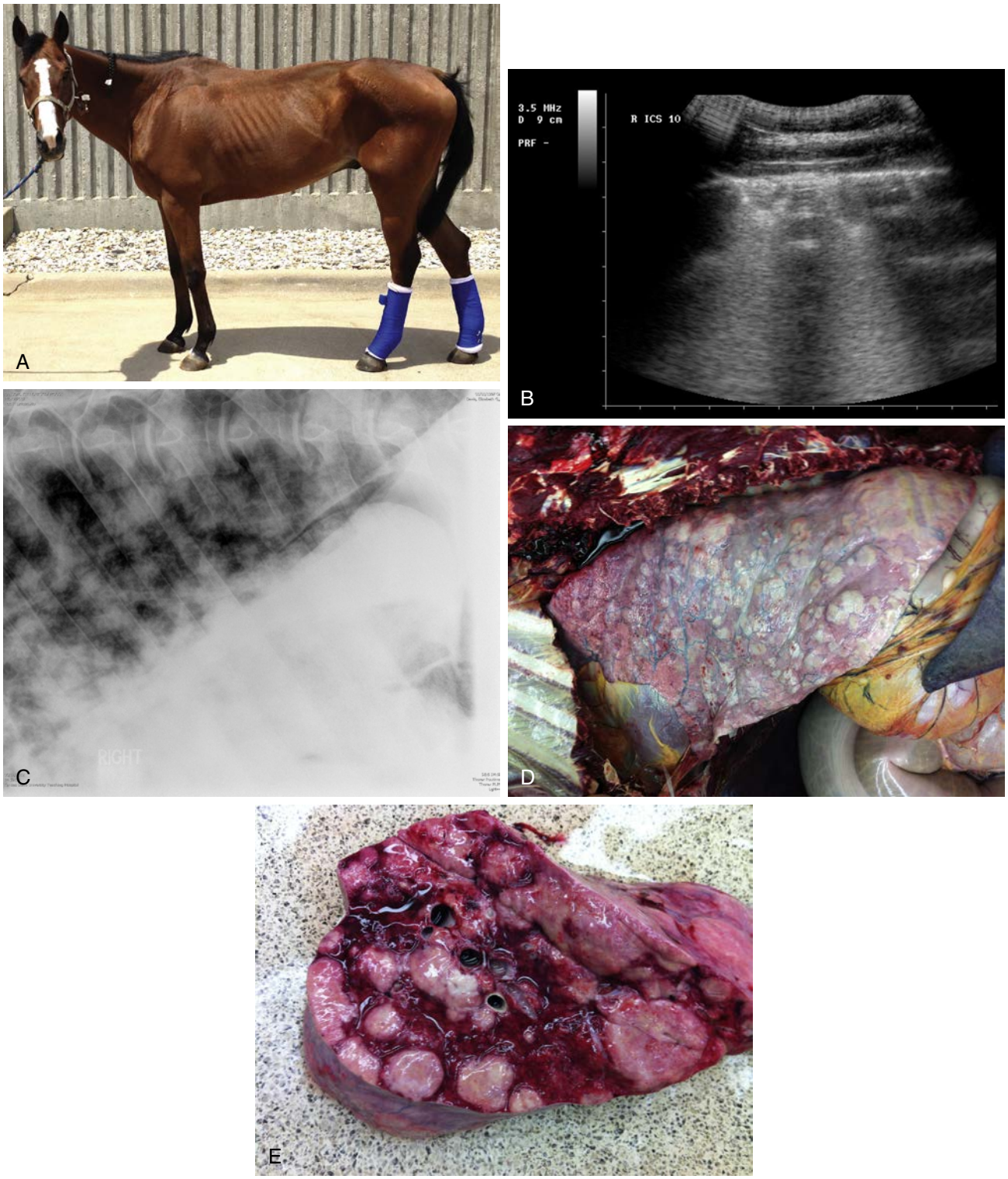


FIG. **8.15** (A) A 15-year-old Thoroughbred gelding presenting for weight loss and poor appetite was diagnosed with equine herpesvirus 5 (EHV-5) associated equine multinodular pulmonary fibrosis. (B) Thoracic ultrasound revealed multiple peripherally located nodular lesions ranging from approximately 2 to 4 cm in diameter. (C) Thoracic radiographs confirmed the presence of a diffuse nodular interstitial radiographic pattern. (D) Failure to respond to therapy necessitated euthanasia, which revealed gross pulmonary changes consistent with those identified antemortem on thoracic ultrasound and radiograph. (E) Cross section of nodular pulmonary fibrosis associated with EHV-5 infection.

under investigation for this particular viral infection, there is evidence to support this dose for this particular condition. A serious and potentially fatal complicating factor for horses suffering from EMPF includes the development of pulmonary hypertension, which may be suspected based on ultrasonographic and radiographic evaluation. The presence of pulmonary hypertension is considered a poor prognostic indicator for EMPF-affected horses and was observed postmortem in one EMPF-affected horse.[311]

In cases suspected to have elevated pulmonary arterial pressure, pulmonary artery diameter should be measured and compared with aortic diameter during echocardiographic evaluation to aid in the establishment of pulmonary hypertension.[311] In addition, pulmonary artery pressure should be measured in such cases whenever possible. An individual report of management of an EMPF-affected horse with associated pulmonary hypertension involved administration of the phosphodiesterase type V inhibitor sildenafil with furosemide.[312] Additional therapies that have been included in the treatment protocol for EMPF include nebulized *N*-acetylcysteine, pentoxifylline, and doxycycline.

Based on the current body of literature regarding EHV-5 in horses, this appears to be an important pathogen that at least in part plays a role in the development of pulmonary fibrosis in horses, whereas AHV-5 appears to play a similar role in disease development among donkeys. EMPF is a distinct disease process that results in nodular pulmonary disease of equids; there is marked remodeling of the pulmonary architecture with eosinophilic intranuclear viral inclusion bodies that may be present within intraluminal macrophages.[304] Early diagnosis and aggressive therapeutic management provide the most favorable prognosis. When severe or advanced, EMPF remains a serious infectious disease of horses.

EQUINE ARTERITIS VIRUS INFECTION

Although most commonly recognized for its association with the equine reproductive tract, equine arteritis virus (EAV) causes a mild to moderate vasculitis and associated respiratory disease termed *equine viral arteritis* (EVA). The virus is maintained in equine populations in carrier stallions because testosterone is required for persistence and maintenance of the virus in vivo. Carrier stallions maintain the virus within the ampulla and vas deferens.

Clinical Signs and Viral Transmission

Clinical manifestations of EVA are similar to those of other viral respiratory tract diseases. Variations in the severity of clinical signs result from strain differences in virulence, pathogen dose, and host immune function. Incubation requires several days to 2 weeks, with a more rapid course of disease after venereal transmission. Clinical signs associated with respiratory tract infection include serous nasal discharge, submandibular lymphadenopathy, mild to moderate cough, and ventral edema. Vasculitis is evident based on clinical signs of edema and occurs secondary to viral damage of the tunica media of small arteries and venules. Edema of the prepuce, scrotum, and limbs is commonly observed. The two most important consequences of EVA infection include abortion of the pregnant mare and induction of the carrier status in a breeding stallion. Clinical signs may persist for 2 to 9 days. Most infections are self-limiting, although edema may be severe and respiratory distress evident. Abortion typically occurs within a month of exposure and occurs during late clinical disease or early convalescence. Typically the fetus is nonviable at the time of abortion and is autolyzed by the time of expulsion. Rarely, neonatal foals infected with the virus demonstrate respiratory difficulty and rarely recover from viral infection.[313-315] Hematologic evidence of EVA includes leukopenia characterized by a lymphopenia, and thrombocytopenia, which may be severe.

EAV is spread by aerosolization of respiratory secretions and venereal transmission. Virus-laden droplets can be disseminated in the environment for up to 2 weeks following recovery from disease. Immunologically naive intact stallions exposed to the virus following sexual maturity maintain the virus in the ampulla of the vas deferens. Carrier stallions remain seropositive, and their semen contains live virus whether used for natural breeding, artificial insemination, or frozen semen. The virus can be maintained for many years in frozen semen with little negative influence on fertility.

Susceptible mares bred to EAV-positive stallions commonly develop respiratory disease (80%–100% cases) and suffer from early embryonic death but do not sustain a persistent EVA infection. Most cases of EVA are self-limiting with low risk of complicating factors. Standardbred horses have a high frequency for seropositivity and carrier infection compared with other horse breeds.

Prevention

The greatest challenge for disease prevention is the presence of a persistently infected stallion. Prevention from infection and induction of the carrier state can be achieved through careful vaccination. Once determined to be serologically negative (requirement for international equine transport), stallions can be successfully vaccinated with the commercially available MLV vaccine to prevent the carrier state. Colts should be vaccinated <280 days of age and isolated for approximately 21 days to minimize the risk of viral shedding in the vaccinated individual and to allow for immunity to develop and prevent viral exposure before the immunity is maximally induced. All vaccinated horses should be separated from nonvaccinated horses for approximately 21 days following vaccination. Infected horses should be separated from herdmates for approximately 3 weeks following infection to reduce the risk of viral spread. Similarly, any individual that has been infected with the virus should not have contact with young horses or broodmares. All at-risk individuals should be vaccinated annually to minimize the risk of disease. Broodmares should be vaccinated before breeding; the modified live vaccine is not recommended to be administered to pregnant broodmares.

EQUINE PICORNAVIRUS INFECTION

The equine picornaviruses are categorized into two serogroups: equine rhinitis virus A and B (ERAV and ERBV). Former classification referred to these viruses as rhinoviruses. ERAV (formerly equine rhinovirus 1) is grouped in the *Aphthovirus* genus based on genotype and similarity with other members of this genus, such as foot and mouth virus, as well as the characteristic viremia and persistent shedding that occurs following infection with the virus.[316,317] Equine rhinitis B virus ERBV1 (formerly equine rhinovirus 2), ERBV2 (formerly equine rhinovirus 3), and ERBV3 (formerly acid-stable picornavirus) are members of the genus

Erbovirus.[318,319] Both ERAV and ERBV have been implicated in association with respiratory disease in horses.[268,320,321] Most recent investigations have provided evidence for the virus-neutralizing capability of antibodies against these viruses and their impact on clinical respiratory disease in horses.[322] Persistent shedding has been identified in respiratory and urinary secretions.[323]

Viral detection of picornaviruses has been identified in horses with respiratory disease and healthy horses.[324,325] It has been suggested that infection happens early in life via aerosol transmission. Notably ERAV is shed in respiratory secretions and urine,[326] whereas ERAB1 and ERAB2 appear to be restricted to the respiratory tract (secretions) only. Experimentally induced respiratory disease with ERAV resulted in upper and lower airway disease and viremia, which lasted 4 to 5 days.[268] Additional clinical signs following experimental challenge included pyrexia, lymphadenopathy, changes on thoracic auscultation, evidence of airway inflammation with mucosal hyperemia, and tracheal mucous accumulation that persisted for 21 days.[327]

Clinical manifestations of disease typically include pyrexia, serous to seromucous nasal discharge, coughing, depression, anorexia, pharyngitis, and submandibular lymphadenopathy.[327] Mild lymphopenia and increased plasma fibrinogen concentrations have been reported in affected horses.[319,328]

Diagnosis is based on serology or molecular detection of the virus. The application of PCR and real-time PCR have become more commonplace because of the variability in serologic titers and prolonged turnaround time for virus isolation.

Preventative strategies have primarily focused on hygiene and overall health maintenance. Recently, a commercially available ERAV vaccine was approved for use in horses and may be considered for high-risk situations.

AFRICAN HORSE SICKNESS

African horse sickness (AHS) is a foreign animal disease that has never been reported in the United States. From a global perspective the disease has major implications on the equine industry. Historically this is a viral disease affecting horses in Sub-Saharan Africa, but outbreaks in India and the Middle East were reported in the mid-1950s,[329] and cases in Spain were reported in the 1980s.[330]

The primary vector for viral transmission is *Culicoides imicola.* This is a serious worldwide pathogen because morbidity and mortality remain high in naive equine populations. Disease manifestation ranges from fulminant pulmonary edema, subcutaneous edema, and myocardial failure to horse sickness depending on the level of host immunity at the time of viral challenge.

There are four clinical syndromes: peracute, acute, subacute, and mild. AHS is caused by a virus within the family Reoviridae, genus *Orbivirus* (double-stranded RNA virus), and is related to the causative agent of bluetongue.

Although endemic in parts of Africa, all equidae are susceptible, with horses having the greatest sensitivity to the virus, followed by mules, donkeys, and zebras.[331]

Viral spread is directly influenced by the insect vector *C. imicola.* This biting midge prefers claylike moisture-retaining soils. In years with abundant rainfall midge numbers increase dramatically. The vector is moving northward, which is why there has been movement into the Middle East and parts of Europe.

As stated previously, disease manifestation is a reflection of host immunity. The peracute syndrome is the pulmonary form with a mortality rate of approximately 95%. With this, there is a brief incubation period that may last up to 5 days. Clinical signs include fever and hemorrhagic pulmonary fluid exudate characteristic of pulmonary edema. Postmortem examination reveals evidence of marked pulmonary edema and hydrothorax. Acute or mixed AHS is a more common manifestation with pulmonary and cardiac abnormalities. The incubation period is slightly longer and may be up to 7 days. Mortality for the acute form is 50% to 95%. Clinical signs reveal a combination of cardiac (edema of head and neck) and pulmonary disease (cough, respiratory distress, and pulmonary edema) manifestations. Postmortem examination reveals marked subcutaneous edema of the head and neck. Subacute AHS demonstrates clinical signs of myocardial dysfunction, fever, edema, and lethargy. Edema of the supraorbital fossa is highly characteristic of AHS. Incubation of the subacute form is 50%. Horse sickness fever is the mildest form of the disease, and this manifestation is displayed by horses with preexisting immunity and those species with inherent resistance to disease such as donkeys and zebras. Mortality tends to be low for this form of the disease.

Diagnosis is based on serology and virus isolation.[332] An inactivated vaccine is available and is used in endemic regions to minimize loss in high-risk regions.[333] During an epidemic, the primary goal is to eradicate disease though implementation of quarantine, vector control,[334] and euthanasia of exposed horses. If these efforts fail, perimeter vaccination is implemented to limit the spread of the disease.[13]

HENDRA VIRUS INFECTION

Hendra virus, formerly referred to as equine morbillivirus, was first identified in Hendra, Australia, in 1995 and was recognized as a zoonotic disease of horses.[335] Hendra virus causes fatal pneumonia and encephalitis in horses and humans.[336] Clinical signs of Hendra virus are similar to AHS. Fruit bats are the reservoir of infection. Disease transmission requires close contact with infected horses or bat excrement. Hendra virus is classified as a Hazard Group 4 pathogen, which requires the highest level of biosecurity procedures.

In an initial outbreak involving Hendra virus, 14 of 21 horses died.[337] Two primary caregivers for the horses became ill, and one did not survive.[338] Morbillivirus was identified from renal tissue from one of the nonsurviving humans, which was identical to the virus cultured from the lungs of five affected horses.[339] All human cases have been reported in association with equine cases. Although Hendra virus is not considered directly contagious, very close contact with horses, particularly in the environment in which they were infected, increases the risk of human disease. Among 157 humans that had casual contact with infected horses and humans, there was no evidence of seroconversion. Gray-headed fruit bats seroconvert and demonstrate mild clinical disease when experimentally infected.[340] However, subclinical disease or seroconversion have not been identified in horses exposed to the virus. Horse are exposed via the upper respiratory or oral cavity and once infected will excrete virus in their urine, saliva, and respiratory secretions.[341] The virions are highly labile and can be destroyed by heat, lipid solvents, detergents, formaldehydes, and oxidizing agents.

Horses that are infected with virus develop severe and typically fatal respiratory disease that is characterized by dyspnea, vasculitis, and severe pulmonary edema. Nonspecific signs include lethargy, anorexia, fever, respiratory distress, ataxia, tachycardia, and frothy nasal discharge. Encephalitic and marked subcutaneous edema may be observed in some cases. The incubation period is 8 to 12 days, with clinical progression characterized as peracute. Death typically occurs 1 to 3 days after the onset of clinical signs. Clinical manifestation is similar to AHS, and diagnosis is based on serology aimed at detecting virus-neutralizing antibodies and immunofluorescence. Virus isolation is performed in Vero cell culture with immunofluorescence used to detect viral antigens within infected tissues. Because of the extreme danger and zoonotic potential of this virus, sample collection should be performed under Biosafety Level (BSL)-4 conditions; this is the highest level of biosecurity implementation.

BACTERIAL PNEUMONIA AND PLEUROPNEUMONIA

Bacterial pneumonia is not usually a spontaneous disease in adult horses. Most often it is a primary disease or risk factor that leads to bronchopneumonia in the adult horse. Events that lead to suppressed endogenous immunity may result in bacterial colonization of the lower respiratory tract. A primary viral upper respiratory tract infection may lead to loss of the mucociliary clearance mechanism leading to bacterial colonization of the lower airways or marked aspiration, potentially in combination with some of the previously mentioned mechanisms. General anesthesia may contribute to immunosuppression and in some cases may result in low-grade (or marked) aspiration. Long-distance transport, particularly with head elevation for extended periods of time or esophageal obstruction can all result in various levels of aspiration into the lower airways. Although anatomic and immunoprotective mechanisms are likely to aid in protection from lower airway colonization, in some instances bacterial challenge overwhelms the host protective mechanisms, and bronchopneumonia may result.

The importance of risk factors cannot be overemphasized because bacterial pathogens isolated from the lower respiratory tract are opportunistic, environmental, or commensal organisms that are unable to initiate primary invasion. The most frequently isolated organism from horses suffering from primary pneumonia is *S. equi* subsp. *zooepidemicus*. Initial colonization involves the primary airways, resulting in bronchitis, whereas disease progression leads to extension beyond the airways to the surrounding pulmonary parenchyma. Further disease dissolution leads to the most advanced progression of bacterial colonization with extension to the pleural space and associated structures and is termed *pleuropneumonia*. In mild or early cases of airway colonization, frequently disease resolution can be achieved with broad-spectrum antibiotic administration and general supportive care to facilitate optimal immune function. However, when disease is severe or chronic or involves marked immunosuppression the prognosis may be guarded. Identification of risk factors and severity of disease will aid the clinician in determining the ideal diagnostic and therapeutic options.

Primary pleuritis is a rare event in horses. Traumatic penetrating wounds that contaminate the pleural cavity commonly result in septic pleuritis. Pneumonia is not a common secondary event to traumatic wounds but may occur in the event that the primary pleuritis is not effectively managed. The clinical approach to equine patients with marked pleural effusion should include consideration for septic, traumatic (hemorrhage), neoplastic causes; secondary to congestive heart failure; and parasitic disease (hydatid cyst). In North America, pleuropneumonia remains the most common cause of pleuropneumonia, whereas in other parts of the world reduced frequency of this disease is observed. Notably, in the UK, some reports indicate that pleuropneumonia only makes up approximately 30% of the cases of infectious lower airway disease.

At the time of diagnosis of pneumonia or pleuropneumonia, relevant history includes information that may support the primary etiology, such as recent viral respiratory infection, long-distance transport, strenuous exercise, immunosuppressive therapy, exercise-induced pulmonary hemorrhage, severe esophageal obstruction, or marked age. Endogenous pulmonary defense mechanisms include cough, mucociliary clearance, respiratory (or bronchial)-associated lymphoid tissue, and alveolar macrophages.

The mucociliary escalator provides an active mechanism for removal of particulate material from the respiratory tract and is an important component of the nonspecific endogenous host mechanisms for clearance of pathogen challenge. The mucociliary escalator is present from the level of the pharynx to the respiratory bronchioles. Particles that accumulate on the epithelial surface are moved in an oral direction under the action of ciliary waves moving the double layer of superficial sol-gel mucus. Once in the pharynx the constituents are swallowed. Anatomic airway division or size impacts respiratory clearance efficiency as ciliary beating frequency is greater in the larger airways compared with the lower bronchioles; host benefit from this effect is prevention of excessive mucous accumulation in the trachea and larger conducting airways. This also contributes to the impact of head position on mucous clearance; prolonged head elevation is a known impediment to respiratory clearance mechanisms primarily mediated by the mucociliary escalator. In the larger airways mucus originates from goblet cells, whereas in the bronchioles mucus is produced in the nonciliated Clara cells. In the bronchi, submucosal bronchial glands produce serous and mucous secretions. Mucous secretion is under autonomic regulation; importantly, transepithelial water and electrolyte constituents may fluctuate, resulting in changes in mucus composition. Changes in volume and composition of mucus can lead to respiratory disease or may be the result of pathogen or toxin exposure. Cough is an important clearance mechanism mediated in part by stimulation of the subepithelial irritant or stretch receptors, which are located with the greatest density in the larger bronchi. Cough is an effective way to clear mucoid secretions from the intrathoracic trachea and large bronchi but not from peripheral bronchi and bronchioles. Alveolar macrophages provide an essential layer of protection within the alveoli. Alveolar macrophages will either move up the mucociliary system or leave the alveoli and enter lymphatics. Once in lymphoid tissues, these macrophages play an important role in antigen presentation. Physiologic factors that impair macrophage function include a hypoxic environment brought about by disease or reduced Fc receptor expression and subsequent phagocytosis secondary to therapeutically administered corticosteroids. Alveolar function is suppressed by viral infection. Therefore, in addition to viral respiratory disease leading to impaired mucociliary clearance mechanisms, viral challenge also impairs direct killing by alveolar macrophages and antigen presentation.

Etiologic Agents

Because the majority of cases of equine bacterial pneumonia result from aspiration, the bacterial flora of the upper respiratory tract is commonly involved with lower airway colonization. Notably, *S. equi* subsp. *zooepidemicus* remains the leading bacterial pathogen of the lower airways of adult horses suffering from pneumonia.[342,343] In addition to *S. equi* subsp. *zooepidemicus,* other pathogens may co-colonize the lower airways. As mentioned previously, a variety of stress and physiology factors may put horses at risk for lower airway infection.

Classification of pathogens that may colonize the lower airways include gram-positive and gram-negative aerobes and anaerobes. In addition to *S. equi* subsp. *zooepidemicus, Staphylococcus* spp. may lead to bronchopneumonia. In rare instances, typically in association with immunodeficiency, *Rhodococcus equi* may be identified. Human pathogens are uncommonly identified in the airways of horses, with *Streptococcus pneumoniae* more commonly being identified among racehorses in the UK and occasionally in the United States.[344,345] Because of the pathogenesis of equine bronchopneumonia, aspiration may result in the colonization with mixed isolates. Anaerobic bacteria may include *Bacteroides fragilis, Prevotella heparinolytica, Clostridium* spp., *Peptostreptococcus* spp., and *Fusobacterium* spp. Historically, anaerobic bacteria have been reported to compose approximately 30% of the isolates identified in horses with pneumonia.[346,347] Identification of the bacterial population present can provide prognostic information, with the identification of anaerobes yielding a higher overall mortality, particularly if there has been a delay from the onset of disease to the time of diagnosis and treatment.[348]

Clinical Manifestation of Disease

Clinical manifestation of lower airway infection frequently indicates severe systemic infection. Although somewhat nonspecific, when combined with relevant history, signalment, and examination findings, the diagnosis is generally not overwhelmingly challenging. Clinical signs often include lethargic attitude, reduced appetite, fever, and increased respiratory effort. Cough may be present but may be blunted as a result of the thoracic pain experienced on induction of the cough reflex.

Diagnosis

Equine patients suffering from pleuropneumonia commonly have a fever and lethargic mentation. Variation in clinical signs at presentation will be influenced by severity and chronicity of disease. Early in the course of infection, in addition to pyrexia and lethargy, horses may experience pleural pain demonstrated by forelimb abduction at the level of the elbow. Physical examination should include complete thoracic auscultation, and particular attention should focus on the identification of breath sounds in all lung fields. If lung sounds are attenuated, particularly in ventral pulmonary fields, examination should include thoracic ultrasonography. Although rebreathing examination may be a reasonable approach, the procedure is contraindicated if there is any evidence of respiratory distress or impairment.

Thoracic ultrasonography should be performed in a thorough and comprehensive manner with particular attention paid to the evaluation of the entire thorax, bilaterally. Examination should be performed from dorsal to ventral either cranial to caudal or vice versa. The region of the mediastinum can be imaged through the triceps and is an important aspect to examine, particularly if abundant fluid and fibrin are identified on ultrasound examination. Advantages of ultrasonography are that location and approximate quantity (depth) of pleural fluid can be identified. Additionally, ultrasonography provides the clinician with the ability to thoroughly examine peripheral lung fields. If fluid is present, once the pleural fluid has been removed thoracic radiography will provide additional imaging information to aid with patient assessment of disease severity. Axial disease, such as abscess formation or lymphadenopathy, will not be apparent with ultrasonography alone.

Thoracic ultrasound will enable identification of not only fluid accumulation but also gas echoes, fibrin, loculation, or highly cellular fluid, which will provide prognostic information regarding the severity of disease. Pleural fluid with high protein content and low cellularity will appear anechoic (Fig. 8.16), whereas fluid containing greater cellularity will have a greater degree of echogenicity. The identification of gas echoes within the pleural space may be identified in patients with anaerobic-containing fluid, rupture of a bronchopleural fistula, or following iatrogenic introduction of air, which may result during thoracocentesis. Pulmonary atelectasis, consolidation, or abscess formation may be identified if located in peripheral lung fields. Ultrasound waves are unable to penetrate gas; therefore, air-filled lung or lung overlapping areas of disease will not be visible with ultrasound imaging alone. Marked pulmonary consolidation identified in combination with serosanguineous pleural fluid, particularly when combined with the identification of epistaxis, suggests the presence of pulmonary infarction, which carries a guarded prognosis.[349] Added benefits of thoracic ultrasound include the ability to determine whether pleural adhesions are present. The clinical importance of identifying where adhesions are located is so they can be avoided if thoracentesis is performed or if indwelling thoracic drains are going to be placed. Pneumothorax can be diagnosed with thoracic ultrasonography, but the extent of disease cannot be fully determined. Once the diagnosis of pneumothorax is made with ultrasound, further patient assessment should be completed with thoracic radiography. Cranial thoracic imaging should be completed to determine whether mediastinal fluid or abscess formation are present. Imaging through the triceps can be performed with most 3- to 5-mHz imaging probes. When examination of the equine patient with pleuropneumonia is

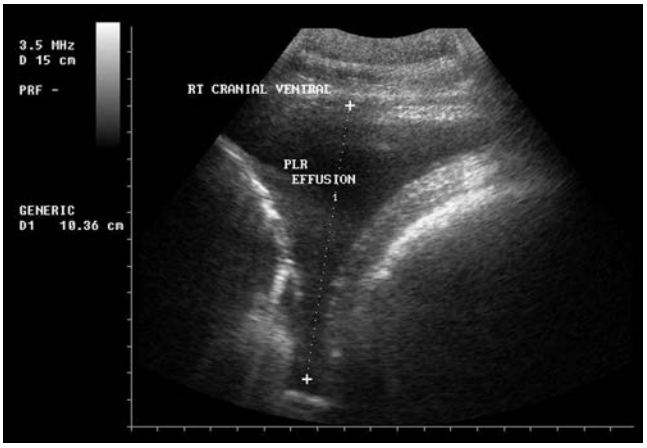


FIG. **8.16** A 25-year-old Arabian gelding with severe pleuropneumonia following esophageal obstruction and associated aspiration pneumonia. Thoracic ultrasound examination with a 3.5-mHz probe revealed the presence of marked anechoic pleural effusion that surrounds consolidated pulmonary parenchyma measuring greater than 10 cm in depth.

completed, the advantage of ultrasound is to not only determine the presence and character of pleural fluid but also to determine the ideal location for performing pleurocentesis and potential indwelling chest tube placement. The goal of sample collection and tube placement is to determine the area of greatest depth (volume) and a safe location that will minimize the potential for adverse outcome such as diaphragmatic or cardiac puncture. Subsequent to making the diagnosis of pleuropneumonia and evacuation of pleural fluid, daily thoracic ultrasound is a mainstay of patient monitoring. Daily ultrasound examinations will allow the clinician to determine evidence of disease progression or resolution, based on volume of fluid present, effectiveness of indwelling thoracic tubes, the presence and extent of pleural fibrin production, and the assessment of peripheral pulmonary parenchymal disease.

Once fluid has been identified within the pleural space, if the volume measures greater than 3 cm in depth, then thoracocentesis is indicated for therapeutic and diagnostic purposes (Fig. 8.17). Location for fluid collection should be determined based on the most ventrally accessible location. Anatomic locations may be altered by large-volume fluid accumulation, and this should be considered when planning the site for thoracocentesis or tube placement. The procedure for thoracocentesis should be performed using sterile technique. Once the location has been determined, subcutaneous infiltration of 2% lidocaine is performed. A no. 15 blade is an ideal choice for making a small skin incision, which is generally 0.5 cm in length. A blunt teat cannula is an ideal instrument for fluid collection. If large-volume fluid is present an indwelling thoracic tube can be placed, which is generally sized between 16 and 32 Fr. Pleural fluid collection will provide a diagnostic sample for Gram stain and aerobic and anaerobic culture. If an indwelling tube is placed, fluid evacuation provides improved patient stability in addition to diagnostic sample collection (Fig. 8.18). Large-volume fluid removal should be completed with caution over a period of 30 to 45 minutes with concurrent isotonic crystalloid fluid administration. Rapid fluid removal from the pleural space can lead to re-expansion pulmonary edema and hypotension.

Normal equine pleural fluid is transparent with a clear to pale yellow color. It is odorless and has a protein concentration of <2.5 g/dL with a nucleated cell count of <8000/μL.[350] Bacterial pleuropneumonia commonly leads to an increase in protein concentration and nucleated cell concentration. As previously mentioned, pulmonary infarction results in serosanguineous pleural fluid. Cytologic examination of pleural fluid is typically represented by a large percentage (>80%) of neutrophils, which are characterized by degenerate changes. Often intracellular and extracellular bacteria are visible. Additional evidence for septic disease will include an elevated pleural fluid lactate concentration (greater than found in circulation), pH <7.1, and low glucose concentration (<40 mg/dL). Pleural fluid with a fetid odor often suggests the presence of anaerobic bacteria. A recent report that described five cases of intrathoracic esophageal rupture leading to the development of severe fibrinous pleuropneumonia[351] highlights the importance of a thorough examination of pleural fluid cytology. The presence of feed material in pleural fluid is not a normal



FIG. **8.17** Teat cannula placement with a polyethylene extension set for evacuation of purulent pleural fluid associated with pleuropneumonia that developed in a 13-year-old Arabian mare that had recently been transported over approximately 1600 miles.



FIG. **8.18** Indwelling thoracic tube placement should be determined with ultrasound as indicated by tube placement in this individual with a dorsally located loculated pocket of pleural fluid accumulation. Note the unusually dorsal location of tube placement in this individual. Abundant septic pleural fluid can be observed to fill this thoracic tube.

finding and should alert the clinician to potential esophageal damage and rupture. Particular case details that may provide the clinician with evidence to support a possible esophageal rupture includes a history inconsistent with the development of pleuropneumonia, such as a horse that has not had recent long-distance transport or esophageal obstruction. Examination of pleural fluid should also include Gram stain, which is beneficial to determine the class of bacterial organisms present, which can direct initial therapeutic strategies until bacterial culture results are available.

In addition to pleural fluid culture for aerobic and anaerobic bacterial pathogens, percutaneous transtracheal aspiration should be performed. Although pleural fluid is a readily accessible sample, based on the pathogenesis of disease resulting from aspiration, sampling from the primary site is likely to provide superior diagnostic information, which may differ from pleural fluid analysis.[13] Cytologic examination will provide supportive evidence of septic inflammation characterized by a predominance of degenerate neutrophils. Gram stain evaluation will provide diagnostic information that may aid with therapeutic decision making while waiting for final culture results to be returned.

Etiologic agents associated with equine pleuropneumonia commonly originate from the upper respiratory tract, with polymicrobial infection as a predominant and consistent feature of equine pneumonia.[280,342,348,352] Although the most common isolate remains *S. equi* subsp. *zooepidemicus,* gram-negative pathogens such as *Escherichia coli* are also recognized.[353] When a *Klebsiella* spp. is isolated from the lower airways of adult horses with pneumonia, a particularly guarded prognosis may be indicated and likely reflects the high degree of antibiotic resistance that has been reported.[354] Obligate anaerobes are normal inhabitants of the oral cavity and intestinal tract of horses and for this reason may be isolated from the lower airways of horses with aspiration pneumonia. A recent investigation that used a novel molecular method of detection (referred to as the 16S rRNA gene sequencing) recognized that *Bacteroides* spp. and *Prevotella* spp. are dominant anaerobes of the lower airways with antibiotic susceptibility patterns that include metronidazole, imipenem, and clindamycin.[355]

Pleuropneumonia may be effectively managed with evacuation of the pleural space via teat cannula placement followed by primary closure of the entrance made into the thoracic cavity. Alternatively and particularly with large-volume pleural fluid, indwelling thoracic drains are commonly left in place until marked improvement or complete resolution of the septic process. Once placed the chest tube can be maintained in place by using a snare-type suture pattern along the 3 to 5 cm of thoracic tube that is exiting from the body wall. A one-way (Heimlich) valve is required to provide a mechanism for fluid evacuation and reduce the potential for introduction of air or additional pathogens into the pleural space. Patient assessment should include daily thoracic ultrasound examination to determine the effectiveness of pleural drainage and identify any changes that may develop, such as the production of fibrin accumulation within the pleural cavity. Loculated parapneumonic effusion with fibrin deposition has been reported to have a more guarded prognosis in horses with pleuropneumonia and is an important characteristic that should be carefully monitored through the course of disease management.[356] A sampling of pleural fluid should be cultured every 10 to 14 days to identify changes in microbial populations and associated antimicrobial susceptibility patterns. Fibrin and cellular debris may occlude the end of the thoracic drainage tube, and ultrasound can aid in determining the efficacy of thoracic tube drainage based on the amount of fluid exiting the tube and the amount of fluid remaining in the pleural space. If needed, a 60-mL catheter-tip syringe can be used in a sterile fashion to remove debris from the indwelling tube. In addition sterile isotonic fluid can be introduced into the thoracic tube to lavage the pleural space. Approximately 5 to 10 L of warm sterile fluid can be introduced and removed by gravity flow. When fluid is introduced patient monitoring should be continued throughout instillation. If at any time the patient appears uncomfortable or anxious the fluid should be allowed to drain and relieve any built-up pressure. If during the instillation of fluid any fluid is noted to exit from the nostrils, coughing observed, or pleural fluid exiting from the nostrils, lavage should be immediately discontinued, and consideration should be given to the presence of a bronchopleural fistula. Continued pleural lavage in the presence of a bronchopleural fistula can lead to dissemination of septic material to previously healthy regions of lung. If suspected, a bronchopleural fistula can be confirmed by the instillation of fluorescein dye into the pleural space. The presence of dye at the level of the nostrils will confirm open communication between the septic pleural space and the central airways. Additional complications that may develop include kinking of the indwelling tube or cellulitis surrounding the site in which the tube is located. In instances where cellulitis develops locally, applied warm compress material can aid with reduced swelling and inflammation. Marked pain or swelling indicate more severe disease necessitating tube removal.

Thoracic ultrasonography is an essential diagnostic modality for the ongoing management of an equine patient with pleuropneumonia. In addition to establishing a diagnosis, determining an optimal site(s) for pleural drainage by thoracic ultrasound is essential for the ongoing evaluation and assessment of patient progress and response to therapy. In addition to thoracic ultrasound thoracic radiography is an important imaging modality for determining disease severity. It is paramount that pleural fluid is drained effectively before thoracic radiography so there is optimal visualization of pulmonary parenchymal disease. Thoracic radiography will provide additional information to patient assessment because radiographs are better able to detect central disease such as mediastinal lymphadenopathy or abscess formation, deep parenchymal lesions, mediastinal disease, and the presence and severity of pneumothorax. Serial imaging studies will provide the clinician with diagnostic information regarding response to therapy and disease progression. Disease distribution will differ based on the etiology of pneumonia because most cases of bronchopneumonia are a consequence of aspiration. Cranioventral pulmonary alveolarization is typically identified. In contrast, disease that resulted from hematogenous delivery will be evidenced by a caudodorsal pulmonary distribution.

Treatment

Clinical management of pleuropneumonia requires effective antibiotic therapy, pleural drainage, ideal supportive patient management, and in some cases surgical intervention for the removal of necrotic pulmonary foci.

Broad-spectrum antibiotic therapy is the mainstay of patient management because of the frequency of polymicrobial infection. In addition to antimicrobial therapy frequent patient monitoring is essential to determine patient status

and response to therapy. Patient status is typically dynamic, particularly early in the course of disease. For this reason changes in patient status should be addressed quickly to minimize further complications and morbidity. In many instances fibrin deposition will lead to marked flocculation of pleural fluid. Thoracocentesis may be efficacious in particular regions of disease and should be modified to address current patient status. It is not uncommon that several thoracic drains may be required to effectively drain an individual hemithorax caused by the presence of fibrin loculations and the organization of numerous fluid pockets. In addition to antibiotic therapy and effective pleural drainage, it is paramount that the clinician provide excellent supportive care in the form of maintenance of euhydration with IV fluid therapy, assessment of acid-base status, supplemental oxygen therapy in severe cases, provision of adequate nutritional support, and analgesic antiinflammatory therapy commonly through the judicious administration of NSAIDs. In cases where medical management alone provides incomplete disease resolution, surgical intervention may be indicated for the removal of necrotic pulmonary tissue as well as to facilitate drainage that may be impaired by the use of thoracic tubes alone.

When horses suffering from pleuropneumonia are identified and properly diagnosed early in the course of the disease, effective therapeutic management may lead to disease resolution with limited or individual thoracocentesis to facilitate pleural drainage. More commonly, disease progression is present at the time of presentation, and pleural drainage is required multiple times for management of an individual or multiples, and at times bilateral indwelling thoracic drainage tubes are required.

Combined thoracic drainage and systemic antibiotic therapy are the mainstay treatments required for the resolution of pleuropneumonia in horses. Effective medical therapy for the management of pleuropneumonia required knowledge about the microorganism(s) present and susceptibility to various antimicrobial agents. Broad-spectrum antibiotic coverage is required for the various classes of bacterial pathogens that may be present. A rational approach for initial treatment includes the combination of penicillin (or other β-lactam agent), gentamicin, and metronidazole. This combination will provide a broad level of antibiotic protection against gram-positive (e.g., *Streptococcus* spp.), gram-negative enteric organisms, and anaerobic bacteria, respectively. Although penicillin has good efficacy against many anaerobic organisms, the synthesis of β-lactamase by *Bacteroides* spp. warrants the addition of metronidazole for more complete coverage. Adverse events or reduced patient stability may require modification to this initial protocol. Once bacterial isolate(s) have been identified and antibiotic susceptibility profiles provided, informed changes in antibiotic coverage can be made (Table 8.6).

An essential adjunct therapy in horses suffering from pleuropneumonia involves analgesic and antiinflammatory therapy. In the early stages of disease management, NSAID therapy is indicated to address the issues of systemic inflammation and fever, pain of disease and thoracic tube placement, and endotoxemia that may ensue because of infection with gram-negative pathogens and subsequent lipopolysaccharide (LPS) liberation. Commonly selected NSAID agents aimed at providing analgesia in horses with pleuropneumonia include flunixin meglumine, phenylbutazone, firocoxib, and meloxicam (Table 8.7).[357-361] Caution should be exercised in horses that have evidence of hypovolemia, renal impairment, or loss of gastrointestinal epithelial integrity. Even though NSAIDs are a mainstay of clinical management of equine patients suffering from a variety of inflammatory and painful disorders, the risk for an adverse drug event should be recognized. Renal papillary necrosis, gastric ulceration, and right dorsal ulcerative colitis have been well reported as consequent events that can develop in equine patients.[362] Particular attention should be paid to medications that may have been administered before veterinary evaluation to minimize the potential for concurrent NSAID agent administration, which could potentiate adverse events.[363,364]

Nutritional support should be carefully monitored in horses suffering from marked pleuropneumonia. It is not uncommon for horses that suffer from pain and sepsis to enter into a negative energy balance, which can be potentiated with reduced feed intake. Monitoring body weight at routine intervals will aid the clinician to determine a decline in condition. In addition, monitoring certain clinical

TABLE **8.6** **Antibiotic Therapy**

Antimicrobial	Dose
Amikacin	10–185 mg/kg, IV, q 24 h
Ampicillin sodium	11–22 mg/kg, IV or IM, q 6–8 h
Ceftiofur sodium	2.2 mg/kg, IV or IM, q 12 h
Ceftiofur crystalline-free acid	6.6 mg/kg IM, q 96 h
Chloramphenicol	50 mg/kg, PO, q 6 h
Doxycycline	10 mg/kg, PO, q 12 h
Enrofloxacin	7.5 mg/kg, PO or IV, q 24 h 5.0 mg/kg, PO or IV, q 12 h
Gentamicin	6.6 mg/kg, IV or IM, q 24 h
Metronidazole	15–25 mg/kg, PO, q 6–8 h
Minocycline	4 mg/kg, PO, q 12 h
Oxytetracycline	6.6 mg/kg, IV, q 12–24 h
Potassium penicillin G	22,000 IU/kg, IV, q 6 h
Procaine penicillin G	22,000 IU/kg, IM, q 12 h
Rifampin	5–10 mg/kg, PO, q 12 h
Sodium penicillin G	22,000 IU/kg, IV, q 6 h
Trimethoprim-sulfonamide	15–30 mg/kg, PO or IV, q 12 h

IM, Intramuscularly; *IV,* intravenous; *PO,* orally; *q,* every.

TABLE **8.7**

Non-specific COX inhibitors	
Flunixin meglumine	0.5-1.1 mg/kg, IV or PO, q 12-24 hr
Phenylbutazone	2.2-4.4 mg/kg, IV or PO, q 12-24 hr
Selective COX-2 specific inhibitors	
Firocoxib	0.1 mg/kg, IV, q 24 hr
Meloxicam	0.6 mg/kg, PO, q 24 h

Dosages of non-steroidal antiinflammatory drugs (NSAID) used in the treatment of pleuropneumonia and other severe pulmonary infections in which the administration of corticosteroids might be contraindicated.

chemistry parameters can also aid with the determination of overall nutritional status. Monitoring of blood glucose, electrolyte concentrations, and serum triglyceride levels will help determine whether the horse is entering into a negative energy balance. Particular attention should be paid to horses that are among breeds at risk for lipid mobilization such as ponies, Miniature horses, or those with evidence (or risk) of insulin resistance or pituitary pars intermedia dysfunction. Palatable feeds and fresh grass can often help to encourage feed intake in sick horses. When indicated additional nutritional supplementation can be achieved with the addition of dextrose to IV fluids or the introduction of parenteral nutrition (partial or complete). Enteral nutrition can be achieved by offering commercial supplementation with high-protein amino acid products (Purina Well Gel, Purina Mills, Inc. St. Louis, MO).

Complicated, refractory disease often requires surgical thoracotomy to facilitate removal of fibrinous material and necrotic lung to facilitate pleural drainage. Evidence that a horse is suffering from refractory disease includes lack of pleural fluid resolution over the course of several weeks despite appropriate antimicrobial therapy and repeated pleural drainage. Case selection for thoracotomy is a critical component of case outcome. Timing for thoracotomy in equine pleuropneumonia cases was reported to have a mean time of 60 days since disease development in one report. Surgical intervention too early in the course of disease may result in complications that include bilateral pneumothorax, hemorrhage, and/removal of viable lung, which can result in catastrophic case outcome. When case selection is made, timing for thoracotomy should be based on several factors that include patient stability and approximately 45 to 60 days of medical therapy. In an ideal setting pleural abscess will become walled off so that a pleural to parietal pocket is completely formed and separate from other regions of the thorax. At a minimum, it is important to determine that the mediastinum is complete based on the ability of the patient to handle air introduction into the proposed surgical hemithorax. The indwelling thoracic drain should be left open without a one-way valve for a minimum of 2 hours to determine whether the patient is able to physiologically handle a unilateral pneumothorax. If the patient is noted to become anxious and have altered respiratory rate and pattern or other signs of distress the site should be closed, air removed, and the procedure delayed until the patient is anatomically prepared with a closed mediastinum for the surgical intervention.

The procedure for thoracostomy can be performed using two different methods: an intercostal approach that opens an area in an ICS or a partial rib resection, which removes a section of rib adjacent to the abscessed material. An intercostal approach has the advantage of using a smaller window of approach, which limits postoperative discomfort. In mild cases intercostal thoracotomy can provide a functional area for pleural drainage and removal of fibrin and necrotic pulmonary tissue. In more severe cases, partial rib resection has the advantage of a larger surgical window in which larger, more advanced necrotic lung and debris can be removed. Patient recovery in the postoperative period is further delayed with rib resection because of the size and extent of tissue removal, which delays wound healing. In the immediate postoperative period daily lavage should be performed with warmed sterile isotonic fluids. Lavage will help facilitate the removal of debris and blood clots following surgery. After tissue granulation has been established (by 7–10 days) postoperatively nonsterile fluids (tap water) can be used for ongoing pleural drainage. Drainage should continue until sepsis is resolved and the surgical site has closed. The convalescent period following surgery will depend on the surgical procedure selected and degree of ongoing sepsis at the time of surgery. Generally, horses complete tissue healing and resolution of sepsis within 60 days of thoracotomy.

Complicating factors may prolong or prevent disease resolution. Primary pulmonary conditions that may complicate disease management include the development of pneumothorax, pleural adhesions, pulmonary abscess formation, pulmonary infarction, bronchopleural fistula, or cranial mediastinal abscess formation. Pneumothorax is a relatively common complication that develops with pleuropneumonia. Causes include iatrogenic introduction of air at the time of thoracocentesis or indwelling chest tube placement or secondary to development of a bronchopleural fistula. Mild pneumothorax is commonly well tolerated and may not have clinical consequences. However, more severe pneumothorax requires air evacuation to reestablish adequate pulmonary function and oxygen delivery. Clinical signs that support the necessity of air evacuation in a patient with pneumothorax include respiratory distress and elevated respiratory rate and effort. Placement of an indwelling thoracic tube in the caudodorsal lung field following the confirmation of pneumothorax via radiography will provide the clinician with the ability to remove pleural air to reestablish negative intrathoracic pressure. Pulmonary infarction has been reported to occur following strenuous exercise and is associated with severe pulmonary disease characterized by epistaxis, pulmonary consolidation, and fetid serosanguineous pleural fluid.[349] Bronchopleural fistulae can develop in horses with extensive pulmonary necrosis, which result in an open communication between the pleural cavity and central airways. The presence of a bronchopleural fistula does not impair the prognosis for survival, but it will prevent thoracic lavage and may impact future athletic performance. Occasionally septic pleural disease can have cranial dissemination leading to the organization of a mediastinal abscess. Clinical signs that suggest a mediastinal abscess may be present include apparent preference to stand with forelimbs in a pointed position, evidence of jugular vein occlusion with pronounced jugular pulsation, and marked pectoral edema. Imaging with ultrasound and thoracic radiography may provide additional evidence of mediastinal abscess based on cranial fluid accumulation located cranial to the heart. In such cases, there is also evidence of caudal displacement of the cardiac silhouette. Mediastinal abscess formation can be particularly challenging to manage, often requiring general anesthesia to facilitate effective drainage cranial to the heart.

Extrathoracic complications can develop in association with pleuropneumonia and include laminitis, jugular vein thrombophlebitis, and antibiotic-associated colitis. Similar to other manifestations of laminitis, depending of the severity of laminar damage, recovery may be prolonged and athletic performance limited following recovery despite resolution of pulmonary disease. Horses suffering from pleuropneumonia are commonly critically ill at the time of diagnosis, and hematologic parameters may indicate the onset of disseminated intravascular coagulopathy (DIC). Whether clinical or subclinical, DIC will place patients at greater risk for thrombophlebitis. In some cases catheter site cellulitis may progress

to thrombophlebitis, and for these reasons catheter selection should be aimed at materials with a low likelihood of thrombogenicity with placement and maintenance a clinical priority. Inadvertent or frequent jugular venipuncture should be avoided in horses at high risk for thrombotic disorders. In most instances, if a timely identification of jugular vein disease is established recovery can be favorable with an alternate placement site, and thrombosis is not severe. Antibiotic-associated colitis is an ever-present potential in horses receiving antibiotic therapy. Based on the nature and severity of disease, this should be monitored throughout the course of therapy. If evidence of gastrointestinal disturbance is noted, hydration, acid-base status, and electrolyte concentrations should be corrected. Antibiotic therapy will be required throughout the course of management for pleuropneumonia, so alternate therapy should be selected to target the primary pulmonary pathogen(s).

Prognosis and Outcome

The overall prognosis for horses to recover and resume athletic performance has improved over the past three decades. Evidence to suggest a more guarded prognosis includes the presence of marked fibrin accumulation with an overall survival of 68%. In horses that did not have fibrin accumulation survival was 100%, whereas horses that had fibrinous accumulation survival was only 62%.[21] It was noted that in this population, the nonfibrinous group only had 18% of the patients identified as having necrotic pulmonary parenchyma, whereas 60% of the patients with fibrinous disease exhibited necrotic pulmonary parenchyma. Bacterial isolates in this report were similar to previous reports, with the most common pathogen being *S. equi* subsp. *zooepidemicus* followed by *E. coli, Actinobacillus equuli, Enterococcus,* and *Klebsiella.* The most common anaerobes were *Prevotella* spp. and *Bacteroides* spp.[21] In a previous report, horses that had pleuropneumonia or pneumonia had an overall survival rate of 75%.[342] In that report 38% of the horses with anaerobic bacteria cultured from the lower airways survived, which is similar to 35% survival in the recent report, regardless of the presence of marked fibrin accumulation.[21,346] In a report that looked at pneumonia as secondary to penetrating thoracic wounds, the association of reduced survival with the presence of anaerobic bacteria was not found. This finding may have resulted, in part, because of the different pathogenesis of disease rather than the lack of association with anaerobic bacteria, resulting in a more guarded prognosis.[365] Among the published reports regarding the prognosis for horses with pleuropneumonia the range is 43.3% to 95.7%.[353,366,367] Not only is there a favorable overall prognosis for the horse to survive pleuropneumonia, the likelihood of returning to the previous performance level is also favorable. With appropriate treatment the prognosis is approximately 90% with about 60% returning to performance. As mentioned previously a delay in therapy, the presence of anaerobic bacteria, and the presence of marked fibrin loculations are associated with a more guarded prognosis. Horses that are at risk for the development of lower airway disease and bacterial colonization should be treated appropriately with antimicrobial therapy, monitored closely, and have therapy modified as indicated by the bacteria isolated from the lower airways. Complicating factors such as pneumothorax, laminitis, or colitis may prolong hospitalization and owner expense, which may also limit the ability of the horse to recover from its primary disease.

PULMONARY ABSCESS FORMATION

Pulmonary abscess formation most commonly occurs in weanling age foals in association with *R. equi* infection (Chapter 20). *S. equi* subsp. *zooepidemicus* is the organism most commonly cultured from the lungs of horses with generalized pneumonia and rarely results in abscess formation. Complications from *S. equi* infection include metastatic spread to various organs including possible pulmonary abscess formation. Aspiration is another cause of focal pulmonary infection and abscessation. Aspiration pneumonia is a potential complication of esophageal obstruction or dysphagia in horses. Neonatal foals may suffer from dysphagia in association with hypoxic ischemic encephalopathy or nutritional muscular dystrophy, whereas adult horses may develop aspiration pneumonia after complete esophageal obstruction.

INTERSTITIAL PULMONARY DISEASE

Interstitial pneumonia is a pulmonary disorder that may affect horses of various age groups. Adult horses with interstitial pneumonia have a more guarded prognosis for recovery and survival[280] compared with foals and weanlings. Horses less than a year of age that are provided with appropriate therapy and supportive care tend to have a favorable prognosis for complete recovery.[368]

Etiology

Some cases of interstitial pneumonia result from a primary toxic or infectious insult, but at the time of presentation determination of the exact etiology can be challenging.[300,368-370] Toxic pulmonary disease has been associated with ingestion of crofton weed, pyrrolizidine alkaloids (*Crotalaria, Trichodesma,* and *Senecio*), perilla ketones, silicosis, and prolonged oxygen therapy. Hepatic metabolites of pyrrolizidine alkaloids cause cellular damage and death in the pulmonary endothelium.[370,371] Inhaled irritants or toxins may contribute to direct pulmonary damage such as occurs after inhalation of smoke or agrichemicals.[372-374] Silicosis is a highly specific chronic granulomatous pneumonia of horses and should be considered in horses with compatible clinical signs that originate from the Carmel Valley in California.[280]

The most recent evidence for an infectious etiology associated with interstitial disease in horses is EHV-5–associated multinodular pulmonary fibrosis. Supportive evidence for the association was recently described in which investigators characterized findings of 24 adult horses that suffered from progressive nodular fibrotic lung disease associated with EHV-5.[302] Herpes viral DNA was identified in 79.2% of affected individuals compared with 8.7% of the control animals.[302] Similarly, a case series of five horses suffering from respiratory distress with clinical evidence of interstitial disease were all confirmed to have EHV-5 in pulmonary tissue or secretions, supporting previous findings associated with this disorder and multinodular pulmonary fibrosis secondary to viral disease.[311] An additional report of two horses also confirmed similar clinical manifestation of disease, radiographic and histopathologic evidence of multinodular granulomatous disease, and the presence of EHV-5.[304] It should be noted that the exact association of viral involvement remains speculative in some circumstances. In a prospective investigation of mares and foals in central Kentucky, up to 88% of the foals were positive for the virus in peripheral blood yet they

remained clinically healthy.[375] Although EHV-5 has been isolated from the upper respiratory tract of horses with respiratory disease, similar to a related gammaherpesvirus EHV-2, clinical disease associated with EHV-5 has been suggested to result in lymphadenopathy, immunosuppression, depression, and primary respiratory tract disease. Adult onset of severe respiratory disease characterized by multinodular granulomatous pulmonary lesions should be strongly suspected to be associated with EHV-5 infection.

The initial infectious or toxic agent causes alveolar damage resulting in cell death and increased permeability at the level of the alveoli. Pulmonary congestion, interstitial edema, erythrocyte extravasation, and alveolar edema occur during the exudative phase of the disease. Subsequently, alveolar infiltrates with inflammatory leukocytes and fibrin and increased permeability lead to fluid accumulation, impairing normal gas exchange mechanisms, hyaline membrane formation, and clinical respiratory distress. Acutely affected patients typically demonstrate respiratory distress, injected mucous membranes, and impaired pulmonary function. Subacute to chronic disease results in alveolar regeneration with alveolar type II pneumocyte proliferation to replace damaged type I pneumocytes. Fibroplasia leads to cellular proliferation and septal thickening, fibrous development, and ultimately to reduced pulmonary compliance.

Clinical Findings

Horses presenting with interstitial pneumonia are typically in severe respiratory distress with labored breathing, dark mucous membranes, poor pulse quality, and tachycardia. Some patients are mistakenly considered to have an obstructive disease like heaves, yet interstitial pneumonia is characterized by a restrictive rapid, shallow breathing pattern. Additional clinical features of disease include hypoxemia, a stress or inflammatory leukogram, hyperfibrinogenemia, and hypoxemia that may be severe. More chronic disease may be observed in mildly affected individuals with exercise intolerance and chronic cough.

Diagnosis

Definitive diagnosis of interstitial pneumonia in horses is based on histopathologic evaluation of a pulmonary biopsy. Examination for EHV-5 viral DNA can be performed on a tissue sample or BALF. Thoracic radiographs can be helpful in establishing a preliminary diagnosis of interstitial pneumonia. Two patterns of interstitial disease have been described in horses with interstitial pneumonia: as discrete or diffuse nodules suggestive of neoplasia or mycotic disease or as a diffuse increase in radiographic interstitial pattern (Fig. 8.19). Serum titers may indicate recent exposure or infection with viral respiratory disease. Histopathologic evaluation of lung biopsies or specimens obtained at postmortem will confirm the diagnosis of interstitial pneumonia. If silicosis remains a differential, diagnosis is based on x-ray diffraction techniques on lung tissue preparations.[280]

Treatment

The prognosis for adult horses with interstitial pneumonia remains guarded. In horses that present with mild to moderate disease, treatment should be aimed at improving oxygenation. Intranasal insufflation of oxygen is warranted for patients that are severely hypoxemic. Antiinflammatory therapy should initially include systemic corticosteroids

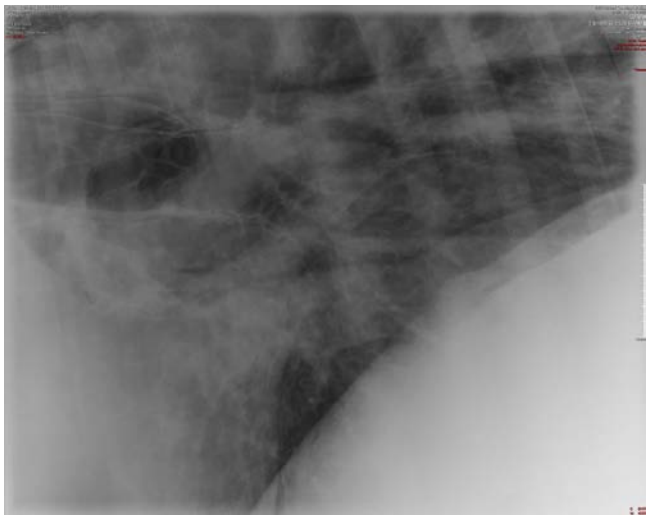


FIG. **8.19** A 19-year-old Quarter Horse mare presented with a 5-day history of fever, anorexia, and respiratory distress. The mare failed to respond to antibiotic and nonsteroidal antiinflammatory drug therapy. Thoracic radiographs were diagnostic for generalized interstitial pneumonia.

(dexamethasone 0.05–0.1 mg/kg IV daily), with transition to aerosolized corticosteroids (beclomethasone 1500 µg intranasally three times a day to twice a day)[376] as clinical improvement is observed. Corticosteroid therapy should be continued until clinical resolution is observed or no further improvement is noted with therapy. Prolonged corticosteroid therapy of several weeks to months should be anticipated because of the severity of lower airway inflammation associated with this condition. Bronchodilator therapy is indicated when severe bronchoconstriction exists. β_2-adrenergic receptor agonists are the drugs of choice for immediate bronchodilation and subsequent improvement of air movement to the lower airways (albuterol 360–720 mg/kg every 3–12 hours).[377,378] Enteral bronchodilator therapy with clenbuterol 0.8 to 3.0 µg/kg orally every 12 hours (Ventipulmin, BIVI) can provide additional support in cases with marked bronchoconstriction. Following initial stabilization, an additional therapeutic option is the use of a combined parasympatholytic agent (ipratropium 360–470 µg/kg every 6–12 hours)[379] with a β_2-adrenergic receptor agonist (albuterol; Combivent, 3M Pharmaceuticals, and Boehringer Ingelheim) to improve oxygen delivery to the lower airways with the added advantage of having an increased half-life compared with β_2-adrenergic agonist therapy alone.

In addition to supportive care and antiinflammatory therapy, specific antiviral therapy may be selected for cases of EHV-5–associated multinodular pulmonary fibrosis, typically in combination with antiinflammatory therapy. Valacyclovir has the greatest oral bioavailability with a protocol that includes 27 mg/kg orally every 8 hours for 48 hours followed by 20 mg/kg every 12 hours thereafter.[309,380,381] Duration of therapy should be based on clinical response to treatment. In some circumstances, this therapeutic protocol may be cost prohibitive or only possible for a limited time. Investigations are ongoing regarding the optimization of antiviral therapeutic protocols for horses that suffer from herpes viral infection. Supportive evidence is provided by the use of such agents in human patients that are at risk for serious cytomegalovirus infection post organ transplantation.[382,383]

Prognosis

The prognosis for return to function is guarded for adult horses and favorable for foals that are managed appropriately. Supportive and antiinflammatory therapy may improve clinical status, yet high-level athletic activity may be impaired.

FUNGAL PNEUMONIA

Respiratory fungal disease is most commonly a result of inhalation of aerosolized fungal pathogens. After inhalation the causative organisms are able to penetrate into the lower pulmonary airways and alveoli as a result of their small particle size. Interestingly, more than 90% of the particles present in stable air samples that are visible with light microscopy are spores of fungi or *Actinomyces*.[384,385] However, in some cases of fungal pneumonia penetration may occur through a compromised gastrointestinal tract in an immunocompromised patient. In one report 86% of the horses diagnosed with mycotic pneumonia developed disease secondary to gastrointestinal disease with disruption of epithelial integrity.[386] For this reason, in addition to horses that demonstrate evidence of pulmonary disease and have appropriate risk factors such as gastrointestinal disease, particularly if immunocompromised (marked neutropenia is present), fungal pneumonia should be considered.

The most common isolate from horses with fungal pneumonia is *Aspergillus* spp., which are commonly present in the equine environment,[387] particularly in moldy feed and bedding materials, such as straw. Predisposing factors that lead to disease development in horses include immunosuppression because *Aspergillus* spp. are opportunistic pathogens. Adequate anatomic and immunologic function provides the healthy individual with effective protection against colonization and disease. Immunosuppression may result from endogenous disease that alters effective immunity or is secondary to the administration of immunosuppressive therapy.[388-392]

Pathogenesis

Enteritis, colitis, and typhlocolitis are proposed to predispose horses to the development of systemic fungal dissemination. Gastrointestinal inflammation results in the disruption of the epithelial barrier, allowing for translocation of fungal organisms and subsequent systemic dissemination. Lesions have been described to center around large blood vessels, which then lead to systemic spread of organisms. Among the reported retrospective cases of invasive pulmonary aspergillosis nearly 84% were associated with enterocolitis.[388,392] Hematogenous dissemination leads to fungal deposition in the lung.

Among the risk factors for mycotic distribution from the gastrointestinal tract, NSAID therapy may contribute to epithelial disruption, enhancing mucosal permeability to luminal microorganisms. Additionally, neutropenia is an important risk factor for humans to develop mycotic pneumonia. Similarly in horses, the majority of cases are reported to suffer from neutropenia even though the exact role that neutropenia plays regarding disease manifestation is not fully elucidated. Dissemination of disease is an important component of disease pathogenesis; 40% of the horses with fungal pneumonia have evidence of invasive fungal disease in other organs including the kidney and brain.[347,388]

Overwhelming exposure to inhaled spores may result in pulmonary fungal disease, particularly in immunosuppressed individuals. Altered immunity may result from iatrogenic administration of corticosteroid therapy, pituitary pars intermedia dysfunction, or other overwhelmingly debilitating disease. Some invasive fungal pathogens may cause primary disease and do not require a predisposing host factor as a precedent for colonization, which includes *Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum,* and *Cryptococcus neoformans.*

Direct inhalation of *B. dermatitidis* conidia can result in pulmonary colonization. These are dimorphic saprophytic fungi that can be detected on cytologic examination of airway secretions. Infection has been reported to result in pulmonary abscess formation, peritonitis, subcutaneous abscesses, and mastitis.[393-395] *Histoplasma capsulatum* is most commonly associated with moist regions, particularly those contaminated with bird or bat fecal waste. The characteristic appearance of yeast includes a 2- to 4-μm organism with a definitive circular halo surrounding the centrally located crescent-shaped cytoplasm. Although horses are considered resistant to infection, disease associated with *H. capsulatum* has included pulmonary granuloma,[396] pneumonia, and abortion.[397] Coccidioidomycosis is an uncommon pathogen of horses, but among horses that live in regions of sandy, alkaline soils disease may occur following inhalation. Marked pulmonary inflammation develops initially, followed by pulmonary and lymphoid localization. Nonspecific clinical signs may involve multiple organ systems including liver, lung, kidney, or spleen and warrant a particularly guarded prognosis. Horses with severe pulmonary disease associated with *C. immitis* similarly are unlikely to survive.

Immunosuppressed Patients

Candida albicans is an uncommon opportunistic pathogen that has been observed as a complicating factor in septic neonatal foals, which rarely can be identified on diagnostic evaluation of the respiratory tract. In suspect cases, fungal culture of transtracheal aspirate samples may be indicated. *Pneumocystis jirovecii* is currently classified as a saprophytic fungus based on the DNA sequence information from the 16S-like ribosomal subunit. Clinical signs of *P. jirovecii* infection typically include an interstitial pneumonia that fails to respond to conventional therapeutics. Historical reports have described Arabian foals with severe combined immunodeficiency as the most likely to succumb to infection. Similarly, in humans, this is a disease of immunosuppressed individuals that commonly suffer from HIV infection that have progressed to develop AIDS. Diagnosis is based on cytologic examination of lower airway secretions obtained by BAL sampling. This organism cannot be cultured.

Clinical Signs

Clinical signs of equine fungal pneumonia include tachypnea, fever, nasal discharge, epistaxis, nasal plaques or erosions, abnormal lung sounds, and pleural friction rubs. Most horses have a history of progression of respiratory disease despite aggressive antimicrobial therapy. Inspection of the nasal passages may reveal nasal plaque formation or ulceration. Although these lesions may be detected in the minority of cases, estimated in 10% of horses, this is highly predictive of concomitant or subsequent invasive pulmonary aspergillosis.

Diagnosis

Low-volume pleural effusion may be detected by thoracic ultrasound, and fibrin plaques may occur on visceral and parietal pleural surfaces. If pleural effusion is identified, pleural

fluid samples should be submitted for cytologic evaluation and culture. Observation of fungal spores on cytologic evaluation and/or culture of fungal elements from transtracheal aspirate samples are not strong indicators of a diagnosis of fungal pneumonia in horses. Culture and histologic evaluation of lung biopsy samples provide definitive evidence of fungal pneumonia and differentiation from neoplasia. Fungal mediastinal granuloma appears similar to mediastinal lymphoma on radiographic examination.[398] The site is difficult to biopsy percutaneously and is best identified via thoracoscopy.

The radiographic appearance of fungal pneumonia can be similar to disseminated pulmonary neoplasia. A miliary or reticulonodular interstitial pattern is common with multifocal, coalescing nodules visualized in peripheral lung fields in both fungal pneumonia and metastatic neoplasia (see Fig. 8.9).

Patient examination will provide the clinician with the opportunity to determine optimal sample collection for diagnostic examination. Thoracocentesis may be performed in cases with evidence of pleural fluid. However, in cases of lower airway disease, with an absence of pleural fluid accumulation, transtracheal aspiration will be a preferred diagnostic sample for collection. Cytologic examination of pleural fluid or tracheal aspirate reveals the presence of fungal organisms. *Aspergillus* spp. are characterized by having broad septate hyphae with parallel sides and discrete 90-degree branches that can be identified on cytologic or histopathologic examination. Sample collection should include fungal culture analysis in suspect cases. Serologic assessment may provide supportive evidence, but specificity is influenced by the frequency of fungal exposure among horses. Sample staining may provide more efficient diagnostic evidence; immunohistochemistry and immunofluorescence are available diagnostic options.

Lesions involving the upper respiratory tract may be sampled using standard biopsy techniques. Uterine biopsy instruments are useful tools for sample acquisition because in some cases sample size can make definitive diagnosis challenging. For lesions involving sinus structures, sample acquisition is generally accomplished with a sinus flap. Fungal pneumonia may be diagnosed via TTW or BAL sampling or lung biopsy acquisition. Risk of pulmonary vascular damage is a potential risk associated with biopsy collection and should be carefully monitored with ultrasound and physical examination (epistaxis) to determine whether hemorrhage is present.[385] Routine staining with hematoxylin and eosin may not provide diagnostic information for all fungal organisms, particularly when chronic lesions contain marked fibrosis; therefore, in suspect cases special staining for fungal organisms should be requested. Staining techniques may include periodic acid–Schiff, Gridley fungus stain, and Grocott-Gomori methenamine silver nitrate staining.[385]

The presence of fungal elements may be identified in cytologic fluid samples or impression smears obtained from tissue biopsy samples, although interpretation of such findings should be carefully considered. The direct association of fungal elements in biologic samples should correlate with the presenting complaint and patient status. Tracheal aspirate and lower airway samples commonly contain *Alternaria* spp. (nonpathogenic *barn fungus*), yet this does not correlate with clinical disease, and they are found in combination with normal cytologic findings in healthy horses.[399] If diagnosis is based on cytologic examination, ideally this finding should be based on clinical pathologic findings of abundant fungal organisms in the presence of marked inflammation characterized by abundant (>20%) and typically degenerate neutrophils present. Clinically relevant findings typically also include intracellular and extracellular organisms in the presence of marked inflammation.

In vitro growth of fungal organisms can be lengthy because of their naturally fastidious growth kinetics. Suspect samples should be applied to prepared culture media and transported to a microbiology laboratory at room temperature. Routine culture media includes Sabouraud dextrose agar, inhibitory mold agar, or a mycobiotic containing cycloheximide and chloramphenicol, which enhance the potential for positive fungal growth. Test interpretation should carefully consider patient status because of the frequency of fungal organisms in the equine environment. Similar to airway cytology findings test specificity is reduced and false positive samples can occur.[385,400]

Serologic diagnostic testing can provide the clinician with valuable diagnostic information and the opportunity to monitor disease progression and response to antifungal therapy. Currently available options include immunodiffusions, radioimmunoassays, complement fixation, and ELISA testing. These tests will aid with the quantification of circulating antifungal antibody titers. For this reason they have diagnostic and patient-monitoring benefits. Immunohistochemistry, fluorescence in situ hybridization, and DNA probes are antigen-detection testing methods. Additionally, a commercial comprehensive real-time PCR assay can be used to detect fungal DNA in biological samples. This can be followed by a species-specific real-time PCR test to determine the particular inciting pathogen.[401]

Although not all fungal pathogens are opportunistic in nature, based on the potential for attenuation of endogenous immune activity, suspect cases that fail to demonstrate an appropriate risk for fungal disease may benefit from immune assessment testing. Overall lymphocyte concentrations can be ascertained on compete blood count analysis, but lymphocyte subset testing and functional analysis can provide the clinician with an improve assessment of host immune function. Testing should include lymphocyte subset immunophenotyping and immunoglobulin concentration assessment.[402-407]

Treatment and Prognosis

Amphotericin B deoxycholate is a polyene antibiotic that combines with ergosterol in the fungal cell wall to enhance membrane permeability. The hazards of drug administration are numerous, and caution should be exercised when administering to equine patients. When administered intravenously the drug is nephrotoxic and quite irritating, potentially leading to phlebitis. Additional adverse events may include anorexia, anemia, cardiac arrhythmias, hepatic dysfunction, and hypersensitivity reactions.[408] Despite the potential for adverse events in some instances, this is considered a reasonable treatment choice and has been used with success to treat systemic diseases such as histoplasmosis, pulmonary aspergillosis, cryptococcosis, and topically for nasal *Conidiobolus coronatus*.[100,387,409-412]

Benzimidazole derivative drugs classified among the azoles provide an effective antimicrobial therapy against fungal pathogens by inhibiting ergosterol synthesis in the cell membrane. These drugs have been used with success topically and as systemically administered drugs (Table 8.8).

TABLE **8.8 Antifungal Agents for Consideration in Equine Patients**

Drug	Dose	Comments
Amphotericin B	0.3–0.6 mg/kg in 1 L 5% dextrose[1]	Nephrotoxicity, phlebitis, hypersensitivity Use caution
AZOLE DRUGS		
Miconazole 2% topical	Topical[2]	Guttural pouch mycosis
Enilconazole	Topical[3]	Guttural pouch mycosis Nebulization for fungal pneumonia, *Scopulariopsis* spp.
Ketoconazole	30 mg/kg NG tube, daily[4,5]	Acidify with 0.2 N HCl
Itraconazole (Sporonox)	5 mg/kg PO, daily[6]	*Aspergillus* spp., *Histoplasma* spp., *Blastomyces* spp. Compounded formulation not recommended
Fluconazole	Load 14 mg/kg PO	Not effective against *Aspergillus* spp. and *Fusarium* spp.
	Then 5 mg/kg PO, daily[7–9]	Compounded formulation is stable
Voriconazole	4 mg/kg PO, daily[10]	Drug of choice for human invasive *Aspergillus* spp. pneumonia
IODIDE THERAPY		Do not administer to pregnant mares, congenital hypothyroidism
Sodium iodide (20%)	20–40 mg/kg/day 7–10 days[11–13]	Monitor for signs of iodonism: lacrimation, dermatitis
Oral formulations		
Inorganic potassium iodide	10–40 mg/kg/day [12,13]	Chemical grade only, unstable in light, heat, humidity
Organic ethylenediamine dihydriodide	0.86–1.72 mg/kg/day[13]	Commercially available

NG, Nasogastric; *PO,* orally.

References

1. Begg LM, Hughes KJ, Kessell A et al., Successful treatment of cryptococcal pneumonia in a pony mare, *Aust Vet J.* 2004;82:686–692.
2. Davis PR, Meyer GA, Hanson RR et al., *Pseudallescheria boydii* infection of the nasal cavity of a horse, *J Am Vet Med Assoc.* 2000;217, 674, 707-709.
3. Stewart AJ, Cuming RS, Update on fungal respiratory disease in horses, *Vet Clin North Am Equine Pract.* 2015;31:43-62.
4. Weinstein WL, Moore PA, Sanchez S et al., In vitro efficacy of a buffered chelating solution as an antimicrobial potentiator for antifungal drugs against fungal pathogens obtained from horses with mycotic keratitis, *Am J Vet Res.* 2006;67:562-568.
5. Ziemer EL, Pappagianis D, Madigan JE et al., Coccidioidomycosis in horses: 15 cases (1975-1984), *J Am Vet Med Assoc.* 1992;201:910-916.
6. Davis JL, Salmon JH, Papich MG, Pharmacokinetics and tissue distribution of itraconazole after oral and intravenous administration to horses, *Am J Vet Res.* 2005;66:1694-1701.
7. Davis JL, Little D, Blikslager AT et al., Mucosal permeability of water-soluble drugs in the equine jejunum: a preliminary investigation, *J Vet Pharmacol Ther.* 2006;29:379-385.
8. Latimer FG, Colitz CM, Campbell NB, et al., Pharmacokinetics of fluconazole following intravenous and oral administration and body fluid concentrations of fluconazole following repeated oral dosing in horses, *Am J Vet Res.* 2001;62:1606-1611.
9. Reilly LK, Palmer JE, Systemic candidiasis in four foals, *J Am Vet Med Assoc.* 1994;205:464-466.
10. Davis JL, Salmon JH, Papich MG, Pharmacokinetics of voriconazole after oral and intravenous administration to horses, *Am J Vet Res.* 2006;67:1070-1075.
11. French DD, Haynes PF, Miller RI, Surgical and medical management of rhinophycomycosis (conidiobolomycosis) in a horse, *J Am Vet Med Assoc.* 1985;186:1105-1107.
12. Zamos DT, Schumacher J, Loy JK, Nasopharyngeal conidiobolomycosis in a horse, *J Am Vet Med Assoc.* 1996;208:100-101.
13. Scott DW. Fungal skin diseases. In: Saunders-Elsevier ed. *Equine Dermatology.* 2nd ed. St. Louis (MO), 2003;311-312.

Although iodide-containing agents have limited evidence to demonstrate efficacy against fungal pathogens, they have apparent effectiveness against some granulomatous diseases and are thought to provide benefit through antiinflammatory properties. Advantages of iodide therapy include that these are cost-effective agents and for this reason are often administered to equine patients with fungal disease, commonly in combination with other therapies (Table 8.8).

The overall prognosis for horses suffering from fungal disease involving the respiratory system is dependent on a variety of factors that include infecting agent, host immune status, primary disease process, the stage of the disease at the time of diagnosis, and owner commitment to invest in newer generation antifungal agents when indicated. Collectively, in some instances horses suffering from mycotic pulmonary disease may demonstrate a positive response to appropriate therapeutics, but because of the multitude of factors that may be involved with host status these must also be considered. In cases where disease is advanced, complicating factors are marked and/or therapeutic options are limited, prognosis for disease resolution may be more guarded.

PARASITIC PNEUMONITIS

Parasitic pneumonia is a condition that may affect foals or adult horses. Parasites associated with this condition include *Parascaris equorum* larvae or the adults of *Dictyocaulus arnfieldi.* Clinically affected horses have obvious evidence of respiratory disease that includes exercise intolerance and coughing that may be accompanied by nasal discharge, fever,

and depression, particularly when secondary bacterial infection has occurred. *P. equorum* infection is most common in foals and weanlings, particularly those raised on breeding farms in which the parasite resides in the environment and soil. *Dictyocaulus* infection may occur in horses of any age, but this parasite requires a donkey as a primary host to complete its life cycle.

Clinical Findings

Chronic coughing, mucoid to mucopurulent nasal discharge, respiratory distress, and poor overall body condition provide nonspecific evidence of parasitic disease in foals. Poor body condition, abnormal pulmonary sounds represented by increased bronchovesicular sounds, crackles, and wheezes are common findings on thoracic auscultation of horses with parasitic pneumonia. Poor body condition is a common finding because of intestinal involvement of parasitic infection. Colic may be a component of the history or may result following therapeutic anthelmintic treatment in severely affected individuals. Frequently the history also includes a poor response to appropriate antimicrobial therapy for suspected bronchopneumonia.

Diagnosis

Hematologic evaluation commonly reveals an inflammatory leukogram consisting of a mature neutrophilia, hyperfibrinogenemia, and hyperglobulinemia. In some instances, particularly early in the course of disease, hematologic evaluation may reveal few abnormalities. Hepatic parasitic migration (*P. equorum*) may result in mild to moderate hepatic enzyme elevation.[413] Thoracic radiography is a useful diagnostic test in affected individuals. A moderate to severe bronchointerstitial pattern is a common finding, whereas granuloma or abscess formation may be detected by radiographs with advanced disease. Thoracic ultrasonographic examination will allow the clinician to detect the presence of pleural fluid or peripheral pulmonary consolidation.

Cytologic examination of a sterile tracheobronchial aspirate will often reveal abundant eosinophils (5–50%, normal <2%), and neutrophilic inflammation may exist concurrently, particularly when secondary bacterial infection is present. Microorganisms are apparent with significant bacterial infection; culture is recommended to determine the presence of bacterial infection and to determine the antimicrobial sensitivity pattern for pathogens of concern. Fecal flotation is indicated to determine the presence of parasite ova being shed from the host. *D. arnfieldi* requires a donkey or mule host for life cycle completion; therefore, parasite eggs will only infrequently be detected in adult horses with lungworm infection. It is difficult to diagnose *P. equorum* infection on fecal flotation because tissue migration occurs during the prepatent period. Therefore, diagnosis is based on clinical signs, lack of evidence of bacterial infection, and tracheal wash cytology indicating eosinophilic pneumonitis. Response to therapy is supportive of the diagnosis, although antibiotic therapy may be required in combination with anthelmintic therapy.

Therapy

Severely hypoxemic patients may require oxygen insufflation. Severe pulmonary inflammation is induced by eosinophilic infiltrates necessitating bronchodilator and potentially aerosolized corticosteroid therapy (see previous recommendations on aerosol therapy). Oral anthelmintics used to treat *P. equorum* infection include an initial low dose of fenbendazole (5 mg/kg). Careful monitoring for approximately 24 hours is recommended to observe the foal for evidence of deterioration or gastrointestinal distress. Laxative therapy may be required if gastrointestinal ascarid impaction is suspected. After the foal has received an initial low dose of fenbendazole without complication, the dose can be increased (10 mg/kg PO daily) and this therapy can be repeated daily for 5 days. This therapy will be effective in killing adult and migrating larvae. Because this is a farm problem, other individuals on the same property of similar age should be managed appropriately, even if clinical evidence of disease is not apparent. Other anthelmintics that have been used include pyrantel pamoate (6.6 mg/kg) and ivermectin (200 μg/kg). *D. arnfieldi* infection can be successfully treated with ivermectin (200 μg/kg), moxidectin (adult horses only, 400 μg/kg), thiabendazole 440 (mg/kg/day twice), or levamisole (10 mg/kg).

Benzimidazole anthelmintic agents inhibit microtubule formation, which impairs the parasite's ability to move and ingest food. Energy metabolism is also impaired because of the inhibition of fumarate reductase.[414] Although many benzimidazoles are efficacious against intestinal larvae, they are not uniformly effective at killing migrating parasite larvae; however, at higher doses fenbendazole is safe and effective at killing intestinal and tissue larvae. Anecdotal and personal observations have suggested this anthelmintic to be highly efficacious, particularly in cases where ivermectin resistance is suspected.

Pyrantel pamoate is an acetylcholine agonist that results in parasite paralysis.[415] At the recommended dose this agent is effective at killing intestinal larvae but not migrating larvae.[413,414] Avermectins are effective because they bind glutamate-gated chloride channels, and this class is effective against both *P. equorum* and *D. arnfieldi* adults and migrating larvae. Ivermectin has a reported efficacy of 76.9% for removal of intestinal *P. equorum* and is 100% effective for removal of pneumonic larvae.[414,416] Overall, ivermectin and moxidectin have similar efficacy as effective anthelmintics in horses for many gastrointestinal parasites other than *Anoplocephala perfoliata*.[417] Based on these reports regarding anthelmintic efficacy, recommendations include combining therapeutic agents to maintain maximal efficacy. Initial treatment with fenbendazole (10 mg/kg orally daily for 5 days) followed in 14 days with an avermectin product at the appropriate dose should clear the individual of both intestinal and pneumonic parasites.

Prognosis

Foals or adult horses with primary parasitic pneumonia and secondary bacterial infection will require concurrent antibiotic and anthelmintic therapy. The prognosis is excellent for recovery from parasitic pneumonitis. It is important to emphasize the need for complete deworming, including donkeys and mules, because they harbor the adult *Dictyocaulus* parasites that serve as a source for parasite contamination and infection of horses in the immediate environment.

RECURRENT AIRWAY OBSTRUCTION

Clinical Presentation

Equine RAO (heaves and severe equine asthma[418]) causes a reproducible series of clinical signs in affected horses. RAO is a common cause of performance impairment characterized by

a chronic cough, serous to mucoid nasal discharge, and episodic respiratory distress. Clinical signs are often seasonal and more intense when horses are housed indoors and exposed to dust, poor-quality hay, ammonia fumes, fungal spores, and other nonspecific stimuli.[418-420] Horses with RAO are typically mature adults, and the average age of horses with RAO is >7 years of age.[421]

There is no breed or gender predilection, but there is an apparent heritable component to disease development, particularly in certain lines of breeds such as Warmblood horses in Europe.[422-424] Although these studies have demonstrated that in some lines the heritability of RAO is more clearly defined, the overall mode of inheritance for RAO is not completely understood and is presumed to be complex. A question that has challenged equine clinicians for many years has involved the potential relationship between pulmonary inflammation in the form of IAD leading to the subsequent development of RAO. Additional information that may contribute to the complexity and understanding of these conditions relates to the current terminology of equine airway inflammation classified as mild to moderate asthma in horses suffering from IAD and severe equine asthma in horses with RAO. An incomplete understanding of disease pathophysiology may lead an individual to assume that these conditions are directly linked. In reality, there is insufficient evidence to provide a direct relationship that a horse with IAD will develop RAO in later life. Current evidence supports that horses with cough associated with pulmonary mucus and inflammatory cell accumulation are at risk for the development of RAO,[425] and there is insufficient evidence to demonstrate that there is a clear transition from IAD to RAO.[418] Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a syndrome similar to heaves or severe equine asthma; however, the clinical signs are triggered by exposure to late summer pollens in the southeastern United States. The primary difference between heaves and summer-associated disease is the local climate with exposure to specific allergens that induce clinical signs of airway obstruction. The presenting complaint for horses with severe equine asthma varies but often includes a chronic cough. Exercise intolerance, respiratory distress, mucopurulent nasal discharge, abnormal pulmonary sounds, and an enlarged field of percussion may also be identified.

Diagnosis

Typically the diagnosis of RAO is based on supportive historical information and physical examination findings. Complete blood count and serum chemistry analysis are generally unremarkable in horses without comorbid disease. Thoracic radiography is not indicated for routine diagnosis of heaves, but if a horse fails to demonstrate expected clinical improvement to appropriate pharmacologic therapy and environmental modification, then additional diagnostics should include thoracic radiography. Thoracic radiography is an important diagnostic modality to determine whether marked interstitial disease exists. Therefore, in horses suspected to have bronchointerstitial pneumonia or multinodular pulmonary fibrosis, thoracic radiography is indicated. Characteristics of the respiratory pattern should provide the clinician with evidence of marked fibrosis or interstitial disease resulting in restrictive pulmonary disease, which would be characterized by marked inspiratory distress compared with the RAO-affected horse that demonstrates marked expiratory effort caused by air trapping and expanded pulmonary fields.

BAL cytology will provide diagnostic confirmation of pulmonary inflammation in horses with mild to moderate disease. BAL is recommended as the appropriate diagnostic method to obtain samples for cytologic analysis. Compared with samples obtained with TTW aspiration, BAL sampling provides more representative diagnostic information regarding the status of cellular infiltrate into the lower airway secretions. There is poor correlation using the criteria for RAO diagnosis of >5% neutrophils in a BAL sample compared with >20% neutrophils in a tracheal aspirate sample. It is well recognized that a great deal of variability can be identified in the cellular composition of tracheal fluid, which is in contrast to samples from the lower airways obtained by BAL sampling.[426] When sampling is performed using the BAL protocol, specifically when marked inflammation is present, there may be a reduction in sample volume obtained resulting from airway collapse distal to the tube or endoscope.[17] In such instances, low aspiration pressures should be applied in an effort to reduce distal airway collapse. In addition to difficulty with sample collection, the quality of sample collected should be inspected. In horses where airway collapse has occurred the amount and quality of surfactant collected may be reduced. Interpretation of cytologic findings support RAO when neutrophilic inflammation (>5% neutrophils) is found on BALF cytology. Cellular morphology is generally normal and classified as nondegenerate without evidence of sepsis. Mucus is commonly abundant in cytologic analysis of horses suffering from RAO and deeply stained casts of inspissated mucus (classified as Curschmann's spirals) may be reported. However, mucus may be identified with other conditions that can lead to pulmonary inflammation, reducing the specificity of this independent finding. The diagnosis of RAO is more difficult when clinical signs are mild and exhibited by an occasional cough or minimal abdominal effort. Rebreathing is a simple procedure that will enhance pulmonary sounds on auscultation, but in horses with less severe disease, ancillary diagnostics are required. When pulmonary function testing is performed on horses with severe equine asthma, they typically demonstrate an increased change in pleural pressure ΔP_{Lmax} (>15 cm H_2O), increased pulmonary resistance (R_L), and decreased dynamic lung compliance (C_{dyn}).[427-430] Although standard pulmonary testing is not sensitive enough to detect mild airway obstruction when RAO patients are in remission, horses with RAO have histologic evidence of pulmonary disease. These findings indicate that although clinical signs are recurrent, and the patient may be in remission, pulmonary disease is permanent and pulmonary function is negatively impacted, particularly when exacerbation is present.

Therapeutic goals of horses suffering from RAO include improved pulmonary function through the attenuation of airway inflammation and bronchial wall hyperresponsiveness leading to marked bronchoconstriction. The central factor for disease control is environmental modification that limits (preferably eliminates) allergen exposure. The removal of the environmental allergen is a key feature to achieving disease remission in horses suffering from heaves. Medical therapy aimed at attenuation of clinical signs associated with pulmonary inflammation and bronchoconstriction will provide temporary clinical benefit. If environmental modification is insufficient, disease exacerbation will occur once drug dosages are reduced. Synthetic corticosteroid administration will aid with reduction in pulmonary inflammation, and

bronchodilator therapy will open airways, improving pulmonary function as a rescue therapy, and should be continued concurrently with corticosteroid therapy until bronchodilation is achieved.

The goal of therapy is to reduce airway inflammation and bronchoconstriction while enhancing mucociliary clearance mechanisms. Corticosteroids and bronchodilator therapy are used regularly for treatment of RAO. The most common environmental allergens leading to exacerbation of RAO signs include airborne particulate material that is in extraordinarily high concentrations in most stable environments. Apparently clean and fresh hay typically contains mold spores such as *Aspergillosis fumigatus, Faenia rectivirgula,* and *Thermoactinomyces vulgaris.*[419,431] In addition to environmental molds, environmental endotoxin and ammonia are recognized to contribute to induction of airway inflammation.[432,433] Round bale hay is exposed to repeated environmental moisture and contains high levels of airway allergens when fed to RAO-affected horses maintained on pasture. Horses that are stabled will have an improved local environment if provided with ample ventilation, a clean stall with low-dust bedding material, and commercial pelleted feed (low dust) that contains minimal allergens and particulate materials. Moistened hay pellets, cubes, or silage may provide alternate roughage sources for horses with airway-related inflammation. Commercial roughage products such as hydration hay are becoming increasingly available with the advent of small-sized baled hay that can be soaked and fed from a 5-gallon bucket. These products have the advantage of high-quality roughage with the introduction of limited allergens and airborne particles. Soaking of long-stem hay is an option for management of mildly affected horses but will not provide sufficient reduction of allergen exposure for highly sensitive individuals. Management of heaves-affected horses should take into account all triggers. Although dust particles are not a specific allergen, horses with airway inflammation will benefit from a low-particle environment.[434] Avoidance of indoor arenas, housing near a rock or dirt road, or maintenance in a dry lot pen should be avoided in an effort to remove all particles from the breathing zone of affected horses.

Antiinflammatory Therapy

The cornerstone of remission of clinical signs in horses suffering from RAO is reduced inflammation. It is well recognized that the inflammatory cytokines released by activated T lymphocytes increase the pulmonary concentration of IL-4 and IL-8.[435,436] The recruitment of neutrophils, particularly in response to the potent chemokine IL-8, maintains a heightened level of immune reactivity in this environment, contributing to enhanced mucous production, bronchospasm, and coughing. Corticosteroids act as direct inhibitors of this inflammatory cycle, resulting in improved clinical signs. The combination of corticosteroids with bronchodilator therapy is the most effective method to induce disease remission. It is important to consider that long-acting potent corticosteroids are more likely to produce detrimental effects such as laminitis and immunosuppression. Therefore, therapeutic recommendations include using the lowest effective dose and avoiding prolonged therapeutic administration. In many cases, a course of therapy for approximately 2 weeks is sufficient to induce clinical remission.

Systemic administration of potent corticosteroids improves clinical signs, reduces airway inflammation, and improves pulmonary function in horses with RAO. Triamcinolone acetonide (0.09 mg/kg intramuscularly) as a single dose relieves lower airway obstruction for up to 4 weeks. However, this protocol also results in adrenal suppression for up to 4 weeks.[437] Triamcinolone has also been demonstrated to induce hyperglycemia and hypertriglyceridemia for up to 4 days following drug administration.[438] Dexamethasone (0.1 mg/kg IV q 24 h) reduces airway obstruction and inflammation within 3 to 7 days with clinical benefit evident for up to 7 days.[439] Administration of dexamethasone also produces adrenal suppression; however, this effect lasts for approximately 3 days after discontinuation of treatment.[18] Oral prednisolone has favorable bioavailability and improves clinical signs of airway inflammation, but compared with IV dexamethasone does not provide the same antiinflammatory effect based on BALF cytologic examination.[440] Oral prednisone demonstrates limited efficacy for resolution of clinical signs associated with RAO. Nor does this therapy reduce pulmonary inflammation in horses with moderate to severe disease.[441] NSAIDs and antihistamines are ineffective for treatment of RAO.[442]

Inhaled corticosteroids provide an effective treatment alternative for mild to moderately affected RAO horses. Beclomethasone (500–1500 µg b.i.d.) reduces pulmonary inflammation, improves pulmonary function, and improves ventilation imaging of horses suffering from RAO.[443] In contrast to rescue bronchodilator therapy, no immediate drug effect is observed with aerosolized corticosteroids.[18] Aerosolized medications can effectively be administered with a spacer device such as the Equine Haler (Jorgensen Labs, Inc.) or the AeroHippus (Trudell Medical International). Investigations aimed at comparison of delivery systems have revealed similar efficacy.[444] Clinical improvement is apparent approximately 24 hours following initiation of therapy. Administration of beclomethasone (3200 µg b.i.d.) using the Equine Haler device improves parameters of pulmonary function and arterial oxygen tension approximately 3 to 4 days after initiation of therapy.[1] Among the variety of available inhaled corticosteroid preparations, fluticasone is the most potent with the advantage of lowest adrenosuppressive effects.[9,11,13] Reduced pulmonary neutrophilia, improved pulmonary function, and reduced airway hyperresponsiveness are significantly improved following fluticasone administration (2000 µg t.i.d.).[445-447] Although inhaled corticosteroids are particularly useful for resolution of airway obstruction, unless environmental modification is implemented, clinical signs of obstructive disease will return within 2 to 4 days after discontinuation of therapy.

Aerosolized corticosteroids are effective in horses with mild to moderate airway obstruction, with clinical signs ranging from exercise intolerance to respiratory distress while at rest. Aerosolized drugs provide the benefit of drug delivery directly to the respiratory tract but represent a more substantial financial investment and may be reserved for valuable or performance horses. Currently, there are three aerosolized corticosteroid preparations available in metered dose inhaler preparations: beclomethasone dipropionate, fluticasone propionate, and flunisolide. The relative potency of these surface-acting corticosteroids are fluticasone > beclomethasone > flunisolide = triamcinolone. When considering dexamethasone as a standard of 1, flunisolide = 1.9, triamcinolone = 2.0, beclomethasone = 13.5, and fluticasone = 18.0. When comparing the commercially available corticosteroid products,

fluticasone is the most potent and the most expensive. Fluticasone is highly lipophilic, which results in the longest pulmonary residence time. Because fluticasone has extremely low oral bioavailability (<2%) and extensive first pass metabolism (99%), fluticasone has the advantage of the least potential for adverse systemic side effects and the most favorable therapeutic index of the commercially available aerosolized corticosteroids. Horses suffering from RAO and receiving fluticasone (2000 μg t.i.d.) demonstrate reduced pulmonary neutrophilia, improved pulmonary function assessment, and reduced responsiveness to histamine challenge during episodes of airway obstruction.[29] When healthy horses are evaluated following administration of fluticasone, serum cortisol levels are reduced by 40% following 1 day of therapy and 65% following 1 week of treatment. Serum cortisol concentrations return to pretreatment levels approximately 24 to 48 hours following discontinuation of drug therapy. Beclomethasone is the first line of aerosolized corticosteroid therapy for moderate to severe allergic airway disease in human patients. Beclomethasone (500–1500 μg t.i.d.) results in reduced pulmonary inflammation, improved parameters of pulmonary function, and improved ventilation imaging of horses with RAO.[1,11,13,18] Although immediate effects are not observed following administration of beclomethasone, clinical signs and pulmonary function begin to improve within 24 hours of administration. Administration of beclomethasone (3750 μg t.i.d.) improves pulmonary function and arterial oxygen tension for about 2 weeks. When housed in an allergen-rich environment, pulmonary function parameters return to pretreatment values within 3 to 7 days following discontinuation of drug therapy.[18] Subsequently, it can be concluded that if environmental management is not addressed, clinical resolution will not be maintained when beclomethasone treatment is used without environmental modification.

Despite the fact that human asthma patients remain on inhaled corticosteroids for extended periods without the detriment of adrenosuppression, horses have increased sensitivity to the adrenosuppressive effects of inhaled corticosteroids. Endogenous cortisol production is reduced in horses treated with beclomethasone (>1000 μg b.i.d.)[18] or fluticasone (2000 μg b.i.d.)[29] with the threshold for beclomethasone of approximately 500 μg b.i.d. The adrenosuppressive effects have been observed to last for approximately 2 days following discontinuation of drug therapy. Interestingly, the therapeutic efficacy of beclomethasone (500 μg b.i.d.) is approximately equal to larger doses, suggesting that horses can be treated at an effective dose with the least adrenal suppression being induced when environmental modification is implemented.[18]

Bronchodilator Therapy

Pulmonary bronchoconstriction associated with RAO requires specific therapy for immediate relief of smooth muscle contraction in the lower airways. Symptomatic bronchodilator therapy will improve clinical signs; however, lower airway inflammation is not altered by bronchodilator administration. Bronchodilator therapy is primarily indicated for immediate relief of bronchoconstriction (rescue therapy).[2,5,19,24] The rapid onset of action of an aerosolized bronchodilator consistently provides immediate relief of bronchoconstriction. The most effective agents available for bronchodilation are β_2-adrenergic agonists and parasympatholytic agents.

Clinical improvement associated with bronchodilator treatment is characterized by reduced pulmonary resistance, increased pulmonary compliance, and decreased maximal change in pleural pressure observed in heaves-affected horses treated with bronchodilator therapy.[2,5,19] The most consistently effective bronchodilators are β_2-adrenergic agonists and anticholinergic agents. Methylxanthines are less reliable for providing positive results and have a narrow therapeutic index. Heaves-affected horses stabled in a dust-free environment demonstrate a positive response to bronchodilator therapy, suggesting that environmental management alone will not completely resolve the bronchoconstrictive component of heaves.[15]

The pathogenesis of bronchospasm in horses with recurrent airway disease appears to be primarily vagally mediated and may involve soluble mediators such as serotonin, histamine, and leukotriene D4.[12,15] Defective inhibitory pathways of airway smooth muscle have been suggested to play a role in the development of bronchospasm. β-Adrenergic agonists serve as inhibitors of smooth muscle contraction through the release of nitric oxide from inhibitory nonadrenergic noncholinergic (iNANC) nerves and release of inhibitory prostanoids such as prostaglandin 2 (PGE_2) from the airway mucosa. During episodes of acute RAO, iNANC-mediated relaxation is not present, and the prostanoid profile shifts from an inhibitory one to a stimulatory one.[15]

Smooth muscle contraction, smooth muscle hypertrophy, and mucosal thickness all contribute to airway narrowing and subsequent air trapping. Pulmonary remodeling in heaves-affected horses includes mucous cell hyperplasia and metaplasia that contributes to the cascade of smooth muscle contraction leading to luminal contraction and narrowing. Bronchodilator therapy has remained a mainstay of heaves treatment, and resolution of severe airway distress requires administration of rapid-acting bronchodilators.[9,11,13]

Increasing luminal diameter should not be the only focus of therapy. Antiinflammatory therapy is required to reduce exudate and prevent bronchoconstriction. Inflammation is recognized to be the underlying pathophysiological process in all cases of bronchoconstriction, and daily antiinflammatory therapy will mitigate potential disease exacerbation and reduce the requirements for bronchodilator therapy. Corticosteroids are recognized to be the only drug class to demonstrate substantial modification of allergic airway disease in human asthmatic patients. Chronic administration of β_2-adrenergic agonists alone produce deterioration in pulmonary function, increased airway responsiveness, and more frequent exacerbations of bronchoconstriction.

Aerosolized, short-acting β_2-adrenergic agonists (albuterol, pirbuterol, and fenoterol) are rapidly effective bronchodilators indicated for "rescue therapy" for horses demonstrating respiratory difficulty at rest.[2,5,19,24] Albuterol sulfate (360 μg) improves pulmonary function by 70% within 5 minutes of administration.[5,19] When severe airway obstruction is present, drug administration at 15-minute increments for up to 2 hours may be implemented for sequential bronchodilation. Beneficial effects from short-acting β_2-adrenergic agonists last approximately 1 hour in severely affected horses, resulting in the need for longer acting preparations. Combination therapy with corticosteroids will reduce the tolerance that develops to these agents in addition to enhancing β_2-adrenergic receptor protein expression.[11,13,18]

Long-acting bronchodilator preparations are ineffective for use as rescue treatment in patients suffering from severe airway obstruction caused by delayed onset of activity and slightly reduced peak activity compared with albuterol sulfate.[10,20] Salmeterol xinafoate is a chemical analog of albuterol

with an elongated side chain providing a longer duration of action. Salmeterol xinafoate (210 μg) improves pulmonary function by 55% within 60 minutes of administration with efficacy lasting for up to 8 hours, even in severely affected horses.[10,11] Ipratropium bromide is a surface-acting antimuscarinic agent that exhibits little to no systemic absorption (quaternary ammonium structure) from the respiratory or gastrointestinal system. Ipratropium (90–180 μg) improves pulmonary function by 50% within 1 hour, and the duration of the effect is approximately 4 to 6 hours in severely affected patients. Oral clenbuterol (0.8 μg/kg, PO, b.i.d.) is a systemic alternative to the aerosolized, long-acting bronchodilators for management of RAO-affected horses. Clenbuterol is prepared for oral administration as a syrup (Ventipulmin, BIVI). A significant advantage to clenbuterol is a longer duration of action (12 hours). Clenbuterol is approved by the Food and Drug Administration (FDA) in the United States for use in horses with airway constriction. It should be noted that clenbuterol is illegal for use in food-producing animals.[1,2] Dosing protocols for clenbuterol should be followed carefully and in accordance with manufacturer recommendations. Initial therapy at the lower end of the dosing range is generally well tolerated, and incremental dose increases may be tolerated. However, inadvertent initial high-dose therapy (3.2 μg/kg) may result in tachycardia, tremors, sweating, and apparent anxiety. Clenbuterol administration is indicated for temporary smooth muscle airway relaxation in association with airway disease; prolonged therapy is not indicated and may result in adverse effects.

Alternate Bronchodilatory Options

N-butylscopolammonium bromide (NBB; Buscopan) is a quaternary ammonium that serves as a peripherally acting antimuscarinic, anticholinergic agent similar in action to atropine. This formulation is marketed by BIVI as Buscopan for administration to horses. NBB is FDA approved in the United States for use in horses that demonstrate colic signs resulting from gas, spasms, or mild impactions. Recent work has focused on the potential utility of NBB administration for the treatment of marked airway obstruction that may occur with conditions of airway inflammation such as RAO. Current evidence provides support for the use of NBB as an immediate bronchodilator.[429,448] Although tachycardia and reduced gastrointestinal motility are valid considerations, based on the short duration of activity and evidence to support improved ventilatory function it is considered an appropriate therapeutic option when managing equine patients with severe airway obstruction. Additional evidence to support the use of NBB in horses suffering from airway obstruction is supported be a crossover investigation in RAO-affected horses in which atropine and NBB provided similar bronchodilatory effects, yet the systemic effects, such as pupillary dilation and one horse developing colic signs, were only observed following atropine administration.[449] From this study it was concluded that NBB bromide was associated with fewer systemic side effects and is therefore a preferred treatment for reversible airway obstruction in horses. Atropine (0.005–0.01 mg/kg horse, IV) is a rapid and powerful bronchodilator in horses suffering from heaves. Because several adverse effects (ileus, CNS toxicity, tachycardia, increased viscosity of mucous secretions, and impaired mucociliary clearance) may develop following the administration of atropine, use of this agent is recommended as a rescue therapy for severe and life-threatening airway obstruction.

SPAOPD is a condition similar to RAO regarding clinical signs and management; however, a marked difference in disease development involves the triggering aeroallergen involving molds and pollens from pasture grasses. Disease development of SPAOPD is similar with respect to the development of hypersensitivity; however, the antigenic stimulus involves pasture forage pollens that have been recognized in the Gulf Coast states in the United States and in England.[450,451] Weather conditions that favor exacerbation for sensitized horses include hot, humid weather that most commonly occurs in late summer.[452,453] Evidence has demonstrated that specific environmental conditions that contribute to the exacerbation of SPAOPD include not only temperature but also humidity, which affect concentration of fungal spores, seasonal vegetative growth, and pollination of plants in the pasture environment.[453,454]

Bronchodilator and antiinflammatory therapy are recommended in combination with relocation to a clean, low-dust environment. Eliminating or reducing access to an antigen commonly requires a well-ventilated stable environment. Clinical signs are similar to those associated with traditional RAO. The clinical management of SPAOPD involves removal from pasture, whereas in traditional RAO the preferred environment is full-time pasture access. Interestingly, some horses manifest airway obstruction under specific environmental conditions such as traditional RAO in which a closed barn environment favors disease exacerbation, whereas in the southeastern United States a warm humid outdoor environment results in clinical signs of respiratory distress. The particularly challenging component of managing any horse with allergic airway disease is to specifically determine the inciting cause of disease so antigen avoidance can be used. In horses that react to a multitude of environments with clinical signs of airway obstruction, maintaining a horse in a state of consistent disease remission can be particularly challenging. The similarity among disease development and the recognition that SPAOPD may also demonstrate sensitivity to allergens in a confined environment such as hay and straw dust and molds support the suggestion that minimizing these exposures should also be avoided.

INFLAMMATORY AIRWAY DISEASE

Clinical Presentation

IAD (mild to moderate equine asthma[418]) can develop in horses of all ages. In contrast to RAO, clinical signs of IAD are typically subtle, particularly at rest. Horses with IAD tend to be younger horses compared with horses that suffer from RAO, but clinical signs of IAD are dependent on the extent of physiologic exertion. The presenting complaint may include poor performance, particularly at exertion, with occasional cough. Frequency of cough in horses with IAD is variable. In some reports cough has been used as a measure of inclusion criteria,[455] which complicates the determination of the overall frequency of cough in horses with confirmed IAD.[419] At rest, horses with IAD do not demonstrate any discernable evidence of respiratory dysfunction. Pulmonary inflammation in horses that suffer from IAD is mild, requiring advanced pulmonary function testing to detect alteration in gas exchange mechanisms. However, similar to RAO, horses with IAD typically have airway mucus accumulation, which can be marked in severe cases. Current recommendations have focused on

the identification of lower airway cytologic characteristics that define the inflammatory milieu of the lower airway in combination with pulmonary function testing.[418] Collectively these criteria provide the clinician with direct evidence with respect to the origin of reduced exercise performance.[32,456-458] Different phenotypes of airway inflammation have been described for horses with IAD and have apparent correlation with disease severity. Mast cell inflammation has been observed in association with airway hyperreactivity[459] and subclinical airway obstruction, whereas airway neutrophilia has been observed in association with cough and airway mucus accumulation.[418,459]

Diagnosis

Diagnosis of IAD (mild to moderate equine asthma) is based on clinical signs, airway inflammation with excess mucus, abnormal lower airway cytologic evaluation, or abnormal pulmonary function. It must be confirmed that horses with IAD do not suffer from RAO or infectious pulmonary disease. Identification of clinical signs consistent with lower airway inflammation are characterized by reduced athletic performance and mild cough and airway mucus identified on endoscopy (Table 8.9). Other consideration should be given to causes for poor performance such as upper airway disease or musculoskeletal disease or injury.

BALF cytology is the diagnostic test sampling procedure of choice for a confirmatory diagnosis of IAD. Although the cytologic profile of BALF analysis in horses with RAO is markedly neutrophilic, horses with IAD typically have a cytologic profile characterized by mild to moderate increase in neutrophil, eosinophil, and/or mast cell percentages.[418,457,460] Variability among sampling techniques and instrumentation prevents the application of universal cutoff limits for the classification of IAD. Based on the availably body of literature, when 250 mL of sterile saline is infused the approximate findings should include a total cell count of ≤530 cell/μL, neutrophils ≤5%, eosinophils ≤1%, and metachromatic (mast) cells ≤2%. When interpretation of cytologic values are based on total percentages, regardless of volume of fluid instilled, findings consistent with IAD include neutrophils >10%, mast cells >5%, and eosinophils >5%.[418,460-463] Collectively the interpretation of BALF cytology needs to be based on the patient history, clinical signs, physical examination, and findings on airway endoscopic examination in combination with the cytologic analysis.

It is important to note that the interpretation of cytologic findings should be carefully considered in association with the patient. It has been long recognized that age, vocation, and environment can play a role regarding cellular changes in the respiratory tract. In a recent study young horses transitioning to a race-training environment were recognized to have abnormal BALF cytologic analysis after 4 weeks in race training.[433] Although the horses were not tested for pulmonary function changes, the actual clinical implications for these cellular changes cannot be made. This investigation highlights the importance of environment, particularly in young horses entering race training that may not have any measurable changes in training performance but may have cytologic changes occurring in their lower airways. In other words, looking at lower airway BALF cytology independent of other factors may lead to overinterpretation of clinical association. Sample collection is also an important consideration, which has been well reported in the literature. When examination of a suspect IAD horse is performed, sample collection must be restricted to the use of BALF analysis. Although in some settings collection of a transtracheal aspirate may provide greater ease and efficiency of collection, based on the lack of correlation between TTW and BALF cytology,[426,464,465] TTW sample collection for cytologic analysis for the diagnosis of IAD is not recommended.[418]

Additional diagnostics that may be completed for a horse with suspect IAD may include complete hematologic and serum chemistry profile analysis, pulmonary function testing, and thoracic imaging to include ultrasound and radiography. Hematologic changes are rarely abnormal in horses with IAD with the exception of horses suffering from eosinophilic IAD, which may also be associated with peripheral eosinophilia,[466] particularly if the horse is suffering from idiopathic eosinophilic pneumonia, although circulating changes in eosinophilic concentrations are not always present in horses with airway eosinophilia.[433] Sensitive pulmonary function testing that includes forced expiration and impulse oscillometry provides evidence that horses with IAD suffer from airway obstruction.[33,460] Because advanced pulmonary function testing requires specialized instrumentation and training, diagnostic testing is limited to specialty services that provide such testing. Reproducible characteristics of eosinophilic and mast cell–associated IAD include clinical evidence of airway hyperresponsiveness to airway particles. Clinical manifestation of airway reactivity includes bronchoconstriction, airway reactivity, and cough, which represent the physiologic response to irritants that alter pulmonary homeostasis, resulting in reduced pulmonary function, particularly at speed. Based on the recognition that there are similarities and differences between IAD and RAO, clinically practical observations can be made to aid the clinicianin discriminating between these two airway disorders.[418] Specifically, a horse with evidence of airway disease can be placed in a challenge environment of moldy hay and observed for evidence of respiratory distress or difficulty. Although horses that suffer from IAD will have cytologic changes and increased mucous production in their lower airways, they will not demonstrate evidence of respiratory distress. In contrast, a horse that suffers from severe equine asthma will demonstrate increased expiratory effort, cough, and nostril flare when placed in the challenge environment.

TABLE **8.9 Tracheal Mucus Grading Scale**[1,2]

Grade	Mucus Accumulation
0	None
1	Multiple small
2	Moderate larger, non-confluent
3	Marked mucus
	Marked - confluent
	Marked - stream forming
4	Large pool-forming
5	Extreme, profuse amounts

1. Gerber V, Lindberg A, Berney C, et al., Airway mucus in recurrent airway obstruction—short-term response to environmental challenge, *J Vet Intern Med.* 2004;18: 92-97.
2. Couetil LL, Cardwell JM, Gerber V, et al., Inflammatory airway disease of horses-revised consensus statement, *J Vet Intern Med.* 2016;

Treatment

Clinical management of horses suffering from IAD has some similarities to the management of horses with RAO. Interestingly, published evidence to support therapeutic protocols are more limited when demonstrated efficacy for the management of IAD is compared with the efficacy of therapy for RAO.[418,419] As stated, IAD is a milder condition; horses do not demonstrate respiratory distress and appear normal at rest. For these reasons disease characterization can be more challenging; therefore, objective measures that define disease outcome and response to therapy are limited. Nonetheless, clinical management is aimed at reducing pulmonary inflammation through the use of antiinflammatory therapy and environmental management.

Once the diagnosis of IAD has been established, antiinflammatory therapy combined with environmental management[467] is aimed at reducing pulmonary cellular infiltrates and airway hyperresponsiveness.[467]

Treatment goals of horses suffering from IAD include immediate control of horses that demonstrate evidence of bronchoconstriction and cough that impairs performance, reduced mucous production and associated airway obstruction, and sustained pulmonary quiescence to allow for continued athletic performance. Despite the limited evidence to support the efficacy of corticosteroids for improvement of IAD in horses, anecdotal reports support recommendations that antiinflammatory therapy is an important component of therapy in horses with performance limitation caused by IAD. Systemically administered corticosteroids have a profound and global effect on inflammation and associated eicosanoid expression. The decision for route of drug delivery for systemically administered drug compared with aerosolized medication is based on factors that include severity of disease and financial availability, because aerosolized medications can be cost prohibitive in some settings. Additionally, potential consequences associated with corticosteroid administration must be factored into the decision-making process. Systemic responses following corticosteroid administration include some degree of immunosuppression, altered protein synthesis, and reduced adrenal responsiveness. Unpredictable idiosyncratic reactions may have deleterious patient effects, such as the development of laminitis.

Using RAO as a model to determine drug efficacy, several studies have been designed to assess drug efficacy based on clinical signs, BALF cytology, and pulmonary function. Systemically administered dexamethasone appears to provide superior antiinflammatory effects as evidenced by improvement in pulmonary cellular infiltrate compared with prednisolone[468] in an antigen-controlled investigation. In an independent investigation aimed at determining drug efficacy in an antigen-rich environment, oral prednisolone and dexamethasone were compared with improved clinical signs observed in all treated horses; however, dexamethasone-treated horses demonstrated improvement in pulmonary function. Although prednisolone has historically been a treatment of choice because of the ease of oral administration, response to therapy has not been consistently observed and likely relates to variability in bioavailability among horses. Adverse events appear to be more common with systemically administered triamcinolone, and for this reason this agent is not recommended for the management of airway inflammation. An investigation that demonstrated similar clinical efficacy when dexamethasone was compared with isoflupredone also demonstrated hypokalemia, making this a less than ideal drug selection as well.[469]

Use of aerosolized corticosteroids has become commonplace for the management of equine patients with lower airway inflammation (Table 8.10). In most clinical settings when treating a patient for airway inflammation associated with IAD (similar to the management of RAO), in all but the most mild of cases initial therapy will include systemically administered medication; once airway inflammation is believed to be reduced drug administration will typically include aerosolized therapy. As stated, finances may impact the ability of the clinician to use the airway for drug delivery. Options for the administration of synthetic corticosteroid delivery are beclomethasone and fluticasone. Available spacer devices include the AeroHippus and Equine Haler devices, with drug delivery and efficacy among the two devices being similar.[444]

Efficacy of aerosolized corticosteroid therapy in horses with RAO was evidenced by the administration of fluticasone propionate (2000 µg q 12 h) with improvement of clinical signs and pulmonary function without evidence of impairment of immune system function.[446] Beclomethasone when administered at moderate dosages (≥500 µg) results in adrenosuppression.[470] Similarly, fluticasone (1500 µg b.i.d.) administration

TABLE **8.10** **Systemically Administered Therapeutic Agents Used for the Treatment of Non-Infectious Airway Disease**[1]

Medication	Dose
Corticosteroids	
Dexamethasone	0.04-0.1 mg/kg, IV or IM, q 24 h
	0.05 mg/kg, PO, q 24-48 h[2]
Prednisone	1-2 mg/kg, PO, q 24 h
Bronchodilators	
Clenbuterol	0.8-3.2 µg/kg, PO, q 12 h[3]
Aminophylline	2-5 mg/kg, IV, q 12 h
	5-10 mg/kg, PO, q 12 h
Theophylline	5-10 mg/kg, PO, q 12 h
Other	
Interferon alpha	50-150 U, PO, q 24 h x 5 days[4]
Omega-3 polyunsaturated fatty acids	1.5 g DHA, PO, q 24 h x 60 days[5]

Continued

TABLE **8.10 Systemically Administered Therapeutic Agents Used for the Treatment of Non-Infectious Airway Disease[1]—cont'd**

Aerosolized medications for the management of non-infectious airway disease[1]

Medication	Device	Dose
Corticosteroids		
Fluticasone	AeroHippus, Equine Haler	1-6 μg/kg, q 12 h[6–11]
Beclomethasone	AeroHippus, Equine Haler	1-8 μg/kg, q 12 h[12–17]
Bronchodilators		
Albuterol	AeroHippus, Equine Haler	1-2 μg/kg, q 1–3 h[18,19]
Ipratropium	AeroHippus, Equine Haler	0.2-0.4 μg/kg, q 8–12 h[18,20,21]
	Ultrasonic nebulizer	2-3 μg/kg 0.02% solution for nebulization, q 24 h
Chromones		
Cromolyn sodium	Jet nebulizer	200 mg 0.02% solution for nebulization, q 12 h[22–26]
	Ultrasonic nebulizer	80 mg 0.02% solution for nebulization, q 24 h

1. Couetil LL, Cardwell JM, Gerber V, et al., Inflammatory Airway Disease of HorsesRevised Consensus Statement, *J Vet Intern Med.* 2016.
2. Grady JA, Davis EG, Kukanich B et al., Pharmacokinetics and pharmacodynamics of dexamethasone after oral administration in apparently healthy horses, *Am J Vet Res.* 2010;71:831-839.
3. Erichsen DF, Aviad AD, Schultz RH, et al., Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD), *Equine Vet J.* 1994;26:331-336.
4. Moore BR, Krakowka S, Cummins JM, et al., Changes in airway inflammatory cell populations in standardbred racehorses after interferon-alpha administration, *Vet Immunol Immunopathol.* 1996;49:347-358.
5. Nogradi N, Couetil LL, Messick J, et al., Omega-3 fatty acid supplementation provides an additional benefit to a low-dust diet in the management of horses with chronic lower airway inflammatory disease, *J Vet Intern Med.* 2015;29:299-306.
6. Couetil LL, Chilcoat CD, DeNicola DB, et al., Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction, *Am J Vet Res.* 2005;66:1665-1674.
7. Couetil LL, Chilcoat CD, DeNicola DB, et al., Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction, *Am J Vet Res.* 2005;66:1665-1674.
8. Dauvillier J, Felippe MJ, Lunn DP, et al., Effect of long-term fluticasone treatment on immune function in horses with heaves, *J Vet Intern Med.* 2011;25:549-557.
9. Giguere S, Viel L, Lee E et al., Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate, *Vet Immunol Immunopathol.* 2002;85:147-158.
10. Laan TT, Westermann CM, Dijkstra AV, et al., Biological availability of inhaled fluticasone propionate in horses, *Vet Rec.* 2004;155:361-364.
11. Robinson NE, Berney C, Behan A et al., Fluticasone propionate aerosol is more effective for prevention than treatment of recurrent airway obstruction, *J Vet Intern Med.* 2009.
12. Couetil LL, Art T, de MB, et al., Effect of beclomethasone dipropionate and dexamethasone isonicotinate on lung function, bronchoalveolar lavage fluid cytology, and transcription factor expression in airways of horses with recurrent airway obstruction, *J Vet Intern Med.* 2006;20:399-406.
13. Rush BR, Raub ES, Thomsen MM, et al., Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction, *J Am Vet Med Assoc.* 2000;217:359-364.
14. Rush BR, Trevino IC, Matson CJ, et al., Serum cortisol concentrations in response to incremental doses of inhaled beclomethasone dipropionate, *Equine Vet J.* 1999;31:258-261.
15. Rush BR, Worster AA, Flaminio MJ, et al., Alteration in adrenocortical function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively, *Am J Vet Res.* 1998;59:1044-1047.
16. Rush BR, Raub ES, Rhoads WS, et al., Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively, *Am J Vet Res.* 1998;59:1039-1043.
17. Rush BR, Flaminio MJ, Matson CJ, et al., Cytologic evaluation of bronchoalveolar lavage fluid from horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively, *Am J Vet Res.* 1998;59:1033-1038.
18. Bayly WM, Slocombe RF, Schott HC, et al., Effects of inhalation of albuterol sulphate, ipratroprium bromide and frusemide on breathing mechanics and gas exchange in healthy exercising horses, *Equine Vet J.* 2001;33:302-310.
19. Bertin FR, Ivester KM, Couetil LL, Comparative efficacy of inhaled albuterol between two hand-held delivery devices in horses with recurrent airway obstruction, *Equine Vet J.* 2011;43:393-398.
20. Bayly WM, Duvivier DH, Votion D, et al., Effects of inhaled ipratropium bromide on breathing mechanics and gas exchange in exercising horses with chronic obstructive pulmonary disease, *Equine Vet J.* 2002;34:36-43.
21. Robinson NE, Derksen FJ, Berney C, et al., The airway response of horses with recurrent airway obstruction (heaves) to aerosol administration of ipratropium bromide, *Equine Vet J.* 1993;25:299-303.
22. Beech J, Principles of therapy, *Vet Clin North Am Large Anim Pract*. 1979;1:73-88.
23. Hare JE, Viel L, O'Byrne PM, et al., Effect of sodium cromoglycate on light racehorses with elevated metachromatic cell numbers on bronchoalveolar lavage and reduced exercise tolerance, *J Vet Pharmacol Ther.* 1994;17:237-244.
24. Murphy JR, McPherson EA, Lawson GH, The effects of sodium cromoglycate on antigen inhalation challenge in two horses affected with chronic obstructive pulmonary disease (COPD), *Vet Immunol Immunopathol.* 1979;1:89-95.
25. Soma LR, Beech J, Gerber NH, Jr., Effects of cromolyn in horses with chronic obstructive pulmonary disease, *Vet Res Commun.* 1987;11:339-351.
26. Thomson JR, McPherson EA, Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse, *Equine Vet J.* 1981;13:243-246.

attenuated serum cortisol concentrations following 1 week of therapy.[471] Notably, there are no reports that describe clinical manifestation of Addisonian crisis or evidence of adrenal dysfunction in horses following treatment, but it should be advised that because of systemic effects judicious drug administration should implemented.

Current protocol recommendations from a recent review suggest that treatment is ideally initiated with systemically administered corticosteroid therapy using a tapering dose protocol for approximately 4 weeks.[419] Drug dosages are typically reduced at 25% increments at weekly intervals or as clinical response to therapy is observed. Clinical improvement of

IAD can subjectively be difficult to determine because clinical signs are typically mild, particularly at rest. Therefore, a protocol aimed at systemically administered corticosteroids tapered over 4 weeks and followed by aerosolized therapy is a rational approach aimed at improved pulmonary inflammation. Transition from systemically administered medications to fluticasone or beclomethasone is determined by disease severity and response to therapy. Some individuals apparently respond more favorably to one preparation over another, and this factor should be considered with drug selection and follow-up examination to determine response to therapy.

The overall goal of therapy for horses suffering from airway hyperresponsiveness is the reduction in pulmonary inflammation as well as attenuation of tissue airway remodeling, which is a consequence of chronic airway inflammation. Application of environmental modification used in combination with anti-inflammatory therapy with fluticasone in combination will provide the greatest likelihood of achieving these therapeutic goals.

Bronchodilator therapy is aimed at improving airway mechanics and airflow, particularly with severe airway obstruction. Therapeutic agents available to provide benefit include β-adrenergic type 2 receptor agonists and parasympatholytic agents. Among the β-adrenergic receptor type 2 agonists, aerosolized albuterol, and oral clenbuterol syrup (Ventipulmin, BIVI) are the agents of preference. Among drugs that target muscarinic receptors, aerosolized ipratropium or IV NBB[429,449] (Buscopan, BIVI) are the agents of preference. Atropine used at low dosages provides bronchodilation but carries the potential risk of iatrogenically induced ileus, and for that reason it is not recommended for routine use. Albuterol is indicated for immediate rescue treatment of bronchoconstriction, whereas clenbuterol is indicated for prolonged bronchodilation. Chronically administered clenbuterol (0.8 μg/kg, PO, b.i.d.) was reported to lead to tachyphylaxis evidenced by an investigation that determined 21 days of clenbuterol therapy resulted in increased airway hypersensitivity and impaired bronchodilatory activity.[472] As stated, clenbuterol administration is indicated for temporary bronchodilation; prolonged therapy is not recommended.

Aerosolized albuterol is indicated for bronchoconstriction observed in horses with severe airway disease. Although rescue therapy is not commonly required for the management of patients with IAD, in some instances airway obstruction may benefit from bronchodilatory therapy. In addition to bronchodilation, therapeutic strategies must also incorporate corticosteroid therapy and environmental modification to reduce dust and aeroallergen materials. Albuterol is generally cost-effective and is readily available for use in equine patients. Uncommon events such as paradoxical bronchoconstriction may result in patient responsiveness to the racemic mixture of albuterol preparations. In instances where an alternate approach is desired, the (R)-enantiomer can be administered solely in the form of levalbuterol. Although levalbuterol has historically been cost prohibitive for use in equine patients, current costs have come down considerably. Longer acting β_2-adrenergic agonist therapy is available in the form of salmeterol. With a modification of molecular design, this agonist can sustain longer activity compared with albuterol. Frequency of administration is approximately every 8 hours.[473] Additional classes of aerosolized bronchodilator therapy may provide added benefit for patients that have diminished sensitivity to β_2-adrenergic receptor agonists. Ipratropium is a surface-acting parasympatholytic agent that has similar activity to atropine. Low to negligible bioavailability provides the advantage of not inducing unwanted side effects of systemically administered parasympatholytic agents. Horses suffering from mast cell IAD may benefit from the administration of sodium cromoglycate, which blocks calcium channels, resulting in mast cell stabilization rather than degranulation, inhibiting the release of histamine and tryptase, which potentiate leukotriene and PGE expression and bronchoconstriction. Client compliance is essential because the drug must be administered for 1 to 2 weeks to produce clinical benefit. For some caregivers this therapeutic protocol is not practical.

Although mild to moderate equine asthma is not nearly as debilitating as severe equine asthma, consideration must be made for environmental influences that may potentiate airway reactivity and hyperresponsiveness. Low-dust environments and avoidance of any feeding from hay nets or elevated mangers should be practiced. Areas in which dust is present should be wet down before cleaning and whenever possible, horses removed from the environment before cleaning. Use low-dust bedding material, avoid stabling in an area with an indoor arena, and avoid overhead storage of hay or straw.

EXERCISE-INDUCED PULMONARY HEMORRHAGE

Definition

EIPH is defined as the presence of blood in the airways following strenuous exercise. Hemorrhage originates from the pulmonary capillary vasculature into the alveolar spaces. The predominant regions of hemorrhage are localized in the caudodorsal lung fields. At the microscopic level, tissue changes include edema, pulmonary capillary hemorrhage, and alveolar hemorrhage.

Blood may be identified by gross examination with airway tracheobronchoscopy or by the identification of erythrocytes or hemosiderophages on tracheal or BAL cytologic examination. EIPH may result in occult hemorrhage, in which blood is not obviously visible on examination but can be identified with airway endoscopy or may be evidenced by epistaxis following exertional exercise. A recent systematic review was compiled on this subject and published as an ACVIM consensus statement and serves as a valuable reference on this particular disease process.[474]

Epidemiology

EIPH is a disease the affects all disciplines of horses that work at intensity. The characterization of the frequency of EIPH among performance horses is somewhat hindered by the use of different criteria to define the disease. When the diagnosis was made with tracheobronchoscopy within 2 hours of racing, approximately 43% to 75% of racing Thoroughbreds were identified with blood within the tracheal lumen.[475-478] However, when horses have been repeatedly examined the frequency of disease increases to 85%.[478,479] The dramatic changes in pulmonary pressure are a direct effect of cardiac output generated during the high-intensity exercise of athletic horses. Performance events other than racing have variable prevalence of disease ranging from 10% to 70%, with the greatest frequency in horses that work at speed such as racing Quarter Horses and those with the lowest frequency on horses that work at lesser intensity such as pleasure ponies. The relevance of use is

associated with the intensity of exercise rather than the duration of work. In one investigation, polo ponies were examined for the presence of tracheal hemorrhage, with approximately 11% to 46% of the horses demonstrating evidence of pulmonary hemorrhage.[480] The primary lesion associated with pulmonary hemorrhage is pulmonary capillary stress failure. More severe grades (3–4) of EIPH are associated with reduced racing career length.

Pathogenesis

The pathophysiologic mechanism leading to stress failure is a result of high pulmonary capillary pressure and very low inspiratory alveolar pressures that occur during intense exercise.[481] Consequently, the pulmonary capillary transmural pressure is extreme at times of intense, strenuous exercise leading to tissue failure and hemorrhage. Pulmonary capillary pressure at rest is very low, yet with high-intensity exercise pressures may exceed 95 mm Hg during maximal exercise.[482,483] The critical point at which stress failure occurs appears to be when capillary pressure exceeds 75 to 100 mm Hg. There is good indirect evidence that these numbers are exceeded by most galloping horses.[484] Ultrastructural pulmonary capillary stress failure and changes have been identified in horses tested using a treadmill challenge and in a perfused lung model system. Characteristic changes that are identified in association with EIPH include disruption of the capillary endothelium and alveolar epithelium, with resulting erythrocytes within the alveoli and pulmonary interstitial spaces and subsequent erythrocytes, platelets, and macrophages in the region of tissue failure. A characteristic change occurring in some horses that suffer from EIPH involves the development of venoocclusive remodeling associated with pulmonary veins. This remodeling takes place in the intralobar pulmonary veins with the presence of collagen deposition, smooth muscle hypertrophy, and intimal hyperplasia within the pulmonary veins. The end result of this venoocclusive remodeling is increased stiffness with reduced vascular compliance. It is believed that these changes are the physiologic consequence to repeated bouts of strenuous exercise. The pathologic effect on the horse with continued high-intensity exercise is that with increased cardiac output and continued high pressure from the pulmonary arterial vasculature, tissue failure can continue and potentially worsen over time, leading to propagation of tissue failure and hemorrhage into the alveolar spaces and surrounding interstitium. Evidence to support this postulated series of events is in part a reflection of the finding that racehorses that have suffered from EIPH can have increased fibrosis in their caudodorsal lung fields.[485] The specific amount of exercise required to lead to these tissue changes has not been defined. The complete understanding of the impact that venoocclusive remodeling has on the development and propagation of EIPH continues to be under investigation.

Among the historical proposed risk factors for the development of EIPH, lower airway inflammation has been suggested to play a role in disease development. The suggested pathway has included persistent pulmonary inflammation with associated bronchoconstriction leading to increased negative alveolar pressure that would further potentiate pulmonary hemorrhage.[486,487] More recent investigations, however, do not support this suggestion,[488] and airway inflammation as a proposed mechanism for EIPH disease development is not supported. Although it is clear that following an episode of pulmonary hemorrhage there is an indication and recruitment for leukocyte infiltration, specifically macrophages that will provide the phagocytic capacity needed for clearance of erythrocytic debris, there is no evidence to demonstrate that this inflammation was the proximal mediator in the cascade that induced the primary event of hemorrhage. Instillation of whole blood into the airways will consistently induce inflammation and associated alveolar macrophage activation to clear erythrocyte byproducts evidenced by the presence of hemosiderophages, which will clear the region of hemorrhage within 14 days.[489,490] Further supportive evidence for the lack of primary inflammation serving as an initiating factor for the development of EIPH is based on the recognition that when a model system using the instillation of blood to determine clearance mechanisms was investigated, not only was there efficient and effective removal of blood, there were no caudodorsal pulmonary lesions characteristic of horses with EIPH.[491,492] Although some investigations have examined the role of experimentally induced airway inflammation with the propensity for pulmonary hemorrhage to occur, there is insufficient evidence from larger scale investigations that have examined racehorses to make a direct correlation between cough as a measure of inflammation and the presence of hemosiderophages[493] or between endoscopic scoring of tracheal mucus and tracheal hemorrhage.[494] Collectively, when considering pathogenic events that my lead a horse to experience pulmonary hemorrhage with intense exercise, the current understanding is that the level of evidence to support inflammation of the airways or pulmonary parenchyma as a cause of EIPH is low.[474]

Clinical Signs

Identification of a horse that has suffered a bout of EIPH will most consistently be determined with airway endoscopy. In a small proportion of racehorses (approximately 5%) epistaxis may be observed.[475,476,495,496] Physical examination of horses with EIPH is within normal limits for most parameters evaluated; in one small-scale investigation (n = 10) there were changes identified with thoracic percussion in 50% of the horses with EIPH but no auscultable abnormalities in the caudodorsal lung fields.[497] The recent systematic review provided that there was no evidence of changes in examination parameters, such as changes in respiratory rate, respiratory effort, or evidence of distress, or changes in behavior to provide clinical evidence of EIPH following exercise. The quality of evidence suggesting that consistent clinical abnormalities can be detected in horses with EIPH (with the exception of epistaxis following exercise) is also low.[474]

Although epistaxis is an uncommon finding, when present it is an indication of the severity of hemorrhage. The clinician may consider differential possibilities for the presence of epistaxis following exercise, but current evidence supports the suggestion that when epistaxis is observed soon after exercise, EIPH is the likely cause.[474] Several retrospective studies have further characterized the frequency of epistaxis among performance horses with EIPH. In Japan, when horses were examined 30 minutes following race completion that included both flat and steeplechase racehorses, epistaxis was identified in 0.13% of the Thoroughbreds examined and 0.10% of Anglo-Arabs.[498] A similar frequency of epistaxis has been identified in South African Thoroughbred racehorses.[499] Once a horse suffers from epistaxis, the recurrence rate was reported to be 13%, supporting the persistent nature of the disease. In one study, there was an apparent association with horses that were

TABLE **8.11 Grading Scale for EIPH Endoscopic Examination**[1]

0	No blood in pharynx, larynx or anywhere along the tracheal lumen
1	Few specs of blood, fever than 2 short areas
2	Specs throughout, < 10% tracheal surface stream
3	Multiple distinct streams, covers > 30% tracheal surface, but not pooling at thoracic inlet
4	Multiple coalescing streams, covers > 90% tracheal surface with obvious pooling at thoracic inlet

1. Hinchcliff KW, Jackson MA, Brown JA, et al., Tracheobronchoscopic assessment of exercise-induced pulmonary hemorrhage in horses, *Am J Vet Res.* 2005;66:596-598.

older and that raced over jumps. The evidence to support an age-associated phenomenon is lacking. Although age may play a role with the development of disease, the number of races that horses have competed in needs to be recognized because this directly impacts the number of high-intensity events that a horse has endured and therefore, the degree of pulmonary vasculature remodeling that exists.

Some early indicators suggested that acute death may be associated with EIPH in racehorses. However, the overall frequency of EIPH is notably high among racehorses at 85% or higher in some populations. In contrast, the development of acute death among racehorses is remarkably low at 0.08 to 0.29 horses per 1000 starts.[500] Although some reports suggest that pulmonary hemorrhage may account for up to 35% of acute deaths in which postmortem was successful with the confirmatory cause of death,[501] other reports do not consistently provide evidence to support this finding. Currently, the level of evidence to support that EIPH is causally associated with acute death in racehorses is low.[474]

The overall influence of EIPH on the performance level of horses has long been questioned. Although the standards for athletic performance differ among breeds, when racehorses are examined for their racing career longevity the evidence demonstrates that horses with low to moderate (grades 1–3/4) level EIPH do not have shortened race careers.[502] In contrast, racehorses with severe EIPH are likely to have shortened race careers.[474] When considering the impact that EPIH has on racing performance using finishing position as a measure of racing performance,[477,503] horses that are EIPH grade 1 or less (no blood) as defined by endoscopic examination are more likely to win or at least finish in the top three finishing positions.[504] With the currently available and analyzed evidence, the consensus is that horses with more severe EIPH will have inferior performance compared with horses that have minimal to no pulmonary hemorrhage.[474] Horses with more severe forms of EIPH have reduced career utility compared with horses that have lower levels of hemorrhage associated with EIPH.

Diagnosis

The diagnosis of EIPH is based on the identification of hemorrhage within the airways. Although tracheobronchial endoscopy can be performed between 30 and 90 minutes following exertional exercise and will identify frank hemorrhage in most EIPH-affected horses, BAL remains a valuable diagnostic technique. An endoscopic scoring system reflecting the presence of tracheal blood following exercise has been established (Table 8.11) and has consistent interpretation among observers.[477] Commonly, tracheobronchoscopic examination following intense exercise will provide confirmatory evidence of pulmonary hemorrhage. However, the absence of blood does not eliminate the possibility of pulmonary hemorrhage. The presence of hemorrhage will be evident following exercise, but the clearance of erythrocyte constituents may not be complete for up to 14 days. Because of this, BAL can be valuable for the diagnosis of EIPH.

BAL cytologic assessment can aid with the diagnosis of EIPH. Although total nucleated cell counts in BALF are similar among horses in race training and yearlings that have not entered training,[505] erythrocytes and hemosiderophages in the BALF are greater in horses in training compared with horses not being trained. Although the identification of erythrocytes within the BALF is used to provide the diagnosis of EIPH, variability among samples has led to the concern that enumeration of BALF erythrocytes alone may not provide accurate evidence to allow the clinician to classify the severity of EIPH.[506] When horses confirmed with EIPH are compared with healthy horses, total hemosiderin score (cytoplasmic calculation of iron within alveolar macrophages) correlates with EIPH status. Total hemosiderin score >75 has a reported sensitivity and specificity of 94% and 88%, respectively.[507] Notably, hemosiderin scores should be carefully interpreted because pulmonary hemorrhage, regardless of etiology, such as traumatic pneumonia/pleuropneumonia or abscess formation will increase the hemosiderin score.

Thoracic radiographs obtained from horses with EIPH will have characteristic changes in the caudodorsal pulmonary fields. Radiographic changes may include alveolar or mixed interstitial opacities in the caudodorsal lung fields. Clinical management of EIPH-affected horses is unlikely to be impacted by the identification of radiographic changes. Lesions can be identified with radiography and have been consistently identified in horses at postmortem.

Treatment

Furosemide is the recommended therapy for the prophylaxis of hemorrhage associated with EIPH. Although hemorrhage is not eliminated, it is evident that furosemide administration (0.5–1.0 mg/kg, IV) approximately 4 hours before exertion reduces the severity of pulmonary hemorrhage in racehorses.[477] Efficacy of furosemide to reduce the severity of pulmonary hemorrhage is believed to be an effect of reduced pulmonary arterial pressure and left atrial (pulmonary wedge) pressures.[508-515] The effect of lowering pulmonary arterial pressure is a reduction in capillary and transmural pressure during exercise, which reduces the impact on the capillary alveolar interface and associated tissue stress failure and disruption. There is strong evidence that furosemide is an effective mitigating therapy for horses that suffer from EIPH.

In addition to the administration of furosemide as a prophylactic treatment for the attenuation of pulmonary hemorrhage associated with EIPH, additional therapeutic agents have been tested for their efficacy as a prophylactic agent or treatment for pulmonary hemorrhage. Agents have included antiinflammatory therapeutics, bronchodilators, phosphodiesterase inhibitors, procoagulants (synthetic and herbal remedies), and application of nasal dilator strips.

Two independent investigations aimed to determine the effectiveness of aminocaproic acid for the treatment of EIPH. Both were randomized, controlled studies that used a treadmill model to induce high-intensity exercise. These

investigations failed to identify the difference between aminocaproic acid administration and saline placebo administration 2 to 4 hours before high-intensity exercise. No difference in BALF erythrocyte number was identified between treatment groups. These were low-powered investigations based on sample size and methods applied for outcome assessment.[516,517] There is insufficient evidence to support the recommendation for the use of aminocaproic acid in horses with EIPH.[474] Alternate agents that have been considered for the treatment of EIPH include bronchodilators for the potential of subclinical bronchoconstriction that may further reduce negative alveolar pressure. Clenbuterol administered independently (intravenously) or in combination with furosemide failed to influence hemodynamics among study subjects.[508,518] Additionally, clenbuterol was investigated for the potential beneficial effect of enhancing pulmonary clearance of red blood cell debris following experimental inoculation, but no benefit was observed in clenbuterol-treated horses.[519] Atropine and ipratropium have also been investigated for their potential benefit in EIPH patients, but no clear benefit has been identified.[520] Based on these investigations without any demonstrated benefit, bronchodilators are not recommended for the treatment of EIPH-affected horses. Among the potential treatments that may provide benefit, intuitively corticosteroids have been reasonably considered for the therapeutic benefit of reduced potential inflammation and subsequent remodeling that may contribute to the development of lower airway stiffness. Limited numbers of studies have been undertaken to test dexamethasone, beclomethasone, or oral prednisolone, but among the outcomes examined there was no evidence of benefit or improvement of EIPH severity.[519,521] Nonsteroidal agents have similarly been investigated in small-scale treadmill investigations; neither phenylbutazone with furosemide nor flunixin meglumine has demonstrated any improvement with the presence of blood on endoscopic examination.[514,522] Vasodilator therapy and modification of erythrocyte rheologic properties have been postulated to potentially influence the severity of EIPH, resulting in two treadmill investigations testing the efficacy of pentoxifylline as a potential treatment strategy for EIPH. When administered alone or in combination with furosemide there was no evidence of change in pulmonary hemodynamics.[511,523] Using nasal dilator strips for the prevention of pulmonary hemorrhage has been tested in several investigations. One treadmill study found that there was no benefit from using nasal dilator strips on the amount of blood in the airways following exercise. Four additional investigations with a limited number of horses revealed that there was a reduction in the number of BALF erythrocytes following exercise with the application of nasal strips.[524-527] These data show low-quality evidence to support the efficacy of nasal dilator strips for the prevention of EIPH.[474]

TUMORS OF THE RESPIRATORY SYSTEM

Equine patients suffering from pulmonary neoplasia present with nonspecific signs that may not clearly indicate the primary disease process. Careful history and physical examination combined with thorough diagnostic assessment will enable the clinician to establish the diagnosis of pulmonary neoplasia.

Primary Thoracic Neoplasia

Primary lung tumors are less common than metastatic pulmonary neoplasms and constitute less than 10% of all pulmonary tumors.[528] Granular cell tumor is the most frequently reported primary pulmonary tumor of horses.[529] Despite being the most common type, only approximately 30 cases have been reported in the literature.[530-537] These tumors have been described as myoblastomas and appear to originate from Schwann cells.[538] They occur as single or multiple masses adjacent to the bronchi and bronchioles and are locally invasive with no reports of metastasis. The mass typically extends into a large-caliber airway, resulting in partial or complete occlusion of the lumen. Although some authors suggest an increased frequency for the right hemithorax, there is no apparent predisposition to right-sided or left-sided lesions.[537] Bilateral disease is uncommon and occurs in less than 20% of the case reports. There is no breed predilection, yet mares more commonly develop these tumors. Despite their size and propensity to occlude large airways, granular cell tumors may result in minimal clinical signs and represent an incidental necropsy finding in some horses.

In clinically affected horses, the presenting complaints are chronic cough, exercise intolerance, tachypnea, and weight loss. Horses with advanced disease demonstrate an increased effort of respiration during expiration. Depending on tumor size, auscultation may reveal decreased breath sounds over one hemithorax. Based on age (mean age at onset = 13 years old) and clinical signs, horses with granular cell tumor are often assigned a presumptive diagnosis of recurrent airway disease (heaves).[537] Failure to respond to appropriate therapy results in further diagnostic testing. Focal pneumonia may develop in the pulmonary parenchyma distal to the mass because of poor clearance of respiratory secretions and inhaled particulate material. Horses with focal pneumonia present with fever, depression, abnormal lung sounds, and leukocytosis, in addition to coughing. There are no reports of pleural effusion, epistaxis, or mediastinal lymphadenopathy in horses with granular cell tumor. Hypertrophic osteopathy (HO) has been reported as a paraneoplastic complication of granular cell tumor in some horses.[535,539-541]

Routine blood work provides little evidence to advance the diagnosis of granular cell tumor. Thoracic radiography identifies a single, large mass or multiple pulmonary masses near or caudal to the hilus.[529] Evaluation of magnification in left and right radiographic projections can identify the hemithorax affected by the tumor. Rarely, the tumor cannot be visualized via thoracic radiography. In some cases, the mass may be obscured by focal pneumonic lung. Focal pneumonia in an atypical pulmonary location (i.e., central or dorsal lung fields) should trigger a suspicion of tumor or inhaled foreign body. Using a tracheobronchial map of the lung,[542] the approximate location of the tumor in the pulmonary tree can be determined for endoscopic examination.

There is no report of visualization of the tumor mass via ultrasonographic examination; however, decreased movement of the pleural surface over the affected hemithorax has been reported in horses with large granular cell masses.[537]

Gross assessment of granular cell tumors reveals them to be smooth pink to white in color and occludes or nearly occludes a large-caliber airway. A mainstem bronchus is the most commonly affected airway.[543] Biopsy confirmation is difficult and may not be necessary because the endoscopic appearance of the tumor is distinctive. The external surface of the mass consists of normal respiratory epithelium; therefore, a

biopsy sample obtained via endoscopy may be non-diagnostic because of insufficient size and depth.[537,544] To obtain a larger tissue sample, with a greater chance of achieving a diagnosis, a biopsy instrument (such as uterine biopsy forceps) can be passed through a tracheotomy incision at the level of the thoracic inlet.[544]

On histopathological examination, neoplastic cells are benign and appear rounded to polyhedral with hyperchromatic nuclei, numerous eosinophilic cytoplasmic granules, and indistinct cytoplasmic margins.[537,543] Histochemical and immunohistochemical staining results of these tumors are well described and suggest they are composed primarily of neural crest cells, which are likely myelinating Schwann cells.[538] Morphologic features of the equine pulmonary granular cell tumors are similar to those of endobronchial granular cell tumors of humans.[538,543] In other species (dogs, cats, and humans), granular cell tumors are reported to occur at many other sites along with the thoracic cavity, including the oral cavity and CNS.

Granular cell tumors are presumably slow growing and may be an incidental finding at postmortem examinations. Conservative treatment may produce an acceptable clinical outcome for years in horses with stable clinical signs.[537] The presenting complaint for the horse is indicative of focal pneumonia and includes cough, fever, and depression. The granular cell tumor obscured a third-generation bronchus and compressed only a small portion of pulmonary parenchyma. The mare was treated with a 2-week course of broad-spectrum antibiotics and managed for several years with minimal clinical signs. Ohnesorge et al.[545] removed the intraluminal portion of a granular cell tumor mass via transendoscopic electrosurgery. The remaining tumor surface was irradiated using an Nd-YAG laser to coagulate and kill residual tumor cells. In most cases, the tumor observed in the airway represents only a small proportion of the total tumor mass. Horses with large tumor masses require more aggressive therapy. Facemire et al.[544] removed the entire right lung, which was affected by multiple large masses. In the reports by Ohnesorge et al.[545] and Facemire et al.,[544] there was no tumor recurrence after a 2-year period of follow-up.

Necropsy examination reveals a single large mass or, more commonly, a large mass with multiple small nodular masses, which compress the surrounding parenchyma and invade the lumen of a large airway. There are no reports of metastasis to other organs, primary neoplasia originating from other sites, metastasis to regional lymph nodes, or invasion of nonpulmonary tissue.[529]

Thymic Tumors

Thymic tumors are classified as benign or metastatic, based on evidence of tissue invasiveness, even though they uniformly appear benign histologically. These tumors are derived from epithelial reticular cells of the thymus. Tumor classification has historically included those with a lymphocytic component, epithelial, or mixed composition. More recently, the World Health Organization (WHO) has provided classification recommendations to include A, AB, B1, B2, and B3.[546] The equine case report that used this classification scheme describes a type A tumor characterized by the proliferation of spindle cells without nuclear atypia that contain no more than a few nonneoplastic lymphocytes and inconspicuous nuclei. [547] Thymic tumors are rare in horses. In an original report of two cases, they were described as incidental postmortem findings without evidence of metastasis.[548]

Malignant thymoma has rarely been reported to affect horses.[499,547,549] A mediastinal mass, associated lymphadenopathy, and marked pericardial involvement was reported in one case.[499] In addition to cranial thoracic disease, the affected Percheron mare had evidence of pulmonary lymph node involvement, extensive parenchymal pulmonary disease, and abdominal and retroperitoneal lesions. A separate report described a mixed-breed mare affected by a squamous cell thymoma that demonstrated a markedly more aggressive nature.[549] This latter report described the lesions to extend from the intermandibular space to the thoracic inlet. Postmortem examination revealed the mass to involve the mediastinum and pericardial sac. Pulmonary nodules were diffusely present and confirmed on histopathology.

A recent report describes an 18-year-old Tennessee Walking Horse that suffered from acute death during a trail ride.[547] Postmortem examination revealed that the pleural cavity and pericardial sac contained a combined volume of 300 to 500 mL of serosanguineous fluid. A large mass was found to occupy the cranial thoracic cavity and was adherent to the thoracic wall. The architecture of the right atrium was significantly distorted, which also involved the tricuspid valve. Histopathological evaluation of the mass revealed few characteristics of neoplasia consistent with previous reports of thymic tumors in horses. This tumor was classified in accordance with the WHO classification system as a type A thymoma with proliferation of spindle-shaped cells with oval to elongated nuclei, lack of nuclear atypia, and a lack of neoplastic lymphocytes with inconspicuous nuclei. A predominant feature of this neoplasm and the findings in this reported case is the lack of a neoplastic nature identified on histopathology, yet there was aggressive behavior of the tumor in the host. In the reported case, cardiac invasion was attributed to lymphatic drainage or direct local invasion, yet hematogenous spread was also considered a possibility.[547]

Other Tumor Types

Additional primary thoracic neoplasms originate from various pulmonary tissues and are primarily reported as single case reports: pulmonary and bronchial carcinoma and adenocarcinoma,[550-554] bronchogenic squamous cell carcinoma,[555] bronchial myxoma,[556] pulmonary chondrosarcoma,[557] pulmonary leiomyosarcoma,[558] and pleuropulmonary blastoma.[559]

Clinical signs of primary pulmonary neoplasms are dependent on the tumor type and location. Chronic cough, weight loss, anorexia, fever, and respiratory difficulty are common clinical findings in horses with pulmonary neoplasia regardless of the tissue of origin. Ventral edema, pleural effusion, and epistaxis are not unusual in horses with pulmonary neoplasia. Most case reports involve aged horses (>12 years of age), although pleuropulmonary blastoma has been reported in a neonate and a young adult horse.[559] Similar to humans, this tumor is characterized by mixed epithelial and mesenchymal elements and aggressive malignancy. The clinical presentation of pulmonary leiomyosarcoma is similar to granular cell tumor, with the exception of the presence of epistaxis.[558]

Mesothelioma is a rare primary pleural tumor arising from the mesothelium of the pleura, pericardium, and peritoneum. In humans, this malignancy is associated with asbestos exposure. The clinical presentation in horses includes weight loss, respiratory difficulty, and large-volume pleural effusion.

Differentiation of neoplastic mesothelial cells from reactive mesothelial cells can be challenging based on cytologic examination of pleural effusion. Ultrasonographically, the tumor is observed as multiple small nodules on a thick serosal surface, and pleural biopsy is diagnostic. There is no treatment and the prognosis is grave.[560-567]

Most cases of primary thoracic neoplasia have an extended history of cough and nonspecific signs of weight loss and anorexia.[529] The most common first-opinion diagnosis in horses with pulmonary neoplasia is heaves, followed by low-grade pneumonia and pleuropneumonia. In some cases, the definitive diagnosis is not identified for months to years. With the exception of granular cell tumor, there are limited options for therapeutic intervention, and the prognosis is grave at the time of diagnosis.[528]

Metastatic Pulmonary Neoplasia

Equine lymphoma is the most common hematopoietic neoplasm in horses and can present with a variety of the clinical signs that have been previously described.[554,568-573] Classification involves four main manifestations of lesions: mediastinal, multicentric, alimentary, and cutaneous. When present in the thoracic cavity, this manifestation of lymphoma is not considered a true primary neoplasm because it originates from an extrathoracic site.[554] Lymphoma is usually a disease of adult horses without a specific breed or gender predilection.[572]

Clinical signs of lymphoma typically relate to the primary organ system involvement. Common clinical features of the disease include chronic weight loss, lethargy, anorexia, subcutaneous edema, lymphadenopathy, colic, bleeding tendency, and diarrhea.[574-576] Although the disease has an insidious progression, it is not uncommon for cases to present with a relatively acute change or deterioration in condition. It is likely that the time of presentation relates to a stage of disease in which the clinical manifestation has become pronounced.

Ventral edema, particularly when in the pectoral region, is a common clinical finding in association with lymphoma, resulting from lymphatic obstruction. Coughing and labored respiratory effort are often apparent in individuals suffering from mediastinal masses. In such instances, pleural effusion may result in severe pulmonary atelectasis, and pulmonary function is significantly compromised.

Hematologic and serum biochemical testing are nonspecific regarding the provision of diagnostic evidence for the presence of thoracic lymphoma. Thoracic radiography may identify a pleural fluid line, and in some instances it reveals the presence of a mediastinal mass. Thoracic ultrasonography provides specific evidence regarding the presence, depth, and character of pleural fluid. Thoracocentesis is an important diagnostic procedure that will provide the clinician with evidence of the nature of fluid. Cytologic examination provides evidence of lymphoma, when the tumor is exfoliative. In some instances concurrent sepsis may be identified. Therefore, careful examination of lymphocyte morphology should be performed. When overlapping conditions exist, such as sepsis with mediastinal lymphoma, a lack of response to appropriate medical therapy should alert the clinician to the potential for complicating factors rather than a primary pleuropneumonia. Thoracoscopy may be particularly helpful in these cases. When enlarged peripheral lymph nodes are present, biopsy is strongly recommended to aid in diagnostic confirmation of lymphoma. Fine-needle aspirates typically do not provide diagnostic information regarding lymph node architecture and are difficult to cytologically differentiate from reactive lymph nodes.

Peripheral lymphadenopathy is an uncommon clinical feature of disease,[577] yet when lymphadenopathy develops, the prescapular or submandibular nodes are usually enlarged. Postmortem examination typically reveals conclusive evidence of lymphoid pathology. When examining a patient with suspected lymphoma, rectal palpation is an important component of the physical examination to determine whether abdominal lymphadenopathy is present. A recent report of lymphoma described an 18-year-old mare with a presenting complaint of severe pruritus, alopecia, pyrexia, mammary gland enlargement, and lymphadenopathy.[578] This mare was determined to be suffering from primary T-cell lymphoma that primarily affected the mammary gland. Hematologic dyscrasias that may be present in lymphoma cases include hyperglobulinemia, hypercalcemia, and anemia. Rarely leukemia may be identified, which typically represents bone marrow involvement. The mare of the previously mentioned report suffered from pyrexia and anemia, which resulted from paraneoplastic complications. Pruritus has been previously described to occur as a paraneoplastic condition in horses[579] and has been identified in approximately 25% of human patients suffering from Hodgkin's lymphoma.

A recent report described a mare with significant submandibular lymphadenopathy and respiratory distress that was later diagnosed with lymphomatoid granulomatosis.[580] Although the mare had clinical findings to suggest a more typical case of lymphoma, this mare had evidence of marked lymphocytosis on hematologic examination. Morphologic evaluation of these cells revealed that approximately 98% of the cells were classified as atypical T cells. Additional clinical findings included anemia and thrombocytopenia. On postmortem examination unencapsulated nodules were scattered throughout all the lung lobes, without obvious involvement of other organs. However, microscopic examination of the skin revealed perivascular and mural infiltrates of neoplastic mononuclear cells closely associated with an affinity for vascular elements. Neoplastic cells were characterized to have oval or cleaved nuclei with characteristics of neoplasia represented by multiple nucleoli and evidence of mitosis among cells. Lymphomatoid granulomatosis is a rare form of lymphoma that has been previously identified in humans,[581] dogs,[582-586] and cats.[587] The findings in this mare were supportive of this diagnosis for the first time in a horse. Interestingly, her lesions involved primarily the submandibular lymph nodes, pulmonary tissue, and skin. The leukemic nature of disease in this horse was believed to represent a consequence of the angioinvasive progression of the disease. This differential consideration should be considered for clinical disease that presents in a similar fashion with clinical and histopathologic findings to support this diagnosis.

Classification of tumor type has become a routine component of the clinical evaluation of lymphoma. Specifically, diagnostic modalities used for tumor classification include immunophenotypic examination with flow cytometry and immunohistochemical staining on tissue samples. Immunophenotypic evaluation of pleural fluid can be performed to determine lymphocyte surface marker expression, which can aid with the characteristic nature of neoplasia. Flow cytometry of fluid effusions is analyzed for surface expression of MHC II, CD4, CD5, CD8α, or CD8/α/β.[588] When tissue samples

are available, immunohistochemistry is used to establish cell-surface expression, which provides criteria for tumor classification.[577,589] Cellular staining provides diagnostic evidence for the distinction between T- and B-cell lineage neoplasms. Standard staining protocols involve identification of CD3 for T-cell lesions and CD20, CD21, and/or CD79α for B-lymphocyte surface marker. Several veterinary laboratories offer diagnostic services that can aid in the specific identification of tumor type. Examination of DNA ploidy may aid in characterization of neoplastic cell populations in some clinical investigations.[403,590]

Classification of equine lymphoma has been hindered by the documentation of relatively few cases. An original report examined 31 horses with diagnostic confirmation of lymphoma. Among these cases, 24 (77%) horses had lymphoma derived from B lymphocytes with an infiltration of nonneoplastic T lymphocytes and is termed *T-cell–rich B-cell lymphoma* (TCRBCL).[589] This investigation concluded that not all equine lymphomas can be classified; however, among equine tumors that can be classified, there is an apparent tendency toward TCRBCL.

In contrast, a more recent investigation that used immunophenotyping classified 37 cases of equine lymphoma.[577] Among all tumors, 34 (91%) of the neoplasms involved multiple lymphoid tissues in addition to abdominal or thoracic organs. Twenty-six (70%) of the cases were identified to be of T-cell origin, 7 cases B-cell origin, and 4 cases were not able to be classified. Immunophenotyping was performed on effusions and was found to be consistent with the immunohistochemical findings in six tumors.[577] The investigation of this population concluded that most horses had large T-cell tumors, a concurrent inflammatory response was common, and many horses had mediastinal masses. This population of affected horses was uncharacteristically young, less than 5 years old. Anemia was the most common cytopenia and was frequently associated with agglutination and hyperglobulinemia. Thrombocytopenia and neutropenia were identified in association with myelophthisis.[577] Although equine lymphoma is relatively uncommon, an effort to establish a diagnosis early in the course of disease would be expected to enhance the chance for a favorable case outcome should chemotherapy be a therapeutic option.

Postmortem examination typically reveals large-volume pleural effusion and associated ventral pulmonary atelectasis. Cranial mediastinal masses are commonly present and are often composed of coalescing enlarged lymph nodes. Such masses may occlude the thoracic inlet contributing to obstruction of blood flow and lymphatic drainage. Regional and local lymph nodes are commonly enlarged. In some instances, the pulmonary parenchyma may be infiltrated with neoplastic lesions as well as other organs that may be involved including liver, kidney, spleen, and potentially gastrointestinal tract.[554,591]

Hemangiosarcoma appears to be the second most common metastatic thoracic neoplasm in horses. In a series of 35 cases, pulmonary parenchyma and pleura were involved in 77% of the horses.[592] The age distribution of affected horses is clustered around middle-aged adults (mean age 12 years, range 3–27 years). Pulmonary hemangiosarcoma is not unusual in 6- to 7-year-old horses.[593-595] Disseminated hemangiosarcoma is aggressive and rapidly progressive. Most horses present in good body condition with an abbreviated history of anorexia and depression. The clinical presentation often includes tachypnea; pale or icteric mucous membranes; respiratory distress; epistaxis; and subcutaneous, cutaneous, or intramuscular masses.[596] Anemia, thrombocytopenia, and neutrophilia are the most common abnormalities on routine blood work.[592] A large volume of hemorrhagic pleural effusion is present in approximately 20% of the cases, accompanied by ventral edema and marked respiratory distress. Thoracocentesis provides relief for horses with large-volume pleural effusion. Pleural fluid is typically serosanguineous in color and may be characterized as hemothorax. Cytologic examination may or may not reveal neoplastic cells. Trauma is the most common first-opinion diagnosis in horses with hemothorax caused by hemangiosarcoma.[592]

Antemortem diagnosis is uncommon[592] but has been achieved via cytologic evaluation of pleural fluid and pleuroscopic-guided biopsy.[595] Biopsy of a hemangiosarcomatous mass can result in further hemorrhage. Plans to manage hemostasis should be considered before completing a biopsy in cases of suspected hemangiosarcoma. At postmortem examination, neoplastic tissue is widely distributed to many tissues including the heart, spleen, kidney, skeletal muscle, and CNS. The spleen is the most common organ of origin, although many other tissues have been reported to be the site of primary tumor formation. Occasionally, the thoracic cavity is considered the primary tumor with disseminated metastasis to distant sites.

Disseminated hemangiosarcoma should be differentiated from focal hemangiosarcoma of the distal limb in horses less than 3 years of age.[597] These tumors do not demonstrate the same aggressive biological activity and are unlikely to metastasize at the same rate. Surgical resection can be curative, and, in some cases, the tumor may resolve spontaneously.

Other tumor types that metastasize to the thoracic cavity include adenocarcinoma,[598,599] squamous cell carcinoma,[600] fibrosarcoma,[601] metastatic melanoma,[602,603] mastocytoma,[604] or undifferentiated sarcoma.[528,554] The clinical features of these tumors are generally nonspecific and often relate more to the primary site of tumor formation. Some metastatic tumors will produce neoplastic effusion and/or damage to intrathoracic structures.[601,602] Cytologic evaluation may or may not identify neoplastic cells. Thoracoscopy is an important diagnostic tool to obtain a tissue sample to confirm neoplasia and identify the tissue type.[605,606]

Paraneoplastic Disease

Paraneoplastic conditions develop in association with neoplasia yet are often unrelated to the primary site of tumor development. Recent reviews will provide the reader with a comprehensive understanding of this condition and specific manifestations in equine patients.[607]

Fever is a common paraneoplastic disorder observed in association with equine lymphoma resulting from cytokine production by the tumor. IL-1, IL-6, and TNF are pyrogens that are believed to increase PGE production by hypothalamic endothelial cells. When pyrexia is identified in combination with anemia, the differential list must include potential infectious etiologies such as equine infectious anemia and piroplasmosis. Immune-mediated disorders should also be considered as a primary disease or manifestation of paraneoplastic disease that may also involve intermittent or persistent fevers.

Pruritus has been clearly demonstrated to develop as a paraneoplastic condition associated with equine lymphoma.[579] Altered T-cell function is believed to result in modified cytokine synthesis and secretion. Additional etiologies for pruritus

include nerve entrapment or compression, tumor growth, and hepatic involvement leading to bile duct obstruction.[607]

Anemia may result from immune-mediated mechanisms or reduction of bone marrow production, such as with myelophthisis. Anemia of chronic disease is well recognized and occurs secondary to a variety of inflammatory conditions. Changes in bone marrow function occur secondary to altered cytokine synthesis. Cytokines that have been implicated in this mechanism include transforming growth factor B, IL-11, IL-6, and interferon gamma, which upregulate hepcidin synthesis. Hepcidin will antagonize gastrointestinal iron uptake and utilization. In addition, erythropoietin antagonism results from TNF affecting bone marrow production and function.[607]

Hypercalcemia (calcium >14 mg/dL) may result from a paraneoplastic syndrome, although the clinician should consider other differential possibilities as well such as chronic renal failure, iatrogenic hypervitaminosis D, consumption of wild or day-blooming jasmine *(Estrum diurnum)* particularly in the southeastern United States, rapid administration of calcium-containing solutions, laboratory error, and hyperparathyroidism.

The mechanisms of hypercalcemia occurring in patients suffering from neoplastic disease may include lytic bone metastases, malignant hyperparathyroidism, ectopic tumor production of parathyroid hormone-like hormone (PTHLH, PTHLP), tumor-produced PGEs (PGE_1 and PGE_2), and tumor-produced osteoclast activating factor.[608-610] The cDNA sequence for equine PTHLP has been reported (Accession NP_001157453). Although this protein has been clearly defined to result in hypercalcemia associated with canine tumors such as with lymphoma and anal sac adenocarcinoma,[611] further investigations are required to more clearly define the relationship of PTHLH in the horse. Reported cases of hypercalcemia in equine patients suffering from neoplasia are somewhat limited to include disseminated lymphoma and lymphoid leukemia.[579,612,613]

Hypertrophic osteopathy (aka Marie's disease) has been reported in domestic species and is most commonly associated with primary and metastatic pulmonary tumors.[614-616] The pathogenesis of HO remains incompletely understood. The primary syndrome involves periosteal proliferation on the cortices of long bones. The condition is classically described to occur secondary to an intrathoracic mass yet may develop secondary to extrathoracic lesions as well. Although HO is more common in humans and dogs, it also occurs in horses. In horses, it has been reported in association with a variety of pulmonary conditions including infection, neoplasia, and trauma.[540,617] Thoracic neoplasia is an uncommon disease in horses, with HO being rarely reported in affected individuals. This finding is in contrast to the findings in humans in which up to 10% of patients with thoracic neoplasia develop HO, often characterized by clubbing of the fingers, dermal changes, limb swelling, and arthropathy.[616] In canine patients suffering from HO, the most common predisposing factor is consistently pulmonary neoplasia.[614] The presence of HO in an equine patient should alert the clinician to be aware of the potential for intrathoracic disease.

Although HO is a relatively uncommon condition in horses, it has been described to occur in association with pulmonary and extrapulmonary disease. Previous retrospective reports that described HO in horses include a total of 42 cases.[540,617,618] These reports are divided among individual case reports that date back to 1944 and a more recent retrospective that describes the clinical features of 24 cases.[540] In this report, 71% of the horses were ultimately diagnosed with pulmonary disease. Among those with pulmonary lesions, 40% were neoplastic in origin. In cases with a treatable pulmonary condition, resolution of HO occurred following clearance of the primary pulmonary disease. Limb swellings were present on all four limbs and typically occurred in a bilaterally symmetric fashion. In some instances, the limbs were cool and comfortable, whereas in others they were warm and sensitive to palpation. Head lesions were present in two of the cases that involved the mandible and maxilla.[540] Stiffness and pain were commonly reported in affected individuals. When intrathoracic disease was present, clinical signs also included cough and dyspnea. The radiographic findings associated with HO included periosteal new bone formation in a palisade fashion. Similar to findings in humans and canine patients, in those individuals with a treatable condition, clinical signs resolved on resolution of the primary disorder. Interestingly, in this report three cases were not identified to have a primary disorder, yet clinical signs resolved following symptomatic therapy with phenylbutazone. In humans, indomethacin is an NSAID that has been reported to provide clinical benefit when used as the primary therapy for the management of HO.[619]

Other Considerations

Differentiating a thoracic tumor from a number of infectious conditions of the thorax can be surprisingly difficult. Middle-aged and aged horses with solid pulmonary tumors are often mistaken as affected with heaves. Hydatid cysts and fungal pneumonia are less common conditions that may mimic signs of thoracic neoplasia.

Equine cases of hydatid cyst may present with a large volume of pleural effusion. In Europe, hydatidosis *(Echinococcus equinus)* is generally well tolerated in horses, and cysts in the liver and lung may be an incidental finding at postmortem examination.[19] In the United States *Echinococcus* spp. and an unidentifiable aberrant, acephalic metacestode have been identified in the liver and lungs of horses. Occasionally the cyst will rupture, resulting in clinical respiratory difficulty caused by large-volume pleural effusion. In the United States, this clinical syndrome has been associated with the acephalic metacestode.

Affected horses may have intermittent fever, depression, rapid shallow respiration, pectoral edema, and nonspecific laboratory findings indicative of inflammation.[19] A large volume of pleural effusion is a consistent finding in horses with pulmonary and pleural metacestode infection. The effusion has low to moderate cellularity (5000–80,000 cells/μL), 20% to 80% neutrophils, and markedly increased protein concentration (5.0–8.0 g/dL) and may be difficult to differentiate from neoplastic effusion.[529] Bacterial and fungal culture of the pleural fluid is negative. Ultrasound examination may reveal a large fluid-filled cyst within the pulmonary parenchyma, on the surface of the diaphragm, and/or within the hepatic parenchyma. Metacestodes may be attached to a thickened pleural surface, or hypoechoic cysts (1 × 4 mm) may be seen floating within the pleural or peritoneal fluid.

Treatment of a horse with an unidentified aberrant, acephalic metacestode utilized albendazole (10 mg/kg, PO, s.i.d. × 30 days), thoracic drainage, and surgical debridement of the pleura and cyst (10 × 10 × 17) on the surface of the diaphragm. Disruption of a cyst by centesis or surgery may result in an anaphylactic reaction or seeding of daughter metacestodes

within the thoracic cavity. Surgical intervention was performed in this case after 2 weeks of antiparasitic therapy. The horse was asymptomatic 6 weeks after treatment and returned to athletic performance for several years.

REFERENCES

1. Rossdale PD, Hopes R, Digby NJ, et al. Epidemiological study of wastage among racehorses 1982 and 1983. *Vet Rec*. 1985;116: 66–69.
2. Perkins NR, Reid SWJ, Morris RS. Profiling the New Zealand Thoroughbred racing industry. 2. Conditions interfering with training and racing. *New Zeal Vet J*. 2005;53:69–76.
3. Long MT. Mechanisms of Infectious Disease. In: Reed SM BW, Sellon DC, eds. *Equine Internal Medicine*. 2nd ed. St. Louis: Saunders; 2004:59–74.
4. Bailey GD, Love DN. Oral associated bacterial infection in horses: studies on the normal anaerobic flora from the pharyngeal tonsillar surface and its association with lower respiratory tract and paraoral infections. *Vet Microbiol*. 1991;26:367–379.
5. Raidal SL, Love DN, Bailey GD. Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 hours of confinement with the head elevated. *Aust Vet J*. 1995;72:45–50.
6. Raidal SL, Love DN, Bailey GD. Effect of a single bout of high intensity exercise on lower respiratory tract contamination in the horse. *Aust Vet J*. 1997;75:293–295.
7. Raidal SL, Love DN, Bailey GD. Effects of posture and accumulated airway secretions on tracheal mucociliary transport in the horse. *Aust Vet J*. 1996;73:45–49.
8. Jones AL, Sutcliffe IC, Goodfellow M. Prescottia equi gen. nov., comb. Nov.: a new home for an old pathogen Antonie Van Leeuwenhoek. *Int J Gen Microbiol Biotechnol*. 2013;103:655–671.
9. Kotlikoff MI, Gillespie JR. Lung sounds in veterinary medicine part II. Deriving clinical information from lung sounds. *Compend Contin Educ Pract Vet*. 1984;6:462.
10. Forgacs P. *Lung Sounds*. London: Bailliere Tindall; 1978.
11. Bakos Z, Voros K. Thoracic percussion to determine the caudal lung border in healthy horses. *J Vet Intern Med*. 2007;21: 504–507.
12. Allen KJ, Franklin SH. Comparisons of overground endoscopy and treadmill endoscopy in UK Thoroughbred racehorses. *Equine Vet J*. 2010;42:186–191.
13. Franklin SH, Naylor JR, Lane JG. Videoendoscopic evaluation of the upper respiratory tract in 93 sport horses during exercise testing on a high-speed treadmill. *Equine Vet J Suppl*. 2006: 540–545.
14. Morrow KL, Park RD, Spurgeon TL, et al. Computed tomographic imaging of the equine head. *Vet Radiol Ultrasound*. 2000;41:491–497.
15. Kraft SL, Gavin PR. Physical principles and technical considerations for equine computed tomography and magnetic resonance imaging of equine musculoskeletal conditions. *Vet Clin North Am Equine Pract*. 2001;17:115–121.
16. Barakzai SZ, Barnett TP. Computed tomography and scintigraphy for evaluation of dental disease in the horse. *Equine Vet Educ*. 2015;27:323–331.
17. Manso-Diaz G, Dyson SJ, Dennis R, et al. Magnetic resonance imaging characteristics of equine head disorders: 84 cases (2000-2013). *Vet Radiol Ultrasound*. 2015;56:176–187.
18. Koblinger K, Hecker K, Nicol J, et al. Bronchial collapse during bronchoalveolar lavage in horses is an indicator of lung inflammation. *Equine Vet J*. 2014;46:50–55.
19. Koblinger K, Nicol J, McDonald K, et al. Endoscopic assessment of airway inflammation in horses. *J Vet Intern Med*. 2011;25:1118–1126.
20. Blutke A, Hamel D, Huttner M, et al. Cystic echinococcosis due to Echinococcus equinus in a horse from southern Germany. *J Vet Diagn Invest*. 2010;22:458–462.
21. Perricone G, Mazzarelli C. Images in clinical medicine. Reexpansion pulmonary edema after thoracentesis. *N Engl J Med*. 2014;370:e19.
22. Tomlinson JE, Reef VB, Boston RC, et al. The Association of Fibrinous Pleural Effusion with Survival and Complications in Horses with Pleuropneumonia (2002-2012): 74 Cases. *J Vet Intern Med*. 2015;29:1410–1417.
23. Reef VB. Advances in diagnostic ultrasonography. *Vet Clin North Am Equine Pract*. 1991;7:451–466.
24. Reef VB, Whittier M, Allam LG. Thoracic ultrasonography. *Clin Tech Equine Pract*. 2004;3:284–293.
25. Reef VB. *Thoracic ultrasonography. Equine Diagnostic Ultrasound*. Philadelphia: W. B. Saunders; 1998:187–214.
26. Reef VB. Thoracic ultrasonography: noncardiac imaging. In: Reef VB, ed. *Equine Diagnostic Ultrasound*. Philadelphia: W. B. Saunders; 1998:187–214.
27. Dunkel B, Gibbs C, Weller R. A fresh approach to equine thoracic radiography. In *Practice*. 2013;35:589–596.
28. Robinson NE. Tests of equine airway function. in *Proc Am Coll Vet Intern Med Forum*. 1992:284.
29. Robinson NE. The physiologic basis of pulmonary function tests. *Proc Am Coll Vet Intern Med*. 1992:403.
30. Young SS, Hall LW. A rapid, non-invasive method for measuring total respiratory impedance in the horse. *Equine Vet J*. 1989;21:99–105.
31. van EE, Votion D, Art T, et al. Measurement of respiratory function by impulse oscillometry in horses. *Equine Vet J*. 2004;36:21–28.
32. Hare JE, Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. *J Vet Intern Med*. 1998;12:163–170.
33. Hoffman AM, Mazan MR, Ellenberg S. Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. *Am J Vet Res*. 1998;59:176–181.
34. Couetil LL, Rosenthal FS, DeNicola DB, et al. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am J Vet Res*. 2001;62:538–546.
35. Mazan MR, Hoffman AM, Manjerovic N. Comparison of forced oscillation with the conventional method for histamine bronchoprovocation testing in horses. *Am J Vet Res*. 1999;60: 174–180.
36. Art T, Anderson L, Woakes AJ. Mechanics of breathing during strenuous exercise in Thoroughbred horses. *Respir Physiol*. 1990;82:279.
37. Raphel CF, Gunson DE. Percutaneous lung biopsy in the horse. *Cornell Vet*. 1981;71:439–448.
38. Venner M, Schmidbauer S, Drommer W, et al. Percutaneous lung biopsy in the horse: comparison of two instruments and repeated biopsy in horses with induced acute interstitial pneumopathy. *J Vet Intern Med*. 2006;20:968–973.
39. Savage CJ, Traub-Dargatz JL, Mumford EL. Survey of the large animal diplomates of the American College of Veterinary Internal Medicine regarding percutaneous lung biopsy in the horse. *J Vet Intern Med*. 1998;12:456–464.
40. Pusterla N, Watson JL, Madigan JE. Technique and diagnostic value of percutaneous lung biopsy in 66 horses with diffuse pulmoanry diseases using an automated biopsy device. *Equine Vet Educ*. 2007;19:157–202.
41. Moorthy AR, Spradbrow PB. Isolation of mycoplasmas from the respiratory tract of horses in Australia. *Vet Rec*. 1976;98: 235–237.
42. Tan RM, DeFrancisco AL, Singh K. Pathology in practice. Severe chronic diffuse pyogranulomatous, necrohemorrhagic and eosinophilic rhinitis caused by Conidiobolus. *J Am Vet Med Assoc*. 2010;236:831–833.
43. Arun S, Neubauer H, Gurel A, et al. Equine glanders in Turkey. *Vet Rec*. 1999;144:255–258.

44. Malik P, Singha H, Goyal SK, et al. Incidence of Burkholderia mallei infection among indigenous equines in India. *Vet Rec Open*. 2015;2:e000129.
45. Greet T. Differential diagnosis of nasal discharge in the horse. In *Practice*. 1986;8:49–57.
46. Korenek NL, Legendre AM, Andrews FM, et al. Treatment of mycotic rhinitis with itraconazole in three horses. *J Vet Intern Med*. 1994;8:224–227.
47. Cruz VC, Sommardahl CS, Chapman EA, et al. Successful treatment of a sinonasal cryptococcal granuloma in a horse. *J Am Vet Med Assoc*. 2009;234:509–513.
48. Deegen E. The causes of rhinorrhea in horses. *Tierarztl Prax*. 1973;1:169–176.
49. Riley CB, Bolton JR, Mills JN, et al. Cryptococcosis in seven horses. *Aust Vet J*. 1992;69:135–139.
50. Ziemer EL, Pappagianis D, Madigan JE, et al. Coccidioidomycosis in horses: 15 cases (1975-1984). *J Am Vet Med Assoc*. 1992;201:910–916.
51. Tremaine WH, Dixon PM. A long-term study of 277 cases of equine sinonasal disease. Part 1: details of horses, historical, clinical and ancillary diagnostic findings. *Equine Vet J*. 2001;33:274–282.
52. Fiske-Jackson AR, Pollock PJ, Witte TH, et al. Fungal sinusitis resulting in suspected trigeminal neuropathy as a cause of headshaking in 5 horses. *Equine Vet Educ*. 2012;24:126–133.
53. Henninger W, Frame EM, Willmann M, et al. CT features of alveolitis and sinusitis in horses. *Vet Radiol Ultrasound*. 2003;44:269–276.
54. Probst A, Henninger W, Willmann M. Communications of normal nasal and paranasal cavities in computed tomography of horses. *Vet Radiol Ultrasound*. 2005;46:44–48.
55. Swor TM, Skirpstunas RT, Hines MT, et al. What is your diagnosis? Soft tissue opacification of the right maxillary sinus and lysis of the maxillary bone. *J Am Vet Med Assoc*. 2001;218:347–348.
56. Freeman DE. Sinus disease. *Vet Clin North Am Equine Pract*. 2003;19:209–243. viii.
57. Davis PR, Meyer GA, Hanson RR, et al. Pseudallescheria boydii infection of the nasal cavity of a horse. *J Am Vet Med Assoc*. 2000;217:707–709. 674.
58. Dixon PM, Parkin TD, Collins N, et al. Equine paranasal sinus disease: a long-term study of 200 cases (1997-2009): ancillary diagnostic findings and involvement of the various sinus compartments. *Equine Vet J*. 2012;44:267–271.
59. Tessier C, Bruhschwein A, Lang J, et al. Magnetic resonance imaging features of sinonasal disorders in horses. *Vet Radiol Ultrasound*. 2013;54:54–60.
60. Perkins JD, Windley Z, Dixon PM, et al. Sinoscopic treatment of rostral maxillary and ventral conchal sinusitis in 60 horses. *Vet Surg*. 2009;38:613–619.
61. Dixon PM, Parkin TD, Collins N, et al. Historical and clinical features of 200 cases of equine sinus disease. *Vet Rec*. 2011;169:439.
62. Sullivan M, Burrell MH, McCandlish IA. Progressive haematoma of the maxillary sinus in a horse. *Vet Rec*. 1984;114:191–192.
63. Laing JA, Hutchins DR. Progressive ethmoidal haematoma in horses. *Aust Vet J*. 1992;69:57–58.
64. Greet TR. Outcome of treatment in 23 horses with progressive ethmoidal haematoma. *Equine Vet J*. 1992;24:468–471.
65. Tremaine WH, Dixon PM. A long-term study of 277 cases of equine sinonasal disease. Part 2: treatments and results of treatments. *Equine Vet J*. 2001;33:283–289.
66. Dixon PM, Parkin TD, Collins N, et al. Equine paranasal sinus disease: a long-term study of 200 cases (1997-2009): ancillary diagnostic findings and involvement of the various sinus compartments. *Equine Vet J*. 2012;44:267–271.
67. Tucker R, Windley ZE, Abernethy AD, et al. Radiographic, computed tomographic and surgical anatomy of the equine sphenopalatine sinus in normal and diseased horses. *Equine Vet J*. 2016;48:578–584.
68. Marriott MR, Dart AJ, Hodgson DR. Treatment of progressive ethmoidal haematoma using intralesional injections of formalin in three horses. *Aust Vet J*. 1999;77:371–373.

68a Blazyczek I, Hamann H, Ohnesorge B, et al. Population genetic analysis of the heritability of gutteral pouch tympany in Arabian purebred foals. *Dtsch Tierarztl Wochenschr*. 2003;110:417–419.

68b Dixon PM, Rowlands AC. Atlanto-occipital joint infection associated with guttural pouch mycosis in a horse. *Equine Vet J*. 1981;13:260–262.

69. Frees KE, Gaughan EM, Lillich JD, et al. Severe complication after administration of formalin for treatment of progressive ethmoidal hematoma in a horse. *J Am Vet Med Assoc*. 2001;219:950–952. 939.
70. Specht TE, Colahan PT, Nixon AJ, et al. Ethmoidal hematoma in nine horses. *J Am Vet Med Assoc*. 1990;197:613–616.
71. Hanselka DV, Young MF. Ethmoidal hematoma in the horse. *Vet Med Small Anim Clin*. 1975;70:1289–1291.
72. Schumacher J, Yarbrough T, Pascoe J, et al. Transendoscopic chemical ablation of progressive ethmoidal hematomas in standing horses. *Vet Surg*. 1998;27:175–181.
73. Freeman DE. Update on disorders and treatment of the guttural pouch. *Vet Clin North Am Equine Pract*. 2015;31:63–89.
74. Dyce KM, Sack WO, Wensing T. The head and ventral neck of the horse: the guttural pouch. In Textbook of Veterinary Anatomy. 2nd ed. Philadelphia: W. B. Saunders; 1996:501–504.
75. McCue PM, Freeman DE, Donawick WJ. Guttural pouch tympany: 15 cases (1977-1986). *J Am Vet Med Assoc*. 1989;194:1761–1763.
76. Blazyczek I, Hamann H, Deegen E, et al. Retrospective analysis of 50 cases of guttural pouch tympany in foals. *Vet Rec*. 2004;154:261–264.
77. Blazyczek I, Hamann H, Ohnesorge B, et al. Population genetic analysis of the heritability of gutteral pouch tympany in Arabian purebred foals. *Dtsch Tierarztl Wochenschr*. 2003;110:417–419.
78. Blazyczek I, Hamann H, Ohnesorge B, et al. Gutteral pouch tympany in German warmblood foals: influence of sex, inbreeding and blood proportions of founding breeds as well as estimation of heritability. *Berl Munch Tierarztl Wochenschr*. 2003;116:346–351.
79. Blazyczek I, Hamann H, Ohnesorge B, et al. Inheritance of guttural pouch tympany in the arabian horse. *J Hered*. 2004;95:195–199.
80. Zeitz A, Spotter A, Blazyczek I, et al. Whole-genome scan for guttural pouch tympany in Arabian and German warmblood horses. *Anim Genet*. 2009;40:917–924.
81. Freeman DE, Hardy J. Guttural Pouch. In: Auer JA, Stick JA, eds. *Equine Surgery*. 4th ed. St. Louis: Elsevier Saunders; 2012:623–642.
82. Cook W. *Diseases of the Ear, Nose and Throat in the Horse*. Bristol, UK: John Wright and Sons; 1971:12.
83. Ludwig A, Gatineau S, Reynaud MC, et al. Fungal isolation and identification in 21 cases of guttural pouch mycosis in horses (1998-2002). *Vet J*. 2005;169:457–461.
84. Archer RM, Knight CG, Bishop WJ. Guttural pouch mycosis in six horses in New Zealand. *N Z Vet J*. 2012;60:203–209.
85. Caron JP, Fretz PB, Bailey JV, et al. Balloon-tipped catheter arterial occlusion for prevention of hemorrhage caused by guttural pouch mycosis: 13 cases (1982-1985). *J Am Vet Med Assoc*. 1987;191:345–349.
86. Church S, Wyn-Jones G, Parks AH, et al. Treatment of guttural pouch mycosis. *Equine Vet J*. 1986;18:362–365.
87. Cook WR, Campbell RS, Dawson C. The pathology and aetiology of guttural pouch mycosis in the horse. *Vet Rec*. 1968;83:422–428.
88. Freeman DE, Ross MW, Donawick WJ, et al. Occlusion of the external carotid and maxillary arteries in the horse to prevent hemorrhage from guttural pouch mycosis. *Vet Surg*. 1989;18:39–47.
89. Greet TR. Outcome of treatment in 35 cases of guttural pouch mycosis. *Equine Vet J*. 1987;19:483–487.

90. Freeman DE, Long-term. follow-up on a large number of horses that underwent transarterial coil embolisation (TCE) for guttural pouch mycosis (GPM). *Equine Vet J*. 2006;38:271.
91. Walmsley JP. A case of atlanto-occipital arthropathy following guttural pouch mycosis in a horse. The use of radioisotope bone scanning as an aid to diagnosis. *Equine Vet J*. 1988;20: 219–220.
92. Dixon PM, Rowlands AC. Atlanto-occipital joint infection associated with guttural pouch mycosis in a horse. *Equine Vet J*. 1981;13:260–262.
93. Millar H. Guttural pouch mycosis in a 6-month-old filly. *Can Vet J*. 2006;47:259–261.
94. Ryan JA, Modransky PD, Welker B. Guttural pouch mycosis in a 3-month old foal. *Equine Practice*. 1992;14:21–22.
95. Cook WR. The clinical features of guttural pouch mycosis in the horse. *Vet Rec*. 1968;83:336–345.
96. Colles CM, Cook WR. Carotid and cerebral angiography in the horse. *Vet Rec*. 1983;113:483–489.
97. Lane JG. The management of guttural pouch mycosis. *Equine Vet J*. 1989;21:321–324.
98. Eichentopf A, Snyder A, Recknagel S, et al. Dysphagia caused by focal guttural pouch mycosis: mononeuropathy of the pharyngeal ramus of the vagal nerve in a 20-year-old pony mare. *Ir Vet J*. 2013;66:13.
99. Lepage OM, Piccot-Crezollet C. Transarterial coil embolisation in 31 horses (1999-2002) with guttural pouch mycosis: a 2-year follow-up. *Equine Vet J*. 2005;37:430–434.

99a. Holcombe SJ. Medical Treatment of Upper Airway Dysfunction. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. 5th ed. Philadelphia: Saunders; 2003:398–400.

100. McMullan WC, Joyce JR, Hanselka DV, et al. Amphotericin B for the treatment of localized subcutaneous phycomycosis in the horse. *J Am Vet Med Assoc*. 1977;170:1293–1298.
101. Davis JL. The use of antifungals. *Comp Equine*. 2008;3:128–133.
102. Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue distribution of itraconazole after oral and intravenous administration to horses. *Am J Vet Res*. 2005;66:1694–1701.
103. Davis EW, Legendre AM. Successful treatment of guttural pouch mycosis with itraconazole and topical enilconazole in a horse. *J Vet Intern Med*. 1994;8:304–305.
104. Benredouane K, Lepage O. Trans-arterial coil embolization of the internal carotid artery in standing horses. *Vet Surg*. 2012;41:404–409.
105. Freeman DE, Staller GS, Maxson AD, et al. Unusual internal carotid artery branching that prevented arterial occlusion with a balloon-tipped catheter in a horse. *Vet Surg*. 1993;22:531–534.
106. Bacon MC, Wilson DA, Martin DD, et al. Complications of balloon catheterization associated with aberrant cerebral arterial anatomy in a horse with guttural pouch mycosis. *Vet Surg*. 1998;27:450–453.
107. Lepage OM, Piccot-Crezollet C. Transarterial coil embolisation in 31 horses (1999-2002) with guttural pouch mycosis: a 2-year follow-up. *Equine Vet J*. 2005;37:430–434.
108. Judy CE, Chaffin MK, Cohen ND. Empyema of the guttural pouch (auditory tube diverticulum) in horses: 91 cases (1977-1997). *J Am Vet Med Assoc*. 1999;215:1666–1670.
109. Rashmir-Raven AM, DeBowes RM, Gift LJ. What's your diagnosis? Upper airway obstruction in a horse caused by pharyngeal perforation during nasogastric intubation. *J Am Vet Med Assoc*. 1991;198:1991–1992.
110. Sweeney CR, Timoney JF, Newton JR, et al. Streptococcus equi infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med*. 2005;19:123–134.
111. Verheyen K, Newton JR, Talbot NC, et al. Elimination of guttural pouch infection and inflammation in asymptomatic carriers of Streptococcus equi. *Equine Vet J*. 2000;32:527–532.
112. Seahorn TL, Schumacher J. Nonsurgical removal of chondroid masses from the guttural pouches of two horses. *J Am Vet Med Assoc*. 1991;199:368–369.
113. Gehlen H, Ohnesorge B. Laser fenestration of the mesial septum for treatment of guttural pouch chondroids in a pony. *Vet Surg*. 2005;34:383–386.
114. Hawkins JF, Frank N, Sojka JE, et al. Fistulation of the auditory tube diverticulum (guttural pouch) with a neodymium: yttrium-aluminum-garnet laser for treatment of chronic empyema in two horses. *J Am Vet Med Assoc*. 2001;218:405–407. 361.
115. Holcombe SJ. Medical Treatment of Upper Airway Dysfunction. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. 5th ed. Philadelphia: Saunders; 2003:398–400.
116. Moore BR, Krakowka S, Cummins JM, et al. Changes in airway inflammatory cell populations in standardbred racehorses after interferon-alpha administration. *Vet Immunol Immunopathol*. 1996;49:347–358.
117. Moore BR. Clinical application of interferons in large animal medicine. *J Am Vet Med Assoc*. 1996;208:1711–1715.
118. Palmer SE. The use of lasers for treatment of upper respiratory tract disorders. *Vet Clin North Am Equine Pract*. 2003;19:245–263.
119. Ainsworth D, Hackett RP. Disorders of the respiratory system. In: Fathman L, Merchant T, eds. *Reed, Bayly and Sellon: Equine Internal Medicine*. 2nd ed. St. Louis: Saunders; 2004:289–353.
120. Lane JG, Bladon B, Little DR, et al. Dynamic obstructions of the equine upper respiratory tract. Part 1: observations during high-speed treadmill endoscopy of 600 Thoroughbred racehorses. *Equine Vet J*. 2006;38:393–399.
121. Tan RH, Dowling BA, Dart AJ. High-speed treadmill videoendoscopic examination of the upper respiratory tract in the horse: the results of 291 clinical cases. *Vet J*. 2005;170:243–248.
122. Ducharme NG. *Larynx. Equine Surgery*. 3rd ed. St. Louis (MO): Elsevier Saunders; 2006:592–622.
123. Dart AJ, Dowling BA, Hodgson DR, et al. Evaluation of high-speed treadmill videoendoscopy for diagnosis of upper respiratory tract dysfunction in horses. *Aust Vet J*. 2001;79: 109–112.
124. Barakzai SZ, Johnson VS, Baird DH, et al. Assessment of the efficacy of composite surgery for the treatment of dorsal displacement of the soft palate in a group of 53 racing Thoroughbreds (1990-1996). *Equine Vet J*. 2004;36:175–179.
125. Parente EJ, Martin BB, Tulleners EP, et al. Dorsal displacement of the soft palate in 92 horses during high-speed treadmill examination (1993-1998). *Vet Surg*. 2002;31:507–512.
126. Lane JG, Bladon B, Little DR, et al. Dynamic obstructions of the equine upper respiratory tract. Part 2: comparison of endoscopic findings at rest and during high-speed treadmill exercise of 600 Thoroughbred racehorses. *Equine Vet J*. 2006;38: 401–407.
127. Rehder RS, Ducharme NG, Hackett RP, et al. Measurement of upper airway pressures in exercising horses with dorsal displacement of the soft palate. *Am J Vet Res*. 1995;56:269–274.
128. Cook WR. Some observations on form and function on the upper airway in health and disease. *Proc Am Assoc Eq Pract*. 1981:355–392.
129. Fogel RB, Trinder J, White DP. The effect of sleep onset on upper airway muscle activity in patients with sleep apena versus controls. *J Physiol*. 2005;564:549.
130. Gumery PY, Roux-Buisson H, Meignen S, et al. An adaptive detector of genioglossus EMG reflex using Berkner transform for time latency measurement in OSA pathophysiological studies. *IEEE Trans Biomed Eng*. 2005;52:1382–1389.
131. Tessier C, Holcombe SJ, Derksen FJ, et al. Effects of stylopharyngeus muscle dysfunction on the nasopharynx in exercising horses. *Equine Vet J*. 2004;36:318–323.
132. Holcombe SJ, Derksen FJ, Stick JA, et al. Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. *Am J Vet Res*. 1998;59:504–508.
133. Holcombe SJ, Derksen FJ, Stick JA, et al. Effect of bilateral tenectomy of the tensor veli palatini muscle on soft palate function in horses. *Am J Vet Res*. 1997;58:317–321.

134. Ducharme NG, Hackett RP, Woodie JB, et al. Investigations into the role of the thyrohyoid muscles in the pathogenesis of dorsal displacement of the soft palate in horses. *Equine Vet J*. 2003;35:258–263.
135. Holcombe SJ, Beard WL, Hinchcliff KW, et al. Effect of sternothyrohyoid myectomy on upper airway mechanics in normal horses. *J Appl Physiol (1985)*. 1994;77:2812–2816.
136. Malhotra A, Pillar G, Fogel RB, et al. Pharyngeal pressure and flow effects on genioglossus activation in normal subjects. *Am J Respir Crit Care Med*. 2002;165:71–77.
137. Brennick MJ, Ogilvie MD, Margulies SS, et al. MRI study of regional variations of pharyngeal wall compliance in cats. *J Appl Physiol (1985)*. 1998;85:1884–1897.
138. Franklin SH, Naylor JR, Lane JG. The effect of a tongue-tie in horses with dorsal displacement of the soft palate. *Equine Vet J Suppl*. 2002;34:430–433.
139. Ducharme NG. *Pharynx. Equine Surgery*. 3rd ed. St. Louis (MO): Elsevier Saunders; 2006:569–591.
140. Harrison IW, Raker CW. Sternothyrohyoideus myectomy in horses: 17 cases (1984-1985). *J Am Vet Med Assoc*. 1988;193:1299–1302.
141. Anderson JD, Tulleners EP, Johnston JK, et al. Sternothyrohyoideus myectomy or staphylectomy for treatment of intermittent dorsal displacement of the soft palate in racehorses: 209 cases (1986-1991). *J Am Vet Med Assoc*. 1995;206:1909–1912.
142. Ahren TJ. Oral palatopharyngoplasty. *J Equine Vet Sci*. 1993;13:670–672.
143. Barakzai SZ, Dixon PM. Conservative treatment for thoroughbred racehorses with intermittent dorsal displacement of the soft palate. *Vet Rec*. 2005;157:337–340.
144. Woodie JB, Ducharme NG, Kanter P, et al. Surgical advancement of the larynx (laryngeal tie-forward) as a treatment for dorsal displacement of the soft palate in horses: a prospective study 2001-2004. *Equine Vet J*. 2005;37:418–423.
145. Woodie JB, Ducharme NG, Hackett RP, et al. Can an external device prevent dorsal displacement of the soft palate during strenuous exercise? *Equine Vet J*. 2005;37:425–429.
146. Goulden BE, Anderson LG. Equine laryngeal hemiplegia. Part III. Treatment by laryngoplasty *N Z Vet J*. 1982;30:1–5.
147. Lane JG, Ellis DR, Greet TR. Observations on the examination of Thoroughbred yearlings for idiopathic laryngeal hemiplegia. *Equine Vet J*. 1987;19:531–536.
148. Dixon PM, McGorum BC, Railton DI, et al. Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses. *Equine Vet J*. 2001;33:452–458.
149. Brown JA, Hinchcliff KW, Jackson MA, et al. Prevalence of pharyngeal and laryngeal abnormalities in Thoroughbreds racing in Australia, and their association with performance. *Equine Vet J*. 2005;37:397–401.
150. Brakenhoff JE, Holcombe SJ, Hauptman JG, et al. The prevalence of laryngeal disease in a large population of competition draft horses. *Vet Surg*. 2006;35:579–583.
151. Harrison GD, Duncan ID, Clayton MK. Determination of the early age of onset of equine recurrent laryngeal neuropathy. 1. Muscle pathology. *Acta Neuropathol*. 1992;84:307–315.
152. Dixon P, Robinson NE, Wade JF. *Workshop summary* Proceedings of a workshop on Equine Recurrent Laryngeal Neuropathy. Stratford-upon-Avon, United Kingdom, R & W Publications (Newmarket) Ltd; 2003.
153. Rakestraw PC, Hackett RP, Ducharme NG, et al. Arytenoid cartilage movement in resting and exercising horses. *Vet Surg*. 1991;20:122–127.
154. Ducharme NG. *Four-grade system of equine laryngeal function*. Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy. Stratford-upon-Avon, United Kingdom R & W Publications (Newmarket) Ltd; 2003.
155. Hammer EJ, Tulleners EP, Parente EJ, et al. Videoendoscopic assessment of dynamic laryngeal function during exercise in horses with grade-III left laryngeal hemiparesis at rest: 26 cases (1992-1995). *J Am Vet Med Assoc*. 1998;212:399–403.
156. Chalmers HJ, Cheetham J, Yeager AE, et al. Ultrasonography of the equine larynx. *Vet Radiol Ultrasound*. 2006;47:476–481.
157. Quinlan TJ, Goulden BE, Barnes GR, et al. Innervation of the equine intrinsic laryngeal muscles. *N Z Vet J*. 1982;30:43–45.
158. Duncan ID, Griffths IR, McQueen A, et al. The pathology of equine laryngeal hemiplegia. *Acta Neuropathol*. 1974;27:337–348.
159. Gunn HM. Histochemical observations on laryngeal skeletal muscle fibers in "normal" horses. *Equine Vet J*. 1972;4:144.
160. Cahill JI, Goulden BE. The pathogenesis of equine laryngeal hemiplegia—a review. *N Z Vet J*. 1987;35:82–90.
161. Dattilo DJ, Drooger SA. Outcome assessment of patients undergoing maxillofacial procedures for the treatment of sleep apnea: comparison of subjective and objective results. *J Oral Maxillofac Surg*. 2004;62:164–168.
161a. Ducharme NG, Horney FD, Partlow GD, et al. Attempts to restore abduction of the paralyzed equine arytenoid cartilage. I. Nerve-muscle pedicle transplants. *Can J Vet Res*. 1989;53:202–209.
161b. Fulton IC, Derksen FJ, Stick JA, et al. Treatment of left laryngeal hemiplegia in standardbreds, using a nerve muscle pedicle graft. *Am J Vet Res*. 1991;52:1461–1467.
162. COLE CR. Changes in the equine larynx associated with laryngeal hemiplegia. *Am J Vet Res*. 1946;7:69–77.
163. Derksen FJ, Stick JA, Scott EA, et al. Effect of laryngeal hemiplegia and laryngoplasty on airway flow mechanics in exercising horses. *Am J Vet Res*. 1986;47:16–20.
164. Brown JA, Derksen FJ, Stick JA, et al. Laser vocal cordectomy fails to effectively reduce respiratory noise in horses with laryngeal hemiplegia. *Vet Surg*. 2005;34:247–252.
165. Shappell KK, Derksen FJ, Stick JA, et al. Effects of ventriculectomy, prosthetic laryngoplasty, and exercise on upper airway function in horses with induced left laryngeal hemiplegia. *Am J Vet Res*. 1988;49:1760–1765.
166. Tetens J, Derksen FJ, Stick JA, et al. Efficacy of prosthetic laryngoplasty with and without bilateral ventriculocordectomy as treatments for laryngeal hemiplegia in horses. *Am J Vet Res*. 1996;57:1668–1673.
167. Ehrlich PJ, Seeherman HJ, Morris E, et al. The effect of reversible left recurrent laryngeal neuropathy on the metabolic cost of locomotion and peak aerobic power in thoroughbred racehorses. *Vet Surg*. 1995;24:36–48.
168. Kidd JA, Slone DE. Treatment of laryngeal hemiplegia in horses by prosthetic laryngoplasty, ventriculectomy and vocal cordectomy. *Vet Rec*. 2002;150:481–484.
169. Hawkins JF, Tulleners EP, Ross MW, et al. Laryngoplasty with or without ventriculectomy for treatment of left laryngeal hemiplegia in 230 racehorses. *Vet Surg*. 1997;26:484–491.
170. Kraus BM, Parente EJ, Tulleners EP. Laryngoplasty with ventriculectomy or ventriculocordectomy in 104 draft horses (1992-2000). *Vet Surg*. 2003;32:530–538.
171. Dixon PM, McGorum BC, Railton DI, et al. Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 2: owners' assessment of the value of surgery. *Equine Vet J*. 2003;35:397–401.
172. Brown JA, Derksen FJ, Stick JA, et al. Effect of laryngoplasty on respiratory noise reduction in horses with laryngeal hemiplegia. *Equine Vet J*. 2004;36:420–425.
173. Schumacher J, Wilson AM, Pardoe C, et al. In vitro evaluation of a novel prosthesis for laryngoplasty of horses with recurrent laryngeal neuropathy. *Equine Vet J*. 2000;32:43–46.
174. Radcliffe CH, Woodie JB, Hackett RP, et al. A comparison of laryngoplasty and modified partial arytenoidectomy as treatments for laryngeal hemiplegia in exercising horses. *Vet Surg*. 2006;35:643–652.
175. Davenport CL, Tulleners EP, Parente EJ. The effect of recurrent laryngeal neurectomy in conjunction with laryngoplasty and unilateral ventriculocordectomy in thoroughbred racehorses. *Vet Surg*. 2001;30:417–421.

176. Strand E, Martin GS, Haynes PF, et al. Career racing performance in Thoroughbreds treated with prosthetic laryngoplasty for laryngeal neuropathy: 52 cases (1981-1989). *J Am Vet Med Assoc*. 2000;217:1689–1696.
177. Russell AP, Slone DE. Performance analysis after prosthetic laryngoplasty and bilateral ventriculectomy for laryngeal hemiplegia in horses: 70 cases (1986-1991). *J Am Vet Med Assoc*. 1994;204:1235–1241.
178. Ducharme NG, Horney FD, Partlow GD, et al. Attempts to restore abduction of the paralyzed equine arytenoid cartilage. I. Nerve-muscle pedicle transplants. *Can J Vet Res*. 1989;53:202–209.
179. Fulton IC, Derksen FJ, Stick JA, et al. Treatment of left laryngeal hemiplegia in standardbreds, using a nerve muscle pedicle graft. *Am J Vet Res*. 1991;52:1461–1467.
180. Fulton IC, Derksen FJ, Stick JA, et al. Histologic evaluation of nerve muscle pedicle graft used as a treatment for left laryngeal hemiplegia in standardbreds. *Am J Vet Res*. 1992;53: 592–596.
181. Barnes AJ, Slone DE, Lynch TM. Performance after partial arytenoidectomy without mucosal closure in 27 Thoroughbred racehorses. *Vet Surg*. 2004;33:398–403.
182. Russell T, Wainscott M. Treatment in the field of 27 horses with epiglottic entrapment. *Vet Rec*. 2007;161:187–189.
183. McClure SR, Schumacher J, Snyder JR. Transnasal incision of restrictive nasopharyngeal cicatrix in three horses. *J Am Vet Med Assoc*. 1994;205:461–463.
184. Perkins JD, Hughes TK, Brain B. Endoscope-guided, transoral axial division of an entrapping epiglottic fold in fifteen standing horses. *Vet Surg*. 2007;36:800–803.
185. Lumsden JM, Stick JA, Caron JP, et al. Surgical treatment for epiglottic entrapment in horses: 51 cases (1981-1992). *J Am Vet Med Assoc*. 1994;205:729–735.
186. Ross MW, Gentile DG, Evans LE. Transoral axial division, under endoscopic guidance, for correction of epiglottic entrapment in horses. *J Am Vet Med Assoc*. 1993;203:416–420.
187. Greet TR. Experiences in treatment of epiglottal entrapment using a hook knife per nasum. *Equine Vet J*. 1995;27:122–126.
188. Tate LP, Sweeney CL, Bowman KF, et al. Transendoscopic Nd: YAG laser surgery for treatment of epiglottal entrapment and dorsal displacement of the soft palate in the horse. *Vet Surg*. 1990;19:356–363.
189. Tulleners EP. Correlation of performance with endoscopic and radiographic assessment of epiglottic hypoplasia in racehorses with epiglottic entrapment corrected by use of contact neodymium: yttrium aluminum garnet laser. *J Am Vet Med Assoc*. 1991;198:621–626.
190. Tulleners EP. Transendoscopic contact neodymium: yttrium aluminum garnet laser correction of epiglottic entrapment in standing horses. *J Am Vet Med Assoc*. 1990;196:1971–1980.
191. Ross MW, Gentile DG, Evans LE. Transoral axial division, under endoscopic guidance, for correction of epiglottic entrapment in horses. *J Am Vet Med Assoc*. 1993;203:416–420.
192. Kemper T, Spier S, Barratt-Boyes SM, et al. Treatment of smoke inhalation in five horses. *J Am Vet Med Assoc*. 1993;202:91–94.
193. Haynes PF. Dorsal displacement of the soft palate and epiglottic entrapment: diagnosis, management and interrelationships. *Compend Contin Educ Pract Vet*. 1983;5:379.
194. Blikslager AT, Tate LP, Tudor R. Transendoscopic laser treatment of rostral displacement of the palatopharyngeal arch in four horses. *J Clin Laser Med Surg*. 1999;17:49–52.
195. Klein HJ, Deegen E, Stockhofe N, et al. Rostral displacement of the palatopharyngeal arch in a seven-month-old Hanoverian colt. *Equine Vet J*. 1989;21:382–383.
196. Goulden BE, Anderson LJ, Davies AS, et al. Rostral displacement of the palatopharyngeal arch: a case report. *Equine Vet J*. 1976;8:95–98.
197. Crabill M, Schumacher J, Walker M. What is your diagnosis? Rostral displacement of the palatopharyngeal arch. *J Am Vet Med Assoc*. 1994;204:1347–1348.
198. Schumacher J, Hanselka DV. Nasopharyngeal cicatrices in horses: 47 cases (1972-1985). *J Am Vet Med Assoc*. 1987;191:239–242.
199. Chesen AB, Whitfield-Cargile C. Update on diseases and treatment of the pharynx. *Vet Clin North Am Equine Pract*. 2015;31:1–11.
200. Chesen AB, Rakestraw PC. Indications for and short- and long-term outcome of permanent tracheostomy performed in standing horses: 82 cases (1995-2005). *J Am Vet Med Assoc*. 2008;232:1352–1356.
201. Dean PW, Cohen ND. Arytenoidectomy for advanced unilateral chondropathy with accompanying lesions. *Vet Surg*. 1990;19:364–370.
202. Norman TE, Chaffin MK, Bisset WT, et al. Association of clinical signs with endoscopic findings in horses with nasopharyngeal cicatrix syndrome: 118 cases (2003-2008). *J Am Vet Med Assoc*. 2012;240:734–739.
203. Slater JD. Strangles, bastard strangles, vives and glanders: archaeological relics in a genomic age. *Equine Vet J*. 2003;35:118–120.
204. Bannister MF, Benson CE, Sweeney CR. Rapid species identification of group C streptococci isolated from horses. *J Clin Microbiol*. 1985;21:524–526.
205. Harrington DJ, Sutcliffe IC, Chanter N. The molecular basis of Streptococcus equi infection and disease. *Microbes Infect*. 2002;4:501–510.
206. Timoney JF, Artiushin SC, Boschwitz JS. Comparison of the sequences and functions of Streptococcus equi M-like proteins SeM and SzPSe. *Infect Immun*. 1997;65:3600–3605.
207. Jorm LR, Love DN, Bailey GD, et al. Genetic structure of populations of beta-haemolytic Lancefield group C streptococci from horses and their association with disease. *Res Vet Sci*. 1994;57:292–299.
208. Sweeney CR, Whitlock RH. Streptococcus equi infection in horses: part I. *Compend Contin Educ Pract Vet*. 1987;9:689.
209. Prescott JF, Srivastava SK, deGannes R, et al. A mild form of strangles caused by an atypical Streptococcus equi. *J Am Vet Med Assoc*. 1982;180:293–299.
210. Galan JE, Timoney JF, Lengemann FW. Passive transfer of mucosal antibody to Streptococcus equi in the foal. *Infect Immun*. 1986;54:202–206.
211. Sweeney CR, Benson CE, Whitlock RH, et al. Description of an epizootic and persistence of Streptococcus equi infections in horses. *J Am Vet Med Assoc*. 1989;194:1281–1286.
212. Todd TG. Strangles. *J Comp Pathol Ther*. 1910;23:212.
213. Timoney JF. The pathogenic equine streptococci. *Vet Res*. 2004; 35:397–409.
214. Hamlen HJ, Timoney JF, Bell RJ. Epidemiologic and immunologic characteristics of Streptococcus equi infection in foals. *J Am Vet Med Assoc*. 1994;204:768–775.
215. Piche CA. Clinical observations on an outbreak of strangles. *Can Vet J*. 1984;25:7–11.
216. George JL, Reif JS, Shideler RK, et al. Identification of carriers of Streptococcus equi in a naturally infected herd. *J Am Vet Med Assoc*. 1983;183:80–84.
217. Whelchel DD, Chaffin MK. Sequelae and complications of Streptococcus equi subspecies equi infections in the horse. *Equine Vet Educ*. 2009;21:135–141.
218. Lindahl S, Baverud V, Egenvall A, et al. Comparison of sampling sites and laboratory diagnostic tests for S. equi subsp. equi in horses from confirmed strangles outbreaks. *J Vet Intern Med*. 2013;27:542–547.
219. Taylor SD, Wilson WD. Streptococcus equi subsp. equi (strangles) infection. *Clin Tech Equine Pract*. 2006;5:211–217.
220. Duffee LR, Stefanovski D, Boston RC, et al. Predictor variables for and complications associated with Streptococcus equi subsp equi infection in horses. *J Am Vet Med Assoc*. 2015;247: 1161–1168.
221. Newton JR, Wood JL, Dunn KA, et al. Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with Streptococcus equi. *Vet Rec*. 1997;140:84–90.

222. Waller AS, Sellon DC, Sweeney CR, et al. Streptococcal infections. In: Sellon DC, Long MT, eds. *Equine Infectious Diseases*. 2nd ed. St. Louis: Elsevier; 2014:265–277.
223. Sweeney CR, Timoney JF, Newton JR, et al. Streptococcus equi infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med*. 2005;19: 123–134.
224. Jorm LR. Factors affecting the survival of Streptococcus equi subsp equi on surfaces. *Proc 6th Intnatl Conf Equine Infectious Diseases*. 1991:39–43.
225. Weese JS, Jarlot C, Morley PS. Survival of Streptococcus equi on surfaces in an outdoor environment. *Can Vet J*. 2009;50: 968–970.
226. Sechi LA, Roger F, Diallo A, et al. Molecular characterization of Streptococcus equi subspecies equi isolated from an Ethiopian camel by ribotyping and PCR-ribotyping. *New Microbiol*. 1999;22:383–387.
227. Yigezu LM, Roger F, Kiredjian M, et al. Isolation of Streptococcus equi subspecies equi (strangles agent) from an Ethiopian camel. *Vet Rec*. 1997;140:608.
228. Breiman RF, Silverblatt FJ. Systemic Streptococcus equi infection in a horse handler—a case of human strangles. *West J Med*. 1986;145:385–386.
229. Holden MT, Heather Z, Paillot R, et al. Genomic evidence for the evolution of Streptococcus equi: host restriction, increased virulence, and genetic exchange with human pathogens. *PLoS Pathog*. 2009;5:e1000346.
230. Anzai T, Sheoran AS, Kuwamoto Y, et al. Streptococcus equi but not Streptococcus zooepidemicus produces potent mitogenic responses from equine peripheral blood mononuclear cells. *Vet Immunol Immunopathol*. 1999;67:235–246.
231. Timoney JF. Strangles. *Vet Clin North Am Equine Pract*. 1993;9:365–374.
232. Evers WD. Effect of furaltadone on strangles in horses. *J Am Vet Med Assoc*. 1968;152:1394–1398.
232a. Muhktar MM, Timoney JF. Chemotactic response of equine polymorphonuclear leucocytes to Streptococcus equi. *Res Vet Sci*. 1988;45:225.
232b. Hamilton A, Robinson C, Sutcliffe IC, et al. Mutation of the maturase lipoprotein attenuates the virulence of Streptococcus equi to a greater extent than does loss of general lipoprotein lipidation. *Infect Immun*. 2006;74:6907.
232c. Tiwari R, Qin A, Artiushin S, et al. Se18.9, an anti-phagocytic factor H binding protein of Streptococcus equi. *Vet Microbiol*. 2007;121:105.
232d. Kelly C, Bugg M, Robinson C, et al. Sequence variation of the SeM gene of Streptococcus equi allows discrimination of the source of strangles outbreaks. *J Clin Microbiol*. 2006;44:480.
232e. Sheoran AS, Sponseller BT, Holmes MA, et al. Serum and mucosal antibody isotype responses to M-like protein (SeM) of Streptococcus equi in convalescent and vaccinated horses. *Vet Immunol Immunopathol*. 1997;59:239.
233. Grant ST, Efstratiou A, Chanter N. Laboratory diagnosis of strangles and the isolation of atypical Streptococcus equi. *Vet Rec*. 1993;133:215–216.
234. Timoney JF, Timoney PJ, Strickland KL. Lysogeny and the immunologically reactive proteins of Streptococcus equi. *Vet Rec*. 1984;115:148.
235. Kaese HJ, Valberg SJ, Hayden DW, et al. Infarctive purpura hemorrhagica in five horses. *J Am Vet Med Assoc*. 2005;226:1893–1898. 1845.
236. Newton JR, Verheyen K, Talbot NC, et al. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of Streptococcus equi. *Equine Vet J*. 2000;32: 515–526.
237. Waller AS, Jolley KA. Getting a grip on strangles: recent progress towards improved diagnostics and vaccines. *Vet J*. 2007;173:492–501.
238. Davidson A, Traub-Dargatz JL, Magnuson R, et al. Lack of correlation between antibody titers to fibrinogen-binding protein of Streptococcus equi and persistent carriers of strangles. *J Vet Diagn Invest*. 2008;20:457–462.
239. Boyle AG, Rankin SC, Duffee L, et al. Streptococcus equi Detection Polymerase Chain Reaction Assay for Equine Nasopharyngeal and Guttural Pouch Wash Samples. *J Vet Intern Med*. 2016;30:276–281.
240. Ramey D. Does early antibiotic use in horses with "strangles" cause metastatic Streptococcus equi bacterial infections? *Equine Vet Educ*. 2007;19:14.
241. Sweeney CR, Benson CE, Whitlock RH. Streptococcus equi infection in horses. Part 2. *Compend Contin Educ Pract Vet*. 1987;9:845–850.
242. Rumbaugh GE, Smith BP, Carlson GP. Internal abdominal abscesses in the horse: a study of 25 cases. *J Am Vet Med Assoc*. 1978;172:304–309.
243. Spoormakers TJ, Ensink JM, Goehring LS, et al. Brain abscesses as a metastatic manifestation of strangles: symptomatology and the use of magnetic resonance imaging as a diagnostic aid. *Equine Vet J*. 2003;35:146–151.
244. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118–121.
245. Heath SE, Geor RJ, Tabel H, et al. Unusual patterns of serum antibodies to Streptococcus equi in two horses with purpura hemorrhagica. *J Vet Intern Med*. 1991;5:263–267.
246. Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of Streptococcus equi. *J Immunol*. 1985;135:3134–3137.
247. Yelle MT. Clinical aspects of Streptococcus equi infection. *Equine Vet J*. 1987;19:158–162.
248. Todhunter RJ, Brown CM, Stickle R. Retropharyngeal infections in five horses. *J Am Vet Med Assoc*. 1985;187:600–604.
249. Golland LC, Hodgson DR, Davis RE, et al. Retropharyngeal lymph node infection in horses: 46 cases (1977-1992). *Aust Vet J*. 1995;72:161–164.
250. De CD, van LG, Nollet H, et al. Percutaneous puncture technique for treating persistent retropharyngeal lymph node infections in seven horses. *Vet Rec*. 2003;152:169–172.
251. Todhunter RJ, Brown CM, Stickle R. Retropharyngeal infections in five horses. *J Am Vet Med Assoc*. 1985;187:600–604.
252. Rigg DL, Ramey DW, Reinertson EL. Tracheal compression secondary to abscessation of cranial mediastinal lymph nodes in a horse. *J Am Vet Med Assoc*. 1985;186:283–284.
253. Bell RJ, Smart ME. An unusual complication of strangles in a pony. *Can Vet J*. 1992;33:400–401.
254. Finno C, Pusterla N, Aleman M, et al. Streptococcus equi meningoencephalomyelitis in a foal. *J Am Vet Med Assoc*. 2006;229:721–724.
255. Lewis SS, Valberg SJ, Nielsen IL. Suspected immune-mediated myositis in horses. *J Vet Intern Med*. 2007;21:495–503.
256. Galan JE, Timoney JF. Mucosal nasopharyngeal immune responses of horses to protein antigens of Streptococcus equi. *Infect Immun*. 1985;47:623–628.
257. Borst LB, Patterson SK, Lanka S, et al. Evaluation of a commercially available modified-live Streptococcus equi subsp equi vaccine in ponies. *Am J Vet Res*. 2011;72:1130–1138.
258. Kemp-Symonds J, Kemble T, Waller A. Modified live Streptococcus equi ('strangles') vaccination followed by clinically adverse reactions associated with bacterial replication. *Equine Vet J*. 2007;39:284–286.
259. Prescott JF, Timoney JF. Could we eradicate strangles in equids? *J Am Vet Med Assoc*. 2007;231:377–378.
260. Timoney JF, Gillespie JH, Scott FW, et al. The Orthomyxoviridae. In: Timoney JF, Gillespie JH, Scott FW, et al., eds. *Hagan and Bruner's Microbioloby and Infectious Diseases of Domestic Animals*. 8th ed. Ithaca: Comstock Publishing Associates; 1988:775–789.

261. Wood JM. Antigenic variation of equine influenza: a stable virus. *Equine Vet J*. 1988;20:316–318.
262. Morley PS, Townsend HG, Bogdan JR, et al. Descriptive epidemiologic study of disease associated with influenza virus infections during three epidemics in horses. *J Am Vet Med Assoc*. 2000;216:535–544.
263. Newton JR, Verheyen K, Wood JL, et al. Equine influenza in the United Kingdom in 1998. *Vet Rec*. 1999;145:449–452.
264. Sovinova O, Tumova B, Pouska F, et al. Isolation of a virus causing respiratory disease in horses. *Acta Virol*. 1958;2:52–61.
265. Waddell GH, Teigland MB, Sigel MM. A new influenza virus associated with equine respiratory disease. *J Am Vet Med Assoc*. 1963;143:587–590.
266. Mumford JA, Hannant D, Jessett DM. Experimental infection of ponies with equine influenza (H3N8) viruses by intranasal inoculation or exposure to aerosols. *Equine Vet J*. 1990;22: 93–98.
267. Willoughby R, Ecker G, McKee S, et al. The effects of equine rhinovirus, influenza virus and herpesvirus infection on tracheal clearance rate in horses. *Can J Vet Res*. 1992;56:115–121.
268. Gilkerson JR, Bailey KE, Diaz-Mendez A, et al. Update on viral diseases of the equine respiratory tract. *Vet Clin North Am Equine Pract*. 2015;31:91–104.
269. Livesay GJ, O'Neill T, Hannant D, et al. The outbreak of equine influenza (H3N8) in the United Kingdom in 1989: diagnostic use of an antigen capture ELISA. *Vet Rec*. 1993;133:515–519.
270. Mumford JA, Rossdale PD. Virus and its relationship to the "poor performance" syndrome. *Equine Vet J*. 1980;12:3–9.
271. Kirkland PD, Davis RJ, Gu X, et al. Application of high-throughput systems for the rapid detection of DNA and RNA viruses during the Australian equine influenza outbreak. *Aust Vet J*. 2011;89(suppl 1):38–39.
272. Quinlivan M, Dempsey E, Ryan F, et al. Real-time reverse transcription PCR for detection and quantitative analysis of equine influenza virus. *J Clin Microbiol*. 2005;43:5055–5057.
273. Cullinane A, Gildea S, Weldon E. Comparison of primary vaccination regimes for equine influenza: working towards an evidence-based regime. *Equine Vet J*. 2014;46:669–673.
274. El-Hage CM, Savage CJ, Minke JM, et al. Accelerated vaccination schedule provides protective levels of antibody and complete herd immunity to equine influenza. *Equine Vet J*. 2013;45:235–239.
275. Davison AJ, Eberle R, Ehlers B, et al. The order Herpesvirales. *Arch Virol*. 2009;154:171–177.
276. Allen GP, Bryans JT. Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infections. *Prog Vet Microbiol Immunol*. 1986;2:78–144.
277. Vandekerckhove AP, Glorieux S, Gryspeerdt AC, et al. Equine alphaherpesviruses (EHV-1 and EHV-4) differ in their efficiency to infect mononuclear cells during early steps of infection in nasal mucosal explants. *Vet Microbiol*. 2011;152:21–28.
278. Patel JR, Heldens J. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4) - epidemiology, disease and immunoprophylaxis: a brief review. *Vet J*. 2005;170:14–23.
279. O'Keefe JS, Alley MR, Jones D, et al. Neonatal mortality due to equid herpesvirus 4 (EHV-4) in a foal. *Aust Vet J*. 1995;72:353–354.
280. Wilkins PA. Lower airway diseases of the adult horse. *Vet Clin North Am Equine Pract*. 2003;19:101–121. vii.
281. Carr E, Schott H, Pusterla N. Absence of equid herpesvirus-1 reactivation and viremia in hospitalized critically ill horses. *J Vet Intern Med*. 2011;25:1190–1193.
282. Foote CE, Love DN, Gilkerson JR, et al. Detection of EHV-1 and EHV-4 DNA in unweaned Thoroughbred foals from vaccinated mares on a large stud farm. *Equine Vet J*. 2004;36: 341–345.
283. Slater JD, Borchers K, Field HJ. Equine herpesvirus-1: a neurotropic alphaherpesvirus. *Vet Rec*. 1994;135:239–240.
284. Slater JD, Borchers K, Thackray AM, et al. The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. *J Gen Virol*. 1994;75:2007–2016.
285. Allen GP. Antemortem detection of latent infection with neuropathogenic strains of equine herpesvirus-1 in horses. *Am J Vet Res*. 2006;67:1401–1405.
286. Gilkerson JR, Whalley JM, Drummer HE, et al. Epidemiology of EHV-1 and EHV-4 in the mare and foal populations on a Hunter Valley stud farm: are mares the source of EHV-1 for unweaned foals. *Vet Microbiol*. 1999;68:27–34.
287. Gilkerson JR, Whalley JM, Drummer HE, et al. Epidemiological studies of equine herpesvirus 1 (EHV-1) in Thoroughbred foals: a review of studies conducted in the Hunter Valley of New South Wales between 1995 and 1997. *Vet Microbiol*. 1999;68: 15–25.
288. Burrows R, Goodridge D. Experimental studies on equine herpesvirus type 1 infections. *J Reprod Fertil Suppl*. 1975:611–615.
289. Foote CE, Gilkerson JR, Whalley JM, et al. Seroprevalence of equine herpesvirus 1 in mares and foals on a large Hunter Valley stud farm in years pre- and postvaccination. *Aust Vet J*. 2003;81:283–288.
290. Nugent J, Birch-Machin I, Smith KC, et al. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. *J Virol*. 2006;80:4047–4060.
291. Perkins GA, Goodman LB, Tsujimura K, et al. Investigation of the prevalence of neurologic equine herpes virus type 1 (EHV-1) in a 23-year retrospective analysis (1984-2007). *Vet Microbiol*. 2009;139:375–378.
292. Lunn DP, vis-Poynter N, Flaminio MJ, et al. Equine herpesvirus-1 consensus statement. *J Vet Intern Med*. 2009;23:450–461.
293. Pusterla N, Mapes S, Wilson WD. Diagnostic sensitivity of nasopharyngeal and nasal swabs for the molecular detection of EHV-1. *Vet Rec*. 2008;162:520–521.
294. Browning GF, Bulach DM, Ficorilli N, et al. Latency of equine herpesvirus 4 (equine rhinopneumonitis virus). *Vet Rec*. 1988; 123:518–519.
295. Palfi V, Christensen LS. Analyses of restriction fragment patterns (RFPs) and pathogenicity in baby mice of equine herpesvirus 1 and 4 (EHV-1 and EHV-4) strains circulating in Danish horses. *Vet Microbiol*. 1995;47:199–204.
296. Borchers K, Wolfinger U, Ludwig H, et al. Virological and molecular biological investigations into equine herpes virus type 2 (EHV-2) experimental infections. *Virus Res*. 1998;55: 101–106.
297. Craig MI, Barrandeguy ME, Fernandez FM. Equine herpesvirus 2 (EHV-2) infection in thoroughbred horses in Argentina. *BMC Vet Res*. 2005;1:9.
298. Johnson PJ. An emerging role for the equine and asinine gamma-herpesviruses? 2013.
299. Murray MJ, Eichorn ES, Dubovi EJ, et al. Equine herpesvirus type 2: prevalence and seroepidemiology in foals. *Equine Vet J*. 1996;28:432–436.
300. Kleiboeker SB, Schommer SK, Johnson PJ, et al. Association of two newly recognized herpesviruses with interstitial pneumonia in donkeys (Equus asinus). *J Vet Diagn Invest*. 2002;14:273–280.
301. Fortier G, van EE, Fortier C, et al. Herpesviruses in respiratory liquids of horses: putative implication in airway inflammation and association with cytological features. *Vet Microbiol*. 2009;139:34–41.
302. Williams KJ, Maes R, Del PF, et al. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. *Vet Pathol*. 2007;44:849–862.
303. Wong D, Scarratt WK. Equine herpes myeloencephalopathy in a 12-year-old American quarter horse. *Vet Clin North Am Equine Pract*. 2006;22:177–191.

304. Niedermaier G, Poth T, Gehlen H. Clinical aspects of multinodular pulmonary fibrosis in two warmblood horses. *Vet Rec*. 2010;166:426–430.
305. Vengust M, Wen X, Bienzle D. Herpesvirus-associated neurological disease in a donkey. *J Vet Diagn Invest*. 2008;20:820–823.
306. Hart KA, Barton MH, Williams KJ, et al. Multinodular pulmonary fibrosis, pancytopenia and equine herpes virus 5 in the Thoroughbred gelding. *Equine Verterinary Education*. 2008;20:470–475.
307. Marenzoni ML, Passamonti F, Lepri E, et al. Quantification of Equid herpesvirus 5 DNA in clinical and necropsy specimens collected from a horse with equine multinodular pulmonary fibrosis. *J Vet Diagn Invest*. 2011;23:802–806.
308. Bentz BG, Maxwell LK, Erkert RS, et al. Pharmacokinetics of acyclovir after single intravenous and oral administration to adult horses. *J Vet Intern Med*. 2006;20:589–594.
309. Maxwell LK, Bentz BG, Bourne DW, et al. Pharmacokinetics of valacyclovir in the adult horse. *J Vet Pharmacol Ther*. 2008;31:312–320.
310. Schwarz B, Schwendenwein I, van den Hoven R. Successful outcome in a case of equine multinodular pulmonary fibrosis (EMPF) treated with valacyclovir. *Equine Vet Educ*. 2012;2:1–4.
311. Wong DM, Belgrave RL, Williams KJ, et al. Multinodular pulmonary fibrosis in five horses. *J Am Vet Med Assoc*. 2008;232:898–905.
312. Wilkins PA. Equine multinodular pulmonary fibrosis. 2004;178–180.
313. Timoney PJ, McCollum WH. Equine viral arteritis. *Vet Clin North Am Equine Pract*. 1993;9:295–309.
314. Timoney PJ. Equine viral arteritis: a disease of emerging significance? *Equine Vet J*. 1986;18:166–168.
315. Del Piero F, Wilkins PA, Lopez JW, et al. Equine viral arteritis in newborn foals: clinical, pathological, serological, microbiological and immunohistochemical observations. *Equine Vet J*. 1997;29:178–185.
316. Li F, Browning GF, Studdert MJ, et al. Equine rhinovirus 1 is more closely related to foot-and-mouth disease virus than to other picornaviruses. *Proc Natl Acad Sci U S A*. 1996;93:990–995.
317. Hartley CA, Ficorilli N, Dynon K, et al. Equine rhinitis A virus: structural proteins and immune response. *J Gen Virol*. 2001;82:1725–1728.
318. Diaz-Mendez A, Viel L, Hewson J, et al. Surveillance of equine respiratory viruses in Ontario. *Can J Vet Res*. 2010;74:271–278.
319. Diaz-Mendez A, Hewson J, Shewen P, et al. Characteristics of respiratory tract disease in horses inoculated with equine rhinitis A virus. *Am J Vet Res*. 2014;75:169–178.
320. Quinlivan M, Maxwell G, Lyons P, et al. Real-time RT-PCR for the detection and quantitative analysis of equine rhinitis viruses. *Equine Vet J*. 2010;42:98–104.
321. Plummer G. An equine respiratory virus with enterovirus properties. *Nature*. 1962;195:519–520.
322. Black WD, Wilcox RS, Stevenson RA, et al. Prevalence of serum neutralising antibody to equine rhinitis A virus (ERAV), equine rhinitis B virus 1 (ERBV1) and ERBV2. *Vet Microbiol*. 2007;119:65–71.
323. Dynon K, Varrasso A, Ficorilli N, et al. Identification of equine herpesvirus 3 (equine coital exanthema virus), equine gammaherpesviruses 2 and 5, equine adenoviruses 1 and 2, equine arteritis virus and equine rhinitis A virus by polymerase chain reaction. *Aust Vet J*. 2001;79:695–702.
324. Fukunaga Y, Kumanomido T, Imagawa H, et al. Isolation of picornavirus from horses associated with Getah virus infection, Nihon Juigaku. *Zasshi*. 1981;43:569–572.
325. Fukunaga Y, Kumanomido T, Kamada M. Equine Picornavirus Isolation of Virus from the Oral Cavity of Healthy Horses. 1983;20:103–109. Bulletin of Equine Research Institute.
326. Lynch SE, Gilkerson JR, Symes SJ, et al. Persistence and chronic urinary shedding of the aphthovirus equine rhinitis A virus. *Comp Immunol Microbiol Infect Dis*. 2013;36:95–103.
327. Klaey M, Sanchez-Higgins M, Leadon DP, et al. Field case study of equine rhinovirus 1 infection: clinical signs and clinicopathology. *Equine Vet J*. 1998;30:267–269.
328. Diaz-Mendez A, Viel L, Shewen P, et al. Genomic analysis of a Canadian equine rhinitis A virus reveals low diversity among field isolates. *Virus Genes*. 2013;46:280–286.
329. Howell PG. The 1960 epizootic of African Horse Sickness in the Middle East and S. W. Asia. *J S Afr Vet Assoc*. 1960;31:329–334.
330. Lubroth J. African horse sickness and the epizootic in Spain 1987. *Equine Practice*. 1988;10:26–33.
331. Mellor PS, Hamblin C. African horse sickness. *Vet Res*. 2004;35:445–466.
332. Rubio C, Cubillo MA, Hooghuis H, et al. Validation of ELISA for the detection of African horse sickness virus antigens and antibodies. *Arch Virol Suppl*. 1998;14:311–315.
333. Lelli R, Molini U, Ronchi GF, et al. Inactivated and adjuvanted vaccine for the control of the African horse sickness virus serotype 9 infection: evaluation of efficacy in horses and guinea-pig model. *Vet Ital*. 2013;49:89–98.
334. Lincoln VJ, Page PC, Kopp C, et al. Protection of horses against Culicoides biting midges in different housing systems in Switzerland. *Vet Parasitol*. 2015;210:206–214.
335. Middleton D. Hendra virus. *Vet Clin North Am Equine Pract*. 2014;30:579–589.
336. Mahalingam S, Herrero LJ, Playford EG, et al. Hendra virus: an emerging paramyxovirus in Australia. *Lancet Infect Dis*. 2012;12:799–807.
337. Tulsiani SM, Graham GC, Moore PR, et al. Emerging tropical diseases in Australia. Part 5. Hendra virus. *Ann Trop Med Parasitol*. 2011;105:1–11.
338. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis*. 2010;16:219–223.
339. Field H, Schaaf K, Kung N, et al. Hendra virus outbreak with novel clinical features, Australia. *Emerg Infect Dis*. 2010;16:338–340.
340. Field H, de JC, Melville D, et al. Hendra virus infection dynamics in Australian fruit bats. *PLoS One*. 2011;6:e28678.
341. Martin G, Plowright R, Chen C, et al. Hendra virus survival does not explain spillover patterns and implicates relatively direct transmission routes from flying foxes to horses. *J Gen Virol*. 2015;96:1229–1237.
342. Sweeney CR, Holcombe SJ, Barningham SC, et al. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *J Am Vet Med Assoc*. 1991;198:839–842.
343. Davis E, Rush BR, Herr LG, et al. Enrofloxacin use in a long-distance transport model of equine respiratory disease. *Vet Ther*. 2006;7:232–242.
344. Wood JL, Newton JR, Chanter N, et al. Association between respiratory disease and bacterial and viral infections in British racehorses. *J Clin Microbiol*. 2005;43:120–126.
345. Blunden AS, Hannant D, Livesay G, et al. Susceptibility of ponies to infection with Streptococcus pneumoniae (capsular type 3). *Equine Vet J*. 1994;26:22–28.
346. Sweeney CR, Holcombe SJ, Barningham SC, et al. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *J Am Vet Med Assoc*. 1991;198:839–842.
347. Sweeney CR, Divers TJ, Benson CE. Anaerobic bacteria in 21 horses with pleuropneumonia. *J Am Vet Med Assoc*. 1985;187:721–724.
348. Racklyeft DJ, Love DN. Bacterial infection of the lower respiratory tract in 34 horses. *Aust Vet J*. 2000;78:549–559.
349. Carr EA, Carlson GP, Wilson WD, et al. Acute hemorrhagic pulmonary infarction and necrotizing pneumonia in horses: 21 cases (1967-1993). *J Am Vet Med Assoc*. 1997;210:1774–1778.
350. Wagner AE, Bennett DG. Analysis of equine thoracic fluid. *Vet Clin Pathol*. 1982;11:13–17.

351. Hepworth-Warren KL, Wilgenbusch CM, Wong DM, et al. Intrathoracic oesophageal perforation and secondary pleuropneumonia: five cases. *Equine Vet Educ*. 2015;27:283–290.
352. Sweeney CR, Divers TJ, Benson CE. Anaerobic bacteria in 21 horses with pleuropneumonia. *J Am Vet Med Assoc*. 1985;187:721–724.
353. Reuss S, Giguere S. Update on Bacterial Pneumonia and Peuropneumonia in the Adult Horse. *Vet Clin North Am Equine Pract*. 2015;31:105–120.
354. Estell KE, Young A, Kozikowski T, et al. Pneumonia Caused by Klebsiella spp. in 46 Horses. *J Vet Intern Med*. 2016;30:314–321.
355. Kinoshita Y, Niwa H, Katayama Y. Use of loop-mediated isothermal amplification to detect six groups of pathogens causing secondary lower respiratory bacterial infections in horses. *Microbiol Immunol*. 2015;59:365–370.
356. Tomlinson JE, Reef VB, Boston RC, et al. The Association of Fibrinous Pleural Effusion with Survival and Complications in Horses with Pleuropneumonia (2002-2012): 74 Cases. *J Vet Intern Med*. 2015;29:1410–1417.
357. Knych HK, Stanley SD, Arthur RM, et al. Detection and pharmacokinetics of three formulations of firocoxib following multiple administrations to horses. *Equine Vet J*. 2014;46:734–738.
358. Kvaternick V, Pollmeier M, Fischer J, et al. Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther*. 2007;30:208–217.
359. Vander Werf KA, Davis EG, Kukanich B. Pharmacokinetics and adverse effects of oral meloxicam tablets in healthy adult horses. *J Vet Pharmacol Ther*. 2013;36:376–381.
360. Vivancos M, Barker J, Engbers S, et al. Pharmacokinetics and bioequivalence of 2 meloxicam oral dosage formulations in healthy adult horses. *Can Vet J*. 2015;56:730–736.
361. Foreman JH, Ruemmler R. Phenylbutazone and flunixin meglumine used singly or in combination in experimental lameness in horses. *Equine Vet J Suppl*. 2011:12–17.
362. McConnico RS, Morgan TW, Williams CC, et al. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *Am J Vet Res*. 2008;69:1496–1505.
363. Reed SK, Messer NT, Tessman RK, et al. Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. *Am J Vet Res*. 2006;67:398–402.
364. Keegan KG, Wilson DA, Wilson DJ, et al. Evaluation of mild lameness in horses trotting on a treadmill by clinicians and interns or residents and correlation of their assessments with kinematic gait analysis. *Am J Vet Res*. 1998;59:1370–1377.
365. Collins MB, Hodgson DR, Hutchins DR. Pleural effusion associated with acute and chronic pleuropneumonia and pleuritis secondary to thoracic wounds in horses: 43 cases (1982-1992). *J Am Vet Med Assoc*. 1994;205:1753–1758.
366. Raphel CF, Beech J. Pleuritis secondary to pneumonia or lung abscessation in 90 horses. *J Am Vet Med Assoc*. 1982;181:808–810.
367. Seltzer KL, Byars TD. Prognosis for return to racing after recovery from infectious pleuropneumonia in thoroughbred racehorses: 70 cases (1984-1989). *J Am Vet Med Assoc*. 1996;208:1300–1301.
368. Nout YS, Hinchcliff KW, Samii VF, et al. Chronic pulmonary disease with radiographic interstitial opacity (interstitial pneumonia) in foals. *Equine Vet J*. 2002;34:542–548.
369. Britton AP, Robinson JH. Isolation of influenza A virus from a 7-day-old foal with bronchointerstitial pneumonia. *Can Vet J*. 2002;43:55–56.
370. Buergelt CD, Hines SA, Cantor G, et al. A retrospective study of proliferative interstitial lung disease of horses in Florida. *Vet Pathol*. 1986;23:750–756.
371. Nobre D, Dagli ML, Haraguchi M. Crotalaria juncea intoxication in horses. *Vet Hum Toxicol*. 1994;36:445–448.
372. Buergelt CD. Interstitial pneumonia in the horse: a fledgling morphological entity with mysterious causes. *Equine Vet J*. 1995;27:4–5.
373. Donaldson MT, Beech J, Ennulat D, et al. Interstitial pneumonia and pulmonary fibrosis in a horse. *Equine Vet J*. 1998;30:173–175.
374. Dungworth DL. Interstitial pulmonary disease. *Adv Vet Sci Comp Med*. 1982;26:173–200.
375. Bell SA, Balasuriya UB, Gardner IA, et al. Temporal detection of equine herpesvirus infections of a cohort of mares and their foals. *Vet Microbiol*. 2006;116:249–257.
376. Rush BR, Raub ES, Thomsen MM, et al. Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction. *J Am Vet Med Assoc*. 2000;217:359–364.
377. Derksen FJ, Olszewski MA, Robinson NE, et al. Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. *Am J Vet Res*. 1999;60:689–693.
378. Rush BR, Hoskinson JJ, Davis EG, et al. Pulmonary distribution of aerosolized technetium Tc 99m pentetate after administration of a single dose of aerosolized albuterol sulfate in horses with recurrent airway obstruction. *Am J Vet Res*. 1999;60:764–769.
379. Leguillette R. Recurrent airway obstruction—heaves. *Vet Clin North Am Equine Pract*. 2003;19:63–86, vi.
380. Garre B, Baert K, Nauwynck H, et al. Multiple oral dosing of valacyclovir in horses and ponies. *J Vet Pharmacol Ther*. 2009;32:207–212.
381. Garre B, Gryspeerdt A, Croubels S, et al. Evaluation of orally administered valacyclovir in experimentally EHV1-infected ponies. *Vet Microbiol*. 2009;135:214–221.
382. Razonable RR. Cytomegalovirus infection after liver transplantation: current concepts and challenges. *World J Gastroenterol*. 2008;14:4849–4860.
383. Razonable RR, Emery VC. Management of CMV infection and disease in transplant patients. 27-29 February 2004. *Herpes*. 2004;11:77–86.
384. Clarke AF. A review of environmental and host factors in relation to equine respiratory disease. *Equine Vet J*. 1987;19:435–441.
385. Stewart AJ, Cuming RS. Update on fungal respiratory disease in horses. *Vet Clin North Am Equine Pract*. 2015;31:43–62.
386. Sweeney CR, Habecker PL. Pulmonary aspergillosis in horses: 29 cases (1974-1997). *J Am Vet Med Assoc*. 1999;214:808–811.
387. Guillot J, Sarfati J, de BM, et al. Comparative study of serological tests for the diagnosis of equine aspergillosis. *Vet Rec*. 1999;145:348–349.
388. Slocombe RF, Slauson DO. Invasive pulmonary aspergillosis of horses: an association with acute enteritis. *Vet Pathol*. 1988;25:277–281.
389. Blomme E, Del Piero F, La Perle KM. Aspirgillosis in horses: a review. *Equine Vet Educ*. 1998;10:86–93.
390. Johnson PJ, Moore LA, Mrad DR, et al. Sudden death of two horses associated with pulmonary aspergillosis. *Vet Rec*. 1999;145:16–20.
391. Tunev SS, Ehrhart EJ, Jensen HE, et al. Necrotizing mycotic vasculitis with cerebral infarction caused by Aspergillus niger in a horse with acute typholocolitis. *Vet Pathol*. 1999;36:347–351.
392. Sweeney CR, Habecker PL. Pulmonary aspergillosis in horses: 29 cases (1974-1997). *J Am Vet Med Assoc*. 1999;214:808–811.
393. Toribio RE, Kohn CW, Lawrence AE, et al. Thoracic and abdominal blastomycosis in a horse. *J Am Vet Med Assoc*. 1999;214:1357–1360, 1335.
394. Dolente BA, Habecker P, Chope K. Disseminated blastomycosis in a miniature horse. *Equine Vet Educ*. 2003;15:139–142.
395. Wilson JH, Olson EJ, Haugen EW, et al. Systemic blastomycosis in a horse. *J Vet Diagn Invest*. 2006;18:615–619.
396. Katayama Y, Kuwano A, Yoshihara T. Histoplasmosis in the lung of a race horse with yersiniosis. *J Vet Med Sci*. 2001;63:1229–1231.
397. Rezabek GB, Donahue JM, Giles RC, et al. Histoplasmosis in horses. *J Comp Pathol*. 1993;109:47–55.

398. Rush Moore B, Reed S, Kowalski JJ, et al. Aspergillosis granuloma in the mediastinum of a nonimmunocompromised horse. *Cornell Vet*. 1993;83:97–104.
399. Sweeney CR, Humber KA, Roby KA. Cytologic findings of tracheobronchial aspirates from 66 thoroughbred racehorses. *Am J Vet Res*. 1992;53:1172–1175.
400. Sweeney CR, Beech J, Roby KA. Bacterial isolates from tracheobronchial aspirates of healthy horses. *Am J Vet Res*. 1985;46:2562–2565.
401. Pusterla N, Holmberg TA, Lorenzo-Figueras M, et al. Acremonium strictum pulmonary infection in a horse. *Vet Clin Pathol*. 2005;34:413–416.
402. Flaminio MJ, Rush BR, Cox JH, et al. CD4+ and CD8+ T-lymphocytopenia in a filly with Pneumocystis carinii pneumonia. *Aust Vet J*. 1998;76:399–402.
403. Davis EG, Wilkerson MJ, Rush BR. Flow cytometry: clinical applications in equine medicine. *J Vet Intern Med*. 2002;16:404–410.
404. Perron Lepage MF, Gerber V, Suter MM. A case of interstitial pneumonia associated with Pneumocystis carinii in a foal. *Vet Pathol*. 1999;36:621–624.
405. Franklin RP, Long MT, MacNeill A, et al. Proliferative interstitial pneumonia, Pneumocystis carinii infection, and immunodeficiency in an adult Paso Fino horse. *J Vet Intern Med*. 2002;16:607–611.
406. MacNeill AL, Alleman AR, Franklin RP, et al. Pneumonia in a Paso-Fino mare. *Vet Clin Pathol*. 2003;32:73–76.
407. Perryman LE, McGuire TC, Crawford TB. Maintenance of foals with combined immunodeficiency: causes and control of secondary infections. *Am J Vet Res*. 1978;39:1043–1047.
408. Chandna VK, Morris E, Gliatto JM, et al. Localised subcutaneous cryptococcal granuloma in a horse. *Equine Vet J*. 1993;25:166–168.
409. Begg LM, Hughes KJ, Kessell A, et al. Successful treatment of cryptococcal pneumonia in a pony mare. *Aust Vet J*. 2004;82:686–692.
410. Cornick JL. Diagnosis and treatment of pulmonary histoplasmosis in a horse. *Cornell Vet*. 1990;80:97–103.
411. French DD, Haynes PF, Miller RI. Surgical and medical management of rhinophycomycosis (conidiobolomycosis) in a horse. *J Am Vet Med Assoc*. 1985;186:1105–1107.
412. Zamos DT, Schumacher J, Loy JK. Nasopharyngeal conidiobolomycosis in a horse. *J Am Vet Med Assoc*. 1996;208:100–101.
413. Burks BS. Parastitic Pneumonitis in Horses. *Compend Contin Educ Pract Vet*. 1998;20:378–383.
414. Austin SM, DiPietro JA, Foreman JH, et al. Comparison of the efficacy of ivermectin, oxibendazole, and pyrantel pamoate against 28-day Parascaris equorum larvae in the intestine of pony foals. *J Am Vet Med Assoc*. 1991;198:1946–1949.
415. Pardridge WM. Blood-brain barrier drug targeting enables neuroprotection in brain ischemia following delayed intravenous administration of neurotrophins. *Adv Exp Med Biol*. 2002;513:397–430.
416. DiPietro JA, Todd Jr KS. Anthelmintics used in treatment of parasitic infections of horses. *Vet Clin North Am Equine Pract*. 1987;3:1–14.
417. Costa AJ, Barbosa OF, Moraes FR, et al. Comparative efficacy evaluation of moxidectin gel and ivermectin paste against internal parasites of equines in Brazil. *Vet Parasitol*. 1998;80:29–36.
418. Couetil LL, Cardwell JM, Gerber V, et al. Inflammatory Airway Disease of Horses-Revised Consensus Statement. *J Vet Intern Med*. 2016;30:503–515.
419. Mazan MR. Update on noninfectious inflammatory diseases of the lower airway. *Vet Clin North Am Equine Pract*. 2015;31:159–185.
420. Davis E, Rush BR. Equine recurrent airway obstruction: pathogenesis, diagnosis, and patient management. *Vet Clin North Am Equine Pract*. 2002;18:453–467, vi.
421. Couetil LL, Ward MP. Analysis of risk factors for recurrent airway obstruction in North American horses: 1,444 cases (1990-1999). *J Am Vet Med Assoc*. 2003;223:1645–1650.
422. Gerber V, Bailey E. Genetics and disease in the horse. *Equine Vet J*. 1995;27:400–401.
423. Marti E, Gerber H, Essich G, et al. The genetic basis of equine allergic diseases. 1. Chronic hypersensitivity bronchitis. *Equine Vet J*. 1991;23:457–460.
424. Gerber V, Baleri D, Klukowska-Rotzler J, et al. Mixed inheritance of equine recurrent airway obstruction. *J Vet Intern Med*. 2009;23:626–630.
425. Bosshard S, Gerber V. Evaluation of coughing and nasal discharge as early indicators for an increased risk to develop equine recurrent airway obstruction (RAO). *J Vet Intern Med*. 2014;28:618–623.
426. Derksen FJ, Brown CM, Sonea I, et al. Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. *Equine Vet J*. 1989;21:23–26.
427. Tesarowski DB, Viel L, McDonell WN. Pulmonary function measurements during repeated environmental challenge of horses with recurrent airway obstruction (heaves). *Am J Vet Res*. 1996;57:1214–1219.
428. Robinson NE, Olszewski MA, Boehler D, et al. Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. *Equine Vet J*. 2000;32:393–400.
429. Couetil L, Hammer J, Miskovic FM, et al. Effects of N-butylscopolammonium bromide on lung function in horses with recurrent airway obstruction. *J Vet Intern Med*. 2012;26:1433–1438.
430. Couetil LL, Rosenthal FS, Simpson CM. Forced expiration: a test for airflow obstruction in horses. *J Appl Physiol (1985)*. 2000;88:1870–1879.
431. Pirie RS, McLachlan G, McGorum BC. Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves. 1: preparation and composition of HDS. *Equine Vet J*. 2002;34:332–336.
432. Pirie RS, Dixon PM, McGorum BC. Endotoxin contamination contributes to the pulmonary inflammatory and functional response to Aspergillus fumigatus extract inhalation in heaves horses. *Clin Exp Allergy*. 2003;33:1289–1296.
433. Ivester KM, Couetil LL, Moore GE, et al. Environmental exposures and airway inflammation in young thoroughbred horses. *J Vet Intern Med*. 2014;28:918–924.
434. Millerick-May ML, Karmaus W, Derksen FJ, et al. Local airborne particulate concentration is associated with visible tracheal mucus in Thoroughbred racehorses. *Equine Vet J*. 2013;45:85–90.
435. Ainsworth DM, Grunig G, Matychak MB, et al. Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. *Vet Immunol Immunopathol*. 2003;96:83–91.
436. Cordeau ME, Joubert P, Dewachi O, et al. IL-4, IL-5 and IFN-gamma mRNA expression in pulmonary lymphocytes in equine heaves. *Vet Immunol Immunopathol*. 2004;97:87–96.
437. Chen CL, Goldberg J, Gronwall RR. Pharmacokinetics of intravenously administration of prednisolone in the horse as determined by radioimmunoassay. *Chin J Physiol*. 1995;38:1–6.
438. French K, Pollitt CC, Pass MA. Pharmacokinetics and metabolic effects of triamcinolone acetonide and their possible relationships to glucocorticoid-induced laminitis in horses. *J Vet Pharmacol Ther*. 2000;23:287–292.
439. Rush BR, Worster AA, Flaminio MJ, et al. Alteration in adrenocortical function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. *Am J Vet Res*. 1998;59:1044–1047.

440. Courouce-Malblanc A, Fortier G, Pronost S, et al. Comparison of prednisolone and dexamethasone effects in the presence of environmental control in heaves-affected horses. *Vet J*. 2008;175:227–233.
441. Peroni DL, Stanley S, Kollias-Baker C, et al. Prednisone per os is likely to have limited efficacy in horses. *Equine Vet J*. 2002;34:283–287.
442. Gray PR, Derksen FJ, Robinson NE, et al. The role of cyclooxygenase products in the acute airway obstruction and airway hyperreactivity of ponies with heaves. *Am Rev Respir Dis*. 1989;140:154–160.
443. Rush BR, Raub ES, Rhoads WS, et al. Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. *Am J Vet Res*. 1998;59:1039–1043.
444. Bertin FR, Ivester KM, Couetil LL. Comparative efficacy of inhaled albuterol between two hand-held delivery devices in horses with recurrent airway obstruction. *Equine Vet J*. 2011;43:393–398.
445. Couetil LL, Chilcoat CD, DeNicola DB, et al. Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction. *Am J Vet Res*. 2005;66:1665–1674.
446. Dauvillier J, Felippe MJ, Lunn DP, et al. Effect of long-term fluticasone treatment on immune function in horses with heaves. *J Vet Intern Med*. 2011;25:549–557.
447. Giguere S, Viel L, Lee E, et al. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. *Vet Immunol Immunopathol*. 2002;85:147–158.
448. Couetil L, Hammer J, Miskovic FM, et al. Effects of N-butylscopolammonium bromide on lung function in horses with recurrent airway obstruction. *J Vet Intern Med*. 2012;26:1433–1438.
449. de LM, Rodrigues N, Chevigny M, et al. N-butylscopolammonium bromide causes fewer side effects than atropine when assessing bronchoconstriction reversibility in horses with heaves. *Equine Vet J*. 2014;46:474–478.
450. Seahorn TL, Beadle RE, McGorum BC, et al. Quantification of antigen-specific antibody concentrations in tracheal lavage fluid of horses with summer pasture-associated obstructive pulmonary disease. *Am J Vet Res*. 1997;58:1408–1411.
451. Mair TS. Obstructive pulmonary disease in 18 horses at summer pasture. *Vet Rec*. 1996;138:89–91.
452. Seahorn TL, Groves MG, Harrington KS, et al. Chronic obstructive pulmonary disease in horses in Louisiana. *J Am Vet Med Assoc*. 1996;208:248–251.
453. Costa LR, Johnson JR, Baur ME, et al. Temporal clinical exacerbation of summer pasture-associated recurrent airway obstruction and relationship with climate and aeroallergens in horses. *Am J Vet Res*. 2006;67:1635–1642.
454. Ward MP, Couetil LL. Climatic and aeroallergen risk factors for chronic obstructive pulmonary disease in horses. *Am J Vet Res*. 2005;66:818–824.
455. Christley RM, Hodgson DR, Rose RJ, et al. A case-control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. *Equine Vet J*. 2001;33:256–264.
456. Sanchez A, Couetil LL, Ward MP, et al. Effect of airway disease on blood gas exchange in racehorses. *J Vet Intern Med*. 2005;19:87–92.
457. Couetil LL, Rosenthal FS, DeNicola DB, et al. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am J Vet Res*. 2001;62:538–546.
458. Couetil LL, DeNicola DB. Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease. *Equine Vet J Suppl*. 1999:77–82.
459. Bedenice D, Mazan MR, Hoffman AM. Association between cough and cytology of bronchoalveolar lavage fluid and pulmonary function in horses diagnosed with inflammatory airway disease. *J Vet Intern Med*. 2008;22:1022–1028.
460. Richard EA, Fortier GD, Denoix JM, et al. Influence of subclinical inflammatory airway disease on equine respiratory function evaluated by impulse oscillometry. *Equine Vet J*. 2009;41:384–389.
461. Robinson NE, Berney C, Eberhart S, et al. Coughing, mucus accumulation, airway obstruction, and airway inflammation in control horses and horses affected with recurrent airway obstruction. *Am J Vet Res*. 2003;64:550–557.
462. Fogarty U, Buckley T. Bronchoalveolar lavage findings in horses with exercise intolerance. *Equine Vet J*. 1991;23:434–437.
463. Moore BR, Krakowka S, Robertson JT, et al. Cytologic evaluation of bronchoalveolar lavage fluid obtained from standardbred racehorses with inflammatory airway disease. *Am J Vet Res*. 1995;56:562–567.
464. Fraipont A, van EE, Ramery E, et al. Subclinical diseases underlying poor performance in endurance horses: diagnostic methods and predictive tests. *Vet Rec*. 2011;169:154.
465. Malikides N, Hughes KJ, Hodgson DR, et al. Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses. 2. Evaluation of the diagnostic significance of neutrophil percentage. *Aust Vet J*. 2003;81:685–687.
466. Hare JE, Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. *J Vet Intern Med*. 1998;12:163–170.
467. Ivester KM, Couetil LL. Management of chronic airway inflammation in the horse: a systematic review. *Equine Vet Educ*. 2014;26:647–656.
468. Courouce-Malblanc A, Fortier G, Pronost S, et al. Comparison of prednisolone and dexamethasone effects in the presence of environmental control in heaves-affected horses. *Vet J*. 2008;175:227–233.
469. Picandet V, Leguillette R, Lavoie JP. Comparison of efficacy and tolerability of isoflupredone and dexamethasone in the treatment of horses affected with recurrent airway obstruction ('heaves'). *Equine Vet J*. 2003;35:419–424.
470. Rush BR, Worster AA, Flaminio MJ, et al. Alteration in adrenocortical function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. *Am J Vet Res*. 1998;59:1044–1047.
471. Laan TT, Bull S, van Nieuwstadt RA, et al. The effect of aerosolized and intravenously administered clenbuterol and aerosolized fluticasone propionate on horses challenged with Aspergillus fumigatus antigen. *Vet Res Commun*. 2006;30:623–635.
472. Read JR, Boston RC, Abraham G, et al. Effect of prolonged administration of clenbuterol on airway reactivity and sweating in horses with inflammatory airway disease. *Am J Vet Res*. 2012;73:140–145.
473. Henrikson SL, Rush BR. Efficacy of salmeterol xinafoate in horses with recurrent airway obstruction. *J Am Vet Med Assoc*. 2001;218:1961–1965.
474. Hinchcliff KW, Couetil LL, Knight PK, et al. Exercise induced pulmonary hemorrhage in horses: American College of Veterinary Internal Medicine consensus statement. *J Vet Intern Med*. 2015;29:743–758.
475. Pascoe JR, Ferraro GL, Cannon JH, et al. Exercise-induced pulmonary hemorrhage in racing thoroughbreds: a preliminary study. *Am J Vet Res*. 1981;42:703–707.
476. Raphel CF, Soma LR. Exercise-induced pulmonary hemorrhage in Thoroughbreds after racing and breezing. *Am J Vet Res*. 1982;43:1123–1127.
477. Hinchcliff KW, Jackson MA, Morley PS, et al. Association between exercise-induced pulmonary hemorrhage and performance in Thoroughbred racehorses. *J Am Vet Med Assoc*. 2005;227:768–774.

478. Hinchcliff KW, Morley PS, Guthrie AJ. Efficacy of furosemide for prevention of exercise-induced pulmonary hemorrhage in Thoroughbred racehorses. *J Am Vet Med Assoc*. 2009;235: 76–82.
479. Sweeney CR, Soma LR, Maxson AD, et al. Effects of furosemide on the racing times of Thoroughbreds. *Am J Vet Res*. 1990;51:772–778.
480. Voynick BT, Sweeney CR. Exercised-induced pulmonary hemorrhage in polo and racing horses. *J Am Vet Med Assoc*. 1986;188:301–302.
481. West JB, Mathieu-Costello O, Jones JH, et al. Stress failure of pulmonary capillaries in racehorses with exercise-induced pulmonary hemorrhage. *J Appl Physiol (1985)*. 1993;75: 1097–1109.
482. Manohar M, Goetz TE. Pulmonary vascular pressures of strenuously exercising Thoroughbreds during intravenous infusion of nitroglycerin. *Am J Vet Res*. 1999;60:1436–1440.
483. Manohar M, Goetz TE. Pulmonary vascular pressures of exercising thoroughbred horses with and without endoscopic evidence of EIPH. *J Appl Physiol (1985)*. 1996;81:1589–1593.
484. Birks EK, Mathieu-Costello O, Fu Z, et al. Very high pressures are required to cause stress failure of pulmonary capillaries in thoroughbred racehorses. *J Appl Physiol (1985)*. 1997;82: 1584–1592.
485. Stack A, Derksen FJ, Sordillo LM, et al. Effects of exercise on markers of venous remodeling in lungs of horses. *Am J Vet Res*. 2013;74:1231–1238.
486. O'Callaghan MW, Pascoe JR, Tyler WS, et al. Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VIII. Conclusions and implications. *Equine Vet J*. 1987;19:428–434.
487. O'Callaghan MW, Hornof WJ, Fisher PE, et al. Exercise-induced pulmonary haemorrhage in the horses: results of a detailed clinical, post mortem and imaging study. VII. Ventilation/perfusion scintigraphy in horses with EIPH. *Equine Vet J*. 1987;19:423–427.
488. Williams KJ, Derksen FJ, De Feijter-Rupp H, et al. Regional pulmonary veno-occlusion: a newly identified lesion of equine exercise-induced pulmonary hemorrhage. *Vet Pathol*. 2008;45:316–326.
489. Derksen FJ, Williams KJ, Uhal BD, et al. Pulmonary response to airway instillation of autologous blood in horses. *Equine Vet J*. 2007;39:334–339.
490. McKane SA, Slocombe RF. Sequential changes in bronchoalveolar cytology after autologous blood inoculation. *Equine Vet J Suppl*. 1999:126–130.
491. McKane SA, Slocombe RF. Alveolar fibrosis and changes in equine lung morphometry in response to intrapulmonary blood. *Equine Vet J Suppl*. 2002:451–458.
492. Williams KJ, Derksen FJ, Defeijter-Rupp HL, et al. Repeated blood instillation into the airway of the horse does not cause pulmonary fibrosis. *Equine Vet J*. 2011;43:354–358.
493. Christley RM, Hodgson DR, Rose RJ, et al. Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. *Vet Rec*. 2001;148:99–104.
494. Chapman PS, Green C, Main JP, et al. Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Vet Rec*. 2000;146:91–95.
495. Speirs VC, van Veenendaal JC, Harrison IW, et al. Pulmonary haemorrhage in standardbred horses after racing. *Aust Vet J*. 1982;59:38–40.
496. Hillidge CJ, Whitlock TW. Sex variation in the prevalence of exercise-induced pulmonary haemorrhage in racing quarter horses. *Res Vet Sci*. 1986;40:406–407.
497. Doucet MY, Viel L. Clinical, radiographic, endoscopic, bronchoalveolar lavage and lung biopsy findings in horses with exercise-induced pulmonary hemorrhage. *Can Vet J*. 2002;43: 195–202.
498. Takahashi T, Hiraga A, Ohmura H, et al. Frequency of and risk factors for epistaxis associated with exercise-induced pulmonary hemorrhage in horses: 251,609 race starts (1992-1997). *J Am Vet Med Assoc*. 2001;218:1462–1464.
499. Furuoka H, Taniyama H, Matsui T, et al. Malignant thymoma with multiple metastases in a mare, Nippon Juigaku. *Zasshi*. 1987;49:577–579.
500. Boden LA, Charles JA, Slocombe RF, et al. Sudden death in racing Thoroughbreds in Victoria, Australia. *Equine Vet J*. 2005;37:269–271.
501. Lyle CH, Uzal FA, McGorum BC, et al. Sudden death in racing Thoroughbred horses: an international multicentre study of post mortem findings. *Equine Vet J*. 2011;43:324–331.
502. Sullivan SL, Anderson GA, Morley PS, et al. Prospective study of the association between exercise-induced pulmonary haemorrhage and long-term performance in Thoroughbred racehorses. *Equine Vet J*. 2015;47:350–357.
503. de Mello Costa MF, Thomassian A, Gomes TS. Study of exercise induced pulmonary hemorrhage (EIPH) in flat racing Thoroughbred horses through 1889 respiratory endoscopies after races. *Rev Bras Cien Vet*. 2005;12:89–91.
504. Hinchcliff KW, Jackson MA, Brown JA, et al. Tracheobronchoscopic assessment of exercise-induced pulmonary hemorrhage in horses. *Am J Vet Res*. 2005;66:596–598.
505. McKane SA, Canfield PJ, Rose RJ. Equine bronchoalveolar lavage cytology: survey of thoroughbred racehorses in training. *Aust Vet J*. 1993;70:401–404.
506. Hinchcliff KW. Counting red cells—is it an answer to EIPH? *Equine Vet J*. 2000;32:362–363.
507. Doucet MY, Viel L. Alveolar macrophage graded hemosiderin score from bronchoalveolar lavage in horses with exercise-induced pulmonary hemorrhage and controls. *J Vet Intern Med*. 2002;16:281–286.
508. Manohar M, Goetz TE, Rothenbaum P, et al. Clenbuterol administration does not enhance the efficacy of furosemide in attenuating the exercise-induced pulmonary capillary hypertension in Thoroughbred horses. *J Vet Pharmacol Ther*. 2000;23:389–395.
509. Manohar M, Goetz TE, Sullivan E, et al. Pulmonary vascular pressures of strenuously exercising Thoroughbreds after administration of varying doses of frusemide. *Equine Vet J*. 1997;29:298–304.
510. Manohar M. Furosemide attenuates the exercise-induced increase in pulmonary artery wedge pressure in horses. *Am J Vet Res*. 1993;54:952–958.
511. Manohar M, Goetz TE, Rothenbaum P, et al. Intravenous pentoxifylline does not enhance the pulmonary haemodynamic efficacy of frusemide in strenuously exercising thoroughbred horses. *Equine Vet J*. 2001;33:354–359.
512. Magid JH, Manohar M, Goetz TE, et al. Pulmonary vascular pressures of thoroughbred horses exercised 1, 2, 3 and 4 h after furosemide administration. *J Vet Pharmacol Ther*. 2000;23: 81–89.
513. Gleed FD, Ducharme NG, Hackett RP, et al. Effects of frusemide on pulmonary capillary pressure in horses exercising on a treadmill. *Equine Vet J Suppl*. 1999:102–106.
514. Manohar M. Pulmonary vascular pressures of strenuously exercising thoroughbreds after administration of flunixin meglumine and furosemide. *Am J Vet Res*. 1994;55:1308–1312.
515. Olsen SC, Coyne CP, Lowe BS, et al. Influence of furosemide on hemodynamic responses during exercise in horses. *Am J Vet Res*. 1992;53:742–747.
516. Buchholz BM, Murdock A, Bayly WM, et al. Effects of intravenous aminocaproic acid on exercise-induced pulmonary haemorrhage (EIPH). *Equine Vet J Suppl*. 2010:256–260.
517. Epp TS, Edwards KL, Poole DC. Effects of conjugated oestrogens and aminocaproic acid upon exercise induced pulmonary hemorrhage (EIPH). *Compative Exerc Physiol*. 2008;5:95–103.

518. Manohar M, Goetz TE, Rothenbaum P, et al. Clenbuterol administration does not attenuate the exercise-induced pulmonary arterial, capillary or venous hypertension in strenuously exercising Thoroughbred horses. *Equine Vet J*. 2000;32:546–550.
519. Walker HJ, Evans DL, Slocombe RF, et al. Effect of corticosteroid and bronchodilator therapy on bronchoalveolar lavage cytology following intrapulmonary blood inoculation. *Equine Vet J Suppl*. 2006:516–522.
520. Sweeney CR, Soma LR, Bucan CA, et al. Exercise-induced pulmonary hemorrhage in exercising Thoroughbreds: preliminary results with pre-exercise medication. *Cornell Vet*. 1984;74: 263–268.
521. Manohar M, Goetz TE, Hassan AS, et al. Anti-inflammatory agent, dexamethasone, does not affect exercise-induced arterial hypoxemia in Thoroughbreds. *J Appl Physiol (1985)*. 2002;93:99–106.
522. Manohar M, Goetz TE, Griffin R, et al. Pulmonary vascular pressures of strenuously exercising thoroughbreds after administration of phenylbutazone. *Am J Vet Res*. 1996;57:1354–1358.
523. Manohar M, Goetz TE, Rothenbaum P, et al. Intravenous pentoxifylline does not affect the exercise-induced pulmonary arterial, capillary or venous hypertension in Thoroughbred horses. *J Vet Pharmacol Ther*. 2000;23:317–322.
524. Geor RJ, Ommundson L, Fenton G, et al. Effects of an external nasal strip and frusemide on pulmonary haemorrhage in Thoroughbreds following high-intensity exercise. *Equine Vet J*. 2001;33:577–584.
525. Kindig CA, McDonough P, Fenton G, et al. Efficacy of nasal strip and furosemide in mitigating EIPH in Thoroughbred horses. *J Appl Physiol (1985)*. 2001;91:1396–1400.
526. Kindig CA, Poole DC, McDonough P, et al. Nasal strips and EIPH in the exercising Thoroughbred racehorse. *J Appl Physiol (1985)*. 2001;91:1908–1910.
527. Valdez SC, Nieto JE, Spier SJ, et al. Effect of an external nasal dilator strip on cytologic characteristics of bronchoalveolar lavage fluid in Thoroughbred racehorses. *J Am Vet Med Assoc*. 2004;224:558–561.
528. Sweeney CR, Gillette DM. Thoracic neoplasia in equids: 35 cases (1967-1987). *J Am Vet Med Assoc*. 1989;195:374–377.
529. Mair T, Rush BR, Tucker RL. Clinical and diagnostic features of thoracic neoplasia in the horse. *Equine Vet Educ*. 2004;16:30–36.
530. Misdorp W, Nauta-van Gelder HL. Granular-cell myoblastoma' in the horse. A report of 4 cases *Pathol Vet*. 1968;5:385–394.
531. Parker GA, Novilla MN, Brown AC, et al. Granular cell tumour (myeloblastoma) in the lung of a horse. *J Comp Pathol*. 1979;89:421–430.
532. Nickels FA, Brown CM, Breeze RG. Myoblastoma. Equine granular cell tumor. *Mod Vet Pract*. 1980;61:593–596.
533. Turk RHH, Breeze RG. Histochemical and ultrastructural features of an equine pulmonary granular cell tumour. *J Comp Pathol*. 1981;91:471–481.
534. Scarratt WK, Crisman MV, Sponenberg DP, et al. Pulmonary granular cell tumour in 2 horses. *Equine Vet J*. 1993;25:244–247.
535. Sutton RH, Coleman GT. A pulmonary granular cell tumour with associated hypertrophic osteopathy in a horse. *N Z Vet J*. 1995;43:123.
536. Goodchild LM, Dart AJ, Collins MB, et al. Granular cell tumour in the bronchus of a horse. *Aust Vet J*. 1997;75:16–18.
537. Pusterla N, Pesavento PA, Smith P, et al. Idiopathic granulomatous pneumonia in seven horses. *Vet Rec*. 2003;153:653–655.
538. Bouchard PR, Fortna CH, Rowland PH, et al. An immunohistochemical study of three equine pulmonary granular cell tumors. *Vet Pathol*. 1995;32:730–734.
539. Alexander JE, Keown GH, Palotay JL. Granular cell myoblastoma with hypertrophic pulmonary osteoarthropathy in a mare. *J Am Vet Med Assoc*. 1965;146:703–708.
540. Mair TS, Dyson SJ, Fraser JA, et al. Hypertrophic osteopathy (Marie's disease) in equidae: a review of twenty-four cases. *Equine Vet J*. 1996;28:256–262.
541. Heinola T, Heikkila M, Ruohoniemi M, et al. Hypertrophic pulmonary osteopathy associated with granular cell tumour in a mare. *Vet Rec*. 2001;149:307–308.
542. Smith BL, Aguilera-Tejero E, Tyler WS, et al. Endoscopic anatomy and map of the equine bronchial tree. *Equine Vet J*. 1994;26:283–290.
543. Kelley LC, Hill JE, Hafner S, et al. Spontaneous equine pulmonary granular cell tumors: morphologic, histochemical, and immunohistochemical characterization. *Vet Pathol*. 1995;32:101–106.
544. Facemire PR, Chilcoat CD, Sojka JE, et al. Treatment of granular cell tumor via complete right lung resection in a horse. *J Am Vet Med Assoc*. 2000;217:1522–1525.
545. Ohnesorge B, Gehlen H, Wohlsein P. Transendoscopic electrosurgery of an equine pulmonary granular cell tumor. *Vet Surg*. 2002;31:375–378.
546. Dadmanesh F, Sekihara T, Rosai J. Histologic typing of thymoma according to the new World Health Organization classification. *Chest Surg Clin N Am*. 2001;11:407–420.
547. Shahriar F, Moore J. Thymic epithelial tumor with heart metastasis in a horse. *Vet Med Int*. 2010;2010.
548. Migaki G. Hematopoietic neoplasms of slaughter animals. *Natl Cancer Inst Monogr*. 1969;32:121–151.
549. Whiteley LO, Leininger JR, Wolf CB, et al. Malignant squamous cell thymoma in a horse. *Vet Pathol*. 1986;23:627–629.
550. Dill SG, Moise NS, Meschter CL. Cardiac failure in a stallion secondary to metastasis of an anaplastic pulmonary carcinoma. *Equine Vet J*. 1986;18:414–417.
551. Uphoff CS, Lyncoln JA. A primary pulmonary tumour in a horse. *Equine Pract*. 1987;9:19–20.
552. van Rensburg IB, Stadler P, Soley J. Bronchiolo-alveolar adenocarcinoma in a horse. *J S Afr Vet Assoc*. 1989;60:212–214.
553. Anderson JD, Leonard JM, Zeliff JA, et al. Primary pulmonary neoplasm in a horse. *J Am Vet Med Assoc*. 1992;201:1399–1401.
554. Mair TS, Brown PJ. Clinical and pathological features of thoracic neoplasia in the horse. *Equine Vet J*. 1993;25:220–223.
555. Schultze AE, Sonea I, Bell TG. Primary malignant pulmonary neoplasia in two horses. *J Am Vet Med Assoc*. 1988;193:477–480.
556. Murphy JR, Breeze RG, McPherson EA. Myxoma of the equine respiratory tract. *Mod Vet Pract*. 1978;59:529–532.
557. Clem MF, O'Brien TD, Christopher MM. Pulmonary chondrosarcoma in a horse. *Compend Contin Educ Pract Vet*. 1986;8:964–969.
558. Rossdale PD, Greet TR, McGladdery AJ. Pulmonary leiomyosarcoma in a 13-year-old Thoroughbred stallion presenting as a differential diagnosis recurrent airway obstruction (RAO). *Equine Vet Educ*. 2004;16:21–28.
559. Perez-Ecija RA, Mendoza FJ, Zafra R, et al. Clinical, pathological and immunohistochemical features of a pulmonary blastoma in a horse. *Vet Rec*. 2009;164:182–183.
560. Straub R, von TC, Pauli B, et al. Pleural mesothelioma in a horse. *Schweiz Arch Tierheilkd*. 1974;116:207–211.
561. Kramer JW, Nickels FA, Bell T. Cytology of diffuse mesothelioma in the thorax of a horse. *Equine Vet J*. 1976;8:81–83.
562. Carnine BL, Schneider G, Cook JE, et al. Pericardial mesothelioma in a horse. *Vet Pathol*. 1977;14:513–515.
563. Wallace SS, Jayo MJ, Maddux JM, et al. Mesothelioma in a horse. *Compend Contin Educ Pract Vet*. 1987;9:210–216.
564. Colbourne CM, Bolton JR, Mills JN, et al. Mesothelioma in horses. *Aust Vet J*. 1992;69:275–278.
565. Mair T, Hillyer MH, Brown PJ. Mesothelioma of the pleural cavity in a horse: diagnostic features. *Equine Vet Educ*. 1992;4:59–61.
566. Frye FL, Knight HD, Brown SI. Hemangiosarcoma in a horse. *J Am Vet Med Assoc*. 1983;182:287–289.
567. Fry MM, Magdesian KG, Judy CE, et al. Antemortem diagnosis of equine mesothelioma by pleural biopsy. *Equine Vet J*. 2003;35:723–727.
568. Cotchin E. A general survey of tumours in the horse. *Equine Vet J*. 1977;9:16–21.

569. East LM, Savage CJ. Abdominal neoplasia (excluding urogenital tract). *Vet Clin North Am Equine Pract*. 1998;14:475–4vi.
570. Neufeld JL. Lymphosarcoma in a mare and review of cases at the Ontario Veterinary College. *Can Vet J*. 1973;14:149–153.
571. Neufeld JL. Lymphosarcoma in the horse: a review. *Can Vet J*. 1973;14:129–135.
572. Platt H. Observations on the pathology of non-alimentary lymphomas in the horse. *J Comp Pathol*. 1988;98:177–194.
573. van den Hoven R, Franken P. Clinical aspects of lymphosarcoma in the horse: a clinical report of 16 cases. *Equine Vet J*. 1983;15:49–53.
574. Reef VB, Dyson SS, Beech J. Lymphosarcoma and associated immune-mediated hemolytic anemia and thrombocytopenia in horses. *J Am Vet Med Assoc*. 1984;184:313–317.
575. Rebhun WC, Bertone A. Equine lymphosarcoma. *J Am Vet Med Assoc*. 1984;184:720–721.
576. Adams R, Calderwood-Mays MB, Peyton LC. Malignant lymphoma in three horses with ulcerative pharyngitis. *J Am Vet Med Assoc*. 1988;193:674–676.
577. Meyer J, DeLay J, Bienzle D. Clinical, laboratory, and histopathologic features of equine lymphoma. *Vet Pathol*. 2006;43:914–924.
578. Mendes LCN, de Araujo MA, Bovino F, et al. Clinical, histological and immunophenotypic findins in a mare with ammary lymphoma associated with anemia and pruritis. *Equine Vet Educ*. 2011;23:177–183.
579. Finley MR, Rebhun WC, Dee A, et al. Paraneoplastic pruritus and alopecia in a horse with diffuse lymphoma. *J Am Vet Med Assoc*. 1998;213:102–104.
580. Keen JA, Swain JM, Rhind SM, et al. Lymphoproliferative disease resembling lymphomatoid granulomatosis in a thoroughbred mare. *J Vet Intern Med*. 2004;18:904–906.
581. Liebow AA, Carrington CR, Friedman PJ. Lymphomatoid granulomatosis. *Hum Pathol*. 1972;3:457–558.
582. Lucke VM, Kelly DF, Harrington GA, et al. A lymphomatoid granulomatosis of the lungs in young dogs. *Vet Pathol*. 1979;16:405–412.
583. Postorino NC, Wheeler SL, Park RD, et al. A syndrome resembling lymphomatoid granulomatosis in the dog. *J Vet Intern Med*. 1989;3:15–19.
584. Berry CR, Moore PF, Thomas WP, et al. Pulmonary lymphomatoid granulomatosis in seven dogs (1976-1987). *J Vet Intern Med*. 1990;4:157–166.
585. Leblanc B, Masson MT, Andreu M, et al. Lymphomatoid granulomatosis in a beagle dog. *Vet Pathol*. 1990;27:287–289.
586. Fitzgerald SD, Wolf DC, Carlton WW. Eight cases of canine lymphomatoid granulomatosis. *Vet Pathol*. 1991;28:241–245.
587. Valentine BA, Blue JT, Zimmer JF, et al. Pulmonary lymphomatoid granulomatosis in a cat. *J Vet Diagn Invest*. 2000;12:465–467.
588. Roberts MC. Equine lymphoma: What are the prospects for cellular differentiation, early daignosis and intervention strategies? *Equine Vet Educ*. 2008;20:464–466.
589. Kelley LC, Mahaffey EA. Equine malignant lymphomas: morphologic and immunohistochemical classification. *Vet Pathol*. 1998;35:241–252.
590. Ross JS. DNA ploidy and cell cycle analysis in cancer diagnosis and prognosis. *Oncology (Williston Park)*. 1996;10:867–882, 887.
591. Scarratt WK, Crisman MV. Neoplasia of the respiratory tract. *Vet Clin North Am Equine Pract*. 1998;14:451–473, v.
592. Southwood LL, Schott HC, Henry CJ, et al. Disseminated hemangiosarcoma in the horse: 35 cases. *J Vet Intern Med*. 2000;14:105–109.
593. Valentine BA, Weinstock D. Metastatic testicular embryonal carcinoma in a horse. *Vet Pathol*. 1986;23:92–96.
594. Johnson JE, Beech J, Saik JE. Disseminated hemangiosarcoma in a horse. *J Am Vet Med Assoc*. 1988;193:1429–1431.
595. Rossier Y, Sweeney CR, Heyer G, et al. Pleuroscopic diagnosis of disseminated hemangiosarcoma in a horse. *J Am Vet Med Assoc*. 1990;196:1639–1640.
596. Jean D, Lavoie JP, Nunez L, et al. Cutaneous hemangiosarcoma with pulmonary metastasis in a horse. *J Am Vet Med Assoc*. 1994;204:776–778.
597. Johns I, Stephen JO, Del PF, et al. Hemangiosarcoma in 11 young horses. *J Vet Intern Med*. 2005;19:564–570.
598. Prater PE, Patton CS, Held JP. Pleural effusion resulting from malignant hepatoblastoma in a horse. *J Am Vet Med Assoc*. 1989;194:383–385.
599. East LM, Steyn PF, Dickinson CE, et al. Occult osseous metastasis of a colonic adenocarcinoma visualized with technetium tc 99m hydroxymethylene diphosphate scintigraphy in a horse. *J Am Vet Med Assoc*. 1998;213:1167–1173.
600. Ford TS, Vaala WE, Sweeney CR, et al. Pleuroscopic diagnosis of gastroesophageal squamous cell carcinoma in a horse. *J Am Vet Med Assoc*. 1987;190:1556–1558.
601. Jorgensen JS, Geoly FJ, Berry CR, et al. Lameness and pleural effusion associated with an aggressive fibrosarcoma in a horse. *J Am Vet Med Assoc*. 1997;210:1328–1331.
602. Murray MJ, Cavey DM, Feldman BF, et al. Signs of sympathetic denervation associated with a thoracic melanoma in a horse. *J Vet Intern Med*. 1997;11:199–203.
603. MacGillivray KC, Sweeney RW, Del PF. Metastatic melanoma in horses. *J Vet Intern Med*. 2002;16:452–456.
604. Tan RH, Crisman MV, Clark SP, et al. Multicentric mastocytoma in a horse. *J Vet Intern Med*. 2007;21:340–343.
605. Peroni JF, Robinson NE, Stick JA, et al. Pleuropulmonary and cardiovascular consequences of thoracoscopy performed in healthy standing horses. *Equine Vet J*. 2000;32:280–286.
606. Vachon AM, Fischer AT. Thoracoscopy in the horse: diagnostic and therapeutic indications in 28 cases. *Equine Vet J*. 1998;30:467–475.
607. Hollis AR. Paraneoplastic syndromes. *Equine Vet Educ*. 2011;23:184–185.
608. Blackman MR, Rosen SW, Weintraub BD. Ectopic hormones. *Adv Intern Med*. 1978;23:85–113.
609. Dascanio JJ, Zhang CH, Antczak DF, et al. Differentiation of chronic lymphocytic leukemia in the horse. A report of two cases. *J Vet Intern Med*. 1992;6:225–229.
610. Weir EC, Burtis WJ, Morris CA, et al. Isolation of 16,000-dalton parathyroid hormone-like proteins from two animal tumors causing humoral hypercalcemia of malignancy. *Endocrinology*. 1988;123:2744–2751.
611. Weir EC, Norrdin RW, Barthold SW, et al. Primary hyperparathyroidism in a dog: biochemical, bone histomorphometric, and pathologic findings. *J Am Vet Med Assoc*. 1986;189:1471–1474.
612. Jaeschke G, Rudolph R. Clinical diagnostic keys and special manifestations in equine leukosis. *Berl Munch Tierarztl Wochenschr*. 1991;104:303–307.
613. Jaeschke G, Rudolph R. Clinical chemistry in leukosis of horses (review). *Berl Munch Tierarztl Wochenschr*. 1992;105:114–122.
614. Brodey RS. Hypertrophic osteoarthropathy in the dog: a clinicopathologic survey of 60 cases. *J Am Vet Med Assoc*. 1971;159:1242–1256.
615. Brodey RS, Craig PH, Rhodes WH. Hypertrophic osteoarthropathy in a dog with pulmonary metastases arising from a renal adenocarcinoma. *J Am Vet Med Assoc*. 1958;132:232–237.
616. Fawthrop FW, Russell RGG. Hypertrophic pulmonary osteoarthropathy. In: Nordin BEC, Need AG, Morris HA, eds. *Metabolic Bone and Stone Disease*. 3rd ed. Edinburgh: Churchill Livingstone; 1993;330.
617. Mair TS, Tucker AL. Hypertrophic osteopathy (Marie's disease) in horses. *Equine Vet Educ*. 2004;16:308–311.
618. Sweeney CR, Stebbins KE, Schelling CG, et al. Hypertrophic osteopathy in a pony with a pituitary adenoma. *J Am Vet Med Assoc*. 1989;195:103–105.
619. Leung FW, Williams AJ, Fan P. Indomethacin therapy for hypertrophic pulmonary osteoarthropathy in patients with bronchogenic carcinoma. *West J Med*. 1985;142:345–347.

CHAPTER 9

Disorders of the Cardiovascular System

Colin C. Schwarzwald*

Cardiovascular (CV) system function is critical to exercise, thermoregulation, and the blood flow–dependent functions of the brain, heart, lungs, kidneys, gut, and reproductive system. Heart and vascular diseases are common in horses, but fortunately the underlying lesion is often minor and well tolerated. However, clinically significant CV disease can develop in horses with clinical signs that can include arrhythmia, exercise intolerance, congestive heart failure, weakness or collapse, systemic infection, or sudden death.

The clinical evaluation of the equine CV system is often perplexing. Horses are renowned for a variety of physiologic murmurs and arrhythmias. Furthermore, the equine CV system has an enormous compensatory capacity, and the limits of normal CV function can only be reached by horses undergoing exercise at the highest levels. Clinical assessment is best served by an awareness of normal variation, the appreciation of relevant diseases, diagnostic studies and prognostic indicators, and an understanding of available management options. Communication of these issues constitutes the focus of this chapter.

Equine cardiology has advanced from a study of physiologic variation and speculation to one of accurate diagnosis and focused therapy, though many clinical issues are unresolved. Much of the important groundwork in clinical equine cardiology can be attributed to the studies of Detweiler and colleagues,[1-4] Hamlin, Smith, and Smetzer,[5-20] and Holmes and his trainees.[21-51] The initial information provided by these cardiologists regarding normal CV physiology, cardiac catheterization, pathology, cardiac auscultation, and electrocardiography was pivotal, and we still make clinical decisions based on data these groups provided. There has been steady progress in cardiac assessment by many other investigators over the past decades. Many of these advances relate to the various modalities of echocardiography, which (along with auscultation) is the most important of available diagnostic studies. Other examinations of importance to CV assessment include ambulatory electrocardiography, functional exercise testing, and biochemical tests of cardiac injury. Appropriate selection and interpretation of these tests allows the clinician to identify and quantify most diseases of the heart and circulation.

A variety of sources are available providing a comprehensive overview of the current knowledge of CV diseases in horses.[52-67] A recent consensus statement formulated by a group of experts in the field of equine CV medicine summarizes the current recommendations for management of equine athletes with CV abnormalities.[68] These recommendations are included in the respective sections later in this chapter.

Heart murmurs and arrhythmias are commonly detected in horses, even in the absence of clinical signs of CV disease.[69-82] A variety of studies describe the prevalence of CV disease in horses, indicating that mitral regurgitation, aortic regurgitation, tricuspid regurgitation, and atrial fibrillation are the most common cardiac abnormalities detected.[33,83,84] Else and Holmes noted myocardial fibrosis in 14.3% of horses examined at necropsy and evidence of chronic valvular disease in approximately 25% of the hearts examined.[27-29] Various CV lesions were considered important in 8.5% of 480 consecutive losses in a necropsy study conducted by Baker and Ellis.[85] Cardiovascular diseases are probably the third most common cause of poor performance following musculoskeletal and respiratory diseases.[86-90] Occult heart disease, cardiac arrhythmias, and vascular lesions are considered important reasons for unexplained sudden death.[85,91-106] Certainly, CV abnormalities are clinically relevant, and most clinicians encounter manifestations of CV disease or dysfunction on a regular basis.

The assessment of CV disease in a horse is predicated on a competent clinical examination, the clinician's knowledge, and the ability to order and evaluate diagnostic studies. Incomplete information may impede an accurate diagnosis, foster miscommunication of risks to the client, or delay the proper course of management. Fundamentals of CV anatomy, physiology, and electrophysiology are reviewed elsewhere.[9,20,53,57,107-117] This chapter offers a framework for understanding the lesions, pathophysiology, diagnosis, and management of important congenital and acquired conditions of the heart and major vessels. Clinical aspects of circulatory shock are described in this volume. The reader is referred elsewhere for the management of cardiopulmonary arrest.[58,118]

ANATOMIC CORRELATES OF CARDIOVASCULAR DISEASES

Most diagnostic techniques, including cardiac auscultation, electrocardiography, cardiac catheterization, and echocardiography, are predicated on an understanding of cardiac anatomy and physiology. The CV system is divided into two separate circulations—systemic and pulmonary. The systemic circulation has a greater venous capacitance, ventricular pumping

*The editors and authors acknowledge and appreciate the contributions of John D. Bonagura and Virginia B. Reef as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

pressure, arterial pressure, and vascular resistance.[57,110,112-114,116] Despite these differences, the functions of these two circulations are interdependent as the following examples illustrate. Systemic and pulmonary circulations are arranged in series; therefore cardiac output (CO) from the left ventricle (LV) and the right ventricle (RV) must be equivalent. Accordingly, failure of either ventricle limits CO. Isolated left ventricular failure, as with severe mitral regurgitation, can cause right-sided failure. This is explained by the increased pulmonary venous pressure causing pulmonary vascular remodeling and pulmonary hypertension and imparting a pressure load on the RV. A third example is the case of isolated RV failure with marked right ventricular dilation. Here the leftward bulging of the ventricular septum impairs filling of the LV. This last situation also can develop in chronic pericarditis. Arrhythmias also affect both sides of the heart, so that the development of atrial fibrillation (AF) in the setting of severe structural heart disease often promotes biventricular heart failure.

The heart consists of unique active and passive components, and different diagnostic methods are needed to evaluate these structures and associated functions. Normal heart action requires coordination of electrical activity, muscular contraction and relaxation, and valve motion. When reviewing heart anatomy, and subsequently cardiac pathology, it is useful to consider the anatomic integrity of the pericardium, myocardium, endocardium and valves, specialized impulse-forming and conduction systems, and blood vessels.[107,109,111] Using this approach, the causes of CV disease can be conveniently subdivided into anatomic, physiologic (functional), and etiologic diagnoses (Boxes 9.1 and 9.2).

Pericardial Disease

The pericardium limits cardiac dilation, acts as a barrier against contiguous infection, and contributes to the diastolic properties of the heart. The pericardial space is formed by the reflection of the two major pericardial membranes, the parietal pericardium and the visceral pericardium (epicardium), and normally contains such a small amount of serous fluid that it cannot be seen by echocardiography. Pericardial effusion leading to cardiac compression (tamponade) impairs ventricular filling and diastolic function, typically causing right-sided congestive heart failure (CHF). Some cases of pericarditis progress to constrictive pericardial disease, which severely limits ventricular filling.

Pericardial effusion can develop as a primary disorder or secondary to pleuropneumonia. Infective pericarditis can produce an effusion sufficient to cause cardiac tamponade or eventual constriction of the heart.[88,119-137] Sterile, idiopathic pericardial effusion also has been reported in horses. The volume of effusion can be substantial and can lead to cardiac decompensation.[126] Cardiac mass lesions and intrapericardial tumors have been reported sporadically.[128,131,138,139] Cranial mediastinal tumors (lymphosarcoma) or abscesses secondary to pleuropneumonia also can compress the heart and mimic pericardial disease.[140] Clinical aspects of pericardial disease are discussed later in this chapter.

Myocardial Disease

The myocardium forms the bulk of the atrial and ventricular muscular walls. The right atrium (RA) communicates with the RV inlet through the right atrioventricular (AV) or tricuspid valve. The RV appears crescent shaped on cross-sectional echocardiographic examination and is functionally U shaped. The RV inlet is located in the right hemithorax and the outlet, pulmonary valve, and main pulmonary artery (PA) on the left side of the chest. The left atrium (LA) is caudal to the RA and separated by the atrial septum. The LA is dorsal to the LV through which it communicates across the left AV (mitral) valve. The LV is circular in cross section when viewed by echocardiography and separated from the RV by the ventricular septum. The septum and free walls are thicker than the RV free wall (by approximately 2.5–3 times). Persistent embryologic openings in the cardiac septa are known as septal defects, with the ventricular septal defect (VSD) representing the most common cardiac anomaly in most equine practices (see Box 9.2). The LV is functionally V shaped, with an inlet and outlet separated by the cranioventral (septal or according to the human nomenclature "anterior") leaflet of the mitral valve (Fig. 9.1). The aorta originates in the LV outlet, continuous with the ventricular septum cranially and in fibrous continuity with the septal mitral leaflet caudally. This great vessel exits from near the center of the heart and to the right of the main PA.

The myocardium may dilate or hypertrophy in response to exercise,[141,142] in response to increased work caused by

BOX 9.1 Cardiac Diagnoses

ANATOMIC DIAGNOSIS
- Cardiac malformation
- Valvular (endocardial) disease
- Myocardial disease
- Pericardial disease
- Cor pulmonale (pulmonary disease leading to secondary heart disease)
- Disorder of the impulse-forming or conduction system
- Vascular disease

PHYSIOLOGIC DIAGNOSIS
- Systemic: pulmonary shunting
  - Left to right
  - Right to left
- Valvular insufficiency
- Valvular stenosis
- Myocardial (systolic) dysfunction
- Diastolic dysfunction
- Cardiac rhythm disturbance
- Cardiac-related syncope
- Heart insufficiency or failure (limited cardiac output)
- Congestive heart failure
- Shock
- Sudden cardiac death
- Cardiopulmonary arrest

ETIOLOGIC DIAGNOSIS
- Malformation (genetic)
- Degenerative disease
- Metabolic or endocrine disease
- Neoplasia
- Nutritional disorder
- Inflammatory disease
  - Infective or parasitic
  - Noninfective
    - Immune-mediated
    - Idiopathic
- Ischemic injury
- Idiopathic disorder
- Iatrogenic disease
- Toxic injury
- Traumatic injury

BOX 9.2 Causes of Cardiovascular Disease

CONGENITAL CARDIAC MALFORMATION

Simple systemic-to-pulmonary shunts (left to right)
- Atrial septal defect
- Ventricular septal defect
  - Paramembranous defect
  - Ventricular inlet defect
  - Subarterial (subpulmonic) defect
  - Muscular defect
- Patent ductus arteriosus

Patent foramen ovale (permitting right-to-left shunting)
Valvular dysplasia
- Mitral stenosis/atresia
- Pulmonary atresia (leading to a right-to-left shunt)
- Tricuspid stenosis/atresia (leading to a right-to-left shunt)
- Aortic stenosis/insufficiency (bicuspid or quadricuspid valve)

Subaortic rings with stenosis
Tetralogy of Fallot
Pulmonary atresia with ventricular septal defect (pseudotruncus arteriosus)
Double-outlet right ventricle
Subaortic stenosis
Hypoplastic left side of the heart
Other complex malformations

VALVULAR HEART DISEASE CAUSING VALVE INSUFFICIENCY OR STENOSIS

Congenital valve malformation
Semilunar valve fenestrations causing valve insufficiency
Degenerative (fibrosis) or myxomatous disease causing valve insufficiency
Valvular prolapse
Bacterial endocarditis causing valve insufficiency with or without stenosis
Rupture of a chorda tendinea causing mitral or tricuspid valve insufficiency
Rupture of a valve leaflet causing flail leaflet and valve insufficiency
Noninfective valvulitis
Valvular regurgitation following dilation of the heart or a great vessel
Papillary muscle dysfunction causing valvular insufficiency

MYOCARDIAL DISEASE

Idiopathic dilated cardiomyopathy: ventricular dilation and myocardial contractility failure
Myocarditis
Myocardial fibrosis
- Ischemic (embolic?) myocardial fibrosis
- Parasitic (*Strongylus*) embolization

Myocardial degeneration/necrosis
- Myocardial ischemia/hypoxia
- Toxic injury (e.g., ionophores, plant toxins, cantharidin, snake venom)
- Nutritional deficiencies (e.g., vitamin E, selenium)
- Atypical myopathy
- Trauma

Infiltrative myocardial disease (e.g., myocardial neoplasia, amyloidosis)
Tachycardia-induced cardiomyopathy
Chronic hypertension (e.g., chronic pain, laminitis, metabolic syndrome, chronic kidney disease)

PERICARDIAL DISEASE

Pericardial effusion with or without cardiac tamponade
- Infective: bacterial, viral, or fungal
- Immune-mediated
- Trauma
- Neoplasia
- Idiopathic pericardial effusion

Constrictive pericardial disease
Mass lesion (intrapericardial or extrapericardial) compressing the heart

PULMONARY HYPERTENSION AND COR PULMONALE

Pulmonary hypertension following left-sided heart disease
Pulmonary vascular disease following left-to-right shunt
Immature pulmonary circulation
Primary bronchopulmonary or pulmonary vascular disease
Alveolar hypoxia with reactive pulmonary arterial vasoconstriction
Severe acidosis
Pulmonary thromboembolism

CARDIAC ARRHYTHMIAS (see Box 9.12)

Atrial arrhythmias
Junctional (nodal) arrhythmias
Ventricular arrhythmias
Conduction disturbances

VASCULAR DISEASES

Congenital vascular lesions
Rupture of the aorta, pulmonary artery, or systemic artery
Aneurysm of the aortic sinus of Valsalva
Aortopulmonary fistula
Aortic or aortoiliac degenerative disease
Arteritis
- Infective
- Immune-mediated

(Jugular) venous thrombosis/thrombophlebitis
Pulmonary embolism
Mass lesion or tumor obstructing blood flow

structural cardiac disease, or as a consequence of a noncardiac disorder. Ventricular or atrial dilation is recognized echocardiographically or at necropsy by distention and rounding of the affected chambers, including a "double-apex" sign when there is marked RV enlargement. Lesions causing systolic pressure overload lead to concentric hypertrophy.[143] More common in horses are lesions such as incompetent valves or shunts that cause ventricular volume overload with dilation and eccentric ventricular hypertrophy. Increased cardiac work also occurs in response to exercise, severe anemia, and infections. In these situations, compensatory increases in CO, sympathetic activation, and peripheral vasodilation occur to maintain oxygen delivery to the tissues.[57,110,112-114,116,117,144]

The overall prevalence of myocardial disease is unknown; however, multifocal areas of fibrosis are commonly found at necropsy.[28,29,97,145-150] Whether these areas indicate prior inflammation, toxic injury, or ischemic necrosis caused by intramural coronary disease is uncertain. Cases of multifocal or diffuse

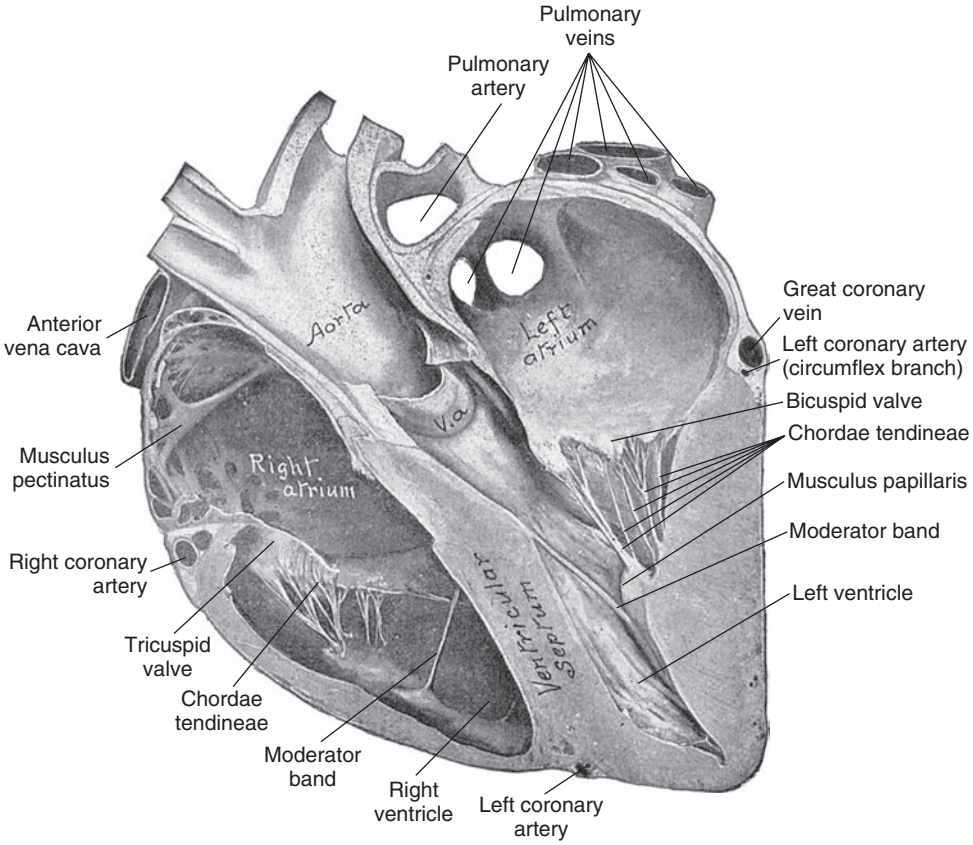


FIG. **9.1** Sagittal view of the equine heart. The thicknesses of the ventricles, the position of the atria relative to the ventricles, and the relationship of the left ventricular (LV) inlet and outlet are evident. The bicuspid valve referred to in this figure is the mitral valve. The circular appearance of the left atrium (LA) and the relationship of the septal cusp of the mitral valve to the LV inlets and outlets are notable. These aspects are important when examining the heart by echocardiography. *v.a.,* Segment of aortic valve. (From Sisson S, Grossman JD: *Anatomy of the domestic animals,* ed 4, Philadelphia, 1953, WB Saunders.)

myocarditis have been observed. Myocardial inflammation and myocardial failure can lead to cardiac arrhythmias and heart failure.[4,151,152] Idiopathic dilated cardiomyopathy develops sporadically and is recognized echocardiographically as a dilated, hypokinetic left or right ventricle.[129] As in other species, echocardiographic features of dilated cardiomyopathy can also be tachycardia induced and may resolve following successful control of heart rate (HR) and heart rhythm.[153] Ingestion of monensin or other ionophores can cause mild to severe myocardial injury.[154-163] Vitamin E and selenium deficiency have been described as causes of myocardial degeneration and dysfunction.[164,165] Neoplastic infiltration is considered rare.[93,138,139,166] Impaired myocardial function as a consequence of regional ischemia has been sought using stress echocardiography immediately following treadmill exercise or pharmacologic stress, but this diagnosis requires further definition[167,168] (see later discussion). Myocardial contraction is dictated by electrical activity of the myocardium; accordingly, cardiac arrhythmias—especially atrial fibrillation or ventricular tachycardia (VT)—can limit CO and cause exercise intolerance in performance animals (see later discussion). Clinical aspects of myocardial disease are discussed later in this chapter.

Valvular and Endocardial Diseases

The cardiac chambers are lined by the endocardium, which also covers the four cardiac valves and is continuous with the endothelium of the great vessels. Normal valves govern the one-way flow of blood through the heart by preventing significant regurgitation of blood from higher to lower pressure zones. The AV inlet valves—the tricuspid and the mitral—are anchored by the collagenous chordae tendineae and papillary muscles and are supported by a valve annulus and the caudal atrial walls (Fig. 9.2; also see Fig. 9.1).[107,109,111] The mitral valve consists of two major cusps and several accessory cusps.[45] The tricuspid valve is the largest valve and consists of three well-defined leaflets. Lesions of any portion of the AV valve apparatus or dilation of the ventricle can lead to valvular insufficiency (see Box 9.2). The aortic and pulmonary valves each consist of three semilunar leaflets that close during diastole to protect the ventricles from the higher arterial blood pressure (BP). Aortic valvular tissue in horses is not simply passive, and it will contract in response to a number of adrenergic and vascular agonists, such as angiotensin II and endothelin.[169] The left and right main coronary arteries originate within the aortic valve sinuses (of Valsalva).

Valvular disorders in horses are common. Congenital valve stenosis, dysplasia, or atresia are recognized sporadically in foals.[4,151,170-190] Degenerative diseases of the aortic, mitral, and tricuspid valves are very common in mature horses,[4,123,148,151,191,192] and endocarditis can develop on any cardiac valve[4,31,129,151,191,193-206] (see Box 9.2). Valvular lesions of obscure etiology, including nonseptic valvulitis, have been recognized sporadically. Tricuspid and mitral regurgitation, of unspecified etiology, is often detected in high-performance animals, including Standardbred and National Hunt horses.[70,72,75,207-211] Mitral regurgitation



FIG. **9.2** Anatomy of the left atrioventricular (mitral) valve. Opened left atrium (LA) and LV viewed from the caudal perspective. The large anterior (cranioventral or septal) leaflet in the center of the figure is notable. Chordae tendineae attach the valve to the papillary muscles. The ventricle has been cut so that the multiple cusps of the posterior (caudodorsal or mural) leaflet are visible to the left and the right of the anterior leaflet.

(MR) due to rupture of a chorda tendineae is recognized in both foals and mature animals.[38,212-214] Clinical aspects of valvular heart disease are discussed later in this chapter.

Disease of the Impulse-Forming and Conduction Systems

The specialized cardiac tissues consist of the sinoatrial (SA) node, internodal pathways, AV node, bundle of His, bundle branches, fascicles, and Purkinje system (Fig. 9.3). The SA node, a relatively large, crescent-shaped structure, is located subepicardially at the junction of the right auricle and cranial vena cava. Well-documented sinus node disease, although suggested,[215] is rare; in contrast, vagally induced sinus arrhythmias are common.[9,215-217] The equine atrial muscle mass is large and predisposes the horse to development of reentrant rhythms and fibrillatory conduction.[218] The AV node, situated in the ventral atrial septum, and the bundle of His, which continues on into the bundle branches, are sites for AV block, both physiologic (vagal) and, infrequently, pathologic in nature. Conduction is slow across the normal AV node.[112,219-221] The His-Purkinje system in the ventricular septum and ventricular myocardium can act as substrates for junctional and ventricular ectopic impulses and tachycardias. Because the horse has relatively complete penetration of Purkinje fibers in the ventricles—except for a small portion of the LV free wall—the substantial equine ventricles are electrically activated in a relatively short time (approximately 110 msec).[7]

The autonomic nervous system extensively innervates the heart and influences cardiac rhythms.[4,151,222-227] Interplay between the sympathetic and parasympathetic branches normally controls HR and heart rhythm in response to changes in arterial BP.[9,228] The vagus innervates supraventricular tissues extensively and probably affects proximal ventricular tissues to

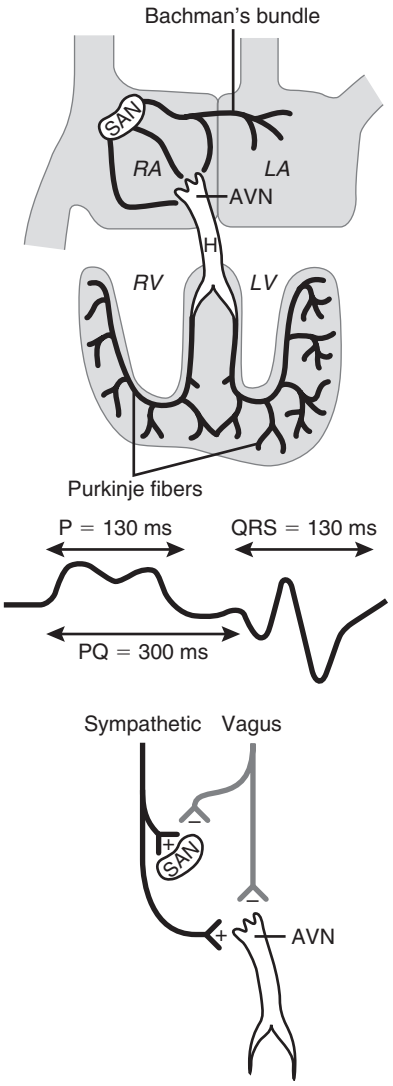


FIG. **9.3** Impulse-forming and conduction systems of the heart. The impulse originates in the sinoatrial node (SAN) and is propagated across the right atrium (RA) and left atrium (LA), generating the P wave. Specialized internodal and interatrial (Bachmann''s bundle) pathways facilitate impulse conduction. The impulse is delayed in the atrioventricular node (AVN) and rapidly conducted through the bundle of His (H), bundle branches and Purkinje network *(top).* Electrical activation of ventricular myocytes generates the QRS complex. The automaticity of the SA node and conduction across the AV node are modulated by the autonomic nervous system *(bottom).* (Courtesy Dr. Robert L. Hamlin. From Schwarzwald CC, Bonagura JD, Muir WW: The cardiovascular system. In Muir WW, Hubbell JA, editors: *Equine anesthesia: monitoring and emergency therapy,* ed 2, St. Louis, 2009, WB Saunders.)

a minor extent. Vagal influence generally depresses HR (chronotropism), AV conduction (dromotropism), excitability (bathmotropism), and myocardial contractile state (inotropism). However, because vagotonia also shortens the action potential and refractory period of atrial myocytes, high vagal activity is a predisposing factor in the development of AF.[112,229] Innervation of the stimulatory sympathetic nervous system is extensive throughout the heart and has effects generally opposite to those of the parasympathetic system. β_1-Adrenergic receptors dominate in the equine heart,[230] but presumably there are other autonomic subtype receptors, including α-adrenergic receptors and small numbers of β_2-adrenoceptors.[112,231] The notable increase in HR that attends exercise is related to increased

sympathetic efferent activity and withdrawal of parasympathetic tone.[9] Increases in HR to 220 to 240 beats/min are not uncommon with maximal exercise.[232-236] The exact role of dysautonomia in the genesis of cardiac arrhythmias has not been determined; however, infusion of autonomic receptor agonists and antagonists can be associated with direct or baroreceptor-induced changes in HR and rhythm.[237-247] Cardiac arrhythmias are discussed later in this chapter.

Vascular Diseases

There are three major subdivisions of the circulation: systemic, coronary, and pulmonary. The arteries and veins consist of three layers: adventitia, media, and intima. The overall structure and function of each layer vary with the vessel and location. Vascular receptors[20,112,114,116] and anatomic lesions influence vascular resistance and blood flow. α-Adrenoceptors dominate in the systemic vasculature, and BP is generally raised by vasoconstriction following stimulation of postsynaptic α-adrenergic receptors by norepinephrine, epinephrine, or infused α-adrenergic receptor agonists such as phenylephrine.[246,248-254] The presence of vasodilator β_2-adrenergic receptors is clinically relevant, insofar as infused β_2-agonists cause vasodilation in circulatory beds that contain high β-agonist adrenergic receptor density. Many vascular beds also dilate following the production of local vasodilator substances, such as nitric oxide, released during exercise, stress, or metabolic activity.[112,114,116] Dopaminergic receptors, when present in vascular walls, may be stimulated, causing vasodilation, provided vasoconstricting α-adrenergic activity does not dominate. Stimulation of histamine (H_1) receptors or serotonin (5-HT) receptors causes arteriolar dilation, venular constriction, and increased capillary permeability.[112,114,116] Infusion of endothelin[255-257] or of calcium salts causes arterial vasoconstriction,[258] whereas administration of calcium channel antagonists (e.g., verapamil, diltiazem) causes vasodilation of vascular smooth muscle.[259]

Various vascular lesions have been reported in horses (see Box 9.2). Rupture of the aorta, PA, or middle uterine artery is devastating and often lethal.[4,34,92,151,260-262] The aorta may also rupture into the heart, creating an aortic to cardiac fistula.[263,264] Aortopulmonary fistulation in conjunction with aortic rupture is relatively common in Friesians, possibly caused by a connective tissue disorder.[265,266] Although parasitic arteritis may predispose to vascular injury, the cause of most vascular lesions, including aortoiliac thrombosis, is unknown.[267-272] Causes of vasculitis include *Strongylus vulgaris* infestation of the cranial mesenteric artery, infective thrombophlebitis of the jugular veins, equine viral arteritis, and suspected immune-mediated disease.[145,147,273] Neoplasms can obstruct blood flow by external compression or through invasion, more often affecting the right side of the circulation. Examples include obstruction of the PA by a lung tumor and obstruction of venous return by neoplastic compression or invasion of the vena cava. Clinical features of vascular disease are discussed later in this chapter.

CLINICAL CARDIOVASCULAR PHYSIOLOGY

The clinician must appreciate elementary aspects of normal heart function in order to perform a clinical CV examination and understand the abnormalities associated with heart disease and CHF. Central to this are the electrical-mechanical correlates of Wiggers' cardiac cycle.

Cardiac Cycle

The association between electrical and mechanical events of the heart first described by Wiggers has been reviewed in standard physiology textbooks (Fig. 9.4).[57,110,112-115] From a study of this cycle, it is evident that cardiac electrical activity precedes pressure and volume changes; therefore arrhythmias can exert deleterious hemodynamic effects, especially during exercise, illness, or anesthesia. Relevant aspects of this cycle are now considered.

The P wave of the electrocardiogram (ECG) stems from electrical activation of the atria, late in ventricular diastole, and after the ventricles have been largely filled. During the ensuing atrial contraction the *atrial sound* (fourth heart sound or S_4) is generated and the ventricle is filled to its end-diastolic volume. The increase in atrial pressure, the atrial *a wave,* is reflected as a normal *jugular pulse* in the ventral cervical region. The magnitude of the atrial contribution to ventricular filling generally placed at 15% to 20% at rest but increases dramatically during high HRs. Therefore atrial tachyarrhythmias such as AF have the greatest impact on CO during exercise or tachycardia.

The QRS complex heralds ventricular systole. After depolarization of the ventricular myocytes, calcium enters the cell to trigger release of calcium stores in the sarcoplasmic reticulum. Increased cytosolic calcium interacts with the cardiac troponin complex on actin and myosin filaments to shorten the myofilaments and develop tension. These events are enhanced by sympathetic activity or drugs such as digoxin or dobutamine and depressed by anesthetics and drugs that impair calcium entry into cells. The abrupt increase in ventricular wall tension and chamber pressure closes the AV valves (coinciding with the vibrations of the *first heart sound;* S_1) and increases intraventricular pressure *(isovolumic period)* until the semilunar valves open.[23,25] At this instant the ventricular walls move inward and blood is ejected into the great vessel as the ventricular pressure increases to peak value and creates a similar peak arterial BP (Figs. 9.4A, 9.5C and D, and 9.6). The contracting heart twists during systole, and the left ventricle strikes the chest wall caudal to the left olecranon causing the *cardiac impulse* or *apex beat.* This early systolic movement, coincident with opening of the aortic valve, is a useful timing clue for cardiac auscultation and for identifying the mitral valve area for auscultation. The delay between the onset of the QRS and the opening of the semilunar valves, termed the *preejection period,* can be measured by Doppler echocardiography and is an index of ventricular myocardial contractility such that sympathetic activation and positive inotropic drugs shorten the preejection period.[46,241,274-279] Blood is ejected into the aorta and PA with an initial velocity that generally peaks near 1 m/sec and can be measured by Doppler echocardiography (see Fig. 9.5D).[280,281] The aortic ejection time usually exceeds 400 msec in a horse at rest, and reductions of either ejection velocity or ejection time are suggestive of reduced LV function. A *functional systolic ejection murmur* is often heard during ejection (Fig. 9.4A). Such murmurs, by definition, must begin after the first sound and end before the second sound. The difference between the diastolic and systolic pressure (pulse pressure) and rate of rise of pressure contribute to a palpable *arterial pulse* during midsystole (see Figs. 9.4A and 9.6). The precise timing of the pulse depends on the proximity of the palpation site relative to the heart. At the end of the *ejection period,* as ventricular pressures fall below those of the corresponding arteries, the semilunar valves close coincident with the high-frequency *second heart sound* (S_2) and the incisura of the arterial pressure curves (see Figs. 9.4A and 9.6).[19,23,25] The pulmonary valve may close either after or before the aortic valve.[3,4,282] Asynchronous valve closure may lead to

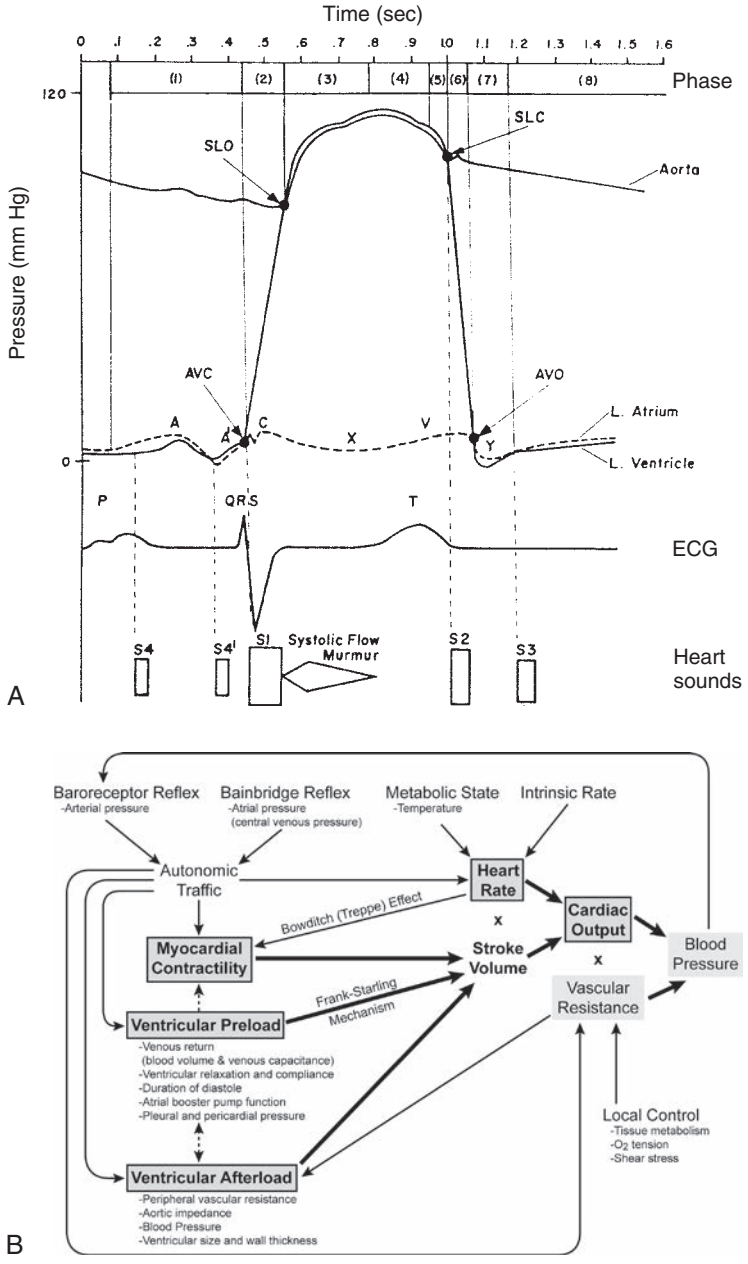


FIG. **9.4** A, The cardiac (Wiggers) cycle of the horse. This drawing integrates the electrical, pressure, mechanical, and flow events of diastole and systole and demonstrates the origins of the heart sounds. Electrical activity precedes mechanical events. See the text for a full description. *AVC,* Closure of the mitral (atrioventricular) valve; *AVO,* opening of the mitral (atrioventricular) valve; *SLO,* opening of the aortic (semilunar) valve; *SLC,* closure of the aortic (semilunar) valve. B, Determinants of cardiac output (CO) and blood pressure (BP). (A, Modified from Detweiler DK, Patterson DF: The cardiovascular system. In Cattcott EJ, Smithcors JF, editors: *Equine medicine and surgery,* ed 2, Santa Barbara, CA, 1972, American Veterinary Publications. B, From Muir WW, Hubbell JA: *Equine anesthesia,* ed 2, St. Louis, 2009, Saunders.)

audible splitting of S_2, which is normal but can be extreme in some horses with lung disease and pulmonary hypertension. During the ejection period the ventricular volume is markedly reduced from the end-diastolic volume: this volume ejected is defined as the *stroke volume.* The ratio of the stroke volume to the end-diastolic volume is the *ejection fraction,* a commonly used index of systolic heart function and correlated to the often-used *shortening fraction* of the M-mode echocardiogram (see Fig. 9.5C). Contraction of the ventricles causes the AV valves to bulge toward the atrium, leading to the early-systolic positive *c wave* of the atrial pressure curve, and is accompanied by a downward motion of the mitral and tricuspid annulus toward the apex, causing the subsequent *x descent* of the atrial pressure curve and a brief systolic collapse of the jugular vein. Subsequent to atrial filling during ventricular systole, a positive pressure wave, the *v wave,* occurs in the atrial and venous pressure curves. Severe TR accentuates this wave and may lead to pathologic systolic pulsations extending up the jugular furrow. Finally there is a decline in ventricular pressure *(isovolumic relaxation)* related to off-loading of calcium from the troponin apparatus

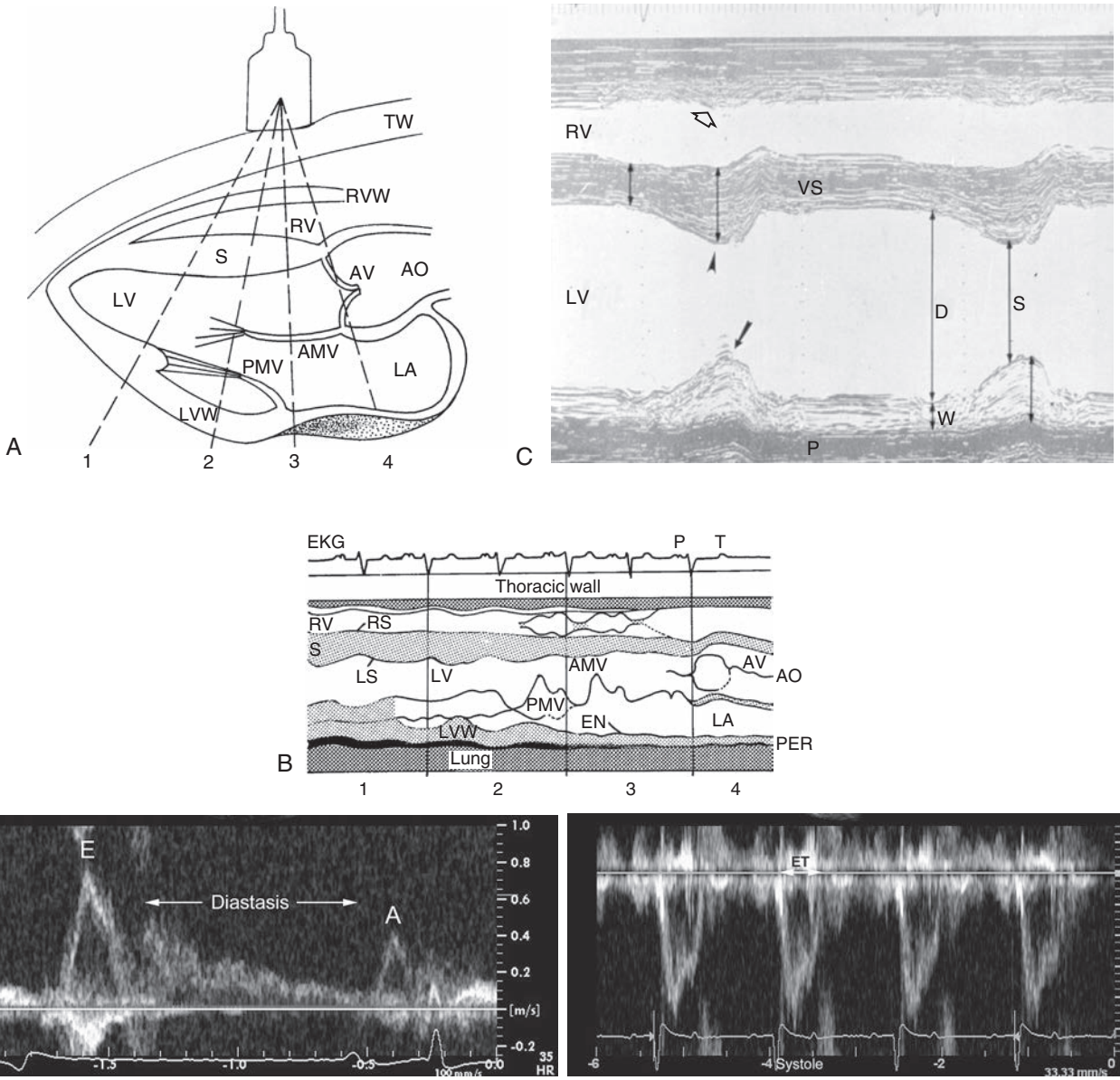


FIG. **9.5** Ventricular function and echocardiography. A, Derivation of the M-mode echocardiogram. The lines demonstrate typical paths of M-mode recording planes (*1,* ventricular/papillary muscle; *2,* chordae tendineae; *3,* anterior mitral valve [AMV]; *4,* aortic root and left atrium [LA]/auricle; *TW,* thoracic wall; *RVW,* right ventricular wall; *RV,* right ventricle; *S,* septum; *LV,* left ventricle; *AV,* aortic valve; *AO,* aortic outflow tract; *LVW,* left ventricular wall; *PMV,* posterior mitral valve; *LA,* left atrium). B, The drawing demonstrates the appearance of the M-mode echocardiogram at each level (*PER,* pericardium; *RS, LS,* right and left sides of the ventricular septum; *EN,* endocardium). C, M-mode echocardiogram demonstrating the method of measuring left ventricular shortening fraction (LVSF), in which *D* is diastolic dimension and *S* is systolic dimension (LVSF = D – S/D). The prominent thickening of the walls during systole is notable. The end-systolic excursion of the LV wall is visible *(arrow).* In practice, the systolic dimensions of the ventricular septum and the LV wall generally are measured along the same line as demonstrated for the LV lumen in systole *(S)* (*W,* LV wall; *VS,* ventricular septum). D, *Left:* Doppler echocardiographic recordings of LV filling *(left)* and ejection *(right).* Transmitral inflow is characterized by an early diastolic rapid-filling wave *(E),* low-velocity middiastolic filling (diastasis), and a presystolic atrial filling wave *(A). Right:* The velocity profile of aortic ejection is characterized by a roughly triangular appearance with rapid acceleration of blood flow into the ascending aorta in early to midsystole and termination of flow at the time of aortic valve closure. The area under the velocity spectrum curve (velocity-time-integral) correlates directly with ventricular stroke volume. The preejection period (the time between the start of the QRS and beginning of ejection) and the ejection times *(ET, arrows)* are load-dependent indices of LV function.

and resequestration into the sarcoplasmic reticulum. This active ventricular relaxation is associated initially with closure of the semilunar valves and eventually by opening of the AV valves (see Fig. 9.4A). Relaxation in healthy hearts is enhanced by sympathetic activity. Conversely, myocardial ischemia can impair active relaxation, and it is likely that subendocardial ischemia combined with reduced diastolic filling time contributes to the marked elevations in left atrial pressures observed during galloping or other high-intensity exercise.[283,284]

Ventricular filling commences just as the AV valves open. As shown in Fig. 9.5D, ventricular diastole can be subdivided into three general phases: rapid ventricular filling, diastasis, and atrial contraction.[110,112,113,115] These phases are readily observed using pulsed-wave Doppler echocardiography or Doppler tissue imaging.[168,285-287] Once the ventricles have relaxed and the atrial pressure exceeds the corresponding ventricular pressure, the AV valves open. At that instant, rapid filling ensues, with a peak velocity of about 0.5 to 1 m/sec but varying directly with the HR.[280] The ventricular pressures increase only slightly during this phase, whereas the ventricular volume curves change dramatically from the venous return. Rapid filling may be associated with a *functional protodiastolic murmur,* which is concluded by the *third heart sound* (S_3), the low-frequency vibrations occurring near the termination of rapid ventricular filling (see Fig. 9.4A). The loss of atrial volume and corresponding decline in the atrial pressure (the *y descent*) is reflected in the jugular furrow as the vein collapses. Following rapid filling, a period of greatly reduced low-velocity filling, diastasis, ensues. This period may last for seconds during vagal arrhythmias such as sinus bradycardia, pronounced sinus arrhythmia, or second-degree AV block. With markedly exaggerated pauses, the jugular vein may begin to fill prominently. The last phase of diastole is the contribution to ventricular filling caused by the atrial contraction. A functional *presystolic murmur* has been associated with this period between the fourth and first heart sounds.

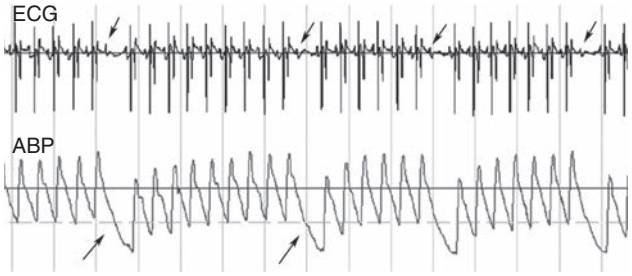


FIG. **9.6** Compressed electrocardiogram (ECG) with simultaneous arterial blood pressure (ABP) recording in a horse with second-degree atrioventricular block. The progressive increase in ABP triggers a baroreceptor reflex leading to atrioventricular conduction block *(upper arrows)* and a corresponding fall in the ABP *(lower arrows)*. Presumably this mechanism, along with sinus arrhythmia and sinus arrest, represents vagally induced mechanisms for controlling ABP in the standing horse.

During the cardiac cycle the atrium functions as a reservoir for blood (ventricular systole), a conduit for venous return (early to middiastole), and as a pressure pump (atrial systole).[288] Mechanical atrial function of the LA can be studied using two-dimensional (2D) echocardiography and advanced Doppler echocardiographic methods. Impaired electrical and mechanical function of the atria may predispose to recurrent atrial arrhythmias such as AF.[218,287,289-291]

Ventricular Function

The ability of the ventricles to eject blood depends on both systolic and diastolic ventricular function as well as HR and heart rhythm (Box 9.3; see also Fig. 9.4B). The most commonly used

BOX 9.3 Determinants of Cardiac Function

SYSTOLIC FUNCTION: DETERMINANTS OF VENTRICULAR STROKE VOLUME (see also Fig. 9.4B)

Preload [+]—ventricular end-diastolic volume
- Plasma (blood) volume
- Determinants of diastolic function (see below)

Contractility [+]—intrinsic myocardial ability to contract
- Sympathetic activity
- Loading conditions
- Myocardial perfusion (ischemia)
- Myocardial disease
- Drugs (positive or negative inotropic agents)

Afterload [–]—wall tension required to eject blood
- Aortic impedance
- Vascular resistance
- Ventricular volume (tension increases with dilation)
- Ventricular wall thickness (thin walls have higher tension)

Cardiac lesions increasing workload [–]
- Valvular regurgitation (common)
- Valvular stenosis (rare)
- Septal defects and shunts

DIASTOLIC FUNCTION: DETERMINANTS OF VENTRICULAR FILLING

Pleural/mediastinal factors
- Intrapleural pressure (ventilation, effusion)
- Mass lesions

Pericardial function
- Intrapericardial pressure (effusion)
- Constriction

Myocardial recoil (passive elastic properties)

Myocardial relaxation (active process)
- Myocardial perfusion (ischemia)

Ventricular wall distensibility (chamber and myocyte compliance)

Venous pressure and venous return (must be matched with compliance)
- Plasma volume
- Venous capacitance

Diastolic filling time
- Heart rate

Atrial contribution to filling (“booster pump”)
- Heart rate
- Cardiac arrhythmias (atrial fibrillation)

Atrioventricular contraction sequencing
- Cardiac arrhythmias

Atrioventricular valve function
- Stenosis

measurements of overall ventricular performance and circulatory function are HR, invasively or noninvasively determined arterial BP, rate of ventricular pressure change (dp/dt), cardiac output (CO), stroke volume (SV), LV ejection fraction, LV shortening fraction, systolic time intervals, central venous pressure, PA and pulmonary capillary wedge pressures, and arteriovenous oxygen difference (A-V DO_2).[57]

Cardiac output, the amount of blood pumped by the left (or right) ventricle in 1 minute (L/min), is the product of ventricular SV (mL/beat) multiplied by HR (beats/min) (see Fig. 9.4B). Cardiac index refers to the CO divided by (indexed to) the body surface area (or body mass). Cardiac output coupled to systemic vascular resistance determines the mean arterial BP; an increase in either variable raises mean arterial pressure. Values for CO vary widely with the size and activity of the horse and are often influenced by drug therapy or anesthesia.[292,293] Ventricular *stroke volume* depends on myocardial contractility, preload, and afterload (see Fig. 9.4B and Box 9.3).[24,26,110,112-115] Although traditionally considered independent determinants of myocardial function, these three variables are all interconnected and influence force, velocity, and duration of ventricular contraction and thereby SV.[110,112,114] Invasive and noninvasive estimates of SV and CO can be obtained in standing and anesthetized horses using a large variety of methodologies.[276,294-318] In clinics, SV is often estimated noninvasively by 2D and Doppler echocardiography (see later discussion).[168,297,306,307,309,311-313]

Myocardial *contractility (inotropy)* is defined as the maximum velocity of fiber shortening at zero load.[110,112-115] Ultimately, it is determined by the availability of calcium to the sarcomere and the sensitivity of troponin C for calcium, which are modulated by the autonomic input, the initial myocardial stretch (preload; see later discussion), and the HR (Bowditch "treppe" effect) (see Fig. 9.4B). Contractility is increased by catecholamines, calcium, digitalis glycosides, and phosphodiesterase inhibitors.* True contractility is difficult to measure in the clinical setting because of the influence of HR and loading conditions on clinical measurements of systolic ventricular function. Overall systolic ventricular function can be estimated noninvasively by observing directional changes in load-dependent preejection or ejection phase indices of ventricular function. These include LV shortening and ejection fractions by M-mode and 2D echocardiography; preejection period, ejection time, flow acceleration, velocity time integral of aortic or pulmonic ejection, and peak myocardial velocity by tissue Doppler echocardiography; and myocardial deformation (strain) or strain rate by computerized analysis of 2D echocardiograms or tissue Doppler studies (see Fig. 9.5).† Measured variables will be influenced by physiologic state, altered mildly by day-to-day variation[350,351] or sedatives,[274] and affected markedly by exercise[168] and general anesthesia.[292]

Ventricular sarcomere length before contraction or *preload* is a positive determinant of ventricular systolic function that depends on ventricular filling (Box 9.3). The healthy ventricle is highly preload dependent such that increases in preload increase SV. This ability of the heart to change its force of contraction and therefore SV in response to changes in preload is called the *Frank-Starling mechanism* (or Starling's law of the heart).[110,112-115] Dehydration, venous pooling, impairment of ventricular relaxation and compliance, increased intrapleural or intrapericardial pressures, loss of atrial booster pump function (e.g., with AF or atrial stunning after AF) and certain tachyarrhythmias (by shortening filling time and disturbing the atrioventricular conduction sequence) all reduce ventricular filling and decrease SV. Increased preload can be observed in horses with heart disease as a consequence of impaired pump function and fluid retention secondary to activation of the renin-angiotensin-aldosterone system.[117] Moderate to severe valvular insufficiency increases ventricular filling pressures and preload.[144,352-355] The increased ventricular diastolic dimensions serve as a compensatory mechanism that maintains forward stroke volume in the early setting of a failing ventricle or regurgitant heart valve. Ventricular preload can be estimated by determining ventricular end-diastolic dimensions (volume, area, or diameter) using echocardiography[168,344,345,355] or by measuring venous filling pressures using intracardiac catheters.[353,354] The measurement of venous filling pressures (central venous pressure, pulmonary diastolic, or pulmonary capillary wedge pressure)[353-358] provides an accurate gauge of preload provided that HR and ventricular compliance (distensibility) are normal and ventilation is relatively stable. Myocardial ischemia, which impairs myocardial relaxation, and pericardial diseases, which constrict the ventricles, both reduce ventricular compliance; in such cases, the venous filling pressures may not accurately reflect chronic changes in ventricular preload.

Ventricular *afterload* relates to the forces impeding ejection of blood and is closely related to the aortic pressure.[110,112-115] Vascular impedance during ejection is determined by the elastic, resistive, and dynamic properties of the connected great vessel and vascular tree. However, afterload is not solely determined by blood pressure. Ejection against a pressure load causes wall stress, which according to Laplace's law is proportionate to pressure (P) × radius (r) and inversely proportionate to wall thickness (h). Afterload can be expressed as LV wall stress during ejection. While the exact equation depends on cardiac chamber shape, which is subject to changes during the cardiac cycle, the above simplified relationship highlights how pressure, radius, and wall thickness contribute to afterload. Peak wall stress occurs immediately before aortic valve opening. Afterload is difficult to measure clinically, and although BP is not identical to afterload, it may be used to estimate directional changes in afterload. Additionally, relative LV wall thickness can be measured by echocardiography and should be taken into account.[168,344] Increases in ventricular chamber size, thinning of ventricular walls, aortic stenosis, arterial stiffness or resistance (leading to increased pressures), as well as marked increases in hematocrit, increase the impedance to ventricular ejection and reduce SV. Ventricular hypertrophy, by increasing relative wall thickness, can be thought of as a mechanism that reduces wall stress at a given pressure and radius.[114] The failing heart is characterized by poor myocardial contractility, an exhausted preload reserve, and an increased sensitivity to ventricular afterload. Arterial vasodilators such as acepromazine, hydralazine, and angiotensin-converting enzyme inhibitors decrease afterload and are important components of heart failure treatment.[55,58,359-363]

Ventricular *synergy* refers to the normal method of ventricular activation and contraction. Normal electrical activation causes a burst of activation of great mechanical advantage. Cardiac arrhythmias, especially ventricular rhythm disturbances, can cause dyssynergy (dyssynchrony) with a resultant decrease in SV. Coronary occlusions leading to transient myocardial ischemia or ischemic myocardial necrosis also cause dyssynergy but are considered relatively rare.[91,147,364]

Structural and functional *competency of the cardiac valves and the ventricular septa* influence ventricular systolic function.

* References 237, 238, 241, 246, 249-251, 253, 258, 277, 319-339.

† References 49-51, 56, 168, 279, 286, 340-349.

Valvular insufficiency (or the rare stenosis) reduces ventricular stroke volume unless there is adequate compensation from ventricular dilation and hypertrophy. Ventricular remodeling, combined with HR reserve, often allows small septal defects or mild to moderate valvular lesions to be well tolerated even during exercise. However, large defects or severe valvular lesions can create significant volume overload of the left side of the heart, progressive myocardial dysfunction, and heart failure. Development of CHF is particularly likely when an arrhythmia such as AF is superimposed on a serious structural lesion.

Ventricular *diastolic function* determines ventricular filling and preload.[110,112-115] Factors that affect diastolic function are indicated in Box 9.3. When diastolic function is abnormal, there is often greater HR and higher venous pressure dependencies for maintenance of CO. A well-recognized cause of diastolic dysfunction is constriction or compression of the heart due to pericardial disease. Marked ventricular chamber dilation or hypertrophy also decreases ventricular compliance and requires higher ventricular distending pressures for filling. Left ventricular diastolic dysfunction as a consequence of severe RV dilation or hypertrophy can be explained by bulging of the ventricular septum into the left ventricle, which impedes left-sided filling. This impact of *ventricular interdependence* is observed clinically with chronic pericardial disease and severe pulmonary hypertension. Ventricular diastolic function also is affected by arrhythmias. Persistent tachycardia shortens diastole, cardiac filling time, and coronary perfusion. With AF, the atrial contribution to filling is lost. Junctional and ventricular arrhythmias lead to AV dissociation preventing normal AV sequencing and can also create marked dyssynchrony of ventricular contraction. Objective measures of diastolic function are very complicated, and no good clinical indicator of diastolic function is currently available for horses. However, diastolic dysfunction may be assumed when one of the aforementioned conditions is recognized. It is possible to measure transmitral and tricuspid inflow using Doppler techniques, but these methods are unreliable, are crude, and also depend on atrial pressure.[280,281,287,344,365] Assessment of diastolic wall motion velocities by tissue Doppler imaging also may provide more insight into diastolic cardiac function.[161,164,286,349,366]

Imbalance between *myocardial oxygen demand* and delivery can reduce both ventricular systolic and diastolic function and may affect cardiac rhythm as well. This relationship is also relevant when there is airway obstruction or bronchopulmonary disease, which can reduce arterial oxygenation.[90] Myocardial oxygen demand is augmented by increasing myocardial inotropic state, HR, and ventricular wall stress (related to preload and afterload).[110,112-115] Oxygen delivery depends on coronary anatomy and vasomotion (degree of vessel constriction), diastolic arterial BP, diastolic (coronary perfusion) time, and metabolic activity of the myocardium.[10,367-373] Normal coronary flow is highest to the LV myocardium in the ventricular septum and LV wall.[373] The immediate subendocardial layer of myocardium is probably most vulnerable to ischemic injury,[367] and altered ventricular depolarization may develop secondary to an imbalance in oxygen delivery. This probably accounts in part for the ST-T depression and changes in the T waves observed in hypotensive animals and in normal horses during sinus tachycardia. Coronary vasomotion is effective in augmenting coronary perfusion even at high HRs (up to 200/min in ponies); however, coronary autoregulation is not as effective if diastolic perfusing pressure decreases in the aorta.[368,369] The clinician may use the "double product" of arterial BP × HR as a general estimate of myocardial oxygen demand.[112] Persistent ST-segment depression or elevation, especially at rest and normal HRs, suggests deficient myocardial perfusion. However, marked ST-segment and T-wave changes are normal in horses examined during treadmill exercise and are therefore difficult to interpret.

CARDIOVASCULAR EXAMINATION OF THE HORSE

General Approach

A general approach to the recognition and diagnosis of heart disease and an assessment of its severity is summarized in Box 9.4.[61,62] Undoubtedly, history and physical examination are the most important initial evaluation procedures in the CV examination of the horse. With the exception of mild abnormalities in cardiac structure and function, normal physical examination and cardiac auscultation in a horse with good exercise tolerance practically precludes clinically relevant heart disease. The initial CV physical examination should include recording of an accurate resting HR and respiratory rate, evaluation of the arterial pulses (head and limbs), inspection of the veins (mostly jugular veins), evaluation of the mucous membranes for pallor, refill time, and cyanosis (which may develop secondary to a right-to-left cardiac shunt or severe respiratory disease), evaluation for abnormal fluid accumulation, inspection for abnormal ventilatory patterns, palpation of the precordium, a thorough auscultation of the heart at all valve areas, and auscultation of both lung fields. Noninvasive BP measurement can serve to objectify palpatory findings on arterial pulses and identify widening of pulse pressure.[52,68]

It is worth emphasizing that most *serious* cardiac disorders can be detected initially by physical examination and a stethoscope. The presence of a cardiac murmur is the essential finding that leads one to suspect degenerative or infective valvular disease or a congenital heart malformation. Sustained or recurrent cardiac arrhythmias are easily discovered through cardiac auscultation and palpation of the arterial pulse. Pericarditis and cardiac tamponade are characterized by muffled heart sounds or pericardial friction rubs, jugular distention, and often RV failure. Significant myocardial disease is usually associated with heart failure, arrhythmias, or a cardiac murmur, especially when ventricular dilation or dysfunction causes insufficiency of the mitral or tricuspid valves. Mild, subtle, or occult CV disease may be subclinical at rest and require a detailed examination, including exercise testing, before abnormalities can be objectively detected. Laboratory studies, electrocardiography, echocardiography, and cardiac catheterization are additional tests that are particularly useful in recognizing or confirming the underlying basis of CV disease and assessing its severity.

History

CV disease may be suspected from the history or a serendipitous finding during the course of a routine examination. The horse with CHF may be presented for generalized venous distention, jugular pulsations, edema, or rarely, in the case of acute left-sided heart failure, respiratory distress and pulmonary edema.[148,374] Conversely, other cardiac problems such as arrhythmias or murmurs can be incidental findings, detected during a routine physical, prepurchase, or insurance examination. The horse with clinically apparent CV disease may have subtle performance problems that are only apparent at peak performance levels. In many cases, performance

BOX 9.4 Diagnostic Studies for the Evaluation of Heart Disease

HISTORY[a]

- Signalment, presenting complaints, general medical history, past illnesses, past and current medications, weight loss, work history, and exercise tolerance

PHYSICAL EXAMINATION[a]

- Body condition
- Heart rate and rhythm
- Arterial and venous pulses, venous distention, venous refill
- Mucous membrane color and capillary refill time
- Evaluation for abnormal fluid accumulation: subcutaneous edema, pleural effusion (by thoracic percussion)
- Auscultation of the heart (heart sounds, heart murmurs, rhythm) and lungs
- Measurement of arterial blood pressure and pulse pressure (noninvasive)

ELECTROCARDIOGRAPHY

- Resting electrocardiogram—heart rate, rhythm, P-QRS-T configuration, conduction sequence, association of P and QRS-T, ECG time intervals, and electrical axis (axis has limited value in horses)
- Exercise and postexercise electrocardiography[b]
- 24-Hour ambulatory (Holter) electrocardiography[b]

ECHOCARDIOGRAPHY

- Two-dimensional echocardiography—cardiac anatomy, chamber size, and vessel dimensions; valve anatomy and motion; systolic atrial and ventricular function; identification of cardiac lesions or free fluid; estimation of cardiac output
- M-mode echocardiography—ventricular dimensions and systolic ventricular function; cardiac anatomy and valve motion; estimation of cardiac output
- Doppler echocardiography—identification of normal and abnormal flow; estimation of intracardiac pressures and pressure gradients; estimation of cardiac output; assessment of systolic and diastolic ventricular function
- Postexercise echocardiography[c]—identification of regional or global wall dysfunction or valve dysfunction exacerbated by exercise

THORACIC RADIOGRAPHY

- Evaluation of pleural space, pulmonary parenchyma, lung vascularity
- Estimation of heart size

THORACIC AND ABDOMINAL ULTRASONOGRAPHY

- Evaluation of abnormal fluid accumulation: pleural effusion, ascites

CLINICAL LABORATORY TESTS

- Complete blood count, serum amyloid A (SAA), and fibrinogen to identify anemia and inflammation
- Serum biochemical tests including electrolytes (particularly K^+, Mg^{2+}, Ca^{2+}), renal function tests, and muscle enzymes: these studies can be useful for assessment of arrhythmias, identification of low cardiac output (azotemia), and recognition of myocardial cell injury (CK and AST, unspecific)
- Cardiac troponin T or I (cTnT, cTnI): Can be measured at rest and after exercise testing to identify myocardial damage
- Serum proteins to identify hypoalbuminemia and hyperglobulinemia
- Arterial blood gas analysis to evaluate pulmonary function (alternatively: pulse oximetry)
- Venous (or preferably mixed venous) blood gas analysis to assess acid-base status, oxygen delivery, and oxygen extraction in the tissues
- Blood lactate to identify anaerobic metabolism associated with poor tissue oxygenation or impaired oxygen utilization in the tissues
- Blood cultures in cases of thrombophlebitis or suspected endocarditis
- Cytology and culture of pericardial effusates
- Urinalysis to identify renal injury from heart failure or endocarditis
- Serum/plasma assays for digoxin, quinidine, and other cardioactive drugs (therapeutic drug monitoring)

CARDIAC CATHETERIZATION AND ANGIOCARDIOGRAPHY[c]

- Diagnosis of abnormal blood flow and identification of abnormal intracardiac and intravascular pressures

RADIONUCLIDE STUDIES[c]

- Detection of abnormal blood flow or lung perfusion; assessment of ventricular function

[a] Most important part of the cardiac evaluation.
[b] May be needed to identify paroxysmal arrhythmias.
[c] Not routinely performed.

may only deteriorate slightly. In other horses, particularly in cases of AF, racing performance may decline greatly by 20 to 30 seconds or more throughout a race. Horses with sudden-onset AF or with malignant ventricular tachycardia (VT) may stop abruptly or even fall. Horses with CV disease also may demonstrate excessively high heart and respiratory rates during and after exercise or may take a longer than normal time to return to a resting rate (or "cool out"). Coughing, either at rest or during exercise, tachypnea, and exercise-induced pulmonary hemorrhage are respiratory signs reported in some horses with heart disease. Cardiovascular disease must always be considered along with musculoskeletal, respiratory, metabolic, and neurologic problems in the differential diagnosis of poor performance (Box 9.5).[87-90,375-377] Other performance-related problems with CV disease can include weakness, ataxia, collapse, and sudden death (Box 9.6).[85,91-106,261]

Once an abnormality has been found, a complete CV examination is aimed at determining the lesion and the clinical relevance of disease in terms of horse and rider safety, performance capabilities, and expected longevity.

BOX 9.5 Cardiovascular Disorders Associated With Poor Performance

ARRHYTHMIAS
Atrial premature complexes
Ventricular premature complexes
Atrial fibrillation
Supraventricular tachycardia
Ventricular tachycardia
Advanced second-degree atrioventricular block
Complete third-degree atrioventricular block

CONGENITAL, VALVULAR, MYOCARDIAL, OR PERICARDIAL HEART DISEASES ASSOCIATED WITH MURMURS
Ventricular septal defects or other congenital malformations
Mitral regurgitation
Tricuspid regurgitation
Aortic regurgitation
Cardiomyopathy with secondary atrioventricular valvular regurgitation
Pericardial disease causing friction rubs

OCCULT HEART DISEASE
Pericardial disease
Myocardial disease

VASCULAR DISORDERS
Aortoiliac thrombosis
Jugular vein thrombosis/thrombophlebitis (bilateral)
Aortic root rupture (aortocardiac fistula)
Peripheral vein thrombosis/thrombophlebitis

BOX 9.6 Causes of Sudden Cardiovascular Death

DISORDERS OF THE HEART (ARRHYTHMIAS)
Ventricular tachycardia, flutter, or fibrillation
Complete atrioventricular block
Asystole

HEMORRHAGE
Rupture of the heart (with cardiac tamponade)
Rupture of the aorta or pulmonary artery (with or without cardiac tamponade)
Arterial rupture
Middle uterine artery
Mesenteric, omental, or other large arteries
Severe pulmonary hemorrhage
Rupture of the spleen or liver
Brain hemorrhage

ACUTE MYOCARDIAL FAILURE
Acute decompensation of chronic heart failure
Acute onset of severe valvular insufficiency
Acute myocardial ischemia/necrosis (rare)

TOXIC INJURY TO THE HEART
Drug- or toxin-induced arrhythmia
Anesthetics
Toxic plants
Myocardial toxins
Systemic toxin secondarily affecting the heart

CARDIAC TAMPONADE
Bacterial pericarditis
Idiopathic pericarditis
Viral pericarditis
Trauma

EMBOLISM
Carotid air embolism
Coronary embolism or thrombosis

ELECTROCUTION
Lightning
Alternating current electrocution

CARDIAC TRAUMA
Cardiac catheterization or needle puncture of a ventricle leading to ventricular fibrillation
Penetrating thoracic wound

Auscultation

Clinical Method

Cardiac auscultation is the systemic examination of the heart using a stethoscope. Auscultation is expedient and relatively sensitive for detection of serious heart disease when performed by a knowledgeable and experienced examiner. It provides information about HR, persistent arrhythmias, and presence or absence of congenital and acquired heart diseases. Auscultation should be conducted within the context of a medical history and general physical examination.

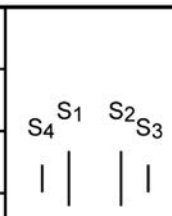
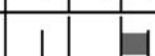
Effective auscultation requires an understanding of anatomy, physiology, pathophysiology, and sound. There is extensive clinical experience regarding cardiac auscultation in the horse,* and experience with Doppler echocardiography has refined the clinician's understanding of heart sounds and murmurs.[71,208,209,211,390,391] Experience and training are also significant factors in effective cardiac auscultation,[392,393] and this examination method should be considered an acquired skill that can be constantly honed. The overall sensitivity of auscultation is high for identification of congenital heart diseases, *significant* valvular disease, and *persistent* cardiac arrhythmias. Sensitivity is lower for primary myocardial or pericardial diseases, unless there are obvious associated abnormalities such as a murmur, arrhythmia, or prominent friction rub. The specificity of auscultation in the horse (e.g., the ability to distinguish a functional from a pathologic murmur or identify a specific flow disturbance) has not been sufficiently studied, but most certainly depends on the clinician's knowledge and experience,[392] as well as one's opinion related to the physiologic versus pathologic nature of valvular regurgitation in some high-performance horses.

A prerequisite for auscultation is an appreciation of the normal heart sounds, the genesis of which has already been described (see Clinical Cardiovascular Physiology). The examiner must be familiar with the causes and clinical features of arrhythmias and murmurs (Tables 9.1 through 9.3) and the areas for auscultation (Fig. 9.7).* Auscultation is best

* References 3, 16, 19, 23, 25, 57, 72, 73, 75, 152, 223, 282, 378-389.

* References 3, 19, 57, 73, 379-382, 385, 386, 393, 394.

TABLE **9.1** **Identification of Heart Sounds and Common Cardiac Murmurs**

Auscultatory finding	Timing°		Point of maximal intensity (valve area)*
Normal Heart Sounds			
First heart sound (S_1)	Onset S		Left apex (mitral valve)
Second heart sound (S_2)	End S		Left base (aortic valve)
Pulmonic component	End S		Left base (pulmonic valve)
Third heart sound (S_3)	Early D		Left apex (mitral valve)
Fourth (atrial) sound (S_4)	Late D		Ventricular inlet or base (Left)
Functional Murmurs†			
Systolic ejection murmur	S		Left base (Aortic/pulmonary valves)
Early (proto-)diastolic	D		Ventricular inlets (Left/Right)‡
Late diastolic (presystolic)	D		Ventricular inlets (Left/Right)‡
Valvular Regurgitation§			
Mitral regurgitation	S		Left apex (mitral valve)
Tricuspid regurgitation	S		Right hemithorax (tricuspid valve)
Aortic regurgitation	D		Left base (aortic valve)
Pulmonary insufficiency	D		Left base (pulmonic valve)
Ventricular Septal Defect ‖	S		Right sternal border/left cardiac base
Patent Ductus Arteriosus	S + D		Dorsal left base over pulmonary artery

°S—systole, the interval between S_1 and S_2; D—diastole, the interval between S_2 and S_1 (see Figs. 9.8 and 9.9).
*Only typical features are considered; "apex" refers to the ventral part of the heart, at the point of the palpable cardiac impulse (apex beat); "base" refers to the craniodorsal part of the heart over the outlet valves (aortic, pulmonic) where the second heart sound is most intense (see Fig. 9.7).
†The exact causes of functional (flow) murmurs have not been proven. The systolic ejection murmur, which begins after the first heart sound and ends before the second heart sound, is the most commonly identified murmur in the horse; the protodiastolic murmur extends from the second to the third heart sound; the presystolic murmur is quite short, spanning the fourth and first heart sounds. Functional murmurs may be musical.
‡Ventricular inlets refer to the parts of the thorax overlying the ventricular inflow tracts. These include the areas just dorsal to the mitral and tricuspid valve areas and extend ventrally to the apical regions of the ventricles.
§Murmurs of AV valve insufficiency are generally heard over the affected valve, project prominently toward the respective ventricular apex, and also radiate dorsally, following the regurgitant jet into the atrium. Occasionally, murmurs of tricuspid valve disease are prominent at the extreme left, cranial heart border. Valve regurgitation murmurs are evident throughout systole or diastole and extend into the second heart sound (holosystolic or pansystolic) or first heart sound (holodiastolic or pandiastolic); however, late systolic murmurs, which may be related to valve prolapse, have been identified with mitral or tricuspid valve insufficiency, and the murmur of aortic insufficiency may not always be holodiastolic. Documented reports of valve stenosis are quite rare.
‖Murmurs caused by defects in the right ventricular inlet septum (paramembranous VSD, common) are heard best above the right sternal border; murmurs from defects in the RV outlet septum (subpulmonic VSD, rare) may be loudest over the pulmonic valve; increased flow across the pulmonic valve can cause left-basilar systolic murmurs of relative pulmonic stenosis in the absence of pulmonic valve pathology; flow across very large nonrestrictive defects can be relatively soft.
From Schwarzwald, C.C., Bonagura, J.D., and Muir, W.W.: The cardiovascular system. In: *Equine Anesthesia—Monitoring and Emergency Therapy* (Ed. W.W. Muir), 2nd ed. Saunders Elsevier, p. 65.

carried out in a very quiet area because extraneous noise makes detection of soft to moderate murmurs quite difficult. The horse or foal should be sufficiently restrained so the examiner can concentrate on listening. The venous pulse should be inspected and then the arterial pulse and the precordium palpated before commencing auscultation. Although uncommonly used today, percussion of precordial area to identify the region of cardiac dullness can be used effectively by practiced examiners to identify heart size.[395]

The stethoscope examination generally commences on the left side. Both stethoscope chest pieces—the diaphragm (applied tightly, for high-frequency sounds and murmurs) and the bell (applied lightly, for low-frequency sounds and murmurs)—should be used. When using a single-piece tunable

TABLE **9.2** **Auscultation of Cardiac Arrhythmias**

Rhythm	Heart Rate per Minute	Heart Sounds[a]	Auscultation
SINUS RHYTHMS			
Sinus rhythm	Variable	S_4–S_1–S_2 (S_3)	Rate and rhythm dependent on autonomic tone
Sinus arrest/block	<26	S_4–S_1–S_2 (S_3)	Irregular, long pauses
Sinus bradycardia	<26	S_4–S_1–S_2 (S_3)	Generally regular unless escape rhythm develops
Sinus arrhythmia	25–50	S_4–S_1–S_2 (S_3)	Irregular, cyclic change in heart rate; often interval varying between S_4 and S_1; often associated with second-degree atrioventricular block or sinus bradycardia
Sinus tachycardia	>50	S_4–S_1–S_2 (S_3)	Typically regular, but second-degree atrioventricular block may develop if sympathetic tone decreases (e.g., during recovery from exercise)
SUSTAINED ATRIAL TACHYARRHYTHMIAS			
Atrial tachycardia and atrial flutter	>30	S_1–S_2 (S_3)	Ventricular regularity and rate dependent on atrioventricular conduction sequence and sympathetic tone; consistent S_4 absent; variable-intensity S_1; may detect independent atrial sounds
Atrial fibrillation	>30	S_1–S_2 (S_3)	Ventricular response irregular; rate related to sympathetic tone; heart rates consistently above 60 beats/min suggestive of significant underlying heart disease or heart failure; absence of consistent S_4
ECTOPIC RHYTHMS			
Junctional rhythm	26–200	S_1–S_2 (S_3)	Heart rate usually regular with idionodal rhythms or junctional tachycardia; heart rate dependent on the mechanism and sympathetic tone; inconsistent independent S_4 at lower rates possibly audible
Ventricular rhythm	26–200	S_1–S_2 (S_3)	Heart rate possibly regular during monomorphic, uniform ectopic rhythm or irregular during polymorphic or multiform ectopic activity; heart rate dependent on the mechanism (e.g., escape rhythm versus ventricular tachycardia); variable intensity and split heart sounds possibly audible
Premature atrial and junctional beats	Varies	Early S_1–S_2	Intensity of S_1 may be louder or softer than normal; the sounds are not usually split; less than compensatory pause often follows premature beat; nonconducted atrial premature complexes result in pauses but not premature first heart sounds
Premature ventricular beats	Varies	Early S_1–S_2	Intensity of S_1 often variable and ventricular beats possibly softer than normal; heart sounds possibly split from asynchronous ventricular activation heart sounds; compensatory pause typically following a premature beat
ATRIOVENTRICULAR BLOCKS			
Incomplete (first- and second-degree block)	<50	S_4–S_1–S_2 (S_3) Isolated S_4	Heart rate variable; cyclic arrhythmia, variable S_4–S_1 interval; some variation in heart sounds; isolated S4 with second-degree AV block
Complete (third-degree block)	<26	S_4/S_1–S_2 (S_3)	Ventricular escape rhythm, usually regular; independent atrial (S_4) sounds; variable-intensity heart sounds

[a]Heart sounds in parentheses may not be audible.

TABLE **9.3** **Causes of Cardiac Murmurs**

Cardiac Murmur	Lesion Identified by Echocardiography, Cardiac Catheterization, or Necropsy
Functional murmurs[a]	No identifiable lesions; occasionally markedly turbulent systolic blood flow identified in great vessels on color Doppler echocardiography
Congenital heart disease murmurs	Defect(s) in the atrial or ventricular septa; patent ductus arteriosus; atresia/stenosis of tricuspid or pulmonic valve; valve stenosis; complex malformations of the heart
Mitral regurgitation[b]	No identifiable lesion (high-level training); degenerative thickening of the valve; bacterial endocarditis; mitral valve prolapse into left atrium; rupture of a chorda tendinea; dilated, hypokinetic ventricle (dilated cardiomyopathy, severe aortic regurgitation); papillary muscle lesion or dysfunction; noninfective valvulitis; malformation

Continued

TABLE **9.3** **Causes of Cardiac Murmurs—cont'd**

Cardiac Murmur	Lesion Identified by Echocardiography, Cardiac Catheterization, or Necropsy
Tricuspid regurgitation[b]	Same as mitral regurgitation; also pulmonary hypertension from severe left-sided heart failure or chronic respiratory disease
Aortic regurgitation[b]	Degenerative thickening and/or prolapse of the valve; congenital fenestration of the valve; bacterial endocarditis[c]; aortic prolapse into a ventricular septal defect; flail aortic valve leaflet; noninfective valvulitis; malformation; ruptured aorta or aortic sinus of Valsalva
Pulmonary regurgitation[b]	No identifiable lesion; bacterial endocarditis[c]; pulmonary hypertension; flail pulmonary leaflet; noninfective valvulitis; malformation; rupture of the pulmonary artery

[a]Functional murmurs may be innocent (unknown cause) or physiologic (suspected physiologic cause); functional murmurs are very common in foals, trained athletes (athletic murmur), and horses with high sympathetic nervous system activity (pain, stress, sepsis); are associated with fever; and are often heard in anemic horses. Functional murmurs are very dependent on physiologic state and can be altered (accentuated or abated) by changing the heart rate. Such dynamic auscultation is very useful in detecting functional murmurs.
[b]"Silent" trivial or mild regurgitation across right-sided (most common) or left-sided (less common) cardiac valves can be identified in some horses by Doppler echocardiography; this is probably a normal finding of no clinical relevance provided that no other findings indicate the presence of cardiac disease. Pulmonary insufficiency is often silent. Many trained athletes have audible murmurs of tricuspid and/or mitral regurgitation that are not associated with poor performance or signs of heart disease (by physical examination and echocardiography) and are not considered clinically relevant.
[c]Large vegetations may result in anatomic stenosis causing a systolic murmur, which is usually associated with a diastolic murmur of valve insufficiency; increased flow across a normal valve may generate a murmur of relative valve stenosis (e.g., with aortic regurgitation, there may be a systolic ejection murmur resulting from an increased stroke volume).

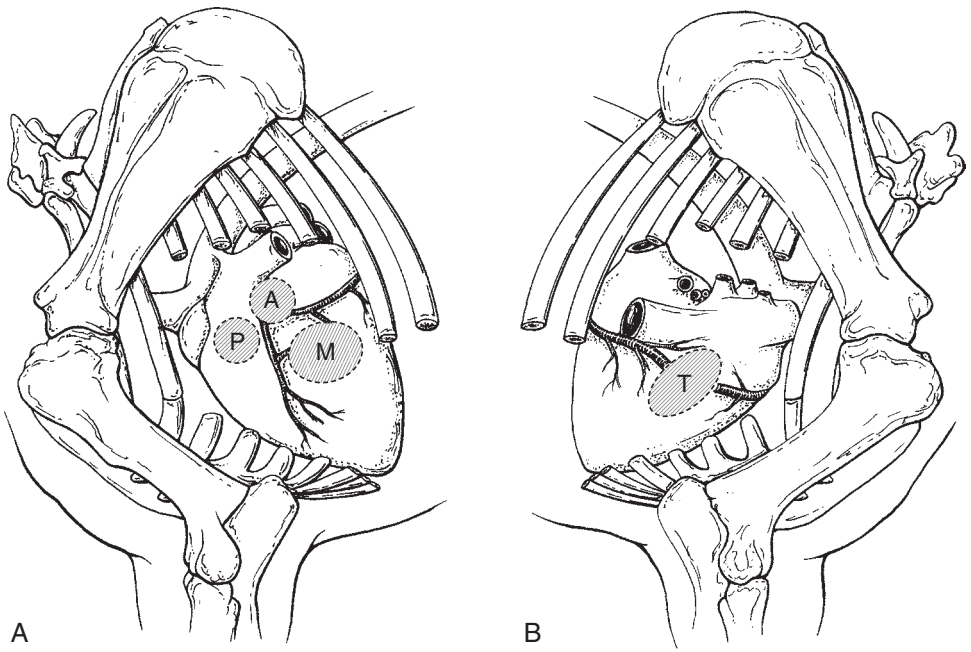


FIG. **9.7** Cardiac auscultation areas in the horse from the left (A) and the right (B) side of the thorax. Note the size and anatomic position of the heart within the thorax. The cardiac apex (apical area) is usually located slightly above the level of the olecranon and can be identified by palpation of the apical beat. The cardiac base (basilar area) is located more cranially and at the level of the scapulohumeral joint. The shaded areas represent the respective valve areas (*P,* pulmonic; *A,* aortic; *M,* mitral; *T,* tricuspid). The right atrium (RA) *(above T),* tricuspid valve, and right ventricular (RV) inlet (inflow region, *below T*) are located on the right side of the thorax. Paramembranous ventricular septal defects (VSDs) that communicate with the RV inlet (below the tricuspid valve) are usually heard best along the lower right hemithorax *(below T)* and typically radiate from the right ventricle (RV) into the pulmonary artery (left hemithorax). Most (but not all) murmurs of tricuspid valve disease are heard best over the right chest wall, usually heard best more dorsally as they radiate into the RA *(above T).* The RV outlet projects to the left side of the thorax and continues into the pulmonary artery, which is located at the left dorsal cardiac base *(above P).* Thus murmurs originating in the RV outlet (e.g., murmurs from subpulmonic VSDs), systolic murmurs of relative pulmonic stenosis (from cardiac shunting), murmurs of pulmonic insufficiency (rare), and functional pulmonary arterial murmurs are heard best over the left chest wall *(P).* The aortic valve is located centrally, and diastolic murmurs of aortic insufficiency may be heard at either hemithorax, although they are usually loudest on the left *(A).* Functional murmurs generated in the ascending aorta are heard over the cranial basilar region of the heart. The systolic murmur of mitral regurgitation (MR) radiates to the left apex and is usually heard across the left ventricular (LV) inlet (area caudoventral of *M*). The regurgitant mitral jet can be directed craniodorsal or caudodorsal into the left atrium (LA) and can account for variability in murmur radiation. The functional protodiastolic murmurs associated with ventricular filling are usually evident over the ventricular inlets and may be heard on either side of the thorax. (From Reef VB: Cardiovascular system. In Orsini JA, Divers TJ, editors: *Manual of equine emergencies: treatment and procedures,* ed 2, Philadelphia, 2003, WB Saunders.)

diaphragm, the pressure on the chest piece should be gradually increased to optimize the sounds of interest. A variety of digital stethoscopes are available today that are able to amplify heart sounds and murmurs across selected frequency ranges, reduce or eliminate external noise and artifacts, record sound, and display phonocardiograms on a computer or a smartphone. These devices can be helpful for diagnostic and teaching purposes. However, the clinician should be aware that digitally processed sound quality is different compared with traditional stethoscopes. The diagnostic agreement may only be moderate, and the sensitivity to detect abnormal sounds and murmurs may not necessarily be improved with digital techniques.[396,397] It is therefore advisable to use digital stethoscopes complementary to and not in place of conventional stethoscopes.

All auscultatory areas should be examined (see Fig. 9.7). The locations of the cardiac valve areas can be identified using the following method:

- The left apical impulse located at the left thoracic wall is adjacent to or under the olecranon near the fifth intercostal space; this location identifies the ventral region of the LV inlet. The mitral valve is dorsal to the cardiac impulse, but mitral sounds and murmurs often project well to the apex while also radiating dorsally into the left atrium. The first heart sound (S_1) is best heard at this location and the ventricular filling or third heart sound (S_3) is also heard well over this point.
- The aortic valve area is located one (or two) intercostal spaces cranially and is dorsal to the left apical impulse. The second sound (S_2) is loudest at this point, and aortic valve murmurs are heard best over this valve. The murmur of mitral regurgitation may also radiate dorsally and cranially to this area. Because of the central location of the aortic valve and the orientation of the ascending aorta to the right, aortic flow murmurs and the murmur of aortic regurgitation generally can be heard bilaterally, just medial to the triceps muscles.
- The pulmonic valve is located slightly cranioventral (generally one intercostal space) to the aortic valve, and the pulmonary component of S_2 is loudest at this point. This is also the location where splitting of the second sound is most obvious. The main PA extends dorsally from the pulmonic valve, high on the left-cranial base. Murmurs that radiate into the PA are heard best at this location, including some functional murmurs and the murmur of patent ductus arteriosus. The murmur associated with a subarterial (subpulmonic) VSD, as well as the rare murmurs of an atrial septal defect or pulmonic stenosis, are typically loudest over the pulmonary valve and PA.
- The tricuspid valve area is located over a wide area on the right hemithorax, dorsal to the sternum and just cranial to the mitral valve; sounds and murmurs associated with tricuspid valve disease are usually heard best over the right hemithorax. Murmurs of paramembranous VSDs are often detected ventral and slightly cranial to the tricuspid valve area, dorsal to the sternum.

It is worthwhile to concentrate first on the individual heart sounds when assessing the heart rhythm, because the generation of cardiac sounds depends on the underlying electrical rhythm and heart murmurs are timed relative to the heart sounds (Fig. 9.8). The atrial sound (S_4) is heard after the P wave. The first and second heart sounds (S_1 and S_2) encompass systole, indicating the presence of a QRS complex. A murmur detected between the first and second sounds is termed *systolic*. In contrast, a murmur heard after S_2 is designated as *diastolic*. The distinctive atrial sound (S_4) is absent in AF. Normal variation in the PR interval causes gradual changes in the S_4–S_1 interval. Absence of a QRS complex, which occurs with second-degree AV block and with nonconducted atrial premature complexes, causes a pause in which the first and second heart sounds are absent. The heart sounds and precordial movements often can be palpated, especially over the left thoracic wall. Frequently the cardiac movements and low-frequency vibrations corresponding to the atrial contraction (S_4), onset of ventricular contraction (S_1), and closure of the semilunar valves (S_2) can be detected. With ventricular volume overloading or vigorous normal filling, an accentuated apex beat and third sound (S_3) will be palpable. Cardiac enlargement or displacement of the heart by an intrathoracic mass may lead to an abnormal location of the cardiac impulse. A loud cardiac murmur is often associated with a palpable vibration or *precordial thrill*.

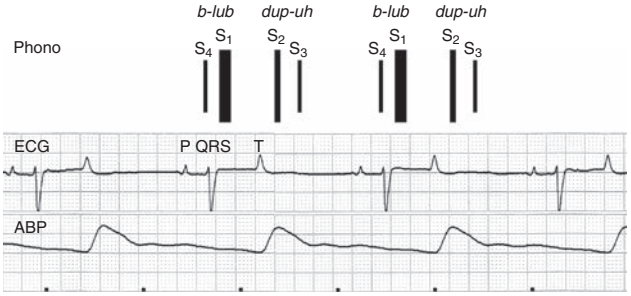


FIG. **9.8** Schematic diagram of the normal heart sounds (Phono) in relation to a surface electrocardiogram (ECG) and an arterial blood pressure tracing (ABP) recorded from the transverse facial artery. S_1, First (systolic) heart sound; S_2, second (diastolic) heart sound; S_3, third heart sound; S_4, fourth (atrial) heart sound. *B-lub dup-uh* describes the sounds heard on auscultation. Note the timing of the heart sounds and the (peripheral) pulse pressure wave relative to the ECG. (From Orsini JA, Divers TJ, editors: Manual of equine emergencies: treatment and procedures, ed. 2, Philadelphia, 2003, Saunders.)

Heart Sounds

Heart sounds should be readily heard on each side of the thorax, although there is some variability based on body type, and sounds are louder over the left thoracic wall. All four heart sounds can be detected in healthy horses, but all may not be present or evident at the same location (Figs. 9.8 and 9.9; see Table 9.1).[3,23,25,382] The intensity of the heart sounds should be consistent when the rhythm is regular, but variation of heart sound intensity occurs with arrhythmias (irregular cardiac filling). Muffled heart sounds are heard with pericardial effusions or pericardial abscesses (the muffling may only be on one side of the thorax) but also occur in some horses with large pleural effusions and cranial mediastinal masses. Accentuation of all heart sounds, especially the third sound, may be detected with volume-loaded ventricles or with marked sympathetic activity. Projection of heart sounds over a wider area is sometimes evident in cases of pleuropneumonia with pulmonary consolidation.

The first heart sound (S_1) varies with arrhythmias and often becomes louder (or sometimes softer) after prolonged diastolic periods. This in itself is not diagnostic of an abnormality. Splitting of the first heart sound, if pronounced, may indicate abnormal ventricular electrical activation or ventricular premature complexes.[3,282] Close splitting of the first sound may be more obvious when there is AF, and in the absence of an atrial sound (S_4), the split S_1 may be misinterpreted as a closely timed S_4–S_1 complex.

The second sound (S_2) is loudest normally over the aortic valve area and may be audibly split over the pulmonic valve

area.[3,282] This sound can be very soft or absent following a premature beat, and it may be obscured by a holosystolic murmur. Audible splitting of S_2 occurs commonly in normal horses, varies with HR or respiration, and only infrequently is associated with pulmonary hypertension. The relative closure of the semilunar valves probably varies with the HR and PA pressure,[3,282] although the pulmonic component is most often detected after the aortic component in the healthy, resting horse. If the pulmonic component of the second sound develops a tympanic quality, becoming equal to or louder than the aortic component of S_2, the clinician should suspect pulmonary hypertension. Identification of a loud pulmonic S_2 is a useful clinical finding in cases of right-sided heart failure because it often indicates that a lesion on the *left* side of the heart has led to pulmonary hypertension and right-sided CHF.

Diastolic transient sounds are normal.[3,16,17,19,387] The atrial sound (S_4) should be detected in virtually all horses, and this sound may be quite loud in some cases. Isolated atrial sounds are commonly auscultated at slow resting HRs due to second-degree AV block; however, multiple isolated S_4 sounds indicate high-grade AV block. The ventricular filling sound (S_3) is most localized and variable and may be difficult to detect unless the bell is placed lightly over the left apex. This sound is normal and has a low-pitched sound that often increases transiently when there is sinus tachycardia and enhanced rapid filling. This sound also achieves greater intensity when there is ventricular dilation and elevated filling pressures as with heart failure.

Other sounds may be detected with heart disease. Systolic clicks are uncommonly heard over the great vessels where they are thought to be benign. A systolic click loudest over the mitral area may be a marker for mitral valve disease or prolapse of the valve into the left atrium. Constrictive pericardial disease can create a loud early diastolic ventricular filling sound, a ventricular "knock," indicating abrupt termination of rapid filling under high venous pressures. In addition to muffling of sounds, pericarditis is often associated with a pericardial friction rub. These rubs are classically detected during three portions of the cardiac cycle (systole, early diastole, late diastole). These sounds must be distinguished from pleural friction rubs or respiratory mediated sounds, which are occasionally associated with the cardiac cycle. Pleural and pulmonary rubs tend to be heard only during one or two phases of the cardiac cycle and may not correlate closely.

Heart Rate and Rhythm

HR can change rapidly and dramatically, varying with autonomic efferent traffic and level of physical activity. Changes in the rate and rhythm are reflected in cardiac auscultation and palpation of the pulse (see Table 9.2). The normal arterial pulse and cardiac rhythm are regular or cyclically irregular. HR in mature horses varies between 28 and 44 beats/min, although slightly slower HRs can be detected in some very fit racehorses, and higher HRs are often present in foals,[386] yearlings, draft horses,[398] ponies,[399] and healthy but nervous horses. An elevated resting HR is commonly detected with late pregnancy, fever, pain, hypovolemia, severe anemia, infection, or shock. Persistent, otherwise unexplained tachycardia is also typical of heart failure wherein the standing HR usually exceeds 60 beats/min.

Physiologic arrhythmias associated with high resting vagal tone must be differentiated from pathologic arrhythmias. Vagal induced arrhythmias are most often observed in healthy relaxed

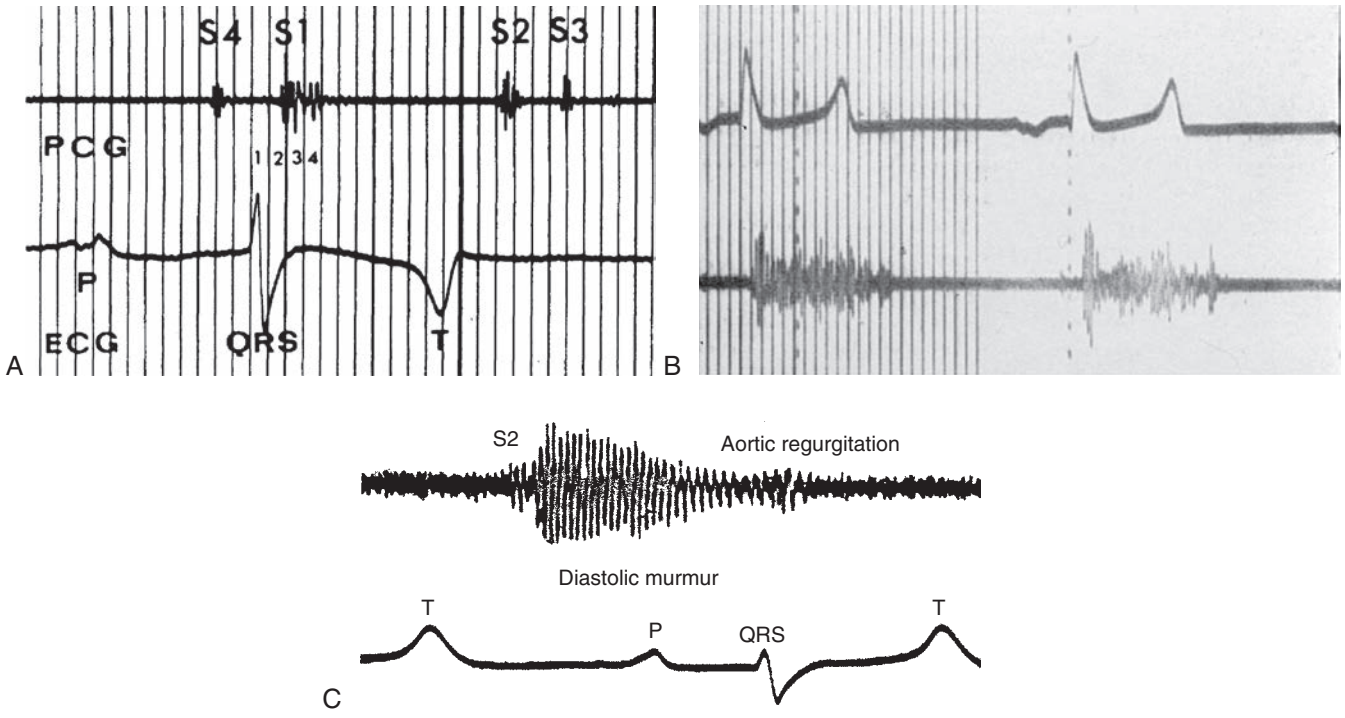


FIG. **9.9** Phonocardiograms (PCG). A, The four normal heart sounds (S_1 through S_4) are evident in this recording. (The numbers *1* through *4* indicate the various components of the first heart sound; *ECG,* electrocardiogram.) B, Systolic murmur recorded from a yearling with a ventricular septal defect (VSD) (P waves on the ECG are negative in this tracing). C, Decrescendo, musical, holodiastolic murmur recorded from a horse with aortic regurgitation (AR). The murmur begins in early diastole (after the T wave) and ends at the QRS complex. (*A,* Recording courtesy D. Smetzer, R.L. Hamlin, and C.R. Smith.)

horses. The most common of these are sinus arrhythmia and second-degree AV block (see Fig. 9.6), which is reported in 15% to 18% of normal horses at rest and has been detected in up to 44% of normal horses with 24-hour continuous electrocardiographic monitoring.[222,227,400] This arrhythmia is most common in fit racehorses or in other high-performance animals and disappears with stimulation of sympathetic nervous system activity. Sinus arrhythmia also occurs regularly in horses and is often associated with sinus bradycardia and resting HRs of 24 to 28 beats/min. Sinus arrhythmia may wax and wane with respiration; however, synchronization with ventilation is not a consistent finding in the horse, and it is more likely related to changes in baroreceptor activity. Sinoatrial block and SA arrest occur sporadically in fit horses. Because these physiologic arrhythmias are associated with high vagal tone, it is typical for the HR to range from a low-normal value to overt bradycardia. Maneuvers that reduce vagal activity and increase sympathetic tone can be undertaken to ensure that the rhythm becomes normal. Successful methods include leading the horse through three or four tight circles, jogging, or lunging. It should be recognized, however, that some horses redevelop physiologic AV block within 10 to 60 seconds following such maneuvers. If the arrhythmia persists after exercise, or if the auscultatory findings suggest another arrhythmia, an ECG should be obtained (Table 9.4; also see Table 9.2).

The arrhythmias associated with heart disease occur most often with normal to increased HRs, with the exception of advanced second-degree and complete (third-degree) AV block.[33,84,383,401,402] Atrial premature beats are characterized by a regular cardiac rhythm, which is suddenly interrupted by earlier than normal beats. Because the sinus node is reset during an atrial premature beat, the following pause is usually incomplete or less than compensatory (provided the underlying sinus rhythm is regular). Premature atrial beats can also be blocked in the AV node, leading to a sudden pause in the rhythm without a premature first heart sound. A ventricular premature beat is often followed by a fully compensatory pause unless the extrasystole is interpolated between two normal sinus beats. Pulse deficits (more first heart sounds than arterial pulses) are common with premature beats due to inadequate ventricular filling and subsequent low stroke volume.

Atrial fibrillation is characterized by an irregularly irregular pulse and ventricular rhythm, with some beats occurring sooner than expected, and with pauses in the rhythm demonstrating no consistent diastolic interval. The atrial contraction and hence the fourth sound (S_4) are absent in AF. Infrequently, AF develops with recurring periodicity of cycle lengths that can cause difficulty in distinguishing this from second-degree AV block.[219,221,229,403] At higher rates, such as during exercise or in a horse in heart failure, the cycle lengths shorten and the rhythm appears to become (more) regular. However, when listening carefully, an irregularly irregular rhythm is still evident, distinguishing AF from second-degree AV block. Another diagnostic pitfall can be the presence of a split first sound, because the clinician may mistake this for a closely timed S_4–S_1 complex.

Atrial tachycardia can be conducted with patterns leading to a rapid but regular heart rhythm. However, most horses with atrial tachycardia develop irregular rhythms owing to frequent block of atrial impulses in the AV node, especially at rest. HRs are usually higher than normal at rest. Auscultation of the horse in a quiet area may reveal isolated audible fourth heart sounds associated with the second-degree AV block.

Junctional or nodal tachycardia and ventricular tachycardia (VT) are usually manifested by a rapid regular rhythm, although some VTs (particularly polymorphic or multiform VT) cause an irregular rhythm. Owing to concomitant AV dissociation or abnormal ventricular activation, the heart sounds may sound split or variable in intensity.

Cardiac Murmurs

Cardiac murmurs are prolonged audible vibrations developing in a usually quiet portion of the cardiac cycle. In general, murmurs are manifestations of either normal (functional) or abnormal (pathologic) blood flow in the heart and blood vessels. Although many heart murmurs are functional (physiologic, athletic, innocent), other murmurs provide evidence of heart disease and may require further investigation (see Tables 9.1, 9.3, and 9.4). One of the challenges of physical diagnosis is determination of the cause and clinical relevance of a cardiac murmur. With knowledge and experience the clinician can accurately determine the *likelihood* that a murmur is physiologic or pathologic in origin, and efficiently select animals requiring additional studies. The clinician should describe the *timing, duration, quality or pitch, grade, point of maximal murmur intensity,* and *radiation* of a murmur (Box 9.7), as well as the effect of changing HR on the sounds. Determination of timing is made relative to the heart sounds so that murmurs are designated as systolic, diastolic, or continuous. When the overall timing is not obvious, the clinician can either palpate the apical impulse, which occurs in early systole, or the systolic pulse in the brachial or median artery to identify the timing of the murmur (consider the slight time delay between ventricular contraction and occurrence of a peripheral pulse wave). It is also helpful to remember that at a normal (low) HR, ventricular diastole (i.e., the time interval between S_2 and the following S_1) is markedly longer than ventricular systole (i.e., the time interval between S_1 and the following S_2) (see Fig. 9.8). Hence, long-lasting murmurs or brief murmurs occurring during the long S_2–S_1 interval are likely to be diastolic murmurs. Skilled auscultators subdivide the timing of murmurs into *proto-* (early), *meso-* (middle), or *tele-* (late) systole or diastole because the timing and duration of the murmur often correlate with specific flow disturbances. Shorter murmurs, especially those occurring in early systole or protodiastole, are more likely to be functional, although this is not always the case. Experience also teaches that relatively loud or long murmurs are more likely to be associated with cardiac pathology, but again there are exceptions. The pitch or quality of the murmur provides additional insight. For example, mixed frequency (harsh or blowing) murmurs are typical of cardiac disease. A brief vibratory or musical murmur is typically functional; however, a prolonged musical murmur is suggestive of a valvular lesion. Applying the characteristics of murmurs to the clinical setting requires study, an organized scheme (see Table 9.4), and practice.

Functional, innocent, or physiologic heart murmurs are very common and are not attributed to cardiac pathology. Such murmurs may be related to the large size and tremendous inflow and outflow volumes of the equine heart. They are caused by vibrations that attend the *ejection* of blood from the heart during systole or the *rapid filling* of the ventricles during early diastole.* Physiologic causes of functional murmurs include fever, high sympathetic activity (e.g., colic,

* References 3, 18, 19, 32, 72, 207, 223, 380, 382, 384, 388, 404-407.

TABLE **9.4 Diagnostic Algorithm for Cardiac Auscultation in the Horse**

Auscultation		Considerations		Further Observations		Probable Assessment		Other Tests
Irregular rhythm	→	Vagally mediated or abnormal rhythm	⇒	Induce increased sympathetic tone[a] ⇓				
				↑ Rate and *regular* rhythm	→	Vagally mediated (physiologic) rhythm[b]	→	None
				↑ Rate and *irregular* rhythm	→	Abnormal cardiac rhythm (see Table 9.2 for details)	→	ECG, ± echo, electrolyes, cardiac troponin I (cTnI)
Regular tachycardia <90 beats/min[c]	→	Sympathetic activation (stress/anxiety, pain, volume depletion, infection, anemia, heart failure, etc.) or ectopic cardiac rhythm	⇒	Manage underlying disorder ⇓				
				Appropriate HR reduction with normal heart sounds	→	Physiologic sinus tachycardia	→	None
				Persistent tachycardia, abnormal heart sounds or murmurs	→	Persistent sinus or ectopic tachycardia,[d] underlying organic heart disease	→	ECG, ± echo, electrolyes, cardiac troponin I (cTnI)
Loud cardiac murmur with a precordial thrill	→	Organic heart disease	⇒	Determine timing and PMI (see below)	→	Congenital or acquired heart disease	→	Echo
Systolic murmur PMI: left thorax	→	Functional or organic	⇒	PMI: pulmonic or aortic valve Crescendo-decrescendo				
				Grade: 1–3/6[e]	→	Functional ejection murmur	→	None[g]
				Grade: 4–6/6	→	Congenital heart defect[f]	→	Echo
				PMI: mitral valve or left apex Any grade or configuration	→	Mitral regurgitation	→	Echo[g,h]
Systolic murmur PMI: right thorax	→	Organic murmur	⇒	PMI: tricuspid valve Any grade or configuration	→	Tricuspid regurgitation	→	Echo[g,h]
				PMI: cranioventral thorax Holosystolic	→	Ventricular septal defect (VSD)	→	Echo
Diastolic murmur PMI: left or right thorax	→	Functional or organic	⇒	Brief (protodiastolic or presystolic)	→	Functional murmur (or trivial AR)	→	None[g]

				PMI: aortic or pulmonic valve Holodiastolic	→	Aortic regurgitation (AR) or pulmonic regurgitation with pulmonary hypertension	→	Echo[g,h]
Continuous murmur PMI: left or right thorax	→	Organic murmur	⇒	PMI: pulmonary artery Foal or other signs of CHD	→	Patent ductus arteriosus (PDA) or a systemic to pulmonary shunt	→	Echo
				Mature horse	→	Aortic to cardiac fistula or aortic to pulmonary artery fistula	→	Echo
Pericardial friction rub[i]	→	Organic murmur	⇒	Systolic or multiphasic Distant (muffled) heart sounds Jugular venous distention	→	Pericarditis	→	Echo

[a]Endogenous sympathetic activity can be transiently increased by suddenly leading the horse in four or five tight circles or through trotting or lunging; the horse should be examined *immediately* after the activity has stopped.
[b]Vagally induced rhythms include sinus bradycardia, pause or arrest, sinoatrial block, sinus arrhythmia, and second-degree atrioventricular block.
[c]Resting tachycardia exceeding 90/min should be evaluated with an ECG to qualify the rhythm; the cutoff rate is arbitrary but clinically useful.
[d]Ectopic rhythms include ectopic atrial tachycardia, junctional (nodal) tachycardia, and ventricular tachycardia.
[e]Functional murmurs due to ejection of blood into the great vessels often become transiently louder following an increase in sympathetic activity.
[f]Loud ejection murmurs over the semilunar valves and great vessels may indicate pulmonic stenosis or complex defects such as tetralogy of Fallot.
[g]Judgment should be exercised regarding the need for echocardiography. An echocardiogram might be indicated in the prepurchase examination of any valuable foal or horse with a cardiac murmur; however, echocardiography may not be indicated in an older horse with a soft murmur of aortic regurgitation with normal arterial pulses or in a fit racehorse with a typical murmur of tricuspid regurgitation.
[h]According to the 2014 Joint ACVIM/ECEIM Consensus Statement on Recommendations for Management of Equine Athletes with Cardiovascular Abnormalities (Reef VB, et al., J Vet Intern Med 28:749-761, 2014), an echocardiogram is specifically indicated if a left-sided murmur compatible with mitral regurgitation or aortic regurgitation is of grade 3–6/6 or a right-sided systolic murmur compatible with tricuspid regurgitation is of grade 4–6/6.
[i]Classic pericardial friction rubs are heard during systole, early diastole, and late diastole. Occasionally a functional presystolic murmur exhibits a frequency quality similar to a brief friction rub.

Abbreviations and Terminology:

Configuration = the phonocardiographic "shape" of a murmur; for example, a "plateau-shaped" murmur that extends from the first sound through the second sound is termed *holosystolic* or *pansystolic* (e.g., MR or VSD); a "diamond-shaped" murmur is crescendo-decrescendo in configuration (e.g., functional ejection); a "blowing" murmur that starts suddenly but dissipates gradually is decrescendo (e.g., aortic regurgitation); and a murmur that becomes progressively louder and then stops is crescendo in configuration (e.g., MR from mitral valve prolapse)
ECG = perform an electrocardiogram to determine heart rhythm
Echo = perform an echocardiogram to identify abnormal structure, cardiac size, or function; Doppler studies are added to assess normal and abnormal blood flow patterns and are optimal for evaluating horses with cardiac murmurs; Doppler studies must be interpreted relative to auscultatory findings
Grade = intensity of the murmur on a 1 through 6 scale; in most systems a grade 5 or 6 murmur is accompanied by a precordial thrill
HR = heart rate
Organic = related to a structural lesion in the heart or great vessels
PMI = point of maximal murmur intensity
Precordial thrill = the palpable thoracic vibration associated with a loud cardiac murmur
Troponin I = serum/plasma concentration of inhibitory cardiac troponin or cTnI, a biomarker used to detect cardiomyocyte injury or necrosis
Valve = refers to the general valve area of auscultation (see text for details)

BOX 9.7 Characterization of Heart Murmurs

TIMING AND DURATION (see Figs. 9.8 and 9.9 and Tables 9.1 and 9.4)

- Systolic, diastolic, or continuous
- Early, mid, or late systolic or diastolic
- Holosystolic (S_1 to S_2), holodiastolic (S_2 to S_1)

QUALITY

- Frequency of sound—high pitch, low pitch, mixed-pitch
- Character of murmur—harsh, coarse, rumbling, musical, honking, blowing

GRADE

- Grade 1/6—very quiet, only heard over a very localized area after careful auscultation in quiet environment, may be inconsistent
- Grade 2/6—quiet, heard consistently over the point of maximal intensity
- Grade 3/6—moderately loud, heard immediately and consistently, small area of radiation
- Grade 4/6—loud, radiating over a wider area, no palpable thrill
- Grade 5/6—very loud, radiating over widespread area, palpable precordial thrill
- Grade 6/6—extremely loud, also heard with the stethoscope held just above the skin surface
- Variations in grade can be described as crescendo (increasing in grade), decrescendo (decreasing in grade), or crescendo-decrescendo ("diamond-shape")

POINT OF MAXIMAL INTENSITY

- Apical—location of the thoracic wall cardiac impulse (apical beat), approximately at or slightly above the level of the elbow (ventral region of the left ventricular inlet)
- Basilar—area above the elbow and slightly more cranial, below the triceps muscle (region of the ventricular outflow tracts, semilunar valves, and great vessels)
- Mitral, aortic, pulmonic, tricuspid valve area (see Fig. 9.7)

RADIATION

- Dorsal-ventral, cranial-caudal, left-right

exercise, pain), moderate to severe anemia, and peripheral vasodilation. These murmurs are typically soft (grades 1–3/6), localized, relatively short, crescendo-decrescendo in timing, and labile. Increasing HR usually increases the intensity and duration of a functional murmur, although in some horses the murmur becomes less intense. Functional murmurs are neither holosystolic nor holodiastolic, and therefore the heart sounds should still be evident.

The most common functional murmur is the systolic ejection murmur heard over the aortic and pulmonic valves and projecting into their respective arteries at the left cardiac base (see Fig. 9.7 and Table 9.1).[3] The functional ejection murmur is generated by flow into the great vessels and by definition must start after S_1 and end before S_2. Nonetheless, in some horses, the functional ejection murmur can achieve substantial intensity (rarely, a grade 4–5/6) and may seem holosystolic at higher HRs. At times there is a remarkable change in functional murmur intensity from one day to another. An example of this is the varying murmurs identified in some horses with emergent colic. Because a loud functional murmur can radiate caudally, it may be confused with mitral regurgitation. In terms of diagnostic considerations, the clinician should not mistake a functional ejection murmur for that of aortic or pulmonic stenosis. Pulmonic stenosis is rare in the horse and aortic stenosis even less common. Accordingly, these valvular malformations are rarely entertained in the differential diagnosis. The murmurs resulting from subpulmonic (subarterial) VSDs or the stenosis associated with tetralogy of Fallot may be loudest over the great vessels, but such murmurs are usually very loud and prompt echocardiographic investigation.

Functional diastolic murmurs are also common, especially in young horses and in the Thoroughbred breed. These murmurs are generally soft and are detected over the LV or RV inlets—from the dorsal atrium to the ventricular apex. The functional protodiastolic murmur is an early diastolic murmur detected between S_2 and S_3. The murmur may be musical, vibratory, or "squeaky" and typically accentuates with increased HR.[3] It most reasonably represents the rapid early filling of the ventricles. The presystolic functional murmur is heard between S_4 and S_1, and the vibrations may sound like a "rub" or long heart sound following the atrial contraction. It can be confused with an early systolic murmur or a friction rub.

Causes of *abnormal (pathologic) murmurs* include *structural or organic heart disease* such as incompetent cardiac valves, septal defects, vascular lesions, and (very rarely) valvular stenosis. The continuous murmur of patent ductus arteriosus is a normal finding in full-term foals for up to 3 days after parturition but occasionally persists for almost a week after foaling.[382,386,408,409] The most commonly detected pathologic murmurs in horses are those generated by tricuspid regurgitation, mitral regurgitation, aortic regurgitation, and ventricular septal defect. Typical causes and auscultatory features of these murmurs are indicated in Tables 9.1, 9.3, and 9.4 and described in detail later in this chapter.

When a murmur is believed to represent underlying cardiac pathology, further investigation is required. The overall relevance of the hemodynamic abnormality should include consideration of the work history, general physical examination, presence or absence of heart enlargement or cardiac failure, and results of ancillary tests such as echocardiography, electrocardiography, and exercise performance (or testing). Certainly, the history should be considered, because the horse with excellent performance and good exercise tolerance is unlikely to have serious heart disease. Auscultation, however, is insufficient to distinguish trivial from significant heart disease in the poorly performing horse or in a horse with a cardiac arrhythmia. Echocardiography is helpful in these cases to quantify heart size and objectively assess ventricular function. Underlying lesions such as a valve vegetation, a ruptured valve chorda tendineae, or dilated cardiomyopathy can be identified or discounted. Although there are no well-defined echocardiographic or Doppler correlates to functional heart murmurs, these studies can document abnormal blood flow and can pinpoint the cause of a pathologic murmur.

Pulmonary Auscultation

Auscultation of the lungs should reveal normal breath sounds with the horse at rest, while ventilating into a rebreathing bag, and after exercise. Decreased or absent airway sounds or large airway sounds in the ventral portions of the thorax indicate a pleural effusion, a common finding in biventricular or



FIG. **9.10** Peracute pulmonary edema in a horse with a ruptured chorda tendinea. The photo was taken immediately after euthanasia. An important note is that tracheal froth is often a postmortem artifact, particularly when observed hours after death.

right-sided heart failure. Moist or bubbling (fluid) sounds or crackles (i.e., rales) are uncommonly auscultated in the lungs of horses with pulmonary edema and left-sided heart failure. Instead, tachypnea associated with harsh bronchovesicular breath sounds is usually heard, as horses with chronic left-sided CHF seem to develop more interstitial than alveolar pulmonary edema. When alveolar edema does develop, respiratory distress may be severe, crackles may become evident, and free fluid may be auscultated in the trachea. On rare occasion, primarily with peracute left-sided heart failure, froth will be visible at the nares and the horse will cough and expel large quantities of pulmonary edema (Fig. 9.10). Such horses demonstrate severe respiratory distress (marked tachypnea and dyspnea), anxiety, and agitation.

Examination of the Peripheral Vasculature and Blood Pressure Measurement

An evaluation of the peripheral vasculature is part of a CV workup and should include examination of the arteries and veins in the head, forelimbs, and hindlimbs and assessment of the mucous membranes. When the equipment is available, arterial BP also should be measured. The genesis of the arterial and venous pulses has been described previously. The heart sounds should be correlated with both the jugular and arterial pulses.

Arterial Pulses and Blood Pressure Measurement

Normal quality *arterial pulses* should be palpable in the facial, median, carotid, great metatarsal, coccygeal, and digital arteries. The arterial pulse represents the pulse pressure (i.e., the difference between peak systolic and diastolic pressures), the rate of rise of arterial pressure, and the physical characteristics of the artery and surrounding tissues. HR and heart rhythm, as well as altered hemodynamic states, may be identified by palpating the facial artery pulse. The arterial pulse can be described as normal, hypokinetic (weak), hyperkinetic, or variable. Irregularity often indicates a cardiac arrhythmia.

Thready or hypokinetic arterial pulses are associated with reduced stroke volume and peripheral vasoconstriction and therefore may be detected in CHF and in diseases associated with volume depletion or profuse hemorrhage. Thready arterial pulses may also be detected only in the hindlimbs with aortoiliac thrombosis. The pulse in septic animals may be weak, owing to reduced pulse pressure, or normal to rapidly declining if there is peripheral vasodilation. Bounding, hyperkinetic arterial pulses are palpable with clinically relevant aortic regurgitation, patent ductus arteriosus, and aortic to pulmonary or aortic to right-sided heart fistulas. Marked variation in the intensity of the pulse usually occurs with arrhythmias, particularly AF and rapid or multiform VT. A pulse deficit (first heart sound without palpable pulse) occurs when developed LV pressure does not exceed aortic pressure. Deficits are likely to be palpated in association with pathologic arrhythmias, particularly premature beats or following very short diastolic periods of tachyarrhythmias when ventricular filling is insufficient to generate a significant stroke volume.

Arterial pressure is determined by the interplay among SV, HR, and vascular resistance (see Box 9.3 and Fig. 9.4B). Palpation of the arterial pulse mainly provides information on pulse pressure but cannot quantify the actual *arterial BP,* which (if required) must be measured directly by arterial puncture or cannulation (invasive blood pressure [IBP]) or indirectly using various auscultatory, Doppler, or oscillometric techniques (noninvasive blood pressure [NIBP]).[259,410-440] Percutaneous placement of an arterial catheter in the facial, transverse facial, transverse tibial, or metatarsal artery is frequently used to monitor pressure invasively in critically ill or anesthetized horses and foals. Indirect methods have been used successfully to monitor pressure in the coccygeal, metacarpal, metatarsal, and median artery. However, NIBP measurements are less reliable compared with IBP measurements. Although different study designs (including varying patient populations, hemodynamic interventions, devices, cuff sizes and placements, and correction factors) make direct comparison difficult, overall agreement between IBP and NIBP measurements tends to be better in anesthetized animals compared with standing conscious horses. The mean arterial pressure (MAP), which is derived from the maximum oscillation amplitude, is more accurately detected by oscillometric monitors than the algorithm-dependent systolic arterial pressure (SAP) and diastolic arterial pressure (DAP).[441,442] No information is available on the accuracy and reliability of noninvasive pulse pressure measurements, although in clinical practice quantification of pulse pressures is considered very useful to detect or confirm hyperkinetic pulses, particularly in horses with AR (see later discussion).[406]

Attention must be directed to placement and diameter of the occluding cuff when indirect methods are used.[427] A cuff width to tail circumference ratio of 0.4 to 0.6 is often recommended by the manufacturers and has been used in horses when measuring pressure in the middle coccygeal artery.[412,436,437,439,440] However, a large range of ratios from 0.2 to 0.9 have been described in the literature,[412,434-440,443] and some authors recommend an optimal cuff width between 0.2 to 0.35 of the circumference of the tail.[421,425,444] Generally, if wide cuffs tend to cause underestimation then small cuffs tend to cause overestimation of NIBP.[427,443]

The effect of gravity and the vertical distance between site of cuff placement and the heart base also need to be considered. Measurements of NIBP in standing horses using a tail cuff will underestimate true pressures because the coccygeal artery is located above the heart base. Generally, a correction

factor of 0.77 mm Hg/cm of vertical distance between the base of the tail and the point of the shoulder is applied or, if not, measurements should be described as "uncorrected" values.[427]

NIBP measurements may be less sensitive in hypotensive animals and may lag in response during rapid changes in BP.[413,415,417] The slow HR in horses may represent another source of inaccuracy for NIBP measurements, because the rate of the cuff deflation can be a source of error if the deflation rate is too rapid or the HR is too slow.[445] The arterial pulse wave itself varies, depending on the site of measurement, due to the wave reflection when the pulse wave is entering from central to the periphery. The distal arterial systolic pressure may be higher, and the diastolic pressure lower, than the corresponding central aortic pressures. However, due to compensation of the increase in peak systolic pressure by narrowing of the pressure wave, the MAP is usually unaffected by wave reflection.[444,446]

Arterial BP monitoring includes determination of SAP, DAP, MAP, and pulse pressures (difference between SAP and DAP). Normal reported values for NIBP in conscious standing adult horses (corrected for vertical distance of cuff position above heart base) are around 135 ± 15, 110 ± 15, and 90 ± 15 mm Hg (average ± standard deviation [SD]) for SAP, MAP, and DAP, respectively, with a calculated pulse pressure of approximately 45 ± 6 mm Hg.[423,431,436,439] BP is lowest in neonates and rises during the first month of life to the normal adult range.[409,415,426] There is some variation among breeds. Draft breeds tend to have lower pressures than racehorses, and Standardbreds have lower pressures than Thoroughbreds.[431] Posture imposes a significant influence on arterial pressure, because raising the head from the feeding position necessitates a higher aortic pressure to maintain cerebral perfusion. Obviously, when the head is lowered, the hydrostatic pressure imposed by an elevated head position is minimized. MAP measured in the middle coccygeal artery can vary approximately 20 mm Hg with the changing head position.[428] Marked increases in CO can lead to significant increases in arterial BP, with systolic pressures exceeding 200 mm Hg.[447-449] Sedatives, anesthetics, and adrenergic drugs cause significant dose- and time-dependent increases or decreases in systemic BPs.[292]

The *systolic arterial pressure* is generated by the left ventricle and is consequently affected by the interplay between the stroke volume, HR, aortic compliance, and previous diastolic BP.[46] Arterial *pulse pressure* is highly dependent on stroke volume and the peripheral arteriolar resistance that determines the runoff of diastolic pressure. Ventricular failure or reduced venous return reduces pulse pressure, creating a hypokinetic pulse, whereas abnormal diastolic runoff (aortic regurgitation, generalized vasodilation) widens the pulse pressure, producing a stronger peripheral pulse. *Diastolic and mean pressures* are better estimates of perfusion pressure in anesthetized or critical equine patients; these variables are increased by arteriolar vasoconstriction or by higher CO.[57] Arterial pressures fluctuate slightly with ventilation and significantly under positive pressure ventilation or during cyclic changes in HR (see Fig. 9.6) related to variation in ventricular filling. However, marked differences can be associated with cardiac tamponade, and the dramatic fall in pressure during inspiration is termed *pulsus paradoxus*.

Mucous Membranes

The color of the mucous membranes and the capillary refill time (CRT) are determined by cardiac output, systemic blood pressure, peripheral vasomotor tone, PCV and hemoglobin concentrations, and plasma bilirubin concentrations. Although assessment is largely subjective, normal mucous membranes should be moist and pink, with a CRT of less than 2 seconds. Dry, congested membranes suggest dehydration and CV disturbance. Pale membranes indicate anemia or poor peripheral perfusion and vasoconstriction, and dark red (injected) or gray-bluish membranes may indicate septicemia or endotoxemia and peripheral vasodilation or vasoconstriction, respectively. Capillary refill time is prolonged with poor CO, hypovolemia, hypotension, or peripheral vasoconstriction; conversely, it may be shortened when there is vasodilation. Cyanosis because of right-to-left cardiac or vascular shunting is a rare finding. It is important to realize that the detection of hypoxemia by inspection of the mucous membranes is influenced by the blood hemoglobin concentration, because the bluish color of the mucous membranes directly relates to the absolute concentration of deoxygenated hemoglobin. Hence, anemic horses may be hypoxemic without showing any cyanosis, whereas polycythemic horses might appear cyanotic without being markedly hypoxemic.

Peripheral Veins

The cutaneous veins should be examined for distensibility, refill, thrombosis, and estimated venous pressure. The jugular veins in normal horses are collapsed, but the veins of the limbs and torso are visible and somewhat filled. Venous pressure in the jugular vein is normally less than 10 cm of water above the phlebostatic point (i.e., 0 [zero] reference point, usually taken to be the right atrium), and *jugular pulsations* are normally confined to the thoracic inlet and the ventral one third (10 cm) of the neck. These pulsations are reflections of the right atrial pressure changes that range from positive to subatmospheric over the cardiac cycle (discussed previously). Pulsations are normally visible along the entire length of the jugular vein when the head is lowered below the level of the heart and should not be misinterpreted. A pathologic jugular pulse also can be misdiagnosed if there is transmission of carotid arterial pressure into the jugular furrow. Occluding the jugular vein ventrally can demonstrate if the pulses are actually originating within the right atrium or not, because venous pulse transmission, or prominent collapse, will be prevented by light digital pressure over the vein. Pronounced jugular pulsations are observed occasionally in excited but otherwise normal horses with high sympathetic tone. This might be due to either vigorous right atrial contraction. Alternatively, an exaggerated venous collapse can create a "jugular pulse" that is simply a reflection of normal venous refilling during periods of positive right atrial pressure. When there is doubt, this finding can be verified by simple ultrasound imaging of the ventral portion of the vein.

Jugular abnormalities include abnormal pulses or elevated venous pressure. An abnormal jugular pulse is one that extends proximally up the jugular vein for more than 10 cm in systole (with the horse's head held in a normal position) or that demonstrates retrograde filling from the heart when the vein is occluded dorsally. Duplex Doppler studies of the jugular vein can distinguish a prominent pulse caused by retrograde flow from an apparent pulse caused by prominent collapse and normal filling. Abnormal jugular pulses are observed with arrhythmias causing AV dissociation (nonconducted atrial premature beats, junctional tachycardia, VT); with diseases of the tricuspid valve (moderate to severe tricuspid regurgitation; tricuspid

stenosis); and with RV failure from any cause including cardiac tamponade or constrictive pericardial disease. Elevated jugular venous pressure generally indicates right-sided CHF, pericardial disease, or hypervolemia. Generalized venous distention, particularly when accompanied by subcutaneous edema, is characteristic of CHF (Figs. 9.11 and 9.12). Isolated distention of the veins cranial to the thoracic inlet is suggestive of a cranial mediastinal or pulmonary mass (or abscess) with obstruction of the cranial vena cava.[140] Prolonged refill of the saphenous vein indicates the possibility of aortoiliac thrombosis and decreased arterial supply to the affected limb.[267-272,450-455] A distended vein that is firm to palpation and associated with marked distention of associated veins indicates probable venous thrombus. Pain or heat associated with venous swelling is suggestive of thrombophlebitis.[456-458]

Diagnostic and Laboratory Studies

Exercise Testing

Because most horses function as athletes, exercise testing represents an important method for identifying CV and other disorders in horses with exercise intolerance or poor performance. In horses with clinically important structural lesions, intermittent premature complexes, or AF, exercise testing can determine whether the HR is appropriate for the work performed or if an arrhythmia deteriorates over the course of the test.[53,54,61,68,459] Exercise testing is also indicated during a prepurchase examination when a nonfunctional heart murmur or sporadic arrhythmia is identified. Having advanced this recommendation, it must be acknowledged that the reliability and overall sensitivity, specificity, and predictive values of exercise testing (as it pertains to detection of arrhythmia and organic heart disease) are incompletely defined, and more data and information are needed to establish the clinical value of such studies relative to CV disease. Contraindications for an exercise test include CHF; severe valvular regurgitation with secondary AF, PHT, or severely reduced systolic function; or presence of a ventricular arrhythmia of dangerous complexity.[68]



FIG. **9.11** Jugular venous distention in a Shire foal with biventricular congestive heart failure (CHF) caused by congenital heart disease.



FIG. **9.12** Ascites, ventral edema, and weight loss are evident in this mare with right-sided congestive heart failure (CHF). The distended lateral thoracic vein *(arrow)* is evident caudal to the triceps.

High-speed treadmill exercise and various forms of field exercise testing are increasingly used to detect subtle clinical abnormalities that limit peak performance or may lead to collapse, syncope, or sudden death.[53,460,461] Treadmill exercise is particularly useful when evaluating the horse at risk of collapse, because the cardiac response to exercise can be evaluated without risk to a rider. Although an exercise test at lower levels of work effort (e.g., lunging exercise) might still provide some valuable information, the work intensity during an exercise test should generally be at or slightly exceeding the horse's customary or anticipated activities. This is particularly important when the history indicates performance problems only at the peak of exercise. Some method of inducing unexpected sympathetic stimulation should be included in the exercise test to identify an inappropriate HR, aberrant conduction, or ectopy associated with adrenergic stimulation.[68] If the animal is too young to perform a regular exercise test, evaluation after a period of free exercise is recommended.

The exercise examination provides valuable information about musculoskeletal diseases, airway diseases, and cardiovascular disorders. There are a number of components to a standardized treadmill examination, which should include continuous recording of an ECG using a device that has permanent storage and playback capabilities.[54,68,462] Specific cardiac assessments include (1) the effects of exercise on auscultation (rate, rhythm, and murmurs); (2) peak HR during exercise; and (3) HR and heart rhythm during the different phases of the exercise test and during recovery. Optionally, echocardiography can be performed before and after exercise (stress echocardiogram). Additional tests that might be indicated are gait analyses, assessment of airway dynamics by videoendoscopy, evaluation of pulmonary function by arterial blood gas analyses, and clinical laboratory tests such as preexercise and postexercise serum creatine kinase (CK) activities to identify subclinical myopathy. The "standardized" exercise test may not be identical across equine medical centers so it may be difficult to compare results. The examiner's own reference for normal variation further qualifies the examination results.

Changes in HR and ECG with exercise have been well studied.* The normal horse develops sinus tachycardia, shortening of conduction intervals, and marked ST-T alterations associated with exercise. The maximal HR achieved depends

* References 53, 54, 61, 233, 448, 459-461, 463-471.

on the level of exercise performed. At HRs of less than 100 to 120 beats/min, SA node discharge is very labile and subject to psychological influences. Once exercise has commenced, the HR should accelerate and then stabilize at a level appropriate for the work being performed. As a general guideline: on the flat, HRs of 70 to 140 beats/min are normal at the trot, 120 to 160 beats/min at the canter, 150 to 180 beats/min at the gallop, and more than 180 beats/min when galloping hard. When exercising on slopes, the highest rates associated with each gait increase, and it is possible to approach maximal rates at the trot when on gradients of greater than 20%. The maximal HR for most horses is between 210 and 240 beats/min, although some younger horses develop higher rates, and aged horses can have maximum rates below 210 beats/min. There is a linear relationship between HR and the velocity or work effort of exercise when the HR is between 120 and 210 beats/min. HR usually recovers rapidly and falls to less than 100 beats/min within 2 to 5 minutes of cessation of exercise. The recovery of HR to the resting level is influenced by many factors, including the humidity, ambient temperature, training fitness, work performed, psychological factors, and state of CV health. After maximal work, such as a race, an hour may pass before the HR completely recovers to the resting rate. An inappropriately high HR for a given level of exercise may simply denote a healthy, but unfit, horse; however, this finding can also indicate the presence of pulmonary, musculoskeletal, or CV disease.[68,472] Information about the HR is particularly useful when a horse has the HR monitored routinely during exercise.

Exercise-induced arrhythmias may be observed during or after exercise. An exercising ECG is necessary to determine whether an arrhythmia is present during exercise. An HR monitor may provide some indication regarding arrhythmias during exercise; however, the detectable maximum HR (or shortest RR interval) might be limited by inherent filtering algorithms, and the detection of erratic heart "blips" or "beeps" does not adequately characterize the rhythm disturbance. Exercise may induce supraventricular premature complexes, AF, ventricular premature complexes, or VT. Exercise-induced (paroxysmal) AF should be included in the differential diagnosis of horses with poor performance during high-intensity exercise.[39,54,68,88,472-474] Exercise-induced ventricular arrhythmias (VAs) are a particular concern because ventricular rhythm disturbances can lead to poor performance, sudden stopping, or even falling and have been suspected as a common cause of sudden death.* Repetitive ventricular activity (paroxysmal ventricular tachycardia) with short R-R intervals or R-on-T phenomenon is particularly worrisome and may indicate preexisting myocardial disease or myocardial ischemia with altered cardiac electrical activity. Although AF per se is not a fatal rhythm, sudden-onset AF during maximal exercise may rarely cause a horse to collapse. One recent study further showed that the prevalence of QRS broadening and R-on-T phenomenon was high in horses with AF even at lower levels of exercise, indicating that some horses with AF might in fact be at risk for fatal ventricular rhythms.[472] AF associated with exercise-induced VA resulting in sudden cardiac death has been documented in at least one horse.[54]

Undoubtedly, CV disorders can be induced or worsened by the stress of exercise, and the likelihood of detecting disease is increased. However, there are challenges to this assessment because physiologic variability is also magnified. Horses demonstrate marked fluctuations in sympathetic and parasympathetic tone during the recovery period following exercise, often termed *autonomic instability*.[471,475-478] Arrhythmias are especially common during this time,* even if the resting and peak exercise heart rhythms were normal. Marked sinus arrhythmia, second-degree AV block, supraventricular ectopy, and ventricular premature complexes can be evident in the immediate postexercise period. With the exception of vagally mediated arrhythmias, postexercise arrhythmias should be considered suspicious. Atrial fibrillation, ectopic supraventricular tachycardias, and ventricular tachycardias are considered abnormal at any point in the exercise test. However, the importance of single (isolated) ectopic complexes identified only in the immediate postexercise phase is less certain,* which is not surprising considering similar issues persist in assessing human athletes. In assessing these horses, attention also should be directed to baseline auscultation and echocardiographic examinations, the heart rhythm during exercise, and measurements of plasma cardiac troponin I (cTnI) concentrations before and after exercise. The presence of coexisting respiratory disease and associated arterial hypoxemia needs to be considered, because it creates the potential for secondary rhythm disturbances, unrelated to primary cardiac disease.[90]

Echocardiography immediately following the stress of exercise (the so-called stress echo) may be used in an attempt to identify global and regional myocardial dysfunction.[90,167,168,459,480-482] This examination must occur within 2 to 3 minutes after high-intensity exercise and with the HR in excess of 100 beats/min.[168] However, the optimal HRs and the influence of rapidly decreasing HRs on echocardiographic measurements such as shortening fraction require better definition.[168] Dynamic changes can be viewed from the short-axis tomograms, and prominent thickening of the ventricular septum and LV free-wall identified from long-axis images. Persistent abnormalities in regional myocardial contraction are suggestive of vascular disease with ischemia, preexistent myocardial fibrosis, or a localized conduction disturbance. Left ventricular shortening fraction and LV fractional area change—two estimates of LV systolic function—are commonly measured during stress echocardiography but have recently been shown to poorly track stress-induced changes in LV function.[168] Errors in cursor placement (e.g., too dorsal) or inconsistent orientation of the M-mode cursor placement between examinations can produce misleading results. Volumetric estimates of stroke volume and LV ejection fraction by 2D echocardiography are more reliable to assess global LV systolic function after exercise.[168] Other methods used for stress echocardiographic assessment of LV function are the manual tracking of endocardial motion and subjective evaluation of regional wall motion. Newer echocardiographic modalities such as 2D strain imaging (2D speckle tracking) may have some advantages over conventional methods and could prove useful for quantitative assessment of regional and global LV function during stress echocardiography.[168] However, the quantitative and objective detection of stress-induced hypokinesia, akinesia, and dyssynchrony in diseased horses requires additional investigations, and more data and standardizations are needed before methods can be widely accepted. Finally, the assessment of cardiac valve function following exercise requires much better definition before any conclusions can be made.

* References 54, 94, 96-99, 104, 106, 150, 475.

* References 54, 77-80, 82, 90, 475, 478, 479.

Although the standardized treadmill examination is the most commonly used cardiac "stress test" at equine centers, some attention has been directed on performing pharmacologic stress testing. This examination involves increasing the HR and myocardial contractile force with dobutamine, either with or without atropine or glycopyrrolate, followed by echocardiographic assessment of ventricular contraction.[243,247,481,483,484] Pharmacologic stress echocardiography is used to identify regions of reduced myocardial perfusion or ischemic myocardial injury in human patients with coronary heart disease; the study is used in people who cannot perform physical exercise to stress the heart and increase myocardial oxygen demand. Whether this form of stress testing should be used in horses is subject to debate. First, it must be emphasized that there is no compelling evidence from pathology studies that horses develop significant coronary arterial disease. Admittedly, myocardial fibrosis is found in some horses at necropsy[29,485]; however, the pathogenesis or clinical relevance of these often microscopic lesions is unresolved. In human patients, the diagnostic target is well-defined extramural coronary artery disease, and stress echocardiography generally predicts normal or abnormal findings at coronary arteriography. There is no evidence at this time for a similar situation in horses, and this represents a major contextual issue when considering "stress echo" with either exercise- or pharmacologic-induced cardiac work. Furthermore, pharmacologic stress testing may not achieve the identical exercise endpoints in terms of HR or altered vascular resistances. The combination of dobutamine and atropine involves the use of proarrhythmic drugs, making the heart rhythm assessment—a pivotal component of the exercise test—quite problematic. Pharmacologic stress testing does not provide an opportunity for dynamic assessment of the gait and airways.[482] Last, assessment of left heart valve function is complicated by changes in systemic arterial BP that may develop during drug-induced tachycardia (but have been poorly characterized in studies thus far). Thus, although pharmacologic stress testing carries the benefit of a stationary diagnostic setup not requiring a treadmill, a number of outstanding issues must be addressed before this examination can be advocated for wider use.

Electrocardiography

Normal Cardiac Cell Electrical Activity. Cardiomyocytes are excitable and capable of responding to electrical stimuli regardless of extrinsic innervation. Specialized cardiac tissues such as those in the sinoatrial node and the His-Purkinje system demonstrate spontaneous depolarization and can serve as pacemakers for the heart. The electrical processes responsible for the ECG are caused by ion fluxes across the cell membrane (Fig. 9.13).[57,110,112-114,116] A general understanding of cellular activity, generation, and spread of the cardiac electrical impulse; effects of autonomic innervation; and electrocardiographic lead systems is required to interpret the equine ECG.

The partially selective nature of the cardiac cell membrane and the presence of various cell membrane pumps lead to an unequal partitioning of ions across the cell membrane.[57,110,112-114,116] This results in very high potassium and relatively low sodium concentrations intracellularly compared with the extracellular fluid. Other ions including chloride and magnesium, but most notably calcium, are important to cellular electrical activity. Calcium also is essential for contraction of the cardiomyocytes. Sudden changes in cardiac cell membrane permeability or conductance to sodium, calcium, potassium, and chloride are responsible for the processes of depolarization, muscular contraction, and repolarization (see Fig. 9.13). These processes, in turn, are affected by serum electrolyte concentration, acid-base status, autonomic traffic, myocardial perfusion and oxygenation, heart disease, and drugs. The basic processes of cell depolarization, calcium influx, and cell repolarization form the basis for, respectively, the P and QRS complexes of the ECG, myocardial contraction, and the ST-T wave of the ECG.

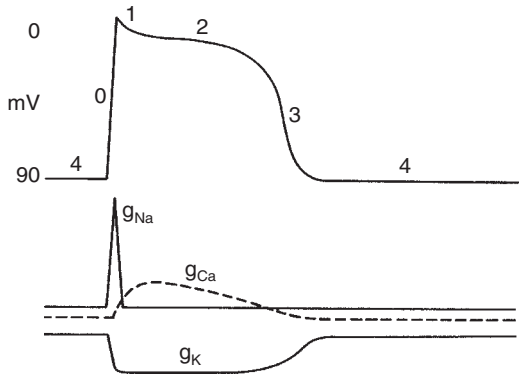


FIG. **9.13** The cardiac action potential. The diagram demonstrates the phases of depolarization and repolarization, as well as membrane conductance (g) to important ions (see the text for details). (From Berne RM, Levy MN: *Cardiovascular physiology,* St. Louis, 1986, CV Mosby.)

The resting membrane potential is determined primarily by the partitioning of potassium ions and proteins across the cell membrane and the relative impermeability of the membrane to sodium. *Depolarization* of atrial and ventricular myocytes and Purkinje fibers is caused by the rapid influx of extracellular sodium into the cell.[57,110,112-114,116] This current is represented by phase 0 of the cardiac cell action potential recording. Normal cardiac tissues depolarized in this manner conduct electrical impulses at high velocity. In contrast, the cells of the SA and AV nodes, as well as ischemic cells, demonstrate less negative diastolic membrane potentials. Nodal cells depend on a slow-inward current of depolarization that is carried mainly by calcium ions across the transient and long-lasting calcium channels. In ischemic cells, due to the less negative resting potential, some sodium channels remain in the inactivated state and thus are unavailable for activation and membrane depolarization during phase 0. Therefore cells in the SA node, AV node, and ischemic tissue conduct electrical impulses very slowly and can be involved in blocks, abnormal automatic mechanisms, or reentrant pathways that promote cardiac arrhythmias. Antiarrhythmic drugs and calcium channel blockers act differently on tissues that are "fast" versus "slow" current dependent (see Cardiac Arrhythmias later in this chapter).

The influx of extracellular calcium into the cell during the *plateau phase* (phase 2) of the action potential triggers the release of intracellular calcium from the sarcoplasmic reticulum. Increases in cytosolic calcium cause myocardial contraction. Hypoxia, acidosis, anesthetics, and many other drugs can affect myocardial contractility by interfering with calcium entry into cells, with the release of calcium from the sarcoplasmic reticulum, or with the binding of calcium to contractile filaments.[57,110,112-114,116] Conversely, digitalis glycosides and dobutamine increase calcium influx and myocardial contractility.

Cellular *repolarization* is initiated by decreases in the cell membrane conductance to sodium and to calcium coincident with increased conductance to potassium. As intracellular potassium moves out of the cell, along its concentration gradient, phase 3 of the cardiac action potential occurs. Repolarization is complex, involves a number of ion currents,[486] and is affected by genetics and drugs such as quinidine or amiodarone. Sympathetic stimulation may activate currents that facilitate repolarization at higher HRs, explaining the shortening of the action potential under sympathetic drive. Paradoxically, vagotonia also enhances repolarization by opening potassium channels, an effect that is very prominent in the atria and may predispose to development of AF. Hypoxia and abnormalities in serum potassium or calcium also alter repolarization. Hyperkalemia accelerates repolarization and shortens the ST-T wave of the ECG. This occurs because hyperkalemia *increases* membrane permeability to potassium, and the high intracellular potassium (exceeding 100 mmol/L) is more than adequate to drive potassium out of the cell (because extracellular potassium rarely exceeds 10 mmol/L).

Spontaneous depolarization or automaticity is a property of select cardiac tissues. Such activity is most prominent in the SA node but may also be encountered in cells around the AV node and in the His-Purkinje network. Normal automaticity is observed in these cells during phase 4 of the cardiac action potential. Spontaneous *pacemaker activity* is generated in normal pacemaker tissues by the background inward sodium current and a time-related decrease in membrane permeability to potassium ion efflux, and a transient inward calcium current.[57,110,112-114,116] Once membrane threshold is reached, ion flow across the long-lasting calcium channels ("slow current") predominates and leads to cell depolarization (phase 0). Spontaneous depolarization is modified markedly by autonomic traffic. Vagal activity opens potassium channels and hyperpolarizes the membrane, depressing automaticity. With sympathetic stimulation or membrane hyperpolarization, the depolarizing "funny current" (I_F) becomes activated, enhancing pacemaker activity.

Autonomic innervation has profound effects on electrical activity[9,217,487] but is not distributed equally through the heart. The right and left vagus have preferential innervation to SA and AV nodes, and parasympathetic traffic is more extensive in supraventricular than ventricular myocardium. Sympathetic activity derived from paired ganglia are also not bilaterally symmetric but do innervate both atria and ventricles extensively. Parasympathetic activity depresses SA nodal activity, enhances intraatrial conduction by shortening atrial action potential duration, and slows AV nodal conduction. Conversely, sympathetic efferent traffic increases HR and shortens AV conduction time.[112] Sympathetic activity also increases cellular excitability, predisposes to some cardiac arrhythmias, and increases myocardial oxygen consumption by augmenting the HR, force of myocardial contraction, and myocardial wall tension. Parasympathetic efferent traffic dominates in the resting, standing horse and frequently fluctuates with changes in BP. The pronounced sinus arrhythmia, sinoatrial block, and second-degree AV block so often encountered in the normal horse are caused by changing vagal tone and serve to regulate arterial BP at rest (see Fig. 9.6).[108]

Depression of normal SA automaticity or increased activity in other tissues in the atria, in the Purkinje system, or in cells around the AV node may lead to an abnormal or "ectopic" cardiac rhythm. This situation is often observed in healthy horses under general anesthesia. When the principal abnormality resides in depression of SA node function or blockage of the impulse in the AV node, the slow discharge of a subsidiary pacemaker is termed an *escape* mechanism. The purpose of these latent cardiac pacemakers is to rescue the heart from extreme bradycardia or asystole. However, ectopic pacemakers also can be enhanced abnormally by drugs, inflammatory mediators, abnormal sympathetic activity, electrolyte disturbances, or ischemia, leading to *ectopic* rhythms that manifest as premature complexes or tachycardias. Additional abnormal electrophysiologic mechanisms, including abnormal automaticity in tissues other than nodal and conduction tissues (i.e., ischemic myocardium), afterdepolarizations, and reentry account for the development of other arrhythmias.[57,110,112-114,116]

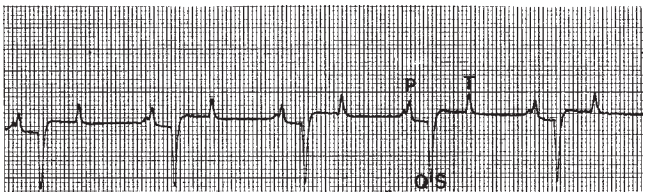


FIG. **9.14** A normal equine base-to-apex lead electrocardiogram (ECG) (25 mm/sec; 1 cm = 1 mV). The waveforms are indicated. The normal P wave in this lead is positive and notched (bifid), and a depression occurs in the baseline after the P wave that indicates atrial repolarization (Ta wave). The QRS complex often lacks an R wave in this lead (QS complex). The T wave is labile and may be negative, biphasic, or positive. Increases in heart rate or sympathetic tone usually lead to positive T waves in this lead.

Genesis of the Electrocardiogram. The SA node is located near the right auricle; therefore initial cardiac muscle depolarization crosses the right atrium (see Fig. 9.3). Activation waves spread through the atrial myocardial cells to the left atrium and also in the direction of the AV node. Specialized atrial muscle cells comprising internodal pathways and Bachman's bundle facilitate transmission of current across the atria.[8,488] These specialized pathways—as well as the SA node—are relatively resistant to high serum potassium concentrations and may function during hyperkalemia to cause a sinoventricular rhythm, even while normal atrial myocytes are inexcitable (atrial standstill).

Conduction continues across the AV tissues by first entering the AV node. Current transmission across the AV nodal cells is especially slow because this tissue depends on inward calcium currents and is subject to physiologic blockade from vagal efferent traffic.[220] Conduction proceeds at a greater velocity through the bundle of His and bundle branches. Propagation of the impulse through the ventricles is enhanced by a rapidly conducting Purkinje system that penetrates relatively completely through the ventricular myocardium.[6,7]

The ECG graphs the time-voltage activity of the heart. The average electrical potential generated by the heart muscle is recorded throughout the phases of the cardiac cycle with time displayed along the X-axis and electrical potential inscribed vertically (Fig. 9.14). The normal waveforms are the P wave (atrial depolarization), the PR (or PQ) interval that is due mostly to slow AV nodal conduction, the QRS complex (ventricular depolarization), and the ST-T wave (ventricular repolarization). A prominent atrial repolarization wave (Ta wave) is often noted in the PR segment of the equine ECG, particularly at faster HRs. The QT interval represents total electrical activation-repolarization time.

BOX 9.8 Electrocardiographic Leads

BASE-APEX MONITOR LEAD (MOST COMMONLY USED)

[+] Electrode over the left apex (left chest, at the level of the olecranon)
[–] Electrode over the right jugular furrow
The most common method to obtain the base-apex lead is to place the left arm (LA) electrode over the left apex, the right arm (RA) electrode over the jugular furrow, and select "lead I" on the electrocardiograph.
This lead is the preferred choice for monitoring cardiac rhythm.

BIPOLAR LEADS (EINTHOVEN)[a]

Lead I = Left foreleg (LA) [+] – Right foreleg (RA) [–]
Lead II = Left rear leg (LL) [+] – Right foreleg (RA) [–]
Lead III = Left rear leg (LL) [+] – Left foreleg (LA) [–]

UNIPOLAR AUGMENTED LIMB LEADS (GOLDBERGER)[a]

Lead aVR = Right foreleg [+] – Left foreleg and Left rear leg [–]
Lead aVL = Left foreleg [+] – Right foreleg and Left rear leg [–]
Lead aVF = Left rear leg [+] – Right foreleg and Left foreleg [–]

UNIPOLAR PRECORDIAL CHEST LEADS

[+] Electrode over selected precordial site
[–] Electrode composed of a compound electrode of left and right foreleg and left rear leg (Wilson central terminal)
The lead is named based on the location of the exploring (V or C) electrode.
V_{10} = [+] over the dorsal spine. Precordial leads other than V_{10} are not commonly used.

MODIFIED ORTHOGONAL LEAD SYSTEM

Lead X = Lead I, right [–] to left [+]
Lead Y = Lead aVF, cranial [–] to caudal [+]
Lead Z = Lead V_{10}, ventral [–] to dorsal [+]

[a] Frontal plane leads.

Clinical Electrocardiography. The clinical application of *electrocardiography* to the horse has been studied extensively.* The principles of recording and interpreting the equine ECG are similar to those used for humans, dogs, and other species. The lead systems employed are identical, although some modified leads, such as the base-to-apex lead, are most useful for monitoring the cardiac rhythm. A number of semiorthogonal lead systems have been evaluated experimentally but are rarely used in clinical practice. The modified Einthoven's lead system, consisting of leads frontal planes I, II, III, $_a$VR, $_a$VL, and $_a$VF, and the precordial lead V_{10}, is quite applicable for ECG studies of horses (Box 9.8). The base-apex lead or chest leads are most often used for rhythm analysis.

The value of continuous, *ambulatory (Holter) ECG* monitoring is obvious for identification of infrequent rhythm disturbances, quantifying the severity of an arrhythmia, or objectively evaluating drug therapy (Fig. 9.15). In one study it was observed that many horses that were historically and clinically without evidence of cardiac disease had supraventricular or (less often) ventricular arrhythmias on Holter ECG.[400,541] Ambulatory monitoring can be performed with self-adhesive contact electrodes using a bipolar lead system similar to that used for the equine HR monitors. Electrodes are often placed over the left saddle area (positive [right arm] electrode), sternum (negative [left arm] electrode), and right thorax (neutral [left leg] electrode), with the ground (right leg) electrode placed anywhere around the girth area. However, alternative electrode placements might be used to record an ambulatory ECG of diagnostic quality (e.g., right arm, right saddle area; left arm and left leg, left thorax 20 cm and 5 cm, respectively, above olecranon; right leg (neutral), left saddle area). To facilitate ECG pattern reading and optimize the ECG for automated processing using dedicated software, the electrode arrangement should provide an ECG with a distinct negative QRS complex and positive P and T waves (see Fig. 9.14). If ECG recordings are analyzed using human Holter analysis systems, it may be beneficial to record an ECG with positive QRS complexes. In any case, the T waves should not be bigger than the QRS complexes. Clipping may not be necessary as long as the hair coat is not too long and the self-adhesive electrodes can be kept moist (e.g., by additionally adding a drop of 0.9% saline or electrode contact gel onto the contact area) and in tight contact with the horse using a surcingle and padding material. This type of continuous 24-hour rhythm monitor can be useful to evaluate the horse with a history of syncope or an arrhythmia but in which an arrhythmia cannot be induced during resting, exercise, and postexercise ECG examinations. An alternative is an ECG event recorder that continuously records the rhythm and stores the ECG following an observed event, such as a sudden fall. These event recorders can be worn for longer periods of time.

Telemetry-based ECG recordings are commonly used in exercise testing (see Exercise Testing earlier in this chapter) and in monitoring of critical patients in hospital settings.[58,90,459,534] The lead systems used are often similar to those employed for Holter ECG recordings. However, the degree of artifact that occurs during exercise is considerable. Those interested in consistent

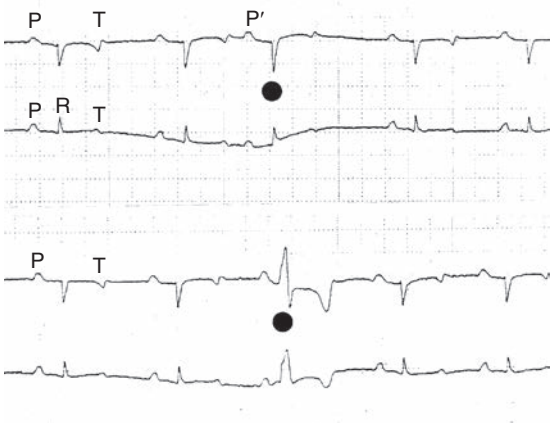


FIG. **9.15** Examples of extrasystoles. Atrial (P′ at *top*) and ventricular (at *bottom*) extrasystoles in a mare with heart failure, sinus tachycardia, and premature beats. The recordings are from a 24-hour tape-recorded (Holter) electrocardiogram (ECG). Two transthoracic leads recorded simultaneously. The waveforms are indicated. The dark circles indicate the premature complexes. The P-R interval of the APC is longer because of physiologic refractoriness in the atrioventricular node. The ventricular ectopic follows the sinus P wave (late diastolic) but discharges the ventricle before the sinus impulse can cause a normal QRS complex. The T wave of the ventricular extrasystole is abnormal (secondary T-wave change). Paper speed is 25 mm/sec.

* References 1, 6-8, 11, 30, 35, 40, 43, 44, 57, 77-79, 82, 216, 222, 224, 378, 383, 399, 426, 470, 479, 489-541.

BOX 9.9 Evaluation of the Electrocardiogram

TECHNICAL ASPECTS
- Paper speed—standard: 25 or 50 mm/sec
- Calibration—standard: 1 cm/mV
- Lead(s)—see Box 9.8

ARTIFACTS
- Electrical, motion, twitching, muscle tremor, equipment (ventilator)

HEART RATE
- Atrial and ventricular rate

CARDIAC RHYTHM
- Regular, regularly irregular, irregularly irregular
- Atrial, AV conduction sequence, ventricular, ventricular conduction
- Arrhythmias
  - Site or chamber of abnormal impulse formation, myocardial fibrillation, or conduction disturbance
  - Rate of abnormal impulse formation
  - Conduction of abnormal impulses
  - Patterns or repeating cycles

WAVES AND COMPLEXES
- P wave—morphology, duration, amplitude, variation
- PQ (PR) interval—duration, variation, conduction block (of P wave)
- QRS—morphology, duration, amplitude, frontal plane mean electrical axis
- ST segment—depression or elevation
- T wave—changes in morphology or size
- QT interval—duration (consider heart rate)

MISCELLANEOUS
- Electrical alternans, synchronous diaphragmatic contraction

stress ECG recordings must experiment with various electrode positions and lead systems. One proven possibility is placement of the right arm and ground electrodes next to each other on the left dorsum above the scapula, cranial to the saddle or the lunging girth, and the left arm and left leg electrodes next to each other on the left ventral thorax, caudal to the saddle girth or lunging girth. The electrodes should be place sufficiently distant to the tack and the rider's legs to minimize motion artifacts.

The orientation, amplitude, and duration of the ECG waveforms depend on many factors, including the age and size of the horse,[386,399,519] the lead examined, the size of the cardiac chambers, the degree of training, and even the phase of ventilation.[492,493] The principal use of the ECG is to diagnose the heart rhythm, because in horses this examination is insensitive for detecting cardiomegaly, especially in horses with mild to moderate heart enlargement. A normal ECG does not exclude heart disease; moreover, the ECG is not a test of myocardial function. A systematic approach to ECG analysis should be undertaken (Box 9.9) and compared with reference values (see Table 9.5). Although the magnitude of absolute differences may be small, body weight needs to be considered when comparing HR and ECG time intervals to reference values, because small equine breeds have slightly faster HRs and shorter ECG time intervals (i.e., PR interval, QRS duration, QT interval) compared with larger equine breeds (Fig. 9.16).[399] Furthermore, the HR dependence of repolarization needs to be considered when measuring QT intervals,[399,536,537,539] acknowledging that the best method for QT correction in individual horses is ill defined.

Atrial depolarization generates the P wave. Normal activation proceeds from right to left and craniad to caudad, leading to positive P waves in left-right lead I and also in craniocaudal leads II and aVF.[8,11] The normal P wave is notched or bifid; however, single peaked, diphasic, and polyphasic P waves may be encountered in normal horses. A negative/positive P wave is often recorded if the focus of pacemaker activity shifts to the caudal right atrium near the coronary sinus (Fig. 9.17). The initial peak of the common bifid P wave is reportedly caused by depolarization of the middle and caudal one third of the right atrium.[6,8] The second peak represents activation of the atrial septum and the medial surface of the left atrium. The P wave peaks can be subdivided, with P_1 reported to be as

TABLE 9.5 Normal Heart Rates and ECG Time Intervals for Horses at Rest

Breed	HR (beats/min)	PQ (msec)[a]	QRS (msec)[b]	QT (msec)[c]
ADULTS[399,470,499,500,512,532,533]				
Large breeds	26–50	200–500	80–140	360–600
Small breeds, ponies	30–54	160–320	60–120	320–560
FOALS[426,519,546]				
Large breeds	100–140	100–180	50–80	200–350
1–7 d	80–130	100–190	60–80	230–350
14 d				
Ponies	70–145	90–130	25–70	180–370
1–30 d	60–95	110–150	30–70	220–420
60 d	50–85	130–170	50–80	310–390
90 d				

[a]Also termed PR, because the Q wave is often not visible. Interval varies with autonomic input, longer when vagal tone is high.
[b]QRS duration is often difficult to determine due to the normal slur in the ST segment. Duration can vary with size of the heart and lead positioning (often longer in the base-apex lead).
[c]Inversely related to heart rate.

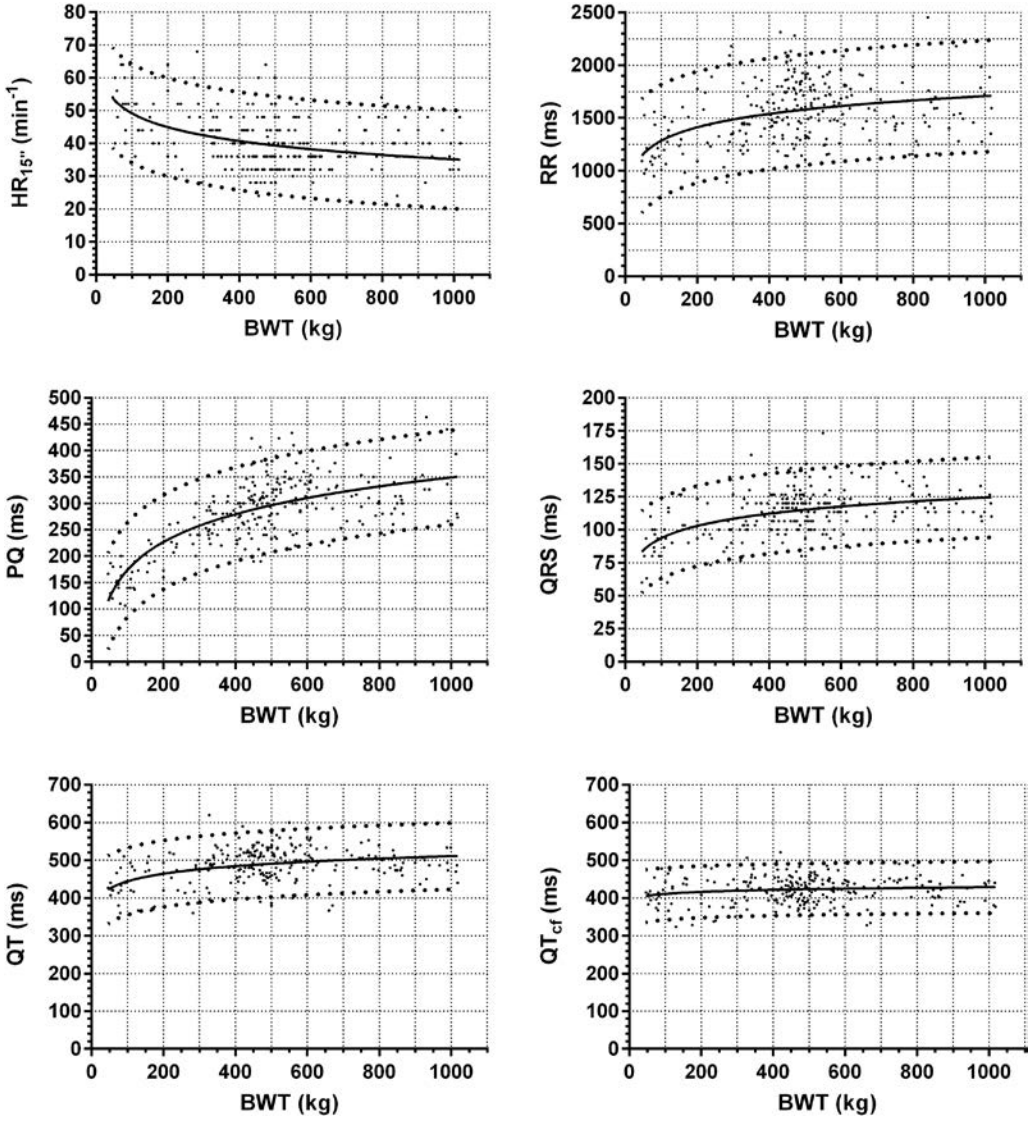


FIG. **9.16** Scatter plots showing the relationship between body weight (BWT), heart rate (HR), and ECG time intervals in healthy adult horses of different breeds (n = 250). Reference intervals for HR and ECG time intervals for a specific BWT can be estimated based on the prediction band (dotted line). $HR_{15"}$, mean HR calculated over 15 sec; *RR,* RR interval; *PQ,* PQ (PR) interval; *QRS,* QRS duration; *QT,* QT interval; QT_{cf}, rate-corrected QT interval, Fridericia's correction. (Modified from Schwarzwald CC, et al.: Relationship of heart rate and electrocardiographic time intervals to body mass in horses and ponies. *J Vet Cardiol* 2012, 14:343-350.)

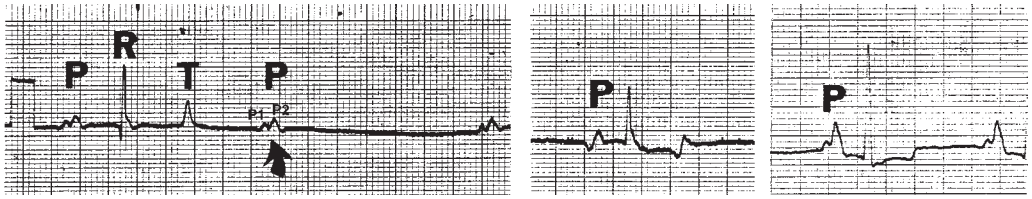


FIG. **9.17** P waves of the horse are demonstrated. On the left is a normal, bifid P wave morphology with two distinct peaks (designated as P_1 and P_2) and also a physiologic second-degree atrioventricular block *(arrow).* The center panel demonstrates a negative/positive P wave of coronary sinus origin. This is a normal variation. The right panel shows increased amplitude P waves recorded in a horse after conversion from atrial fibrillation (AF). The second peak is particularly large and may indicate atrial enlargement; however, such voltage criteria correlate poorly with cardiomegaly in horses. Echocardiography is a more accurate method for evaluating atrial size. Lead 2 paper speed is 25 mm/sec.

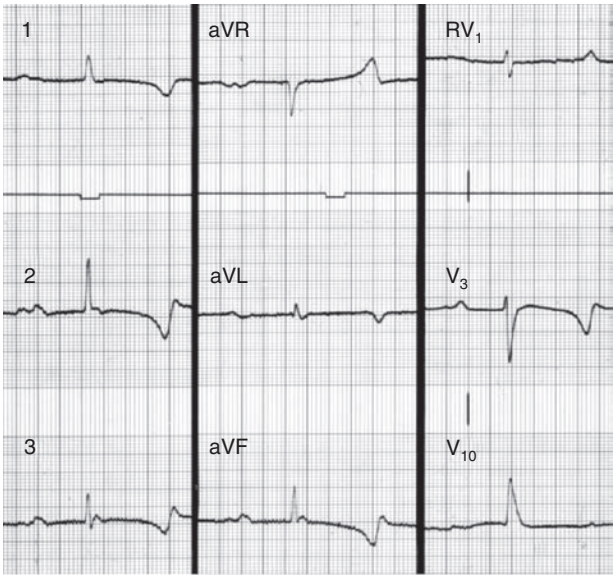


FIG. **9.18** Multiple lead recordings in a normal horse. The varying configuration of the P-QRS-T complexes across the leads is notable.

high as 0.25 mV (mean of 0.14 mV) and the second peak, P_2, reported to be as high as 0.5 mV (mean of 0.28 mV).[499] There are even subtle differences among breeds of horses. The P-wave morphology can change cyclically with waxing and waning of vagal tone during sinus arrhythmia. During tachycardia, the P wave shortens, becomes more peaked, and is followed by a prominent atrial repolarization (T_a) wave that deviates the PQ segment downward. Such features make the diagnosis of atrial enlargement by electrocardiography very difficult.

The time required for conduction across the AV node and His-Purkinje system is estimated by measuring the PR interval. Because physiologic AV block is so common, there is significant variation in the PR interval even within the same horse; thus, normal maximal values for the PR interval are difficult to state. Values that persistently exceed 0.5 seconds are probably abnormal (see Table 9.5). Variation in the PR interval is not usually related to changes in ventilation[42] but is often correlated with changes in BP and baroreceptor activation.[108]

The morphology of the QRS complex is variable. The relatively complete penetration of the conduction system into the free walls of the ventricle causes these chambers to be activated simultaneously with a burst of depolarization, which cancels much of the divergent electromotive forces.[6,7] Consequently, the normal electrical axis may vary, and the amplitude of the QRS complex can be quite small in the frontal plane leads. A substantial dorsally oriented vector causes a prominent positive terminal deflection in lead V_{10}, while the (– electrode) right base to (+ electrode) left apex lead exhibits a prominent S-wave (Figs. 9.14 and 9.18). The normal slur in the ST segment makes determination of QRS duration difficult in many horses.[6,35,542] The mean amplitude for the R wave in lead II for normal racehorses is about 0.8 to 1.1 mV.[1,4,151,499-501,522] Clinical experience suggests that R wave amplitudes exceeding 2.2 mV in lead II or 1.7 mV in lead I are often abnormal. But occasionally, the R wave amplitude in a normal horse exceeds even these limits. The frontal plane leads can be inspected to estimate the mean electrical axis of depolarization, the "average" wave of depolarization. Abnormal axis deviations have been observed with cardiomegaly, cor pulmonale, conduction disturbances, and electrolyte imbalance.[522]

Following ventricular activation, repolarization of the ventricles is recorded. This period is measured from the end of the QRS complex (the "J point") and extends to the end of the T wave.[35,505,543] The T wave vector is most often directed toward the right caudal quadrant, resulting in a positive T wave in lead III and a negative or isoelectric T wave in lead I in resting horses (Fig. 9.18).[499] Although some clinical surveys have suggested that abnormalities of the ST-T indicate cardiac dysfunction in performance animals, marked deviation of the ST segment and increased amplitude of the T wave are anticipated in healthy horses during exercise or even with excitement-induced tachycardia. There is simply no compelling evidence that T-wave abnormalities can be interpreted consistently. Progressive J-point or ST-segment deviation in the horse with hypovolemia or shock may indicate myocardial ischemia, whereas enlargement of the T wave may develop with myocardial hypoxia or hyperkalemia.

Diagnosis and management of cardiac arrhythmias are discussed later in this chapter.

Thoracic Radiography

There are severe limitations to the use of thoracic radiography in the evaluation of the equine heart because of the large size of the mature horse and the ability to obtain only a standing lateral thoracic radiograph in all except the smallest foals.[544] Although recumbent lateral and on occasion ventrodorsal or dorsoventral projections can be obtained on neonatal foals, the stress of restraint or requirement for heavy sedation can make such positioning contraindicated in foals with cardiac disease. Thoracic radiographs may be useful to identify areas of pulmonary or pleural disease and assist in the differential diagnosis of respiratory problems.

Gross changes in cardiac size and shape may be detected in a lateral thoracic radiograph of a foal or horse with significant cardiomegaly.[544-548] A normal thoracic radiograph does not necessarily indicate that the heart is normal in size. Mild to moderate increases in the size of the cardiac chambers may go undetected, particularly in adult horses. Generalized enlargement of the cardiac silhouette is observed in cases of significant pericardial effusion or with CHF (Fig. 9.19). Dorsal displacement of the trachea may be detected in some horses with left atrial (LA) and LV enlargement. In some horses with LA enlargement, the caudodorsal border of the cardiac silhouette bulges caudally. Increased contact between the ventral border of the heart and the sternum may be detected with RV enlargement but is usually difficult to appreciate. A 50% decrease in the spinotracheal angle (the angle between the dorsal border of the trachea and the ventral border of the adjacent thoracic vertebrae) was demonstrated in young horses with cardiomegaly caused by congenital cardiac disease.[544]

Evaluation of the pulmonary vasculature and pulmonary parenchyma is also difficult and very dependent on radiographic technique. Enlarged pulmonary vessels associated with pulmonary overcirculation can occasionally be observed in left-to-right shunts; the opposite is also true in congenital right-to-left shunts. Pulmonary edema causes generalized increased radiopacity, particularly in the hilar regions; the characteristic air bronchograms are more readily identified there when alveolar edema is present.

Angiocardiography is only practical in foals less than about 115 kg and usually requires general anesthesia, which may be contraindicated in the foal with a severely compromised cardiovascular system. Both selective and nonselective positive contrast angiocardiograms have been performed in foals and adult horses (see Fig. 9.19D),[171,545,549] but these techniques have been almost exclusively replaced by echocardiography.

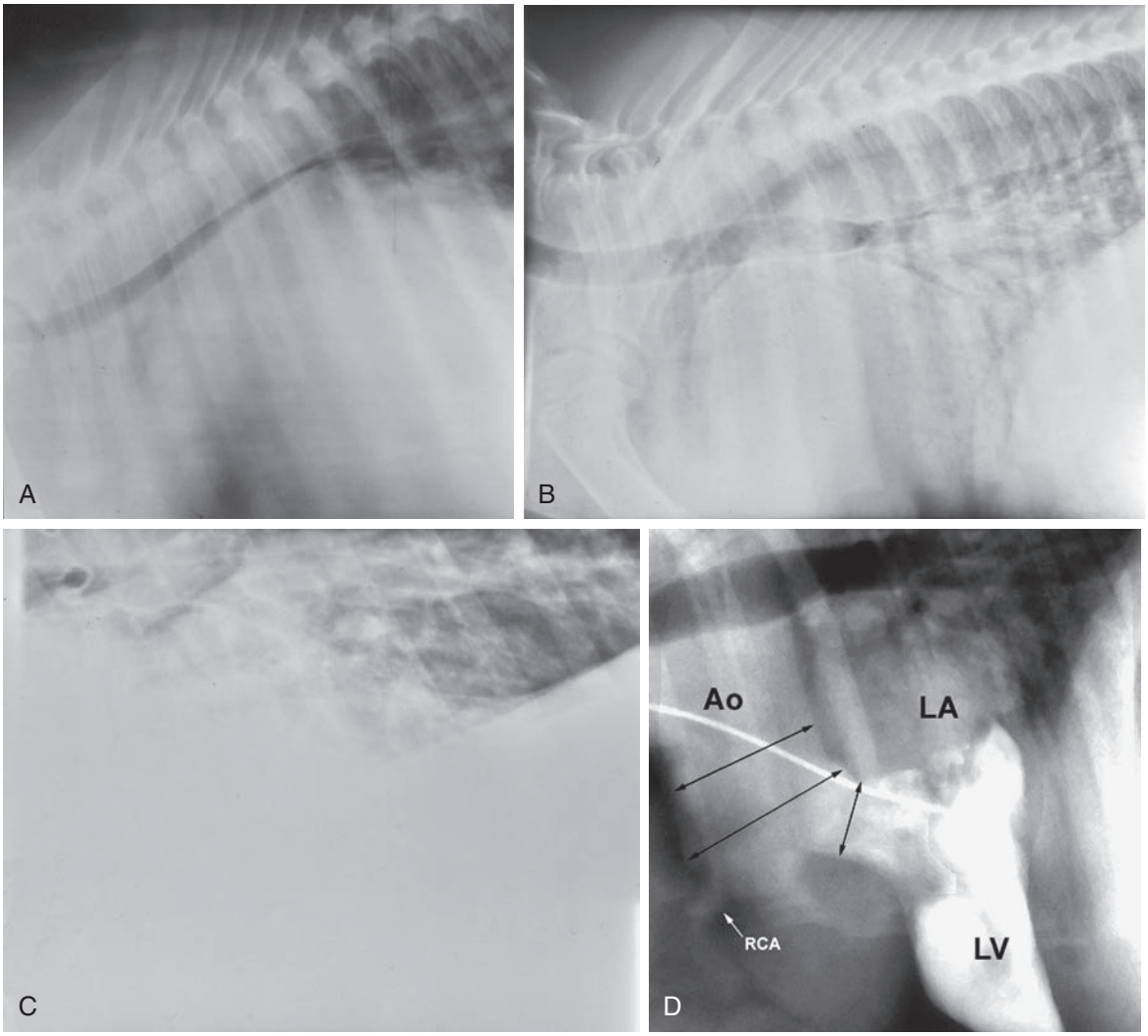


FIG. **9.19** Thoracic radiography. A, Significant cardiomegaly in a Quarter Horse foal with complex congenital heart disease, including multiple ventricular septal defects (VSDs). B, Cardiomegaly and alveolar pulmonary edema in a filly with mitral regurgitation (MR) and left-sided congestive heart failure (CHF). C, Increased pulmonary density and pleural effusion in a Standardbred gelding with atrial fibrillation (AF), atrioventricular valvular regurgitation, myocardial failure, and biventricular CHF. D, Angiocardiogram obtained from an Arabian foal with pulmonary atresia and a large VSD (pseudotruncus arteriosus). Contrast medium was injected in the left ventricle (LV), and this medium opacifies a dilated, overriding aorta. Right-to-left shunting across the VSD results in dilution of contrast medium. The right arrow shows the subaortic LV outflow tract; the widened aorta is delineated by the arrows at the left. *Ao,* Aorta; *LA,* left atrium; *LV,* left ventricle; *RCA,* right coronary artery.

Nuclear medicine imaging has been developed in some equine referral institutions and has been used to assess cardiac function. First-pass nuclear angiocardiography permits the visualization of the cardiac chambers during sequential phases of the cardiac cycle.[544] Application of first pass studies may be useful for identification of cardiac shunting, but again, this diagnosis is more simply attained by echocardiography.

Echocardiography

Over the past 35 years, echocardiography has evolved to become the most important diagnostic study currently available for evaluation of the equine heart. Transthoracic echocardiography, including 2D, M-mode, and Doppler studies, provides the basis for comprehensive evaluation of internal cardiac structures, chamber and vessel dimensions, blood flow characteristics, mechanical function of the heart, presence and nature of heart lesions, and the relevant hemodynamics of a cardiac lesion.* Transesophageal echocardiography (TEE) has been used in horses[241,276,277,309,312,566] but (except in foals) requires custom-made transducers and is only practical in anesthetized or heavily sedated horses due to the fragile (and expensive) nature of the TEE probe.

Despite the recent technical advances that allowed great improvement of image quality and temporal resolution of echocardiographic images, accurate and reliable assessment of chamber dimensions and mechanical function of the heart remains challenging and is limited by a variety of technical, anatomic, and physiologic issues that need to be considered

* References 14, 49, 51, 56, 70, 71, 74-76, 122, 124, 129, 130, 142, 148, 161, 167, 168, 209, 211, 243, 244, 274, 276, 280, 281, 285-287, 289, 297, 306, 311-313, 340-350, 365, 390, 391, 426, 481, 484, 550-593.

when performing echocardiographic examinations.[344,594,595] Knowledge of the technical principles of ultrasonography, technical skills with imaging and instrumentation, strict adherence to a routine protocol in order to obtain high-quality standard sonographic images, comprehensive knowledge of normal cardiac anatomy and physiology, and thorough appreciation of equine cardiac diseases are prerequisites to the successful use of echocardiography.

The echocardiographic examination should take place in a location where the horse can be safely restrained. If possible, the patient should not be sedated before the examination, because cardiac dimensions, indices of cardiac function, and color Doppler signals of regurgitant flow or shunt flow might be altered because of drug effects on preload, afterload, contractility, HR, and rhythm.[274,584,587,596] However, some horses do not tolerate the echocardiographic procedure and require sedation to allow conducting the examination safely, with sufficient quality, and at normal HRs.

Examination findings are relatively consistent when performed by experienced examiners, but there is day-to-day, subject, and examiner variation that can be significant (greater than 10%) for some variables and must be considered when performing serial examinations.* Unfortunately, an imaging standard for equine echocardiography that is unanimously acknowledged and followed by all centers and clinicians is currently lacking. Most clinicians follow some kind of standard, but the methods differ. Similarly, the methods described in the literature vary and are often not sufficiently described when it comes to technical details, machine settings, imaging planes, exact anatomic landmarks, and—importantly—timing of measurements. Reference values for echocardiographc measurements are often insufficiently defined and are based on individual papers or small studies in selected populations. Some representative data are provided in Table 9.6.

A recent survey showed that despite the large number of publications and the great amount of collective experience among experts, there is hardly anything in equine echocardiography everybody does entirely the same way when it comes to modalities, imaging planes, landmarks, timing, and scaling for differences in body mass.[597] It will be important in the near future to define standard imaging guidelines based on expert consensus, better characterize repeatability and reproducibility of echocardiographic methods and measurements, and provide normal reference intervals for echocardiographic measurements considering differences in breed, size, and body weight. This said, it is important to realize that despite the availability of quantitative imaging methods, subjective assessment of recordings is a cornerstone of echocardiography and must not be neglected. Finally, independent of the methods used, the findings obtained during echocardiography must be critically assessed in the light of medical history and clinical findings.

Indications and Clinical Use of Echocardiography in Horses. Echocardiography can be used to identify cardiac disorders, assess hemodynamic and structural consequences of disease, and monitor response to treatment and progression of disease. Echocardiography is primarily indicated in horses with heart murmurs and allows differentiation of physiologic flow murmurs from pathologic murmurs and assessment of their clinical relevance. However, there are other clinical situations when echocardiography also provides valuable information (Box 9.10).[68] Furthermore, ultrasound can also identify other lesions of the lung, pleural space, or mediastinum that may masquerade as heart disease.[140,598]

A complete echocardiographic study should address morphologic lesions, motion abnormalities, cardiac chamber and great vessel size, cardiac valve function, blood flow disturbances, global and regional ventricular systolic function, estimates of hemodynamic variables including pressure gradients and volumetric flow, and ventricular diastolic function and filling pressures (realizing that these two are challenging to measure in mature horses).[68] A normal echocardiogram and Doppler study in a horse with a cardiac murmur is a favorable finding, suggesting a functional basis for the murmur. Conversely, identification of an abnormal flow pattern with associated cardiomegaly or abnormal ventricular function may indicate a high risk or limitation for work. As with all diagnostic studies, there can be ambiguous results, particularly regarding the tricuspid valve, because physiologic tricuspid regurgitation, silent to auscultation, is detectable by Doppler studies in many clinically normal horses. Box 9.11 provides an overview on interpretation of echocardiographic and Doppler studies. Clinical applications of echocardiography are illustrated later in this chapter.

Technical Considerations. The ultrasonographic equipment should include a phased-array sector transducer working at frequencies between 1.5 and 3.5 MHz. Tissue harmonic imaging often improves the image quality, particularly in the far field and in large horses, by providing a higher signal-to-noise ratio, better contrast, and higher spatial resolution. The depth of penetration should reach at least 25 to 30 cm to scan the heart of an adult horse. The image is usually displayed, by convention, with that part of the heart closest to the transducer at the top of the screen. Simultaneous recording of a surface ECG is required and allows exact timing of flow events and echocardiographic measurements. Modern echocardiography systems offer digital raw data storage of still frames and cine loop recordings. This is extremely useful because it reduces the contact time with the patient and allows postprocessing and off-line analysis of the stored data.

Two imaging modalities, the motion-mode (M-mode) and two-dimensional (2D, B-mode, or cross-sectional) formats, are in widespread use. When Doppler studies or contrast echocardiography are added, blood flow can be detected relative to the 2D and M-mode images (Box 9.11, Figs. 9.20–9.25; see also Fig. 9.5).[56,344,590,591]

The *M-mode echocardiogram* is a single-crystal icepick image of the heart (see Figs. 9.5, 9.20, and 9.23).[56,344,590,591] The movement of the cardiac structures (vertical axis) is displayed over time along the horizontal axis. The ECG is recorded to provide a timing reference, and the depth of the cardiac structures from the transducer is displayed in centimeters. Visualization of the characteristic movements of cardiac structures permits the experienced viewer to evaluate and quantify cardiac anatomy and function. The high sampling rate of the M-mode study makes it excellent for visualizing rapidly vibrating structures, such as the oscillating mitral leaflet in aortic regurgitation (see Fig. 9.23D).[554]

The *two-dimensional echocardiogram (2DE)* generates an image by sweeping an ultrasound beam across the heart to produce sector frames (see Figs. 9.20–9.22).[56,344,590,591] In conventional transthoracic echocardiography, the operator hand-directs the imaging probe to achieve a suitable tomographic plane.

* References 286, 287, 340, 341, 346, 350, 351, 567, 569, 577, 592, 593.

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
LV STUDY—SHORT-AXIS LINEAR MEASUREMENTS							
$LVID_d$ (cm)	M-mode	11.2 ± 0.9	(9.9–13.4)[a]	WB Jump/Dress	15	589 ± 47	19
LV internal diameter at end-diastole	Right parasternal short-axis view—chordal level	11.6 ± 0.7		WB Dressage	15	602 ± 38	1
		12.1 ± 1.0		WB Jumping	14	587 ± 40	1
		11.5 ± 0.7		WB Untrained	15	549 ± 44	1
		11.6 ± 1.0	(10.3–12.5)[a]	WB Eventing	5	529 ± 48	16
		11.1 ± 0.9	(9.3–12.9)[c]	WB	31	500[§]	20
			(9.9–12.9)[d]	WB	13	550 ± 59	22
		12.0 ± 0.9	(9.7–13.4)[c]	WB	30	570 ± 53	23
		11.2 ± 0.7	(9.8–12.6)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		12.6–14.0[f]		TBH (trained, var. levels)	483	446–497	9
		11.9 ± 0.7	(10.5–13.4)[a]	THB and THB cross	21	432–648	2
		11.3 ± 1.4	(8.0–14.0)[a]	THB and THB cross	18	482 ± 45	3
		11.9 ± 0.8	(9.7–13.1)[a]	THB	37	420–617	4
		11.5 ± 0.7	(10.3–13.0)[a]	THB (untrained/trained)	8	560 ± 50	7
			(10.9–13.6)[d]	THB	7	548 ± 59	22
		11.5 ± 0.9	(9.8–13.2)[b]	THB	26	n/a	11
		11.5 ± 0.7	(10.1–12.9)[b]	STB (untrained)	8	477–540	5
		10.9 ± 0.8	(9.4–12.4)[b]	STB (untrained)	103	441 ± 42	6
		12.2 ± 0.8	(10.6–13.8)[b]	STB (trained)	103	481 ± 36	6
		11.5 ± 1.1	(10.0–13.3)[a]	STB (untrained)	9	510 ± 37	7
		11.7 ± 0.6	(10.8–12.4)[a]	STB (trained)	7	490 ± 33	7
			(10.2–13.2)[d]	STB	15	539 ± 31	22
		11.8 ± 0.7	(10.4–13.1)[b]	STB (in training)	13	411 ± 10	27
		10.5 ± 0.5	(9.6–11.5)[b]	STB (deconditioned)	13	411 ± 10	27
		10.7 ± 0.9	(8.9–12.5)[b]	Arabian Endurance	24	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
$LVID_s$ (cm) LV internal diameter at peak systole		6.8 ± 0.9	(5.9–9.2)[a]	WB Jump/Dress	15	589 ± 47	19
		8.2 ± 0.6		WB Dressage	15	602 ± 38	1
		8.6 ± 0.8		WB Jumping	14	587 ± 40	1
		8.2 ± 0.7		WB Untrained	15	549 ± 44	1
		7.4 ± 1.5	(5.9–8.9)[a]	WB Eventing	5	529 ± 48	16
		6.7 ± 0.8	(5.0–8.4)[c]	WB	31	500[§]	20
			(5.5–8.3)[d]	WB	13	550 ± 59	22
		6.9 ± 1.1	(4.7–9.1)[c]	WB	30	570 ± 53	23
		6.4 ± 0.8	(4.8–8.0)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		7.6–9.0[f]		TBH (trained, var. levels)	483	446–497	9
		7.4 ± 0.7	(6.1–8.7)[a]	THB and THB cross	26	432–648	2
		7.3 ± 0.8	(5.9–9.1)[a]	THB and THB cross	18	482 ± 45	3
		7.5 ± 0.6	(5.8–8.8)[a]	THB	37	420–617	4
		7.0 ± 1.0	(5.1–8.7)[a]	THB (untrained/trained)	8	560 ± 50	7
			(6.6–8.7)[d]	THB	7	548 ± 59	22
		7.2 ± 0.7	(5.7–8.9)[b]	THB	26	n/a	11
		7.7 ± 0.6	(6.5–8.8)[b]	STB (untrained)	8	477–540	5
		7.5 ± 1.2	(5.4–8.8)[a]	STB (untrained)	9	510 ± 37	7
		7.5 ± 0.7	(6.9–8.7)[a]	STB (trained)	7	490 ± 33	7
			(5.3–8.9)[d]	STB	15	539 ± 31	22
		8.1 ± 0.7	(6.7–9.5)[b]	STB (in training)	13	411 ± 10	27
		7.1 ± 0.6	(5.8–8.3)[b]	STB (deconditioned)	13	411 ± 10	27
		6.8 ± 0.7	(5.4–8.2)[b]	Arabian Endurance	24	423 ± 31	25

IVS_d (cm) Interventricular septal thickness at end-diastole	3.2 ± 0.4	(2.6–4.1)[a]	WB Jump/Dress	15	589 ± 47	19
	3.5 ± 0.4		WB Dressage	15	602 ± 38	1
	3.0 ± 0.3		WB Jumping	14	587 ± 40	1
	3.3 ± 0.5		WB Untrained	15	549 ± 44	1
	3.4 ± 0.3	(2.9–3.6)[a]	WB Eventing	5	529 ± 48	16
	3.0 ± 0.3	(2.3–3.7)[c]	WB	31	500[§]	20
		(2.7–3.8)[d]	WB	13	550 ± 59	22
	3.0 ± 0.3	(2.4–3.5)[c]	WB	30	570 ± 53	23
	2.6–2.8[f]		TBH (trained, var. levels)	483	446–497	9
	3.0 ± 0.4	(2.4–3.7)[a]	THB and THB cross	26	432–648	2
	3.8 ± 0.3	(3.4–4.4)[a]	THB and THB cross	18	482 ± 45	3
	2.9 ± 0.3	(2.3–3.4)[a]	THB	38	420–617	4
	3.3 ± 0.3	(2.9–3.6)[a]	THB (untrained/trained)	8	560 ± 50	7
		(3.0–3.7)[d]	THB	7	548 ± 59	22
	2.6 ± 0.3	(2.0–3.2)[b]	THB	26	n/a	11
	2.6 ± 0.1	(2.3–2.8)[b]	STB (untrained)	8	477–540	5
	3.1 ± 0.3	(2.4–3.5)[a]	STB (untrained)	9	510 ± 37	7
	3.3 ± 0.5	(2.5–3.8)[a]	STB (trained)	7	490 ± 33	7
		(2.5–3.8)[d]	STB	15	539 ± 31	22
	3.2 ± 0.3	(2.5–3.9)[b]	STB (in training)	13	411 ± 10	27
	2.9 ± 0.3	(2.3–3.5)[b]	STB (deconditioned)	13	411 ± 10	27
	3.1 ± 0.3	(2.5–3.7)[b]	Arabian Endurance	24	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
IVS_s (cm) Interventricular septal thickness at peak systole		4.4 ± 0.4	(3.6–5.2)[a]	WB Jump/Dress	15	589 ± 47	19
		4.3 ± 0.7		WB Dressage	15	602 ± 38	1
		4.0 ± 0.5		WB Jumping	14	587 ± 40	1
		4.0 ± 0.6		WB Untrained	15	549 ± 44	1
		4.5 ± 0.7	(3.5–5.2)[a]	WB Eventing	5	529 ± 48	16
		4.4 ± 0.4	(3.5–5.2)[c]	WB	31	500[§]	20
			(4.5–5.2)[d]	WB	13	550 ± 59	22
		4.5 ± 0.4	(3.6–5.4)[c]	WB	30	570 ± 53	23
		3.9–4.3[f]		TBH (trained, var. levels)	483	446–497	9
		4.6 ± 0.6	(3.3–5.6)[a]	THB and THB cross	26	432–648	2
		4.7 ± 0.5	(3.9–5.7)[a]	THB and THB cross	18	482 ± 45	3
		4.2 ± 0.5	(3.2–5.2)[a]	THB	38	420–617	4
		4.6 ± 0.3	(3.9–4.9)[a]	THB (untrained/trained)	8	560 ± 50	7
			(4.0–5.2)[d]	THB	7	548 ± 59	22
		4.0 ± 0.3	(3.4–4.5)[b]	THB	26	n/a	11
		3.5 ± 0.3	(2.9–4.1)[b]	STB (untrained)	8	477–540	5
		4.3 ± 0.4	(3.5–4.9)[a]	STB (untrained)	9	510 ± 37	7
		4.4 ± 0.5	(3.4–5.0)[a]	STB (trained)	7	490 ± 33	7
			(3.8–5.5)[d]	STB	15	539 ± 31	22
		4.3 ± 0.3	(3.7–5.0)[b]	STB (in training)	13	411 ± 10	27
		4.3 ± 0.4	(3.5–5.1)[b]	STB (deconditioned)	13	411 ± 10	27
		4.2 ± 0.6	(3.0–5.4)[b]	Arabian Endurance	24	423 ± 31	25

$LVFW_d$ (cm) LV free wall at end-diastole	2.5 ± 0.4	(1.6–3.0)[a]	WB Jump/Dress	15	589 ± 47	19
	3.0 ± 0.3		WB Dressage	15	602 ± 38	1
	2.6 ± 0.4		WB Jumping	14	587 ± 40	1
	2.7 ± 0.6		WB Untrained	15	549 ± 44	1
	2.7 ± 0.2	(2.4–2.8)[a]	WB Eventing	5	529 ± 48	16
	2.5 ± 0.3	(1.9–3.1)[c]	WB	30	500[§]	20
		(1.9–2.9)[d]	WB	13	550 ± 59	22
	2.6 ± 0.4	(1.8–3.4)[c]	WB	30	570 ± 53	23
	2.5 ± 0.3	(1.8–3.1)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
	2.2–2.3[f]		TBH (trained, var. levels)	483	446–497	9
	2.3 ± 0.4	(1.7–3.4)[a]	THB	37	420–617	4
	2.7 ± 0.3	(2.2–3.1)[a]	THB (untrained/trained)	8	560 ± 50	7
		(2.2–2.7)[d]	THB	7	548 ± 59	22
	2.0 ± 0.2	(1.6–2.4)[b]	THB	26	n/a	11
	2.4 ± 0.1	(2.1–2.7)[b]	STB (untrained)	8	477–540	5
	2.3 ± 0.2	(2.0–2.5)[a]	STB (untrained)	9	510 ± 37	7
	2.8 ± 0.4	(2.2–3.2)[a]	STB (trained)	7	490 ± 33	7
		(1.7–3.1)[d]	STB	15	539 ± 31	22
	2.5 ± 0.3	(1.9–3.2)[b]	STB (in training)	13	411 ± 10	27
	2.2 ± 0.2	(1.8–2.6)[b]	STB (deconditioned)	13	411 ± 10	27
	2.4 ± 0.3	(1.8–3.0)[b]	Arabian Endurance	24	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	Normal Values Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
$LVFW_s$ (cm) LV free wall at peak systole		4.5 ± 0.6	(3.0–5.7)[a]	WB Jump/Dress	15	589 ± 47	19
		3.6 ± 0.5		WB Dressage	15	602 ± 38	1
		3.2 ± 0.5		WB Jumping	14	587 ± 40	1
		3.2 ± 0.6		WB Untrained	15	549 ± 44	1
		4.4 ± 0.3	(4.1–4.8)[a]	WB Eventing	5	529 ± 48	16
		4.4 ± 0.4	(3.7–5.1)[c]	WB	31	500[§]	20
			(3.5–4.9)[d]	WB	13	550 ± 59	22
		4.7 ± 0.5	(3.6–5.8)[c]	WB	30	570 ± 53	23
		4.5 ± 0.5	(3.4–5.5)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		4.0 ± 0.6	(3.0–5.4)[a]	THB and THB cross	9	432–648	2
		3.9 ± 0.4	(3.0–4.6)[a]	THB	37	420–617	4
		4.4 ± 0.3	(3.9–4.8)[a]	THB (untrained/trained)	8	560 ± 50	7
			(3.9–4.7)[d]	THB	7	548 ± 59	22
		3.7 ± 0.4	(3.0–4.2)[b]	THB	26	n/a	11
		3.5 ± 0.3	(2.9–4.0)[b]	STB (untrained)	8	477–540	5
		3.8 ± 0.4	(3.1–4.4)[a]	STB (untrained)	9	510 ± 37	7
		4.2 ± 0.4	(3.7–4.9)[a]	STB (trained)	7	490 ± 33	7
			(2.7–4.3)[d]	STB	15	539 ± 31	22
		3.7 ± 0.5	(2.7–4.8)[b]	STB (in training)	13	411 ± 10	27
		4.0 ± 0.4	(3.2–4.9)[b]	STB (deconditioned)	13	411 ± 10	27
		3.7 ± 0.4	(2.9–4.5)[b]	Arabian Endurance	24	423 ± 31	25
$LVID_d$/AAD $LVID_d$ normalized to aortic annular diameter		1.7 ± 0.1	(1.5–1.9)[a]	WB Jump/Dress	15	589 ± 47	19
		1.7 ± 0.2	(1.4–2.1)[c]	WB	31	574 ± 58	20
		1.7 ± 0.2	(1.3–2.0)[c]	WB	30	570 ± 53	23

RWT_d Relative LV wall thickness at end-diastole = $(LVFW_d + IVS_d)/LVID_d$	0.51 ± 0.07	(0.35–0.63)[a]	WB Jump/Dress	15	589 ± 47	19
	0.53 ± 0.05	(0.49–0.61)[a]	WB Eventing	5	529 ± 48	16
	0.51 ± 0.05	(0.40–0.62)[c]	WB	31	574 ± 58	20
		(0.41–0.63)[d]	WB	13	550 ± 59	22
	0.49 ± 0.06	(0.36–0.62)[c]	WB	29	570 ± 53	23
	0.36–0.38[f]		TBH (trained, var. levels)	483	446–497	9
	0.40 ± 0.05		THB (untrained)	7	463 ± 28	8
	0.45 ± 0.08		THB (trained)	7	461 ± 22	8
	0.52 ± 0.05	(0.44–0.56)[a]	THB (untrained/trained)	8	560 ± 50	7
		(0.40–0.56)[d]	THB	7	548 ± 59	22
	0.43 ± 0.04	(0.35–0.51)[b]	STB (untrained)	8	477–540	5
	0.40 ± 0.04	(0.32–0.48)[b]	STB (untrained)	103	441 ± 42	6
	0.40 ± 0.04	(0.32–0.48)[b]	STB (trained)	103	481 ± 36	6
	0.46 ± 0.02	(0.43–0.50)[a]	STB (untrained)	9	510 ± 37	7
	0.53 ± 0.07	(0.42–0.62)[a]	STB (trained)	7	490 ± 33	7
		(0.39–0.56)[d]	STB	15	539 ± 31	22
	0.5 ± 0.1		Arabian Endurance	24	423 ± 31	25
MWT_d (cm) Mean LV wall thickness at end-diastole = $(LVFW_d + IVS_d)/2$	2.9 ± 0.3	(2.1–3.2)[a]	WB Jump/Dress	15	589 ± 47	19
	2.9 ± 0.3	(2.3–3.5)[b]	WB Eventing	5	529 ± 48	18
	2.8 ± 0.2	(2.4–3.2)[c]	WB	31	500[§]	20
		(2.4–3.2)[d]	WB	13	550 ± 59	22
	2.8 ± 0.2	(2.3–3.3)[c]	WB	30	570 ± 53	23
	2.4–2.5[f]		TBH (trained, var. levels)	483	446–497	9
	2.4 ± 0.2		THB (untrained)	7	463 ± 28	8
	2.7 ± 0.1		THB (trained)	7	461 ± 22	8
	3.0 ± 0.2	(2.7–3.2)[a]	THB (untrained/trained)	8	560 ± 50	7
		(2.7–3.2)[d]	THB	7	548 ± 59	22
	2.2 ± 0.2	(1.8–2.6)[b]	STB (untrained)	103	441 ± 42	6
	2.4 ± 0.2	(2.1–2.8)[b]	STB (trained)	103	481 ± 36	6
	2.7 ± 0.2	(2.2–2.9)[a]	STB (untrained)	9	510 ± 37	7
	3.1 ± 0.4	(2.4–3.4)[a]	STB (trained)	6	490 ± 33	7
		(2.2–3.5)[d]	STB	15	539 ± 31	22
	2.7 ± 0.2	(2.3–3.1)[b]	Arabian Endurance	24	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
LV Mass (g) LV mass $= 1.04 \times [(LVID_d + LVFW_d + IVS_d)^3 - LVID_d^3] - 13.6$ (see Ref. 9)		3575 ± 677	(2539–5225)[a]	WB Jump/Dress	15	589 ± 47	19
		4087 ± 727	(3175–4878)[a]	WB Eventing	5	529 ± 48	16
			(2482–4649)[d]	WB	13	550 ± 59	22
		3567 ± 490	(2546–4589)[c]	WB	29	570 ± 53	23
		3358–4322[f]		TBH (trained, var. levels)	483	446–497	9
		2866 ± 333		THB (untrained)	7	463 ± 28	8
		3783 ± 240		THB (trained)	7	461 ± 22	8
			(3262–4733)[d]	THB	7	548 ± 59	22
		2350 ± 383	(1584–3116)[b]	STB (untrained)	103	441 ± 42	6
		3263 ± 478	(2307–4219)[b]	STB (trained)	103	481 ± 36	6
			(2100–5477)[d]	STB	15	539 ± 31	22
LV FS (%) LV fractional shortening $= (LVID_d - LVID_s)/LVID_d$		40 ± 5	(32–49)[a]	WB Jump/Dress	15	589 ± 47	19
		29 ± 5		WB Dressage	15	602 ± 38	1
		29 ± 5		WB Jumping	14	587 ± 40	1
		28 ± 5		WB Untrained	15	549 ± 44	1
		36 ± 9	(24–46)[a]	WB Eventing	5	529 ± 48	16
		40 ± 5	(40–50)[c]	WB	31	574 ± 58	20
			(33–46)[d]	WB	13	550 ± 59	22
		40 ± 6	(27–54)[c]	WB	30	570 ± 53	23
		43 ± 6	(31–55)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		39 ± 5	(29–47)[a]	THB and THB cross	21	432–648	2
		36 ± 4	(26–44)[a]	THB and THB cross	18	482 ± 45	3
		37 ± 4	(29–45)[a]	THB	37	420–617	4
		40 ± 9		THB (untrained)	7	463 ± 28	8
		31 ± 3		THB (trained)	7	461 ± 22	8
		39 ± 6	(33–50)[a]	THB (untrained/trained)	8	560 ± 50	7
			(32–42)[d]	THB	7	548 ± 59	22
		37 ± 4	(30–45)[b]	THB	26	n/a	11
		34 ± 5	(24–44)[b]	STB (untrained)	103	441 ± 42	6
		31 ± 4	(23–39)[b]	STB (trained)	103	481 ± 36	6
		36 ± 6	(29–47)[a]	STB (untrained)	9	510 ± 37	7
		35 ± 4	(30–39)[a]	STB (trained)	7	490 ± 33	7
			(31–49)[d]	STB	15	539 ± 31	22
		31 ± 4	(24–39)[b]	STB (in training)	13	411 ± 10	27
		33 ± 4	(24–42)[b]	STB (deconditioned)	13	411 ± 10	27
		37 ± 5	(25–49)[b]	Arabian Endurance	24	423 ± 31	25

LV STUDY—SHORT-AXIS AREA MEASUREMENTS							
$LVI_{sx}A_d$ (cm^2) LV internal area at end-diastole	2DE Right parasternal short-axis view—chordal level	102 ± 18	(66–137)[b]	WB Eventing	5	529 ± 48	18
		95 ± 12	(70–119)[c]	WB	28	570 ± 53	23
		94 ± 12	(70–119)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		97–120[f]		TBH (trained, var. levels)	483	446–497	9
		101 ± 11	(81–124)[a]	THB	37	420–617	4
		102 ± 13	(77–127)[b]	THB	26	n/a	11
		84 ± 9	(67–101)[b]	STB (in training)	13	411 ± 10	27
		70 ± 7	(56–83)[b]	STB (deconditioned)	13	411 ± 10	27
$LVI_{sx}A_d/AAD^2$ $LVI_{sx}A_d$ normalized to the second power of aortic annular diameter		2.00 ± 0.35	(1.27–2.73)[c]	WB	29	570 ± 53	23
$LVI_{sx}A_s$ (cm^2) LV internal area at peak systole		33 ± 11	(12–54)[b]	WB Eventing	5	529 ± 48	18
		32 ± 8	(15–49)[c]	WB	28	570 ± 53	23
		33 ± 8	(18–48)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		68 ± 10		THB and THB cross	18	482 ± 45	3
		41 ± 7	(29–57)[a]	THB	37	420–617	4
		38 ± 9	(21–55)[b]	THB	26	n/a	11
		37 ± 7	(24–51)[b]	STB (in training)	13	411 ± 10	27
		29 ± 6	(18–40)[b]	STB (deconditioned)	13	411 ± 10	27
$LV_{sx}MyoA_d$ (cm^2) LV myocardial area at end-diastole		107 ± 9	(89–125)[b]	WB Eventing	5	529 ± 48	18
		116 ± 9	(97–134)[c]	WB	28	570 ± 53	23
		223 ± 15	(193–260)[a]	THB	37	420–617	4
		137 ± 10	(113–158)[b]	STB (in training)	13	411 ± 10	27
		120 ± 7	(105–135)[b]	STB (deconditioned)	13	411 ± 10	27
$LV_{sx}MyoA_s$ (cm^2) LV myocardial area at peak systole		148 ± 7	(135–161)[b]	WB Eventing	5	529 ± 48	18
		152 ± 11	(129–176)[c]	WB	29	570 ± 53	23
		191 ± 17	(157–244)[a]	THB	37	420–617	4
		142 ± 12	(118–166)[b]	STB (in training)	13	411 ± 10	27
		130 ± 11	(109–151)[b]	STB (deconditioned)	13	411 ± 10	27
RWT_{sxAd} Area-based relative LV wall thickness at end-diastole $= [\sqrt{(LVE_{sx}A_d/\pi)} - \sqrt{(LVI_{sx}A_d/\pi)}]/\sqrt{(LVI_{sx}A_d/\pi)}$		0.44 ± 0.05	(0.34–0.54)[b]	WB Eventing	5	529 ± 48	18
		0.50 ± 0.06	(0.37–0.63)[c]	WB	30	570 ± 53	23
MWT_{sxAd} Area-based mean LV wall thickness at end-diastole $= \sqrt{(LVE_{sx}A_d/\pi)} - \sqrt{(LVI_{sx}A_d/\pi)}$		2.5 ± 0.1	(2.3–2.7)[b]	WB Eventing	5	529 ± 48	18
		2.7 ± 0.2	(2.3–3.2)[c]	WB	30	570 ± 53	23

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
LV_{sx} FAC (%)		69 ± 6	(56–81)[b]	WB Eventing	5	529 ± 48	18
LV fractional area change		67 ± 6	(54–79)[c]	WB	30	570 ± 53	23
$= (LVI_{sx}A_d - LVI_{sx}A_s)/LVI_{sx}A_d$		65 ± 7	(51–78)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		60 ± 5	(47–69)[a]	THB	37	420–617	4
		63 ± 6	(51–75)[b]	THB	26	n/a	11
LV STUDY—LONG-AXIS AREA MEASUREMENTS AND VOLUME ESTIMATES							
$LVIA_d$ (cm^2)	2DE	159 ± 16	(127–191)[b]	8 WB, 2 STB	10	541 ± 49	17
LV internal area at end-diastole	Right parasternal long-axis 4-chamber view optimized for the LV	145 ± 20	(106–185)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		167 ± 12	(149–197)[a]	WB Jump/Dress	13	589 ± 47	19
		184 ± 15	(152–216)[c]	WB	29	570 ± 53	23
$LVIA_d/AAD^2$		3.75 ± 0.47	(3.07–4.69)[a]	WB Jump/Dress	13	589 ± 47	19
LV $Area_d$ normalized to the second power of aortic annular diameter		3.86 ± 0.49	(2.85–4.87)[c]	WB	30	570 ± 53	23
$LVIA_s$ (cm^2)		76 ± 14	(48–104)[b]	8 WB, 2 STB	10	541 ± 49	17
LV internal area at peak systole		68 ± 12	(43–93)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26
		72 ± 10	(58–102)[a]	WB Jump/Dress	13	589 ± 47	19
		81 ± 12	(57–106)[c]	WB	29	570 ± 53	23
LV FAC (%)		57 ± 4	(48–66)[a]	WB Jump/Dress	13	589 ± 47	19
LV fractional area change		55 ± 5	(45–66)[c]	WB	30	570 ± 53	23
$= (LVIA_d - LVIA_s)/LVIA_d$		53 ± 6	(41–66)[b]	30 WB, 1 THB, 1 STB	32	563 ± 49	26

$LVIV_d$ (cm^3) LV internal volume at end-diastole	2DE Right parasternal long-axis 4-chamber view optimized for the LV	1195 ± 52	(1091–1299)[b]	8 WB, 2 STB	10	541 ± 49	17
		1288 ± 229	(829–1746)[b]	WB Eventing	5	529 ± 48	18
		1283 ± 176	(1041–1729)[a]	WB Jump/Dress	13	589 ± 47	19
		1475 ± 201	(1057–1893)[c]	WB	29	570 ± 53	23
		1108 ± 134	(840–1376)[b]	Arabian Endurance	26	423 ± 31	25
$LVIV_d/AAD^3$ $LVIV_d$ normalized to the third power of aortic annular diameter		4.30 ± 0.75	(3.26–5.58)[a]	WB Jump/Dress	13	589 ± 47	19
		4.50 ± 0.89	(2.65–6.36)[c]	WB	30	570 ± 53	23
$LVIV_s$ (cm^3) LV internal volume at peak systole	Simpson's method of disks, single plane	318 ± 101	(116–520)[b]	8 WB, 2 STB	10	541 ± 49	17
		337 ± 87	(162–511)[b]	WB Eventing	5	529 ± 48	18
		352 ± 86	(278–615)[a]	WB Jump/Dress	13	589 ± 47	19
		412 ± 82	(241–582)[c]	WB	28	570 ± 53	23
		389 ± 80	(229–549)[b]	Arabian Endurance	26	423 ± 31	25
LV EF (%) LV ejection fraction = $(LVIV_d - LVIV_s)/LVIV_d$		68 ± 2	(64–72)[b]	8 WB, 2 STB	10	541 ± 49	17
		74 ± 4	(67–81)[b]	WB Eventing	5	529 ± 48	18
		73 ± 4	(64–79)[a]	WB Jump/Dress	13	589 ± 47	19
		71 ± 5	(61–81)[c]	WB	30	570 ± 53	23
		65 ± 5	(55–75)[b]	Arabian Endurance	26	423 ± 31	25
LV SV (mL) LV stroke volume = $LVIV_d - LVIV_s$		838 ± 123	(592–1084)[b]	8 WB, 2 STB	10	541 ± 49	17
		951 ± 159	(633–1269)[b]	WB Eventing	5	529 ± 48	18
		931 ± 120	(711–1139)[a]	WB Jump/Dress	13	589 ± 47	19
		1065 ± 139	(774–1356)[c]	WB	28	570 ± 53	23
		719 ± 93	(533–905)[b]	Arabian Endurance	26	423 ± 31	25
CO (L) Cardiac output = $(LVIV_d - LVIV_s) \times HR$		32.9 ± 3.2	(26.5–39.3)[b]	WB Eventing	5	529 ± 48	18
		40.0 ± 11.2	(25.2–59.3)[a]	WB Jump/Dress	13	589 ± 47	19
		41.8 ± 7.9	(25.1–58.4)[c]	WB	25	570 ± 53	23
		27.2 ± 4.0	(19.2–35.2)[b]	Arabian Endurance	26	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	Normal Values Interval	Mode[a]	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
LV STUDY—SYSTOLIC TIME INTERVALS								
LVPEP (msec)	Refs. 11, 15, 22, 27:		(37–61)[d]	MM	WB	8	550 ± 59	22
Preejection period	M-mode	76 ± 18	(40–112)[b]	MM	THB and THB cross	24	n/a	11
Ref. 11: = time (R–O)	Right parasternal	75 ± 18	(40–110)[a]	PWD	THB and THB cross	40	428–648	12
R—peak of the electrocardiographic R wave	short-axis view—aortic valve level	70 ± 10	(50–90)[b]	PWD	THB	7	490–600	13
O—point at which aortic valve fully opened	Refs. 12, 13, 15:	71 ± 10	(50–90)[a]	MM	THB and THB cross	112	308–480	14
	PWD	68 ± 9	(58–88)[a]	PWD	THB and THB cross	112	308–480	14
Others: = time (Q—valve opening) or time (Q—onset flow)	Left-parasternal long-axis view—aortic outflow		(52–104)[d]	MM	THB	4	548 ± 59	22
			(50–110)[d]	MM	STB	4	539 ± 31	22
Q—Onset of electrocardiographic Q wave	Ref. 14:	44 ± 13		MM	STB	13	411 ± 36	15
	M-mode and PWD	35 ± 6		PWD	STB	13	411 ± 36	15
	Left-parasternal	44 ± 12		MM	STB (in training)	13	411 ± 10	27
		75 ± 7		MM	STB (deconditioned)	13	411 ± 10	27
LVET (msec)			(388–532)[d]	MM	WB	8	550 ± 59	22
Ejection time		407 ± 30	(347–467)[b]	MM	THB and THB cross	24	n/a	11
Ref. 11:		467 ± 31	(410–550)[a]	PWD	THB and THB cross	40	428–648	12
= time (O–C)		480 ± 19	(442–518)[b]	PWD	THB	7	490–600	13
O—point at which aortic valve fully opened		532 ± 97	(550–790)[a]	MM	THB and THB cross	112	308–480	14
C—closure point		527 ± 76	(400–700)[a]	PWD	THB and THB cross	112	308–480	14
Others:			(458–538)[d]	MM	THB	4	548 ± 59	22
= time (valve opening-closing) or time (onset–end of flow)			(386–547)[d]	MM	STB	4	539 ± 31	22
		448 ± 16		MM	STB	13	411 ± 36	15
		467 ± 25		PWD	STB	15	411 ± 36	15
		448 ± 14		MM	STB (in training)	13	411 ± 10	27
		432 ± 17		MM	STB (deconditioned)	13	411 ± 10	27
LVPEP/LVET			(0.07–0.13)[d]	MM	WB	8	550 ± 59	22
LVPEP:LVET ratio		0.19 ± 0.04	(0.11–0.27)[b]	MM	THB and THB cross	24	n/a	11
		0.14 ± 0.03	(0.09–0.24)[a]	MM	THB and THB cross	112	308–480	14
		0.13 ± 0.01	(0.09–0.31)[a]	PWD	THB and THB cross	112	308–480	14
			(0.10–0.23)[d]	MM	THB	4	548 ± 59	22
			(0.10–0.29)[d]	MM	STB	4	539 ± 31	22
		0.10 ± 0.03		MM	STB	13	411 ± 36	15
		0.08 ± 0.01		PWD	STB	13	411 ± 36	15
		0.10 ± 0.03		MM	STB (in training)	13	411 ± 10	27
		0.17 ± 0.00		MM	STB (deconditioned)	13	411 ± 10	27

LV STUDY—TISSUE DOPPLER IMAGING							
PEP_m (msec) Preejection period	PW TDI Right parasternal short-axis view—chordal level—LV free wall (radial motion)	96 ± 15	(72–123)[a]	WB	14	589 ± 47	19
		121 ± 14	(96–149)[e]	WB	30	570 ± 53	21
		127 ± 25		STB, THB	6	517–606	24
$IVCT_m$ (msec) Isovolumic contraction time		88 ± 18	(58–123)[a]	WB	14	589 ± 47	19
		87 ± 16	(65–107)[e]	WB	30	570 ± 53	21
		91 ± 25		STB, THB	5	517–606	24
ET_m (msec) Ejection time		420 ± 28	(365–482)[a]	WB	14	589 ± 47	19
		421 ± 25	(374–475)[e]	WB	30	570 ± 53	21
		404 ± 19		STB, THB	6	517–606	24
PEP_m/ET_m PEP_m:ET_m ratio		0.23 ± 0.03	(0.17–0.29)[a]	WB	14	589 ± 47	19
		0.29 ± 0.03	(0.24–0.36)[e]	WB	30	570 ± 53	21
		0.32 ± 0.07		STB, THB	6	517–606	24
$IVCT_m/ET_m$ $IVCT_m$:ET_m ratio		0.21 ± 0.04	(0.15–0.28)[a]	WB	14	589 ± 47	19
		0.21 ± 0.03	(0.15–0.26)[e]	WB	30	570 ± 53	21
		0.23 ± 0.07		STB, THB	5	517–606	24
$IVRT_m$ (msec) Isovolumic relaxation time		60 ± 19	(29–92)[a]	WB	14	589 ± 47	19
		52 ± 15	(36–90)[e]	WB	30	570 ± 53	21
		48 ± 17		STB, THB	6	517–606	24
IMP_m Index of myocardial performance (Tei)		0.35 ± 0.07	(0.24–0.47)[a]	WB	14	589 ± 47	19
		0.33 ± 0.05	(0.25–0.42)[e]	WB	30	570 ± 53	21
		0.35 ± 0.09		STB, THB	5	517–606	24
S_1 (cm/sec) Wall motion velocity during isovolumic contraction		9 ± 2	(5–15)[a]	WB	14	589 ± 47	19
		9 ± 3	(6–18)[e]	WB	30	570 ± 53	21
		10 ± 1		STB, THB	5	517–606	24
S_m (cm/sec) Wall motion velocity during LV ejection		12 ± 2	(10–14)[a]	WB	14	589 ± 47	19
		12 ± 1	(10–15)[e]	WB	30	570 ± 53	21
		12 ± 2		STB, THB	6	517–606	24
E_1 (cm/sec) Wall motion velocity during isovolumic relaxation		7 ± 2	(4–10)[a]	WB	14	589 ± 47	19
		8 ± 2	(4–13)[e]	WB	30	570 ± 53	21
		7 ± 1		STB, THB	6	517–606	24

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
E_m (cm/sec) Wall motion velocity during early diastole (passive filling)		33 ± 6	(25–44)[a]	WB	14	589 ± 47	19
		33 ± 4	(24–41)[c]	WB	28	574 ± 58	20
		35 ± 6	(27–45)[e]	WB	30	570 ± 53	21
		29 ± 3		STB, THB	6	517–606	24
A_m (cm/sec) Wall motion velocity during late diastole (atrial contraction)		11 ± 3	(4–18)[a]	WB	14	589 ± 47	19
		11 ± 2	(7–14)[c]	WB	28	574 ± 58	20
		12 ± 3	(8–20)[e]	WB	30	570 ± 53	21
		10 ± 2		STB, THB	6	517–606	24
E_m/A_m E_m:A_m ratio		3.4 ± 1.4	(1.6–7.1)[a]	WB	14	589 ± 47	19
		3.1 ± 0.8	(1.4–4.7)[c]	WB	28	574 ± 58	20
		3.1 ± 0.8	(2.0–4.6)[e]	WB	30	570 ± 53	21
		3.0 ± 0.8		STB, THB	6	517–606	24
LA STUDY—LINEAR AND AREA MEASUREMENTS							
$LA_{IIx}D_{min}$ (cm) LA diameter at end-diastole	2DE **Left** parasternal long-axis view Midatrium	11.0 ± 0.8	(9.4–12.3)[a]	THB and THB cross	18	482 ± 45	3
		12.8 ± 0.8	(11.3–14.5)[a]	THB	36	420–617	4
		12.4 ± 0.7	(10.0–13.8)[b]	THB	26	n/a	11
		max. 13.5		THB and STB	n/a	n/a	10
		max. 14.0		Larger horses	n/a	n/a	10
$LA_{IIx}D_{max}$ (cm) LA diameter at end-systole		13.6 ± 1.1	(11.6–14.6)[a]	WB Jump/Dress	15	589 ± 47	19
		13.1 ± 0.5	(12.7–13.6)[a]	WB Eventing	5	529 ± 48	16
		12.9 ± 0.5	(11.8–14.0)[c]	WB	31	500[§]	20
		12.6 ± 1.3	(10.8–15.7)[a]	THB and THB cross	18	482 ± 45	3
		12.9 ± 0.8	(11.2–14.5)[a]	THB	28	420–617	4
LAD_{max} (cm) LA diameter at end-systole (before opening of mitral valve)	2DE **Right** parasternal long-axis 4-chamber view, optimized for LA Midatrium $_{max}$ = end-systole (before opening of mitral valve) $_a$ = onset of atrial contraction (P wave) $_{min}$ = end-diastole (closure of mitral valve)	12.3 ± 0.7	(10.9–13.5)[a]	WB Jump/Dress	15	589 ± 47	19
		12.7 ± 0.9	(11.4–13.8)[a]	WB Eventing	5	529 ± 48	16
		11.9 ± 0.7	(10.5–13.2)[c]	WB	31	500[§]	20
		12.7 ± 0.7	(11.4–14.1)[c]	WB	29	570 ± 53	23
		12.6 ± 1.3	(10.9–14.6)[a]	THB (untrained/trained)	8	560 ± 50	7
		12.1 ± 0.9	(10.8–13.7)[a]	STB (untrained, old)	9	510 ± 37	7
		12.4 ± 1.4	(10.4–13.8)[a]	STB (trained, young)	5	490 ± 33	7
		11.3 ± 0.8	(9.7–12.9)[b]	Arabian Endurance	21	423 ± 31	25

LAD_{max}/AAD LAD_{max} normalized to aortic annular diameter	1.8 ± 0.1	(1.7–2.0)[a]	WB Jump/Dress	15	589 ± 47	19
	1.9 ± 0.2	(1.7–2.1)[a]	WB Eventing	5	529 ± 48	16
	1.9 ± 0.1	(1.6–2.1)[c]	WB	31	574 ± 58	20
	1.8 ± 0.1	(1.6–2.1)[c]	WB	29	570 ± 53	23
	1.8 ± 0.2	(1.5–2.1)[a]	THB (untrained/trained)	8	560 ± 50	7
	1.9 ± 0.2	(1.7–2.1)[a]	STB (untrained, old)	9	510 ± 37	7
	1.9 ± 0.2	(1.6–2.1)[a]	STB (trained, young)	5	490 ± 33	7
LAD_{max}/$LVID_d$ LAD_{max}:$LVID_d$ ratio	1.1 ± 0.1	(1.0–1.3)[a]	WB Jump/Dress	15	589 ± 47	19
	1.1 ± 0.1	(0.9–1.3)[c]	WB	31	574 ± 58	20
	1.1 ± 0.1	(0.9–1.3)[c]	WB	30	570 ± 53	23
LAA_{max} (cm^2) Left-atrial area at end-systole (before opening of mitral valve)	102.8 ± 8.5	(90.6–117.4)[a]	WB Jump/Dress	15	589 ± 47	19
	103.5 ± 13.4	(82.1–117.6)[a]	WB Eventing	5	529 ± 48	16
	92.8 ± 5.0	(82.3–103.2)[c]	WB	31	500[§]	20
	109.7 ± 7.1	(94.7–124.6)[c]	WB	29	570 ± 53	23
	99.5 ± 11.3	(87.2–121.2)[a]	THB (untrained/trained)	8	560 ± 50	7
	92.7 ± 9.1	(79.9–108.8)[a]	STB (untrained, old)	9	510 ± 37	7
	94.5 ± 12.6	(73.8–105.9)[a]	STB (trained, young)	5	490 ± 33	7
	82.3 ± 11.1	(60.1–104.5)[b]	Arabian Endurance	21	423 ± 31	25
LAA_{max}/AAD^2 LAA_{max} normalized to the second power of aortic annular diameter	2.7 ± 0.4	(2.2–3.4)[a]	WB Jump/Dress	15	589 ± 47	19
	2.6 ± 0.4	(2.1–3.1)[a]	WB Eventing	5	529 ± 48	16
	2.3 ± 0.3	(1.7–2.8)[c]	WB	30	574 ± 58	20
	2.3 ± 0.2	(1.8–2.8)[c]	WB	29	570 ± 53	23
	2.0 ± 0.3	(1.6–2.5)[a]	THB (untrained/trained)	8	560 ± 50	7
	2.3 ± 0.4	(1.8–3.0)[a]	STB (untrained, old)	9	510 ± 37	7
	2.2 ± 0.3	(1.9–2.5)[a]	STB (trained, young)	5	490 ± 33	7
LAA_{max}/$LVIA_d$ LAA_{max}:$LVIA_d$ ratio	0.61 ± 0.05	(0.53–0.70)[a]	WB Jump/Dress	15	589 ± 47	19
	0.59 ± 0.06	(0.47–0.72)[c]	WB	30	570 ± 53	23
Passive LA FAC (%) Passive LA fractional area change = (LAA_{max} – LAA_a)/LAA_{max}	25 ± 5	(15–32)[a]	WB Jump/Dress	15	589 ± 47	19
	24 ± 5	(18–30)[a]	WB Eventing	5	529 ± 48	16
	23 ± 5	(13–33)[c]	WB	31	574 ± 58	20
	24 ± 4	(17–29)[a]	THB (untrained/trained)	7	560 ± 50	7
	27 ± 5	(17–32)[a]	STB (untrained, old)	9	510 ± 37	7
	20 ± 5	(11–24)[a]	STB (trained, young)	5	490 ± 33	7
	24 ± 6	(12–36)[b]	Arabian Endurance	21	423 ± 31	25

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
Active LA FAC (%) Active LA fractional area change = (LAA_a – LAA_{min})/LAA_a		18 ± 7	(10–29)[a]	WB Jump/Dress	15	589 ± 47	19
		15 ± 5	(9–21)[a]	WB Eventing	5	529 ± 48	16
		20 ± 7	(6–33)[c]	WB	31	574 ± 58	20
		19 ± 3	(14–23)[a]	THB (untrained/trained)	7	560 ± 50	7
		20 ± 5	(11–28)[a]	STB (untrained, old)	9	510 ± 37	7
		14 ± 6	(8–22)[a]	STB (trained, young)	5	490 ± 33	7
		17 ± 6	(5–29)[b]	Arabian Endurance	21	423 ± 31	25
Total LA FAC (%) Total LA fractional area change = (LAA_{max} – LAA_{min})/LAA_{max}		39 ± 4	(33–48)[a]	WB Jump/Dress	15	589 ± 47	19
		35 ± 5	(29–42)	WB Eventing	5	529 ± 48	16
		38 ± 3	(35–43)[a]	THB (untrained/trained)	7	560 ± 50	7
		41 ± 4	(35–49)[a]	STB (untrained, old)	9	510 ± 37	7
		32 ± 2	(30–35)[a]	STB (trained, young)	5	490 ± 33	7
		37 ± 5	(27–47)[b]	Arabian Endurance	21	423 ± 31	25
Active:total LA AC Ratio of active to total LA area change		0.35 ± 0.13	(0.18–0.60)[a]	WB Jump/Dress	15	589 ± 47	19
		0.33 ± 0.11	(0.17–0.48)[a]	WB Eventing	5	529 ± 48	16
		0.39 ± 0.12	(0.13–0.65)[c]	WB	31	574 ± 58	20
		0.38 ± 0.08	(0.27–0.53)[a]	THB (untrained/trained)	7	560 ± 50	7
		0.35 ± 0.12	(0.19–0.54)[a]	STB (untrained, old)	9	510 ± 37	7
		0.37 ± 0.18	(0.20–0.64)[a]	STB (trained, young)	5	490 ± 33	7
LA RI (%) LA reservoir index = (LAA_{max} – LAA_{min})/LAA_{min}		64 ± 12	(48–92)[a]	WB Jump/Dress	15	589 ± 47	19
		55 ± 11	(40–72)[a]	WB Eventing	5	529 ± 48	16
		62 ± 11	(39–86)[c]	WB	30	574 ± 58	20
		63 ± 7	(54–76)[a]	THB (untrained/trained)	7	560 ± 50	7
		72 ± 12	(55–96)[a]	STB (untrained, old)	9		7
		47 ± 4	(44–53)[a]	STB (trained, young)	5		7
$LA_{sx}A_{max}$ (cm^2) LA area at end-systole (closure of aortic valve)	2DE Right parasternal short-axis view—aortic level, optimized for the left atrium and left-atrial appendage	123.6 ± 19.3	(92.4–149.5)[a]	WB Jump/Dress	15	589 ± 47	19
		113.4 ± 18.1	(95.0–138.8)[a]	WB Eventing	5	529 ± 48	16
		108.8 ± 12.2	(83.5–134.1)[c]	WB	31	500[§]	20
		118.4 ± 10.4	(99.3–131.7)[a]	THB (untrained/trained)	8	560 ± 50	7
		106.2 ± 17.5	(65.5–125.0)[a]	STB (untrained, old)	9	510 ± 37	7
		110.2 ± 8.8	(97.4–118.1)[a]	STB (trained, young)	5	490 ± 33	7
$LA_{sx}A_{max}/Ao_{sx}A$ $LA_{sx}A_{max}$ normalized to aortic area		2.6 ± 0.3	(2.1–3.0)[a]	WB Jump/Dress	15	589 ± 47	19
		2.3 ± 0.3	(2.1–2.7)[a]	WB Eventing	5	529 ± 48	16
		2.5 ± 0.3	(2.0–3.2)[c]	WB	31	574 ± 58	20
		2.3 ± 0.3	(1.9–2.7)[a]	THB (untrained/trained)	8	560 ± 50	7
		2.6 ± 0.5	(2.0–3.7)[a]	STB (untrained, old)	9	510 ± 37	7
		2.4 ± 0.2	(2.1–2.6)[a]	STB (trained, young)	5	490 ± 33	7

GREAT VESSELS							
AAD (cm) Aortic annular diameter during systole	2DE Right parasternal long-axis LVOT view	6.8 ± 0.5	(6.1–8.0)[a]	WB Jump/Dress	15	589 ± 47	19
		6.7 ± 0.6	(6.0–7.6)[a]	WB Eventing	5	529 ± 48	16
		6.4 ± 0.4	(5.6–7.2)[c]	WB	31	500[§]	20
		6.9 ± 0.4	(6.1–7.7)[c]	WB	29	570 ± 53	23
		7.0 ± 0.5	(6.4–7.8)[a]	THB (untrained/trained)	8	560 ± 50	7
		6.4 ± 0.5	(5.4–6.9)[a]	STB (untrained)	9	510 ± 37	7
		6.5 ± 0.2	(6.2–6.8)[a]	STB (trained)	5	490 ± 33	7
AoD (cm) Diameter of the aortic sinus (sinus Valsalva) at end-diastole		8.0 ± 0.7	(7.1–9.0)[a]	WB Jump/Dress	15	589 ± 47	19
		7.8 ± 0.7	(7.2–9.0)[a]	WB Eventing	5	529 ± 48	16
		7.6 ± 0.5	(6.5–8.7)[c]	WB	31	500[§]	20
		8.7 ± 0.5	(7.8–9.9)[a]	THB	37	420–617	4
		8.3 ± 0.7	(6.9–9.7)[b]	THB	25	n/a	11
		8.2 ± 0.7	(7.3–9.5)[a]	THB (untrained/trained)	8	560 ± 50	7
		7.4 ± 0.7	(6.2–8.4)[a]	STB (untrained)	9	510 ± 37	7
		7.5 ± 0.6	(7.2–8.5)[a]	STB (trained)	5	490 ± 33	7
		7.7 ± 0.3	(7.1–8.3)[b]	STB (in training)	13	411 ± 10	27
		7.5 ± 0.3	(6.9–8.1)[b]	STB (deconditioned)	13	411 ± 10	27
$Ao_{sx}A$ (cm^2) Internal aortic area at end-systole (closure of aortic valve)	2DE Right parasternal short-axis view—aortic level	44.8 ± 5.5	(33.5–56.2)[c]	WB	31	500[§]	20
PAD (cm) Diameter of the pulmonary sinus at end-diastole	2DE Right parasternal long-axis RVOT view	6.9 ± 0.5	(6.0–7.7)[a]	WB Jump/Dress	15	589 ± 47	19
		6.6 ± 0.6	(5.9–7.4)[a]	WB Eventing	5	529 ± 48	16
		6.5 ± 0.4	(5.6–7.4)[c]	WB	30	500[§]	20
		6.1 ± 0.5	(5.2–6.9)[a]	THB	37	420–617	4
		6.4 ± 0.4	(5.5–6.8)[a]	THB (untrained/trained)	8	560 ± 50	7
		6.1 ± 0.4	(5.6–6.8)[a]	STB (untrained)	9	510 ± 37	7
		6.5 ± 0.5	(5.9–6.9)[a]	STB (trained)	5	490 ± 33	7
PAD_{ann} (cm) Annular diameter of the pulmonary artery (at insertion of the PV) in late systole	2DE Right parasternal long-axis RVOT view	5.5 ± 0.3	(4.9–6.1)[b]	STB (untrained)	8	477–540	5

Continued

TABLE **9.6** **Reference Values of Selected Echocardiographic Measurements in Adult Horses—cont'd**

Measurement Timing	Mode View	Normal Values Mean ± SD	 Interval	Population	*N*	BWT (kg) Mean ± SD or Min-Max	Ref.
AoD/PAD	2DE	1.2 ± 0.1	(1.0–1.3)[a]	WB Jump/Dress	15	589 ± 47	19
AoD:PAD ratio	Right parasternal long-axis views	1.2 ± 0.1	(1.0–1.3)[a]	WB Eventing	5	529 ± 48	16
		1.2 ± 0.1	(1.0–1.4)[c]	WB	31	574 ± 58	20
		1.3 ± 0.1	(1.1–1.6)[a]	THB (untrained/trained)	8	560 ± 50	7
		1.2 ± 0.1	(1.0—1.5)[a]	STB (untrained)	9	510 ± 37	7
		1.2 ± 0.1	(1.1–1.4)[a]	STB (trained)	5	490 ± 33	7
AoD/PAD$_{sx}$	2DE	1.6 ± 0.2	(1.4–1.8)[a]	WB Jump/Dress	15	589 ± 47	19
Ratio of AoD to short-axis PAD measured in same LVOT imaging plane at end-diastole	Right-parasternal long-axis LVOT view	1.5 ± 0.1	(1.3–1.8)[c]	WB	31	574 ± 58	20

Reference intervals:
[a]range (min-max)
[b](mean – 2SD) – (mean + 2SD)
[c]Lower and upper limit reference interval (Reference Value Advisor v2.1, National Veterinary School, Toulouse, France; using standard methods)
[d]2.5th–97.5th percentile
[e]5th–95th percentile
[f]Range of means
BWT, Body weight; *2DE,* two-dimensional echocardiography; *LA,* left atrium; *LV,* left ventricle; *MM,* M-mode; *N,* number of horses; *PWD,* pulsed-wave Doppler; *PW TDI,* pulsed-wave tissue Doppler imaging; *STB,* Standardbred; *THB,* Thoroughbred; *WB,* Warmblood.
[§]Values are allometrically scaled to a body weight of 500 kg.
Note: This summary does not provide a systematic review of the current literature. It is intended to serve as a general guide for assessment of some of the most commonly used echocardiographic indices of cardiac dimensions and cardiac function in horses. Individual measurements may differ depending on echocardiographic techniques and breed, age, body weight, and athletic condition of the animal.
References:

1. Stadler P, Rewel A, Deegen E: M-mode echocardiography in dressage- and show-jumping horses of class "S" and in untrained horses. J Vet Med A 1993;40:292-306.
2. Long K, Bonagura JD, Darke PGG: Standardized imaging technique for guided M-mode and Doppler echocardiography in the horse. Equine Vet J 1992;24:226-235.
3. Voros K, Holmes JR, Gibbs C: Measurement of cardiac dimensions with two dimensional echocardiography in the living horse. Equine Vet J 1991;23:461-465.
4. Patteson MW, Gibbs C, Wotton PR, et al: Echocardiographic measurements of cardiac dimensions and indices of cardiac function in normal adult Thoroughbred horses. Equine Vet J 1995;Suppl 19:18-27.
5. Buhl R, Ersboll AK, Eriksen L, et al: Sources and magnitude of variation of echocardiographic measurements in normal standardbred horses. Vet Radiol Ultrasound 2004;45:505-512.
6. Buhl R, Ersboll AK, Eriksen L, et al: Changes over time in echocardiographic measurements in young Standardbred racehorses undergoing training and racing and association with racing performance. J Am Vet Med Assoc 2005;226:1881-1887.

7. Schwarzwald CC: Unpublished data. 2006. Techniques see: Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of echocardiographic assessment of left atrial size and mechanical function in horses. Am J Vet Res 2007;68:735-747.
8. Young LE: Cardiac responses to training in 2-year-old thoroughbreds: an echocardiographic study. Equine Vet J Suppl 1999;30:195-198.
9. Young LE, Rogers K, Wood JL: Left ventricular size and systolic function in Thoroughbred racehorses and their relationships to race performance. J Appl Physiol 2005; 99:1278-1285.
10. Reef VB. Cardiovascular ultrasonography. In: Reef VB, ed. Equine diagnostic ultrasound, 1st ed. WB Saunders, Philadelphia, 1998:215-272.
11. Patteson MW, Gibbs C, Wotton PR, et al: Effects of sedation with detomidine hydrochloride on echocardiographic measurements of cardiac dimensions and indices of cardiac function in horses. Equine Vet J Suppl 1995:33-37.
12. Blissitt KJ, Bonagura JD: Pulsed wave Doppler echocardiography in normal horses. Equine Vet J Suppl 1995:38-46.
13. Young LE, Scott GR: Measurement of cardiac function by transthoracic echocardiography: day to day variability and repeatability in normal Thoroughbred horses. Equine Vet J 1998;30:117-122.
14. Lightowler C, Piccione G, Fazio F, et al: Systolic time intervals assessed by 2-D echocardiography and spectral Doppler in the horse. Animal Science Journal 2003;74:505-510.
15. Kriz NG, Rose RJ: Repeatability of standard transthoracic echocardiographic measurements in horses. Aust Vet J 2002;80:362-370.
16. Schwarzwald CC, et al: Unpublished data, 2008. Techniques see: Schefer KD, Bitschnau C, Weishaupt MA, Schwarzwald CC. Quantitative analysis of stress echocardiograms in healthy horses with 2-dimensional (2D) echocardiography, anatomical M-mode, tissue Doppler imaging, and 2D Speckle Tracking. J Vet Int Med 2010;24:918-931.
17. Gehlen H, Marnette S, Stadler P: The influence of adrenaline on echocardiographic parameters of left ventricular function in the horse. Equine and Comparative Exercise Physiology 2005;2:89-96.
18. Schefer KD, Bitschnau C, Weishaupt MA, Schwarzwald CC. Quantitative analysis of stress echocardiograms in healthy horses with 2-dimensional (2D) echocardiography, anatomical M-mode, tissue Doppler imaging, and 2D Speckle Tracking. J Vet Int Med 2010;24:918-931.
19. Trachsel DS, Schwarzwald CC, Bitschnau C, et al: Atrial natriuretic peptide and cardiac troponin I concentrations in healthy WB horses and in WB horses with mitral regurgitation at rest and after exercise. J Vet Cardio 2013;15:105-121 (supplementary unpublished data included).
20. Huesler IM, Mitchell KM, Schwarzwald CC: Echocardiographic assessment of left atrial size and function in WB horses: reference intervals, allometric scaling and agreement of different echocardiographic variables. J Vet Intern Med 2016;30:1241-1252.
21. Koenig TR, Mitchell KM, Schwarzwald CC: Echocardiographic assessment of left ventricular function in healthy horses and in horses with heart disease using pulsed-wave tissue Doppler imaging. J Vet Intern Med 2016, submitted.
22. Grenacher PA, Schwarzwald CC: Assessment of left ventricular size and function in horses using anatomical M-mode echocardiography. J Vet Cardio 2010;12:111-121.
23. Berthoud DJ, Schwarzwald CC: Echocardiographic assessment of left ventricular size and systolic function in horses using linear measurements and area-based volume estimates. 2016, in progress.
24. Schwarzwald CC, Bonagura JD, Schober KE: Methods and reliability of tissue Doppler imaging for assessment of left ventricular radial wall motion in horses. J Vet Intern Med 2009;23:643-652.
25. Flethoj M, Schwarzwald CC, Haugaard MM, et al: Left ventricular function after prolonged exercise in equine endurance athletes. J Vet Intern Med 2016;30:1260-1269.
26. Ven S, Decloedt A, Van Der Vekens N, et al: Assessing aortic regurgitation severity from 2D, M-mode and pulsed wave Doppler echocardiographic measurements in horses. Vet J 2016;210:34-38.
27. Kriz NG, Hodgson DR, Rose RJ: Changes in cardiac dimensions and indices of cardiac function during deconditioning in horses. Am J Vet Res 2000;61:1553-1560.

BOX 9.10 Indications for Echocardiography

EVALUATION OF HEART MURMURS

- Previously diagnosed "functional" murmur that is louder on serial examinations[a]
- Grade 3–6/6 left-sided murmur compatible with mitral regurgitation (MR) or aortic regurgitation (AR)[a]
- Grade 4–6/6 right-sided systolic murmur compatible with tricuspid regurgitation (TR)[a]
- Suspected ventricular septal defect (VSD) or other congenital heart lesion
- Continuous or combined systolic-diastolic murmurs
- Murmur associated with poor performance
- Murmur detected on prepurchase examination

EVALUATION OF ARRHYTHMIAS

- Clinically important arrhythmias, whether a murmur is present or not

OTHER INDICATIONS

- Suspected myocardial disease (unexplained tachycardia, supraventricular or ventricular ectopy, increased cardiac troponin I concentrations)
- Unexplained exercise intolerance, poor performance, collapse, or episodic weakness
- Fever of unknown origin or specific suspicion for endocarditis or pericarditis
- Muffled heart sounds (to detect effusion)
- Suspicion of CHF
- Severe respiratory disease (to detect pulmonary hypertension)

[a]Because of the high prevalence of heart murmurs in the healthy horse population, an echocardiographic examination is not strictly recommended in all horses with murmurs. Acknowledging the fact that murmur grade might not be directly related to clinical relevance or severity of disease, the current recommendations are based on murmur grades, on the assumption that murmurs of grade 1–2/6 are less likely to be clinically relevant. In case of doubt, an echocardiogram is always indicated in horses with murmurs.

The pie-shaped image obtained has both depth and breadth but has no significant thickness. Accordingly, different image planes must be used to interrogate the three-dimensional heart. These imaging planes are designated long-axis (sagittal), short-axis (coronal), apical (when the transducer is near the left apex), and angled (hybrid) views. Because of anatomic restrictions, apical images are difficult or impossible to obtain except in foals. By convention, in long-axis and short-axis recordings, the dorsal regions of the heart (atria, heart base) and the cranial regions of the heart (right ventricular outflow tract), respectively, are displayed to the right of the screen.

The 2D field is constantly updated to visualize cardiac motion in real time, and this is done at typical sampling rates (frame rates) of 20 to 40 frames/sec, with the update rate inversely related to penetration depth and angle of field. Fully digital echocardiographs can display much faster frame rates, often exceeding 60/sec. Newer applications such as 2DE-based anatomic M-mode[345] or 2D speckle tracking[161,168,346-348] demand a frame rate between 40 and 90 Hz to achieve sufficient temporal resolution. Human interpretation is generally limited to 32 frames/sec, so that recordings made at higher frame rates require slow motion playback for detailed analysis. 2D echocardiography allows assessment of cardiac anatomy, detection of macroscopic structural lesions, subjective evaluation and measurement of chamber and vessel dimensions, and evaluation of left atrial and left ventricular function.

Contrast echocardiography,[599] whereby saline is used to delineate the path of blood flow, is especially useful for the detection of right-to-left shunts in foals, including patent foramen ovale (see Fig. 9.24D).

Doppler echocardiography relies on the Doppler principle to measure the direction and velocity of red blood cells (RBCs) moving through the heart (see Figs. 9.5 and 9.25).* In Doppler echocardiography, a portion of the ultrasound emitted by the transducer strikes moving RBCs. These targets cause ultrasound to be reflected back to the transducer. Because the RBCs constitute a moving "source" of (reflected) ultrasound, the returning sound waves attain a frequency that is slightly different from that originally transmitted (the "carrier frequency"). When the echocardiograph unit records the Doppler frequency shift, calculation of RBC velocity and direction relative to the transducer is possible. The information is displayed as a Doppler spectrum showing time along the horizontal axis, flow direction relative to the transducer as above (toward) or below (away from) a zero baseline, and calculated RBC velocity along the vertical axis. Disturbed flow, as might be recorded across a regurgitant orifice or ventricular septal defect, results in a high-velocity and broadened velocity spectrum. With all Doppler modalities, parallel alignment with the direction movement of the target is crucial, because excessive angle of interrogation (i.e., greater than 20 degrees) will lead to an underestimation of velocities. Therefore technically, velocities can only be accurately measured for blood flowing parallel to the ultrasound beam (i.e., toward or away from the transducer) but not for blood moving perpendicular to the ultrasound beam. Most echocardiography units offer digital angle correction that can be applied live or during postprocessing to mathematically correct the measured velocities. However, this function should not be applied for Doppler measurements of intracardiac blood flow because the exact true direction of flow in the cardiac chambers is unknown and cannot be derived from the Doppler signals, and angle correction may lead to overestimation of true velocity and severity of the lesion.

Pulsed-wave (PW) Doppler methods measure direction and velocity of RBCs within a discrete area of the heart or circulation, which can be determined by the observer when placing the sample volume within the cardiac chambers or vessels. *Color-coded Doppler imaging* is a more refined example of pulsed-wave technology whereby flow toward the transducer is coded in red and flow away is represented in blue. Calculated velocity is displayed in relative shades of these colors, and green or yellow are added to the flow mapping to identify "turbulence." Color coding permits superimposition of flow information onto the 2D or M-mode image (see Fig. 9.25). Although there are substantial technical challenges in terms of penetration and frame rate, color Doppler imaging is useful in horses because a large area of the heart can be screened for flow disturbances; this is also called "color flow mapping" (CFM). For example, it is much easier to find a jet of mitral regurgitation using color Doppler imaging than with other Doppler

* References 56, 71, 208, 279-281, 285, 297, 311, 313, 340, 344, 391, 558, 560, 563, 590, 591, 600-602.

BOX 9.11 Interpretation of Echocardiographic and Doppler Studies

GENERAL PRINCIPLES OF INTERPRETATION

- Evaluate the electrocardiogram relative to cardiac motion and cardiac rhythm disturbances; arrhythmias can alter measures of ventricular function, cause aortic valvular regurgitation, and alter flow and pressure gradients.
- Remember that persistent tachyarrhythmias can lead to reversible form of dilated cardiomyopathy (tachycardia-induced cardiomyopathy).
- Determine the initial image planes needed for examination based on clinical examination and your standard operating procedures.
- Identify the general situs of the heart and cardiac structures; identify the atria, ventricles, cardiac septa, great vessels, and cardiac valves.
- Note any dilation, attenuation, or absence of the aorta or pulmonary artery, and identify their origins and relationships to the atrioventricular valves and to the ventricles.
- Note the presence of unanticipated or lack of expected structures.
- Identify pleural effusion.
- Rule out extrapericardial mass lesions of the lung or thorax.

PERICARDIUM AND PERICARDIAL SPACE

- Rule out pericardial effusion and pericardial mass lesion.
- Mixed echoic effusions or shaggy tags may indicate highly cellular or inflammatory effusate.
- In pericardial effusion: Identify diastolic collapse of the right atrium or right ventricle indicating cardiac tamponade or a large bilateral pleural effusion; protracted collapse or ventricular or atrial inversion are more reliable signs of tamponade. Exuberant swinging of heart may be seen.
- In pericardial effusion: Identify any heart-related lesions using multiple complementary planes.
- If there is concern about constrictive pericardial disease, evaluate the motion of the ventricular septum (for accentuated flutter or 'septal bounce'), and perform Doppler studies of the left and right sides of the ventricular inlets; large E waves with abrupt termination of filling may be observed; evaluate the effects of ventilation because marked variation may be observed in E waves; tissue Doppler may also be instructive in cases of constrictive disease.

LEFT ATRIUM, PULMONARY VEINS, AND ATRIAL SEPTUM

- Identify pulmonary veins and pulmonary venous entry.
- Examine for attenuation or small size: Rule out volume depletion, right-to-left shunt, or low cardiac output.
- Examine for enlargement or rounding/turgid appearance: Rule out left-to-right shunt, mitral valve disease, left ventricular systolic or diastolic failure, chronic arrhythmia (e.g., atrial fibrillation).
- Measure left atrial diameter and/or left atrial area by 2D imaging.
- Examine the atrial septum for abnormal bowing to the left or right atrium (high atrial pressure).
- Examine the atrial septum for septal defects or patent foramen ovale (perform Doppler and 'bubble' contrast studies if necessary).
- Examine the blood pool for spontaneous contrast.
- Examine the atrioventricular groove for dilated vascular structure: Rule out dilated coronary sinus and left cranial vena cava.
- 2D echocardiography and tissue Doppler can be used to evaluate atrial wall motion and function.

MITRAL VALVE

- Identify two valve leaflets and cusps in long- and short-axis planes; if evidence of mitral regurgitation, also examine mitral valve from left caudal imaging windows in two or more imaging planes.
- Observe motion during cardiac cycle by 2D and M-mode echocardiogram.
- Examine the support apparatus (chordae tendineae, papillary muscles).
- Increased valve echogenicity: Rule out degenerative thickening, valvulitis, vegetation (infective endocarditis), malformation (rare).
- Cleft or common septal leaflet: Rule out endocardial cushion defect/primum atrial septal defect (rare defects).
- M-mode: Reduced diastolic (E-F) slope suggests decreased transvalvular flow.
- Lack of diastolic separation or increased mitral E point to septal distance: Rule out aortic regurgitation (regurgitant jet impinging on the valve), left ventricular dilation with left ventricular failure (reduced transmitral flow), or mitral stenosis/tethering (rare defects or acquired from endocarditis).
- Prolapse of mitral leaflet into left atrium: Rule out degenerative disease, elongated or ruptured chordae tendineae, lesion of papillary muscle.
- Flail leaflet: Rule out ruptured chordae tendineae or avulsion of papillary muscle.
- Double line of mitral closure (mobile): Rule out ruptured chordae tendineae (flail leaflet).
- Diastolic mitral valve fluttering: Rule out aortic regurgitation.
- Systolic mitral fluttering: Rule out mitral regurgitation (musical).
- Chaotic valve motion: Rule out arrhythmia (atrial flutter, premature ventricular complexes), ruptured chordae tendineae.
- Premature (diastolic) closure: Rule out severe semilunar valve insufficiency, long P-R interval, or atrioventricular block.
- Delayed (systolic) closure (B-shoulder): Rule out left ventricular failure and elevated atrial pressure.
- Systolic anterior motion of the mitral valve (systolic anterior motion, systolic mitral–septal contact): Rule out dynamic left ventricular outflow tract obstruction due to valve malformation, subaortic stenosis, volume depletion, pulmonic stenosis, or hypertrophic or infiltrative cardiomyopathy (all very rare).
- Doppler studies can be used to interrogate the mitral valve for regurgitation or abnormal flow; multiple planes should be obtained including short-axis images at the level of the left atrium immediately dorsal to the mitral valve.
- Mitral regurgitation is common. Eccentric jets are often observed. Do not confuse 'backflow' color noise with true

Continued

BOX **9.11** **Interpretation of Echocardiographic and Doppler Studies—cont'd**

regurgitation. Horses can develop mid-to-late systolic mitral regurgitation (as with mitral prolapse). Multiple jets of mitral regurgitation are common.
- Always time flow events of mitral regurgitation (color M-mode and spectral Doppler); do not misdiagnose diastolic mitral regurgitation. Do not overemphasize receiving chamber color coding in mitral regurgitation because red blood cell entrainment and a 'spray effect' can result in overestimation of mitral regurgitation severity, whereas wall-hugging jets underestimate severity of mitral regurgitation. Attempt to measure jet width at origin and correlate to left atrial and left ventricular chamber size.

LEFT VENTRICLE
- Evaluate from long- and short-axis tomograms; inspect contour and walls; dorsal septum is normally slightly thicker than left ventricle free wall.
- Echogenic smoke/contrast: Some spontaneous contrast may be normal; rule out low output states, bradycardia, systemic inflammation (?).
- Evaluate left ventricle dimensions (internal dimensions, thickness of interventricular septum and free wall) and left ventricle systolic function (fractional shortening, ejection fraction) using M-mode or 2D imaging.
- Left ventricular dilation: Rule out causes of volume overload or left ventricular failure, including mitral or aortic valvular regurgitation, dilated cardiomyopathy, myocarditis, left-to-right shunt, persistent tachyarrhythmia.
- Left ventricular wall thinning: Rule out infarct, prior myocarditis, congenital aneurysm (rare).
- Left ventricular wall thickening: Consider "athletic heart"; rule out pseudohypertrophy from volume depletion, acute myocarditis (necrosis/edema; viral, bacterial, toxic, idiopathic), infiltrative cardiomyopathy (amyloid, lymphoma, other neoplasia), chronic systemic hypertension (chronic kidney disease, metabolic syndrome, laminitis, chronic pain), chronic digitalis (foxglove, oleander, yew) poisoning, or left ventricular outflow obstruction (very rare).
- Hyperkinesis of left ventricle: Rule out mitral or aortic insufficiency, bradycardia, sympathetic stimulation.
- Hypokinesis or dyskinesis of the left ventricle: Rule out cardiomyopathy or other myocardial disease, ischemia, infarct, arrhythmia, regional wall infiltration, or myocarditis.
- Hyperechoic myocardium or subendocardium: Rule out recurrent ischemia, fibrosis, infiltration, infarction, myocarditis, amyloidosis.
- Tissue Doppler and 2D speckle tracking (2D strain) may be used to evaluate regional and global left ventricular function; tissue Doppler (E_m/A_m ratio) is useful for assessment of left ventricular diastolic function; transmitral flow can also be obtained but is limited with respect to evaluation of left ventricular diastolic function.

LEFT VENTRICULAR OUTLET, AORTIC VALVE, AND AORTA
- Identify aortic valve leaflets and motion during cardiac cycle: Identify abnormal leaflet morphology (e.g., thickenings, fenestrations) or motion. Examine in both long-axis and short-axis image planes to see all leaflets.
- Thickened leaflets (diffuse or nodular): Rule out degeneration, endocarditis.
- Prolapse of aortic valve into the left ventricle: Rule out degenerative semilunar valve disease (common), ventricular septal defect, or bacterial endocarditis with torn leaflet.
- Diastolic fluttering of the aortic valve: Rule out aortic insufficiency.
- Systolic fluttering or the aortic valve: Normal or high flow state.
- Lack of systolic separation: Rule out valvular cardiac output, arrhythmia, stenosis (rare).
- Decreased aortic diameter: Low cardiac output, hypotension.
- Aortic dilation: Rule out aortic regurgitation tetralogy of Fallot, pulmonary artery atresia, truncus arteriosus, patent ductus arteriosus (rare).
- Aortic sinus of Valsalva aneurysm (focal dilation or ballooning of affected sinus): Evaluate for rupture into ventricular septum or right atrium.
- Doppler studies may be used to identify blood flow patterns in the left ventricle outflow tract, aortic regurgitation, paramembranous ventricular septal defect, and rare cases of aortic stenosis. Doppler studies also can identify abnormal blood flow paths in rare cases of aortic sinus ruptured into the heart.

VENTRICULAR SEPTUM
- Septal hyperkinesis: As per the left ventricle.
- Septal hypokinesis: As per left ventricle; also rule out right ventricular pressure or volume overload.
- Paradoxic ventricular septal motion: Rule out moderate to severe right ventricular volume overload such as atrial septal defect, severe tricuspid regurgitation, severe pulmonary hypertension with tricuspid regurgitation.
- Flat ventricular septum: Rule out right ventricular volume overload (diastolic flattening) or right ventricular pressure overload (systolic flattening).
- Exaggerated and disparate ventricular filling rates with diastolic septal fluttering: Rule out constrictive pericardial disease.
- Examine for ventricular septal defects in both long-axis and short-axis image planes (perform Doppler studies if necessary).
- Echocardiogram dropout or discontinuity of the ventricular septum: Rule out ventricular septal defect (peri/paramembranous adjacent to right/noncoronary cusps of the aortic valve and septal leaflet of tricuspid valve; inlet septal ventricular septal defect (ventral to tricuspid valve); muscular or trabecular ventricular septal defect; subpulmonic ventricular septal defect (also termed outlet, supracristal, subarterial, or doubly committed ventricular septal defect; directly underneath pulmonic and aortic valves); tetralogy of Fallot; pulmonary artery atresia; truncus arteriosus; or a false defect caused by angle of the ascending aorta or aortic dilation.
- Malalignment of the ventricular septum and anterior aortic root: Examine for tetralogy of Fallot, malalignment-type ventricular septal defect.
- Examine for dissection or "track" that may suggest rupture of the aorta into the ventricular septum with subsequent fistula.

BOX 9.11 Interpretation of Echocardiographic and Doppler Studies—cont'd

- High-frequency fluttering of the ventricular septum: Rule out aortic regurgitation.
- Doppler studies, especially color Doppler, are useful for recognizing ventricular septal defects; continuous-wave Doppler should be used to measure velocity across shunts to evaluate for "restrictive" versus unrestrictive septal defects. High-velocity flow across a ventricular septal defect (>4.5 m/sec) generally suggests a restrictive defect more likely to be well tolerated.

RIGHT ATRIUM AND ATRIAL SEPTUM

- Examine for attenuation: If small, rule out volume depletion, external compression, or mass lesion impairing venous return.
- Examine for enlargement: If enlarged, rule out tricuspid regurgitation, tricuspid stenosis or atresia (rare), right-sided heart failure, atrial septal defect, moderate to severe anemia, or chronic arrhythmia.
- Examine the atrial septum for abnormal bowing into the left or right atrium (high atrial pressure).
- Examine the atrial septum for septal defects or patent foramen ovale (double mobile lines in foals).
- Doppler studies can be used to identify atrial septal defect, but streaming of normal flow patterns (i.e., caudal vena cava flow) is confusing.
- "Bubble studies" (by injecting agitated normal saline into the jugular vein) should be used to identify right-to-left shunting of blood. *Caution:* Similar to Doppler studies, streaming of normal blood from the caudal vena cava may produce negative contrast in the right atrium, falsely suggesting left-to-right shunting of blood.

TRICUSPID VALVE

- Identify valve leaflets and cusps in long- and short-axis image planes.
- Observe motion during cardiac cycle.
- Identify support apparatus (chordae tendineae, papillary muscles).
- Increased valve echogenicity: Rule out degenerative thickening, vegetation, malformation, thrombus on valve.
- Common septal leaflet: Rule out endocardial cushion defect/primum atrial septal defect (rare).
- Flail leaflet: Rule out ruptured chordae tendineae or avulsion of papillary muscle; rule out infective endocarditis.
- Chaotic valve motion: Rule out arrhythmia, ruptured chordae tendineae.
- Premature (diastolic) closure: Rule out severe pulmonic insufficiency, long P-R interval, or atrioventricular block.
- Doppler studies can interrogate the tricuspid valve for abnormal flow or regurgitation; most horses have some small jets of tricuspid regurgitation by color Doppler evaluation; use color M-mode and spectral Doppler to evaluate timing and peak velocity. High-velocity tricuspid regurgitation (>3.2 m/sec): Also rule out pulmonary hypertension, large ventricular septal defect, right ventricular outflow obstruction.

RIGHT VENTRICLE

- Dilation: Rule out severe tricuspid insufficiency, chronic right ventricular pressure overload, biventricular congestive heart failure, atrial septal defect, large ventricular septal defect, severe pulmonary insufficiency, persistent tachyarrhythmia.
- Hypertrophy: Rule out large (unrestrictive) ventricular septal defect, pulmonary hypertension, pulmonic stenosis (rare), tetralogy of Fallot (rare), or complex congenital heart disease.
- Midventricular bands: Rule out double-chambered right ventricle (examine for ventricular septal defect); remember that the right ventricular moderator band can be very prominent in horses.
- Hyperechoic tissue: Rule out myocardial fibrosis.

RIGHT VENTRICULAR OUTLET, PULMONARY VALVE, AND PULMONARY ARTERY

- Identify valve leaflets and motion during cardiac cycle; if pulmonary valve lesion is suspected, also interrogate from a left cranial transducer position.
- Thick, fused, or hypoplastic leaflets: Rule out congenital stenosis or dysplasia (rare).
- Diastolic fluttering of pulmonic valve: Rule out valvar insufficiency.
- Systolic fluttering of pulmonic valve: Normal or high flow state.
- Lack of systolic separation of the pulmonic valve: Rule out low cardiac output, arrhythmia, stenosis.
- Pulmonary artery, absence or attenuation: Rule out pulmonary atresia or pulmonary artery hypoplasia.
- Pulmonary artery, dilation of: Rule out pulmonary hypertension, left-to-right shunt, poststenotic dilation of pulmonic stenosis (rare); rupture of the aorta into the right side of the heart or pulmonary artery (rare).
- Doppler studies can be used to identify abnormal flow in the pulmonary artery, including the rare pulmonic stenosis (high velocity), increased velocity flow from a left-to-right shunt, and patent ductus arteriosus in equine neonates. Pulmonary regurgitation is normal; high-velocity pulmonary regurgitation indicates pulmonary hypertension.

methods. A pivotal limitation of color Doppler regards temporal resolution. Depending on the system used, the frame rates of interrogation can be very slow (often less than 10/sec). Thus it is mandatory to time flow events using either spectral Doppler or by invoking the M-mode cursor and recording the event by color-M-mode echocardiography. In many cases, the color M-mode examination is simpler and provides excellent temporal resolution, reducing interpretation errors. This timing of blood flow prevents the clinician from misinterpreting normal "backflow" signals related to valve closure or diagnosing a diastolic flow event as systolic.

PW spectral and color Doppler techniques can provide accurate information about the location of flow disturbances but cannot measure high-velocity flow faithfully. High-velocity flow is encountered as RBCs are ejected from high to lower pressure zones across incompetent valves, stenotic valves, and intracardiac and extracardiac shunts (provided that there is a pressure difference across the defect). In general, once

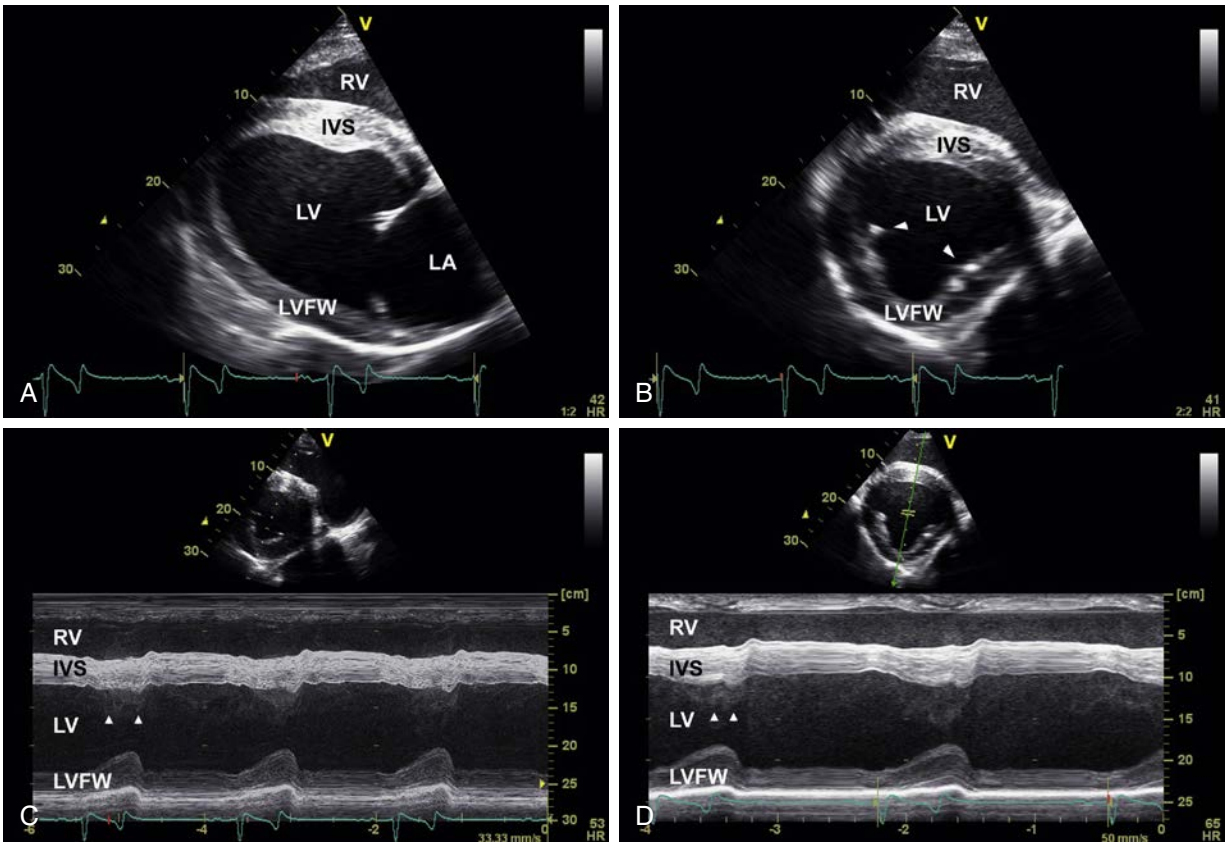


FIG. **9.20** Standard echocardiographic views for assessment of left ventricular size and function. A, Right-parasternal long-axis 4-chamber view centered on the left ventricle (LV). The transducer is positioned in the right 4th intercostal space at a level slightly above the olecranon, angled caudally and rotated clockwise to the 1 o'clock position. Slight changes in transducer placement may be necessary to optimize the image plane. This view is best suited to assess the structures, dimensions, and mechanical function of the LV. Because this image is centered on the LV, the left atrium (LA) may not be imaged in its entirety throughout the cardiac cycle. Assessment of the right side of the heart is limited due to its anatomic position, its complex geometry, and its visualization in the narrow near field of the imaging sector. B, Right-parasternal short-axis view of the LV at the level of the chordae tendineae *(arrowheads).* This view is obtained by rotating the transducer 90 degrees clockwise from a 4-chamber view. It is commonly used for measurement of the diameter and the area, respectively, of the LV and evaluation of LV systolic function. C, Right-parasternal short-axis view *(top)* and corresponding M-mode recording *(bottom)* of the LV at the chordal level. The motion of the interventricular septum (IVS) and the left-ventricular free wall (LVFW) is displayed over time. The right ventricle (RV) and the right-ventricular free wall are displayed in the near field. A small amount of spontaneous echo contrast is frequently seen in the ventricular lumen *(arrowheads).* Measurement of LV wall thickness and internal dimensions, evaluation of septal motion, and calculation of LV fractional shortening allow assessment of LV size and systolic function. D, Anatomic M-mode (AMM) image of the LV *(bottom),* reconstructed from a digitally stored 2D cineloop recording obtained from a right-parasternal short-axis view at the chordal level *(top).* Notice that the AMM cursor *(green line)* can be freely positioned on the 2D image, independent of the sector apex, to bisect the IVS, the LV cavity, and the LVPW into two equal parts throughout the cardiac cycle. (From Schwarzwald CC: Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, et al.: *Atlas of equine ultrasonography,* Wiley, Oxford, UK, 2014. Reproduced with permission of John Wiley & Sons, Ltd.)

velocities exceed about 2.5 m/sec in either direction, part of the returning signal is displayed in the incorrect direction. This problem is called signal "aliasing" (see Fig. 9.25).[56,344,590,591] To quantify high-velocity flow, a third modality must be invoked, either *high pulse-repetition-frequency (HPRF) Doppler* or *continuous-wave (CW) Doppler.* Continuous-wave Doppler has virtually unlimited ability to record very high velocity but does not provide the spatial discrimination found in PW Doppler modalities.

In recent years, more advanced echocardiographic methods such as *tissue Doppler* imaging* and *2D speckle tracking (2D strain) imaging*[161,168,346-348,575,603] (which is based on conventional 2DE cineloop recordings and independent of the Doppler principle) have been investigated in horses to complement the traditional 2D, M-mode, and Doppler echocardiographic methods and to overcome some of their limitations.

* References 161, 168, 286, 289, 349, 572, 574, 575, 603, 604.

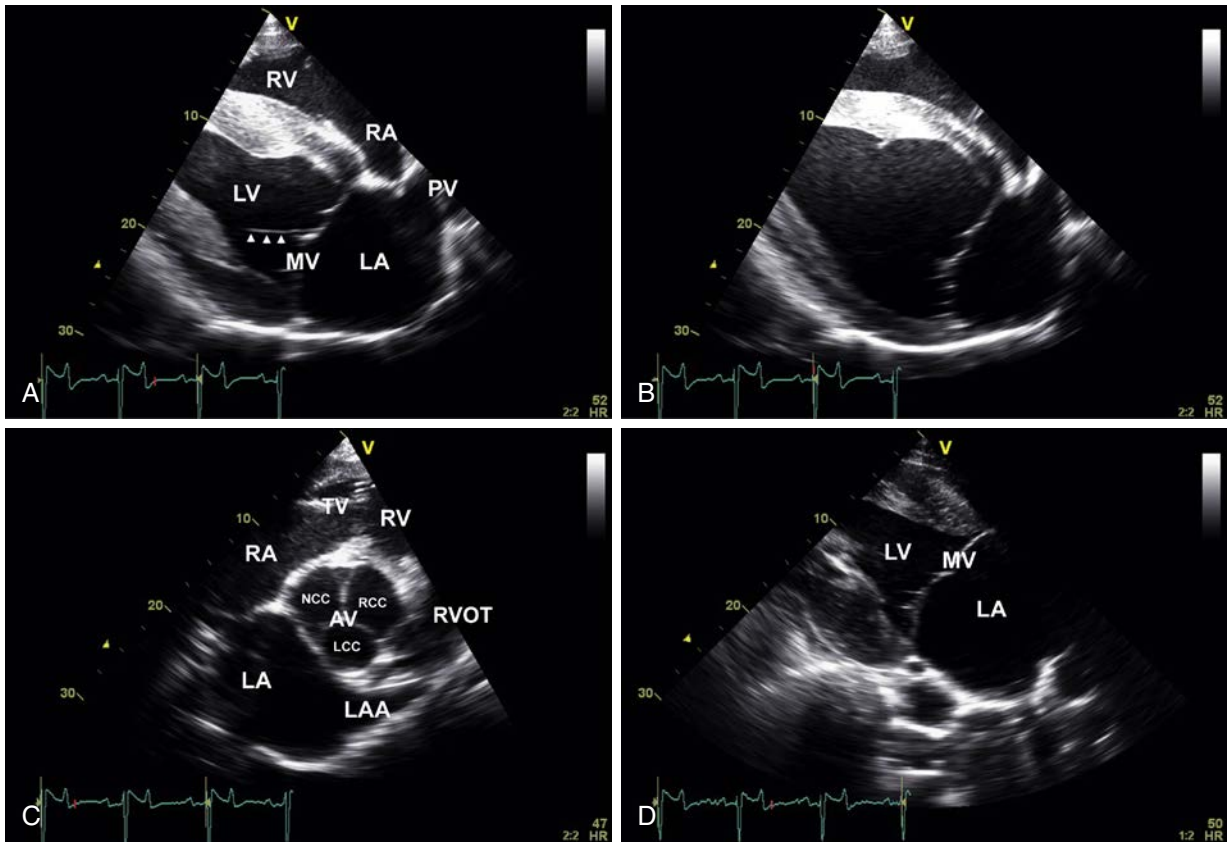


FIG. **9.21** Standard echocardiographic views for assessment of left atrial size and function. A and B, Right-parasternal 4-chamber view centered on the left atrium (LA), to image the LA in its entirety throughout the cardiac cycle. At an imaging depth of 30 cm, this view is best suited to assess the mitral valve (MV) apparatus, LA dimensions, and LA mechanical function. Only in rare cases (e.g., giant draft breeds, horses with severe cardiomegaly) the LA cannot be displayed in its entirety from this window. A, Image recorded at the end of ventricular systole, 1 frame before opening of the MV, when the LA is at its maximum dimensions. Chordae tendineae are seen in the left ventricular (LV) cavity *(arrowheads).* B, Image recorded at the end of diastole, immediately after closure of the MV, when the LA is at its minimum dimensions. Note the obvious change in LA dimensions within a single cardiac cycle (i.e., between A and B), indicating that timing of measurements of LA dimensions is critical. Generally, LA dimensions should be assessed at the end of ventricular systole (A). C, Right-parasternal short-axis view at the level of the aortic valve. This view is obtained by rotating the transducer 90 degrees clockwise from a right-parasternal left-ventricular outflow tract view (Fig. 9.22A). In this view, the aortic valve (AV) with its three cusps is visible in the center of the image. The surrounding structures include right atrium (RA), tricuspid valve, right ventricle (RV), right-ventricular outflow tract (RVOT), LA, and left atrial appendage (LAA). The apparent triangular separation (at the 12 o'clock position) evident between the noncoronary cusp (NCC) and the right coronary cusp (RCC) is a normal finding and does not represent an anomaly. In this view, the end-systolic size of the LA and LAA can be measured and compared with the aortic area. D, Left-parasternal long-axis view of the LA, MV, and LV. The transducer is positioned in the 5th intercostal space slightly above the olecranon, oriented perpendicular to the chest wall and angled dorsally. This view has traditionally been used for assessment of LA dimensions. However, as opposed to the right-parasternal views (A, B), this view often does not allow imaging of the LA in its entirety due to interference with the ventral lung border. Nonetheless, imaging LA and MV from a left thoracic window provides additional information and should complement the right-parasternal views. *LCC,* Left-coronary cusp of the aortic valve; *PV,* pulmonary vein; *TV,* tricuspid valve. (From Schwarzwald CC: Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, et al.: *Atlas of equine ultrasonography,* Wiley, Oxford, UK, 2014. Reproduced with permission of John Wiley & Sons, Ltd.)

These modalities allow quantitative assessment of myocardial motion velocity, deformation (strain), deformation rate (strain rate), displacement, and rotation in longitudinal, radial, and circumferential imaging planes and may provide more sensitive methods for assessing regional or global myocardial function in horses. Tissue Doppler imaging is a promising tool for assessment of LV diastolic function,[164,366,575] and 2D speckle tracking may be helpful for detection and quantification of LV systolic dysfunction in horses with myocardial disease (see Fig. 9.49 later in this chapter)[161,164,575] and for

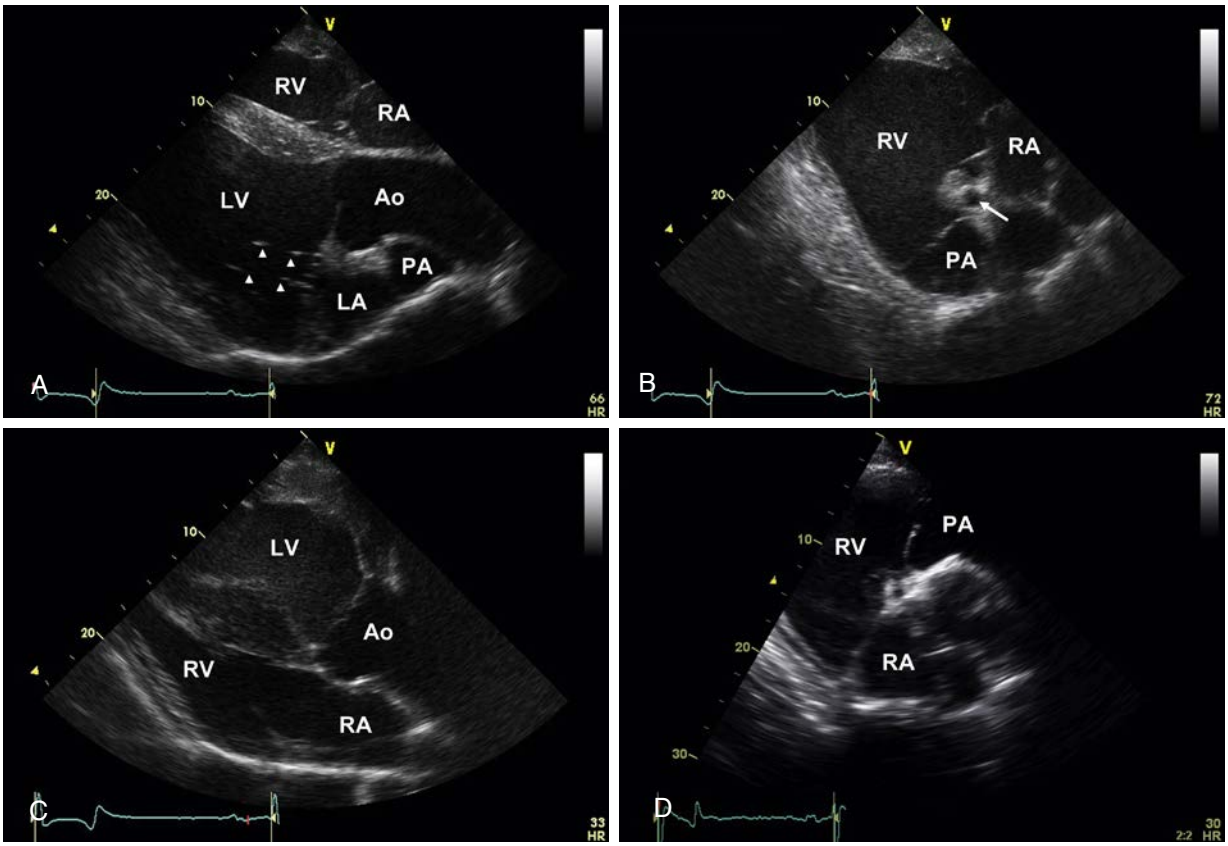


FIG. **9.22** Standard echocardiographic views for assessment of the great vessels. A, Right-parasternal long-axis view of the left ventricular outflow tract (LVOT). Starting from a 4-chamber view, the transducer is angled more cranially, tilted dorsally, and rotated to the 2 o'clock position to obtain this view. It is best suited to assess the structures, dimensions, and function of the aortic valve, the sinus of Valsalva, and the ascending aorta (Ao) and the pulmonary artery (PA). The size of the PA should be smaller than the diameter of the aorta. B, Right-parasternal long-axis view of the right ventricular outflow tract (RVOT). Starting from an LVOT view (A), the transducer is angled more cranially to obtain this view; occasionally the transducer will have to be moved one intercostal space more cranially. The right atrium (RA), the tricuspid valve, the right ventricle (RV), the RVOT, the pulmonic valve, the pulmonary artery (PA), and across section through the right coronary artery *(arrow)* can be visualized. C, Left-parasternal long-axis view of the LVOT. The transducer is positioned in the 5th or 4th intercostal space at a level slightly above the olecranon and angled slightly cranially. D, Left-parasternal long-axis view of the RVOT. Starting from the LVOT view (C), the transducer is moved one intercostal space more cranially to obtain this image. *LA,* Left atrium; *LV,* left ventricle. (From Schwarzwald CC: Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, et al.: *Atlas of equine ultrasonography,* Wiley, Oxford, UK, 2014. Reproduced with permission of John Wiley & Sons, Ltd.)

quantification of stress echocardiograms.[168] However, clinical applications and the added value over traditional echocardiographic methods need further definition.

Assessment of Chamber Dimensions. One of the main goals of echocardiography is the detection of cardiac chamber dilation or hypertrophy and the grading of the enlargement (if present) as mild, moderate, or severe. Chamber dimensions provide important information on hemodynamics, chamber remodeling, and severity of cardiac disease. Assessment of left atrial (LA) and left ventricular (LV) dimensions should be based on an integrative approach combining subjective evaluation and assessment of multiple measurements based on 2D and M-mode echocardiography (see Figs. 9.20–9.23).*

Left-ventricular internal dimensions and LV wall thickness, respectively, are usually measured from a right-parasternal short-axis view or from a right-parasternal four-chamber view using 2DE or M-mode recordings (see Fig. 9.20).[344] Linear measurements of the LV minor dimension may not well describe true LV size and geometry, as asymmetric LV dilation and dimensional changes along the major axis of the ventricle are neglected. Similarly, linear measurement of wall thickness may not be accurate estimates of LV mass, particularly asymmetric thickening is present or if the M-mode cursor crosses a papillary muscle or fails to sample the thickest part of the wall. Furthermore, standardized placement of the M-mode cursor can be challenging and is difficult to verify in all three dimensions. This limitation may be partially overcome by use of anatomic M-mode technology, which allows free orientation of the cursor line within the imaging plane independent of the apex of the imaging sector (see Fig. 9.20D).[345] Measures that consider the short-axis area (instead of diameter) and the length of the LV (i.e., various

* References 168, 287, 289, 344, 345, 576, 591, 595.

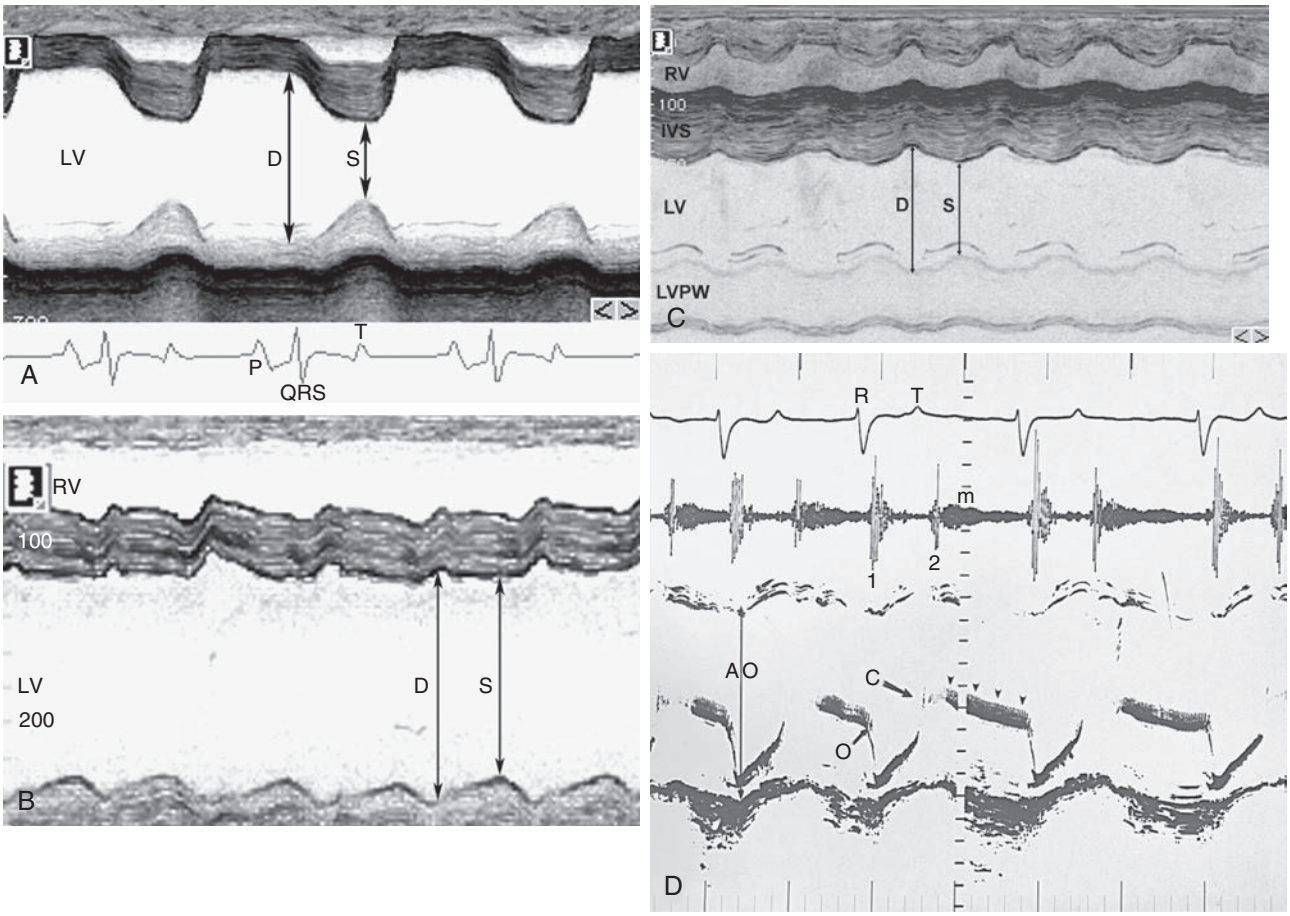


FIG. **9.23** M-mode echocardiography. A, M-mode tracing demonstrating a hyperdynamic left ventricle *(LV)* in a horse with acute mitral regurgitation (MR). The significant change in LV dimensions from diastole *(D)* to systole *(S)* is notable and is typical of volume overloading with preserved ventricular function. Furthermore, the reduced resistance to ejection of blood into the left atrium (LA) enhances ventricular shortening. Depth calibration in millimeters is shown on the left. B, Recording through the ventricles demonstrating decreased systolic function with a reduced LV shortening fraction. This pattern of contraction can be caused by myocarditis, myocardial injury (e.g., monensin), idiopathic dilated cardiomyopathy, chronic volume overload, protracted ventricular tachycardia (VT), or administration of negative inotropic drugs. Depth calibration in millimeters is shown on the left (*RV,* right ventricle). C, M-mode echocardiogram across the ventricles recorded from a horse with cardiac amyloidosis. Global LV systolic function is mildly reduced. The IVS and LVPW are thickened. The IVS also appears hyperechoic relative to the RV wall. The LVPW in the far field is less echogenic owing to attenuation of echoes in the far field. The right ventricle (RV) contained spontaneous echocontrast. Depth calibration in millimeters is shown on the left (*RV,* right ventricle; *IVS,* interventricular septum; *LV,* left ventricle; *LVPW,* left ventricular peripheral wall). D, Recording from a horse with aortic regurgitation (AR) and atrial fibrillation (AF), demonstrating fine diastolic fluttering of an aortic valve leaflet *(small arrows).* The aortic root *(AO),* valve opening *(O),* and valve closing *(C)* are indicated. The murmur *(m)* of AR is evident in the previous phonocardiogram. First *(1)* and second *(2)* heart sounds are labeled. Diastolic fluttering of the mitral valve (most common), aortic valve, ventricular septum, or walls of the aortic root may be observed in horses with this hemodynamic abnormality. The M-mode sampling rate (approximately 1000 pulses/sec) is ideal for detecting these high-frequency events.

area-length models and the Simpson's method of disks) may be more accurate estimates of internal LV dimensions and LV mass (see Fig. 9.20A and B).[168,297,306,344] The accuracy of these methods, on the other hand, is limited by the fact that true LV long axis may be difficult to measure by 2DE because of the LV shape and the translational motion of the LV during contraction and relaxation. Furthermore, all conventional volumetric indices are calculated based on several geometric assumptions and approximations and may therefore be prone to error. Nonetheless, in addition to conventional linear measurements one should consider using area measurements and volume estimates for quantification of chamber dimensions, particularly in cases where the subjective evaluation and conventional measurements provide equivocal results.

Assessment of LA size has traditionally been limited to subjective evaluation and measurement of the LA diameter from a left-parasternal long-axis view (see Fig. 9.21D). However, this view often does not allow imaging of the LA in its entirety due

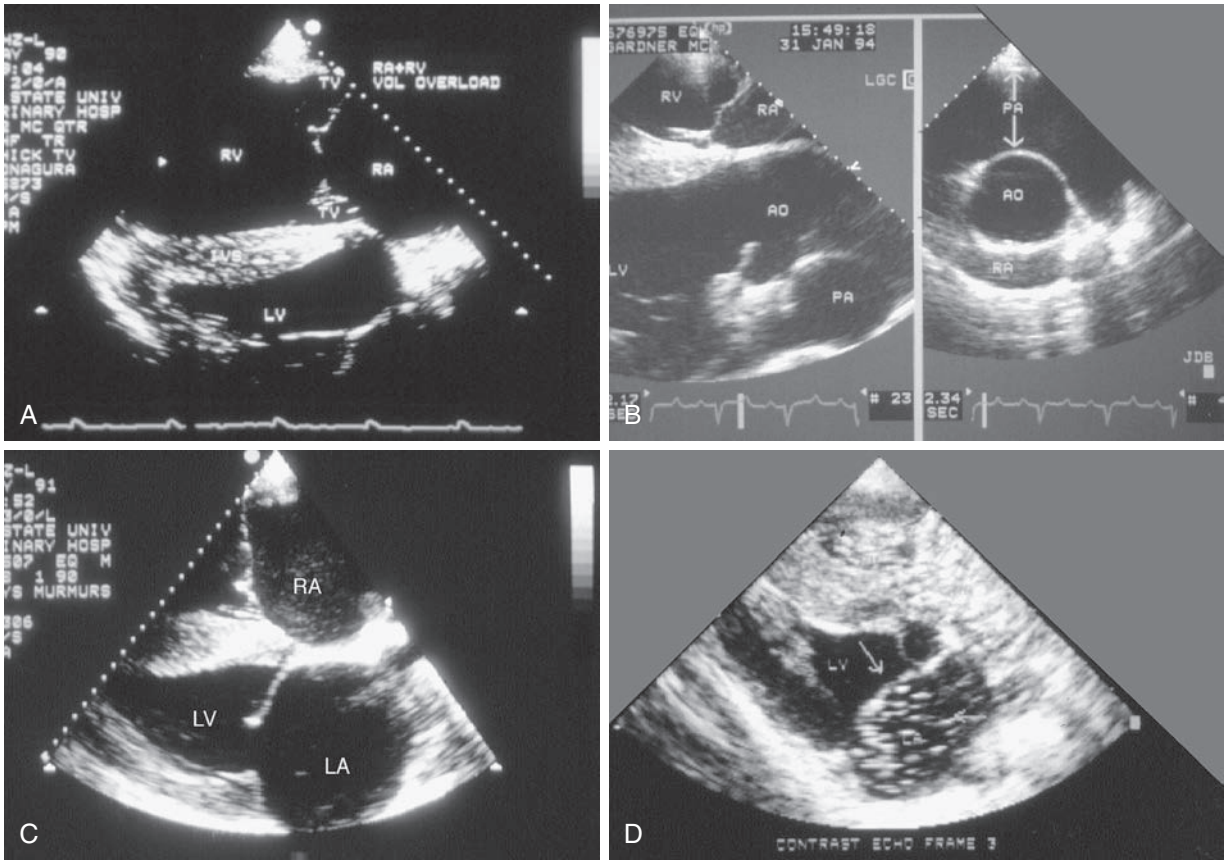


FIG. **9.24** Two-dimensional (2D) echocardiograms. A, Long-axis image from the right thorax in a 12-year-old Quarter Horse gelding demonstrating significant right ventricular (RV) and right atrial (RA) dilation after tricuspid regurgitation (TR) and elevated pulmonary artery pressure. In this case a tumor that obstructed flow in the main pulmonary artery caused the pulmonary hypertension. The ventricular septum is flat and bulges slightly into the left ventricle (LV). B, Images of the ascending aorta and pulmonary artery obtained from the right hemithorax *(left panel)* and left cranial hemithorax. An evaluation of the pulmonary artery diameter relative to the aorta is useful when identifying pulmonary hypertension. C, Biatrial dilation in a Thoroughbred colt with mitral regurgitation (MR), pulmonary hypertension, atrial fibrillation (AF), and congestive heart failure (CHF). Both atria are rounded and appear turgid. The cause of mitral disease in this case was idiopathic lymphocytic-plasmacytic mitral valvulitis (*RA,* right atrium; *LV,* left ventricle; *LA,* left atrium). D, Contrast echocardiogram demonstrating right-to-left shunting at the level of the foramen ovale in a foal with severe respiratory disease. The saline generates echocontrast, opacifies the right atrium (RA) and right ventricle (RV), and visibly fills the left atrium (LA) *(arrows),* although the LV has not yet been opacified. This technique is easy and practical for demonstrating right-to-left shunts across the cardiac septa.

to interference with the ventral lung border; this often results in measurements of the LA diameter that are not parallel to the mitral valve annulus or that are made too close to the annulus and thereby underestimate the true maximum atrial diameter. Also, the timing of LA measurements is often not specified in the equine literature, leading to uncertainties when comparing results from different studies. With contemporary echocardiographic systems, right-parasternal long-axis and short-axis views (Fig. 9.21A–C) are preferred because they allow visualization of the LA in its entirety and provide sufficient anatomic landmarks for consistent and reliable measurement of LA dimensions.[287,289,576] In addition to LA diameter, assessment of LA area should be considered because enlargement may not occur in a uniform fashion and LA geometry may change over time. The LA size is best measured at the end of the ventricular systole, one frame before mitral valve opening, when the LA chamber is at its greatest dimension.

Objective quantification of the right atrial (RA) and right ventricular (RV) dimensions is difficult, because the geometric shape of the right heart is complex and the internal dimensions of the RA and the RV cavity largely depend on transducer placement and imaging plane. Subjective assessment of the right heart chambers in multiple image planes is therefore important.

Assessment of Systolic Left Ventricular Function. The echocardiographic detection of myocardial failure and deterioration of systolic function has great prognostic implications. Most of the indices used in clinical practice serve to evaluate global systolic LV function. It is important to realize that the echocardiographic indices of systolic ventricular function generally do not reflect contractility per se but are (to a variable extent) influenced by preload, afterload, HR, and heart rhythm (see Fig. 9.4).[344 595] Generally, it is advised to use a variety of echocardiographic variables to assess LV systolic function and to interpret them in the context of the clinical findings.

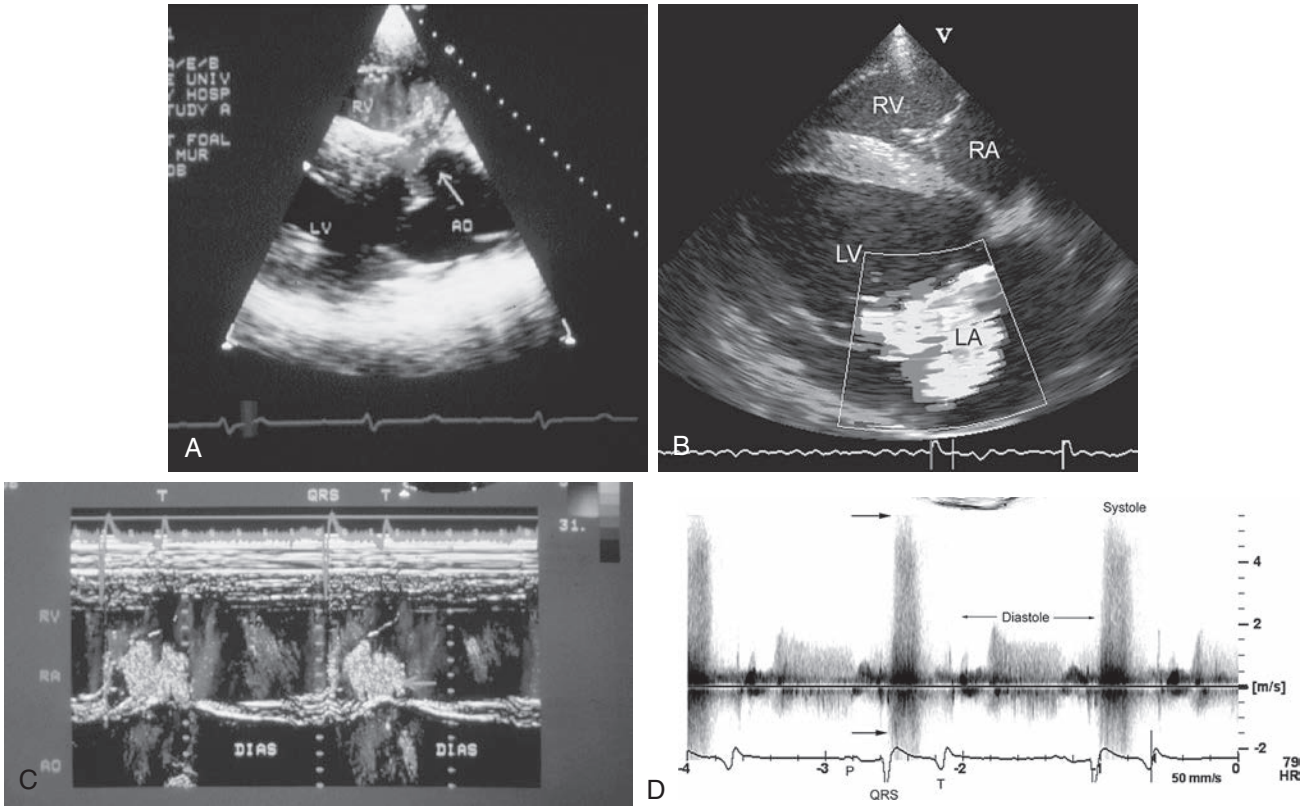


FIG. **9.25** A–C, Black-and-white representations of color Doppler echocardiograms demonstrating mapping of blood through the heart. A, Perimembranous ventricular septal defect (VSD) in a foal *(arrow).* The flow pattern was coded red and aliased as the velocity increased across the defect. B, Wide spray of mitral regurgitation (MR) with flow moving from left ventricle *(LV)* into the left atrium *(LA)* in a horse with mitral valve prolapse (*RV,* right ventricle; *RA,* right atrium). C, Color M-mode study from a horse with tricuspid regurgitation (TR). The bright systolic flow pattern, following the QRS of the electrocardiogram (ECG), was mapped as turbulence in the color study. This is an excellent method for timing abnormal flow events. D, Example of continuous-wave *(CW)* spectral Doppler echocardiogram obtained from a horse with a VSD and mild aortic regurgitation (AR). Time and the ECG waveforms are shown along the x-axis and velocity of flow along the y-axis with a meters per second scale (m/sec). Direction of flow relative to the transducer is also shown; flow toward the transducer is displayed as a positive signal (relative to the zero velocity baseline) and away from the transducer as a negative signal. A low-velocity flow pattern is evident in diastole related to diastolic shunting across the VSD, aortic regurgitant flow moving across the cursor line 'off angle' (with parallel alignment, the AR velocity would be higher [i.e., >3.75 m/sec]), or contaminated transtricuspid flow. In systole a high positive velocity signal is recorded (>5 m/sec) compatible with a small (restrictive) left-to-right shunting VSD. However, the maximum velocity is not evident because the signal reaches the upper limit of the velocity scale *(arrow).* The negative signal *(lower arrow)* is either an aliased signal (if antialiasing filters are off) or VSD turbulence in the other direction. Moving the baseline down or increasing the velocity scale would have allowed for faithful recording of the peak velocity.

The 2D ejection phase indices are based on measurements of LV dimensions. Left ventricular ejection fraction (LV EF = SV/LVEDV × 100, where SV is stroke volume and LVEDV is LV end-diastolic volume) is based on geometric estimates of LV volumes and has traditionally been the standard index of LV systolic function. The LV fractional shortening (LV FS = [LVIDd − LVIDs]/LVIDd × 100; where LVIDd and LVIDs are the LV internal short-axis diameters at end-diastole and at peak systole, respectively), is an approximation of the LV EF and is the most commonly used index of LV systolic function in horses. In fact, often it is the only one used in routine echocardiography, because it can be easily calculated from M-mode recordings (see Fig. 9.23).[306,344,595] However, reliance on this index as a single measurement of LV function may be problematic.[168] The LV FS actually represents the relative shortening of the LV short axis in only one single dimension, disregarding the fact that the LV contracts in all three dimensions. Also, it may lack accuracy when the IVS and the LVFW do not contract synchronously or when the cursor line is not placed optimally. In horses with mitral regurgitation and normal myocardial function, the LV FS can actually be increased due to the increased preload and decreased afterload. Conversely, a normal LV FS in the presence of severe MR may indicate (but does not necessarily prove) myocardial failure.

Area-based measurements such as the LV fractional area change (LV FAC) may be less sensitive to asynchronous wall

motion and allow assessment of shortening in two dimensions. Volume-based measurements (LV EF, stroke volume, cardiac output) can be calculated based on estimates of LV volume as described earlier.[168] They are generally considered more accurate and less affected by altered chamber geometry. However, there are differences and limitations related to the geometric model used.[297,306,344,595] As stated, area- and volume-based measurements of LV systolic function should be considered in cases where the subjective evaluation and conventional measurements give equivocal results.

Doppler-based estimates of stroke volume (SV) and cardiac output (CO) are also indices of global ventricular function. The SV is calculated by multiplying the velocity-time integral (VTI) of the aortic or pulmonic flow signal by the cross-sectional area of the respective vessel.[297,306,307,311,313,344] There are many limitations of this echocardiographic estimate of SV, including the angle-dependency of Doppler-based flow measurements, inaccuracies in the determination of the flow area, and uncertainties as where exactly to measure flow and cross-sectional area (or diameter) of the vessel.[311] In adult horses, adequate alignment of the Doppler beam with blood flow by transthoracic echocardiography is difficult, and Doppler estimates of SV are considered neither very accurate nor reliable. Transesophageal echocardiography (TEE) allows parallel alignment between Doppler beam and aortic blood flow in adult horses, and CO measurements by TEE in adult, anesthetized horses have been shown to be in better agreement with thermodilution CO than CO measurements by transthoracic echocardiography.[309,311,312] In foals, alignment of the ultrasound beam with blood flow can be achieved using apical imaging planes. Although this may improve the accuracy of CO measurements by Doppler echocardiography, volumetric 2DE measurements using the bullet method have been shown to provide better agreement with CO by lithium dilution than Doppler estimates of CO.[306] Generally, measurements of CO by echocardiography may be more accurate for detecting acute changes in CO over time than for obtaining absolute measurements of CO. Assessment of SV and CO by Doppler is not routinely performed in horses with cardiac disease. In fact, myocardial dysfunction generally must be severe for blood flow indices to be affected, because cardiac output is maintained in the failing heart until the compensatory mechanisms are overwhelmed. Therefore estimates of SV and CO may be of limited relevance in most horses with heart disease.

Systolic time intervals (STIs), including LV preejection period (PEP), LV ejection time (LVET), and LV preejection period-to-ejection time ratio (PEP/LVET), can be measured from M-mode images of aortic valve motion and from Doppler tracings of aortic blood flow, respectively.[344,605] The STIs may serve as alternative indicators of LV function that are independent of ventricular shape and geometry and that may be superior to the calculation of the LV FS. However, they are also variably influenced by HR and loading conditions.[344] Accurate time intervals may be difficult to obtain due to inability to clearly identify the onset and the end of ejection on M-mode or Doppler tracings. This limitation may be overcome by use of tissue Doppler imaging for measurement of STIs.[164,286] The clinical value of STIs has not been well established in horses with cardiovascular disease.

Assessment of Diastolic Function. Diastolic ventricular function is more complex than systolic function. Ventricular filling results from the dynamic interplay of active ventricular relaxation, ventricular compliance, filling pressures, pericardial restraint, ventricular interaction, and atrial function (see earlier discussion). Afterload and contractility will further influence diastolic function. Therefore the echocardiographic assessment of diastolic ventricular function and filling pressures (which are commonly assessed together) is difficult. In horses, diastolic ventricular dysfunction may certainly play a role in pericardial and myocardial disease,[161,164,366] but the clinical relevance of diastolic dysfunction in other types of cardiac diseases is not clear, and the prevalence of diastolic heart failure is unknown.

Doppler-derived transmitral flow velocities (E wave, A wave, E/A ratio, E-wave deceleration time and deceleration slope) are commonly used in humans and small animals for assessment of diastolic LV function and filling pressures. However, the usefulness of transmitral flow velocities is limited, because impaired relaxation coexisting with increased filling pressures may lead to "pseudonormalization" of filling patterns.[594] Furthermore, PW Doppler recordings of transmitral flow velocities strongly depend on alignment with blood flow and are sensitive to placement of the sample volume relative to the position of the mitral valve. Transmitral flow velocity profiles can be recorded in horses, but optimal alignment with blood flow from an apical window is not possible in adults. Transmitral flow velocity measurements in horses are relatively unreliable and may not be suitable to detect minor changes in diastolic LV function.[281,287,289] Nonetheless, if marked decrease or reversion of the E/A ratio is present, diastolic dysfunction must be suspected.[164] Tissue Doppler imaging may provide additional velocity-based and time interval–based indices that are easier to obtain and that can be helpful for assessment of LV diastolic function in horses.[161,164,286,366]

Assessment of Left Atrial Function. LA function is rarely considered during routine echocardiography, and the clinical relevance of LA dysfunction is not well known in horses. However, LA function is impaired in horses with atrial fibrillation (AF), and persistent LA contractile dysfunction can be detected in horses after conversion from AF to sinus rhythm, likely attributed to AF-induced atrial remodeling.[289-291] LA size and LA mechanical function can easily be assessed in horses by use of 2DE variables, including LA area and LA fractional area changes (see Fig. 9.21).[287,289-291]Additional studies will be required to determine the clinical relevance of LA mechanical dysfunction in the presence of cardiac disease in horses.[576]

Assessment of Valvular Regurgitation. The Doppler technology of current echocardiographic systems is very sensitive to detect valvular regurgitation, and care must be taken not to overinterpret the echo findings, particularly in otherwise healthy animals without abnormal clinical findings and in the absence of heart murmurs. Assessment of valvular regurgitation should be achieved using an integrated qualitative and quantitative approach, combining clinical examination (including auscultation of a typical murmur) and echocardiographic findings. Measurement of cardiac chamber dimensions provides information on the hemodynamic relevance of chronic valvular regurgitation. Abnormal timing and direction of transvalvular flow as well as flow turbulences can be detected by 2D color Doppler, color M-mode, and spectral Doppler echocardiography. The regurgitant signal in the "receiving chamber" can be interrogated in multiple imaging planes to identify origin, extent, timing, and duration of the regurgitation.[74] However, it is important to realize that

color Doppler echocardiography only describes blood flow direction and velocity but not absolute volumetric flow. The Doppler-derived regurgitant signal is largely influenced by gain settings, direction of flow, orifice size and shape, driving pressure, and characteristics of the receiving chamber. Quantification of regurgitation by assessing signal strength of the spectral Doppler regurgitant signal or measuring the area of regurgitation within the receiving chamber is therefore neither very accurate nor reliable.

Hemodynamic Assessment. The hemodynamic load placed on the heart by cardiac disorders can be estimated by combining echocardiographic information on chamber size, wall thickness, myocardial motion, LV systolic and diastolic function, and intracardiac blood flow. Doppler studies can be used to assess intracardiac pressures and pressure gradients. Normal pressure gradients driving blood flow through heart, valves, and large vessels range from 0.25 to 1.5 m/sec. Abnormally high velocities can be found in many cardiac conditions, including ventricular septal defects and valvular regurgitations, in which pressure gradients drive blood across a restrictive orifice. The pressure gradients are either reflections of normal intracardiac pressures or consequences of pathologically increased pressures. Pressure gradients can be estimated using Doppler echocardiography by employing the simplified Bernoulli equation ($dp = 4 \times v_{max}^2$, where dp is the pressure gradient in mm Hg and v_{max} is the peak velocity in m/sec). The main indications to measure pressure gradients in horses are the assessment of ventricular septal defects (by interrogation of shunt flow) and the diagnosis of pulmonary hypertension (by interrogation of regurgitant flow at the tricuspid valve and the pulmonic valve).

If, for example, a peak velocity of 4.8 m/sec is recorded across a ventricular septal defect, and if the systolic systemic arterial BP is determined noninvasively to be 125 mm Hg, the estimated RV systolic pressure would be calculated as follows:

- Pressure drop across defect = $4 \times 4.8^2 = 92$ mm Hg
- Estimated LV systolic pressure = 125 mm Hg
- Estimated RV systolic pressure = 125 − 92 = 33 mm Hg

These findings indicate a restrictive septal defect, a lesion unlikely to cause difficulties for the horse except at highest levels of performance.

A similar quantitative approach is used to estimate the presence or absence of pulmonary hypertension when tricuspid regurgitation is identified in the absence of right ventricular outflow tract obstruction (i.e., PA ejection velocity of less than 1.5 m/sec). Assuming the jet can be interrogated at nearly parallel to flow, peak regurgitant velocities exceeding 3.2 to 3.4 m/sec are suggestive of elevated PA systolic pressure. For this calculation, peak right atrial pressure must be estimated (around 10 mm Hg in horses without CHF). For example, a peak regurgitant velocity of 3.8 m/sec would results in calculated pressure drop from RV to RA of $4 \times 3.8^2 = 58$ mm Hg. When the RA pressure estimate (10 mm Hg) is added, estimated PA systolic pressure is 68 mm Hg. In cases of right-sided CHF, the RA pressure can be assumed to be at least 20 mm Hg or more accurately measured by a catheter.

Intravascular Pressures and Cardiac Catheterization

There is a large body of literature derived from catheterization studies of the healthy standing, exercising, and anesthetized horse and pony along with limited catheterization data derived from horses with heart disease.* Normal published data relate in part to population and study methods; accordingly, reference intervals vary considerably.[57] Normal values also depend on the head and body positions of the horse, the influence of administered tranquilizers, sedatives, or anesthetic agents, and the size of the animal. Hemodynamic variables that can be measured or calculated by catheterization techniques include systolic, diastolic, and mean BP in the systemic and pulmonary circulations, PA occlusion (or pulmonary capillary wedge) pressure, central venous pressure (in the venae cavae, near the right atrium), intracardiac (atrial and ventricular) pressures, CO, systemic and pulmonary vascular resistances, and arteriovenous oxygen difference.[633]

Cardiac catheterization in the clinical setting has largely been replaced by Doppler echocardiography. However, indications for cardiac catheterization persist, especially for research purposes or in clinical practice, when accurate measurement of PA pressure is needed, the origin of pulmonary hypertension cannot be determined, or the diagnosis of occult constrictive pericardial disease is entertained. Knowledge of the general principles of hemodynamics and catheterization data in health and disease creates a useful framework for understanding clinical assessment of the CV system.

Pressures on the left side of the circulation include systemic arterial, left ventricular, and left atrial pressures. Measurement of systemic arterial BP has been described earlier in this chapter. Left ventricular pressures can be measured by percutaneous retrograde catheterization of the LV via the carotid artery, aorta, and aortic valve.[47,259,449,634,635] Carotid catheterization can be facilitated using ultrasound guidance. For research purposes, subcutaneous translocation of the carotid artery has been described to allow for repeated catheter procedures.[636] Left ventricular pressures are typically measured using microtip pressure-sensing catheters to avoid artifacts that commonly arise when using fluid-filled catheter systems.[47,259,449,635] Left atrial pressure is rarely measured directly but can be estimated during right-sided heart catheterization by a pulmonary capillary wedge pressure as described later.

Systemic arterial pressure is related in a directly positive manner to left ventricular systolic function, impedance to blood flow in the aorta, systemic vascular resistance, and HR. Systolic pressures in the aorta and left ventricle in the standing horse generally peak at approximately 110 to 130 mm Hg (with individual variation).[57,259] Diastolic aortic pressure is usually near 75 mm Hg. An increase in HR as small as 10 beats per minute can increase systemic BP by 20 mm Hg or more over these values. A peak systolic pressure gradient between the LV and central aorta indicates an obstruction to left ventricular outflow, a very rare condition in horses. Left ventricular diastolic pressure reflects diastolic ventricular function and filling pressures as well as ventricular emptying during systole. The diastolic pressure most often reported is the LV end-diastolic pressure (LVEDP), which is higher than the early (often subatmospheric) minimal diastolic LV pressure. The LVEDP typically ranges between 12 and 24 mm Hg in standing horses and ponies and is higher than the LVEDP of either people or dogs.[21,24,57,259,449,615] Exercise increases both left atrial and ventricular end-diastolic pressures.[53,283,284,370,608,610,637] Pathologic elevation of resting LVEDP indicates either reduced myocardial contractility, ventricular failure, LV volume overload

*References 42, 46, 47, 248, 259, 298, 304, 308, 309, 311-314, 317, 318, 321, 352, 354, 355, 367-370, 409, 410, 414, 418, 419, 428, 561, 606-632.

(large VSD, mitral regurgitation, or aortic regurgitation), myocardial infiltration (lymphoma, amyloid), pericardial constraint, or increased ventricular wall stiffness.

Detailed computer-based analysis of a LV pressure tracing allows derivation of several indices of LV systolic and diastolic function. The maximum rate (i.e., the first derivative) of positive pressure change during isovolumetric contraction ($+dp/dt_{max}$) is a classical invasive index of ventricular systolic function. Similarly, the maximum rate of negative pressure change during the isovolumetric relaxation phase ($-dp/dt_{max}$) and the time constant of isovolumetric relaxation (τ) are considered the most reliable and least load-dependent indices of ventricular relaxation.[57,259,633,638] Although these indices are not routinely obtained in clinical cases, they are often used for research purposes.

A right-sided heart catheterization is easily performed in most horses using a percutaneous technique involving local anesthesia, placement of an 8-Fr introducer sheath into the jugular vein, and insertion of a 7-Fr balloon-tipped (Swan-Ganz) catheter of 110 to 120 cm in length through the jugular vein and the cranial vena cava into the RA, RV, and PA. Longer catheters are required to consistently measure pulmonary capillary wedge pressures in large-sized horses, but these have to be custom made for use in horses and are not commercially available on the market. Pressures within the RA, RV, and PA can be obtained by advancing the catheter slowly from the jugular vein into a lobar branch of the PA. This is typically performed in standing horses and guided by pressure measurements and sometimes with 2D ultrasound imaging. Catheter placement in the RV and the PA is considerably more difficult in recumbent (e.g., anesthetized) horses, and if intracardiac pressures are to be measured during anesthesia, it is advised to place all catheters before induction of anesthesia. Once the catheter tip is located in the PA, it may be possible to inflate the balloon tip briefly to occlude PA flow and "wedge" the distal catheter tip. This pulmonary capillary wedge pressure (PCWP, sometimes also referred to as pulmonary artery occlusion pressure) can be used to estimate LV filling pressures that are referred into the pulmonary veins and left atrium.[355,356,409,633,637,639]

Mean RA and central venous pressures estimate the pressures filling the RV and are influenced by plasma volume, venomotor tone, body position, and heart function. Central venous pressure is typically about 5 to 10 mm Hg but increases significantly in recumbent horses, especially during general anesthesia.[57,259] Values frequently double from the standing preanesthetic measurement, and central venous pressure determinations of 20 mm Hg are not uncommon.[612,640] A single measurement of the central venous pressure or of pressure in the RA is difficult to interpret unless the value is severely elevated. Trends are most important in assessment of plasma volume status and cardiac function. Markedly elevated RA pressures are observed with cardiac tamponade, constrictive pericardial disease (along with an abrupt "y" descent; see Fig. 9.4A), and in right-sided CHF. The "x" descent of the RA pressure waveform may be replaced by a positive "c-v" wave in the setting of severe tricuspid regurgitation; this pressure wave corresponds to a prominent jugular pulsation observed during inspection of the neck.

The peak RV systolic pressure is lower than that of the left ventricle and is usually around 40 mm Hg (to up to 60 mm Hg) in standing horses.[52,57,641] A small gradient (usually 10–15 mm Hg) may be measured between the ventricular apex and proximal pulmonary artery during systole in normal animals and relates in part to gravitational influences. The RV end-diastolic pressure is usually between 10 and 14 mm Hg, but values as high as 20 to 28 mm Hg have been reported.[52,57,484,585,642] Hydrostatic effects can affect the RA and the RV end-diastolic pressure if the horse's head is raised or lowered.[427] As with the left ventricle, depression of contractility reduces the rate of systolic pressure development ($+dp/dt_{max}$).[352] Elevated RV systolic pressure is recorded in pulmonary hypertension from any cause, with a large VSD, and with RV outflow obstruction as with pulmonic stenosis, tetralogy of Fallot, or obstructive pulmonary valve vegetation.[200] Pathologic elevations in RV diastolic pressure are encountered with pericardial disease, pulmonary hypertension, severe right-sided valvular disease, and CHF (Fig. 9.26).

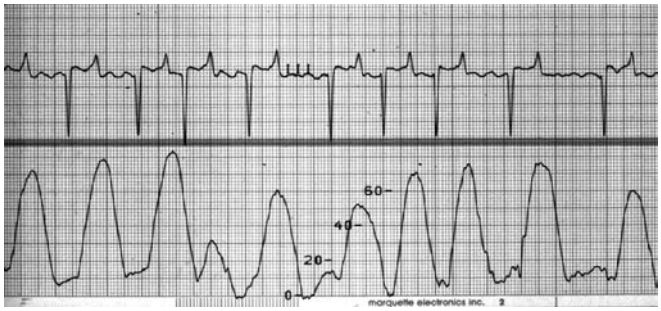


FIG. **9.26** Recording of intravascular pressure during cardiac catheterization. Right ventricular (RV) pressure recordings from a Thoroughbred yearling with atrial fibrillation (AF), pulmonary hypertension, and biventricular congestive heart failure (CHF). The pressure waveforms vary because of ventilation and the arrhythmia. Peak pressures exceed 70 mm Hg. In the absence of pulmonic stenosis or a large ventricular septal defect (VSD), this indicates pulmonary hypertension. The ventricular end diastolic pressure also is elevated and is compatible with heart failure.

Pulmonary artery pressures in standing mature horses are considerably lower than values recorded from the aorta because of the lower resistance encountered in the pulmonary vascular tree. The PA pressure is higher in the newborn foal and decreases significantly during the first 2 weeks of life as pulmonary arteriolar resistance falls.[409] Systolic PA pressures in healthy horses and ponies are approximately 35 to 45 mm Hg (but can be higher or lower depending on CO). Mean PA pressures range around 25 to 30 mm Hg, and diastolic PA pressures are approximately 20 to 25 mm Hg, but again showing some variation.[52,57,259] Pulmonary vascular pressures increase dramatically with increased CO as encountered during sinus tachycardia, and mean PAP can reach (or even exceed) 80 to 90 mm Hg during high-intensity exercise.* The pressure that is developed in the PA depends not only on CO and pulmonary arteriolar resistance but (unlike the systemic circulation) on the pulmonary capillary resistance and on the compliance and pressure in the left atrium. Pulmonary disease can influence these variables; in addition to structural vascular and parenchymal changes,[645] alveolar hypoxia and acidosis can induce reactive vasoconstriction, raising PA pressures.[646] Left ventricular function also directly influences PA pressures because elevation of LA and pulmonary venous pressure places a direct burden on the PA and right ventricle.[449] Left ventricular failure generally

* References 53, 414, 448, 608, 610, 637, 643, 644.

leads to secondary pulmonary hypertension that can be very severe. In other cases, the cause of pulmonary hypertension may not be evident.[647] Accordingly, elevated PA pressures must be assessed in light of HR, CO, and pulmonary capillary wedge, LA, or LV end-diastolic pressure.[352,353] When PA pressure is increased, RV systolic pressure is also elevated to meet the pressure load. If pulmonary hypertension is chronic, right sided cardiac enlargement, tricuspid regurgitation, and right-sided heart failure can ensue. Further details on pulmonary hypertension can be found later in this chapter.

The origin of pulmonary hypertension generally can be determined by conducting a thorough cardiorespiratory examination that includes echocardiographic and Doppler assessment of the left and right sides of the heart. However, catheterization of the PA can be instructive in confusing cases, especially if left-sided heart failure cannot be excluded. The PCWP estimates LV filling pressure and should approximate LVEDP, as long as there are no obstructions in the pulmonary veins or across the mitral valve. The PA diastolic pressure also can estimate PCWP, provided that HR is normal and pulmonary vascular resistance is not increased. Increases in PCWP (or pulmonary diastolic pressure) are measured during exercise as left atrial pressure increases,[283,356,637] in left-sided CHF,[353,639] with moderate to severe MR,[354,355] and in some horses with AF.[357] Overinfusion of crystalloid solutions or marked depression of LV function are other causes of elevated PCWP; conversely, the wedge pressure is reduced in hypovolemia. When pulmonary hypertension is not caused by left heart failure, a near-normal wedge pressure is recorded in the setting of elevated PA diastolic pressure. This indicates increased vascular resistance across the small arteries due to pulmonary vasoconstriction or pulmonary vascular lesions. One caveat is that a pressure gradient between the PA diastolic and PCWP may be observed in normal horses in the setting of resting tachycardia.

Cardiac output (CO) is the volume of blood pumped by the left (or right) ventricle in 1 minute. This can be divided by body weight or body surface area to calculate the cardiac index. The CO can be measured by thermodilution techniques, lithium dilution, arteriovenous oxygen difference (Fick methodology), 2D echocardiographic and Doppler studies (see earlier discussion), and other methods.[168,276,294-318,409,628] Cardiac output in standing adult average-size (500-kg) horses is around 30 to 40 L/min, with reported values for cardiac index in standing horses or ponies ranging between 60 and 80 mL/min/kg.[57] Measurement of CO is most often done clinically when monitoring the effects of anesthetic agents or during fluid and drug therapy of the circulation in critical care situations wherein CO relates to tissue oxygen delivery.[57] However, invasive methods involving cardiac catheterization are largely limited to research purposes. Traditionally, the thermodilution technique has been the most commonly used method for invasive measurement of cardiac output in horses.* Thereby, a thermistor-tipped Swan-Ganz catheter is placed in the pulmonary artery. A cold indicator solution is injected into the right atrium or vena cava either through the proximal injection port of the Swan-Ganz catheter (in foals) or through a second injection catheter placed through the jugular vein into the RA (in larger animals). The indicator solution is usually 5% dextrose or saline cooled to approximately 4°C or, in foals, at room temperature. The resultant change in blood temperature is detected by the thermistor downstream of the heart, and the CO can be calculated from the area under the curve of temperature change over time.

The PA catheter can also be used for sampling of mixed-venous blood. The mixed-venous oxygen content ($C\bar{v}O_2$ in mL oxygen/dL blood), which is determined by hemoglobin concentration (Hb), mixed-venous hemoglobin saturation ($S\bar{v}O_2$), and mixed-venous partial pressure of oxygen ($P\bar{v}O_2$), can be used as an indirect estimate of CO.[57,307,628,630] As CO increases, the tissues extract less oxygen from each aliquot of blood as it passes through the capillaries; consequently, the $C\bar{v}O_2$, $S\bar{v}O_2$, and $P\bar{v}O_2$ increase. As CO decreases, the tissue extraction of oxygen increases, $C\bar{v}O_2$, $S\bar{v}O_2$, and $P\bar{v}O_2$ decrease, and the systemic-to-venous O_2 difference widens.

Systemic and pulmonary vascular resistances strongly influence the mean pressures in their respective vascular systems. Vasoconstriction increases arterial BP. However, resistances cannot be measured directly in the intact animal and are generally calculated using a variation of Poiseuille's or Ohm's laws. The general formula for calculation of static vascular resistance is as follows: Vascular resistance = (Mean arterial pressure − Mean atrial pressure)/CO, where pressure is measured in mm Hg and CO is measured in mL/min. The pressures used are mean aortic pressure and mean right atrial pressure for calculation of systemic vascular resistance and mean PA pressure and mean pulmonary wedge pressure for calculation of pulmonary vascular resistance. The units for vascular resistance are mm Hg × min × mL^{-1}, sometimes abbreviated as peripheral resistance units (PRU). Alternatively, resistance is often expressed in centimeter-gram-second (cgs) units as dynes × sec × cm^{-5}, where 1 mm Hg = 1330 dynes/cm^2 and CO is expressed as cm^3/sec. The resistance value in PRU can be multiplied by 80 for conversion to a corresponding cgs value.[114] It is important to note that SVR can be calculated from pressures and CO, but it is not determined by either of these variables. Physiologically, SVR and CO are the independent variables, and MAP is the dependent variable.[114] Normal systemic vascular resistance in average-size (500-kg) adult horses averages about 265 dynes × sec × cm^{-5}. It is considerably higher in smaller horses, ponies, and foals, because the total length of the circulation and the total cross-sectional area of the resistance vessels, which according to Poiseuille's law determine vascular resistance, are directly related to body size.[57,259] This explains the fact that systemic blood pressures are largely independent of body size despite the differences in CO between larger and smaller individuals. Pulmonary vascular resistance should be about one fifth that of the systemic value, but reported values vary more widely than that.[57] Mechanisms that increase systemic vascular resistance include sympathetic activation, activation of the renin-angiotensin system, and the release of other vasoactive hormones into the blood, including arginine vasopressin (antidiuretic hormone) and epinephrine. The pulmonary vascular resistance is tied to pulmonary vascular anatomy, age, total lung capillary resistance, left atrial pressure, and degree of pulmonary vascular constriction. The latter is controlled by the tension of alveolar oxygen and local mediators, including nitric oxide and endothelin.[57,110,112-117,255-257]

Laboratory Studies

Cardiovascular disorders may develop as a consequence of systemic or metabolic diseases such as electrolyte disturbances or septicemia. Conversely, CV infections, myocardial damage, or

* References 259, 296, 301, 304, 307-309, 311, 312, 314, 317, 648.

circulatory failure may alter routine laboratory tests. Therefore laboratory studies might be indicated during the workup of horses with CV disorders. An overview of the laboratory studies useful in assessment and management of CV diseases is found in Box 9.4.

A complete blood count, measurement of SAA and fibrinogen concentrations, assessment of a biochemical profile, and urinalysis are indicated in horses with arrhythmias, heart failure, or when there is clinical evidence or suspicion of endocarditis, pericarditis, vasculitis, myocardial disease or pleural effusion. Prerenal azotemia and electrolyte disturbances (hyponatremia, hypokalemia, hypomagnesemia) may be detected in the horse with CHF, especially following diuretic therapy. Additional studies including arterial and venous blood gas tensions and oxygen saturation, blood lactate concentrations, blood cultures, and cytology and culture of pericardial effusates are indicated in selected cases. Monitoring of serum or plasma drug concentrations, especially quinidine and digoxin, are appropriate when these drugs are administered.

Cardiac troponins are sensitive and tissue-selective diagnostic biomarkers of myocardial damage in mammals.[649,650] Troponins may be released in the circulation from primary or secondary cardiac muscle injury, as might occur with myocardial infarction, myocarditis, cardiomyopathy, toxic or nutritional myocardial injury, mechanical or electrical myocardial damage, hypotension, ischemia, endothelial injury, DIC, endotoxemia, and septicemia.[161-164,575,651-668] High-intensity or prolonged exercise also induces a small rise in plasma cTnI but does not necessarily indicate underlying structural heart disease and can be observed in healthy horses.[355,484,669-673] Both cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are useful for detection of myocardial injury in horses[674-677] and have replaced the less specific LDH and CK-MB isoenzymes in clinical practice. Troponin concentrations can be obtained from most commercial laboratories or using handheld point-of-care analyzers.[650,656] Normal plasma cardiac troponin concentrations vary depending on assay and laboratory but are often near or below the limit of detection of the respective assay.[656,674,676-679] The average half-life of cTnI in horses is less than 1 hour.[680] Failure to observe a decline in plasma cTnI concentrations over several hours therefore suggests ongoing myocardial damage as opposed to acute, transient myocardial injury.

Natriuretic peptides are released in the blood in response to a variety of stimuli, including myocardial stretch due to volume overload and increases in intracardiac pressures.[110,112-114,116,664,681] Plasma atrial natriuretic peptide (ANP) concentrations increase during exercise,[355,682-687] and they can be elevated in horses with cardiac disease, particularly in the presence of LA enlargement or LA dysfunction.[355,688-690] To date, no assays are available for accurate and reliable measurement of ANP in equine blood in routine clinical practice.[664,691] Therefore the use of ANP as a cardiac biomarker is currently limited to experimental settings, and more work needs to be done to elucidate its clinical value in horses with heart disease.

A variety of other cardiac biomarkers potentially indicating myocyte stress, myocyte injury, neurohormonal activation, myocardial remodeling, inflammation, or oxidative stress are known but are currently not used for routine diagnostic purposes in horses with heart disease.[664] Further application of laboratory studies is discussed under specific diseases later in this chapter.

PULMONARY HYPERTENSION AND COR PULMONALE

Pulmonary hypertension (PHT) is hemodynamically characterized by pulmonary artery pressures exceeding the upper normal limit by more than 10 mm Hg.[692] Current human classifications of pulmonary hypertension include (1) pulmonary arterial hypertension (including idiopathic, drug and toxin induced, and persistent PHT of the newborn); (2) PHT caused by left-sided heart disease (including congenital and acquired heart disease); (3) PHT caused by lung disease and/or hypoxia; (4) chronic thromboembolic PHT; and (5) PHT with unclear multifactorial mechanisms (including hematologic, systemic, metabolic, and other disorders).[693,694]

In horses, clinically relevant PHT most commonly results from severe mitral valve disease, pulmonary overcirculation caused by congenital left-to right shunts (e.g., VSD), or chronic left-sided heart failure. In these cases, PHT can be very severe, with systolic pulmonary artery pressures exceeding 80 mm Hg. Presumably, factors such as reactive vasoconstriction or anatomic changes (structural remodeling) in the pulmonary vascular tree must develop as a consequence of elevated left atrial pressures in order to sustain such high pressures at rest.

Pulmonary hypertension is occasionally diagnosed in foals and adult horses with severe respiratory disease.[695-699] In addition to structural vascular and parenchymal changes,[645] low alveolar oxygen tension is considered a very potent trigger of (reversible) pulmonary vasoconstriction leading to an increase in pulmonary vascular resistance.[700,701] Hypoxia was found to be a contributing factor leading to (relatively mild) pulmonary hypertension in horses with recurrent airway obstruction.[646,697-699] Hypoxic vasoconstriction may also be particularly important in newborn foals, in which vascular resistance is already high.[409]

Persistent pulmonary hypertension of the newborn foal (also called persistent fetal circulation or reversion to fetal circulation) results from failure to successfully make the cardiorespiratory transition to extrauterine life or from reversion to fetal circulatory patterns in response to hypoxia or acidosis.[609,702,703] The fetal circulatory pattern with PHT and right-to-left shunting of blood through the patent foramen ovale (PFO) and the ductus arteriosus are maintained in these cases, and affected foals are in hypoxemic respiratory failure. Factors contributing to persistent PHT in newborns are chronic in utero hypoxia or acidosis, asphyxia, and meconium aspiration, but often the precipitating trigger is unknown.[702]

Pulmonary thromboembolism is rarely diagnosed in horses,[193,704-709] but it is possible that the condition is in fact underrecognized in clinical practice and that its incidence might be higher than supposed.[705] Idiopathic pulmonary hypertension has been reported as a cause of AF in horses; however, left-sided heart dysfunction was not completely excluded in the reported cases.[96]

Exercise-induced PHT, with mean pulmonary artery pressures exceeding 80 to 90 mm Hg, is a unique feature in horses and ponies. It is likely caused by the massive (up to tenfold) increase in pulmonary blood flow, in association with high LV filling pressures (necessary to ensure rapid ventricular filling at maximal HRs), compression of pulmonary vessels during forced expiration and inspiration, greater blood viscosity related to the exercise-induced rise in packed cell volume, and possibly arteriolar vasoconstriction.* Elevated pulmonary

* References 53, 414, 448, 608, 610, 637, 643, 644, 710-716.

vascular pressures during strenuous exercise are thought to be one of the major factors contributing to capillary stress failure and the occurrence of exercise-induced pulmonary hemorrhage.[53,210,644,717]

Pulmonary hypertension increases afterload on the RV and results in elevated end-diastolic and central venous pressures. Acute PHT may result in RV dilation, because there is not enough time for adaptive mechanisms (e.g., RV hypertrophy) to develop. If pulmonary hypertension is chronic, RV hypertrophy will develop over time. Increased RV pressures and alterations in RV geometry often result in tricuspid regurgitation. In severe cases, the interventricular septum bulges into the LV, interfering with filling and thus causing diastolic LV dysfunction. Right-sided or biventricular heart failure can ensue.

Cor pulmonale is characterized by right ventricular enlargement secondary to PHT due to pulmonary parenchymal or vascular disease, in the absence of left ventricular failure, a congenital malformation (e.g., VSD), or an acquired valvular disorder. Diagnosis of cor pulmonale requires evidence of pulmonary hypertension and exclusion of a primary cardiac disease.[57,692] Cor pulmonale is uncommon in horses.[374,695,718-721] Although both acute and chronic airway obstruction can negatively affect right heart and overall cardiac function,[646,696,720,721] there is no compelling evidence at this time to incriminate cor pulmonale as a common cause of clinically important heart disease in horses.

On physical examination, a split-second heart sound with a loud pulmonic component and a right-sided systolic heart murmur consistent with TR may be detected in horses with pulmonary hypertension. In cases of severe PHT, signs of right-sided congestive heart failure including distended jugular veins and peripheral edema will be evident. Pulmonary artery pressures can be estimated echocardiographically by Doppler flow imaging of tricuspid or pulmonic regurgitation velocities. Enlargement of the pulmonary artery (exceeding the aortic root diameter) is considered a fairly specific (but not sensitive) indicator of pulmonary hypertension. Cardiac catheterization allows invasive measurement of pulmonary artery pressures and pulmonary capillary wedge pressures and may aid in the diagnosis of pulmonary hypertension and cor pulmonale. Further details on the diagnostic procedures required to diagnose PHT have been discussed earlier in this chapter.

Horses with PHT are considered unsafe to ride or drive.[68] Treatment of PHT should include alleviation of hypoxia and acidosis to minimize functional hypoxic vasoconstriction. Elimination of the underlying cause may be possible in horses and foals with severe but treatable lung disease. Horses with PHT secondary to severe left heart disease require symptomatic treatment of heart failure (see later discussion). Note that diuretics may be harmful in horses with cor pulmonale because small decreases in preload often worsen clinical signs. Nitric oxide (NO) is a potent, selective vasodilator of the pulmonary circulation, and inhaled NO may have value as a therapeutic agent in foals with PHT.[702,722] The use of other pulmonary vasodilators or of endothelin receptor blockers has not been established for treatment of PHT in horses.

CONGESTIVE HEART FAILURE

Heart failure is a clinical syndrome characterized by cardiac disease leading to systolic dysfunction, diastolic dysfunction,

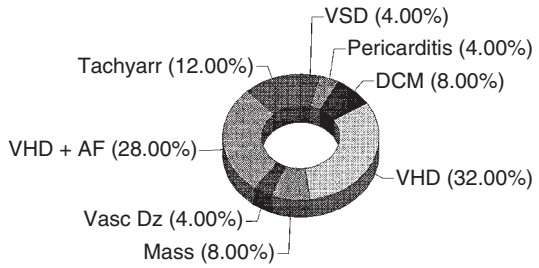


FIG. **9.27** Graph demonstrating the overall causes of congestive heart failure (CHF) in 25 horses at a referral teaching hospital as a percentage of the total number of cases of equine CHF. *Tachyarr,* Tachyarrhythmia; *VSD,* ventricular septal defect; *DCM,* dilated cardiomyopathy; *VHD,* valvular heart disease; *Mass,* mass lesion; *Vasc Dz,* vascular disease; *VHD + AF,* valvular heart disease and atrial fibrillation. Data from Ohio State University Veterinary Hospital.

or a combination of the two, limited CO in the setting of normal to high venous pressures, increased neurohormonal activity, renal sodium retention, accumulation of edema in tissues, and transudation of fluid into serous body cavities. The neurohormonal and renal abnormalities that characterize heart failure have not been extensively studied in horses but are probably similar to those reported in other species and include increase in sympathetic tone, activation of the renin-angiotensin-aldosterone system, and increased release of antidiuretic hormone (vasopressin) and atrial natriuretic peptide.* The most sensitive sign of heart failure is impaired exercise capacity, but this is hardly specific in horses. The most characteristic clinical features of heart failure are tachycardia, increased venous pressures, and fluid accumulation. The typical horse with cardiac failure is recognized in the overtly congested phase termed *congestive heart failure* (CHF) (see Figs. 9.11 and 9.12).

However, most cardiac lesions in horses are insufficient to cause CHF, and this syndrome is not common in equine practice. Nevertheless, CHF does develop in foals and mature horses as a consequence of diverse disorders, including the following: congenital malformation, severe degenerative valvular disease, chordal rupture, valvulitis, bacterial endocarditis, dilated or infiltrative cardiomyopathy, myocarditis, myocardial necrosis, pericardial disease, vascular rupture, pulmonary hypertension, artery obstruction, or persistent tachyarrhythmia.* The most common cause of CHF in horses is valvular heart disease—often complicated by atrial fibrillation, the onset of which can trigger transition from the compensated to the decompensated phase of heart failure (Fig. 9.27). In addition to structural lesions of the heart and blood vessels, primary electrical disturbances, particularly sustained AV dissociation caused by intractable junctional or ventricular tachycardia, can cause tachycardia-induced cardiomyopathy, reduce myocardial function, decrease CO, and lead to CHF.[736] This is especially likely when the HR exceeds 100 beats/min for many days. Resolution of CHF in such cases may be possible if antiarrhythmic therapy and rate control are successful.[153]

* References 57, 110, 112-114, 115, 144, 355, 688-690, 723.
* References 83, 125-127, 144, 148, 152, 192, 204, 212, 213, 262, 265, 352, 374, 546, 563, 724-735.

Clinical Recognition of Heart Failure

Congestive heart failure may develop suddenly or gradually. Peracute heart failure can occur following acute-onset mitral regurgitation due to chordal rupture or papillary muscle ischemia or infarction, consequent to acute endocarditis of a heart valve, or as a result of acute vascular rupture leading to an aortocardiac fistula.[262,265,374] Heart failure may progress rapidly in a foal with a large ventricular septal defect as the pulmonary vascular resistance falls in the weeks following birth.[117,728] Chronic valvular regurgitation can after many years lead to CHF, although only a small number of horses with degenerative valvular disease develop CHF.[583] Some lesions, which might otherwise be well tolerated, can cause heart failure if there are increased demands for CO. Examples include strenuous work, severe anemia, or persistent fever. Pregnancy is another example: the volume expansion and increased demands for CO in the latter stages of gestation may precipitate CHF in a mare with previously compensated heart disease. Development of AF in a horse with underlying structural disease, such as severe mitral or aortic regurgitation, can precipitate CHF. Such cases are distinguished from the more typical case of AF by the presence of persistent, resting tachycardia and identification of structural lesions and cardiomegaly during echocardiography.

The clinical features of CHF are easily recognized,[724] but determination of the underlying cause of heart disease may be more difficult. The etiology can be established if careful auscultation is combined with echocardiography, ECG, laboratory tests, and ultrasound examination of other body cavities.* Presenting clinical signs can vary but often include exercise intolerance, poor recovery after exercise, lethargy, anorexia, weight loss, loss of body condition, coughing, tachypnea, ventral edema, and colic-like signs.

The clinical features of right-sided CHF include resting tachycardia (generally at 60/min or greater) and prominent third (ventricular) sound along with generalized ventral, preputial, pectoral, and limb edema. Scrutiny of the jugular and other superficial veins generally makes the recognition of right-sided CHF straightforward, because affected horses demonstrate elevated venous pressure and pathologic jugular pulses and filling (see Figs. 9.11 and 9.12). Isolated limb edema, which is common in hospitalized horses, or ventral edema in the absence of generalized venous distention are not a sign of CHF and should prompt consideration of other disorders causing edema including hypoproteinemia, vasculitis, or severe pleural or abdominal effusion.

The most common causes of "isolated" right-sided CHF are tricuspid or pulmonic valve lesions as with valvular endocarditis, diffuse pulmonary vascular disease, and pericardial disease. In most cases a prominent murmur of tricuspid regurgitation will be evident over the right thorax. When there is pulmonary hypertension underlying right-sided failure, the tricuspid murmur may be especially loud and the pulmonic component of the second heart sound tympanic. Because right-sided heart failure often develops consequent to pulmonary hypertension caused by left-sided heart failure, concurrent left-sided murmurs are not uncommon. Another common association of right-sided CHF is persistent cardiac arrhythmia, such as AF, superimposed on structural heart disease of any form. Cranial mediastinal lymphoma and cranial thoracic masses including large pulmonary abscesses must be distinguished from RV failure.[140,737] Differential diagnosis is facilitated by examination with 2D echocardiography and thoracic ultrasonography.

The recognition of isolated left-sided heart failure, which causes pulmonary venous congestion and pulmonary interstitial or, uncommonly, alveolar edema, is more difficult in horses. Tachypnea and respiratory distress are predominant clinical signs. In these cases, heart sounds and murmurs may be difficult to identify owing to loud airway sounds or even pulmonary crackles. If a cardiac murmur is present but missed, an erroneous diagnosis of pneumonia or severe equine asthma (recurrent airway obstruction) may be entertained. Again, resting tachycardia and loud third heart sound are typical in these cases, and echocardiographic examination of the horse will be particularly useful. Typical findings in acute left-sided CHF are dilation and rounding of the left atrium and ventricle and often dilation of the PA caused by pulmonary hypertension. Careful examination may demonstrate anatomic lesions of the aortic root, ventricular septum, left heart valves, papillary muscles, or chordae tendineae. Fulminant left-sided CHF can lead to coughing and expectoration of edema—a grave sign in most horses (see Fig. 9.10).

Biventricular CHF is most commonly observed when severe left-sided disease results in chronic CHF and is complicated by AF. The clinical signs of advanced biventricular heart failure include resting tachycardia (usually >60 beats/min), loud ventricular filling sound, subcutaneous edema, tachypnea (from pulmonary congestion or pleural effusion), varying amounts of ascites and pleural effusion, a small pericardial effusion, jugular distention, and abnormal jugular pulsations. Chronic biventricular CHF is frequently characterized by lethargy and loss of body condition. In most cases there will be a left-sided heart murmur typical of mitral or aortic valve regurgitation and a murmur of tricuspid regurgitation on the right, which in advanced cases may become more prominent than the left-sided murmurs. Infrequently, biventricular CHF is due to congenital heart disease such as a VSD or a complex malformation.

It is important to understand that *chronic* left-sided CHF in the horse is likely to cause interstitial lung edema and pulmonary hypertension in the absence of overt clinical signs of left-sided heart failure. The magnitude of pulmonary hypertension is often impressive, with systolic PA pressures exceeding 80 mm Hg in many cases. The mechanisms by which the lung accommodates such severe and chronic elevations in pulmonary venous pressures without development of alveolar edema is speculative. It is likely that pulmonary arterial resistance increases, possibly related to remodeling in pulmonary arteries from chronic elevation of LA pressure. Chronic pulmonary hypertension subsequently leads to RV dilation and hypertrophy, tricuspid regurgitation, and RV failure, which limits CO and results in predominant clinical signs of right-sided CHF. Thus the clinician should anticipate signs of right-sided CHF even when the primary cardiac lesion is located on the left side of the heart. Clinical findings of severe pulmonary hypertension caused by left-sided CHF include a loud, tympanic, pulmonary component of the second heart sound (heard cranioventral to the aortic valve area), a systolic murmur and jugular pulse of tricuspid insufficiency, and dilation of the PA, which can be identified by 2D echocardiography. Thoracic radiographs may demonstrate increased pulmonary vascularity, pulmonary infiltration near the hilus, pleural

* References 83, 125-127, 144, 148, 152, 192, 204, 212, 213, 262, 265, 352, 374, 546, 563, 725-735.

effusion, and rounding or enlargement of the cardiac silhouette. Further scrutiny of the echocardiogram identifies LA dilation and lesions affecting the LV and usually the mitral valve, aortic valve, or aorta. Doppler echocardiography can document abnormal flow patterns such as valvular regurgitation and predict PA pressures (using the Bernoulli relationship discussed previously). An ECG is needed to evaluate cardiac arrhythmias.

Therapy for Congestive Heart Failure

Therapy for CHF is realistic for potentially reversible disorders such as acute papillary muscle ischemia/dysfunction, myocarditis, pericarditis or sustained ventricular tachyarrhythmia. It may also be feasible for valuable breeding stallions and mares, mares that develop CHF during gestation, or horses and ponies kept as pets.[724,727] The goal of treatment is to reduce the hemodynamic consequences of heart failure, restore cardiac output, and reduce congestion. Before therapy can commence, an accurate diagnosis is needed. For example, pericardiocentesis (not cardiac drugs) would be the appropriate initial management of cardiac tamponade; thereafter, surgical drainage of the effusion (using drainage tubes) or a pericardiectomy could be considered.[125-127,133,137,738-742] Antibiotics would be essential in the treatment of bacterial endocarditis or infective pericarditis. Sustained junctional and ventricular tachyarrhythmias may lead to low CO and a potentially reversible dilated cardiomyopathy. Antiarrhythmic therapy with lidocaine, magnesium sulfate solution, quinidine, procainamide, propafenone, sotalol, or amiodarone may be effective for treatment of some of these arrhythmias,[55,58] and with resumption of normal rhythm, CHF may be reversed.[153]

Horses in CHF should be brought to a quiet environment, and any type of stress should be avoided or at least minimized as much as possible. This also means that diagnostic interventions, although necessary, should be limited to those that are required to make an initial decision about the most appropriate initial treatment.

Diuretics are used in the treatment of CHF not associated with cardiac tamponade in order to reduce volume overload and edema and prevent their recurrence. Loop diuretics such as furosemide are preferred due to their superior fluid clearance and dose-dependent effects. They act by inhibiting the $Na^+/K^+/2\ Cl^-$ cotransporter in the ascending limb of the loop of Henle.[743] Furosemide is administered to horses with CHF at initial dosages of 1 to 2 mg/kg IV, given as repeated boluses to effect.[55,58,327,744-748] Because the site of action is reached intraluminally after excretion of furosemide by the proximal tubule, higher or more frequent doses might be required when renal function is impaired.[743] Bolus administration followed by constant rate infusion is thought to be more effective than intermittent bolus administration and can be chosen when profound diuresis is needed[747] (Table 9.7). Respiratory effort should improve when pulmonary edema is evident, and doses should be adapted as dictated by respiratory rate and effort.

Text continued on page 461

TABLE **9.7** **Drug Therapy of Heart Disease**

Drug	Dose Recommendations	Comments Therapeutic Drug Monitoring Adverse/Toxic Effects	Indications (I) Contraindications (CI)
ANTIARRHYTHMICS			
Sodium Channel Blocker (Class I_A)			
Quinidine sulfate (PO) or gluconate (IV)	22 mg/kg quinidine sulfate PO by NGT q 2 h for 4 (–6) doses until converted, adverse or toxic effects, or plasma quinidine concentration >4 μg/mL. Do not exceed 6 doses PO q 2 h. Continue q 6 h until converted, adverse or toxic effects, or total dose of 180 mg/kg. 1–2.2 mg/kg quinidine gluconate IV q 10 min or 0.1–0.22 mg/kg/min CRI up to 12 (–24*) mg/kg total dose (*Caution: Can result in severe adverse effects!)	IV administration for AF of recent onset (<2 weeks) without underlying structural heart disease or AF occurring during anesthesia. PO administration preferred for long-standing AF (>2–4 weeks). Therapeutic drug monitoring: therapeutic range: 2–5 μg/mL [6.2–15.4 μmol/L] 1–2 h after PO administration. Adverse effects: Commonly depression, diarrhea, colic, nasal mucosal swelling, and ataxia. Rarely paraphimosis, urticaria, and laminitis. Cardiovascular effects include acceleration of AV conduction and tachycardia (most common), prolonged QRS and QT interval, VT or torsades de pointes, hypotension, neg. inotropism, exacerbation of heart failure, cardiovascular collapse, sudden death.	I: AF, SVT (ventricular arrhythmias). CI: Ventricular tachyarrhythmias, torsades de pointes, untreated heart failure, preexisting prolonged QRS or QT interval, complete AV block, digitalis intoxication. Use with caution in patients with uncorrected hypokalemia, hypomagnesemia, hypoxia, or acid-base disorders.
Procainamide	25–35 mg/kg PO q 8 h 1 mg/kg/min IV, up to 20 mg/kg total dose	Adverse effects: Hypotension, QRS and QT prolongation, neg. inotropism, arrhythmias, GI and neurologic disorders. Similar but generally less severe than with quinidine.	I: Ventricular and supraventricular arrhythmias. CI: See quinidine.

Continued

TABLE **9.7 Drug Therapy of Heart Disease—cont'd**

Drug	Dose Recommendations	Comments Therapeutic Drug Monitoring Adverse/Toxic Effects	Indications (I) Contraindications (CI)
Sodium Channel Blocker (Class I_B)			
Lidocaine	0.25–0.5 mg/kg slow IV, repeat in 5–10 min to effect, up to 1.5 mg/kg total dose; followed by 0.03–0.05 mg/kg/min CRI	Adverse effects: Low incidence at therapeutic doses. Occasionally depression and muscle fasciculations. Overdoses may lead to ataxia, CNS excitement, seizures, hypotension, VT, collapse, and sudden death.	I: Ventricular arrhythmias, (potentially vagally sustained AF). CI: SA, AV, or intraventricular block, bradycardia. Caution with hypovolemia, liver disease, shock, and heart failure.
Phenytoin	20 mg/kg PO q 12 h for 3–4 doses or until signs of sedation, followed by 10–15 mg/kg PO q 12 h maintenance dose 5–10 mg/kg IV, followed by 1–5 mg/kg IM q 12 h or 10–15 mg/kg PO q 12 h	Maintenance dose varies considerably. Therapeutic drug monitoring: therapeutic range 5–10 μg/mL [19.8–39.6 μmol/L]. Adverse effects: Sedation, lip and facial twitching, gait deficits, excitation seizures, arrhythmias. Hepatotoxicity with chronic therapy. Hypotension and respiratory depression at high doses.	I: Digoxin-induced arrhythmias, other ventricular arrhythmias. CI: SA or AV block, sinus bradycardia.
Sodium Channel Blocker (Class I_C)			
Flecainide	1–2 mg/kg IV, infused at a rate of 0.2 mg/kg/min 4 mg/kg PO q 2 h for 4–6 doses, followed by 4 mg/kg PO q 4 h; adjust dose intervals if signs of toxicity occur	Use with caution. Not generally recommended for treatment of AF. Not considered effective for chronic AF. Adverse effects: depression, colic, neurologic signs, negative inotropism, QRS and QT prolongation, severe ventricular arrhythmias, sudden death.	I: Acute AF, supraventricular and ventricular arrhythmias resistant to other treatments. CI: Structural heart disease, heart failure, SA or AV node dysfunction.
Propafenone	0.5–2.0 mg/kg in 5% dextrose slowly IV over 10–15 min 2 mg/kg PO q 8 h	Not commonly used. Adverse effects: GI and neurologic disorders, bronchospasm, negative inotropism, exacerbation of heart failure, AV block, QRS and QT prolongation, arrhythmias.	I: Supraventricular and ventricular arrhythmias resistant to other treatments. CI: Structural heart disease, heart failure, SA or AV node dysfunction.
β-Adrenoceptor Blocker (Class II)			
Propranolol	0.03–0.16 mg/kg slow IV q 12 h 0.38–0.78 mg/kg PO q 8 h	Unspecific β_1/β_2-blocker. Variable oral bioavailability. Dosage should be individually adjusted. Adverse effects: Depression, lethargy, weakness, bradycardia, AV block, hypotension, neg. inotropism, exacerbation of heart failure, bronchoconstriction (aggravation of recurrent airway obstruction).	I: SVT and AF (rate control, often in combination with digoxin), catecholamine-induced arrhythmias, unresponsive supraventricular and ventricular arrhythmias. CI: Bradycardia, high-degree AV block, untreated heart failure, bronchopulmonary disease.
Potassium Channel Blocker (Class III)			
Sotalol	1 mg/kg PO q 12 h for 1 d, continue at 2–3 mg/kg PO q 12 h	Also class II effects. Good oral bioavailability. Generally well tolerated, even with chronic oral administration. Dosage should be gradually reduced before discontinuing medication. Adverse effects: QT prolongation, ventricular arrhythmias.	I: Prevention of recurrent AF, supraventricular and ventricular arrhythmias CI: Preexisting QT prolongation. Use with caution in patients with uncorrected hypokalemia or hypomagnesemia.

TABLE **9.7 Drug Therapy of Heart Disease—cont'd**

Drug	Dose Recommendations	Comments Therapeutic Drug Monitoring Adverse/Toxic Effects	Indications (I) Contraindications (CI)
Amiodarone	5 mg/kg/h IV for 1 h, followed by 0.83 mg/kg/h for 23 h, and subsequently 1.9 mg/kg/h for 30 h or to effect	Also class I, II, and IV effects. Poor and variable oral bioavailability, long half-life (16 days). Adverse reactions: Hindlimb weakness, weight shifting, torsades de pointes (supposedly low incidence), SA and AV nodal inhibition, bradycardia, hypotension. Prolonged treatment may affect lungs, liver, heart, thyroid gland, GI tract, eyes, skin, and nerves.	I: AF, ventricular arrhythmias (not investigated). CI: Sinus node dysfunction, bradycardia, AV block, cardiogenic shock.
Bretylium tosylate	3–5 mg/kg IV, repeat up to 10 mg/kg	Also indirect antiadrenergic effects. Adverse effects: Excitement, GI disorders, hypotension, tachycardia, arrhythmias.	I: Refractory, life-threatening ventricular tachycardia, ventricular fibrillation.
Calcium Channel Blocker (Class IV)			
Diltiazem Verapamil	0.125 mg/kg over 2 min IV, repeated q 10 min to effect, up to 1.25 mg/kg total dose 0.025–0.05 mg/kg IV q 30 min, up to 0.2 mg/kg total dose	Titrate to effect. Use diltiazem doses >0.5–1.0 mg/kg with caution. Adverse effects: Hypotension, tachycardia, sinus arrhythmia, bradycardia, sinus arrest, high-grade AV block, negative inotropism, exacerbation of heart failure (unless secondary to SVT or AF with rapid ventricular response rate).	I: Supraventricular arrhythmias (ventricular rate control in AF and interruption of SA/AV nodal-dependent SVT). CI: Hypotension, bradycardia, SA or AV block, ventricular systolic dysfunction, severe heart failure, cardiogenic shock, β-blocker.
Physiologic Calcium Channel Blocker, Activator of Membrane Na^+/K^+-ATPase			
Magnesium sulfate	2–6 mg/kg/min IV to effect, up 55 (–100) mg/kg total dose	Adverse effects: Overdoses (rare) may lead to CNS depressant effects, weakness, trembling, bradycardia, hypotension. Very high doses lead to neuromuscular blockade with respiratory depression and cardiac arrest.	I: Ventricular arrhythmias (esp. torsades de pointes and refractory VT), hypomagnesemia associated with cardiovascular disease. CI: Bradycardia, SA and AV block, renal failure.
DIGITALIS GLYCOSIDES			
Digoxin	IV loading dose: 0.0022 mg/kg IV q 12 h, for 2 doses IV maintenance dose: 0.0022 mg/kg IV q 24 h PO maintenance dose: 0.011 mg/kg PO q 12 h	Therapeutic drug monitoring: peak (1–2 h) and trough (12h) concentrations at steady state should fall within 0.8–1.2 (2.0) ng/mL (1–1.5 [2.6] nmol/L). Adverse effects: Depression, anorexia, colic, diarrhea, sinus bradycardia, AV block, supraventricular and ventricular arrhythmias (bigeminy).	I: Heart failure, ventricular rate control in SVT or AF. CI: AV block, diastolic ventricular dysfunction, preexisting digitalis toxicity, myocarditis, ventricular arrhythmias.
ADRENERGIC (SYMPATHOMIMETIC) AGENTS			
Inotropes			
Dopamine	1–5 μg/kg/min CRI, titrate to effect or adverse reactions	β_1-adrenergic, also dose-dependent dopaminergic and α_1-adrenergic. Adverse reactions: Tachycardia, ventricular arrhythmias, vasoconstriction and hypertension can occur at doses >4–5 μg/kg/min.	I: Hypotension, acute heart failure, cardiogenic shock, noncardiogenic shock (after adequate fluid loading), sinus arrest, bradycardia, high-grade or complete AV block, vagally induced bradyarrhythmias. CI: Ventricular arrhythmias, tachycardia, atrial fibrillation (risk of severe tachycardia due to accelerated atrioventricular conduction).
Dobutamine	1–5 μg/kg/min CRI, titrate to effect or adverse reactions	β_1-adrenergic, also β_2- and α_1-adrenergic. Preferred over dopamine. Adverse reactions: Tachycardia, ventricular arrhythmias, vasoconstriction.	

Continued

TABLE **9.7** **Drug Therapy of Heart Disease—cont'd**

Drug	Dose Recommendations	Comments Therapeutic Drug Monitoring Adverse/Toxic Effects	Indications (I) Contraindications (CI)
Epinephrine	0.01–0.05 mg/kg IV 0.1–0.5 mg/kg IT Note different concentrations: 1:1,000 = 1 mg/mL 1:10,000 = 0.1 mg/mL	β-adrenergic, α_1-adrenergic at higher doses. Adverse effects: Tremor, excitability, arrhythmias, ventricular fibrillation. Overdoses can lead to hypertension, arrhythmias, renal failure, pulmonary edema, cerebral hemorrhage.	I: Ventricular asystole, cardiopulmonary resuscitation. CI: Nonanaphylactic shock, arrhythmias, hypertension.
Vasopressors			
Norepinephrine	0.05–1 μg/kg/min CRI, titrate to effect	α_1-adrenergic, norepinephrine also exerts some β_1-adrenergic effects. Usually administered in conjunction with fluid therapy and dobutamine. Adverse effects: Hypertension, reflex bradycardia, reduction in cardiac output due to increased afterload, CNS effects (excitement, restlessness), rarely arrhythmias. Overdosage can lead to seizures, ventricular arrhythmias, cerebral hemorrhage.	I: Hypotension and shock associated with excessive peripheral vasodilation (i.e., septic or endotoxic shock, quinidine toxicity; after adequate volume loading). CI: Hypertension, bradycardia, poor cardiac output, cardiac disease, ventricular tachycardia.
Phenylephrine	0.1–1 μg/kg/min CRI, titrate to effect		
ANTICHOLINERGIC (VAGOLYTIC) AGENTS			
Atropine	0.01–0.02 mg/kg IV, IM	Adverse effects: Constipation, ileus, colic, bradycardia (initially or at very low doses), tachycardia, arrhythmias, CNS effects (stimulation, drowsiness, ataxia, seizures, respiratory depression). Glycopyrrolate is slightly less arrhythmogenic and rarely results in CNS effects.	I: Vagally induced bradyarrhythmias, sinus bradycardia, sinus arrest, high-grade or complete AV block. CI: Tachycardia, tachyarrhythmias, heart failure, GI disease, colic.
Glycopyrrolate	0.005–0.01 mg/kg IV		
ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS			
Benazepril	0.5–1.0 mg/kg PO q 24 h	Potential effects include vasodilation, reduction of volume overload, blunting of mechanism leading to diuretic resistance, cardioprotection by decreasing myocardial remodeling and fibrosis. Optimal doses, safety, and efficacy in horses largely unknown. Benazepril appears most effective at inhibiting serum ACE activity. Monitor renal function and serum potassium concentration. Adverse effects: Cough, impairment of renal function, hypotension, hyperkalemia.	I: Cardiovascular protection in cases with AR or MR, treatment of heart failure. CI: Severe renal failure, hyperkalemia, hypotension, pregnancy.
Ramipril	0.05–0.2 mg/kg PO q 24 h		
Quinapril	0.25 mg/kg PO q 24 h		
Enalapril	0.5 mg/kg PO q 12 h		
VASODILATORS AND INODILATORS			
Acepromazine	0.02–0.06 mg/kg IM q 6–8 h, titrate to effect 0.3–1.0 mg/kg PO q 12 h, titrate to effect	Sedative/tranquilizer, anxiolytic, antiarrhythmic, vasodilator (decreases afterload). Start at lower dose and titrate up to effect. Monitor systemic blood pressure. Adverse effects: Hypotension, protrusion of penis (very low risk of permanent penile dysfunction in intact male horses).	I: Management of CHF CI: Hypotension, hypovolemia, use cautiously and in smaller doses in animals with hepatic dysfunction.

TABLE **9.7 Drug Therapy of Heart Disease—cont'd**

Drug	Dose Recommendations	Comments Therapeutic Drug Monitoring Adverse/Toxic Effects	Indications (I) Contraindications (CI)
Hydralazine	0.5–1.5 mg/kg PO q 12 h 0.5 mg/kg IV q 12 h	Arterial vasodilator (reduces afterload). Adverse effects: Transient weakness, lethargy, hypotension, tachycardia, sodium/water retention (unless given with diuretics).	I: CHF, mitral regurgitation. CI: Hypotension, hypovolemia, severe renal disease.
Nitroglycerine	5–20 μg/kg/min CRI, titrate to effect	Venous vasodilator (reduces preload). Tolerance with long-term treatment (12–24 h). Adverse effects: Severe hypotension, reflex tachycardia, weakness.	I: Acute management of CHF. CI: Hypotension, hypovolemia.
Milrinone	0.2 μg/kg IV bolus, followed by 10 μg/kg/min CRI 0.5–1.0 mg/kg PO q 12 h	Phosphodiesterase inhibitor (inodilator). Adverse effects: Arrhythmias, hypotension, tachycardia, accelerated AV conduction.	I: Acute management of CHF. CI: Normal or low filling pressures, severe renal failure.
DIURETICS			
Furosemide	Initial dose for CHF: 1.0–3.0 mg/kg IV or IM q 8–12 h as needed to produce diuretic effect Alternatively: 1–2 mg/kg IV loading dose, followed by 0.12 mg/kg/h CRI Maintenance dose: 1 mg/kg IV or IM q 12 h	Start at higher/more frequent doses and reduce to minimal effective dose, as dictated by respiratory rate and effort. Oral administration not effective. Adverse effects: At high doses risk of hypovolemia, renal failure, electrolyte and acid-base imbalances.	I: Congestive heart failure, edema. CI: Dehydration, severe electrolyte disturbances.

AF, Atrial flutter/fibrillation; *AR,* aortic regurgitation; *AV,* atrioventricular; *CHF,* congestive heart failure; *CRI,* constant rate infusion; *HF,* heart failure; *IT,* intratracheal; *MR,* mitral regurgitation; *NGT,* nasogastric tube; *RAAS,* renin-angiotensin-aldosterone system; *SA,* sinoatrial; *SVT,* supraventricular tachycardia; *VT,* ventricular tachycardia.

Volume depletion, azotemia, and electrolyte imbalances are the most common adverse effects of furosemide treatment. Renal function should be followed and the dosage controlled to minimize prerenal azotemia. Diuretic therapy can eventually be discontinued in some horses, but others will require once- or twice-daily administration on the farm to prevent fluid retention. Systemic availability of orally administered furosemide is poor and variable,[745] and therefore oral treatment is not recommended. Prolonged diuresis may lead to loss of diuretic potency and diuretic resistance, which can potentially be counteracted by coadministration of angiotensin-converting enzyme inhibitors.[743]

Short-term therapy with intranasal oxygen therapy, if available, at a rate of 5 to 10 L/min using one or two nasal cannulas might be useful for horses with respiratory distress.

Horses in CHF can be cautiously sedated using acepromazine to minimize stress. In addition to its anxiolytic effects, acepromazine also acts as an antiarrhythmic and a potent vasodilator. As such, it reduces afterload, thereby decreasing valvular regurgitant fraction and facilitating ventricular ejection. Acepromazine should be titrated to effect, and if possible, systemic blood pressures should be monitored to avoid severe hypotension (see Table 9.7).[58] Note that α_2-adrenergic agonists such as xylazine and detomidine should not be used for sedation in horses with CHF, because they act as vasoconstrictors that increase afterload and result in increased regurgitant fraction and workload of the heart.

If acute positive inotropic support is required for stabilization of CHF or treatment of cardiogenic shock, dobutamine can be administered as a continuous rate infusion (CRI), taking its proarrhythmic and vasoconstrictive effects into account (see Table 9.7).[55,57,58,743] For chronic inotropic support, digoxin is commonly used. Digoxin therapy is indicated for CHF not associated with pericardial disease or serious ventricular ectopy. It is particularly beneficial when there is concurrent AF superimposed on structural valvular heart disease, because in addition to its inotropic effects it also causes baroreceptor sensitization and increases vagal tone, thereby reducing HR (negative chronotropic effects) and controlling ventricular response rate to AF (negative dromotropic effects).[743] Treatment is initiated by the intravenous route, with most (450–550 kg) horses receiving a total dose of about 1 mg IV (see Table 9.7). The reported elimination half-life of digoxin has not been consistent (7.2–28 hours) and probably relates to the clinical state of the animals studied.* Long-term digoxin therapy involves once- or twice-daily oral administration. Oral doses of digoxin are relatively higher because of lower bioavailability (20%).[320,330,331,334] Chronic digoxin therapy is monitored by measuring the serum digoxin concentration (drawn 8–12 hours after the previous dose). Target therapeutic values range between 0.8 and 1.2 (2.0) ng/mL (1–1.5 [2.6] nmol/L), depending on the time of the blood sample, and drug therapy achieving these serum concentrations is usually well tolerated in terms of appetite and heart rhythm. Periodic cardiac examinations, measurements of serum biochemistries (creatinine, electrolytes), and recordings of ECG

* References 319, 320, 322, 327, 328, 330-332, 334, 727, 749-752.

rhythm strips are warranted during any long-term course of therapy.

The use of acepromazine as a sedative and afterload reducer in horses with CHF has been described earlier in this chapter. There is limited published experience using other vasodilators in horses with CHF (see Table 9.7). Hydralazine[359,743] might be considered in the initial management of severe MR caused by ruptured chordae tendineae or endocarditis, because systemic arterial dilation can reduce the mitral regurgitant fraction. Nitroglycerin is a venous vasodilator that reduces preload and can be effective as an adjunctive treatment in hospital therapy of severe congestive heart failure and pulmonary edema.[743,753] However, clinical experience with the use of nitroglycerin in horses is limited, and the drug should be used with caution. Ointment, sublingual tablets, or transdermal patches have not been used in horses for treatment of heart failure to date. Milrinone is a phosphodiesterase III inhibitor that increases myocardial contractility and causes systemic vasodilation.[328,336,743] It can be used in the acute management of heart failure to provide inotropic support and reduce afterload. Although occasionally recommended for horses, its costs are often prohibitive and the pharmacology, effects, and adverse effects in horses with heart failure are not well studied.

Heart failure leads to an activation of the renin-angiotensin-aldosterone system (RAAS). Angiotensin-converting enzyme (ACE) inhibitors act on the RAAS by inhibiting the angiotensin-converting enzyme (ACE), which is responsible for conversion of angiotensin-I to angiotensin-II (AT-II). Therefore ACE inhibitors decrease renal sodium and water reabsorption, reduce volume overload, blunt the mechanisms leading to diuretic resistance, and cause vasodilation and decrease in myocardial oxygen demand. Furthermore, they are considered cardioprotective by decreasing myocardial remodeling and fibrosis.[743] In humans, ACE inhibitors have been shown to reduce mortality in heart failure. However, the benefits of ACE inhibitors have not been well studied in equine heart failure. There are some data available in horses regarding enalapril,[360,754-757] ramipril,[361,363,756,758,759] quinapril,[362,363,756,760] perindopril,[363] and benazepril (see Table 9.7).[363,756,761] Although effective intravenously,[360] oral enalapril at 0.5 mg/kg PO q 12 h does not demonstrate significant availability, pharmacodynamic effect, or suppression of ACE activity.[754-757] Ramipril administered at 0.1 to 0.3 mg/kg PO daily[363,756,758,759] and quinapril at doses ranging between 0.125 and 0.5 mg/kg PO[363,756,760] suppress ACE activity and reduce indirect BP in healthy horses at rest and during exercise. Ramipril has been used in a horse to treat CHF,[361] and an open-label clinical study of quinapril in horses with MR but without CHF showed some evidence for increased stroke volume and reduced regurgitation fraction.[362] The currently available data suggest that oral benazepril at doses of 0.5 to 1.0 mg/kg PO in healthy horses results in significantly greater serum ACE inhibition compared with the other ACE inhibitors.[363,756,761] Pharmacokinetics is not affected by feeding, and administration of multiple doses does not result in a cumulative effect of the drug on serum ACE inhibition. Unfortunately, the use of benazepril is cost-prohibitive in some countries. Additional studies will be required to assess the pharmacodynamics, clinical efficacy, and safety of benazepril and other ACE inhibitors in horses with volume overload or signs of CHF. Cough, impairment of renal function, hypotension, and hyperkalemia are potential adverse effects of ACE inhibitors that must be considered. Most ACE inhibitors are contraindicated in humans during pregnancy; therefore until appropriately studied, all ACE inhibitors should be considered as contraindicated in pregnant mares because of potential ill effects on the fetus.

Pimobendan is a calcium-sensitizing agent with phosphodiesterase inhibitory effects similar to milrinone. Owing to its positive inotropic and vasodilatory effects, it is increasingly used for treatment of heart failure in small animals.[762-767] A recent study in five healthy mature horses indicated that pimobendan administered intravenously at 0.25 mg/kg of body weight has positive chronotropic and inotropic effects without apparent adverse reactions. There are currently not enough data to allow the clinical use of pimobendan in horses, but this drug warrants further investigation for the treatment of heart failure in horses.[339]

For horses with valvular disease or cardiomyopathy, the development of AF may precipitate CHF. Furosemide and digoxin can effectively control CHF in many of these cases, with ACE inhibitors often being added to the treatment protocol. Quinidine is generally contraindicated in such cases, although horses may sometimes convert to sinus rhythm after the resolution of CHF by medical therapy. Reasonable long-term control of clinical signs may be achieved in some cases of chronic CHF, thus permitting a comfortable existence for the horse and—in the case of breeding animals—continued reproductive service.

Prognosis in CHF is at best guarded when irreversible structural heart disease is the cause of failure; thus the long-term outcome for life is poor. Of course, the horse with CHF requires rest to reduce demands on the heart and should never be worked or ridden owing to the risk of PA rupture, syncope, or fatal arrhythmia.[68] Valuable horses may be used for breeding; however, pregnant mares are likely to be difficult to control in the later stages of gestation related to the volume expansion that accompanies the latter stages of pregnancy.

CONGENITAL HEART DISEASE

The prevalence of congenital heart disease (CHD) in the overall equine population is unknown. In one survey of causes for neonatal death or euthanasia in 608 cases, the prevalence of congenital heart disease was 3.5%.[176] Congenital heart disease is often considered when a foal, weanling, or immature horse is identified with a prominent cardiac murmur, cyanosis, or signs of congestive heart failure.[405] Some defects are lethal to the neonate, whereas other malformations are compatible with life but limit performance or reproductive value.

A wide variety of cardiac malformations have been identified.* Theoretically, a great number of cardiac malformations could occur, including anomalies of (1) venous drainage, (2) atrial situs or septation, (3) atrioventricular connection, (4) atrial or ventricular development (including formation of the two atrioventricular valves and the septum), (5) ventricular outflow tracts, (6) semilunar valves, and (7) great vessels.[797-800] Furthermore, abnormal segmental connections might occur, leading to "discordance" in the path of systemic or pulmonary venous return relative to the pulmonary artery or aorta. These abnormalities include transposition of the great vessels and double-outlet ventricle, wherein both great vessels exit the right or left ventricular cavity.

* References 27, 28, 59, 170-186, 188, 408, 546, 552, 556, 563, 599, 728, 731-733, 768-796.

Hence, complex congenital malformations are often characterized by an unusual arrangement of the cardiac chambers and great arteries and by a markedly altered topographic cardiovascular anatomy. Echocardiographic diagnosis of complex congenital cardiac malformations is perceived as a difficult task by many clinicians and may be challenging even for the most experienced cardiologists. Even at necropsy, the correct and complete diagnosis of congenital heart disease often appears difficult and requires considerable effort. Sequential segmental analysis offers a systematic approach to complex congenital malformations of the heart, facilitating their in vivo diagnosis (using echocardiography, angiography, computed tomography, or magnetic resonance imaging) and postmortem description.[797,799-802]

Despite the wide variety of potential congenital defects, practically speaking, the most common cardiac malformations in horses involve shunting of blood at the atrial or ventricular levels, with the ventricular septal defect (VSD) most often recognized (Fig. 9.28). Isolated malformations leading to valvular stenosis or incompetency are uncommon. Rare lesions such as double-outlet right ventricle,[769,803,804] transposition of the great vessels,[173,182,805,806] persistent right aortic arch,[807-809] aorticopulmonary septal defect,[810] bicuspid pulmonary valve,[772] pulmonic stenosis,[173,184,188,772] subaortic stenosis,[780] mitral valve malformation,[187,793] persistent fetal circulation,[811] hypoplastic left heart syndrome,[174,175] endocardial fibroelastosis,[95,812] total anomalous pulmonary venous return,[813] aortic origin of the pulmonary artery,[731] double-inlet left ventricle,[814] and others have been reported but will not be discussed further. Some of the more frequently recognized defects[59,176,546] are described later in the chapter.

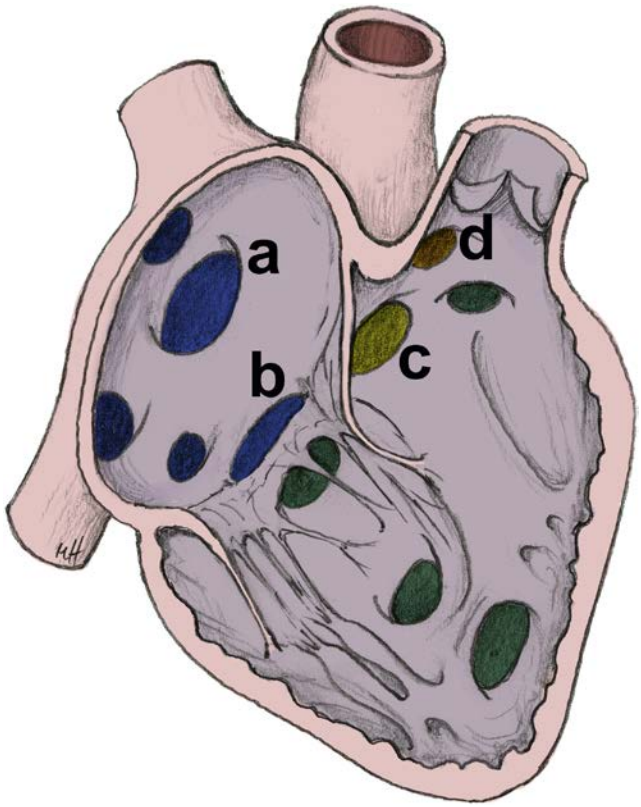


FIG. **9.28** Drawing showing the common locations of atrial septal defects (ASDs) and ventricular septal defects (VSDs). Defects located in the region of the oval fossa are called ostium secundum ASDs or oval fossa defects *(a)*. Other possible locations of interatrial communications include ostium primum *(b)*, sinus venosus, and coronary sinus defects. The most common interventricular communications in horses are the perimembranous (also called membranous or paramembranous) VSDs *(c)*, located in the membranous area of the ventricular septum, adjacent to the aortic root and the tricuspid valve. Less commonly, VSDs can be located in the subpulmonic region adjacent to the pulmonic and aortic valves (*d*, termed subpulmonic, subarterial, supracristal, or doubly committed VSDs) or in the inlet, apical, and outlet portions of the septum (muscular VSDs). (Drawing: Matthias Haab, Equine Department, Vetsuisse Faculty, University of Zurich, Switzerland.)

Pathogenesis of Congenital Heart Disease

The underlying genetic or other etiologic factors responsible for cardiac malformations in horses have not been studied. Potential but unproven causes include drugs, viral infection, environmental toxins, and nutritional disorders. Breed predispositions have been suspected for ventricular septal defects in Arabian horses, Welsh Mountain ponies, and Standardbreds.[190,405,563] Cardiac morphogenesis is complicated, but it is helpful to understand elementary aspects of cardiac development, especially as these pertain to CHD.[802,815-819] Among the fundamentals are septation of the atria, the anatomic components forming the ventricular septum, separation of the great vessels, and the normal fetal circulation.

The right and left atria are separated by incorporation of the right horn of the sinus venosus and through development and fusion of two prominent membranes, septum primum, and septum secundum. The endocardial cushions close the gap between the atrial and ventricular septa. These tissues also contribute to the atrioventricular septum, the septal segment spanning the point of mitral valve septal insertion on the left to the tricuspid valve insertion on the right. The foramen ovale, a normal atrial structure, is located approximately in the middle of the atrial septum, continues almost directly from the entry of the caudal vena cava, and creates a passageway for blood to flow from right to left atrium in the normal fetus.[820] The equine foramen ovale resembles a fenestrated finger cot and can be observed echocardiographically as a mobile septal membrane even in healthy full-term foals. This interatrial path may persist in foals with pulmonary hypertension and elevated right atrial pressures. Failure of normal development can lead to an atrial septal defect (ASD), which is typically designated by the location of the defective membrane (Fig. 9.28).

The ventricular septum is a complicated partition that includes a small membranous portion located adjacent to the aortic root and the tricuspid valve, an inlet septum immediately below the septal tricuspid leaflet, an apically located muscular or trabecular septum, and a dorsal outflow segment that separates the subaortic and the subpulmonic infundibulum (see Fig. 9.28). The ventral atrial septum connects to the dorsal ventricular septum by growth and differentiation of endocardial cushions. These swellings also form major parts of the atrioventricular valves. Insufficient development of any of these embryonic components can lead to a ventricular septal defect (VSD), the most common cardiac malformation in horses. Defective differentiation of the endocardial cushions causes various combinations of an ostium primum (ventral) ASD, an inlet VSD, malformation of the atrioventricular valves, or common atrium with a single atrioventricular valve (see Fig. 9.32B later in this chapter).

During cardiac morphogenesis, the aorta and pulmonary artery begin as a single vessel in the conus arteriosus. This common trunk, or truncus arteriosus, is eventually partitioned by migration of the conus and development of the conotruncal and spiral septa. Twisting of the spiral septum produces appropriate alignment (concordance) of the great vessels with their respective ventricular chambers. The descending aorta and pulmonary artery are connected by the ductus arteriosus Botalli, which carries fetal blood from the pulmonary artery to the descending aorta. Maldevelopment of conotruncal or spiral septal tissues leads to complicated congenital heart defects that can occasionally be seen in the horse, including persistent truncus arteriosus,[181,785,792,821,822] double-outlet right ventricle,[769,803,804] transposition of the great vessels,[173,182,805,806] and aorticopulmonary septal defect.[810] Persistence of the right aortic arch[807-809] and persistent patency of the ductus arteriosus (PDA)[408,731,768,774,809] are both rare in horses.

There are two fetal circulations, one serving the fetus and the other communicating with the placenta.[816] However, unlike the sequentially arranged systemic and pulmonary circulations in the fully developed postpartum organism, the fetal circulations are not completely separate. Functionally there are two right-to-left shunts: one across the foramen ovale and the other across the ductus arteriosus. The fetal lungs are collapsed, pulmonary vascular resistance is high, and pulmonary blood flow is minimal. Desaturated blood returning from the fetal tissues is collected in the cardinal venous system and enters the sinus venosus and right atrium. This blood is largely earmarked for the right ventricle and pulmonary artery. Most pulmonary arterial flow is diverted through the ductus arteriosus to the descending aorta, umbilical arteries, and placenta, where it is oxygenated. Well-saturated blood returning across the umbilical veins is delivered by the caudal vena cava to the right atrium where it preferentially crosses the foramen ovale to enter the left atrium, left ventricle, and ascending aorta, from where it is delivered to the fetal tissues. Note that well-oxygenated blood from the ascending aorta is delivered to the heart (through the coronary arteries) and the brain (through the brachiocephalic trunk), whereas the tissues in the caudal parts of the body receive a mixture of oxygenated blood from the ascending aorta and desaturated blood entering the descending aorta through the ductus arteriosus. These patterns change dramatically with foaling. As the lungs expand, pulmonary vascular resistance falls and pulmonary blood flow increases. The resultant increase in left atrial pressure functionally closes the foramen ovale within the first 24 to 48 hours of life.[820] Similarly, inhibition of local prostaglandins leads to functional closure of the ductus arteriosus within 72 hours after birth in most full-term foals.[408] Persistence of the right-to-left shunts, especially at the level of the foramen ovale, can occur in premature foals or those suffering from severe pulmonary disease with associated pulmonary hypertension. In these cases, shunting across the foramen ovale represents an additional mechanism for arterial desaturation and tissue hypoxia.

Clinical Pathophysiology of Shunts

Fundamental to understanding of cardiac malformations is an appreciation of shunt physiology and the responses of the heart and circulation to a shunt.[59,816,817] Shunting can be defined as an abnormal deviation of blood flow between systemic (left) and pulmonary (right) circulations. Shunting can be left-to-right, right-to-left, and bidirectional. Systemic to pulmonary (left-to-right) shunting is the expected consequence of an ASD, VSD, and PDA as long as systemic pressures and resistances exceed those on the right side and there is no stenosis limiting PA flow. Even in cases of abnormal ventricular-arterial development, as with persistent truncus arteriosus, double-outlet right ventricle, or univentricular heart, the clinical findings of a left-to-right shunt may predominate unless there is obstruction to blood flow or elevated pulmonary vascular resistance.

The actual shunt volume carried to the lungs depends on the caliber (or "restrictive" nature) of the lesion orifice and the relative vascular resistances between systemic and pulmonary circulations. Shunting may not be significant for some weeks after foaling because pulmonary vascular resistance is relatively high, and systemic arterial and left ventricular pressures are relatively low. Eventually left-to-right shunts cause an increase in pulmonary arterial flow and augment pulmonary venous return. Small shunts are easily handled at the expense of mild left-sided dilation and hypertrophy. When the pulmonary-to-systemic flow ratio (Qp:Qs) exceeds about 1.8:1, the shunt is usually considered clinically relevant, and the consequences are obvious volume overload of the LA and LV. The greater the shunt volume, the higher is the potential for left-sided or biventricular CHF from ventricular dysfunction. Pulmonary hypertension can occur in the setting of left-to-right shunting from combinations of increased PA flow, remodeling of the small pulmonary arteries, and left ventricular failure. Thus consequences of significant left-to-right shunting may include any of the following: exercise intolerance, tachypnea, pulmonary edema, respiratory distress, pulmonary hypertension, AF, pleural effusion, jugular venous distention, or ventral edema. The foal may be smaller than expected and may have a history of antibiotic therapy for presumed bouts of "pneumonia."

Right-to-left shunting produces a different clinical presentation. When a shunt is complicated by a right-sided obstruction downstream from the defect, right-to-left shunting will develop once right-sided pressures exceed those on the left. This can occur in a foal with tricuspid valve atresia and ASD or with pulmonary valve atresia and VSD. Conversely, elevated right-sided resistance can develop more chronically from pulmonary vascular disease or vascular remodeling. For example, a large left-to-right shunt can induce medial hypertrophy and intimal thickening of small pulmonary arteries that elevate pulmonary vascular resistance.[645] Though very uncommon, the resultant pulmonary hypertension may become severe and reverse the shunt to right to left (Eisenmenger's physiology). In these cases the left heart chambers are small, and the right ventricle is hypertrophied to generate systemic BP. The entrance of desaturated blood into the left side of the circulation causes arterial hypoxemia with potential consequences of tissue hypoxia, cyanosis, exercise intolerance, mild to moderate polycythemia, hyperviscosity of blood, and stunting of growth. CHF is rare, but sudden death can occur, presumably from arrhythmia. The degrees of hypoxemia and cyanosis in a right-to-left shunt depend on overall pulmonary blood flow and the degree of blood mixing between the circulations. Thus if pulmonary flow is markedly diminished, as with tricuspid atresia, severe cyanosis is likely. However, the impact of right-to-left shunting can be mitigated by overall increases in pulmonary blood flow as with truncus arteriosus or double-outlet right ventricle without pulmonary obstruction. These lesions cause less hypoxemia because the volume of oxygenated blood

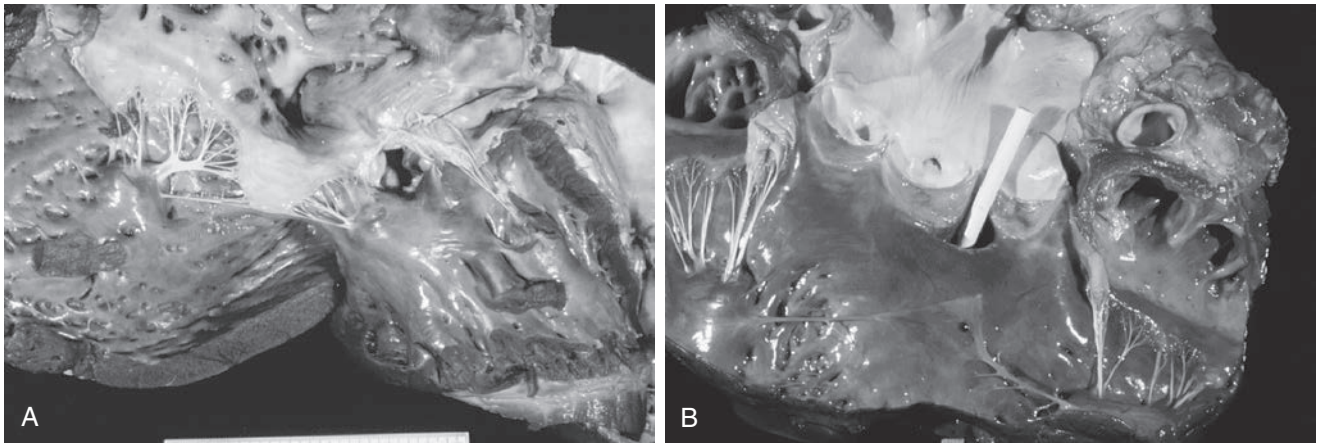


FIG. **9.29** Pathologic evaluation of ventricular septal defects (VSDs). A, Opened right ventricle (RV) from a mare demonstrating a perimembranous septal defect opening just beneath the septal leaflet of the tricuspid valve. Aortic valve cusps are visible through the defect. Congestive heart failure (CHF) occurred late in life, after the development of atrial fibrillation (AF). B, A large VSD in a horse. A probe runs through the defect. The dorsal location immediately beneath the right and the noncoronary cusps of the aortic valve are notable. Ostia of both coronary arteries are also evident.

reaching the left ventricle is increased. The location or "commitment" of a VSD relative to the subaortic or subpulmonic region also influences clinical signs because oxygenated blood from the LV can actually stream preferentially through a VSD into the aorta. In these situations, cyanosis related to any mixing of blood in the RV may be negligible and the clinical condition predominated by left-sided or biventricular CHF. If the increased pulmonary flow is sufficient to minimize arterial hypoxemia but not create heart failure, survival even beyond 5 years of age is possible.

Ventricular Septal Defects

Ventricular septal defect (VSD) is the most important CHD of horses.* A genetic basis is likely in the Arabian breed[190,563] and in Welsh Mountain ponies.[405] In the authors' experience, VSD is also encountered regularly in Standardbred horses[563] and in Quarter Horses. The VSD often accompanies more complicated heart malformations.†

The location of a VSD depends on the embryogenesis of the lesion and influences the designation and even the clinical manifestations of the defect. The nomenclature of VSDs is confusing but can be remembered by considering the main components of the normal ventricular septum (Figs. 9.28, 9.29, and 9.30). In most cases, a VSD is located dorsally ("high") on the ventricular septum, below the right and noncoronary cusp of the aortic valve on the left side, cranial to the septal tricuspid leaflet on the right, and encompassing or contiguous with the fibrous part of the ventricular septum.[563,782] Such defects are generally referred to as "perimembranous," "membranous," or perhaps more correctly, "paramembranous." Most of these holes are also "subcristal" because the VSD is located caudoventral to the supraventricular crest, separating the right ventricular inlet from the outlet. However, a very large paramembranous defect also can extend under the tricuspid valve toward the inlet septum or advance across the supraventricular crest toward the outlet septum. The (conotruncal) septal defects associated with tetralogy of Fallot (see Fig. 9.34 later) and with pulmonary atresia are usually very large and fall into the latter appellation. Sometimes the aortic root is displaced ventrocranially and straddles (or "overrides") the defect, creating a "malalignment" VSD. This is characteristic of the tetralogy of Fallot but also can be seen with large, isolated paramembranous defects. A less common location for a VSD is immediately ventral to the septal tricuspid valve within the muscular septum. Such "inlet" VSDs are typical of a complete endocardial cushion defect and commonly related to a septum primum ASD, common atrioventricular valve leaflet, or persistent atrioventricular canal, which creates a gap between all four cardiac chambers (see Fig. 9.32B later).[773,829,830] A subaortic VSD that communicates with the outlet portion of the ventricular septum directly below the pulmonic valve is variably referred to as a "subpulmonic," "subarterial," "supracristal," or "doubly committed" VSD. This lesion also places the aortic valve at risk for prolapse. Finally, apical muscular (trabecular) defects or multiple VSDs are rare but have been observed in horses. Some of these are small, whereas others have been enormous.

Many VSDs close spontaneously in people. This has also been observed in horses, but whether or not this is common in this species is unknown.[826] However, the flow across a VSD can be diminished by imposition of a cardiac valve. For example, the rim or even a major portion of a VSD may be occluded by fibrotic tissue that ensnares the septal tricuspid leaflet, rendering the defect functionally smaller and possibly creating a hyperechoic aneurysm on the right septal surface. Large defects associated with malalignment of the ascending aorta to the upper border of the remaining septum are often associated with prolapse of the right or noncoronary cusp of the aortic valve (or of the aortic root) into the defect. Aortic prolapse can effectively close even a large VSD, but at the risk of permitting chronic aortic valve insufficiency over time (see Fig. 9.30B).[563]

* References 59, 68, 178, 190, 390, 405, 552, 556, 563, 732, 775, 782, 794, and 823-827.

† References 171-173, 178, 182, 190, 556, 772, 773, 784-787, 803, 806, 809, 814, 828.

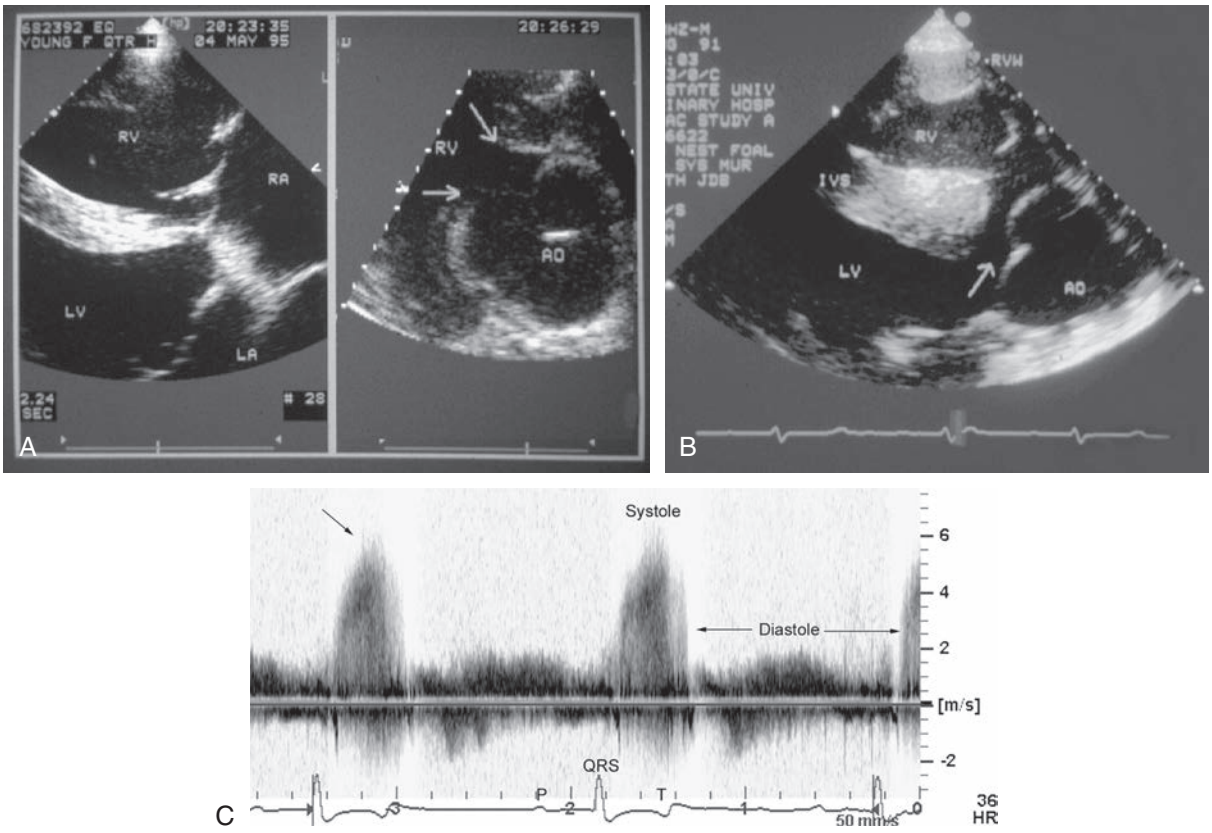


FIG. **9.30** Echocardiograms demonstrating ventricular septal defects (VSDs). A, This subaortic VSD is not evident from the long-axis image *(left panel)* but is visible in the short-axis tomogram *(arrows).* This Quarter Horse gelding also had pulmonic stenosis. The elevated right ventricular (RV) pressures have led to significant RV enlargement with bulging of the septum toward the left ventricle (LV). B, Malalignment and a perimembranous septal defect associated with prolapse of an aortic valve leaflet across the defect *(arrow)* (*RVW,* right ventricular wall). C, Continuous-wave Doppler study recorded from a foal with a small, restrictive VSD. The maximal velocity of nearly 6 m/sec *(arrow)* predicts a pressure difference of up to 144 mm Hg between left and right ventricles.

The pathophysiology of the uncomplicated VSD is that of a left-to-right shunt as described previously (Fig. 9.31). Much of the shunt volume pumped by the left ventricle is ejected immediately into the pulmonary artery. As pulmonary flow increases, there is increased venous return to the left atrium and left ventricle, causing left atrial enlargement and left ventricular dilation and hypertrophy that can be recognized by echocardiography. Thus the left (not the right) ventricle performs most of the extra volume work. This is made more severe if there is aortic valve prolapse with aortic regurgitation or if mitral regurgitation develops owing to left ventricular enlargement. If the shunt is large and pulmonary arteriolar resistance does not increase significantly, left ventricular failure can develop. This is most likely to occur early in life, as the high fetal pulmonary vascular resistance declines, but late cases of CHF (with AF) also have been observed. The degree of RV hypertrophy and enlargement varies, depending on the location and size of the septal defect and pulmonary vascular resistance. Large nonrestrictive defects create a functional common chamber, allowing ventricular pressures to equilibrate and leading to marked RV hypertrophy as well as pulmonary hypertension.

The clinical features of VSD are variable.[59,190,563,775,782,827] Clinical signs may be absent and the defect identified as an incidental finding. A mature horse may be presented for poor performance or with AF. Foals may be symptomatic for pulmonary edema or biventricular heart failure. Commonly, a murmur is detected incidentally during the physical examination for another problem or during a prepurchase examination (see Fig. 9.7B and Tables 9.1 and 9.4). As most defects communicate near the tricuspid valve, the most consistent physical examination finding is a harsh holosystolic or pansystolic murmur that is loudest just below the tricuspid valve region and above the right sternal border. A slightly less intense ejection murmur due to increased flow across the RV outlet is usually evident over the left base (see Fig. 9.31). The second heart sound may be split more widely than normal owing to disparate ventricular ejection times; the pulmonic component of S_2 will be tympanic if there is pulmonary hypertension. In contrast, the murmur of a subpulmonic (subarterial or supracristal) VSD is loudest over the left cranial base as the high-velocity flow enters the main PA. When a VSD is associated with a complex cardiac malformation, the murmur is likely to be loud over each side of the thorax. The severity of the defect cannot be judged based on murmur intensity. In some cases a small defect may be quite loud, whereas a large, less restrictive defect may cause a murmur related entirely to the increased flow (relative pulmonic stenosis). A holodiastolic murmur of

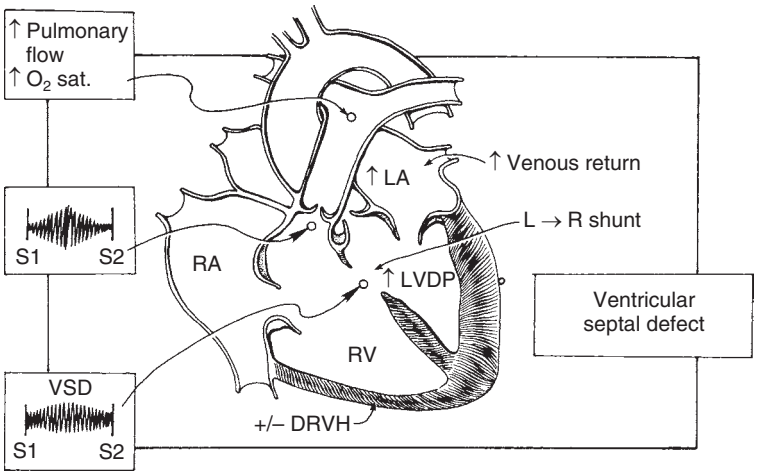


FIG. **9.31** Pathophysiology of ventricular septal defects (VSDs). See text for details. *LA,* Left atrium; *RA,* right atrium; *LVDP,* left ventricular diastolic pressure; *RV,* right ventricle; *RVH,* right ventricular hypertrophy. (From Bonagura JD: Congenital heart disease. In Bonagura JD, editor: *Cardiology,* New York, 1987, Churchill Livingstone.)

aortic regurgitation indicates prolapse of an aortic cusp and increases the likelihood that the lesion is relatively large. Substantial aortic regurgitation is associated with a hyperdynamic arterial pulse and an increased pulse pressure. If there is significant left ventricular volume overload, the mitral valve may become incompetent, and a holosystolic murmur of mitral regurgitation may be evident over the left apex. The rare trabecular (muscular) VSD may also create a systolic murmur over the left or right apex. The VSD associated with pulmonary atresia or persistent truncus arteriosus may not create a substantial murmur, but the increased flow through the dilated single vessel usually generates a loud ejection murmur over each side of the chest. If significant cardiomegaly develops, atrial and ventricular premature complexes, or even AF, may be recognized.

Diagnostic studies are needed to confirm the lesion and determine the severity. The performance history is a useful overall indicator of impact, and the horse with an excellent work history is unlikely to have a large defect. The ECG is unreliable for diagnosing cardiomegaly in horses but is indicated in the setting of an arrhythmia. Thoracic radiography can be useful in foals to demonstrate cardiomegaly (see Fig. 9.19A), the pulmonary circulation, the lungs, and the pleural space. 2D echocardiography and color Doppler imaging establish the diagnosis, and spectral Doppler examinations are useful for assessing the hemodynamic burden of the defect.[344,552,563,590,819] Complex congenital disease must be excluded.

2D echocardiography successfully delineates the VSD in almost every case provided sufficient imaging planes are obtained (see Figs. 9.25 and 9.30). It is important to collect long-axis images of the left ventricular outflow tract and aortic valve, as well as short-axis images at the level of the left ventricular outflow tract, at and just ventral to the aortic leaflets. The typical paramembranous defect appears under the aortic valve and adjacent to the septal leaflet of the tricuspid valve. A true defect is characterized by a relatively echogenic tissue interface, whereas an area of false echo dropout tends to be gradual. Most defects can be imaged in orthogonal (long-axis/short-axis) planes. The ostium of the right coronary artery of the horse is relatively large and may be confused with a subpulmonic VSD in short-axis image planes. It also should be noted that a true inlet VSD of an endocardial cushion defect, located immediately ventral to the septal tricuspid valve, might not be easily seen in standard planes. Tipped or oblique views that show both AV valves may be required. Similarly, finding a muscular, apical, or small subpulmonic defect requires more imaging experience and nonstandard imaging planes and is greatly assisted by color Doppler studies.

Attempts should be made to identify the largest diameter of the defect in complementary planes and compare this with the size of the aortic root, as orifice size is an important prognostic factor. Note that the size of the VSD is often underestimated with 2D echocardiography and overestimated with color-flow Doppler echocardiography. Although there are limitations to echocardiographic sizing of the VSD, a defect exceeding 2.5 cm in diameter or a VSD-to–aortic root diameter ratio of greater than 0.4 identifies a large defect with greater likelihood of clinical signs.[563] There is usually some enlargement of the left side of the heart, but cardiac dimensions fall within the normal range if the shunt is small. However, 2D or M-mode evidence of left-sided cardiac dilation, right ventricular enlargement, or marked dilation of the main PA suggests a hemodynamically relevant VSD and one more likely to affect performance or survival. Moderate to severe enlargement of the LA and LV is concerning and increases the risk for AF, PHT, and CHF. Noticeable systolic enlargement of PA indicates pulmonary overcirculation, whereas marked PA enlargement during both systole and diastole suggests PHT.

Identification of shunting across a VSD is confirmed using color Doppler studies. There will typically be a region of flow acceleration proximal to the defect and a high-velocity, turbulent flow entering the right ventricle distal to the defect during systole, with low-velocity, uniform color shunting noted during diastole. Color Doppler imaging is extremely helpful for identifying a very small VSD or one with an atypical location. Aortic regurgitation is identified in some horses, and the regurgitant flow is often directed into the VSD, resulting in high-velocity diastolic shunting. Continuous-wave Doppler is used to estimate the pressure difference between the two

ventricles, as velocity (in meters per second) is proportional to the instantaneous pressure difference across the ventricles (by the modified Bernoulli equation: $\Delta p = 4 \times v_{max}^2$; see Figs. 9.25D and 9.30C). A relatively small VSD is "restrictive" to flow, and the peak shunt velocity will generally exceed 4.5 m/sec, assuming proper alignment to shunt flow.[563] With good alignment, peak velocities greater than 5 m/sec (corresponding to a left-to-right pressure gradient of greater than 100 mm Hg) are expected. Lower shunt velocities (less than 4 m/sec), along with higher pulmonary artery ejection and mitral inflow velocities, indicate a greater shunt volume. In the setting of pulmonary hypertension, pulmonic stenosis, or systemic hypotension, the velocity of left-to-right shunting will also be lower; when pulmonary hypertension is present, a tricuspid regurgitation jet of greater than 3.4 m/sec may be identified.

Potential outcomes of the isolated VSD include the following: (1) tolerance of the lesion; (2) partial or complete closure of a VSD by adherence of the septal tricuspid leaflet, fibrous tissue, right ventricular hypertrophy, or aortic valve prolapse; (3) progressive aortic regurgitation; (4) progressive mitral regurgitation owing to left-sided chamber enlargement; (5) atrial fibrillation; (6) left-sided or biventricular CHF; (7) pulmonary hypertension (with left-to-right shunting); or (8) reversal of the shunt with development of arterial hypoxemia and cyanosis. The last situation is rare and would be caused by either severe pulmonary vascular disease (Eisenmenger's physiology) or fibromuscular obstruction in the right ventricular outlet leading to subpulmonic stenosis.

The horse with a relatively small-diameter paramembranous defect, high-velocity left-to-right shunt, mild cardiomegaly, relatively normal right ventricular cavity, and normal heart rhythm probably has a restrictive VSD that will be well tolerated. Most of these animals can perform sufficiently in the show ring, as a hunter-jumper, or even as an endurance horse or racehorse. Moderate defects are often well tolerated at rest, but performance in high-intensity sports might be affected. Large defects that are associated with echocardiographic evidence of moderate to severe cardiomegaly, right ventricular hypertrophy, aortic root prolapse, aortic malalignment, marked valvular regurgitation (AR, MR, TR), evidence of pulmonary hypertension, atrial fibrillation, or presence of other comorbidities are prone to complications and carry a less favorable prognosis for performance or life regardless of current clinical signs.

According to the 2014 American College of Veterinary Internal Medicine (ACVIM) consensus statement on the management of equine athletes with CV abnormalities,[68] horses with VSDs should be examined annually. Horses with an isolated small (restrictive) VSD and minimal cardiomegaly can be considered safe to compete, whereas horses with larger defects should be evaluated on a case-by-case basis in consultation with a specialist experienced in equine cardiology. Exercise (ECG) testing is recommended in horses with moderate to large VSDs, in prepurchase situations, or when performance is suboptimal. Breeding of affected animals generally should be discouraged, especially in Arabian horses and Section A Welsh Mountain ponies.

Definitive therapy for VSD would involve cardiopulmonary bypass surgery that is impractical in horses. Surgical banding of the PA elevates right ventricular pressures and reduces left-to-right shunting; however, this procedure also limits CO and is not advised. New "hybrid" procedures involving catheter devices delivered by a transventricular approach and guided by echocardiography or angiography are now possible, though perhaps not practical in the majority of affected patients. Medical management of CHF or of arrhythmias associated with CHD can be considered. However, even if the response to treatment is good, the horse should not be used.

Atrial Septal Defects

Atrial septal defects, including endocardial cushion defects, are quite uncommon in foals.* As indicated earlier, an ASD may involve different portions of the atrial septum (see Fig. 9.28) and is more likely to be observed with complex congenital cardiac defects, particularly with tricuspid or pulmonary atresia. An isolated ASD may be clinically irrelevant, with no significant murmur or clinical signs. Moderate to good exercise capacity may be expected because left-to-right shunting decreases as the systemic to pulmonary vascular resistance ratio declines with exercise. In the case of a large ASD, left-to-right shunting leads to right-sided volume overload and pulmonary overcirculation and potentially to pulmonary vascular injury. Atrial fibrillation has been observed in conjunction with ASD. Large defects are visible echocardiographically (Fig. 9.32A), but thinning, billowing, or flailing of the atrial septum in the region of the oval fossa can lead to an incorrect diagnosis of an ASD, particularly in cases with smaller defects and suboptimal image quality.[344,819] Doppler echocardiography and saline contrast studies (by injection of agitated saline into the jugular vein) can confirm the presence and direction of the shunt and estimate its severity. Thereby, it must be considered that physiologic blood flow entering the RA from the caudal vena cava and the LA from the pulmonary veins can easily be mistaken for transseptal flow on color Doppler recordings. Similarly, streaming of vena cava flow in the RA can cause a distinct negative contrast pattern within the RA during saline contrast studies, mimicking left-to-right transseptal flow.[344,831]

Complete endocardial cushion defects are rarely seen but are serious, usually leading to CHF or AF at an early age. Components of this defect typically include a large ASD involving the primum and the atrioventricular septa, a common atrioventricular valve leaflet, and often an inlet VSD (see Figs. 9.28 and 9.32B). The ventricles may be partitioned normally, unequally with one rudimentary ventricular chamber, or not at all, creating a single ventricle. In the most severe cases there is a common atrioventricular canal, a single common atrioventricular valve, and a single ventricle from which both great vessels exit. The clinical signs of a complete endocardial cushion defect are variable. The foal with two ventricles and an unobstructed outlet to the pulmonary arteries will be hemodynamically similar to one with a large VSD. When a common ventricle is present, varying degrees of cyanosis may be observed. A systolic murmur is typical and may reflect flow across the VSD, ventricular outflow, or atrioventricular valve regurgitation. A 2D echocardiogram can reveal the lesions and Doppler echocardiography the intracardiac shunts and AV valvular regurgitation. CHF may supervene, and the prognosis is poor.

Patent Ductus Arteriosus

Patent ductus arteriosus (PDA) is rare as an isolated congenital cardiac defect in foals and is detected most frequently in combination with other, more complex malformations

* References 175, 177, 180, 599, 733, 773, 784, 829, 830.

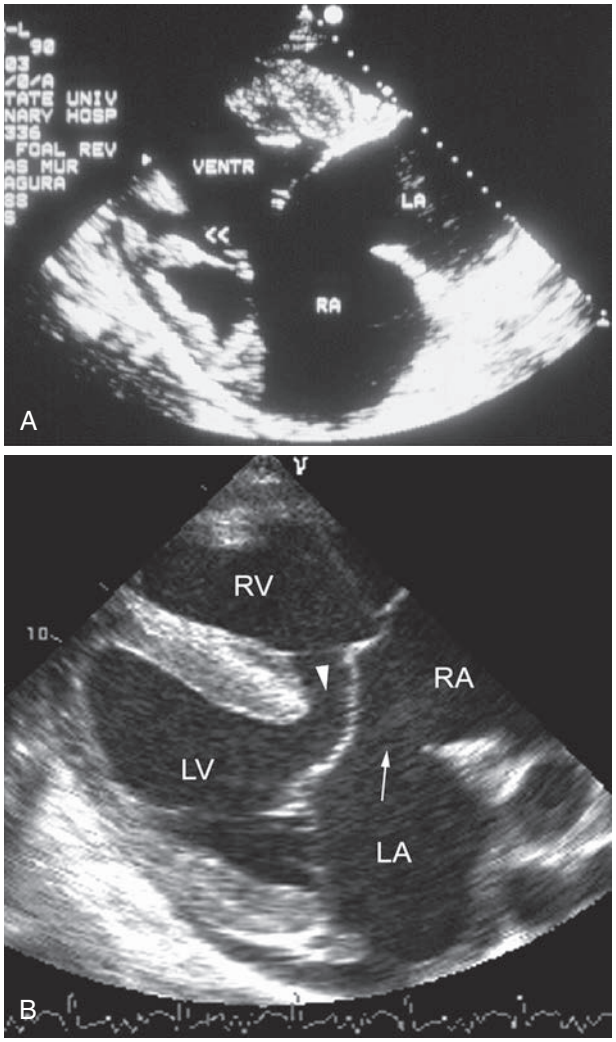


FIG. **9.32** A, Echocardiogram demonstrating a primum atrial septal defect (ASD) in a foal with complex congenital heart disease that included a common *(left)* ventricle, rudimentary *(right)* ventricle, and double-outlet ventricle. The septal defect is evident between the four cardiac chambers. The dorsal secundum septum *(right)* is present, as well as the apical ventricular septum *(left).* B, Long-axis image from a foal with a complete endocardial cushion defect. The primum ASD is evident in the ventral atrial septum *(arrow),* and an inlet ventricular septal defect (VSD) component *(arrowhead)* is observed below the closed common (or straddling) atrioventricular valve. Normal mitral and tricuspid septal leaflets would insert into the septum at slightly different levels (with tricuspid septal leaflet inserting more ventrally than the mitral septal leaflet.)

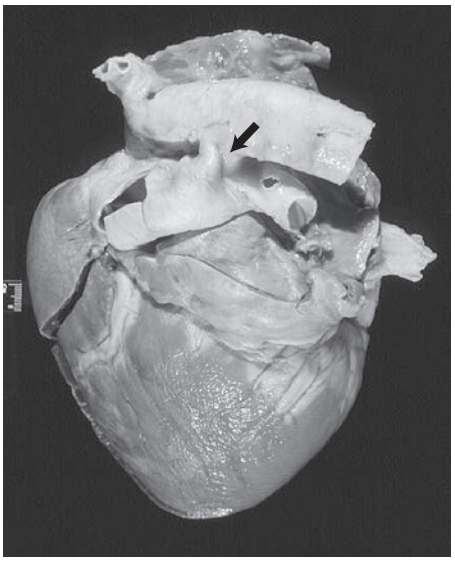


FIG. **9.33** A postmortem demonstration of the ductus arteriosus *(arrow)* between the descending aorta and the pulmonary artery in a foal with complex congenital heart disease.

(Fig. 9.33).* The ductus arteriosus is a fetal vessel, derived from the left sixth aortic arch, that permits shunting from the PA to the descending aorta in the fetus. At birth the ductus arteriosus normally constricts in response to increased local oxygen tension and inhibition of prostaglandins. It is functionally closed 72 hours after birth in the vast majority of foals. If the ductus arteriosus does not close, a left-to-right shunt from the aorta to PA occurs. Although there may be some hereditary predisposition to PDA in other species, this lesion is so rare as an isolated congenital defect that this is not a significant concern. Premature foals, foals with persistent pulmonary hypertension, and foals whose dams have been given prostaglandin inhibitors might be more susceptible to the development of a PDA.

The clinical signs depend on the magnitude of the shunting through the PDA, which is determined by ductal diameter and vascular resistance in the pulmonary circulation. Physical examination findings (with a left-to-right PDA) include a continuous machinery murmur and thrill, usually loudest over the main PA (craniodorsal to the aortic valve area), and bounding arterial pulses. Differential diagnosis includes other systemic-to-pulmonary shunts in association with complex congenital heart disease.

Echocardiography will reveal volume overload of the PA, LA, and LV.[344,819] The severity of these findings depends on the magnitude of the shunt. Direct visualization of the PDA is not always possible by 2D echocardiography because the ductus arteriosus may be obscured by overlying lung. Ductal flow is best identified from the left cranial thorax by Doppler echocardiographic examination of the main PA, which reveals continuous, high-velocity, turbulent flow directed toward the pulmonary valve. Cardiac enlargement and increased pulmonary vascularity may be detected in neonatal foals with a PDA, as well as radiographic evidence of pulmonary edema if the foal has developed CHF. Cardiac catheterization reveals elevated PA and pulmonary capillary wedge pressures and increased PA oxygen saturation. The heart should be evaluated carefully for other congenital cardiac defects before surgical or catheter-based intervention is considered as complex cardiac malformations are likely in a foal with PDA. Late complications of this lesion include rupture of the PA.

Tetralogy of Fallot

The tetralogy of Fallot is one of the more common congenital cardiac anomalies in foals responsible for right-to-left shunting, arterial desaturation, and cyanosis.[171,178,183,190,833-837] The four lesions are (1) large paramembranous-outlet VSD, (2) cranial and rightward (dextro-) positioning of the aorta with overriding of the septal defect, (3) right ventricular outflow

* References 171, 175, 182, 184, 408, 409, 731, 768, 774, 809, 832.

tract obstruction, and (4) right ventricular hypertrophy. Outflow obstruction can be due to subvalvular fibromuscular obstruction, valvular pulmonic stenosis, or hypoplasia of the PA. Ventricular hypertrophy is caused by right ventricular outflow obstruction and the large, unrestrictive, VSD that functionally creates a "common ventricle." Blood leaves the heart along the path of least resistance such that pulmonary flow depends on the severity of right ventricular outflow tract stenosis. As previously discussed, the degree of cyanosis and severity of clinical signs depend on the volume of blood traversing the lungs. In some horses, a PDA is also present (pentalogy of Fallot), and this defect reduces signs by increasing pulmonary flow, left heart filling, and systemic arterial hemoglobin saturation. Tetralogy of Fallot must be distinguished from other causes of cyanotic heart disease, including tricuspid atresia, pulmonary atresia with VSD, transposition of the great vessels, double-outlet right ventricle with pulmonic stenosis, truncus arteriosus, and total anomalous pulmonary venous connection.[816,817]

Affected foals are usually smaller than normal, lethargic, and intolerant of exercise. Cyanosis is most evident after activity and is variably present at rest. Arterial blood gas analysis demonstrates hypoxemia with normal or reduced partial pressure of carbon dioxide (P_{CO_2}). Auscultation is typically characterized by a loud systolic murmur over the pulmonic valve area on the left side caused by (sub-) pulmonic stenosis. The second heart sound is usually unremarkable. Although polycythemia can be significant, it is usually mild, even when arterial oxygen tensions fall to 50 to 70 mm Hg.

Echocardiographic evaluation is diagnostic and reveals a large, unrestrictive VSD, malalignment and overriding of the aortic root, right ventricular outflow tract obstruction, and right ventricular hypertrophy (Fig. 9.34).[344,819] Shunting can be identified by color Doppler or saline contrast echocardiography initiated by injection of agitated saline into the jugular vein. Both studies will demonstrate a right-to-left or bidirectional shunt. Conventional spectral Doppler studies can be used to delineate the shunt (typically bidirectional, low-velocity flow of less than 2 m/sec) and right ventricular outflow obstruction (high-velocity flow exceeding 4 m/sec).

Although it is possible for horses to live for a number of years with tetralogy of Fallot, most affected animals are humanely destroyed due to the poor prognosis for life. Affected horses should not be used or bred if they survive to maturity.

Pulmonary Atresia with Ventricular Septal Defect

Pulmonary atresia with VSD is rare, having been observed most often in Arabian foals (see Fig. 9.34C).[171,172,186,790] This malformation represents the exaggerated form of tetralogy of Fallot, with these findings: (1) the right ventricular outlet does not connect into the pulmonary artery, (2) the right ventricle is hypertrophied, (3) a large malalignment VSD is present (in most cases), and (4) the fetal truncus arteriosus has been partitioned so unequally that the aorta is markedly dilated and the pulmonary trunk atretic or severely hypoplastic. Without careful ultrasound studies (or necropsy dissection) of the pulmonary circulation, the dilated aorta can be mistaken for a persistent truncus arteriosus, hence the moniker "pseudotruncus

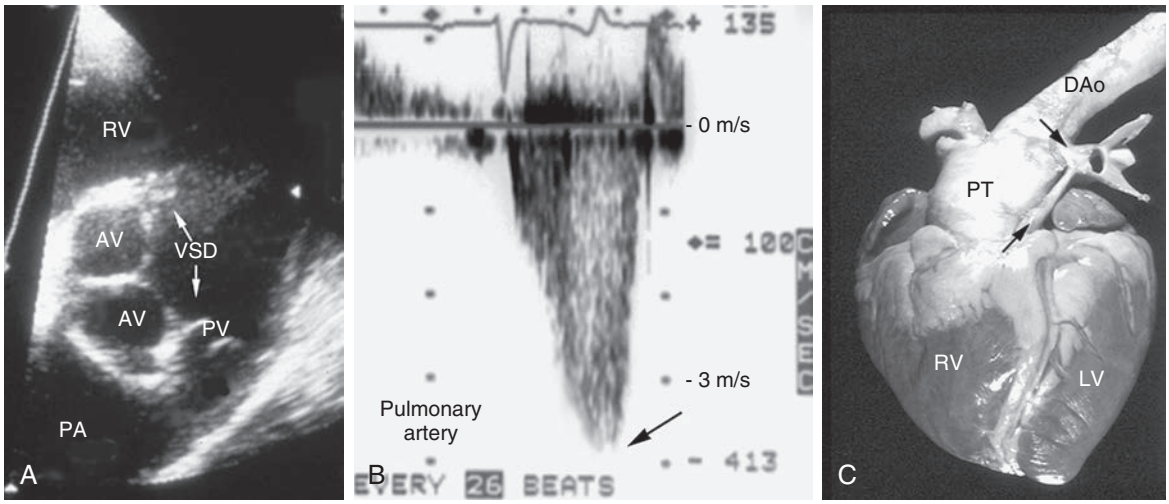


FIG. **9.34** Tetralogy of Fallot. A, Echocardiogram from a filly with tetralogy of Fallot. The image plane shows the right ventricle (RV), right ventricular outlet with stenotic (hypoplastic) pulmonary valves (PV), and poststenotic dilation of the pulmonary artery (PA). There is a very large ventricular septal defect (VSD; *arrows*) connecting the base of the dilated aorta with the right ventricle. Two aortic valve leaflets (AV) are evident in this off-angle plane. B, Continuous-wave Doppler echocardiogram from a horse with tetralogy of Fallot. The Doppler study demonstrates pulmonary stenosis. High-velocity systolic flow is evident, exceeding 4 m/sec. Velocity scale is to the right; ECG at the top. C, Left lateral view of a heart obtained from a foal that died from pulmonary atresia, the "exaggerated" form of tetralogy of Fallot. Because of unequal septation of the truncus arteriosus, a markedly dilated aorta (*PT,* pseudotruncus arteriosus), and a rudimentary, imperforate main pulmonary artery *(lower arrow)* are present. Atresia of the pulmonary valve was evident on opening the heart. Pulmonary artery flow was derived from the ductus arteriosus *(upper arrow),* which serves two underdeveloped pulmonary arteries. *DAo,* Descending aorta; *RV,* right ventricle; *LV,* left ventricle. (From Bonagura JD: Congenital heart disease. In Robinson N, editor: *Current therapy in equine practice,* ed 5, Philadelphia, 2003, WB Saunders.)

arteriosus." Owing to the atretic pulmonary valve, pulmonary blood flow must be derived either from a PDA or the aorta. In the latter instance, the systemic collaterals are usually from bronchial arteries. Pulmonary atresia with an intact ventricular septum has been diagnosed rarely.[791,832]

The diagnosis of pulmonary atresia is usually stimulated by clinical findings of cyanosis, cardiac murmur, and stunting in a foal or weanling. Diagnosis is confirmed by echocardiography.[344,819] Careful imaging can identify the main lesions outlined previously. Of diagnostic importance is the inability to identify the pulmonic valve in the rudimentary right ventricular outflow tract (though a small pouch may be seen). Careful ultrasound examination of the ascending aorta and aortic arch from the right and left sides of the thorax will fail to reveal a normal origin for the pulmonary trunk or an origin of the PA directly from a truncus arteriosus. The bifurcation of the PA may be found from a cranial imaging position, and continuous flow into that vessel documented by Doppler echocardiography suggests that pulmonary blood flow is derived from the ductus arteriosus or a collateral systemic artery.

Truncus Arteriosus Communis (Common Arterial Trunk)

The failure of the fetal truncus arteriosus to partition into the aorta and PA represents a rare anomaly of the equine heart.[181,785,792,821,822,838] In this condition, the fetal truncus never partitions, and both ventricles continue to develop, communicating with the truncus arteriosus across a large malalignment-type VSD. Systemic, coronary, and PA flows each arise from the truncus, which is guarded by a truncal valve (that can be incompetent or stenotic). Pulmonary blood flow originates from one or more pulmonary arteries connected directly to the truncus arteriosus or indirectly from systemic to pulmonary collateral vessels.[802,816,817]

The pathophysiology and clinical findings of this malformation depend largely on the magnitude of pulmonary blood flow. If the PA origins are not stenotic and if pulmonary vascular resistance remains relatively low, the clinical condition resembles a left-to-right shunt, except for right-to-left mixing of blood across the VSD. However, the degree of arterial hypoxemia may not be severe, and cyanosis may not be obvious. Conversely, high pulmonary vascular resistance or obstruction to flow at the truncal origin of the PA is associated with arterial desaturation and findings similar to pulmonary atresia.

Clinical examination usually indicates a systolic cardiac murmur. The mucous membranes may be pink or cyanotic. If there is marked left-to-right shunting, CHF may occur. With careful ultrasound examination, the truncus and origin of the pulmonary arteries may be identified, allowing the condition to be distinguished from pulmonary atresia with VSD.[344,819] Furthermore in some cases an abnormal truncal valve (with four leaflets) may be evident, further supporting the diagnosis. Management is best accomplished by consultation with a cardiac specialist.

Tricuspid Atresia

Another differential diagnosis for cyanotic heart disease is atresia of the tricuspid valve.* This malformation dictates right-to-left shunting of systemic venous blood at the atrial level. The atrial shunt may be across a true ASD or a patent foramen ovale. Because all venous return must mix in the left atrium, this malformation generally causes marked hypoxemia with cyanosis. Affected foals rarely survive to weanling age unless there is also a left-to-right shunting VSD into a functional right ventricular outflow tract that provides good pulmonary flow. Otherwise, pulmonary flow must come from a ductus arteriosus or systemic collaterals (e.g., bronchial arteries). Most foals are stunted, nurse poorly, and exhibit severe exercise intolerance and cyanosis at rest. Arterial oxygen tension can be very low (40–60 mm Hg). Echocardiography reveals a markedly dilated right atrium and coronary sinus, atretic tricuspid valve, and rudimentary right ventricle (larger if there is a functional left-to-right shunting VSD). Atrial shunting that allows systemic venous return to empty into the left atrium must be observed. Abnormal flow patterns can be verified by saline contrast or color Doppler echocardiography. The prognosis is grave.

VALVULAR HEART DISEASE

Healthy cardiac valves maintain normal antegrade flow in the heart and prevent significant regurgitation of blood. Diseased cardiac valves, which can be stenotic or incompetent, limit cardiac output and place an increased workload on the heart. Stenotic valvular lesions in horses are typically congenital in nature and are extremely rare; however, acquired valvular regurgitation (also called valvular insufficiency or valvular incompetency) is common.* The majority of valvular insufficiencies in horses are caused by degenerative valve disease or are related to high-level training.† Infective (bacterial) endocarditis,‡ noninfective valvulitis, and ruptured chordae tendineae[38,212-214,845] are infrequent causes of valvular disease. Box 9.2 summarizes important causes of valvular dysfunction. Degenerative valvular disease and infective endocarditis are the valvular problems most often encountered by the equine practitioner, and are the focus of this section.

The clinical relevance of a valvular lesion depends largely on the severity of regurgitation across the valve. It is clear that many horses adapt to trivial, mild, or even moderate valvular regurgitation with no apparent consequence on performance.* The severity of valvular regurgitation is related to the dynamic cross-sectional area of the regurgitant orifice, the pressure gradient driving blood across the valve, and the time allowed for regurgitation, because not all incompetent valves leak throughout systole or diastole.[74] Regardless of the volume, the movement of blood from a high-pressure to a low-pressure chamber is associated with a high-velocity jet that is proportional to the pressure drop between source and sink. The production of high-velocity jets leads to disturbed flow (with turbulence) and in many cases an audible cardiac murmur, the hallmark clinical feature of valvular heart disease (Fig. 9.35; also see Figs. 9.9 and 9.23D).

Valvular incompetency can be diagnosed by auscultation† or with Doppler echocardiography.‡ Doppler studies are

* References 171, 180, 182, 186, 190, 786, 828, 839-842.

* References 5, 18, 38, 45, 48, 65, 71, 72, 74, 75, 83, 148, 171, 173, 179, 184, 188, 190, 207-212, 214, 355, 390, 391, 406, 407, 554, 557, 583, 602, 772, 793, 843-851.

† References 28, 65, 70, 75, 148, 191, 208-211, 586.

‡ References 31, 191, 193-197, 199-206, 824, 852-860.

* References 70, 71, 75, 208-211, 355, 407, 586.

† References 3, 69, 70, 74-76, 207, 379, 381, 385, 389, 390, 392, 393, 406, and 861.

‡ References 71, 75, 124, 208, 209, 211, 214, 344, 390, 391, 560, 601.

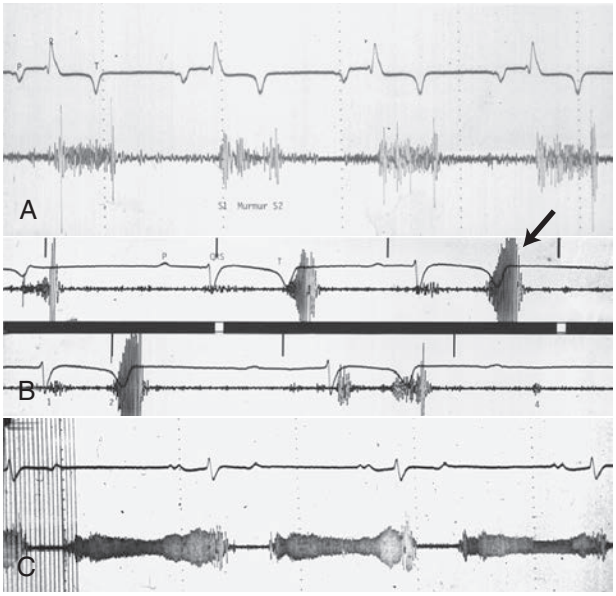


FIG. **9.35** Phonocardiograms of cardiac murmurs caused by valvular heart disease. A, A holosystolic murmur of mitral regurgitation (MR) in a horse with chronic valvular degeneration. B, A variable, late systolic murmur of MR related to mitral valve prolapse. The murmur has a crescendo and peaks at end systole *(arrow)*. Heart sounds are indicated. The murmur obscures the second sound. C, A holodiastolic, vibratory murmur of aortic regurgitation (AR) with presystolic accentuation. The accentuation probably is related to atrial contraction, altered ventricular volume and pressure, and an incremental increase in regurgitant volume.

highly sensitive for identification of valvular incompetency and represent the gold standard for identification of valvular dysfunction. However, many horses with normal auscultation findings also demonstrate valvular regurgitation by Doppler examination. Most cardiologists consider these "silent" valvular leaks as normal,[71,74] especially when observed on the right side of the heart. Even on the left side silent regurgitation is often observed by color Doppler examination.

Some of these flow signals are brief, representing "backflow" or valve closure signals; these are easily misinterpreted unless carefully timed by spectral Doppler or color M-mode examinations. Certainly some cases of silent regurgitation represent the earliest signs of degenerative valvular disease. But there is no clinical benefit to screening horses by Doppler, nor is the approach practical or even predictive of future outcome. Thus cardiac auscultation remains the most clinically important method for identifying clinically relevant valvular disease. Echocardiography including Doppler studies is used to verify the source of a pathologic murmur, identify underlying structural heart lesions, assess atrial and ventricular function, and quantify the degree of cardiac remodeling that has developed in response to the lesion. It is also emphasized that usually there is no echocardiographic or Doppler correlate to the functional ejection murmur.

Although examiners usually discount "silent" regurgitation, the clinical relevance of *audible* valvular regurgitation also must be placed in context. For example, examination of high-performance athletes will demonstrate murmurs of tricuspid, mitral, or aortic valvular insufficiency in many of these horses.[70,75,208-211,586] These findings are readily verifiable by Doppler imaging. In one study of 2-year-olds,[75] the prevalence of tricuspid and mitral regurgitant murmurs increased significantly over a 9-month training period to an incidence of 25.5% and 21.8%, respectively. Similarly, studies on young Standardbreds and Thoroughbreds in training revealed an increase in the prevalence of valvular regurgitation detected by echocardiography without noticing effects on racing performance.[70,208,211] The causes of these changes and the interpretation of these findings are problematic, especially when referenced to the clinical benchmark of "normal." It is possible that high-level training induces changes in ventricular geometry (i.e., physiologic cardiac hypertrophy, athlete's heart)[208,211,588] or in valvular thickness related to cardiovascular work, elevations of BP during training, or other factors. Nevertheless, whether these murmurs are actually "normal" or not, it is clear that the clinical importance of a regurgitant murmur in a horse must be interpreted with caution and certainly with the perspective of clinical and imaging findings.

The most practical evaluations for assessing the clinical relevance of a heart murmur are the age, work history, physical examination findings, and echocardiography. Other studies such as electrocardiography and exercise testing provide further information by which to judge the importance of a murmur. As a general rule, significant regurgitant murmurs tend to be loud and long. However, the intensity of an insufficiency murmur is related not only to the regurgitant volume but also to the driving pressures of blood and the physical characteristics of the thorax. Therefore although the intensity of a heart murmur can be graded by the examiner (see earlier in this chapter), it is not possible to grade the severity of regurgitation by auscultation alone. Relatively loud murmurs may be associated with regurgitant volumes that are inconsequential to an individual horse, especially when the murmur is high-pitched, vibratory, or musical in quality. Thus although clinically significant valvular heart disease is best identified by auscultation (see Tables 9.1, 9.3, and 9.4), the clinical relevance of a valvular regurgitation must be assessed in other ways.* This approach is emphasized below. A recent consensus statement formulated by a group of experts in the field of equine cardiovascular medicine summarizes the current recommendations for management of equine athletes with valvular heart disease, and the reader is referred to this statement for additional information.[68]

Mitral Regurgitation

Mitral regurgitation (MR) is commonly detected in horses.[68,83,407,843] The etiopathologic basis of mitral valve incompetency may involve any of the following: high-level training, degenerative thickening, prolapse of the valve, ruptured chordae tendineae, bacterial endocarditis, noninfective valvulitis, primary or ischemic myocardial disease leading to papillary muscle dysfunction, severe LV dilation, congenital malformation of the valve, and idiopathic disease (Figs. 9.36, 9.37, and 9.38; also see Box 9.2).† Degenerative, fibrotic thickening of the mitral valve has been observed at necropsy in mature horses and is probably the basis for most cases of mild to moderate MR, including those with "normal" 2D echocardiographic imaging. The basis of mitral valve prolapse is uncertain but could involve connective tissue disease of the leaflets, stretched chordae tendineae, minor chordal ruptures, or injury to a papillary muscle. Ruptured mitral valve chordae

* References 70, 71, 73, 75, 208-211, 390, 391, 406, 560, 586, 602, 849.
† References 28, 29, 48, 57, 148, 191, 406, 557, 591.

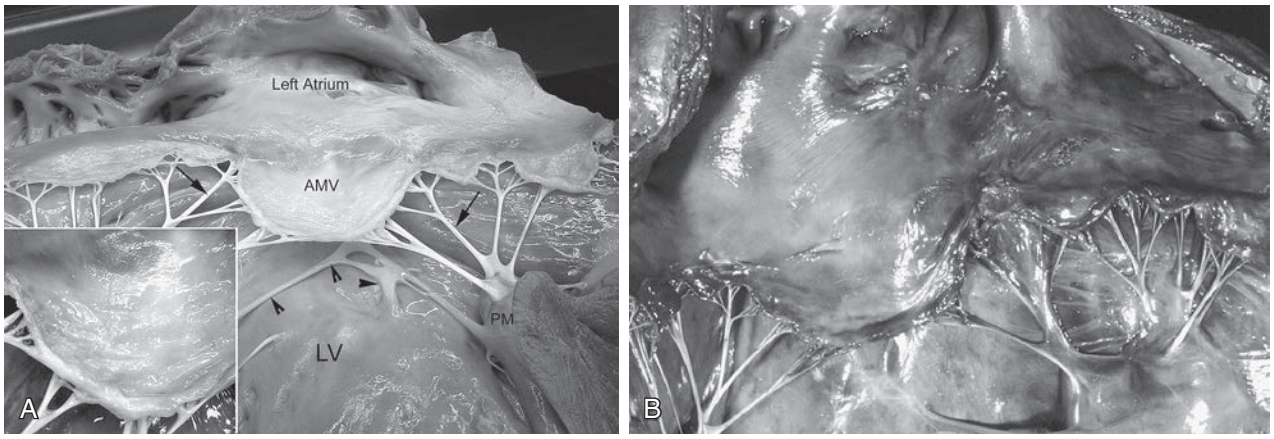


FIG. **9.36** A, Postmortem images from a horse with mitral regurgitation (MR) and congestive heart failure (CHF) caused by severe degenerative valvular disease. The left ventricle (LV) is opened and the septal or anterior mitral valve leaflet (AMV) is shown. The free edges of the valve or cusps are slightly thickened for an equine valve. The body of the valve is very irregular and thick, changes most evident when viewed in close-up (see inset at lower left). The cut surface of a papillary muscle (PM) and intact chordal attachments to the valves are evident *(arrows)*. The *arrowheads* point to the LV moderator band or trabeculae septomarginalis, part of electrical conduction system. Myxomatous degeneration of the valve was evident on histopathology. The cause was unknown. B, Chronic suppurative valvulitis caused by chronic endocarditis has led to scarring, thickening, and distortion of the mitral valve. This horse developed severe left-sided CHF. Noninfective valvulitis also is recognized sporadically in horses, particularly in younger animals.

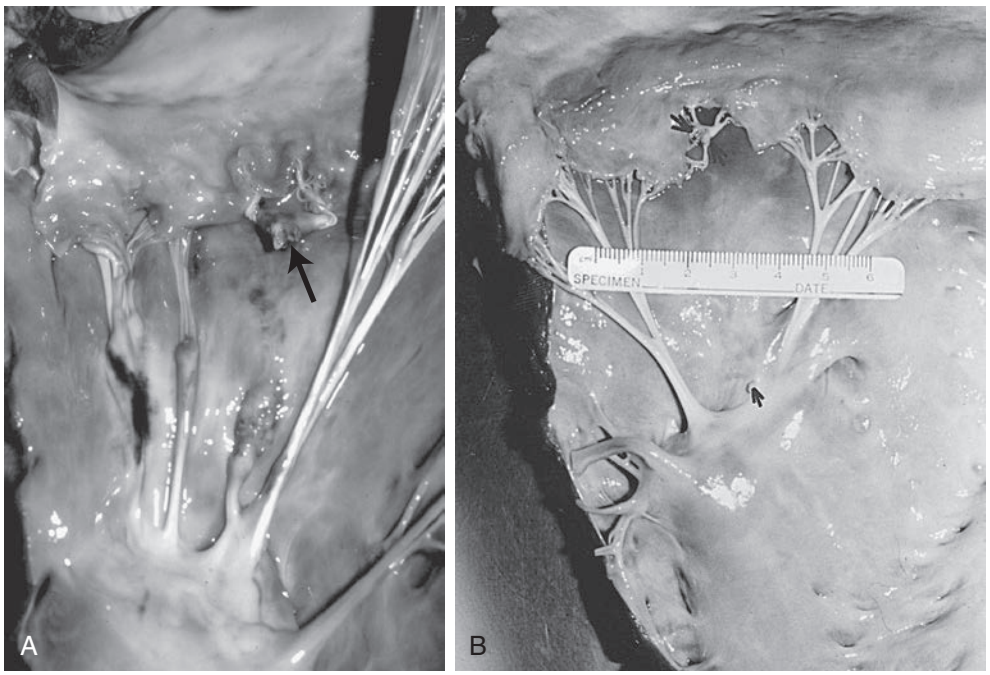


FIG. **9.37** Rupture of the mitral valve chordae tendineae. A, Acute rupture of a chorda tendinea in a horse with lymphocytic plasmacytic valvulitis. The flail mitral cusp actually has twisted because of loss of support. The ventral portion of the tear is obvious adjacent to intact chords. B, Chronic rupture of a chorda tendinea in a horse. The contraction of the scarred segments *(arrow)* is notable.

tendineae with flail mitral leaflet can occur in animals of any age, including foals.[38,212-214,845] Chordal ruptures, which often involve the accessory mitral cusps, may lead to severe MR with fulminant acute CHF. Necropsy findings in these cases often show degenerative thickening of the ruptured strand; rarely endocarditis is involved. Although uncommon, infective endocarditis can lead to ulceration, vegetation, or chordal injury and substantial MR (see later). The authors have observed MR due to severe mitral scarring and thickening in weanlings and young horses. The cause of these lesions is

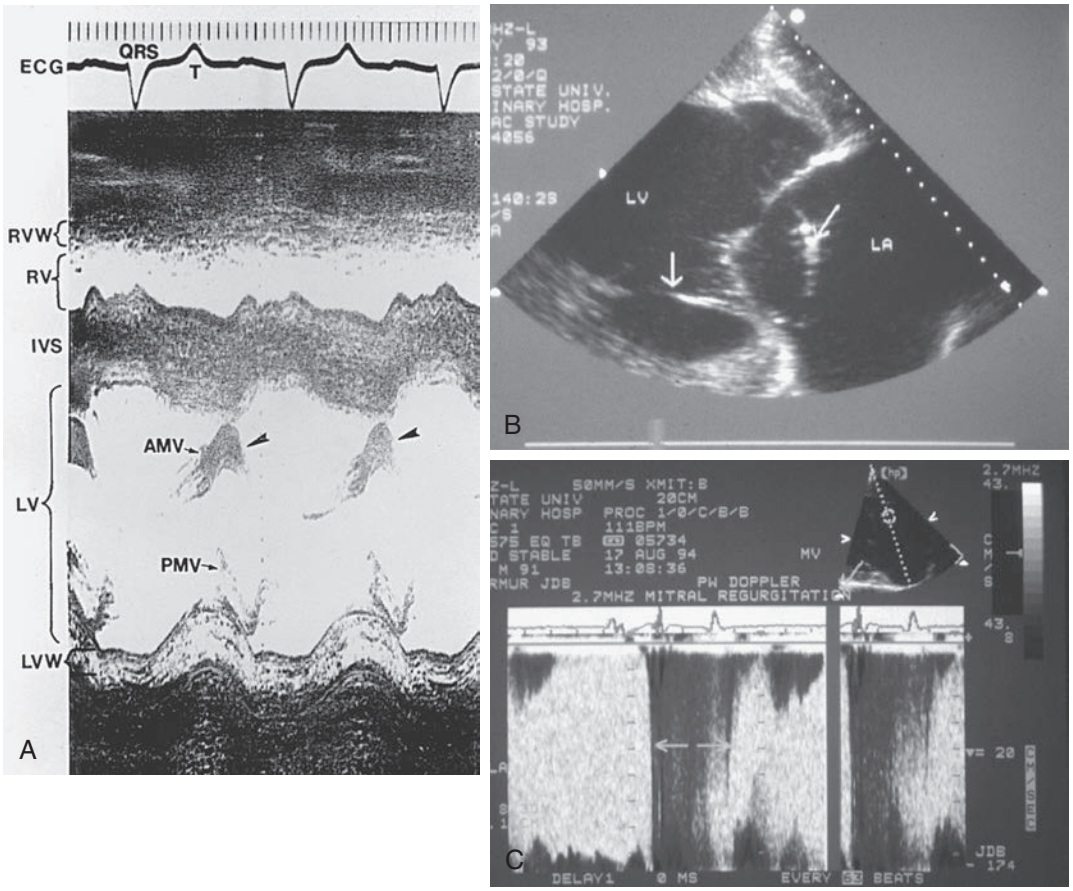


FIG. **9.38** Echocardiograms recorded from horses with mitral regurgitation (MR). A, M-mode study demonstrating significant thickening of the anterior mitral valve (AMV) leaflet *(arrows).* This would be compatible with endocarditis or severe valvulitis (*RVW,* right ventricular wall; *RV,* right ventricle; *IVS,* intraventricular septum; *LV,* left ventricle; *LVW,* left ventricular wall). B, Flail mitral leaflet *(right arrow)* in a horse with multiple chordae ruptures. The prolapsed portion of the mitral valve forms a curved echodense line in the dilated left atrium (LA), whereas the other valve portions are to the left of this echocardiogram. A normal chord is evident *(left arrow).* C, Pulsed-wave Doppler study recorded from the LA demonstrating a turbulent, high-velocity, aliased systolic jet of MR in a Thoroughbred horse. The duration of the event is shown *(arrows).*

unknown, but the nonsuppurative valvulitis identified might be related to an immune-mediated process. Severe aortic insufficiency, nonrestrictive ventricular septal defects, or rarely myocarditis, cardiomyopathy, or myocardial infarction can lead to mitral insufficiency through dilation of the mitral annulus or loss of papillary muscle support.

The clinical presentation of the horse with MR varies. Mitral regurgitation is often an incidental finding detected during a routine examination. In other situations, MR might be identified in a horse with suboptimal performance or overt clinical signs of heart failure. As indicated previously, relatively soft murmurs of MR are common in high-performance horses.[70,75,208-211,586] When MR is only mild to moderate, significant left atrial dilation and LV volume overload are not evident, left atrial pressure (as estimated by pulmonary capillary wedge pressure) increases little compared with healthy controls,[354,355,639] and the horse often performs satisfactorily.[355] Two recent retrospective studies also suggest a good long-term prognosis for cases of mild MR in sport and pleasure horses[583] and in middle-aged to older horses and ponies[407] with left-sided valvular regurgitation. With moderate to severe MR, clinical signs are more likely, including poor performance, exercise-induced pulmonary hemorrhage, or overt CHF. Tolerance of the lesion depends largely on whether the horse is used for vigorous work. Some horses with MR also develop AF, which can further impair cardiac output. When a murmur of MR is identified within the setting of fever, weight loss, polyarthritis, or systemic inflammation, infective endocarditis should be considered. Mitral regurgitation related to chordal rupture is a rare but well-recognized cause of CHF, including peracute disease with fulminating pulmonary edema (see Fig. 9.10). Chronic, hemodynamically relevant MR from any cause can lead to pulmonary hypertension, AF, and biventricular CHF, with clinical signs of right-sided CHF dominating the clinical presentation (see earlier in this chapter).[48,148]

The physical examination of the horse with MR typically reveals a grade 2 to 5/6 holosystolic murmur that is detected most intensely at or dorsal to the palpable left apical impulse and over the mitral valve area (see Figs. 9.7 and 9.35 and Table 9.1). Often the murmur is loud at the aortic valve, probably related to the proximity of the septal mitral leaflet to the aortic valve or to cranial projection of the regurgitant jet. Loud MR murmurs often project quite dorsally and to the right. The typical murmur of MR is long (holosystolic or pansystolic),

extending into the second sound. This timing may cause the listener to misinterpret the third sound as the second, thus preventing a full appreciation of the significance of the murmur. When the third sound is very loud, the clinician should suspect significant volume overload or increased LV diastolic pressure. Because many horses with MR also have concomitant tricuspid regurgitation, echocardiographic examination and Doppler studies may be needed to differentiate bilateral atrioventricular valve insufficiency from isolated MR with radiation to the right. Finally, the murmur of MR can be modified by second-degree AV block, AF, or premature beats.

Two variants of holosystolic MR murmur are the early-to-mid-systolic decrescendo murmur and the mid-to-late systolic crescendo murmur. A decrescendo murmur may be detected with mild MR because coaptation of the leaflets can occur as the ventricular volume decreases during late systole. This type of murmur can be easily confused with a functional ejection murmur, unless the point of maximal murmur intensity is centered near the left apex. Conversely, severe MR with CHF could conceivably cause a decrescendo murmur because atrial and ventricular pressures may equilibrate in late systole. However, this is not a common finding and would be associated with CHF. The other variant of MR, the mid-to-late systolic crescendo murmur (see Fig. 9.35B), presumably is caused by mitral valve prolapse as it starts *after* the left ventricle has begun ejection. Decreasing LV volume predisposes to leaflet prolapse and initiates midsystolic regurgitation that builds through the second heart sound. The resultant murmur can be harsh or musical, and the novice often confuses this flow event with an early diastolic murmur.

Echocardiography including Doppler examinations play a pivotal role in the assessment of the horse with MR* (see Fig. 9.38) and is indicated to examine the anatomy of the valve apparatus (including papillary muscles, chordae tendineae, annulus, and leaflets), estimate the severity of MR, measure the size of the atria, ventricles, and great vessels, and quantify left atrial and ventricular systolic function. The underlying cause of MR may be obvious from the echocardiographic examination. Mild to moderate valvular thickening, although admittedly subjective, is compatible with degeneration or noninfective valvulitis. Prolapse of the mitral valve cusps has been observed in horses with MR, but the limits of "normal" prolapse require further definition. Lesions due to vegetative endocarditis cause the valves to appear irregularly thickened or shortened. In cases of acute endocarditis, there can be evidence of valve thrombus, and a high-frame-rate real-time examination may show oscillation of this tissue. Small, focal lesions are more commonly observed on the atrial surface of the valve that is facing blood flow (see Fig. 9.45B later in this chapter). In chronic endocarditis, the valve may be more echodense or even appear calcified. Chordal rupture is recognized by observing chaotic flutter of a mitral structure (a flail leaflet), prolapse of a large portion of the valve into the atrium, or the contracted chordal remnant flipping into the atrium during systole (see Fig. 9.38B). High-frequency systolic vibrations of the mitral valve may be seen on the M-mode study in horses with a musical murmur of MR. Pulsed- or continuous-wave or color Doppler studies can identify the mitral regurgitant jet (see Fig. 9.38C). The Doppler examination should be performed from both the right- and left-sided thoracic windows. High-velocity or turbulent jets may be difficult to find without a complete and thorough examination of the mitral valve that involves multiple image planes. When color-flow Doppler demonstrates both a wide origin of the regurgitant jet and a pattern of diffuse distribution of turbulence deep into the left atrium, the likelihood of hemodynamically relevant MR is greater. However, receiving chamber (i.e., left atrial) color coding in MR should not be overemphasized because red blood cell entrainment and "spray effects" can result in overestimation of MR severity, whereas wall-hugging jets underestimate severity of MR. Therefore quantitation of cardiac size is instrumental in assessing MR severity. With severe MR and LV volume overload there will be rounding of the LV apex and increased end-diastolic LV dimension. Global LV function may appear normal to exuberant (hyperdynamic) because ventricular preload is increased and afterload decreased in severe MR. However, when MR is both severe and chronic, or if the underlying basis for MR is cardiomyopathy, the ventricular systolic function is normal to decreased. The left atrium often assumes a more circular, almost turgid, appearance when MR is hemodynamically important, and the 2D echocardiographic measure of maximum internal LA dimensions measured from a right-parasternal long-axis view at end-systole often exceed 13.5 cm (for the widest LA diameter parallel to the mitral valve annulus) and 105 cm^2 (for maximum LA area), respectively, in large-breed 500-kg horses.[355,576,689] With acute or chronic MR, the lobar and main PA may be dilated as a consequence of pulmonary hypertension, presumably related to increased LA pressure, interstitial lung edema, vascular remodeling, or other factors.

The prognosis for horses with MR is variable and, as discussed earlier, is related to clinical findings, work history, exercise testing, and results of echocardiographic studies.[68] In one study, MR was the most common valvular disease associated with AF, clinically important ventricular arrhythmias, and CHF.[83] Abnormalities observed during echocardiography, including lesions of the mitral valve leaflets, the degree of LA and LV volume overload, global LV function, and Doppler findings, should be considered when formulating the prognosis. Certainly, when MR is associated with CHF, AF, endocarditis, chordal rupture, marked cardiomegaly, severe valvular thickening, dilated cardiomyopathy, or pulmonary hypertension, the prognosis for life and performance is poor.[148] In the absence of right-sided heart catheterization, increased TR velocity and PA dilation are used as surrogates for the identification of PHT. The detection of PA dilation indicates significant pulmonary hypertension and the low but concrete possibility of PA rupture associated with exercise. Fortunately, the vast majority of horses with MR appear to perform very well, indicating that MR in most cases is not severe enough to be clinically important.[74,355,583] Whether progressive exercise intolerance will develop in a particular case depends on the horse's use and on the progression of the underlying lesion. Generally, when MR is caused by valve degeneration and the heart size is normal, the progression is gradual, the prognosis for life is favorable, and performance is maintained.[74,390,407,583,862] When MR is detected in an untrained colt or filly, the prognosis is less encouraging. The presence of even mild to moderate cardiac dilation in a case of MR recommends a more guarded prognosis, though this assessment is best made by serial examinations. In any case, LA dilation increases the risk for AF. The clinical relevance of trivial to mild MR in the high-performance horse or racehorse is uncertain. In some animals, treadmill exercise is normal whereas

* References 56, 71, 74, 75, 148, 208, 344, 390, 391, 560, 576.

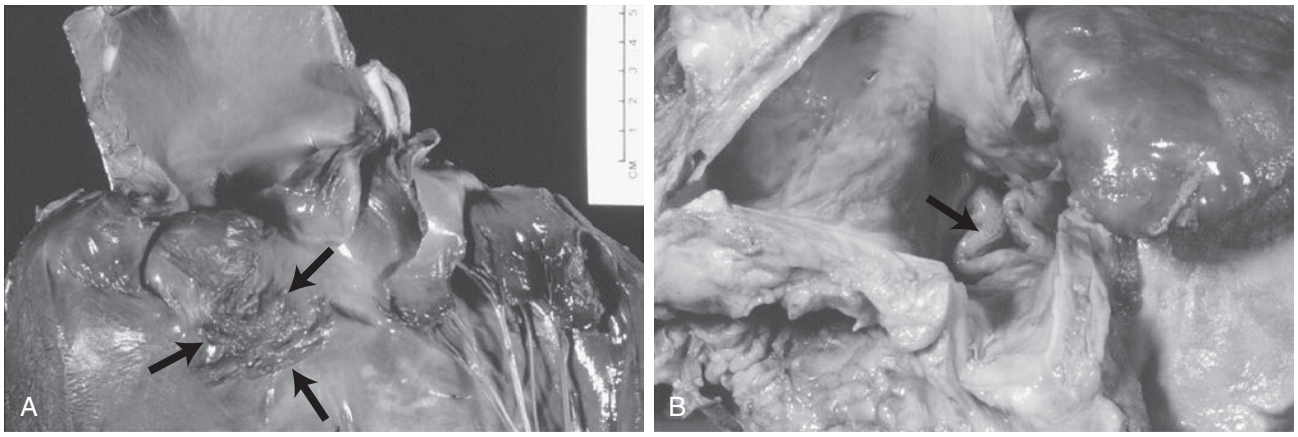


FIG. **9.39** Postmortem lesions of aortic regurgitation (AR; see also Fig. 9.43B). A, A segment of the left ventricular (LV) outflow tract and the ascending aorta. Linear bands are evident on the two valves shown, and a large jet lesion also is visible below the valves between the *arrows*. The lesion is typical in aged horses. B, The aortic valve *(arrow)* viewed from the ascending aorta. Noninfective valvulitis and scarring have caused severe thickening.

others may demonstrate a higher HR for a given level of work than might otherwise be expected. The latter finding may be suggestive of a cardiac limitation to performance.

Regardless of cause or the severity of the condition, the horse diagnosed with MR merits follow-up examinations at least yearly, if not more often, to evaluate progression of the hemodynamic burden and to detect the development of CHF, pulmonary hypertension, or cardiac arrhythmias such as atrial fibrillation (see later discussion).[68] HR and heart rhythm should be monitored on a regular basis with moderate to severe MR. Exercise testing including stress ECG to assess HR response to exercise and occurrence of exercise-induce arrhythmias should be performed (1) in all horses with moderate to severe MR; (2) if AF becomes established (see later discussion); or (3) if MR progresses more rapidly than expected, in the absence of signs of CHF.[68] Treatment of MR involves management of complications of advance disease such as heart failure, endocarditis, or arrhythmias. Limited data have been published about the use of ACE inhibitors in horses (see Therapy for Congestive Heart Failure), and no consensus exists among experts regarding their use in MR in the absence of CHF.[68] These topics are covered elsewhere in this chapter.

Aortic Regurgitation

Aortic regurgitation (AR) is a common valvular insufficiency in horses.[68,83,407] Degeneration of the aortic valve is by far the most common reason for AR.* The degenerative nodular lesions and fibrous bands responsible for AR in older horses have been well described[5] (Fig. 9.39A). Prolapse of the aortic valve is a common echocardiographic finding and probably represents another manifestation of connective tissue degeneration affecting the valve.[194,577] Small fenestrations of the valve also have been identified at necropsy but have uncertain clinical relevance. Other potential causes of AR in horses include infective endocarditis,† congenital valvular disease,[179,189,794] VSD (see earlier in this chapter), noninfective valvulitis, leaflet tearing, and ruptured aortic sinus aneurysm.[34,262-264,777,864-867]

In most cases, AR is an incidental finding encountered during a routine physical, prepurchase, or insurance examination. Most horses with this murmur are older than 10 years of age, and the murmur is especially common in aged horses, a testament to the degenerative nature of the lesion. Careful auscultation in a quiet area may identify a very soft diastolic murmur in a younger horse. Silent AR may also be considered physiologic or trivial, and it is not uncommon to identify a trivial jet of AR by Doppler studies in horses with no identifiable diastolic murmur.[71] Poor performance is an infrequent presenting complaint in horses with AR, and most will continue at their prior performance level, provided that no other clinical or cardiac abnormalities are present. Intermittent fever, weight loss, or lameness should prompt consideration of endocarditis. CHF is infrequently observed in conjunction with isolated AR but may develop in the horses when AR occurs in combination with MR or AF.

Clinically important AR is identified by cardiac auscultation, which reveals a holodiastolic murmur, with the point of maximal intensity over the aortic valve area, and strong radiation to the right and toward the left cardiac apex in many instances (see Table 9.1). The murmur may vary greatly in intensity and character.[18,72,868] The quality is typically harsh and decrescendo with a blowing nature (see Fig. 9.9C), but there may be presystolic accentuation (see Fig. 9.35C). The character can be vibratory, musical, cooing, buzzing, or "dive-bomber" in quality. A precordial thrill is palpable over the aortic valve area when the murmur is loud. A variant of the typical AR murmur is that associated with rupture of an aortic sinus into the right atrium, ventricular septum, or pulmonary artery. An aortic-cardiac fistula leads to a holodiastolic or continuous murmur that is louder over the right side of the thorax.[262-264] However, in the majority of horses with an aortic-cardiac fistula, a continuous machinery-type murmur is detected. A systolic ejection murmur is often present in addition to the typical diastolic murmur of AR, particularly when the regurgitant volume is large, and is explained by ejection of a large stroke volume across the aortic valve. It should be emphasized that there is no evidence for anatomic stenosis due to degenerative aortic valve disease.

* References 5, 28, 29, 57, 83, 169, 191, 406, 843, 850, 863.
† References 194, 195, 205, 554, 852, 853, 855.

The quality of the arterial pulses is a good indicator of the severity of the isolated AR.[406, 554] Bounding, hyperkinetic arterial pulses, usually corresponding to increased pulse pressures (greater than 60 mm Hg), indicate moderate to severe AR and significant (but compensated) LV volume overload.[406] If the myocardium is failing, the arterial pulses will become weak, the pulse pressure will decrease, and tachycardia will develop.

A complete echocardiographic examination including Doppler echocardiography is useful for further evaluation of horses with AR, particularly when a louder (i.e., grade 3–6/6) murmur is identified, the arterial pulse is abnormal, or cardiac-related clinical signs are suspected.* The most common abnormality observed by 2D echocardiography is mild valvular thickening in association with prolapse of one or more aortic leaflets (Fig. 9.40). Fibrous bandlike lesions appearing as an echoic line parallel to the free edge of the left coronary leaflet or, less often, nodular thickening or a generalized increase in echogenicity of the leaflet's free edge are detected.[68,554] These findings are compatible with degenerative valvular disease. Care should be taken not to overdiagnose the presence of aortic valve prolapse, because prolapse can easily be mimicked by ultrasound probe malignment.[577] High-frequency fluttering or vibration of the aortic valve leaflets or aortic walls may be seen, most commonly in horses with musical or vibratory murmurs. The 2D echocardiographic examination also assists with the differential diagnosis. In cases of endocarditis, the leaflets may appear thickened, irregular, and more highly echogenic. The valve may appear to oscillate if there is fresh thrombus within the vegetation. Other rare 2D echocardiographic findings related to AR include fenestrations of the aortic valve leaflets, flail aortic leaflet, aortic sinus aneurysm, and aortic root prolapse into a VSD. Dilation of the aortic root (exceeding 10 cm) may be observed in some horses with AR. A recent study described echocardiographic assessment of the rate of diastolic decrease in aortic diameter, which is associated with the rate of diastolic runoff of blood from the aorta and may serve as an indicator of AR severity.[602]

Left ventricular volume overload that develops with AR can be detected on 2D and M-mode echocardiography and is characterized by increased end-diastolic LV dimensions, decreased relative wall thickness, and increased stroke volume.[554,576,602,849] An exaggerated or swinging septal motion on 2D or M-mode examination indicates a hyperkinetic LV and represents another subjective observation of compensated volume overload (see Fig. 9.40). Left-ventricular shortening fraction and ejection fraction might be high-normal or increased.[554,576,602,849] If myocardial failure develops, septal motion will diminish, shortening fraction and ejection fraction will decrease, and the end-systolic LV dimensions will increase. Inspection of the mitral valve often reveals high-frequency diastolic vibrations of the septal (anterior) leaflet related to an eccentric high-velocity AR jet directed across the LV outflow tract to the left and caudally. Similar vibrations may also be detected on the interventricular septum when the regurgitant jet is oriented in a right or cranial direction. Increased mitral valve E-point to septal separation (EPSS) can indicate that the regurgitant jet is impinging on the valve or herald LV dilation and failure. In the latter case, other markers of severe insufficiency will be evident, including ventricular dilation, rounding of the LV apex, and a wide origin jet of AR on Doppler studies. Premature (presystolic) mitral closure is an uncommon but ominous finding of severe AR with elevated ventricular end-diastolic pressure.

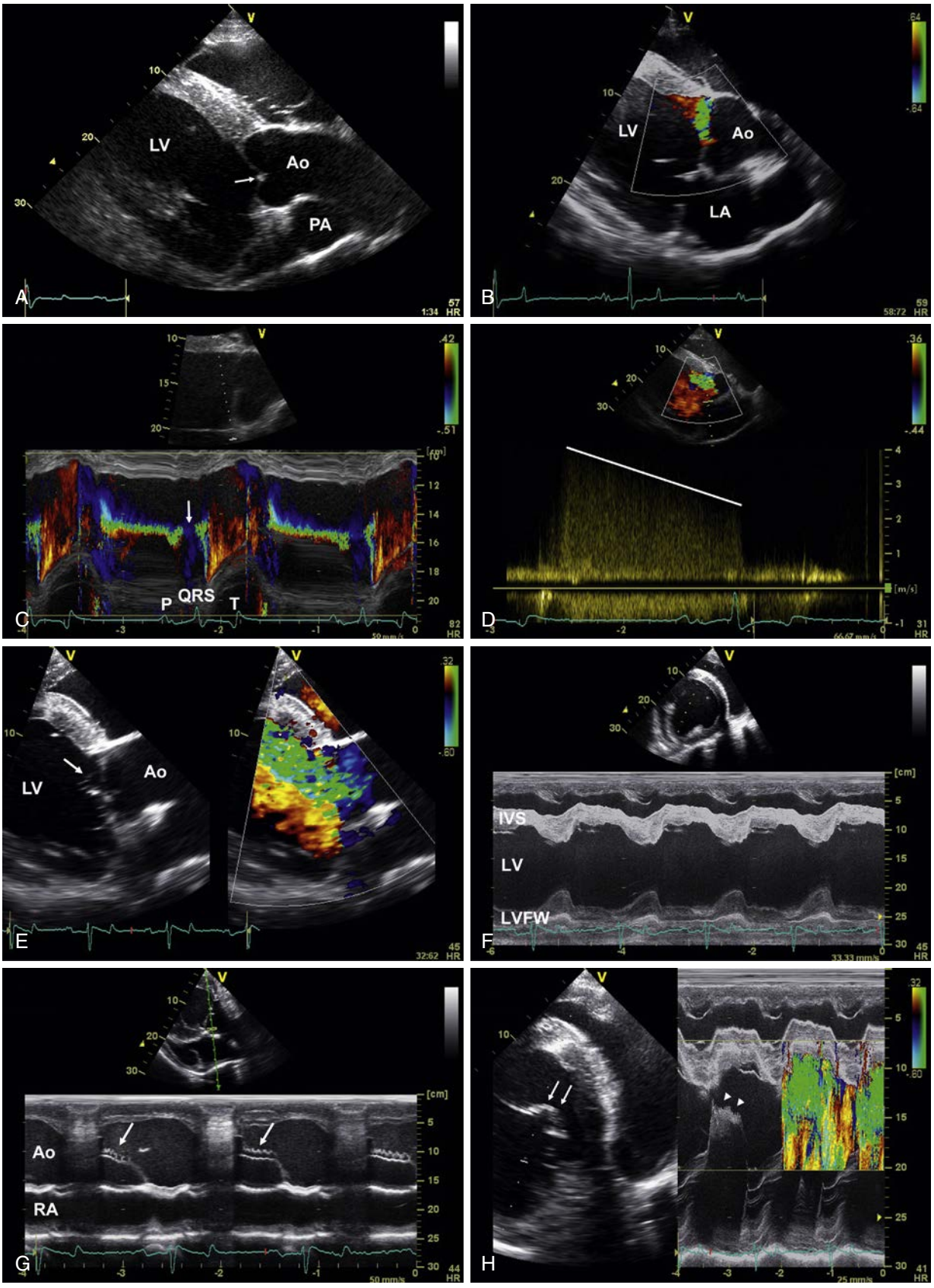
The flow disturbance of AR is confirmed by Doppler studies. The color Doppler examination shows one or more central or eccentric diastolic jets of AR. The timing of the flow disturbance can be verified by spectral Doppler or color-M-mode studies. Assessing the severity of AR by Doppler studies is ripe with pitfalls, and the examiner should use multiple 2DE, M-mode, and Doppler findings to assess severity. Importantly, a wide jet area in the LV outflow tract does not always indicate severe AR. The finding of a small, central perivalvular jet, with a small cross-sectional area, suggests trivial AR. Often these leaks are confined to mid-to-late diastole. Conversely, a strong spectral signal, holdodiastolic timing, and wide-origin color Doppler signal suggests more significant regurgitation. Examination of the short-axis jet area in cross-sectional studies of the aortic root is especially instructive because a wide ventricular spray is often associated with a small origin jet: this suggests mild AR. The CW Doppler spectrum can be assessed for a short pressure half-time (steep AR slope), which indicates LV diastolic pressure is rapidly increasing and that AR is severe. However, the examiner must be certain the ultrasound beam has remained in good and consistent alignment with the regurgitant jet before this assessment can be made.

The clinical relevance and prognosis of AR is most accurately based on the performance history, physical examination, and echocardiogram. As most cases of AR are associated with a slow degeneration of the aortic valve leaflets, and this occurs in older horses without other cardiac problems, the prognosis for life and performance is usually good. Such animals typically have minimal echocardiographic abnormalities or only mild echocardiographic signs of volume overload that are unchanged at follow-up examinations. Epidemiologic studies suggest that in general AR is mild in most cases and, when taking age into account, is not an independent predictor of mortality.[407] However, when AR is moderate to severe or first recognized in a younger horse (less than 10 years of age), the risks for reduced performance life and longevity are higher.[68] The detection of hyperkinetic arterial pulses or a pulse pressure of greater than 60 mm Hg suggests that progression of AR is likely.[68,406] The findings of flail aortic valve leaflet, endocarditis, moderate to severe LV volume overload, or myocardial failure indicate a poor prognosis for life and performance. Concurrent MR and LA enlargement should be anticipated in horses with severe volume overload and increase the risk of atrial fibrillation, pulmonary hypertension, and CHF.

Ventricular dilation can predispose to ventricular arrhythmias. Sudden cardiac death associated with fatal ventricular arrhythmias has been observed in horses with moderate to severe AR and can occur in isolation, without a history of poor performance or CHF.[68] Therefore an exercising ECG and exercise test should be obtained when moderate to severe AR and volume overload are present or when performance issues are evident, to determine whether the horse remains safe to ride.[68] Electrocardiographic evaluation should be focused on identifying exercise-induced premature ventricular complexes and appropriateness of the exercising HR. A Holter ECG can be considered as a potential test to further identify and quantitate ventricular ectopy at rest.[68]

Follow-up examinations including echocardiography and ECG exercise test are indicated for the horse with moderate to severe AR. Affected horses should be reexamined twice yearly and at least annually thereafter if progression has been

* References 68, 344, 391, 406, 554, 576, 602, 849, 869.


A
LV
Ao
PA
B
LV
Ao
LA
C
P
QRS
T
D
E
LV
Ao
F
IVS
LV
LVFW
G
Ao
RA
H

minimal. Longer follow-up intervals are appropriate for horses with mild AR after the first reevaluation.[68] If AF develops in a horse with mild to moderate AR, a reexamination is indicated at that time, including an ECG exercise test. HR and heart rhythm should be monitored on a regular basis in cases of moderate to severe AR; an increased resting HR or an irregularly irregular rhythm suggesting atrial fibrillation or frequent ventricular arrhythmias indicates progression of disease.[68] The detection of exercise-induced ventricular arrhythmias is considered an important negative prognostic indicator; these horses are considered less safe to ride or drive than their age-matched peers.[68] Generally, horses with severe AR should not be ridden or driven by a child, be used as a lesson horse, or participate in a high-risk sport owing to a risk for sudden cardiac death.[68] Limited data have been published about the use of ACE inhibitors in horses (see Therapy for Congestive Heart Failure) and no consensus exists among experts regarding their use in AR in the absence of CHF.[68] These topics are covered elsewhere in this chapter.

Tricuspid Regurgitation

Tricuspid regurgitation (TR) may be the most frequently detected flow disturbance and murmur in the horse.* The prevalence and color Doppler severity of TR increases with age, training, and subtle physiologic increases in RV dimensions, and murmurs consistent with TR are common in horses of racing age.[70,75,208,209] However, the anatomic correlate to this incompetency is uncertain, and there is little information confirming the etiopathogenesis of this condition aside from speculation that it might be related to recurrent physiologic pulmonary hypertension of high-intensity exercise. Tricuspid valve incompetency can further result from any of the following causes: degenerative thickening, prolapse, noninfective tricuspid valvulitis, bacterial endocarditis, ruptured chordae tendineae, pulmonary hypertension (caused by left-sided failure or severe respiratory disease), myocardial disease with secondary cardiomegaly, chronic tachyarrhythmia, and congenital malformation of the valve. Degenerative, fibrotic thickening of the tricuspid valve in mature horses may lead to mild to moderate TR. Endocarditis of the tricuspid valve is in many cases associated with septic thrombophlebitis secondary to jugular venipunctures or catheterization. Compared with the mitral condition, ruptured tricuspid valve chordae tendineae are uncommon and better tolerated unless associated with endocarditis. Pulmonary hypertension, cardiomyopathy, and myocarditis can lead to secondary dilation of the tricuspid annulus or alteration of papillary muscle support, permitting valvular insufficiency. Tricuspid malformation does occur but seems more commonly associated with stenosis or atresia of the valve (see earlier in this chapter).†

Auscultation of the horse with TR typically reveals a grade 2/6 to 5/6 holosystolic murmur with the point of maximal intensity over the right hemithorax at the tricuspid valve area (see Table 9.1). The murmur can be holosystolic, decrescendo, or mid to late systolic. Although the timing and intensity may at times remind the examiner of a functional murmur, the location of greatest murmur intensity argues against that possibility. The murmur usually radiates dorsally and if loud to the extreme left cranioventral thorax. The intensity of the TR murmur in many cases does correlate well to the regurgitant volume, but loudness also depends on PA and RV systolic pressures. A soft, grade 2 to 3/6 systolic murmur of TR is most often an incidental finding detected during a routine examination. The majority of horses with a soft TR murmur perform well; therefore the clinician should first exclude other likely reasons for poor performance before incriminating the tricuspid valve. The murmur is usually more problematic when it is loud. In general, a grade 4 or louder murmur is anticipated

* References 69, 71, 72, 75, 83, 191, 208, 209, 280, 281, 391.

† References 171, 177, 178, 180, 182, 405, 786, 828, 839.

FIG. **9.40** Aortic regurgitation. A, Right-parasternal long-axis view obtained from a 15-year-old Thoroughbred gelding with aortic regurgitation. Nodular thickening of the aortic valve cusps is evident *(arrow).* B, Color Doppler echocardiogram in a right-parasternal long-axis view obtained from a 15-year-old Warmblood mare with aortic regurgitation. The diastolic regurgitant jet is visible as an area of turbulent flow that appears to be directed toward the interventricular septum. Based on receiving chamber analysis, the severity of the regurgitation would be graded as mild. C, Color M-mode echocardiogram in a right-parasternal long-axis view obtained from a horse with aortic regurgitation. The cursor line is placed immediately below the aortic valve *(top).* An ECG is recorded simultaneously for timing. This imaging mode is particularly useful for timing of flow events and identifying brief regurgitant signals or normal valve closure noise. In this case an aortic regurgitant jet is visible as a turbulent flow pattern, starting at the beginning of diastole (i.e., after the T wave) and ending at the onset of systole (i.e., immediately after the QRS complex). Notice the absence of turbulent flow during the PQ-interval *(arrow).* This can be explained by a change in left ventricular pressure occurring after atrial contraction or a reorientation of the regurgitant jet relative to the cursor owing to translational movement of the heart. D, Continuous-wave Doppler recording of a diastolic aortic regurgitant jet from a right-parasternal long-axis view. The absolute velocities are not accurate in this recording because of lack of adequate alignment with blood flow. However, this recording can be used for timing of flow events (an ECG is recorded simultaneously for timing). Furthermore, the change in jet velocity (which can be expressed as "pressure half-time") reflects the rate of decline in pressure gradient between the aorta and the left ventricle and may be useful to assess the severity of regurgitation as long as ventricular relaxation is normal; a relatively flat or gradual slope of the velocity envelope *(white line)* indicates mild aortic regurgitation (AR), a steep slope indicates severe AR. E–H, Echocardiogram of a 21-year-old Hanoverian Warmblood gelding with severe aortic regurgitation and left ventricular volume overload. E, B-mode *(left)* and color Doppler *(right)* echocardiogram of the left ventricular outflow tract in a right-parasternal long-axis view. The aortic valve cusps are irregularly thickened, and aortic valve prolapse is evident during diastole *(arrow).* A large regurgitant jet is visible in the left ventricular outflow tract during diastole *(right panel).* F, M-mode echocardiogram of the left ventricle (LV) in a right-parasternal short-axis view demonstrating LV enlargement and hyperdynamic motion of the interventricular septum (IVS). The LV fractional shortening was 46%. G, M-mode echocardiogram of the aortic valve in a left-parasternal long-axis view. Notice the high-frequency vibrations of the aortic cusps during diastole *(arrows)* caused by the regurgitant blood flow. H, Two-dimensional *(left),* M-mode *(middle),* and color M-mode *(right)* echocardiogram of the mitral valve in a right-parasternal short-axis view. Notice the prolapse *(arrows)* and the high-frequency vibrations *(arrowheads)* of the septal leaflet of the mitral valve, caused by rapid, turbulent regurgitant flow in the left ventricular outflow tract during diastole. *Ao,* Aorta; *LA,* left atrium; *LV,* left ventricle; *LVFW,* left ventricular free wall; *PA,* pulmonary artery; *RA,* right atrium. (From Schwarzwald CC: Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, et al.: *Atlas of equine ultrasonography,* Wiley, Oxford, UK, 2014. Reproduced with permission of John Wiley & Sons, Ltd.)

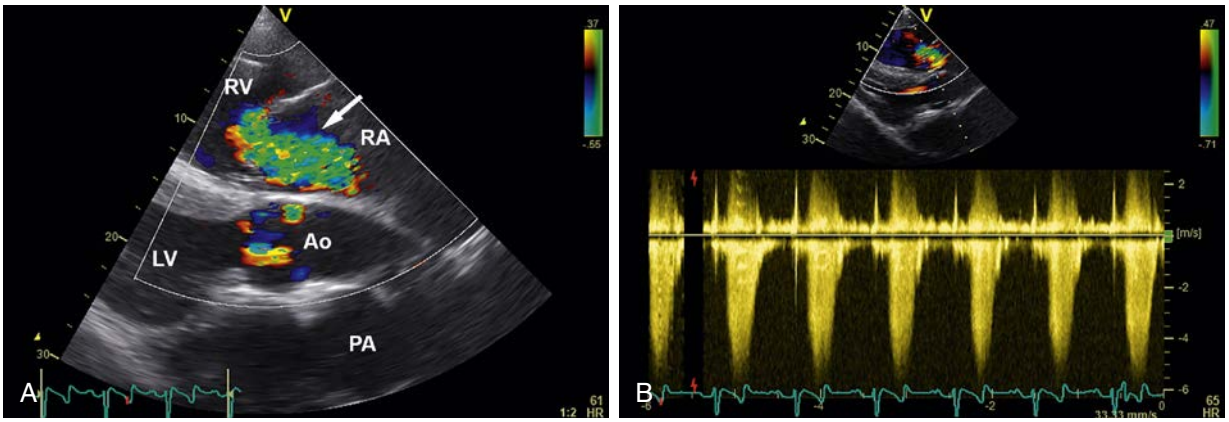


FIG. **9.41** Tricuspid regurgitation (TR) and pulmonary hypertension (PHT) in a 6-year-old Friesian mare with severe mitral regurgitation and congestive heart failure. A, Two-dimensional (2D) color flow Doppler echocardiogram demonstrating marked tricuspid regurgitation *(arrow)*. The right atrium (RA), the right ventricle (RV), and the pulmonary artery (PA) appear enlarged compared with the left ventricle (LV) and the aorta (Ao). B, Continuous-wave Doppler recording of the TR jet. The maximum jet velocity is 4.8 m/sec, corresponding to an RV-to-RA pressure gradient of 92 mm Hg (modified Bernoulli equation: $dp = 4 \times v_{max}^2$). In the absence of RV outflow tract obstruction, the high-velocity TR jet indicates severe pulmonary hypertension. TR jets associated with normal RV pressures are typically less than 3.2 m/sec. An ECG is recorded simultaneously for timing.

when there is moderate to severe TR or when TR is related to pulmonary hypertension. Atrial fibrillation or atrial premature beats are present in some horses with TR, particularly when the right atrium is dilated or the regurgitant jet is large. If chronic, hemodynamically significant TR has developed, peak work effort will suffer and even right-sided CHF may develop. The latter is very uncommon unless the TR is severe, related to pulmonary hypertension or infective endocarditis, or complicated by AF. Prominent jugular pulses (giant c-v waves; see Fig. 9.4) are typical of horses with TR associated with CHF.

Echocardiographic examination is mainly indicated in horses with a grade 4 to 6/6 right-sided systolic murmur compatible with TR in the setting of poor performance, concurrent thrombophlebitis, or fever of unknown origin.[68] However, an echocardiogram may also be performed in horses with softer murmurs in the setting of a prepurchase examination or if the cause or clinical relevance of the murmur is unclear. The tricuspid valve is typically examined from the right side of the thorax, because the tricuspid valve and RV inlet are closer to the right thoracic wall (Fig. 9.41). However, an extreme left cranial transducer location with caudal angulation can also be successful for examining the tricuspid valve. Because there are a number of potential reasons for tricuspid incompetency, careful attention must be directed to the valve and its support apparatus, the size of the PA, and the left side of the heart. In the vast majority of cases, with benign or training-related TR, a clear-cut lesion of the tricuspid valve leaflet is not evident, and the RA and RV are normal in size. With clinically significant TR, there might be structural or motion abnormalities of the TV originating from valve thickening, prolapse, vegetations, chordal rupture, regurgitation secondary to RV dilation, and pulmonary hypertension. As with mitral regurgitation, moderate to severe TR leads to RA and RV volume overload; however, it is more difficult to quantify these chamber volumes owing to their complex geometry.[70,344,573]

Doppler studies can identify the tricuspid regurgitant jet. In trivial or "silent" TR, this jet is typically very narrow at the origin and directed toward the aorta. However, when the jet is wide at its origin, occupies a larger area in the RA, or projects centrally or laterally into the right atrium, cardiomegaly is more likely to be present, and the heart should be examined carefully. The RV (and PA) systolic pressure can be estimated by faithfully recording the peak jet velocity. This requires the examiner to align the CW Doppler cursor parallel to regurgitant flow and may demand a ventral or dorsal placement of the transducer with steep beam angulation. When the jet velocity is less than 2.5 m/sec, pulmonary hypertension is not present. Jets exceeding 3.2 to 3.4 m/sec are indicative of pulmonary hypertension (provided that there is no right ventricular outflow obstruction or VSD; see Fig. 9.41B). The identification of pulmonary hypertension should prompt careful examination of the left side of the heart, because the main cardiac lesion may be centered over the mitral valve, aortic valve, or LV cavity and the right-sided heart changes simply a secondary consequence. If left-sided heart disease can be excluded as a cause for pulmonary hypertension, severe pulmonary disease must be considered, and appropriate diagnostics should be performed.

The prognosis for horses with TR is generally very favorable. A soft murmur of TR in a trained athlete is disregarded as clinically irrelevant; however, defining such a disturbance as purely "functional" would be imprecise. The presence of TR is more likely to cause concern if right-sided cardiomegaly, severe MR, pulmonary hypertension, or AF is evident. Visualization of a vegetation or chordal rupture or clinical signs of CHF would indicate a poor prognosis for performance and a guarded to poor prognosis for life. The width of the regurgitant jet at its origin, recent performance history, and results of exercise testing are useful in developing a prognosis for life and future work. When TR is judged to be moderate or severe, annual reexaminations are indicated to follow the progression of the lesion and to detect cardiomegaly or cardiac arrhythmias if they develop.[68]

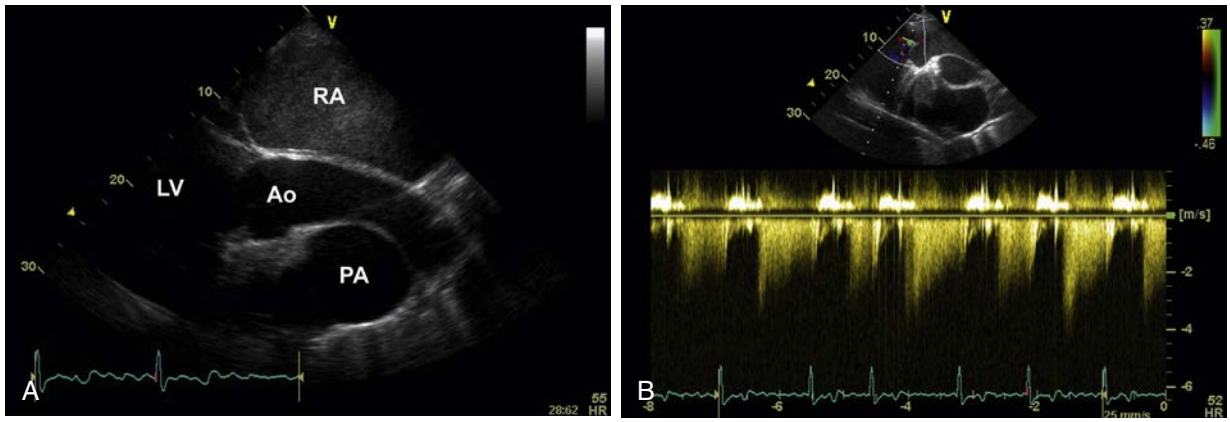


FIG. **9.42** Pulmonic insufficiency and pulmonary hypertension. A, Right-parasternal view of the left ventricular outflow tract obtained from an 11-year-old Paint gelding with severe mitral regurgitation, atrial fibrillation, and congestive heart failure. Enlargement of the right atrium (RA) and severe pulmonary artery (PA) dilation are evident, consistent with pulmonary hypertension. Notice also the marked spontaneous echo contrast (SEC) in the right atrium. B, Continuous-wave Doppler recording of pulmonic insufficiency in a left-parasternal long-axis view of the right ventricular outflow tract, showing diastolic regurgitant flow. The varying peak velocities can be explained by differences in cycle lengths (due to atrial fibrillation), motion of the heart (and the regurgitant jet) relative to the cursor line, and possibly respiratory variations. The highest maximum early-diastolic velocity in this spectral Doppler recording was 3.5 m/sec, corresponding to a transvalvular pressure gradient of 49 mm Hg (modified Bernoulli equation: $dp = 4 \times v_{max}^2$). Assuming a right atrial pressure of 5 mm Hg, the estimated mean pulmonary artery pressure (MPAP) would be at least 54 mm Hg, indicating marked pulmonary hypertension. *Ao,* Aorta; *LV,* left ventricle.

Pulmonic Regurgitation

Trivial and clinically silent, physiologic pulmonic regurgitation (PR) can often be detected by Doppler studies; however, this is a normal finding.[71,209,280,281,391,560] Clinically relevant PR is rare and occurs most frequently with pulmonary hypertension associated with left-sided heart failure or severe respiratory disease. Infective endocarditis, congenital abnormalities of the valve leaflets (bicuspid or quadricuspid valve), and rupture of the pulmonic valve are rare causes of pulmonic insufficiency.[200,730] Murmurs of PR are usually undetectable unless the regurgitant volume is large or is driven by pulmonary hypertension; however, the regurgitant flow can be easily identified using pulsed-wave and color-flow Doppler echocardiography (Fig. 9.42). Dilation of the PA may be detected echocardiographically when pulmonic insufficiency is caused by pulmonary hypertension (see Fig. 9.42A).

When severe pulmonic regurgitation does develop, it is generally a consequence of left-sided heart failure with pulmonary hypertension and accompanied by clinical signs of biventricular failure. Signs of right-sided CHF typically stem from the combination of pulmonary hypertension, pulmonic regurgitation, RV volume overload, and TR. If a murmur of pulmonic regurgitation is detected, it is usually holodiastolic and a decrescendo murmur, with the point of maximal intensity at the pulmonic valve area, radiating toward the right cardiac apex (see Table 9.1). The prognosis for life and performance is usually poor in these cases.

Infective Endocarditis

Infective (bacterial) endocarditis is caused by invasion of the heart valves or endocardium by bacteria. Endocarditis is not common in horses but occurs sporadically in most populations.* Horses of any age may be affected, although the pathogenesis may differ in younger animals or in those that are immunosuppressed. In two reports, the mean age of affected horses was 2.1 and 4.8 years, respectively.[205,855] Numerous bacteria have been associated with bacterial endocarditis. The offending microorganism likely depends on the environment, portal of entry (e.g., respiratory tract, gastrointestinal tract, skin, oral cavity, joint, surgical wound, or intravenous catheter), and the effects of prior antimicrobial therapy that may select for resistant strains. *Streptococcus* spp., *Actinobacillus equuli,* and *Pasteurella* spp. have been isolated most frequently, but *Pseudomonas* spp., *Escherichia coli, Corynebacterium* spp., *Bacillus* spp., *Rhodococcus equi, Erysipelothrix rhusiopathiae,* meningococci, *Staphylococcus* spp., *Candida parapsilosis,* and other organisms (including aspergillosis) have also been reported, with no organism appearing more prevalent than the others.[872]

The pathogenesis involves bacterial invasion from the bloodstream and colonization of the heart valve or endocardial surfaces. Bacteremia is a prerequisite for development of this condition. Direct invasion of a previously normal valve by virulent bacteria, or in the context of overwhelming sepsis, represents the most likely pathogenic mechanism involved in foals with infective endocarditis. Disruption of the endocardial surface by jet lesions associated with congenital intracardiac shunts may also predispose to endocarditis, although this mechanism is not well established in horses. Preexisting valvular heart disease with endocardial changes or high-velocity jets may represent risk factors for bacterial colonization in older horses, but again this is completely unproven.

The most common sites of infective endocarditis are the aortic and mitral valves, although endocarditis lesions have been reported on all cardiac valves. Mural and chordal endocarditis lesions have also been reported but occur much less frequently. The combination of bacterial injury, exposure of

* References 31, 191, 193-206, 824, 852-860, 870, 871.

valve collagen, thrombosis, and host leukocyte response contributes to development of the vegetation (Fig. 9.43), which consists microscopically of bacteria, platelets, fibrin, leukocytes, and varying degrees of granulation or fibrosis. Bacteria may not be evident at necropsy, especially if antimicrobial therapy has sterilized the vegetation.

The pathophysiology of endocarditis in horses is probably similar to that in other species. A host response, as well as the primary cardiac lesions, contributes to the morbidity of this disease. Cardiac manifestations include valvular injury leading to regurgitation, chordal rupture, or rarely to stenosis; secondary cardiomegaly; myocarditis from extension of the infection or through coronary embolization; myocardial infarction if emboli are shed to the coronary arteries; arrhythmias from cardiomegaly, myocarditis, or infarction; and myocardial depression from bacteremia. Recurrent or chronic bacteremia, and hence fever, is characteristic of endocarditis. Metastatic infection, distant thrombosis and infarction, and immune-mediated host responses can occur. Distant infection or immune complex disease can lead to multisystemic clinical signs, including polyarthritis, osteomyelitis, vasculitis, or nephritis. Right-sided thrombi can lead to pulmonary thrombi and/or abscessation of the lungs (see Fig. 9.43).[193, 200]

Clinical features of endocarditis are variable.[205, 855] Affected horses usually have a history of intermittent fever, weight loss, depression, anorexia, lethargy, and often intermittent lameness (Fig. 9.44). Synovial distention may be noted. A predisposing condition or a concurrent infection may be evident, including jugular vein thrombophlebitis, strangles, septic joint, or an abscess. In most horses there is no history of previous illness and no evidence of concurrent infection. The physical examination often reveals fever, and some horses may be tachypneic. The fever is often intermittent. Murmurs of mitral or aortic valvular insufficiency are most commonly detected; those of TR are less common. Systolic murmurs caused by valve destruction must be differentiated from the physiologic flow murmurs that are heard so often in febrile horses. Murmurs of valvular stenosis may also be detected with bacterial endocarditis but occur rarely.[200] Some horses with bacterial endocarditis have no auscultable murmur initially. The quality or intensity of the murmur may change over a number of days. Atrial fibrillation, atrial or ventricular premature depolarizations, and ventricular tachycardia have been observed with endocarditis.[199]

Laboratory studies obtained from horses with infective endocarditis may reveal anemia (related to chronic inflammatory disease), hyperproteinemia (hyperglobulinemia with

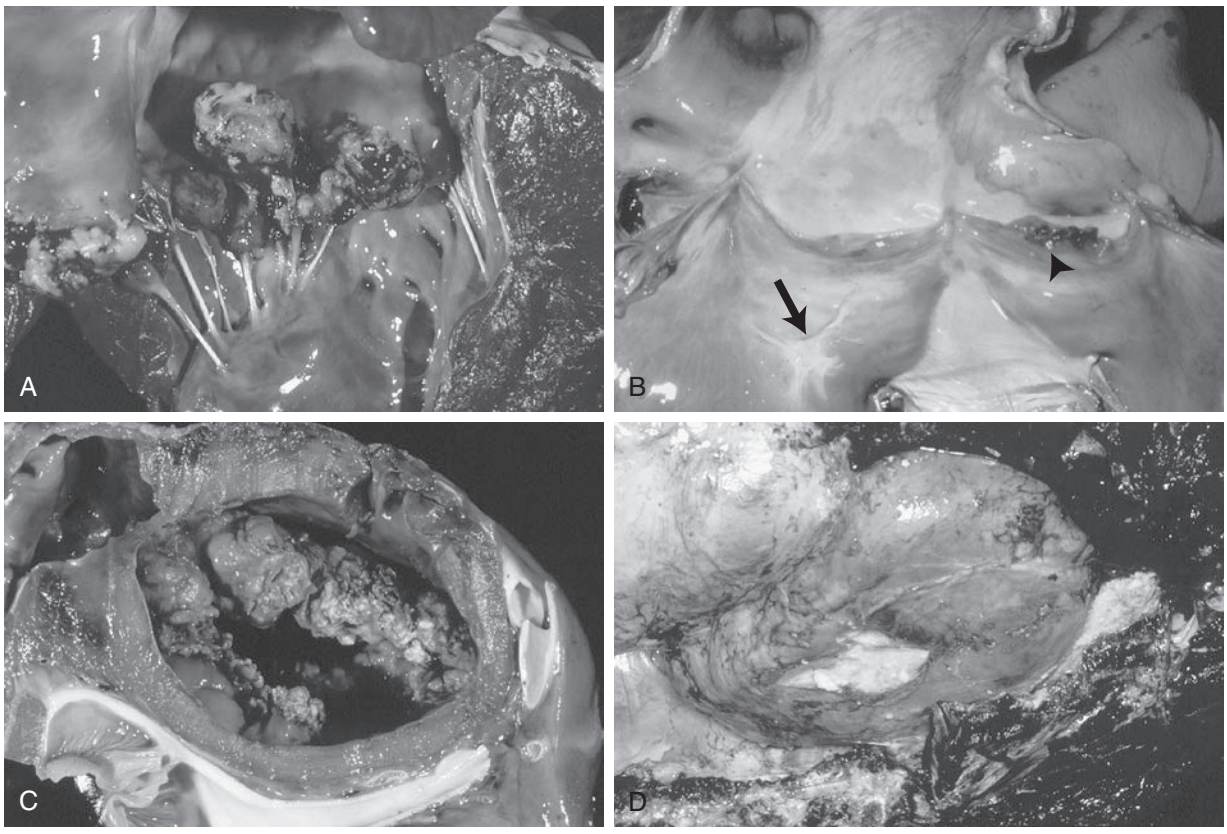


FIG. **9.43** Bacterial endocarditis: postmortem lesions. A, Severe mitral valve endocarditis in a yearling. B, Focal aortic valve endocarditis is evident in this view of the left ventricular (LV) outlet and the ascending aorta. The septal mitral leaflet is at the lower right, and a jet lesion *(arrow)* is visible in the LV outflow tract. Above the mitral leaflet, in the center of the left coronary cusp, is a raised, irregular vegetation *(arrowhead)* that caused aortic regurgitation (AR). C, Tricuspid valve vegetation in a weanling. Although less common than mitral or aortic vegetations, right-sided endocarditis is a definite risk in horses, particularly in animals subjected to repeated jugular venous catheterization. D, A lung abscess in a horse after pulmonary valve endocarditis. The center of the abscess is incised and reveals caseous exudate. Systemic embolization and metastatic infection are recognized complications of valvular infections.

hypoalbuminemia), elevated plasma fibrinogen concentrations, and leukocytosis with a mature neutrophilia.[205,855] Multiple blood cultures should be performed when bacterial endocarditis is suspected. The result of blood cultures may be negative, however, particularly after antimicrobial therapy. A positive blood culture may be more likely if multiple samples are drawn at different times of the day during or near febrile episodes. Antibiotic removal system media might also be of value when culturing blood from horses that have received antibiotics recently, and it can be helpful to consult with a clinical microbiologist regarding optimal broth media used for blood culturing.



FIG. **9.44** Weight loss, loss of condition, and ventral edema in this weanling with endocarditis and right-sided congestive heart failure (CHF; see also Fig. 9.43C).

Diagnosis of infective endocarditis may be definitive in the setting of a compatible clinical history with positive blood cultures or clear echocardiographic demonstration of a vegetation. In the absence of these, the relative likelihood of endocarditis is based on combinations of clinical findings and laboratory tests. Major and minor diagnostic criteria (Duke criteria) have been used for assessment of human patients,[873] and these principles are probably applicable to horses. Endocarditis should be considered in any horse that presents with a fever of unknown origin and one or more of the aforementioned clinical signs, particularly in association with synovial distention and lameness. Endocarditis becomes more likely when the cause of fever cannot be isolated to another body system and concurrent cardiac disease is identified. The diagnosis is confirmed with positive blood cultures in the setting of compatible clinical findings or by echocardiographic detection of vegetative lesions on the valve leaflets or endocardial surface (Fig. 9.45).

Vegetative lesions usually appear echocardiographically as thickened, echogenic to hyperechoic masses with irregular or "shaggy" edges. The valve leaflet often demonstrates diffuse thickening as well. Discrete lesions are generally on the valve surface facing the normal path of blood flow. The typical endocarditis lesion adheres to the valvular endocardium (and therefore moves with the valve). When a fresh thrombus is attached to the vegetation, an oscillatory appearance may be evident with high-frame-rate, real-time imaging. Chronic lesions may contract, calcify, or develop a smooth contour. Rupture of the chordae tendineae or avulsion of a valve leaflet may also be detected echocardiographically, and this complication is not uncommon in mitral or tricuspid valve endocarditis. Pulsed-wave and color-flow Doppler echocardiography can be used to confirm that the valve is incompetent or (rarely) stenotic. Valvular regurgitation often progresses because of continued

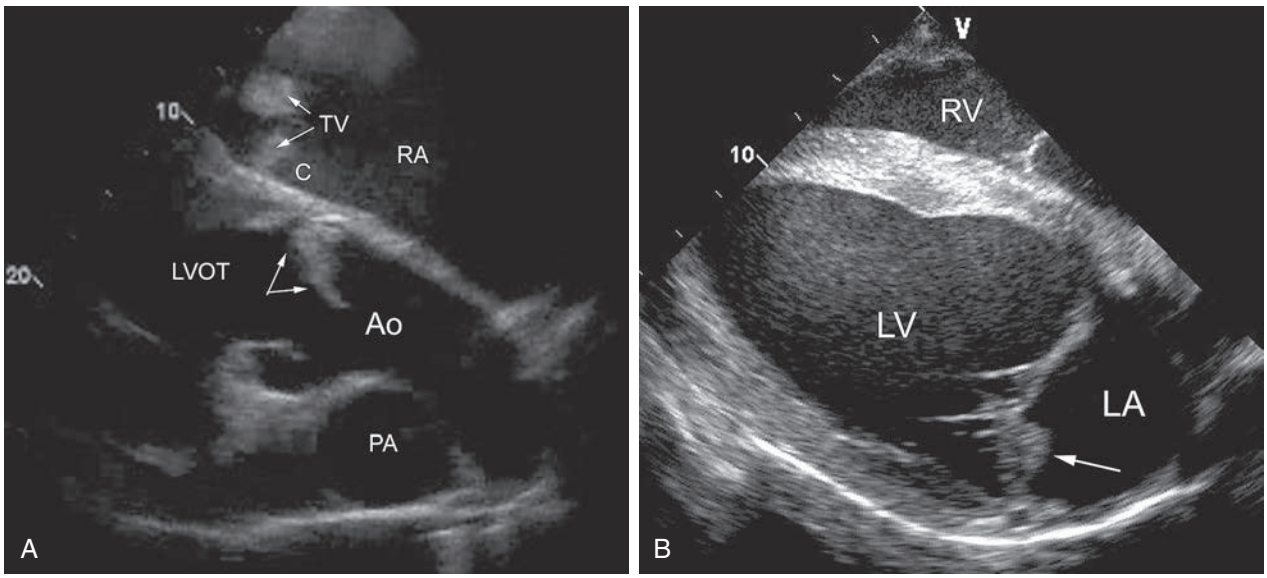


FIG. **9.45** A, Right parasternal long-axis two-dimensional (2D) image from a horse with infective endocarditis affecting multiple valves. Marked thickening of the tricuspid valve (TV) is evident, with spontaneous contrast (C) surrounding the valve. The right leaflet of the aortic valve is markedly thickened *(arrows)* between the left ventricular outflow tract (LVOT) and ascending aorta *(Ao).* Pulmonary artery (PA) and right atrium (RA) are seen in the far and near fields. B, Focal vegetation *(arrow)* is observed on a closed mitral valve in a long-axis image from the right thorax (*RV,* right ventricle; *LA,* left atrium; *LV,* left ventricle).

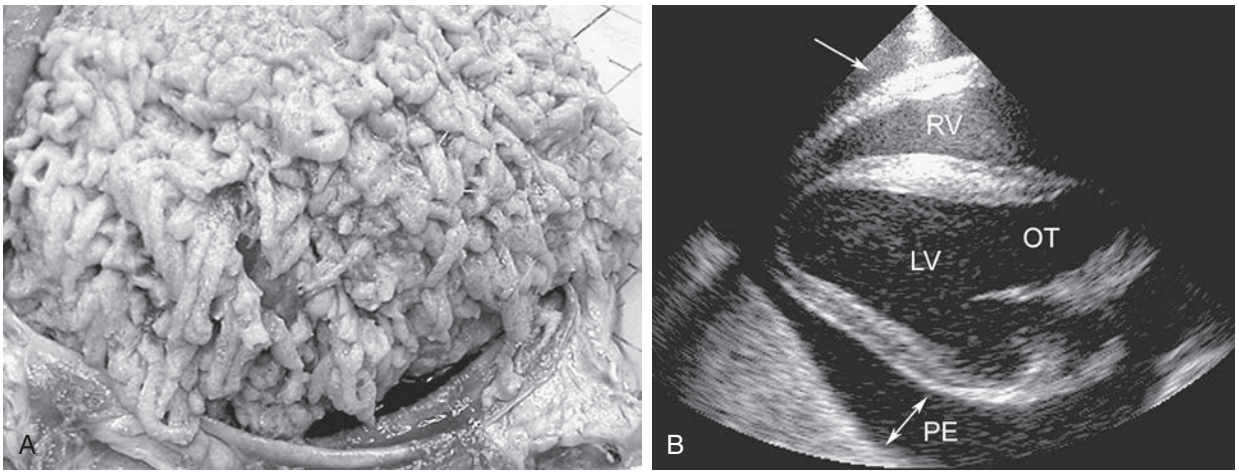


FIG. **9.46** A, Proliferative epicardial reaction in a horse with idiopathic, fibrinopurulent pericarditis. The heart is covered by a layer of organizing fibrin and inflammatory debris creating a shaggy appearance typical of inflammatory pericarditis. B, Two-dimensional (2D) long-axis echocardiogram from another horse demonstrating a moderate pericardial effusion. The effusion (PE) appears more prominent behind the left ventricle (LV) but is also evident *(arrow)* cranial to the right ventricle (RV). *OT,* Left ventricular outflow tract.

damage to the valve leaflets associated with ongoing bacterial infection or subsequent to fibrosis or calcification associated with a bacteriologic cure.

Early diagnosis and aggressive, prolonged treatment are important for successful treatment of infective endocarditis. Treatment should consist of adequate doses of bactericidal antibiotics, ideally based on culture and sensitivity patterns of blood culture isolates. Initial therapy should be broad spectrum, until the results of the blood culture are known or when a positive blood culture cannot be obtained. Intravenous therapy is preferred in the initial stages of treatment. Drugs that penetrate fibrin well, particularly potassium penicillin (22,000–44,000 IU/kg, IV every 6 hours), are reasonable initial choices because bacteria may be sequestered in fibrin and may be unavailable to leukocytes. To extend the antimicrobial spectrum, penicillin is usually combined with gentamicin sulfate (9 mg/kg, IV every 24 hours) or amikacin sulfate (20 mg/kg, IV every 24 hours for adults; 25 mg/kg, IV every 24 hours for foals). Erythromycin estolate (25 mg/kg, PO every 6–8 hours) combined with rifampin (5–10 mg/kg, PO every 12 hours) may be useful in some cases. Nonsteroidal antiinflammatory drugs (flunixin meglumine 1.1 mg/kg, IV q 12 h) may be beneficial to reduce systemic and local inflammatory reactions. Aspirin (10–20 mg/kg, PO every 24–48 hours) and heparin (sodium heparin: 40 IU/kg, SC every 8–12 hours; dalteparin 50–100 IU/kg, SC every 24 hours; or enoxaparin 40–80 IU/kg [0.4–0.8 mg/kg], SC every 24 hours) are used by some clinicians to prevent platelet adhesion, diminish growth of the vegetation, and reduce the risk of thrombotic complications, although the benefits of antithrombotic therapy are uncertain.

With effective antimicrobial therapy, the fever should resolve within 5 to 7 days. Response to treatment should be assessed by repeated examinations, including clinical assessment, laboratory analyses, and serial echocardiograms. Therapy should extend to at least 4 to 8 weeks or until body temperature, plasma fibrinogen concentration, and leukocyte counts have been normal for at least 2 weeks. The duration and type of long-term therapy depend on various factors, and the bacterial isolate, clinical response, cost, and potential toxicosis of the antimicrobial therapy must all be weighed in these decisions.

The expectation for long-term survival is poor in most cases of infective endocarditis causing severe valvular injury. Even in the absence of significant valvular regurgitation, there may be difficulty in achieving a bacteriologic cure, or valvular damage may progress as the vegetation heals and scars the valve. Although some horses have been treated successfully for endocarditis,[31,197,199,205] overall there is a low likelihood of long-term survival with continued use of the horse as a performance animal or in breeding. The absence of an obvious echocardiographic lesion or signs of systemic inflammatory response creates a more favorable situation, provided that a bacteriologic cure can be obtained. Progressive cardiomegaly, CHF, rupture of the PA, development of AF, thromboembolic complications, and sudden death have been reported in horses affected with endocarditis. Accordingly, periodic follow-up examination, including echocardiograms, should be performed in successfully treated cases.

PERICARDIAL DISEASE

Pericardial diseases occur uncommonly and are usually associated with pericardial effusion and fibrinous pericarditis (Fig. 9.46).* Approximately one third of the cases can be attributed to bacterial infection,[872] and an infectious etiology should be assumed until proven otherwise. *Actinobacillus* spp. have most frequently been reported with pericarditis,[872] but *Streptococcus* spp., *Escherichia coli, Enterococcus* spp., *Pseudomonas aeruginosa, Pasteurella* spp., *Corynebacterium pseudotuberculosis, Mycoplasma,* and other microorganisms, including fungal agents, have also been isolated.[126,133-135,739,872,880,881] Outbreaks of pericarditis with largely sterile inflammatory effusates have been described as part of the mare reproductive

* References 67, 119-121, 125-128, 131-137, 140, 191, 738-742, 796, 870, 872, 874-883.

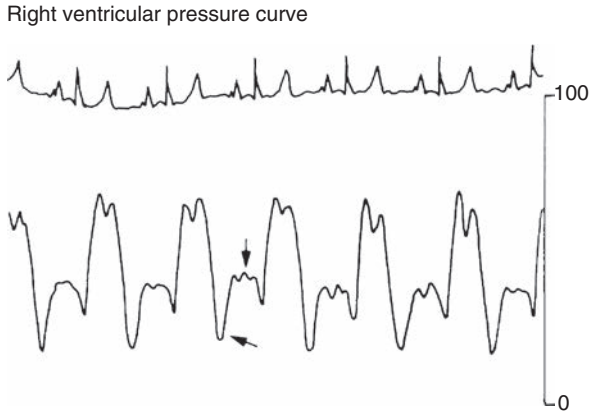


FIG. **9.47** Pressure tracing demonstrating elevated right ventricular (RV) end-diastolic pressure in a horse with constrictive pericarditis and heart failure. A quick rise occurs from the nadir of pressure *(lower arrow)* to a plateau *(upper arrow),* which is typical of constrictive disease and ventricular filling that is limited to early diastole.

loss syndrome, first recognized in Kentucky and subsequently also in other areas.[134,136,884-886] Exposure to eastern tent caterpillars was identified as the greatest risk factor in these outbreaks, and *Actinobacillus* spp. were the principal isolates obtained from necropsy and clinical cases.[134,885] It was proposed that caterpillar-related toxins or hairs of the caterpillars breached the gastrointestinal mucosal integrity and led to secondary opportunistic invasion of commensal bacteria of the mucosal surfaces that appeared to be pericardiotrophic in horses.[885] In many cases, idiopathic pericarditis might in fact be viral in origin, either directly or through immune-mediated mechanisms.[872] Equine influenza, equine viral arteritis, and equine herpes virus infections have been associated with pericarditis, and horses with pericarditis sometimes have a recent or current history of respiratory disease; nonetheless, evidence for a viral etiology is scarce.[872] External thoracic trauma or penetration by a gastric foreign body may lead to bacterial inoculation of the pericardial space.[741,874,877] The pathogenesis of noninfective pericarditis is unknown but might be immune mediated in some situations.[133] Finally, cardiac or pericardial neoplasia can cause pericardial effusion, with mesothelioma and lymphosarcoma being the most common neoplasms affecting the pericardium in horses. Pericardial hernias have also been seen but are rare.

The pathophysiology of pericardial disease in most cases is ascribed to impaired cardiac filling resulting from either external compression (cardiac tamponade) or pericardial constriction.[117] There is typically marked elevation in the venous, atrial, and ventricular end-diastolic pressures (Fig. 9.47). Occasionally a mass lesion compresses the heart or obstructs venous drainage or ventricular outflow and mimics findings of pericardial disease. Other clinical signs may be referable to the underlying cause as with infection or neoplasia.

The *clinical syndrome* of pericardial effusion with tamponade is characterized by reduced cardiac filling despite increases in ventricular filling pressures. The history usually includes systemic signs of illness, such as fever, lethargy, depression, anorexia, tachypnea, ventral edema, colic, and weight loss. A recent history of an upper or lower respiratory tract infection is not uncommon. Physical examination abnormalities include various combinations of tachycardia, fever, pericardial friction rub, muffled heart sounds, tachypnea, pleural effusion, jugular and generalized venous distention, ventral edema, thready pulses, and ascites. In acute cases, clinical signs of depression and weakness may predominate, whereas in chronic cases signs of right-sided CHF are evident. Arterial BP may be decreased in cardiac tamponade, and a pronounced inspiratory fall in BP (pulsus paradoxicus) may be identified by palpation of the pulse or through careful measurement of arterial BP. In chronic disease, BP is normal, owing to fluid retention, elevated venous pressures, vasoconstriction, and tachycardia.

Laboratory studies are contributory to the diagnosis. Clinical laboratory abnormalities are not specific, but the most frequently detected abnormalities include anemia, hyperproteinemia, hyperfibrinogenemia, and a neutrophilic leukocytosis. Other hematologic abnormalities may be observed related to inflammation, CHF, or organ hypoperfusion. Thoracic radiographs usually reveal a globoid cardiac silhouette or pleural effusion and may reveal interstitial pulmonary infiltrates and enlarged pulmonary vessels. The ECG usually demonstrates decreased amplitude of the QRS complexes. If the effusion is large and the heart is swinging, electrical alternans may be observed. Pericarditis may elevate the ST segment in multiple leads, but this change may occur simply as a result of tachycardia. Sinus tachycardia is typical, but ventricular or atrial premature complexes may be detected.

The echocardiographic examination is diagnostic, demonstrating an anechoic or hypoechoic fluid space between the pericardium and epicardial surface of the heart (see Fig. 9.46B) while excluding an extracardiac mass lesion, which can mimic pericardial disease.[140] Fibrin tags frequently are evident on the parietal and visceral pericardial surfaces. Findings of protracted diastolic collapse of the right ventricle or systolic collapse of the right atrium are compatible with a clinical diagnosis of cardiac tamponade. Inflammatory processes may eventually lead to adhesions between the parietal and visceral pericardial layers, causing constrictive pericarditis with minimal or no obvious effusion. Associated pleural effusion is a common ultrasound finding. Echocardiographic diagnosis of constrictive disease without effusion is more challenging but typically reveals thickened pericardium, atrial dilation, systemic venous dilation, exuberant movement of the ventricular septum, and exaggerated inspiratory filling of the heart as documented by pulsed-wave Doppler studies.[121,125] In some cases right-sided heart catheterization is needed to establish the diagnosis. Typical findings are increased central venous pressure, elevated RV diastolic pressure, and possibly a diastolic dip and plateau appearance to the RV waveform (see Fig. 9.47).[125]

Cytologic evaluation of the pericardial effusion is essential to distinguish septic, aseptic, or neoplastic pericardial effusion. Fluid can be obtained in the course of needle pericardiocentesis or during the placement of an indwelling tube for pericardial lavage and drainage. Bacterial culture and sensitivity of the aspirated fluid should be performed so that antimicrobial therapy can be guided in cases of septic pericarditis.

The treatment of pericardial diseases varies, depending on the cause and clinical situation. Even when signs of right-sided CHF dominate the clinical picture, the use of furosemide is generally contraindicated, because aggressive diuresis results in lowered filling pressures, reduced ventricular filling, decreased cardiac output, and possibly syncope. Instead, pericardiocentesis or catheter drainage should be considered as

first-line therapeutic procedure in all cases of pericardial effusion with cardiac tamponade. Because the development of cardiac tamponade depends not only on the volume of pericardial fluid but also on the rate at which it accumulates, the clinician's urgency should be guided by BP, clinical signs, magnitude of pleural effusion, and echocardiographic evidence of cardiac tamponade. Tamponade is an indication for immediate drainage of the pericardial sac. Echocardiography can be used to localize a site for pericardiocentesis and choose an appropriate length of needle, catheter, or drainage tube. Pericardiocentesis should be performed after locally anesthetizing the intercostal muscles and pleura. Electrocardiographic monitoring should be performed continuously during the procedure to monitor for cardiac puncture or in case ventricular arrhythmias develop. Pericardiocentesis is usually performed within the left fifth intercostal space, above the level of the lateral thoracic vein, although it can also be performed at the right hemithorax. Drainage is achieved using a large-bore catheter, teat cannula, or chest tube; the latter is recommended for repeated drainage and lavage of the pericardial sac and is most successful for aggressive management of septic or idiopathic fibrinous pericarditis. After insertion of an indwelling catheter, local therapy is administered that includes pericardial lavage and direct instillation of antimicrobials and possibly anticoagulants such as heparin. Combined with systemic antimicrobials this therapy has been effective in the treatment of septic pericarditis.[133,738] Pericardial lavage should be continued for several days, until there is little accumulation of pericardial fluid (less than 1 L over 12 hours), clinical signs have improved, and the cytologic character of the fluid becomes less inflammatory. Initially, broad-spectrum antibiotics should be used and the antibiotic regimen should then be adapted according to the results of bacterial cultures and antibiotic sensitivity patterns. Nonsteroidal antiinflammatory drugs are indicated to fight inflammation and to reduce the risk of constrictive pericarditis. If the cytologic analysis and culture are negative for bacteria, antiinflammatory doses of dexamethasone may be used for treatment of idiopathic, nonseptic, effusive pericarditis.[126,133] Exudative pericarditis may not respond in all cases to conservative treatment or even to drainage. Surgery is a rarely used option for treatment of pericardial disease but would be most appropriate for constrictive or constrictive-effusive pericarditis.[125] Presumably introduction of minimally invasive thoracoscopic surgical techniques to management of pericardial diseases in horses might prove useful.

The prognosis for survival and maintenance of performance in horses affected by pericardial disease is guarded. The prognosis for cardiac or pericardial neoplasia is quite poor. Caution also must be expressed regarding inflammatory pericarditis because the condition may become chronic, but very good results have been obtained in some reports.[133] The potential of eventual constrictive or fibrotic pericardial disease is also greatest with inflammatory pericardial conditions so that early success may be tempered by later complications of the disease.[126] The best prognosis of horses with fibrinous pericarditis is when treatment includes repeated drainage and lavage. Treated horses should be followed and reevaluated by echocardiography.

MYOCARDIAL DISEASE

Myocardial diseases are probably underrecognized in clinical practice although a variety of reports have been published regarding these disorders.* There is certainly a potential for myocardial injury or inflammation related to drugs and toxins (e.g., ionophore antibiotics, poisonous plants, cantharidin, snake venom), ischemia, hypoxia, infective agents (bacterial, viral, parasitic, fungal), heavy metals, trauma, metabolic disease, or nutritional deficiencies (e.g., vitamin E, selenium). Myocardial injury also can derive from extension of a preexisting infection (pericarditis, pericardial abscess, or endocarditis) or related to sepsis.[652] Infiltrative cardiomyopathies can occur consequent to neoplasia (lymphoma, melanoma, lipoma, hemangioma/hemangiosarcoma, mesothelioma, pulmonary carcinoma)[138,139,166,843,848,907-911] or the very rare amyloidosis[735] (Fig. 9.48; see also Box 9.2). Left ventricular hypertrophy and dysfunction may also develop secondary to systemic hypertension associated with chronic pain, laminitis, chronic kidney disease, or potentially metabolic syndrome.[143,903] Three cases of suspected arrhythmogenic right ventricular cardiomyopathy have recently been described as a cause of episodic collapse and cardiac death.[668,899] Dilated cardiomyopathy (DCM) phenotype has been recognized in the horse, but the underlying cause is generally unknown and the condition is usually considered to be idiopathic. Relentless junctional or ventricular tachycardia developing at a high rate (greater than 100–120 beats/min) and persisting over days can cause tachycardia-induced cardiomyopathy that resembles DCM and is characterized by chamber dilation and systolic dysfunction; control of HR and heart rhythm may lead immediately to more normal LV function, and the cardiomyopathy may resolve following conversion to sinus rhythm.[153]

Clinical Features of Myocardial Diseases

The general manifestations of myocardial disease, regardless of the underlying injury, can be attributed to the following pathophysiologic processes: (1) reduced myocardial contractility and ventricular ejection fraction; (2) diastolic dysfunction with impaired ventricular filling; (3) mitral or tricuspid valve incompetency caused by cardiac dilation or papillary muscle dysfunction; or (4) the development of arrhythmias. The overall cardiac disability engendered by myocardial disease varies greatly. Some horses have no detectable clinical signs; others demonstrate exercise intolerance, life-threatening arrhythmias, low-output CHF, or sudden death.

Persistent ventricular premature depolarizations or ventricular tachycardia can be observed in horses with myocardial disease.[162,164,651,900,912] Atrial premature depolarizations, atrial tachycardia, and AF are more often primary electrical disturbances; however, these arrhythmias also can develop in horses with cardiomyopathies. Although it is tempting to diagnose "myocardial disease" in the setting of any cardiac rhythm disturbance, it should be appreciated that many rhythm abnormalities are "functional," without a gross anatomic substrate. This point is especially germane to horses suffering from electrolyte or other metabolic imbalances, high sympathetic tone, sepsis or toxemia, hypoxemia, or ischemia. In horses examined for sudden death at a racetrack, gross or microscopic myocardial lesions were relatively uncommon findings compared with pulmonary lesions.[103,106]

The onset of clinical signs may lag behind the initial myocardial insult, especially in cases of myocarditis or chronic myocardial injury. For example, a horse that has apparently

* References 28, 29, 66, 97, 138, 145, 149, 150, 154, 155, 160-162, 164, 166, 191, 485, 575, 651, 658, 660, 661, 734, 735, 777, 870, 882, 887-906.

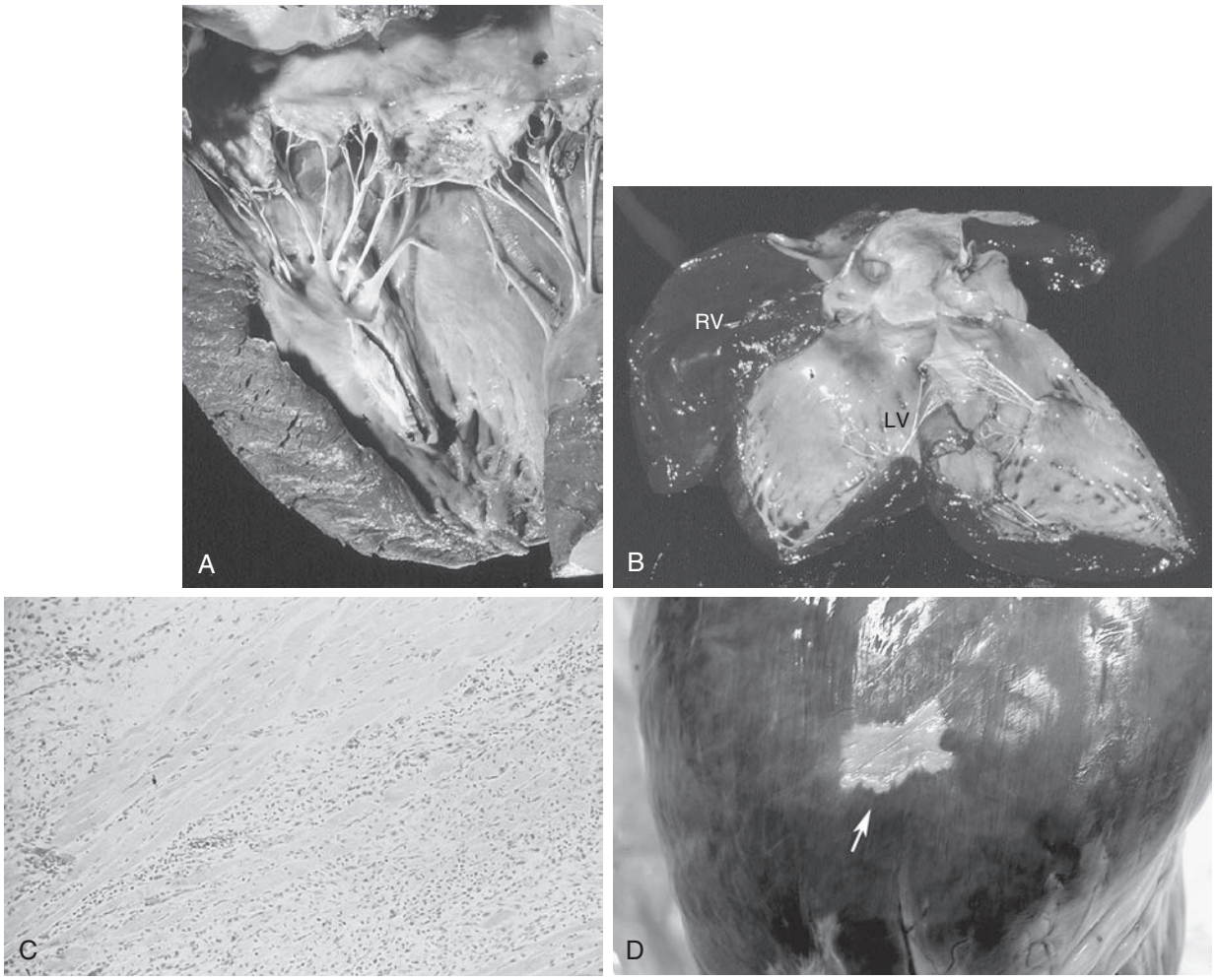


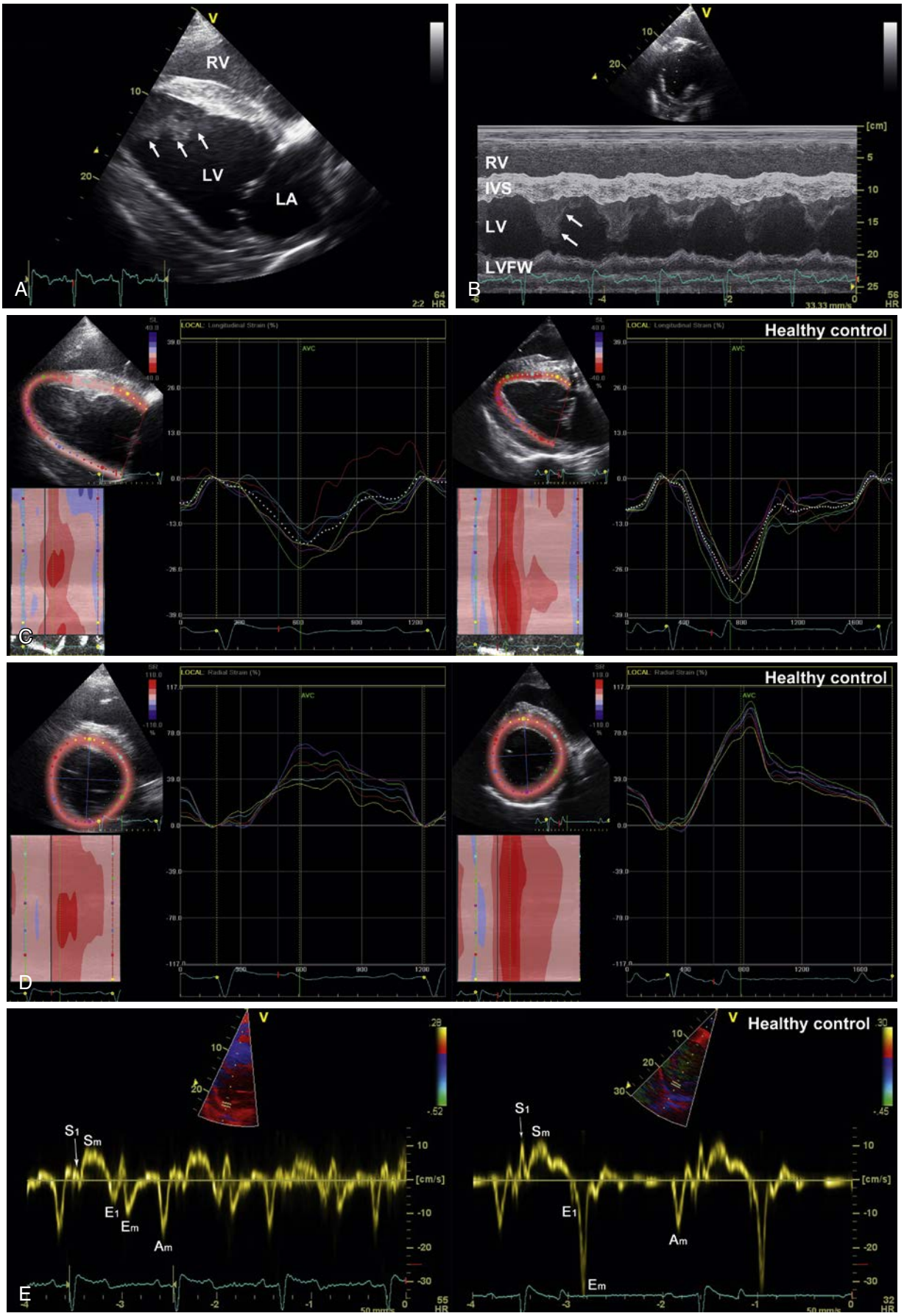
FIG. **9.48** Myocardial diseases. A, An opened left ventricle (LV) revealing a (incised) large, oval area of subendocardial and myocardial fibrosis in a mare. No causative agent was found. B, Significant LV dilation and subendocardial fibrosis in a horse with idiopathic dilated cardiomyopathy. The white discoloration of the LV and left atrium (LA) may result from chronic distention or represent fibrosis after another injury. The opened right ventricle (RV) (to the left) is dilated but is not discolored. C, Myocardial lymphoma. A substantial myocardial infiltration is evident in this photomicrograph. D, Close-up of a left ventricular subepicardial myocardial infarct *(arrow)* of undetermined cause.

recovered from an illness may develop problems once rigorous training is begun. The trainer may complain that the horse is unable to achieve faster speeds or may stop or suddenly slow during hard training. The affected horse may take a long time to "cool out" after a workout. In more severe cases, marked exercise intolerance, weakness, ataxia, or even collapse may occur. Respiratory distress, pulmonary edema, cyanotic mucous membranes, prolonged capillary refill time, and a rapid thready pulse may be detected after exercise. In case of severe myocardial injury, signs such as fever, persistent tachycardia, arrhythmia, murmur, pulmonary or ventral edema, or respiratory distress may be observed. Sudden death may occur without premonitory signs.

Results of the clinical examination in horses with myocardial disease are inconsistent. Resting physical examination findings can be normal or signs of heart disease may be evident. These can include persistent tachycardia, tachypnea, frequent premature beats, sustained arrhythmias, systolic murmurs of AV valvular insufficiency, or CHF. A postexercise examination often detects an abnormally rapid HR, which remains persistently high after exercise is discontinued (note that exercise testing should not be performed in horses with persistent resting tachycardia or tachyarrhythmia that may be attributed to myocardial disease).

An ECG may demonstrate sinus tachycardia or atrial or ventricular arrhythmias. An exercise ECG, in addition to potential exercise-induced arrhythmias, typically records an inappropriately high HR for the level of work undertaken.

Resting echocardiography usually reveals a low normal or unambiguously reduced ventricular systolic function, as demonstrated by low LV shortening fraction or ejection fraction. Novel echocardiographic methods such as tissue Doppler imaging (TDI) or 2D speckle tracking might be more sensitive to detect LV systolic dysfunction compared with conventional 2DE or M-mode echocardiography[161,164] and could be particularly useful to detect subtle myocardial disease in horses (Fig. 9.49). Furthermore, Doppler interrogation of transmitral blood flow (E and A wave) and LV wall motion analysis using TDI (E_m and A_m wave, isovolumic relaxation time) can reveal significant LV diastolic dysfunction in the presence of normal


A
RV
LV
LA
B
RV
IVS
LV
LVFW
C
Healthy control
D
Healthy control
E
S1
Sm
E1
Em
Am
Healthy control

or impaired systolic function,[164] strongly suggesting myocardial disease (see Fig. 9.49). Depending on the type of myocardial disease, the (relative) LV wall thickness can be increased (i.e., infiltrative or hypertensive cardiomyopathy) or decreased (i.e., dilated or tachycardia-induced cardiomyopathy; see Fig. 9.23). Postexercise echocardiography may demonstrate a paradoxic reduction of LV shortening fraction or regional dysfunction characterized by LV wall motion abnormalities.[167,168] Marked increases in left ventricular or left atrial spontaneous contrast may be observed with very poor myocardial function, although this is not a specific finding (see Fig. 9.49). Abnormal areas of myocardial echogenicity have been observed, but myocardial tissue characterization by echocardiography is not well established in horses, and grayscale also depends on technical factors.

Clinical laboratory tests may be useful in the identification of myocardial damage but may not necessarily distinguish myocarditis from myocardial cell injury induced by a toxin or by ischemia. Elevated plasma or serum creatine kinase activity (CK), myocardial fractions of creatine kinase (CK-MB), or lactate dehydrogenase (LDH_{1-2}) suggest myocardial injury.[352] A more specific marker of myocardial is elevation of plasma cardiac troponin I (cTnI) or troponin T (cTnT).* Although normal values or mild elevations do not exclude cardiomyopathy or myocardial infiltration, markedly elevated values (i.e., cTnI greater than 1 ng/mL) point to recent cardiac muscle damage. Persistently elevated plasma cTnI concentrations indicate ongoing damage, because plasma half-life of cTnI is short.[680]

Diagnosis of myocardial disease requires clinical suspicion and integration of findings from clinical and laboratory examinations. Because of the extreme variability of findings, the presumptive diagnosis of myocardial disease can be made only after reviewing the history, physical examination, echocardiogram, ECG, and clinical laboratory tests. Definitive diagnosis of myocarditis requires transvenous endomyocardial biopsy,[913] but this test is currently limited to research purposes and may not identify piecemeal inflammation, degeneration, infiltration, or necrosis.

Treatment of horses affected by myocardial disease is primarily supportive. Prognosis depends on the cause and severity of myocardial injury and the hemodynamic consequences of myocardial disease. All horses should be rested, preferably in a stall, until myocardial function, ECG, and plasma troponin concentrations return to normal or at least remain stable for several weeks. A minimum rest of 1 month (and usually more) should be instituted before a horse is returned to work. Supplementation with vitamin E and selenium may be beneficial, particularly in cases with suspected nutritional deficiencies. Antiarrhythmic therapy is administered when indicated for potentially life-threatening arrhythmias (see later in this chapter). Theoretically, an ACE inhibitor will reduce myocardial remodeling and unload the ventricle, assuming the drug can be sufficiently absorbed and biotransformed to an active state; however, efficacy of this treatment is currently unknown. When CHF has developed, diuretics, peripheral vasodilators, and positive inotropic agents may be prescribed as previously discussed in the section on CHF. Digitalization should be undertaken with caution in horses with ventricular extrasystoles, as the arrhythmia may be aggravated and it is not indicated in ionophore toxicosis (see later). For a suspected bacterial etiology, antibiotic treatment is indicated. If noninfective myocarditis is believed to be the cause of the arrhythmia or clinical signs, corticosteroid therapy may be indicated, although its value is unsubstantiated. When the principal manifestation of myocardial disease is electrical (arrhythmias with otherwise normal myocardial function), the prognosis is fair to good for resolution of the arrhythmias. Horses with decreased myocardial function by echocardiography or those with CHF must be given a guarded prognosis for life and a poor prognosis for

* References 162, 163, 575, 651, 653, 658, 659, 661, 662, 665, 667.

FIG. **9.49** Nutritional myocardial damage. Echocardiographic examination in a 22-year-old Arabian mare suffering from nutritional masseter myopathy with concurrent myocardial damage (cardiac troponin I concentration 11.6 ng/mL; normal <0.06 ng/mL). This case study demonstrates the clinical use of novel echocardiographic indices for the assessment of systolic and diastolic LV function in horses with myocardial disease. A, B-mode recording of the left ventricle in right-parasternal long-axis 4-chamber view. Left atrial (LA), left ventricular (LV), and right ventricular (RV) dimensions appear normal. There is marked spontaneous echo contrast in the LV *(arrows).* This can be found in healthy horses but may become more distinct with low-output states, bradycardia, and possibly systemic inflammation. B, M-mode recording of the LV in a right-parasternal short-axis view. Notice the flat motion of the interventricular septum (IVS) and the LV free wall (LVFW), suggesting poor LV systolic function (LV fractional shortening 22%; normal 30%–45%). Marked spontaneous echo contrast is evident *(arrows).* C, Two-dimensional speckle tracking (2DST) analyses of an LV long-axis recording. Trace screens of the 2DST software are shown, displaying the following information: *top left,* 2D image with the segmented ROI and parametric color coding at the time of aortic valve closure. *Bottom left,* M-mode with parametric color coding. *Right,* Trace display for longitudinal segmental strain (i.e., relative myocardial deformation). The horizontal axis represents the time in msec, the vertical axis represents radial strain in %. The colors of the trace correspond to the colors of the segmented ROI. An ECG is superimposed for timing. The start and the end of the cycle (R waves) are marked on the ECG with yellow dots. Time of aortic valve closure (AVC) is indicated by a green vertical line, dividing the cycle in its systolic and diastolic component. The dotted line indicates the instantaneous average over all segments at the respective time of the cardiac cycle (global strain). Note that peak longitudinal strain in this case *(left)* is reduced compared with the healthy control *(right),* indicating depressed LV systolic function. D, 2DST analyses of an LV short-axis recording. Trace screens of the 2DST software are shown, displaying longitudinal segmental strain (for further explanations see C). Note that peak radial strain *(left)* in this case is reduced compared with the healthy control *(right),* indicating depressed LV systolic function. E, Pulsed-wave tissue Doppler (PW TDI) analysis of the LV free wall recorded at the level of the chordae tendineae in a right-parasternal short-axis view. The horizontal scales of the spectral tracings indicate the time in seconds, the vertical scales indicate the velocity in cm/sec. The E_m/A_m inversion *(left)* indicates diastolic dysfunction and impaired ventricular relaxation. Notice the normal E_m/A_m ratio in the healthy control *(right)* for comparison. S_i, Peak radial wall motion velocity during isovolumic contraction; S_m, peak radial wall motion velocity during ejection; E_i, peak radial wall motion velocity during isovolumic relaxation; E_m, peak radial wall motion velocity during early diastole; A_m, peak radial wall motion velocity during late diastole. (From Schwarzwald CC: Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, et al.: *Atlas of equine ultrasonography,* Wiley, Oxford, UK, 2014. Reproduced with permission of John Wiley & Sons, Ltd.)

future performance. It is notable, however, that some horses with LV hypertrophy and dysfunction and some with acute onset of CHF have recovered completely and returned to their prior performance level. Such horses most likely suffered from acute myocarditis that resolved spontaneously or following antiinflammatory therapy. Other horses may achieve a less spectacular recovery but still serve successfully as breeding animals.

Myocarditis

Myocardial inflammation, or myocarditis, is difficult to diagnose. The diagnosis is often entertained in horses with cardiac arrhythmias, concentric LV hypertrophy (supposedly acute myocarditis with cell infiltration and myocardial edema) or severe LV dilation (supposedly chronic or later stages), and abnormal myocardial function, particularly when cardiac signs occur after another illness and are associated with elevated cardiac isoenzymes or plasma cTnI or cTnT concentrations. Often the prior disorder is a viral, influenza-type condition or an infection caused by *Streptococcus* spp., but parasitic migration, fungal infections, or toxic insults can also result in inflammatory myocardial lesions that may not obviously be associated with preceding clinical disease.* It is logical that immune-mediated myocarditis is operative in many of these cases, but definitive cause-and-effect proof is lacking. Myocarditis may also follow pericarditis or infective endocarditis. Hematogenous spread to the heart may be another mechanism for myocarditis. The signs, prognosis, and treatment of myocarditis are similar to that described in the preceding section.

Toxic Injury of the Myocardium

A number of chemicals and plant toxins are potentially injurious to the myocardium. Ionophore antibiotics are among the most notorious causes of myocardial necrosis in horses.[154-163,656,901,902,915] Toxic myocardial injury also can occur following ingestion of glycoside-containing yew plants (*Taxus* spp.), oleander *(Nerium oleander),* foxglove (*Digitalis* spp.), and summer pheasant's eye *(Adonis aestivalis)*[897,898,912,916-920] or from eating feed contaminated by *Epicauta* species (blister beetles), which contain the toxic element cantharidin.[889,891,921-928] Atypical myopathy (AM) and seasonal pasture myopathy (SPM) that are caused by ingestion of hypoglycin A–containing seeds of maple trees (*Acer* spp.)[575,674-676,929-938] and marshmallow *(Malva parviflora)* toxicosis,[904] through different mechanisms, can cause severe generalized myopathy with myocardial involvement/damage. Myocardial necrosis has also been observed in association with clostridial infection and following endotoxemia, particularly with salmonellosis and torsion of the large colon. Venom of rattlesnakes (*Crotalus* spp.) and of other members of the family of vipers *(Viperidae)* has been associated with cardiac injury and arrhythmias.[659,900,939]

Ionophore Toxicity

Horses are uniquely sensitive to ionophore antibiotics, which are used as a coccidiostat in poultry production and as a growth promoter in cattle. Monensin toxicosis (acute oral LD_{50} 2–3 mg/kg) has occurred most frequently,[154-160,163,901,940] but salinomycin (acute oral LD_{50} 0.6 mg/kg)[915,941,942] and lasalocid (acute oral LD_{50} 21.5 mg/kg)[161,162,943] also have caused myocardial injury and death in horses.[928,944-946] Ionophores react with polar cations to form lipid-soluble complexes, leading to cation transport across myocardial cell membranes. Various ionophores demonstrate particular affinities for different cations, though lasalocid may complex with a variety of ions. Exposure of horses to the ionophore antibiotics usually stems from accidental contamination of equine feedstuffs at the mill or through accidental delivery of poultry or cattle feed to horses. In most outbreaks, a recently acquired ration was fed.

Clinical signs of horses with ionophore toxicity vary with the specific type, quantity, and concentration of ionophore ingested and the preexisting health and body condition of the exposed horses. A wide range of clinical signs has been observed in exposed horses ranging from none to clinical signs involving almost all body systems.[156,157,160,162,163,915] Weakness, lethargy, depression, anorexia, ataxia, colic, diarrhea, profuse sweating, and recumbency have been seen. The cardiac findings are similar to those previously described for myocardial diseases. If sudden death occurs, it is usually within 12 to 36 hours of ingestion of the contaminated feed. A single dose may lead to peracute death from cardiac arrhythmias before the development of myocardial necrosis and associated with only mild signs of colic or neurologic deficits.[901]

Sublethal toxicosis may be well tolerated by some but not all horses, and plasma cTnI concentrations, echocardiography, and exercise testing may help to identify horses affected more severely.[160-163,656] Polyuria and hematuria are other signs that have been reported in ponies following ionophore exposure.

A diagnosis of ionophore toxicity is based on the detection of the ionophore in the feed or stomach contents of exposed horses. Various clinicopathologic abnormalities are observed with monensin toxicity, including decreased serum calcium, potassium, magnesium, and phosphorus and increases in serum urea, creatinine, unconjugated bilirubin, aspartate aminotransferase, and muscle enzymes. Elevated concentrations of plasma cardiac troponin (cTnI, cTnT) and isoenzyme patterns of CK and LDH have indicated cardiac, skeletal, and RBC damage. Elevated packed cell volume and total solids have been associated with dehydration. Echocardiographic evaluation of affected horses has revealed marked decreases in shortening fraction with segmental wall motion abnormalities that range from mild to severe.[161]

Prognostically, horses that exhibit decreased shortening fraction and dyskinesis shortly after exposure to monensin generally are unlikely to survive. Horses with mild decreases in shortening fraction survive and may be useful breeding animals, but most do not return to previous performance levels. Horses with normal echocardiograms typically survive and may return to work at their previous level. The magnitude of increase in plasma cTnI or cTnT concentrations and rate of decrease over time likely has some prognostic implications; however, even in surviving animals concentrations can remain increased for several months, likely because of ongoing release from damaged myocytes.[162] Postmortem findings range from no visible lesions to myocardial pallor and signs of CHF. Severe myocardial necrosis and fibrosis has been observed in horses with decreased shortening fraction or ventricular dyskinesis on echocardiography.

Treatment for affected horses is largely symptomatic (see earlier), unless very recent exposure is known. Vitamin E has been suggested to have a protective effect in other species

* References 149, 658, 870, 900, 902, 905, 914.

and may be beneficial in affected horses. Digoxin and calcium channel blockers are contraindicated in acutely affected horses. If ingestion of the contaminated feedstuff is recent, treatment with activated charcoal or mineral oil is indicated to reduce absorption of the ionophore. Intravenous fluid and electrolyte replacement therapy may be indicated, as well as antiarrhythmic drugs, for any life-threatening arrhythmias. Stall rest in a quiet environment for up to 8 weeks after exposure is most important, because echocardiograms recorded after trivial exercise or excitement can reveal residual disease characterized by marked decreases in fractional shortening and myocardial dyskinesis.

Dilated Cardiomyopathy

Idiopathic dilated cardiomyopathy is a disorder of the myocardium characterized by global reduction of LV systolic function that cannot be explained by valvular, vascular, coronary, or congenital heart disease. The inciting cause of dilated cardiomyopathy is generally undetermined, although myocarditis or prior toxic injury is often suspected. Relentless junctional or ventricular tachycardia also can lead to a DCM state that is reversible with control of the tachyarrhythmia (see earlier discussion).[153] The clinical signs of cardiomyopathy are similar to those described earlier for other myocardial diseases. Echocardiography is diagnostic, revealing cardiomegaly with biatrial and biventricular dilation and depressed shortening fraction (see Fig. 9.23B). Symptomatic therapy with digoxin and diuretics may temporarily stabilize CHF and lead to transient improvement, but most horses deteriorate in the 3 to 12 months after diagnosis and are humanely destroyed. Neither the history nor the postmortem examination reveals the cause, and generally only diffuse or multifocal myocardial degeneration, necrosis, and fibrosis are observed.

A form of dilated cardiomyopathy also can be caused by vitamin E and selenium deficiency and is observed primarily in fast-growing foals from mares with a marginal or deficient selenium status raised in selenium-deficient areas.[178] Affected foals are usually younger than 6 months of age and present with an acute onset of weakness, recumbency, respiratory distress, pulmonary edema, tachycardia, murmurs, and arrhythmias. The prognosis for foals affected with the myocardial manifestations of white muscle disease is poor, and most die within 24 to 48 hours after the onset of clinical signs. Laboratory abnormalities in affected foals include marked elevations of CK (including the MB fraction in foals with myocardial involvement), aspartate aminotransferase (AST), and LDH, as well as hyperkalemia, hyponatremia, and hypochloremia. Myoglobinuria may occur. Echocardiography demonstrates the severity of myocardial involvement. Whole blood selenium, RBC glutathione peroxidase, and vitamin E concentrations may be helpful in the diagnosis; however, tissue samples provide a more accurate indication of selenium stores. Treatment with vitamin E and selenium might be successful, but typically the myocardial necrosis is extensive and incompatible with life. Postmortem findings reveal pale streaking of the myocardium with intramuscular edema, myodegeneration, myocardial necrosis, and fibrosis or calcification.[165] Prevention of white muscle disease is important in selenium-deficient areas. Supplementation of pregnant mares should occur during gestation based on individual blood and tissue selenium concentrations and should be continued during lactation, because more selenium is passed to the foal through the milk than across the placenta.

VASCULAR DISEASES

Acquired disorders of blood vessels include a variety of conditions that vary in etiology and range from subclinical to devastating.* Jugular vein thrombosis or thrombophlebitis is probably the most common vascular problem encountered in clinical practice (Fig. 9.50).[456,458,957-962] Catheter fragmentation and embolization are observed periodically and have been amenable to surgical and percutaneous catheter retrieval.[963-967] Venous aneurysms, vascular and lymphatic malformations, and angiomatous lesions are rare.[952,968-974]

Rupture of the aorta or pulmonary artery is usually a life-threatening event or leads to severe sequelae.[768] Aortic aneurysms, rupture of aortic sinus aneurysm into the ventricular septum or right heart chambers (Figs. 9.51 and 9.52), aortic rupture into the pericardium, and acquired aortic-pulmonary fistula have been reported.[34,63,92,261-266,866,975-977] Rupture of the PA is a potential consequence of long-standing pulmonary hypertension and left-sided heart failure. Periparturient rupture of the middle uterine artery[260,978-982] and rupture of the internal carotid artery, external carotid artery, or maxillary artery with guttural pouch mycosis[983-985] are well recognized and may lead to fatal events. Arteriovenous communications occur rarely and may develop after rupture of smaller arteries or subsequent to growth of vascular tumors. Degenerative lesions (see Fig. 9.52A) including calcification may be observed in the great or peripheral arteries.[947,978,986,987] Degenerative and calcific lesions affecting the bifurcation of the right and left pulmonary arteries also have been reported,[988,989] although the clinical importance of these is not well defined. Arteriosclerosis and arterial thrombosis may be caused by parasite migration in the mesenteric arteries[990] and possibly at the terminal aortic quadrifurcation (Figs. 9.53 and 9.54). Aortic inflammation of unknown cause has been observed.[784,991] Pulmonary vascular disease and pulmonary structural remodeling due to pressure loading (e.g., left-sided heart failure) or increased pulmonary flow (e.g., left-to-right shunting through a VSD) can result in pulmonary hypertension with resulting right heart failure or cor pulmonale (see earlier discussion). Tumors of the great arteries and veins are rare,[952] but neoplasms can compress or invade arteries and veins, including the PA and vena cavae.

The ultrasonographic observation of spontaneous echo contrast within blood vessels and cardiac chambers has been suggested to indicate CV or rheologic disease,[992,993] as well as a normal phenomenon caused by rouleaux formation or platelet aggregates. This observation is common during ultrasound examinations, and contrast may be observed in a variety of vessels, as well as in the heart (see Figs. 9.42, 9.49, and 9.50). Pronounced spontaneous echo contrast is especially likely when there is actual obstruction to blood flow in vessels or when there are low flow rates due to bradycardia, arrhythmia, or myocardial failure. Contrast is often prominent in valvular endocarditis. Lesions, thrombosis, or even venipuncture of the jugular vein can lead to spontaneous echo contrast in systemic veins and in blood flow entering the right side of the heart, often becoming become more obvious if venous return suddenly increases. The identification of spontaneous contrast is also dependent on the operator with respect to transducer, gain, and grayscale contrast settings. The clinical significance

*References 34, 63, 64, 85, 91, 92, 96, 100, 145-147, 260-262, 264, 267-273, 364, 450, 451, 456, 458, 549, 768, 771, 864, 866, 867, 947-956.

of isolated intravascular contrast cannot be stated with certainty, and it has been observed in healthy horses as well as those with CV disease.

Serious vascular disease often can be suspected from the clinical examination, although it may not be obvious until a catastrophic vessel rupture or fatal hemorrhage occurs. In general, significant arterial disease reduces perfusion to the affected vascular bed, impairing tissue metabolism and function. If severe or complete, ischemia may lead to tissue necrosis (infarction) and even perforation of a hollow organ. There may be a bruit—auscultatory evidence of vascular narrowing or rupture—over the affected artery or obvious hemorrhage if a superficial the vessel has ruptured. Ultrasound and Doppler examination can demonstrate serious arterial disease provided there is an acoustic window for examination. Rarely angiography or nuclear scintigraphy is required to demonstrate arterial thrombosis or disruption. In major venous disease, the typical sign is swelling of soft tissues drained by the affected vessel. Palpable evidence of local inflammation and thrombosis may be found in superficial venous disease. Right-sided endocarditis, embolic pneumonia, and pulmonary embolism are potential consequences of systemic venous disease.

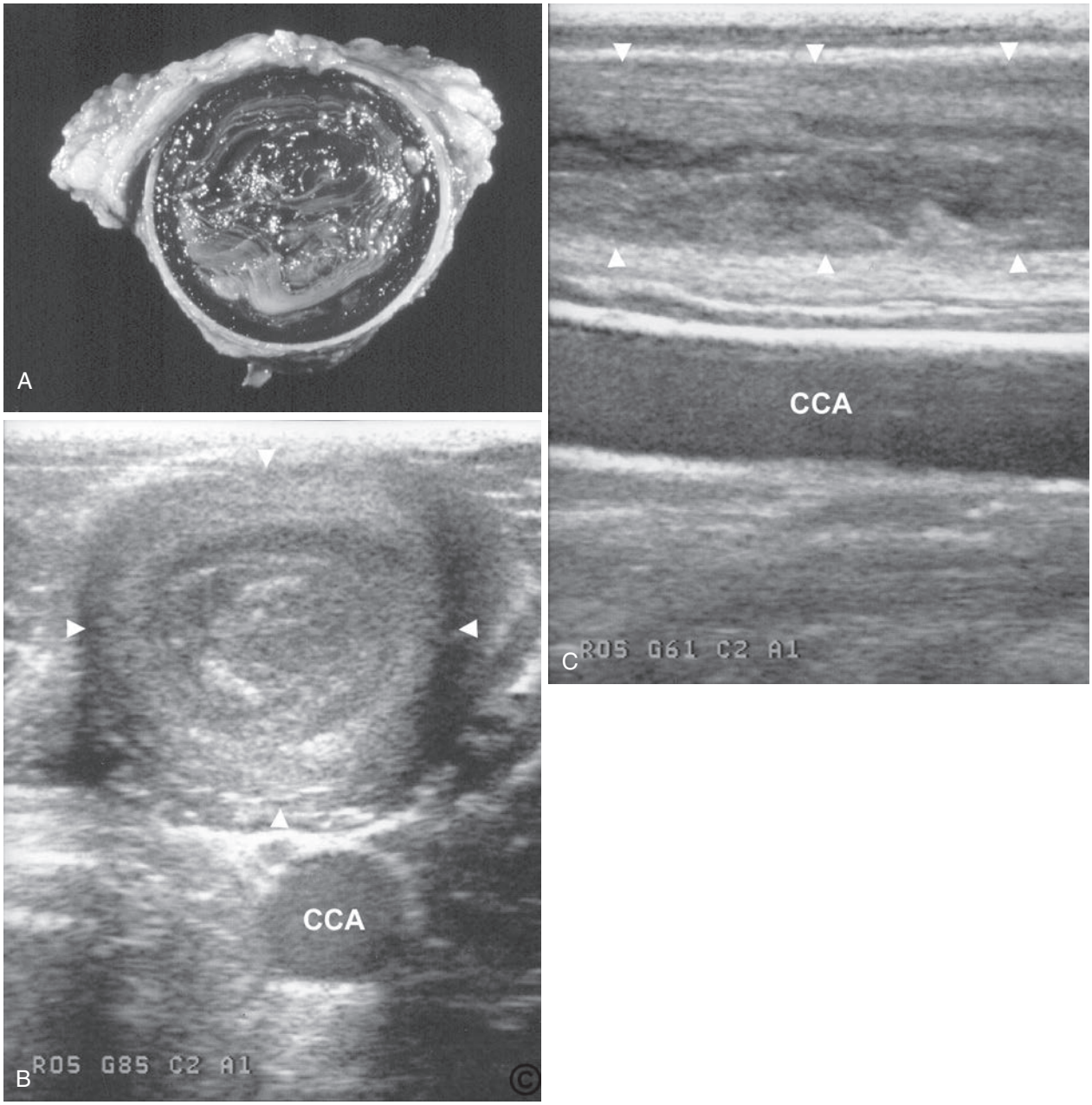


FIG. **9.50** A, Postmortem specimen of a thrombosed jugular vein in cross section. B and C, Ultrasonographic images obtained form a horse with jugular vein thrombosis in a cross-sectional (B) and a longitudinal (C) plane. The images show a mixed echogenic thrombus within the jugular vein *(arrowheads).* The common carotid artery (CCA) is displayed adjacent to the jugular vein. Residual blood flow through the affected area can be evaluated using duplex Doppler ultrasonography or newer vascular imaging technologies that directly visualize blood reflectors (e.g., red blood cells [RBCs]) in a grayscale display.

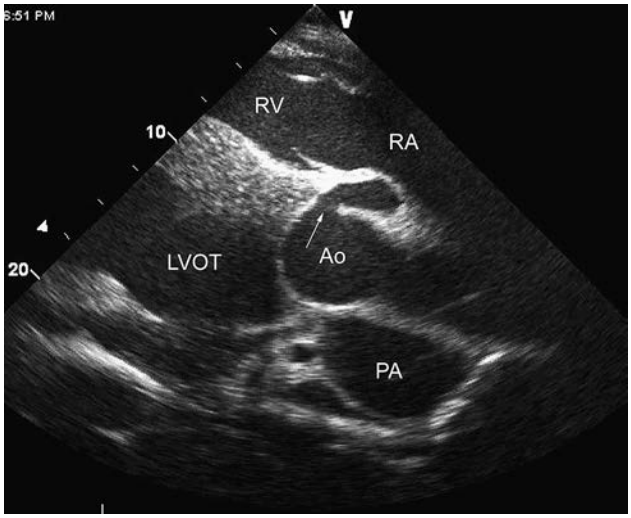


FIG. **9.51** Aortic to cardiac fistula. Off-angle long-axis image of the proximal aorta (Ao) shows the origin *(arrow)* of a ruptured sinus of Valsalva aneurysm. This channel communicated with the right atrium (RA) in another plane. The right ventricle (RV) and pulmonary artery (PA) are slightly dilated because of left-to-right shunting.

Thrombophlebitis

Thrombus formation results from an interplay of multiple factors, including vessel injury or intrinsic vessel wall lesions, blood hypercoagulability, and low-flow states or venous stasis (Virchow's triad).[957] Thrombophlebitis, the inflammation and thrombosis of a vein, in horses is most commonly caused by intravenous catheterization or injection (see Fig. 9.50),[456,458,957-962,994,995] suggesting that local trauma seems to be the most important factor.[959] The jugular veins are most often affected, but cephalic, lateral thoracic, and saphenous veins are sometimes also used for permanent venous access and therefore can be affected as well. The degree of vessel injury and therefore the risk of thrombophlebitis depends on a variety of factors, including technique of catheter placement, stability of the catheter, catheter hygiene and maintenance, training of personnel, and dwell time of the catheter.[457,959,967,996] Catheter material, length, and diameter likely determine the risk of thrombosis or thrombophlebitis. Longer catheters made of materials that are less stiff or rigid are preferable over short, rigid catheters. Polyurethane over-the-wire catheters are thought to carry the least risk of thrombosis and are commonly used for long-term indwelling venous catheterization in horses and foals. Polyurethane over-the-needle catheters are thought to

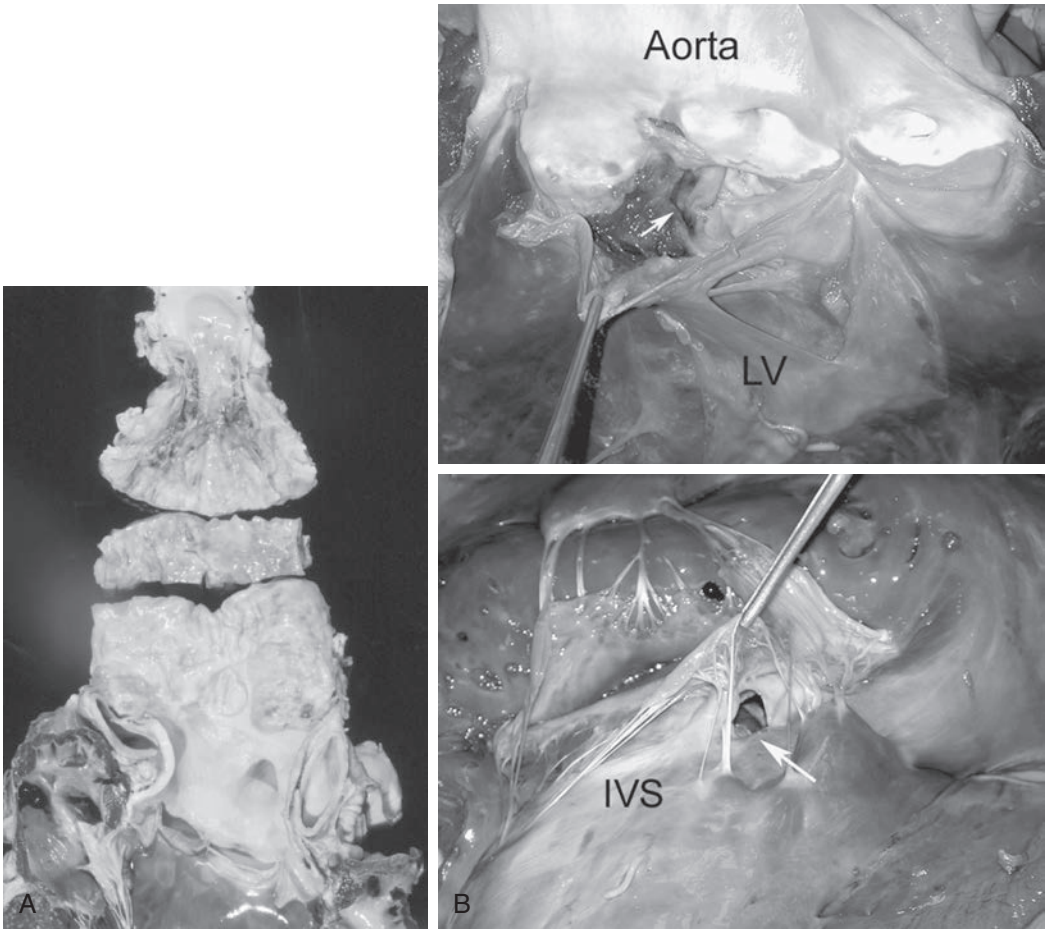


FIG. **9.52** Aortic disease. A, Severe atheromatous change with secondary *Streptococcus* spp. infection of the proximal aorta. The intimal and subintimal change begins just distal to the coronary ostia. Representative sections of the ascending aorta are shown. *Strongylus vulgaris* was not found. The most distal segment was necrotic and severely narrowed flow across the region. B, Dissecting lesion communicating the root of the aorta *(top panel, arrow)* to the right side of the heart across the intraventricular septum (IVS). The distorted tricuspid valve is retracted to demonstrate the lesion on the right *(arrow)*.

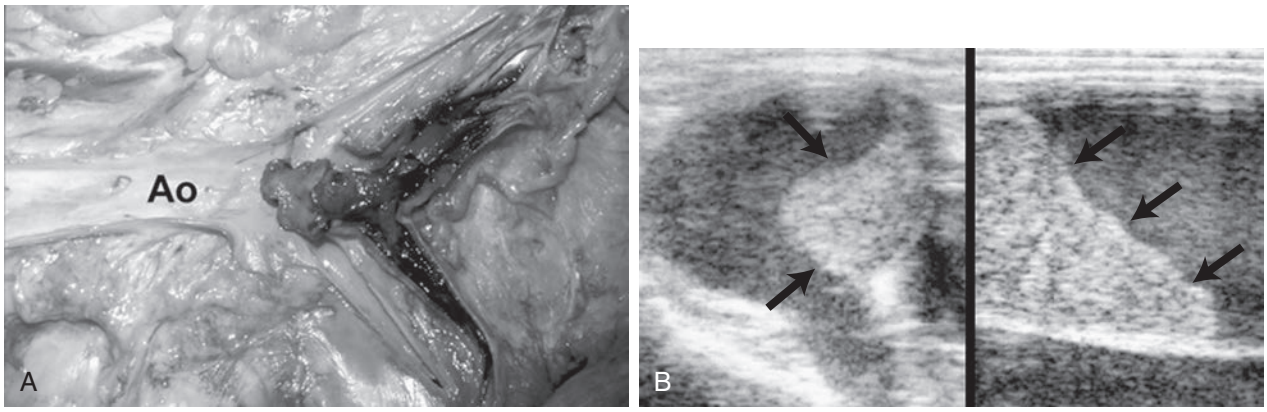


FIG. **9.53** Aortoiliac thrombosis. A, Postmortem image showing the thrombus in the terminal aorta (Ao) and the iliac arteries. B, Images obtained by rectal ultrasonographic examination in cross-sectional *(left)* and longitudinal *(right)* direction. The thrombus is evident as a hyperechoic mass within the vessel *(arrow)*.

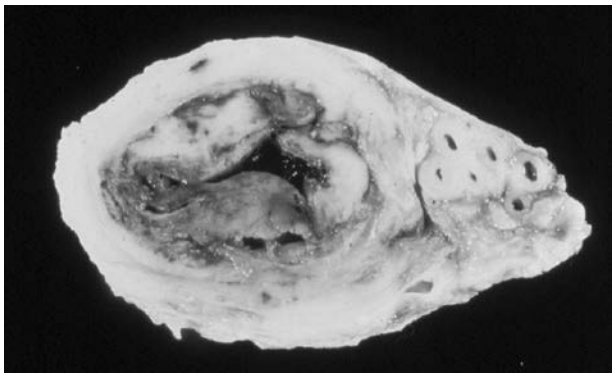


FIG. **9.54** Verminous arteritis and thrombosis in the cranial mesenteric artery caused by *Strongylus vulgaris*.

have a slightly higher risk, and polytetrafluoroethylene (PTFE, Teflon) catheters likely carry the greatest risk of thrombosis.[967,997-999] Some catheters are coated with antibacterial substances to inhibit bacterial growth, but proof of efficacy to prevent catheter-related infection is lacking in horses. The source (i.e., commercially versus locally produced), pH, and osmolality of intravenously administered fluids and the method and rate of IV fluid administration (determining the amount of turbulent blood flow and irritation to the vascular endothelium at the catheter tip) further influence the risk for venous thrombosis or thrombophlebitis.[456,457,967,998,1000] The pharmaceuticals most frequently associated with the development of thrombophlebitis include glyceryl guaiacolate, thiopental, calcium gluconate, oxytetracycline, and phenylbutazone.[957]

Patient factors, including presence of fever, large intestinal disease, hypoproteinemia, salmonellosis, endotoxemia, and other diseases characterized by a hypercoagulable state, are significantly associated with development of catheter-related thrombophlebitis in horses.[457,958,959,967,998,1001] Critically ill patients often stand with their head and neck in a low position, promoting increased turbulence and stagnation of blood flow, which further increases the risk of jugular thrombosis.[998]

Thrombophlebitis can be nonseptic or septic. Migration of skin organisms into the cutaneous catheter tract; direct contamination of catheter or catheter hub by contact with hands, contaminated fluids, or devices or when getting disconnected (e.g., when the horse is moving or rolling in the stall); hematogenous infection from another infectious focus; or contamination of the infusate can result in septic inflammation and thrombosis.[996]

Clinical signs of phlebitis are straightforward, including swelling over the affected vein and pain on palpation of the involved tissues. There is frequently a cellulitis associated with the catheter entrance site causing swelling, pain, and exudation. Note that phlebitis is not always associated with venous thrombosis. Conversely, venous thrombosis can occur without obvious signs of inflammation. Smaller mural thrombi may not cause any obvious clinical signs as long as residual (or collateral) blood flow is sufficient to allow adequate venous drainage. One study showed that approximately 30% of sick horses with long-term indwelling catheters may develop subclinical catheter-related disease.[1001] Partial or complete venous occlusion through a larger thrombus results in distention of the veins and possibly subcutaneous edema adjacent to the affected area. Unilateral complete jugular occlusion is usually well tolerated, but bilateral jugular vein thrombosis can lead to marked head edema and possibly result in poor performance. Marked swelling, heat, and pain on palpation, combined with fever, hyperfibrinogenemia, increased concentrations of serum amyloid A, or a neutrophilic leukocytosis, indicates infection of the thrombus.

The diagnosis and assessment of phlebitis, thrombosis, or thrombophlebitis is based on the detection of the previously described clinical signs and examination by duplex Doppler ultrasonography (see Figs. 9.50B and 9.50C).[458,591,1001] In case of thrombosis, imaging of the affected vein reveals filling of the lumen with an anechoic, hypoechoic, hyperechoic (if gas filled), or mixed echogenic material that partially or completely occludes the vessel. Ultrasonographic evaluation may also reveal thickening of the vessel wall (phlebitis), perivascular swelling, tracts extending from the vein to the subcutaneous tissues or skin, or subcutaneous abscesses. Septate appearance or cavitation in the center of a thrombus is suggestive of infection and represents a target for aspiration and bacterial culture. Anaerobic bacterial infections often result in gas production, creating multiple hyperechoic areas and

an acoustic shadow. Stagnant or turbulent flow may be manifested by increased spontaneous venous contrast. Flow across the affected zone may be evaluated by contrast venography using agitated saline as a contrast agent, with pulsed-wave or color-coded Doppler studies, or with newer vascular imaging technologies that directly visualize blood reflectors (such as red blood cells) in a grayscale or color-coded display. Venous drainage proximal and distal to the area of thrombosis also should be assessed as collateral circulation may still enter the vein distally. A careful evaluation of the communicating large veins and of the heart and lungs should be performed in horses with infective thrombophlebitis, particularly if clinical signs of systemic disease develop. Bacterial endocarditis may develop secondary to embolization of infective thrombus or bacteremia (see earlier discussion). Rarely, embolization of septic thrombi may also lead to pulmonary thromboembolism, pleuropneumonia, and other conditions associated with metastasis of septic thrombi.[961,962]

Early detection and management of phlebitis, thrombosis, or thrombophlebitis are crucial and significantly influence severity and outcome. Therefore at least twice daily careful monitoring of catheter and catheter site is mandatory for all indwelling catheters. Catheter caps and fluid lines should be changed regularly to minimize the risk of bacterial contamination. If a catheter is still in place when a thrombosis or thrombophlebitis is discovered, the catheter should be carefully withdrawn and the tip cultured. If possible, the opposite jugular vein should be preserved to avoid the risk of bilateral thrombophlebitis, and alternative options for fluid and drug administration should be sought.

Local therapy including hot compresses and topical ichthammol (ammonium bituminosulfonate), nonsteroidal antiinflammatory drugs (diclophenac sodium), heparinoids (chondroitin polysulfate), or dimethyl sulfoxide (DMSO) may be helpful in the treatment of thrombophlebitis. Heparin therapy (unfractionated heparin, 40–100 IU/kg of body weight, SC, q 6 h; dalteparin, 50–100 IU/kg of body weight, SC, q 24 h) may be beneficial to decrease progressive thrombosis, particularly if the thrombotic lesion is associated with a generalized coagulopathy.[982,1002,1003] Furthermore, early regional thrombolytic therapy may be used in an attempt to resolve jugular vein thrombosis and to achieve recanalization.[957,1004-1006] However, clear evidence of the efficacy of antithrombotic and thrombolytic treatment is lacking. Infections with multiple organisms should be anticipated in cases of infective thrombophlebitis, and high doses of broad-spectrum antimicrobials should be administered until culture and sensitivity results of the aspirate are obtained. Metronidazole (15 mg/kg PO every 6 hours or 25 mg/kg PO q 12 h) should be considered if anaerobic infection is suspected. Flunixin meglumine (1 mg/kg every 12 hours) may be added to reduce inflammation. If antimicrobial therapy is unsuccessful, surgical resection of the affected tissue or drainage of abscesses may be indicated.

Recanalization of an affected vein often occurs and can be documented by Doppler or contrast ultrasound studies demonstrating blood flow between the vessel wall and the thrombus. Recovery often requires 4 to 8 weeks or longer. Occasionally, loculation may be detected within the ends of the thrombus. Such loculation may result in persistent fibrous webs within the vein that restrict venous return. Venous stricture also may occur due to long-standing thrombophlebitis. Complete fibrous occlusion of the jugular vein may impede venous return from the head such that if adequate collateral circulation does not develop, impaired venous drainage of the head and neck may limit performance or reduce the chance of returning to the previous level of activity in high-level athletes.[961] Bilateral occlusion of the jugular veins with insufficient collateral vascularization may cause swelling of the head and cervical region, leading to dysphagia and airway obstruction that might be performance limiting or even life threatening.[1007,1008]

When jugular vein thrombophlebitis is refractive to medical treatment or causing severe performance-limiting or even life-threatening clinical signs, surgical techniques including phlebotomy, thrombectomy with Fogarty's catheter, or venous grafts may be performed to restore blood flow.[994,995,1009] Alternatively, an expert in interventional radiology or medicine can be consulted regarding other options, such as intravascular stenting.

Prophylaxis for venous thrombosis or thrombophlebitis includes minimizing all of the aforementioned risk factors. Administration of a nonsteroidal antiinflammatory drug while the catheter is in place has been shown to reduce the risk of catheter-related complications.[1001] Antithrombotic prophylaxis is commonly performed using anticoagulants or antiplatelet drugs, but clear evidence of its efficacy is currently lacking.

Aortoiliac Thrombosis

Aortoiliac thrombosis is an uncommon, but potentially serious, disorder. The gross appearance, histologic features, and clinical findings of this vaso-occlusive disorder have been reviewed extensively in the literature.[89,267-272,450-455,950,955,1010-1017] The terminal aorta and the iliac arteries, including their proximal branches, are the focus of involvement in this disease (see Fig. 9.53A). Vessels become partially to completely occluded by multifocal ingrowths of fibrous tissue, laminated thrombi, or fibrous plaques.[267] Histologic lesions include organized, fibrous connective tissue, hemosiderin-laden macrophages, irregular vascular channels, and disruption of the intima and internal elastic lamina. The lesions are generally devoid of fat. Arteriosclerotic and atherosclerotic aortic lesions have also been observed at other sites as well (see Fig. 9.52A). Suggested etiologies of these arterial disorders include *Strongylus vulgaris* infection, systemic infections, embolization, and vasculitis. There is no convincing evidence that aortoiliac thrombosis is consistently caused by *Strongylus* infection, and some have refuted this contention.[269,271]

Although aortoiliac thrombosis is uncommon, it can be associated with severe performance problems. The typical features include exercise-associated, typically (but not uniformly) unilateral, hindlimb lameness, ataxia, or collapse.[267,269,271,272] Aortoiliac thrombosis has also been diagnosed as a cause of breeding failure in stallions.[271,272,955] Physical examination of an affected horse at rest may reveal weak metatarsal arterial pulses or delayed saphenous refill in the affected limb. The temperature of the limb in the resting animal is usually normal, unless complete arterial occlusion has occurred, in which case the limb is cold and painful and may become edematous. Exercise in affected animals results in an exercise-associated gait abnormality (lameness, ataxia, or weakness) with a decreased or absent metatarsal and digital pulse and delayed or absent saphenous refill. This intermittent claudication may cause the horse to become very uncomfortable and reluctant to bear weight on the affected limb. Marked hyperpnea, other signs of distress, and profuse, generalized sweating

are often present with trembling of the affected limb. A rectal examination may reveal fremitus, a weak or absent pulse, or aneurysmal dilation of the affected artery or arteries. These abnormalities may be more evident after exercise and may help to confirm the diagnosis.

The diagnosis can be confirmed by ultrasonographic evaluation of the terminal aorta and iliac arteries (see Fig. 9.53B) with a high-frequency (5–10 MHz) rectal transducer[268,270,451,1016] or by nuclear techniques.[1011,1018] Doppler studies may indicate abnormal blood flow in the femoral arteries.[1019] Essential abnormalities include a hypoechoic to echogenic mass protruding into the arterial lumen. An estimation of the degree of obstruction can be made based on the percentage of the artery occluded. Many cases are longstanding, and hyperechoic areas, even tissues sufficiently echodense to cast acoustic shadows, may be imaged within the aortic or iliac thrombus. These findings suggest mature scar tissue and calcification.

The prognosis for this disorder is guarded. No controlled studies have evaluated therapy for this condition. A case survey[1010] suggests no treatment consistently improves outcome, but there is potential benefit of surgical or catheter-based thrombectomy or anticoagulation. Early diagnosis likely is essential if therapy is to be beneficial. Treatment is aimed at improving collateral circulation and preventing additional thrombus formation. Various medical treatments have been reported including intravenous sodium gluconate, larvicidal dewormings, phenylbutazone, low-molecular-weight dextran, anticoagulants,[455] and a controlled exercise program. There are no reports of successful thrombolytic therapy at this time. Ultrasound-guided balloon thrombectomy has been described and is probably most useful in cases of active thrombosis where the obstruction can be partially relieved.[950,1012,1013,1017,1020] In one study, 65% of the horses regained athletic activity, and 53% of the operated horses performed at their previous level after surgical thrombectomy.[1020] Postoperative complications included severe myopathy (24% of cases), acute thromboembolism on the contralateral limb, wound healing problems, and reocclusion.

Parasitic Vascular Lesions

The migrating larval forms, particularly L4, of *S. vulgaris* are known causes of arterial disease, particularly involving the aorta and the cranial mesenteric artery and its branches (see Fig. 9.54). Lesions have been described as far forward as the bulbous aorta and the aortic sinuses in infected horses. Rounded fibrous plaques and mural thrombi have been reported in the thoracic and cranial abdominal aorta in 9.4% of horses examined immediately after death.[145] These investigators reported a statistical association between the occurrence of proximal aortic *S. vulgaris* lesions and the presence of focal ischemic lesions in the myocardium. They hypothesized that these lesions were consequent to microembolism from parasitic lesions. With the advent of ivermectin and other new anthelmintics, and aggressive deworming programs currently recommended by practicing veterinarians, heavy *S. vulgaris* larval migration damage occurs much less frequently. However, migration of *S. vulgaris* L4 larvae must be considered in horses with poor deworming histories, high potential of exposure, high fecal egg counts, and palpable abnormalities on rectal examination, or when fremitus or aneurysmal dilation of the aorta is found, particularly in the region of the cranial mesenteric artery or iliac system. An ultrasonographic evaluation of the cranial mesenteric artery is possible and can be used to confirm the diagnosis of thrombosis in these vessels.[954,1010,1021] Treatment should consist of larvicidal deworming, combined with a rigorous individual and environmental parasite control program.

Arteriovenous Fistulas

Arteriovenous fistulas occur uncommonly and are most often detected with large vascular tumors (hemangiomas, hemangiosarcomas) or as a rare congenital defect or posttraumatic sequelae.[953,956,969,972] Classic features include a continuous thrill and murmur over the affected area, localized edema, slowing of the HR after occlusion of the shunt, and signs of increased CO if the shunt flow is great. Clinically important fistulas are more likely to develop where large arteries and veins run in parallel, as with the jugular vein and carotid artery. A diagnosis can be made with Doppler ultrasound, including pulsed-wave and color-flow Doppler to demonstrate continuous and pulsatile flow from the artery, through the associated communication, into the distended veins. A complete cardiovascular examination should be performed because a large arteriovenous communication could lead to cardiac failure. Treatment depends on the underlying cause, and an experienced surgeon should be consulted. No treatment may be indicated with small, uncomplicated arteriovenous communications. Large vascular neoplasms are usually inoperable at the time when they are diagnosed or complicated by the possibility of metastasis.

Vascular Rupture

Rupture of the aorta or its branches or of the PA are reported sporadically and often are catastrophic events, with diagnoses made at the necropsy floor. Ruptures of the extrapericardial aorta or other blood vessels leading to fatal hemorrhage or a systemic to pulmonary shunt have been reported.*

Rupture of the middle uterine artery can cause severe periparturient hemorrhage into the broad ligament (hematoma formation) or into the abdomen (acute hemoabdomen). It is more common in older, multiparous mares and is characterized by postpartum colic, tachycardia, anemia, and often sudden death.[260,978-982] Mycotic disease within the guttural pouch is a well-recognized cause of arteritis and rupture of the affected arteries.[983-985] Uterine artery rupture and arterial rupture secondary to guttural pouch mycosis are covered in Chapters 19 and 9, respectively.

Aortocardiac and Aortopulmonary Fistula

Rupture of the aortic root has been commonly reported in older horses, usually older breeding stallions, but it may also occur in older mares or in younger horses.[63,92,262-264,864,866,975] Rupture typically involves the right aortic sinus of Valsalva (see Figs. 9.51 and 9.52B). The aortic root can rupture into the right atrium, right ventricle, interventricular septum, or pericardial space. Dystrophic changes in the media of the aorta, degenerative changes associated with chronic aortic insufficiency, and the hypertension associated with breeding all have been implicated as potential causes of aortic root rupture.[92,263,864,1023] Aneurysms of the right sinus of Valsalva have also been reported and are related to congenital or acquired defects in the media of the aorta near the right coronary sinus.[34,262,864-867,1024]

* References 85, 91, 93, 94, 96, 98-103, 106, 1022.

Rupture of the more distal aorta into the pulmonary artery also can occur.[34] Aortopulmonary fistulation has been mostly reported in Friesian horses.[261,265,266,976,977] The rupture is usually located close to the arterial ligament and concurrent with a circumferential cuff of perivascular hemorrhage.[265] Recent histologic studies suggest a connective tissue disorder affecting elastin or collagen in the aortic media as a potential underlying cause of aortic rupture in Friesian horses.[266,976]

Not all aortic ruptures are immediately fatal. Many horses present with acute onset of poor performance, exercise intolerance, sustained tachycardia, and pain that is often perceived as colic. When aortic rupture or rupture of a sinus of Valsalva aneurysm communicates with the right atrium, right ventricle, or pulmonary artery, right-sided heart failure can occur. Cardiac arrhythmias (usually ventricular tachycardia) may also develop, especially if dissection progresses into the interventricular septum.[263]A continuous machinery murmur loudest on the right side of the thorax with bounding arterial pulses is characteristic of a horse with an aortocardiac fistula.[68,263,264] Friesian horses with aortopulmonary fistulation present with bounding arterial pulses, tachycardia, and a grade 1 to 3/6 holosystolic and early to middiastolic murmur loudest dorsal to the aortic valve; ventricular arrhythmias are rare.[68,265,1025]

Antemortem diagnosis of an aortic sinus of Valsalva aneurysm or aortocardiac fistula is possible by echocardiography and Doppler interrogation (see Fig. 9.51). Because of the more distal location of aortopulmonary fistulas in Friesian horses, echocardiographic imaging is more challenging and requires nonstandard, tilted imaging planes obtained from the left third or fourth intercostal space in order to visualize the aortic rupture and fistula; in addition, right heart and pulmonary artery dilation, tricuspid regurgitation, and pleural and pericardial effusion can be found.[265,1025,1026] Transesophageal ultrasound may allow for better visualization of the aortopulmonary region and for diagnosing aortic rupture in Friesian horses, but the lack of appropriate equipment usually precludes this option in horses.[1026] If echocardiography does not allow definitive diagnosis of an aortopulmonary fistula, cardiac catheterisation may serve to document increased right heart and PA pressures with increased oxygen partial pressures and saturation in the distal pulmonary artery indicating left-to-right shunting of blood.[1025]

Aside from breeding, horses with diagnosed aortic aneurysms should not be used for athletic endeavor, because rupture of the aneurysm could occur at any time. The prognosis for life in horses with aortic rupture is generally guarded to poor, depending on the site and size of the communication. Some horses survive and others may require supportive treatment for CHF. Fatal ventricular arrhythmias can develop any time and lead to sudden cardiac death. Therefore horses with aortocardiac or aortopulmonary fistulas are not considered safe to use.[68]

Pulmonary Artery Rupture

Pulmonary artery rupture is most often caused by chronic pulmonary hypertension and PA dilation.[148,768] This is most commonly detected in conjunction with severe MR and pulmonary hypertension. The potential for PA rupture should be considered whenever a large dilated main PA or left and right PA is detected echocardiographically. Affected horses should be considered unsafe for riding or driving and should not be used, except for breeding, due to the possibility of sudden death. Focal medial calcification of the PA is not uncommon at necropsy[947,989] but is of uncertain clinical significance.

CARDIAC ARRHYTHMIAS

Cardiac arrhythmias are disturbances in HR, rhythm, or conduction and can be classified based on atrial and ventricular rate, anatomic origin of the impulse, method of impulse formation, and conduction sequence. Arrhythmias can develop as isolated electrical disorders or secondary to other etiologic factors, including (1) structural heart disease; (2) metabolic and endocrine disorders; (3) systemic inflammation; (4) hypotension, hemorrhage, anemia, and ischemia; (5) autonomic influences; (6) toxicosis/envenomations; and (7) drugs.[68] A wide variety of cardiac arrhythmias have been recognized.[54,60,736,1027,1028] Of principal concern to the clinician are the hemodynamic consequences of arrhythmias (reduced pressure, flow, perfusion) and the potential for further electrical instability (myocardial fibrillation, sudden death).

The electrophysiologic basis of cardiac arrhythmias will not be discussed here, and the interested reader is referred elsewhere for descriptions of abnormal automaticity, reentry, and triggered activity (early and late afterdepolarizations) as mechanisms of arrhythmogenesis.[57,112,113,117] Cardiac arrhythmias are classified based on the anatomic origin of the manifest ECG mechanism (Box 9.12). The reader should be aware that some arrhythmias, especially those originating in the AV junction, may mimic either atrial rhythm disturbances or "high" ventricular rhythms. For purpose of discussion we have elected to distinguish "atrial" from "junctional"; however, "supraventricular" may also be applied to these rhythm disturbances.

The clinical evaluation of the horse with an arrhythmia is reviewed in Box 9.13. Routine electrocardiography, clinical chemistry, hematology, echocardiography, exercising electrocardiography, and continuous 24-hour electrocardiographic (Holter) monitoring can all play a role in the complete evaluation of a horse suspected of having intermittent cardiac arrhythmias.[68] These points are discussed further later in the chapter. A recent consensus statement formulated by a group of experts in the field of equine cardiovascular medicine summarizes the current recommendations for management of equine athletes with cardiac arrhythmias, and the reader is referred to this statement for additional information.[68]

Sinus Rhythms

A number of physiologic sinus rhythms are recognized. These can be explained by the impact of autonomic nervous system traffic on the SA node (Fig. 9.55). Normal horses at rest demonstrate vagal-mediated sinus bradycardia, sinus arrhythmia, sinus block, and sinus arrest; however, fear or a sudden stimulus may provoke sympathetically driven sinus tachycardia. Exercise leads to pronounced sinus tachycardia, with HRs often exceeding 200 beats/min.[53,54] Atrioventricular (AV) conduction, as a general rule, tends to follow sinus activity. During sinus tachycardia, the PR interval shortens, whereas during periods of progressive sinus node slowing the PR interval generally prolongs. Mobitz type I second-degree AV block (Wenckebach periodicity) often follows a progressive prolongation of the PR interval. Sinus rate and AV conduction usually change in parallel; however, some horses appear to control BP during high sinus tachycardia by blocking impulses in the AV node (see Fig. 9.6). Second-degree AV block is more likely in a standing horse, in a horse that has suddenly stopped submaximal exercise, or after a brief surge of sinus tachycardia in an anxious animal.

Sinus rate and rhythm should be carefully monitored in the critically ill or anesthetized horse because HR is a major

BOX 9.12 Cardiac Rhythms in the Horse

PHYSIOLOGIC RHYTHMS

Sinus Rhythms[a]

Normal sinus rhythm
Sinus arrhythmia
Sinoatrial block/arrest
Sinus bradycardia
Sinus tachycardia

Conduction Disturbances[a]

Atrioventricular block (AV block)
- First-degree (long PQ or PR interval)
- Second-degree (P wave not followed by a QRS complex; commonly Mobitz type I, Wenckebach)

PATHOLOGIC RHYTHMS

Atrial Rhythm Disturbances

Atrial escape complexes[b]
Atrial premature complexes[a]
Atrial tachycardia, nonsustained and sustained
Reentrant supraventricular tachycardia
Atrial flutter, atrial fibrillation[a]

Junctional Rhythm Disturbances

Junctional escape complexes[b]
Junctional escape rhythm[b] (idionodal rhythm)
Junctional premature complexes[a]
Junctional ("nodal") tachycardia
Reentrant supraventricular tachycardia

Ventricular Rhythm Disturbances

Ventricular escape complexes[b]
Ventricular escape rhythm[b] (idioventricular rhythm)
Ventricular premature complexes[a]
Accelerated idioventricular rhythm (idioventricular tachycardia, slow ventricular tachycardia)*
Ventricular tachycardia
Ventricular flutter
Ventricular fibrillation

Conduction Disturbances

Sinoatrial block (high grade or persistent)
Atrial standstill (sinoventricular rhythm caused by hyperkalemia)
Atrioventricular block (AV block)
- Second degree (high grade or persistent)
- Third degree (complete, with AV dissociation)

Ventricular conduction disturbances
Ventricular preexcitation

[a]Most common rhythms and arrhythmias.
[b]Escape complexes develop secondary to another rhythm disturbance.

BOX 9.13 Evaluation of the Horse with a Cardiac Arrhythmia

General medical history, past illnesses, past and current medications
Work history and exercise tolerance
General physical examination
Physical examination: evidence of congestive heart failure
- Subcutaneous edema
- Jugular venous distention and pulsations
- Pulmonary edema
- Pleural and peritoneal effusion
- Weight loss, poor condition

Auscultation of the heart
- Assessment of heart sounds (rhythm, third heart sound)
- Assessment of heart murmurs

Resting electrocardiogram
- Heart rate and cardiac rhythm
- Analysis of P-QRS-T waveforms

Exercise (or treadmill) electrocardiogram
Clinical laboratory studies
- Complete blood count (rule out anemia, infection)
- Blood culture (in cases of suspected endocarditis)
- Serum electrolytes (particularly potassium and possibly magnesium)
- Serum biochemical tests (especially renal function)
- Skeletal muscle enzymes (cardiac isoenzymes)
- Serum troponin concentration
- Red blood cell potassium
- Fractional excretion of potassium

Echocardiogram/Doppler echocardiogram
- Examine for cardiomegaly or reduced myocardial function
- Identify predisposing cardiac lesions and abnormal flow

Serum/plasma concentrations of cardioactive drugs
- Serum digoxin concentration
- Plasma quinidine concentration

Response to medications

determinant of CO and arterial BP (Fig. 9.4B). Sedative drugs and anesthetics can cause sinus bradycardia or sinus arrest. Anesthetic drugs, hypoxia, traction on an abdominal viscus, ocular manipulation, hypothermia, increased intracranial pressure, and hypertension can depress sinus node function. Concurrent depression of the sinus node with stimulation of latent pacemakers in the coronary sinus or AV junction can lead to ectopic rhythms in the anesthetized horse. Conversely, an increasing sinus rate may indicate inadequate depth of anesthesia, pain, hypotension, hypovolemia, hypercarbia and hypoxemia, anemia, endotoxemia or sepsis, pyrexia, systemic inflammatory response syndrome (SIRS), anaphylaxis, or excessive catecholamine administration. Specific therapy for sinus tachycardia is rarely required because it represents a physiologic response to stress. However, when sinus tachycardia is identified, the cause must be sought and treated as appropriate.

Sinus bradycardia is generally a benign rhythm in standing horses; however, when encountered during sedation or anesthesia this rhythm can lead to hypotension. Treatment of symptomatic sinus bradycardia can include the infusion of catecholamines (dobutamine, dopamine, epinephrine) or the administration of anticholinergic drugs (atropine, glycopyrrolate). Dopamine or dobutamine can be infused to increase HR, contractility, and arterial BP; however, reflex AV block may develop in some horses.[237,246,278,327] Excessive administration of catecholamines causes sinus tachycardia and ectopic beats. Anticholinergic drugs may not be effective in the setting of anesthetic-induced depression of SA function, particularly if vagal efferent traffic is low. Gastrointestinal complications including ileus and colic may develop after anticholinergic drug therapy; thus it should not be chosen for trivial rate problems.

Atrial Arrhythmias

Rhythms originating in the atria are common. These disturbances often develop as "functional" disorders with

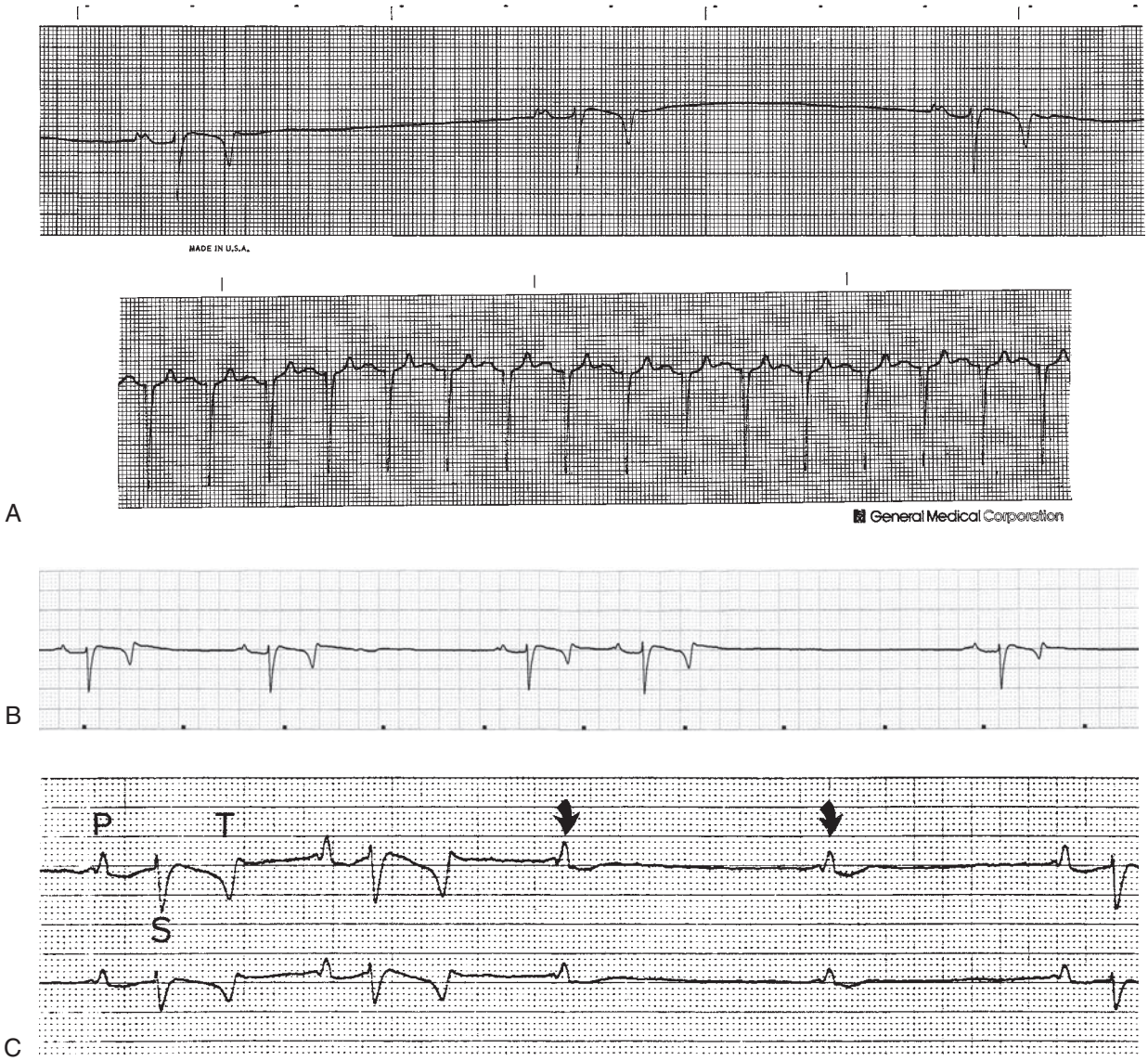


FIG. **9.55** Sinus rhythms. A, Sinus bradycardia *(top)* and tachycardia *(bottom)*. Base-apex lead recorded at 25 mm/sec. B, Sinus arrhythmia with sinus arrest/sinus block recorded at 25 mm/sec paper speed. C, Sinus arrhythmia with second-degree atrioventricular block *(arrows)*. An ambulatory electrocardiogram (ECG) is shown; transthoracic leads recorded at 25 mm/sec. Whereas two consecutively blocked P waves are considered normal on a 24-hour ECG, these phenomena are observed infrequently (in approximately 1% of normal horses).

no overt structural cardiac lesion. Autonomic imbalance (including high sympathetic activity or high vagal tone), hypokalemia, drugs (e.g., catecholamines, anesthetics), infections, fever, anemia, hypoxia, and colic can be associated with atrial premature complexes. Atrial rhythm disturbances are also very common in the setting of structural lesions of the heart valves, myocardium, and pericardium. Cardiac conditions known to predispose to atrial arrhythmias include mitral or tricuspid insufficiency, endocarditis, myocarditis, cardiac (atrial) enlargement, myocardial fibrosis, and myocardial ischemia. Atrial premature complexes can precipitate sustained atrial arrhythmias, including atrial tachycardia, atrial flutter, and atrial fibrillation. The large size of the equine atria and the frequent presence of microscopic atrial fibrotic lesions likely predispose the horse to these sustained arrhythmias. The high resting vagal tone present in most horses serves to shorten the duration of the action potential of atrial myocytes and also facilitates development of sustained atrial tachyarrhythmias, which probably depend on reentry.

Atrial arrhythmias are the most common abnormal rhythms detected in horses (Figs. 9.56 and 9.57).[60,77-84,479,538,1029] Atrial premature complexes are the least complex of these rhythm disturbances; these can be clinically irrelevant or associated with exercise intolerance or other signs of cardiac disease. In contrast, atrial tachycardia, atrial flutter, and AF are more likely to become clinically important. The overall importance of atrial premature complexes is often difficult to ascertain. Interpretation of routine ECG rhythm strips recorded from over 950 horses indicated that atrial arrhythmias, overall, were present in less than 3% of the horses studied (personal communication, JD Bonagura). However, when clinically normal

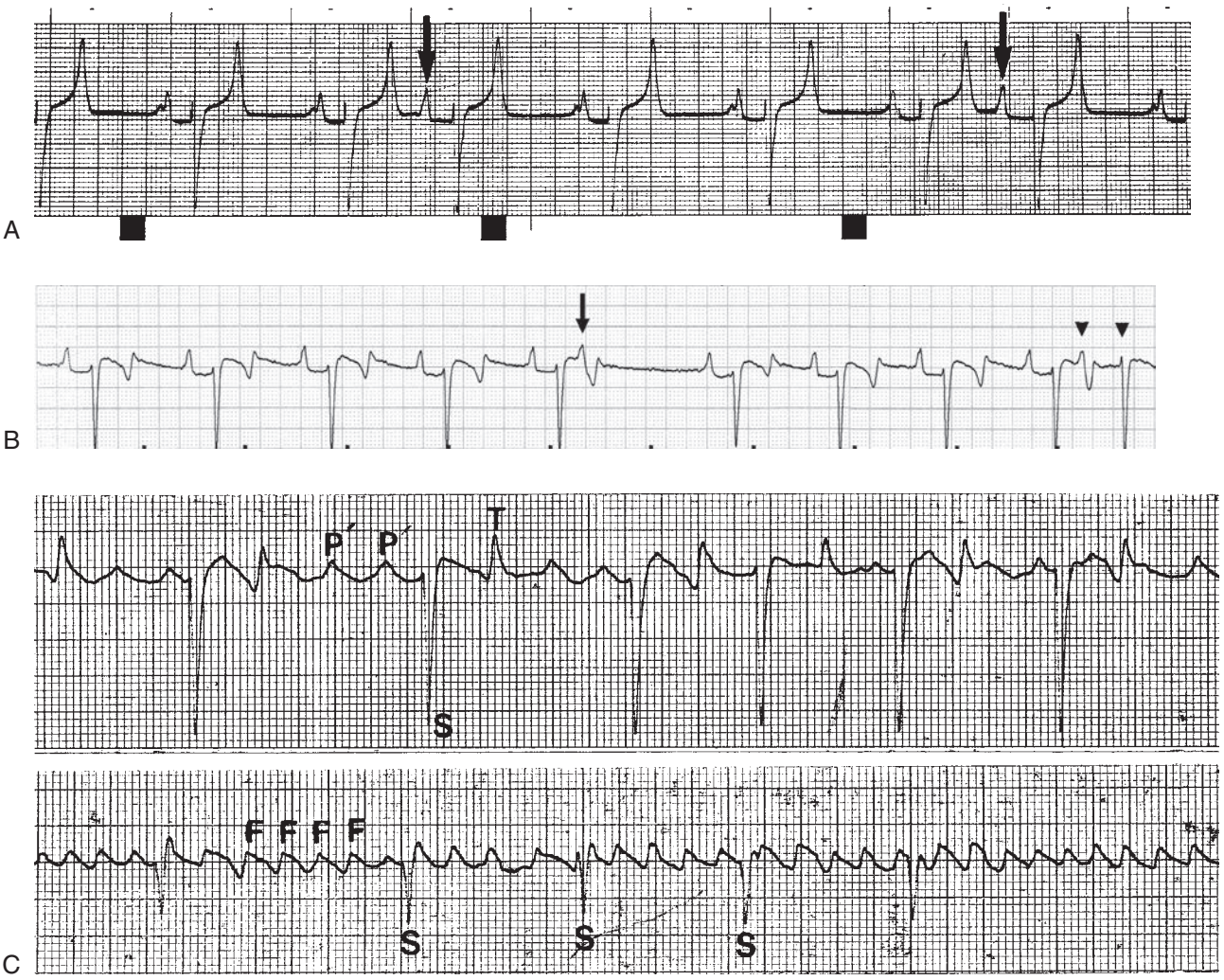


FIG. **9.56** Electrocardiograms (ECGs) with atrial rhythm disturbances (recorded at 25 mm/sec). A, Premature atrial complexes *(arrows).* The premature P waves of different morphology, the slightly prolonged P-R interval indicating atrioventricular nodal refractoriness, and the normal-appearing QRS complex indicating a supraventricular origin are notable. A slight conduction aberrancy is evidence in the second premature QRS complex (see also Fig. 9.15). B, Premature atrial complexes occurring during the QT interval of the preceding complexes. The first premature complex is nonconducted *(arrow),* the second premature complex is conducted with first-degree AV block *(arrowheads).* C, Sustained atrial tachyarrhythmias. The top picture shows atrial tachycardia with rapid, regular P waves (P′) and a variable ventricular rate response to conducted P waves. The base-apex lead recorded at 25 mm/sec. The lower trace shows atrial flutter (F) with variable ventricular response (S). Lead 3 recorded at 25 mm/sec.

horses were examined by Holter monitor, atrial premature complexes were recorded in 28% of the horses (personal communication, VB Reef). The prevalence of atrial arrhythmias during and after exercise has been reported as 29% to 89% and 46% to 62%, respectively,[77-79] with the reservation that it may be difficult to differentiate between supraventricular and ventricular premature beats on exercise ECGs. Thus it appears that the incidence of atrial arrhythmias depends not only on the population examined but also on the methods used for identification. These rhythm disturbances should be assessed in light of other clinical findings.

Atrial Premature Complexes

Atrial premature complexes (APCs) are detected by auscultation and are documented by an ECG. Auscultation usually reveals a regular sinus rhythm that is interrupted by an obviously premature beat or—in case of a blocked APC—by an abnormal pause (see Fig. 9.56A and B). Premature atrial complexes may be fully interpolated (there is continuation of the regular sinus rhythm after the premature atrial contraction), or there may be a pause, if the sinus node is reset or the premature complex is not conducted. The ECG is characterized by a premature (usually narrow) QRS complex, which is preceded by an abnormal, premature P wave (P′ wave) that is often buried within the preceding T wave (see Fig. 9.56B). If the impulse arrives in the AV node before complete repolarization, the PR interval is longer than normal (physiologic first-degree AV block; see Fig. 9.56A). If the ectopic P′ wave is not conducted (physiologic second-degree AV block) a pause will be evident in the ventricular rhythm (see Fig. 9.56). Premature

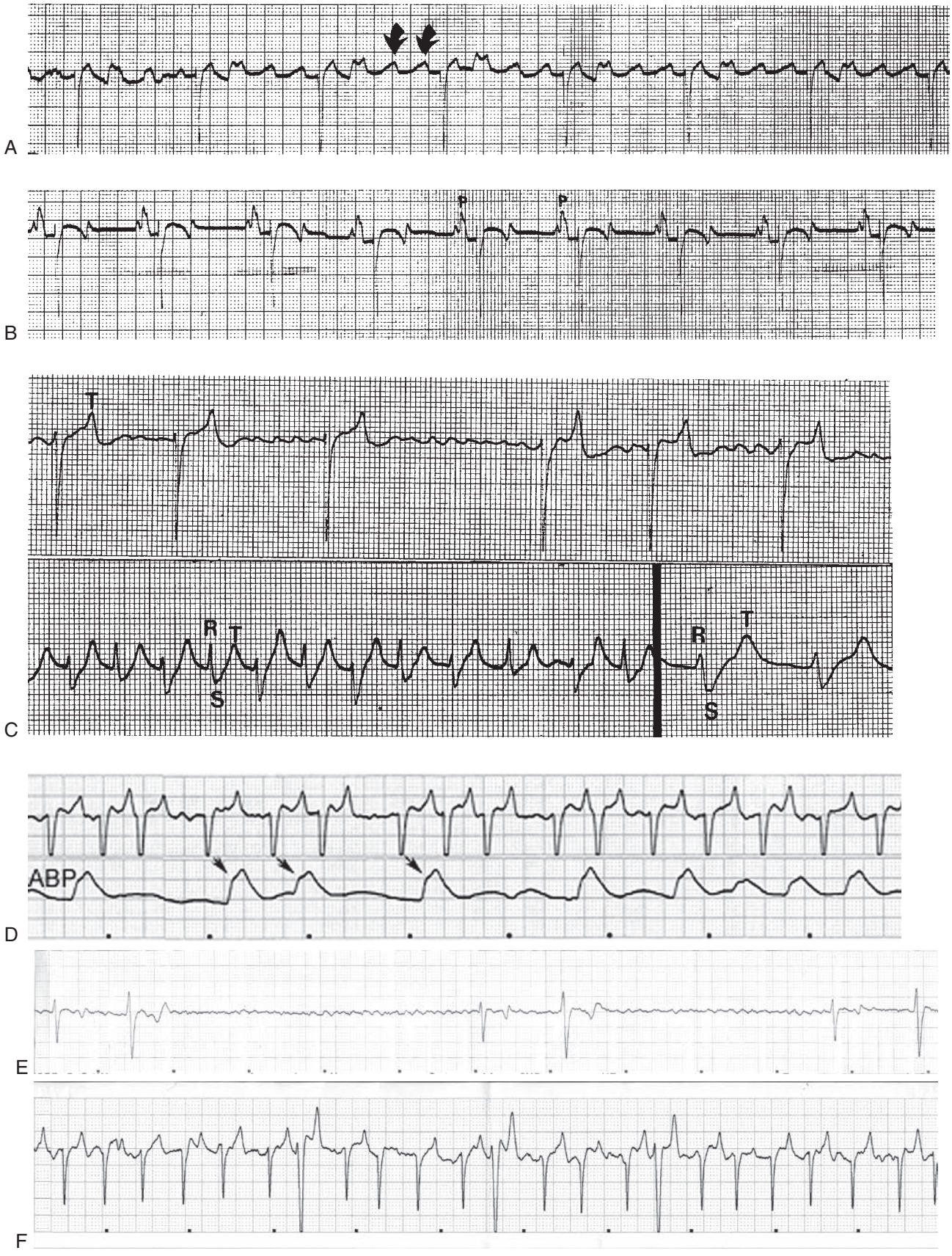

A
B
P
P
C
T
R
T
S
R
T
S
D
ABP
E
F

FIG. **9.57** Atrial arrhythmias. A and B, Successful conversion of atrial fibrillation (AF) using a combination of digoxin and quinidine (see text for details). A, Incomplete conversion of AF to atrial tachycardia with rapid, regular atrial activity *(arrows).* B, Conversion to normal sinus rhythm during combination therapy. Notice the high amplitude of the second part of the P wave, indicating possible atrial enlargement. C, Quinidine toxicosis can be manifested as abnormalities on the electrocardiogram (ECG). In this horse, AF (*top;* base-apex lead recorded at 25 mm/sec) was not converted. The lower recording shows persistent AF with a rapid atrioventricular conduction response, electrical alternans, and widening of the QRS complex (lead 2 recorded at 25 mm/sec; *right panel,* at 50 mm/sec). The rapid rate response is related to the vagolytic effect of quinidine. The electrical alternans is common with regular supraventricular tachycardias with rapid ventricular response rates; this finding indicates varying conduction into the ventricle. The widened QRS is a sign of quinidine toxicosis. An idiosyncratic reaction leading to polymorphous ventricular tachycardia (VT) also has been recognized (see Figs. 9.58 and 9.61). D, Hemodynamic consequences of increasing ventricular response rate in AF. The short QRS-QRS intervals do not generate effective arterial pulsations recorded in the arterial blood pressure (ABP) tracing, which is related to inadequate ventricular filling time during short cycles. Pulse deficits are detectable by physical examination. E, Atrial fibrillation with occasional QRS-T complexes of different morphology (base-apex lead recorded at 25 mm/sec). Note that the large and slightly wider QRS complexes always occur when a short cycle follows a long cycle. These QRS complexes are most likely caused by aberrant ventricular conduction, and this phenomenon is called *Ashman's phenomenon* (or phase 3 aberrancy). F, Atrial fibrillation in a 14-year-old Appaloosa with dilated cardiomyopathy and heart failure (base-apex lead recorded at 25 mm/sec). The heart rate is 132 beats/min. Three ventricular ectopic beats are evident, characterized by different QRS-T morphology.

atrial impulses can also be conducted aberrantly through the ventricle as a result of incomplete repolarization or persistent refractoriness of ventricular conducting tissues; this causes the QRS-T complex to be wider than normal or atypical in configuration. Care must be taken not to overdiagnose atrial premature complexes. Sinus arrhythmia and sinus bradycardia often lead to variations in the P-P intervals, and there is often a "wandering atrial pacemaker" that gradually alters the P wave morphology. Exercise or excitement will abolish such physiologic rhythms.

A continuous 24-hour ECG and an exercising ECG are recommended to assess frequent APCs. Atrial premature complexes are not considered a safety issue. Horses with occasional APCs that are overdriven during exercise and those with occasional APCs during exercise are considered as safe to use as their age-matched peers.[68] The greatest concern about APCs relates to their potential to incite atrial flutter and AF. Atrial premature complexes, as a general rule, are more likely to be clinically relevant in the following circumstances: when they (1) are frequent at rest, (2) are associated with runs of atrial tachycardia, (3) are related to poor performance (if other causes are excluded), (4) precipitate paroxysmal atrial flutter or fibrillation, or (5) develop in conjunction with structural cardiac disease. Documentation of atrial arrhythmias *during* exercise may be critical for determining whether paroxysmal atrial tachycardia, flutter, or fibrillation is likely to be the cause of poor performance.[474] Clinical judgment must be used, however, because supraventricular premature complexes are likely to occur in the immediate postexercise period, probably associated with autonomic imbalance.[77-79,479] If these postexercise arrhythmias are not associated with clinical signs and are not detected during exercise, they are unlikely to be clinically relevant.

Isolated atrial premature complexes generally are not treated. The hemodynamic consequences of these rhythm disturbances are minor, unless sustained abnormal activity develops. Consideration of antiarrhythmic therapy might be appropriate if frequent atrial extrasystoles are documented to precipitate AF or if they occur after conversion of AF to sinus rhythm and pose the horse at a risk of recurrent AF (see later discussion). Unfortunately, neither quinidine nor procainamide is practical for chronic use. Oral administration of sotalol has recently been advocated for chronic antiarrhythmic treatment to reduce the number of APCs, particularly in horses after conversion of AF to sinus rhythm in order to prevent recurrent AF (see Table 9.7).[1030] However, the effectiveness of sotalol and of other antiarrhythmics has not been formally established for this indication. Maintenance of normal serum potassium and magnesium may be important in suppressing atrial ectopic activity. In cases of suspected myocarditis, antiinflammatory doses of dexamethasone might be considered as empiric therapy with consideration of adverse effects of such treatment.

Atrial Tachycardia

Atrial tachycardia can be defined as a series of ectopic atrial premature complexes. Atrial tachycardia may be sustained or nonsustained (paroxysmal) and is generally precipitated by a single atrial premature complex. The atrial rate is rapid and regular; however, because ectopic P′ waves can be blocked physiologically in the AV node, an irregular ventricular rate response often results (see Fig. 9.56C). Atrial rates of 120 to 250 beats/min are typical in horses with sustained atrial tachycardia. At lower atrial rates, 2:1 AV conduction may yield a regular, relentless HR. At the higher atrial rates, the rhythm may be indistinguishable from atrial flutter. Differentiation of atrial tachycardia from flutter is not critical because both arrhythmias carry the same clinical significance and are treated identically. Sustained atrial tachycardia is most often recognized during treatment of horses with quinidine sulfate. Before conversion of AF to sinus rhythm, atrial tachycardia may be observed (see Fig. 9.57A); thus in this setting rhythm indicates a partial therapeutic effect of quinidine on the atrial myocardium. When this atrial tachycardia occurs as an isolated finding, structural or underlying myocardial disease should be suspected and the horse should be considered predisposed to the development or recurrence of AF. Treatment is the same as that described for AF. If the ventricular response rate is especially rapid (greater than 120 per minute) consideration can be given to administration of either digoxin to block AV nodal conduction or possibly to diltiazem if digoxin is ineffective (see Table 9.7). Arterial BP should be monitored carefully if diltiazem is administered.[1031-1033]

Atrial Flutter

Atrial flutter is even less common than atrial tachycardia and represents a form of atrial circuit movement or macroreentry.

The clinical circumstances and assessment of this rhythm disturbance are identical to those of atrial tachycardia. The ECG in atrial flutter is characterized by a very rapid, abnormal, but regular atrial activity that is usually manifested as a "saw-toothed" ECG baseline. The atrial frequency often ranges between 170 and 275 per minute.[68] The RR intervals are usually irregular due to variable AV conduction, and there are fewer QRS and T complexes than flutter waves (see Fig. 9.56C). The ventricular rate depends on the refractory period of the AV node and on the rate and the strength of the atrial stimulus. Treatment of atrial flutter is similar to that for horses with AF.[1034]

Atrial Fibrillation

Atrial fibrillation (AF) is the most common atrial arrhythmia associated with poor performance and exercise intolerance in horses. There is a large body of literature describing different aspects of this important arrhythmia in horses.* Atrial fibrillation is most common in adult horses[82-84,88,1051] and has been reported infrequently in foals,[199,524,733,1065] weanlings, yearlings, or ponies. Several reports indicate a higher incidence in Standardbreds, draft horses, and Warmblood horses compared with the general hospital population,[84,729] and in some Standardbred racehorses it has been identified as a heritable lesion.[1052]

Cardiac output at rest is normal in most horses with AF[621,1045,1066]; however, maximal CO during exercise is limited because the atrial contribution to filling is most important at higher HRs. As expected, exercise intolerance is most common in high-performance horses (e.g., racehorses, advanced combined training horses, polo ponies, eventers, and some Grand Prix jumpers) and less common in show hunters and in pleasure, dressage, and endurance horses. The high LA pressures present in heavily exercising horses[284,637] is further exacerbated by the loss of active atrial transport function[357] and may contribute to exercise intolerance or other associated signs. Exercise-induced pulmonary hemorrhage, respiratory distress, CHF, ataxia, collapse, and myopathy have all been reported with AF; conversely, the arrhythmia is often detected as an incidental finding in horses with no overt clinical signs.[84]

Atrial fibrillation can be acute or chronic, and it can be paroxysmal, persistent, or permanent. Paroxysmal AF is often associated with a single episode of poor performance, with the horse often decelerating suddenly during a race. The arrhythmia usually disappears spontaneously within 24 to 48 hours. Persistent or permanent AF may be less common than paroxysmal AF but is easier to diagnose because the arrhythmia is sustained. Persistent AF is terminating only after treatment, whereas permanent AF is sustained and resistant to therapy. Many horses with paroxysmal and some with sustained AF have no evidence of significant underlying cardiac disease when undergoing physical examination and echocardiography. In these cases, a diagnosis of "lone AF" is often made, implying that AF is the only abnormality present and the etiology of the arrhythmia is purely functional. However, the fact that AF appears to be "lone" in these cases may simply reflect our inability to identify the underlying pathology leading to arrhythmia. Intrinsic factors that are thought to contribute to the risk of developing AF in the horse in general include the (physiologically) large atrial mass and the high resting vagal tone that both can favor development of reentry arrhythmias. Vagosympathetic imbalances during exercise and transient potassium depletion, particularly in horses treated with furosemide or bicarbonate solutions, may further increase the risk of AF. Moreover, ultrastructural and functional myocardial pathology (e.g., focal myocarditis, fibrosis, channelopathies), which cannot be detected using routine diagnostic tests, may be present to promote the substrate that facilitates onset and perpetuation of AF.[1050,1067] Some, often older horses with AF present with underlying structural heart disease that is evident on physical and echocardiographic examination. Left atrial enlargement, most commonly caused by moderate to severe MR, is considered the most important predisposing factor for AF, but RA enlargement or other atrial pathology can contribute in some horses as well.[83,729] Atrial premature complexes likely act as triggers for AF if an adequate substrate for reentry arrhythmias is present.[1050,1067,1068] Finally, once the arrhythmia is established, AF itself can induce atrial electrical, structural, and functional remodeling that may be responsible for the self-perpetuating, progressive, and recurrent nature of AF.[289,290,1050,1062,1067] The duration of AF should be determined when possible because it likely determines the degree of AF-induced atrial remodeling and affects the prognosis for successful conversion and the likelihood of recurrence.[729] Remodeling changes are more likely to be reversible if AF is treated promptly. Recurrent episodes of AF are not uncommon and are more likely in the presence of remodeling and concurrent structural or functional cardiac disease.[291,729,1062]

Horses with AF generally have a normal resting HR, though the rate tends to decrease (and stroke volume tends to increase) following successful conversion of AF to normal sinus rhythm.[621,1048,1066] The presence of resting tachycardia in a horse with AF should give the clinician pause to consider intercurrent cardiac lesions (including marked structural disease or the rare case of an accessory AV conduction pathway[1069]) or a disorder that increases sympathetic tone such as pain, anemia, fever, or infection. A number of factors interact in the prognosis of a horse with AF. Of these, the presence of CHF represents the overall worst prognostic indicator and is invariably associated with resting tachycardia. Severe structural heart disease, recurrent bouts of AF, long-standing AF, and failure to convert with therapeutic serum concentrations of quinidine represent negative prognostic factors inasmuch as affected horses can be more difficult to convert to sinus rhythm or more likely to revert back to AF even when therapy is successful.[291,729,1049,1062]

Diagnostic Workup of Atrial Fibrillation. The diagnosis of AF is initiated by recognition of an irregularly irregular heart rhythm, variable intensity heart sounds, and an absent fourth heart sound during cardiac auscultation. Arterial pulses vary in intensity, and pulse deficits may be present, especially when the ventricular rate is high (see Fig. 9.57D). The ECG is characterized by an absence of P waves; instead, fibrillation or "f" waves are seen in the baseline (see Figs. 9.26, 9.57C, and 9.59). These "f" waves may be coarse (large) or fine (small). The number of atrial impulses per minute cannot be easily counted but usually ranges between 275 and 500 per minute (when analyzed on intracardiac electrograms).[68,572,1062,1063] The QRS-T complexes are normal in morphology and duration, but ventricular rate response is irregularly irregular (chaotic). Infrequently, periodicity leading to a patterned AV conduction sequence may be observed,[219,403] which must be distinguished from second-degree AV block. Although AF often sounds

* References 2, 33, 39, 84, 218, 219, 221, 229, 289-291, 357, 401-403, 472, 572, 621, 647, 721, 729, 1035-1064.

more regular at higher HRs (e.g., immediately after exercise), the rhythm remains irregular and careful auscultation will reveal this. Occasional QRS-T complexes of different morphology may be found, representing aberrant ventricular conduction (e.g., a wide QRS complex following a short RR interval that is preceded by a long RR interval, also called Ashman's phenomenon) or concurrent ventricular premature beats (see Fig. 9.57E and F).

As for all atrial tachyarrhythmias, the ultimate ventricular rate response (and examination HR) depends on the refractory period of the AV node and the frequency and strength of the atrial stimuli.[403] In the otherwise healthy horse in AF, vagal tone will be high and sympathetic tone low when standing; consequently, the ventricular rate will be close to normal or only slightly increased. If sympathetic activity is increased for any reason, or if vagal activity is blocked (as occurs with quinidine sulfate therapy), the ventricular rate response will increase as the AV nodal refractory period shortens. This explains the clinician's simple, but very useful, dependence on measuring pretreatment resting HR in horses with AF.[84,729] Because the horse with structural heart disease will be more likely to require sympathetic support to maintain CO and arterial BP, persistent resting tachycardia, higher than 60 to 70 beats/min, indicates underlying cardiac disease and is associated with a poorer prognosis. Horses with AF also should be examined critically for relevant murmurs that might indicate the presence of valvular regurgitation, atrial enlargement, and remodeling, creating a substrate for AF.

Laboratory studies in horses with AF, including plasma cardiac troponin concentrations, are usually normal. Infrequently, a horse is found to have low serum potassium, urinary fractional excretion of potassium, or RBC potassium. Chest x-rays are usually normal unless there is concurrent pulmonary disease, and this study is not a high-yield procedure in horses without compatible signs of lung disease or structural heart disease.

A complete echocardiogram including Doppler studies should be performed to identify underlying structural heart disease. The echocardiogram is often normal, unless there is concurrent valvular, congenital, or ventricular myocardial disease. Horses in AF often demonstrate a mildly dilated left atrium, even in the absence of significant MR or other structural disease. Significant LA enlargement is usually associated with moderate to severe MR and reduces the likelihood of successful cardioversion and increases the risk for recurrence. Assessment of LV function might be hampered during AF because of ventricular dyssynchrony, tachycardia-induced LV dysfunction and preload, and HR dependence of many of the echocardiographic indices used to assess LV function.[68,344] It is not uncommon for an otherwise normal horse in AF to demonstrate a slightly reduced LV shortening fraction (usually in the 24%–32% range), which returns to normal once the horse has been converted to sinus rhythm.[1070,1071] This decrease in fractional shortening is probably multifactorial in origin and probably related in part to decreased preload from loss of the atrial contribution to ventricular filling and irregular cardiac cycle. Evidence of persistent left ventricular dysfunction, even after the AF has been successfully corrected, might indicate underlying myocardial disease in the horse with AF; these horses often fail to return to their previous level of performance. Echocardiographic evidence of severe volume overload or PHT suggests a grave prognosis.

Although uncommon, horses may collapse after sudden onset of AF during exercise, most likely because of the sudden decrease in CO following the abrupt loss of atrial booster pump function. Recently it was shown that the prevalence of QRS broadening and R-on-T phenomenon is high in horses with AF even at lower levels of exercise. These events are considered risk factors for the development of ventricular arrhythmias and might also explain some of the episodes of weakness and the rare case of collapse or sudden death that have been reported in horses with AF.[472] Atrial fibrillation associated with exercise-induced ventricular arrhythmia resulting in sudden cardiac death has been documented by ECG in at least one horse.[54] An exercise test including an exercise ECG should therefore be performed when a horse is used for any type of performance activity and cardioversion is not an option or could not be attained. The clinician should turn his or her attention to the HR response and maximum HR achieved during exercise and to the occurrence of concurrent ventricular arrhythmias.[472] Horses in AF usually have a slightly higher HR during exercise compared with horses in sinus rhythm. However, the average maximal HR during exercise at an intensity that is at or slightly exceeding the horse's normal activities should not be greater than 220 beats/min. Markedly higher maximal HRs might suggest an increased risk of collapse. Furthermore, ventricular ectopy during exercise or during sympathetic nervous system stimulation indicates a possible risk for sudden cardiac death, particularly when short RR intervals or R-on-T phenomena are observed. Note that conventional HR monitors (as opposed to telemetric ECG devices) may not be able to track HRs above 200 to 220 beats/min and cannot detect arrhythmias and differentiate among different types of arrhythmias (i.e., atrial vs. ventricular). They are therefore not considered suitable for diagnostic workup of horses in AF.

Treatment of Atrial Fibrillation. Cardioversion is generally not performed for the first 24 to 48 hours of a documented, recent onset of AF because spontaneous cardioversion might occur. The most common treatment for AF involves conversion to normal sinus rhythm, unless there is concurrent CHF or the horse is aged and therapy is deemed of little benefit. As a general principle, when AF occurs in the setting of CHF, the treatments should be aimed at controlling CHF and resting HR with furosemide, digoxin, and possibly an ACE inhibitor, as opposed to conversion back to sinus rhythm.

The level of intended activity influences clinical decision making, as sustained AF is likely to limit rigorous athletic work and occasionally also impairs performance at mid to low levels of activity. Other horses with persistent AF are able to perform successfully when used for less intense work. In any case, treatment of AF is recommended for all horses that are still in use for any type of athletic activity when the average maximal HR during sustained maximal exercise exceeds 220 beats/min or when concurrent ventricular arrhythmias are observed (see earlier discussion).[54,68,472] If treatment is not an option in such cases, they should be retired due to safety concerns.[68]

Treatment of AF should only be performed in a controlled setting with continuous (ECG) monitoring, regardless of the treatment method.[68] Conversion of AF to sinus rhythm usually is accomplished using the type I_A sodium channel blocker quinidine* or through electrical cardioversion, generally delivered by intracardiac catheters (transvenous electrical cardioversion [TVEC]).[655,1038,1062,1084-1094] There have been no

* References 58, 401, 729, 752, 1027, 1031, 1034, 1041, 1049, 1070, 1072-1083.

prospective, randomized studies comparing the two methods, and both quinidine and electrical cardioversion are generally safe and effective treatments. Indications for quinidine treatment include lone AF, AF with mild LA enlargement, and comorbidities in which general anesthesia or TVEC is not an option. TVEC can be used to treat lone AF, AF with mild LA enlargement, and horses either intolerant of or unresponsive to quinidine treatment or horses in which quinidine is contraindicated.[68]

Quinidine conversion to sinus rhythm represents the standard therapy for most equine practices and has been used for the longest period of time. Hence, quinidine sulfate is the standard drug against which other drug treatments should be compared, though this agent is becoming more difficult to obtain in some countries. Quinidine cardioversion of AF is successful in many horses, and a number of different treatment plans can be followed (Box 9.14; also see Table 9.7). Based on studies of Reef and colleagues an excellent prognosis for quinidine conversion (greater than 95% conversion rate) may be given for horses with HRs of less than or equal to 60 beats/min, murmurs less than or equal to grade 3/6, and AF of less than 4 months' duration.[729,1070] Recurrences affect approximately 25% of these horses. In a study of transvenous electrical cardioversion, the results were also very positive, with a greater than 98% conversion rate (71 of 72 episodes in 63 horses).[1089] Overall, success rates of 65% to 90% have been reported for both treatment modalities.[68] Young racehorses with lone AF probably have a better prognosis for successful cardioversion, independent of treatment modality. Horses with longer duration of AF, a shorter fibrillation cycle length, or significant structural cardiac disease may be more difficult to convert to sinus rhythm using quinidine or electrical cardioversion and are more likely to have a higher recurrence rate independent of the treatment modality.[729,1062] In one recent study, a recurrence rate of 36%

BOX 9.14 Quinidine Treatment of Atrial Fibrillation (AF)

PREPARATION BEFORE TREATMENT

- Intravenous catheter for rapid venous access in case of an emergency
- Nasogastric tube/transnasal feeding tube for quinidine administration
- (Telemetric) ECG for continuous monitoring of heart rate, rhythm, and conduction times
- Ensure adequate hydration and correct electrolyte and acid-base disturbances
- Prepare emergency drugs and protocols

HORSE WITHOUT HEART FAILURE

Quinidine sulfate PO (by nasogastric tube or feeding tube):

- 22 mg/kg q 2 h until (1) conversion to sinus rhythm, (2) adverse or toxic effects occur, or (3) a total of 4 (to 6) doses have been administered
- If possible, plasma quinidine concentration should be measured if (1) conversion has not occurred 1 hour after the fourth dose, or (2) the patient exhibits adverse or toxic effects
- Therapeutic concentration: 2–5 μg/mL, toxic concentration: >5 μg/mL
- Treatment intervals should be increased to every 6 hours if (1) plasma quinidine concentration is >4 μg/mL or (2) after the fourth dose if concentrations cannot be measured
- Treatment every 6 hours can be continued, until (1) conversion to sinus rhythm, (2) adverse or toxic effects occur, (3) a total cumulative dose of 180 mg/kg is reached
- If conversion to sinus rhythm has not occurred after 24 hours, digoxin at 0.0055–0.011 mg/kg PO q 12 h may be added for 24–48 hours

Quinidine gluconate IV:

- As an alternative to oral treatment if the arrhythmia is recent (<2 weeks) or occurred during anesthesia
- 1–2.2 mg/kg IV as a slow bolus every 10 min or 0.1–0.22 mg/kg/min CRI; to effect
- Total doses exceeding 12 mg/kg are usually not recommended; higher doses (up to 24 mg/kg) may be necessary for conversion but can result in severe adverse effects (hypotension, proarrhythmic effects including ventricular tachycardia and torsades de pointes)

HORSE WITH HEART FAILURE

- Cardioversion using quinidine is usually not attempted; goals of treatment are stabilization of congestive heart failure and ventricular rate control; treat with digoxin to control heart rate and improve myocardial function, furosemide to control edema, and possibly an ACE inhibitor

MONITORING

- Monitor for response to treatment and adverse/toxic effects
- Ensure adequate fluid intake during prolonged quinidine treatment
- Monitor serum electrolytes and BUN/creatinine during prolonged treatment

MANAGEMENT OF QUINIDINE-INDUCED ADVERSE AND TOXIC EFFECTS

- Accelerated ventricular response rate—may occur within therapeutic range:
  - If rate is <100 beats/min and horse is hemodynamically stable, continue treatment with close monitoring
  - If rate is persistently >100 beats/min, administer digoxin (0.0022 mg/kg IV, may repeat dose once)
  - If rate is sustained in excess of 150 beats/min or severe systemic hypotension develops, administer digoxin and $NaHCO_3$ (1 mEq/kg IV)
  - Other options for rate control include diltiazem (see Table 9.7) or alpha-2 agonists; administer to effect, monitor ECG and direct blood pressures
- Prolongation of QRS (>25%)—indication of toxicity, discontinue quinidine
- Severe hypotension—administer phenylephrine (0.1–0.2 μg/kg/min to effect, up to 0.01 mg/kg total dose)
- Ventricular arrhythmia (ventricular tachycardia, torsades de pointes)—discontinue quinidine, administer lidocaine (0.25–0.5 mg/kg slow IV, repeat in 5–10 min, up to 1.5 mg/kg total dose) and $MgSO_4$ (2–6 mg/kg/min IV to effect, up to a total dose of 55 [up to 100] mg/kg)

after first AF episode and an overall recurrence rate of 43% were reported.[291] Long-term recurrence rates after TVEC and quinidine cardioversion are believed to be similar.[68]

Quinidine is typically administered by nasogastric tube or feeding tube because of its irritating effects on the mucous membranes. Intravenous quinidine gluconate can be successful in conversion of horses with AF of recent onset or when nasogastric delivery is not feasible,[1075,1079,1082] but failure to respond does not predict the response to oral treatment (see Box 9.14). Many successful approaches have been used to convert AF in horses, and the clinician should appreciate that the mean quinidine elimination half-life after an oral dose is about 6.7 hours.[327,1082] One treatment approach for AF involves administration of a loading dose of 22 mg/kg quinidine sulfate by nasogastric tube every 2 hours for two to four doses, followed by every-6-hour dosing until the horse converts or develops initial signs of toxicosis. Aggressive q 2 h dosing exceeding 88 to 132 mg/kg is especially likely to induce adverse effects.[1070] Therapeutic serum quinidine concentrations for conversion from AF to sinus rhythm are 2 to 5 μg/mL (6.2–15.4 μmol/L) and should be measured if a horse fails to convert following an appropriate dosing regimen. If conversion to sinus rhythm has not occurred after 24 hours of therapy, digoxin at 0.0055 to 0.011 mg/kg orally twice a day may be added to the treatment regimen for 24 to 48 hours. As in other species, there is a digoxin-quinidine interaction that can effectively double the serum concentration of digoxin.[752] Thus combination therapy beyond 24 hours should be continued only with monitoring of the serum digoxin concentrations (see Table 9.7) and consideration of using the lower end of the digoxin dosage range. Even horses that do not convert on the "standard" administration regimen may convert after the combined use of quinidine with digoxin. The value of using such a treatment plan every 6 hours is that steady-state levels are reached, there is sufficient time to attain myocardial concentrations, and quinidine toxicity is less frequent compared with the every-2-hour regimen. When prolonged treatment over more than 12 to 24 hours is necessary, adequate hydration and electrolyte balance (particularly potassium and magnesium) must be ensured by oral or intravenous administration of crystalloid solutions, because most horses undergoing oral quinidine treatment will become depressed and inappetent, will show reduced water intake, and may develop mild diarrhea.

Careful clinical and continuous electrocardiographic monitoring should be performed on horses with AF during conversion to sinus rhythm. The QRS duration should be monitored and compared with the pretreatment QRS duration before each additional treatment is administered. Prolongation of the QRS duration to greater than 25% of the pretreatment value is an indication of quinidine toxicity and should prompt discontinuation of therapy. The simplest ECG change is an acceleration of AV nodal conduction related to the vagolytic effect of quinidine (see Fig. 9.57C). Rapid supraventricular tachycardias with ventricular rate responses of up to 300 beats/min have been seen in several horses receiving quinidine sulfate. These horses have been treated with intravenous digoxin at 0.002 mg/kg to slow the ventricular response rate, intravenous replacement fluids to improve perfusion, intravenous sodium bicarbonate at 1 mEq/kg to reverse the sodium channel–blocking effects of quinidine (probably by a combined effect of increasing extracellular sodium concentration and alkalinization, leading to increased protein binding of quinidine and decreased extracellular concentrations of ionized calcium), and if needed, a phenylephrine drip to restore BP if there is critical hypotension (see Table 9.7 and Box 9.14). Ventricular arrhythmias (torsades de pointes, multiform VT, and ventricular premature complexes) have also been detected with quinidine toxicosis (Fig. 9.58) and in the rare case may lead to sudden cardiac death during treatment. Intravenous sodium bicarbonate is also indicated in these horses, whereas intravenous magnesium sulfate (up to 25 g in a 450- to 500-kg horse) is the treatment of choice for quinidine-induced ventricular arrhythmias (see Table 9.7). Lidocaine may also be used if needed, starting with an intravenous bolus of 0.5 to 1.5 mg/kg, slowly IV. Conversely, administration of digoxin is contraindicated in horses with ventricular arrhythmias induced by quinidine.

Clinical markers of quinidine toxicosis include ataxia, colic, and nasal edema causing upper respiratory tract stridor. Most toxic reactions to quinidine sulfate are associated with higher serum concentrations of the drug (greater than 5 μg/mL) and can be avoided with careful clinical and electrocardiographic monitoring. Some of the adverse effects of quinidine administration, particularly polymorphic ventricular tachycardia (see Fig. 9.58), are likely an idiosyncratic reaction. These adverse effects should prompt discontinuation of therapy or altering treatment intervals. Depression and paraphimosis occur in most horses treated with quinidine but disappear with the discontinuation of the drug. Diarrhea often develops with the administration of higher doses of quinidine, but this sign also disappears with discontinuation of the drug. Convulsions, hypotension due to vasodilation, CHF, laminitis, urticaria, and sudden death have rarely been reported associated with quinidine sulfate administration. A ventricular rate response in excess of 100 beats/min is an adverse reaction that may be more common in nervous horses, horses that show an excessive ventricular response during exercise, or those prone to the vagolytic effects of quinidine. These horses may benefit from administration of digoxin to blunt the ventricular rate response, particularly if there are no other signs of quinidine toxicosis (that would indicate an excessive serum concentration). If an increased ventricular response rate is anticipated, one may even consider pretreatment with digoxin before initiation of quinidine therapy. Alternatively, alpha-2 agonists (e.g., detomidine)[1063] or diltiazem[259,1031-1033] could be used for ventricular rate control during quinidine treatment, provided that blood pressures can be closely monitored (see Table 9.7). The clinical experience with the use of diltiazem is limited, and doses should be carefully titrated to effect.

Other drugs have been studied for treatment of AF in horses. These might be considered mainly for cases of resistant or recurrent AF or in situations where quinidine or electrocardioversion are unavailable. Procainamide may potentially be effective and could be used at a dose of 1 mg/kg/min IV (up to a maximum of 20 mg/kg) when AF occurs during anesthesia.[327,1095] Based on recent discoveries in dogs, the use of lidocaine could also be attempted to treat acute vagally associated AF that might develop during anesthesia.[1096,1097] However, efficacy of lidocaine for conversion of AF is unknown, and it is certainly not considered the drug of choice for routine treatment of AF in horses. Amiodarone administered as a constant rate infusion has been used for treating experimentally induced and natural AF in horses, but treatment may last up to 54 hours and is expensive, and success rates are considerably lower than those reported for quinidine and TVEC.[1098-1101] Therefore amiodarone cannot be recommended as a first-line therapy at this time. Pharmacokinetics of flecainide, a class IC

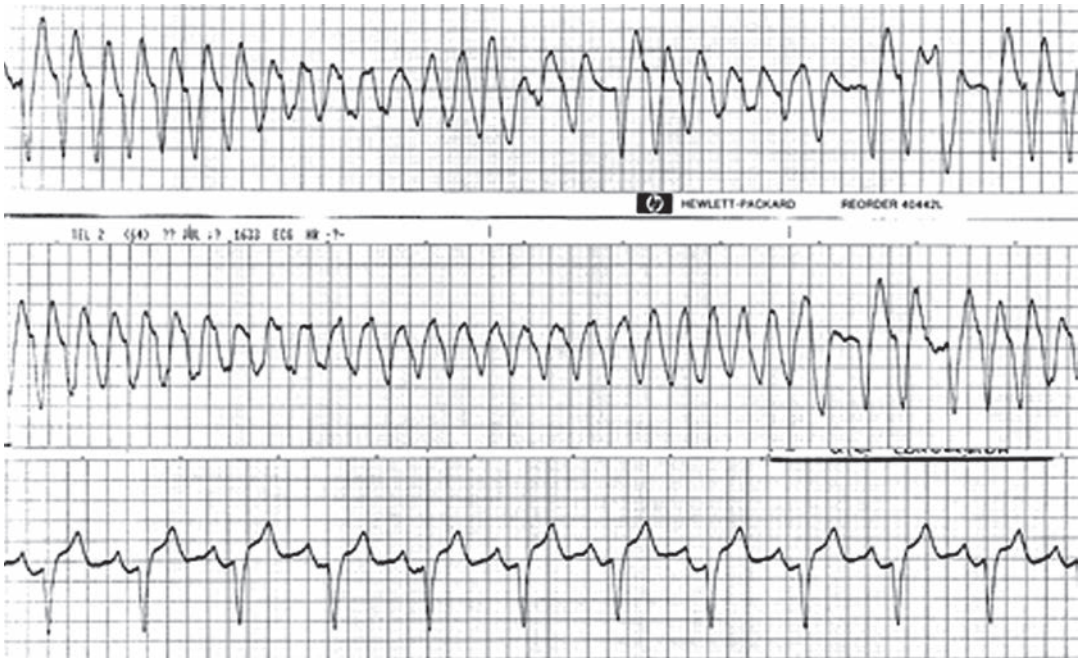


FIG. **9.58** Polymorphic ventricular tachycardia (VT) after administration of quinidine sulfate for attempted conversion of atrial fibrillation (AF). In this case normal sinus rhythm was established *(lower tracing)* after treatment with lidocaine, bicarbonate (to reverse sodium channel–blocking effects of quinidine), and phenylephrine (administered to maintain arterial blood pressure [ABP]).

antiarrhythmic drug, have been studied in horses, and it has been administered for treatment of experimentally induced and naturally occurring AF and atrial tachycardia.[1102-1109] Early studies suggested that flecainide might be a safe and effective alternative to quinidine for treatment of AF. However, intravenous administration of flecainide was not found to be effective to treat chronic naturally occurring AF,[1102,1109] and in an experimental model it failed to show protective properties against immediate reinduction of AF after conversion.[1104] Furthermore, flecainide administration caused widening of the QRS complex and was associated with potentially dangerous arrhythmias and sudden death.[1103,1109] Therefore the use of flecainide is currently not recommended in horses. Intravenous propafenone also has been suggested for conversion of chronic AF[1110,1111] but has recently been shown to be ineffective at a dose of 2 mg/kg IV in horses with naturally occurring and pacing-induced AF, respectively. Therefore the current knowledge does not support the use of this class Ic antiarrhythmic drug for treatment of AF. Potentially, combination therapy using a variety of antiarrhythmic drugs at lower doses might prove useful for treatment of AF in the future. Furthermore, novel ion channel targets and new atrial-selective antiarrhythmic agents are currently under investigation for treatment of AF in horses and other species.[1112,1113] However, more fundamental research has to be conducted before any of the newer strategies can be investigated in clinical studies.

Transvenous electrical cardioversion of AF to sinus rhythm has been used at a number of referral centers as either the primary method of treatment or for management of horses who respond adversely or inadequately to quinidine therapy. As stated, TVEC is a very effective therapy, but it requires special equipment and well-trained, experienced personnel. The procedure involves percutaneous placement of two specialized electrode catheters transvenously with one catheter tip located within the left PA and the other within the right atrial cavity. Catheter placement is done in the standing position under sedation and local anesthesia. The catheters are guided by pressure monitoring through the catheter lumen, 2D echocardiographic imaging, and interrogation of the intracardiac electrogram recorded through the catheter electrodes (Fig. 9.59). Radiography is used to verify the placement of the catheters either before or preferably after induction of general anesthesia. General anesthesia is required because electrical cardioversion is very painful and the shock results in a sudden jolt of the body and limbs. Electrical cardioversion involves a timed shock delivery on the R wave. Proper synchronization of the shock is crucial to avoid shocking on the T wave, which represents the vulnerable period and is susceptible for inducing fatal ventricular arrhythmias. A more detailed description of the specific procedure used for TVEC is beyond the scope of this textbook, and the reader is referred to the respective literature.[655,1038,1062,1084-1094]

The complications associated with electrocardioversion appear to be quite low but are finite related to general anesthesia or electrical shock.[1088,1091,1093] A case of transient complete AV block has been reported,[1092] and depending on catheter position (i.e., placement of the RA catheter close to the AV node) this complication might not be uncommon. Furthermore, fatal arrhythmias can be induced by unsynchronized shock delivery.[1114] The immediate recurrence of AF (IRAF) within the first 24 hours after cardioversion, although infrequent, is more likely than with quinidine cardioversion.[68] Pretreatment with antiarrhythmic drugs before TVEC or administration of an antiarrhythmic drug during and after anesthesia might minimize the likelihood of IRAF.[1038,1090] Amiodarone and sotalol have been used for this purpose (see Table 9.7).

Follow-up Care after Conversion of Atrial Fibrillation. After spontaneous cardioversion, an evaluation is still indicated, including measurement of serum K^+ and Mg^{++}

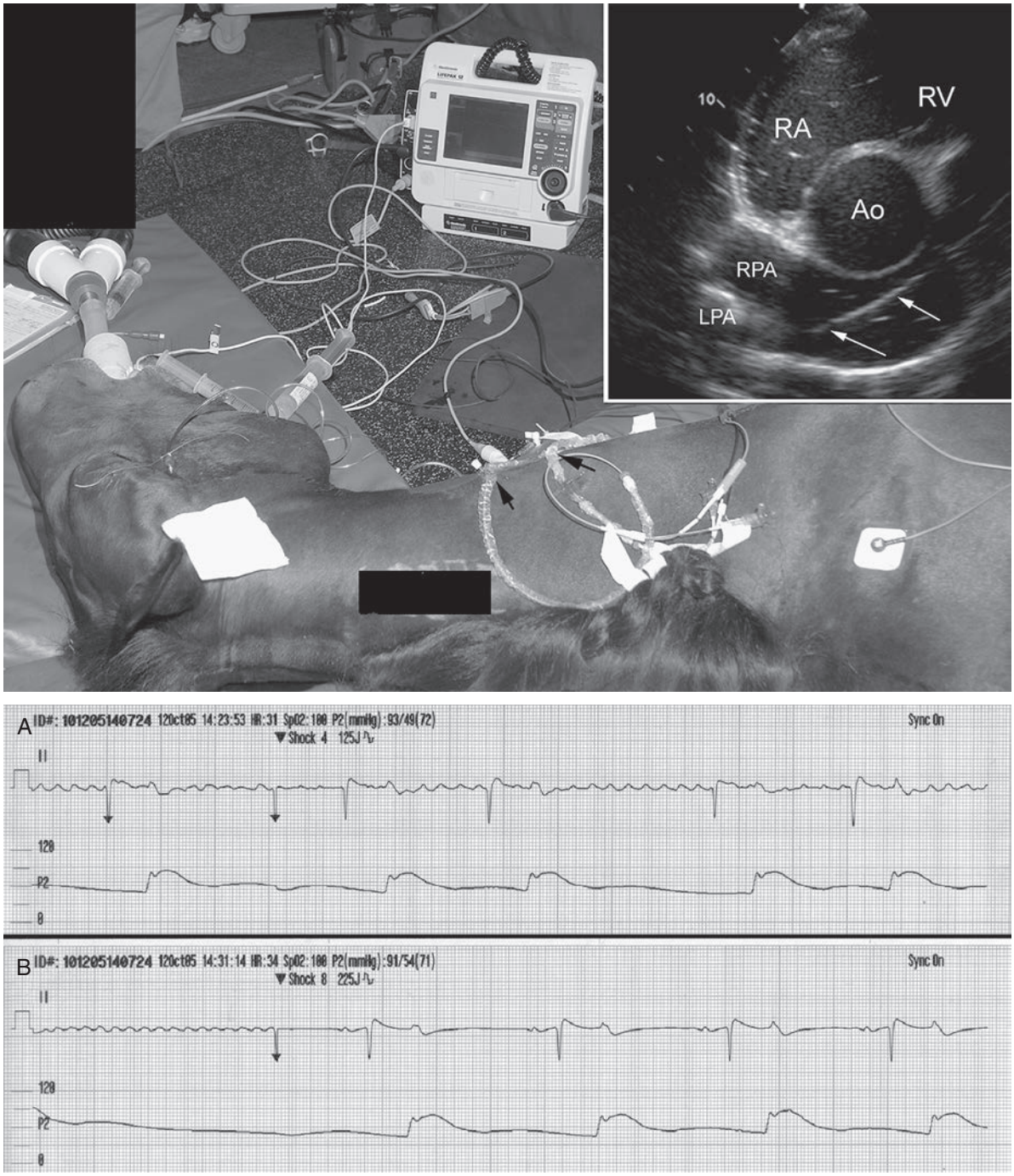


FIG. **9.59** A, Electrocardioversion procedure in a Thoroughbred horse. The horse is under general anesthesia. Two specialized electrode catheters *(arrows)* were previously placed percutaneosly under local anesthesia into the right atrium (RA) and left pulmonary artery under guidance from two-dimensional (2D) echocardiography and intravascular pressure monitoring. The biphasic cardioverter (*top,* center of image) is connected to the catheters and is used to deliver the synchronized cardioversion shock, as well as monitor electrocardiogram (ECG) rhythm, invasive blood pressure (BP), and pulse oximetry. *Inset:* 2D image showing short-axis image at base of heart. The catheter inserted into the left pulmonary artery (LPA) is indicated *(arrows)* (*RA,* right atrium; *RV,* right ventricle; *Ao,* aorta; *RPA,* right pulmonary artery). B, Transvenous electrical cardioversion for treatment of atrial fibrillation (AF) in an 2-year-old Standardbred racehorse under general anesthesia. A surface ECG (25 mm/sec) and an arterial blood pressure (ABP) tracing are displayed. The QRS complexes are automatically detected by the defibrillator unit and marked by small triangles. Biphasic electrical shocks *(larger triangles on top)* are applied at increasing energy levels. Delivery of the shocks is synchronized to the QRS complex to avoid the vulnerable period (T wave) and prevent induction of ventricular arrhythmias. *(A)* Unsuccessful attempt at an energy level of 125 J. *(B)* Successful cardioversion at an energy level of 225 J. Immediately after the shock, the baseline flattens and normal sinus rhythm resumes. No further treatment is required at this point. From Schwarzwald CC, Bonagura JD, Muir WW: The cardiovascular system. In Muir WW, Hubbell JAE, editors: *Equine anesthesia: monitoring and emergency therapy,* ed 2, St. Louis, 2009, WB Saunders.

concentrations, fractional excretion of K^+ (in racehorses), echocardiography, continuous 24-hour ECG, and optimally an exercising ECG test to identify atrial triggers or other arrhythmias. These tests are also appropriate in a horse with normal sinus rhythm if paroxysmal AF is suspected from the clinical history.[68]

A continuous 24-hour ECG to detect recurrent atrial arrhythmias is recommended after successful conversion of AF to sinus rhythm. However, the optimal timing of this examination, the influence of premature atrial complexes on long-term prognosis, and the best approach to management of recurrent atrial ectopy are unknown.[68] A complete echocardiogram after cardioversion can evaluate LV and LA mechanical function and reassess heart size and valvular function. Left ventricular function should return to normal within 3 days.[1048] Recovery of LA contractile function can occur within a few days or might take several weeks when AF has been long-lasting.[287,289,1038] Persistent LA contractile dysfunction can be caused by AF-induced atrial remodeling or underlying primary cardiomyopathy and might portend an increased risk of recurrent AF.[291,1038]

Oral sotalol at an initial dose of 1 mg/kg PO and a maintenance dose of 2 to 3 mg/kg PO q 12 h (see Table 9.7)[1030] is currently used by some clinicians, including the author, in an attempt to suppress atrial ectopy after cardioversion and reduce the risk of recurrent AF. Sotalol is usually weaned before reinitiation of normal training, and only rarely chronic long-term treatment is elected. Although sotalol seems to be well tolerated, its efficacy is currently not well studied. Other antiarrhythmic drugs that, based on human studies or experimental animal models, might potentially be effective against atrial arrhythmias include propafenone,[1110,1111] amiodarone,[1100,1101] and phenytoin.[1115] However, efficacy and safety of these treatments are currently unknown and further studies are needed.[68] Drugs known to predispose to ectopic impulse formation should be avoided, including furosemide, supplements containing sodium bicarbonate, and thyroid hormones. Potassium chloride supplementation is indicated in most horses administered furosemide before racing or in those with low fractional urinary excretion of potassium.[68]

Horses can usually be returned to training within 1 week after conversion with paroxysmal AF or short-duration lone AF, if follow-up examinations after cardioversion are normal. However, horses with long-standing AF or with persistent sinus tachycardia, recurrent atrial premature complexes, or persistent LA dysfunction observed after successful conversion of AF to NSR should be given 4 to 6 weeks to recover.[68] Ideally, rest is enforced until atrial electrical and contractile function has normalized or is nearly normal.

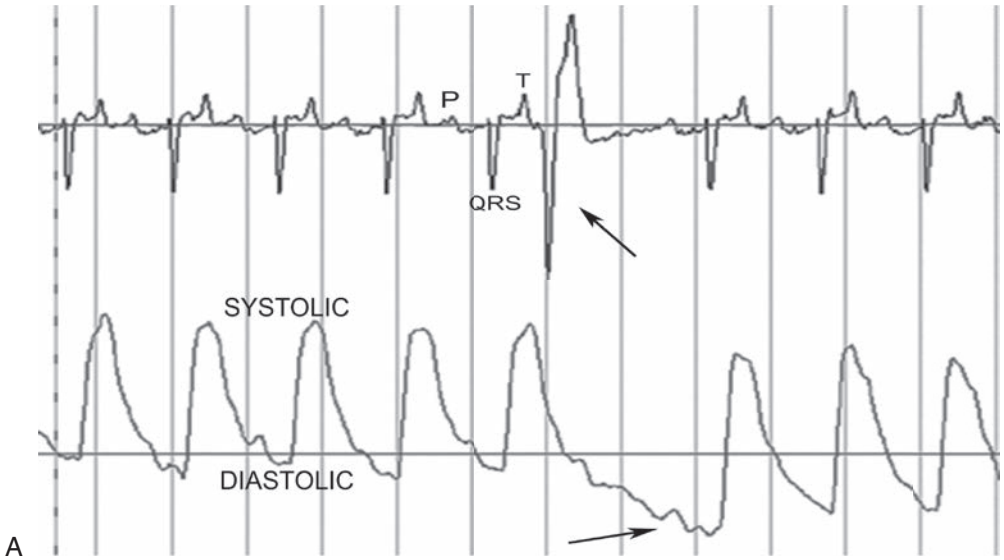
Conversion of horses with AF generally results in a return to their previous performance level.[84,729] Horses with repeated episodes of AF are often converted numerous times with quinidine sulfate or electrocardioversion. Most horses that experience a recurrence of AF do so within 1 year of initial conversion,[291,1038,1070] but much longer intervals have occurred between episodes of AF in some horses. If the duration of sinus rhythm becomes shorter, repeated treatments may no longer be practical and a career change may be indicated. Some horses eventually become refractory to drug or electrical cardioversion, probably due to progressive atrial fibrosis or underlying myocardial disease. However, in the absence of significant, detectable cardiac disease, horses with persistent or permanent AF may still be used at lower levels of exercise (as long as the expected level of performance can be achieved and the results of exercise testing do not suggest an increased risk of collapse or sudden death; see earlier discussion). Alternatively, they can be kept as breeding animals or pasture horses. Importantly, horses with persistent AF should only be ridden or driven by an informed adult and limited to an exercise level considered relatively safe based on an exercising ECG (see previous discussion). The use of an HR monitor might be helpful to track HR during exercise and modify the rigor of the work performed.[68]

Junctional and Ventricular Arrhythmias

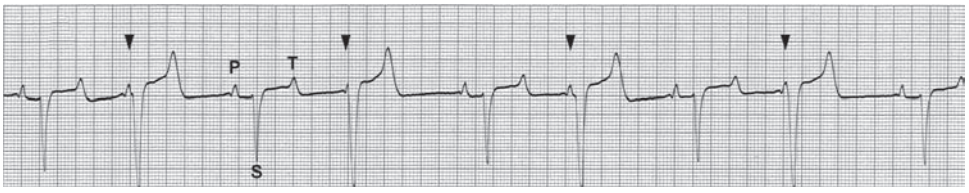
Cardiac arrhythmias that originate within the AV conducting tissues, the ventricular specialized conducting tissues, or ventricular myocardium are classified as "junctional" (AV node and parts of the bundle of His) or ventricular in origin. Unlike sinus or atrial arrhythmias, these arrhythmias are not preceded by a conducted P wave. When sustained, junctional and ventricular rhythms often lead to dissociation between the sinoatrial activity (P wave) and that of the ventricle (QRS-T complex), resulting in AV dissociation.[57,60,502] In these cases, an independent atrial rhythm is superimposed on the ectopic rhythm (Fig. 9.60C and D). Note that AV dissociation in these cases develops because the premature AV junctional or ventricular depolarization causes interference to the conduction of normal SA impulses. It is important to realize that escape rhythms (see later discussion) also cause AV dissociation through a different mechanism. Hence, "AV dissociation" is a purely descriptive term of an ECG finding and neither characterizes the type and pathophysiologic mechanism of the arrhythmia nor determines the therapeutic approach.

With sustained junctional or ventricular tachycardias, P waves may be identified that are not conducted and therefore not consistently associated with a QRS complex (see Fig. 9.60). Some of these P waves may be buried in the ectopic QRS-T complexes (especially at higher rates of ventricular activation), making their identification difficult. The use of ECG calipers helps determine the P-P interval and can greatly facilitate the identification of P waves. Occasionally, atrial impulses may be normally conducted, leading to capture beats or fusion beats. *Capture beats* are characterized by a normal P-QRS-T configuration, resulting from normal ventricular activation occurring before the ectopic focus discharges (see Fig. 9.60D). *Fusion beats* are seen when both the conducted impulse and an ectopic impulse cause simultaneous ventricular activation. The QRS-T morphology of a fusion beat represents the summation of a normal and an ectopic beat (see Fig. 9.60C).

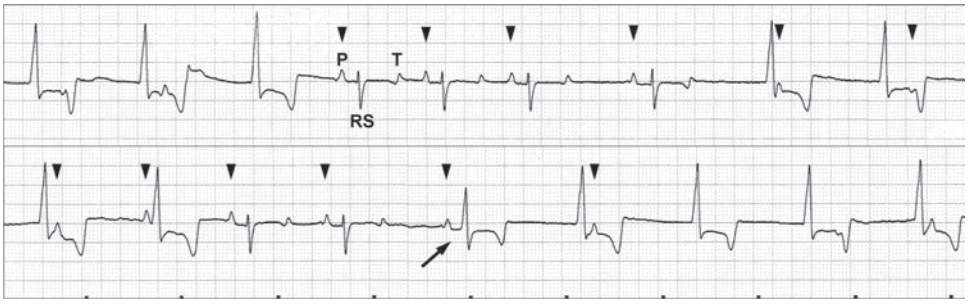
It may be difficult to distinguish between junctional and ventricular arrhythmias and to determine the exact location of the abnormal impulse formation. The differentiation of junctional and ventricular rhythms can sometimes be made by inspection of the QRS complex. Junctional impulses are more likely to result in a narrow, relatively normal-appearing QRS complex with normal initial activation and electrical axis, because they originate above the ventricular myocardium. Complexes of ventricular origin, by contrast, are conducted abnormally and more slowly, resulting in a widened QRS, an abnormal QRS orientation, and abnormal T waves. However, junctional tachycardias also may be conducted aberrantly, resulting in a bizarre and wide QRS complex. When sustained, both types of rhythms cause AV dissociation with an independent atrial rhythm superimposed on the ectopic ventricular rhythm. Advanced echocardiographic methods that identify the sequence of ventricular



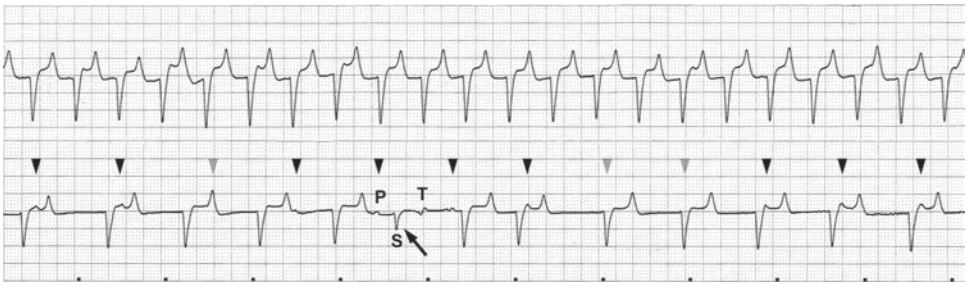
A



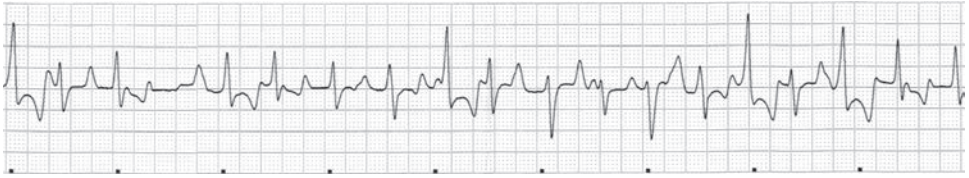
B



C



D



E

FIG. **9.60** Ventricular arrhythmias. A, The ectopic complex *(arrow)* is premature and abnormal in morphology. A compensatory pause follows the extrasystole because the next sinus P wave is blocked in the atrioventricular node. The effect of the premature complex on arterial blood pressure (ABP) in the lower tracing *(arrow)* is noticeable. B, Base-apex lead electrocardiogram (ECG) recorded from a 15-year-old Arab mare with ventricular bigeminy. Normal sinus beats alternate with slightly larger and wider ventricular ectopic beats. SA node discharge is not affected by the ectopic beats, as indicated by the presence of nonconducted P waves immediately before the ectopic beats *(arrowheads)* (paper speed 25 mm/sec). C, Base-apex lead ECG recorded from an 18-year-old Arab mare recovering from acute diarrhea and endotoxemia. The ECG shows an intermittent accelerated idioventricular rhythm at a rate of 50 beats/min. P wave intervals are indicated *(arrowheads).* The recording demonstrates that the ectopic focus is suppressed at higher rates of SA node discharge. The ventricular rhythm only becomes manifest when the sinoatrial (SA) rate drops below the rate of the ventricular pacemaker. SA node discharge is not affected by the ectopic rhythm, resulting in AV dissociation. A fusion beat is present *(arrow),* resulting from summation of a conducted sinus impulse with an ectopic ventricular beat (paper speed 25 mm/sec, voltage calibration 0.5 cm/mV). D, Base-apex lead ECG recorded from a 3-year-old Clydesdale gelding. The top recording shows a regular tachycardia at a rate of 120 beats/min. The appearance of the QRS-T complexes does not allow conclusive distinction between a supraventricular rhythm with rapid ventricular response and a ventricular rhythm. However, as the rate slows down *(bottom strip),* AV dissociation because of a ventricular tachycardia (VT) becomes apparent. P waves *(arrowheads)* and a capture beat *(arrow)* are indicated (paper speed 25 mm/sec, voltage calibration 0.25 cm/mV). E, Base-apex ECG recorded from a 5-year-old Clydesdale stallion with acute myocardial necrosis of unknown cause. The serum cardiac troponin I concentrations were severely elevated (404 ng/mL; normal <0.15 ng/mL). The ECG shows multiform VT at a rate of 120 beats/min (paper speed 25 mm/sec, voltage calibration 0.5 cm/mV). (B to E, From Schwarzwald CC, Bonagura JD, Muir WW: The cardiovascular system. In Muir WW, Hubbell JAE, editors: *Equine anesthesia: monitoring and emergency therapy,* ed 2, St. Louis, 2009, WB Saunders.)

activation and allow detection of ventricular dyssynchrony and advanced electrophysiologic studies may be of value in distinguishing ventricular origin complexes from those starting above the ventricle and using the normal conduction system, but these methods are not established in horses.

Junctional Arrhythmias

Junctional complexes that arise early relative to the normal cardiac cycle are designated as *premature junctional complexes.* These complexes may occur as single or repetitive events and can resemble ectopic rhythms that might originate in the atria but without being related to a P wave. Repetitive ectopic complexes that occur in short bursts or runs are termed *nonsustained* or *paroxysmal junctional tachycardias. Sustained junctional tachycardias* may also occur and can lead to cardiomyopathy and CHF, which are reversible with control of the tachyarrhythmia (see earlier discussion).

The clinical relevance of an occasional junctional premature complex is difficult to ascertain. Persistent or repetitive junctional rhythms are indicative of heart disease, systemic disease, or a drug-induced abnormality of cardiac rhythm. The best management choice, besides treatment of the underlying disorder, is uncertain inasmuch as some junctional rhythms behave more like atrial tachyarrhythmias, whereas others cause AV dissociation and appear to act like ventricular ectopic impulses. Because the mechanism responsible for junctional tachycardias can be either abnormal automaticity or reentry (circuit) movement using the AV node, empiric therapy is usually required to control sustained arrhythmias. If there is obvious AV dissociation, lidocaine, procainamide, quinidine, or sotalol would seem reasonable choices (see Table 9.7). If the mechanism is uncertain but is clearly supraventricular, intravenous digoxin or intravenous diltiazem may either silence the rhythm or slow the rate.

Escape Rhythms

The normal heart contains potential cardiac pacemakers within the AV junctional and ventricular specialized tissues that are usually overdrive suppressed by the sinus node, which discharges at a faster rate than these subsidiary pacemakers. The automaticity of the subsidiary pacemakers may become manifest during periods of sinus bradycardia or AV block, creating *escape complexes* or *escape rhythms* (see Fig. 9.62B later). Escape rhythms are characterized by slow ventricular rates, often in the realm of 15 to 25 beats/min. Specific antiarrhythmic drug suppression of escape rhythms is generally not necessary and is contraindicated because these rhythms serve as rescue mechanisms for the heart. Instead management of escape rhythms should be aimed at resolving the underlying cause of sinus bradycardia or AV block.

Accelerated Idioventricular (Idionodal) Rhythms

Occasionally, the subsidiary nodal or ventricular pacemakers may be enhanced and discharge at a rate that is equal to or slightly above the sinoatrial rate (usually between 60 and 80 beats/min). The resulting rhythm is commonly referred to as *accelerated idioventricular (idionodal) rhythm* or *slow ventricular tachycardia* (see Fig. 9.60C). Conditions that favor the development of these rhythms include gastrointestinal disease (possibly due to a combination of endotoxemia, autonomic imbalance, acid-base disturbances, and electrolyte abnormalities)[504] and administration of anesthetics or catecholamines. When the independent atrial and ventricular pacemaker foci discharge at similar rates, the P waves may appear to "march in and out" of the QRS complex. This phenomenon is called *isorhythmic AV dissociation* and is occasionally observed in adult horses during inhalation anesthesia. Accelerated idioventricular rhythms are often quite regular and may be misdiagnosed as sinus tachycardia on auscultation or palpation of peripheral pulses. Persistent, unexplained mild to moderate tachycardia should therefore prompt an electrocardiographic examination to ascertain a correct rhythm diagnosis. However, accelerated idioventricular rhythms generally are of little clinical (electrophysiologic and hemodynamic) relevance and resolve spontaneously with appropriate treatment of potential underlying conditions and reduction of anesthetic dosages. Electrolyte supplementation (potassium, magnesium) and correction of fluid deficits and acid-base disturbances may be beneficial. Lidocaine (see Table 9.7) is sometimes administered as an intraoperative adjunct to general anesthesia, or it is used as an analgesic, antiinflammatory, and prokinetic drug in

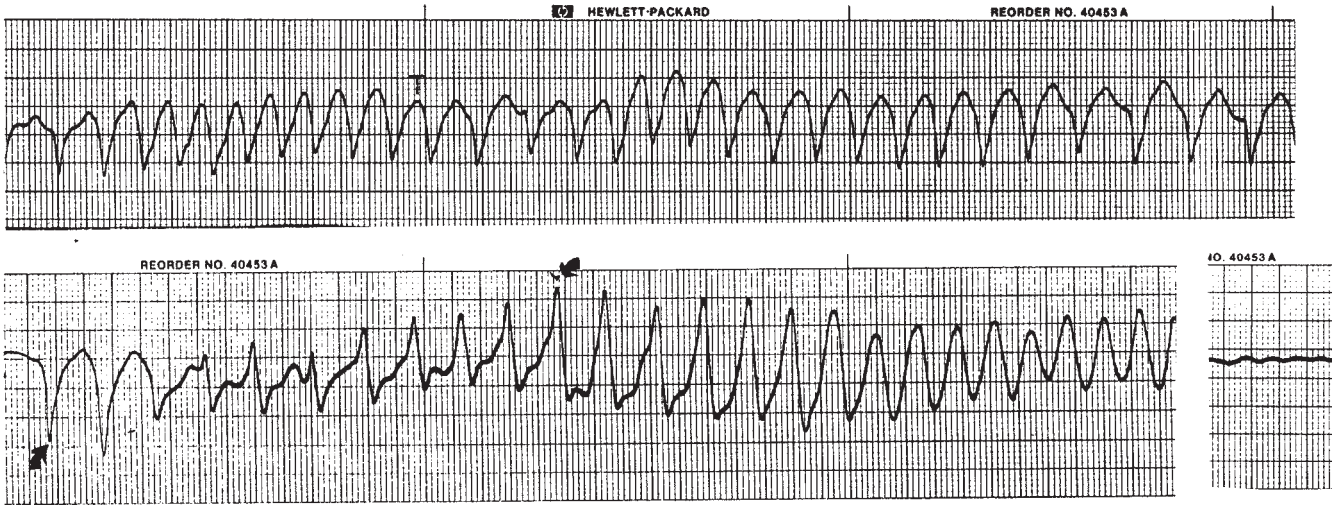


FIG. **9.61** Sustained, rapid ventricular tachycardia (VT) *(top)* of varying rate that develops into a polymorphic ventricular rhythm with varying morphology complexes (torsades de pointes). The horse died of cardiac arrest *(lower right panel).* The arrows indicate QRS complexes of varying polarity (25 mm/sec).

the management of postoperative ileus. In these situations, its antiarrhythmic effects may provide some additional preventive or therapeutic benefits.

In some cases, accelerated idioventricular (idionodal) rhythms can be sustained in an otherwise healthy horse. If the abnormal rhythm is overdriven by exercise, affected horses can be used with caution by an informed adult. However, because of concerns about underlying myocardial or electrical disease and potentially increased risks of exercise-associated collapse and sudden cardiac death, these horses should not be used by a child or as a lesson horse.[68]

Ventricular Arrhythmias

Ventricular arrhythmias (VAs) are less common than atrial arrhythmias but are more likely to be associated with underlying structural cardiac disease, lesions of the myocardium, or a multisystemic disorder, including infections.* VAs may be observed with severe toxemia or sepsis or primary gastrointestinal disorders including proximal enteritis and large bowel disorders. Altered potassium, magnesium, and calcium concentrations can affect myocardial electrophysiology,[1125] and electrolyte and acid-base disorders can induce ventricular ectopy. Other potential causes of VAs include metabolic disorders, ischemia, hypoxia, drugs, toxins, and systemic inflammation. Primary heart diseases, including severe valvular regurgitation, cardiomyopathy, myocarditis, endocarditis, and pericarditis, can induce VAs. This said, VAs are also frequently encountered in apparently healthy athletic horses at rest and during and after exercise.[77-79,82,475,478,479]

Ventricular ectopic rhythms are classified as indicated in Box 9.12. The approach to the horse with VAs should emphasize ruling out the noncardiac causes, correcting them (if possible), and then following if necessary with a complete CV examination, including electrocardiography, echocardiography, and clinical laboratory studies.[68] Plasma troponin concentrations should be measured to identify myocardial injury, although it may be problematic to separate primary from secondary causes of cardiac troponin elevations. An echocardiogram is specifically recommended for horses with ventricular tachycardia or complex VA, when VA is recurrent or persistent, or when VA is associated with poor performance, collapse, a clinically relevant cardiac murmur, or a marked increase in cardiac troponin concentration.[68] Further workup of a horse with VA should include an exercising ECG, unless underlying systemic or myocardial disease, VT, or complex VA is present.[68]

As opposed to atrial arrhythmias, safety of the horse and the rider or driver is more of a concern with VAs, and defining the safety risks is paramount in cases of ventricular ectopy.[68] The complexity of a ventricular arrhythmia (see later discussion) likely relates to the risk of hemodynamic compromise, electrical deterioration, and sudden cardiac death. Although risk stratification for VA is imperfect, recommendations should be biased toward safety as opposed to maintaining athletic activity.[68] A history of collapse, presence of important structural heart disease (and cardiomegaly), or detection of systemic hypotension during a documented run of ventricular tachycardia raises great concern in a horse with VA. In the absence of clinical signs or of serious structural heart disease, the risk of ventricular ectopy is usually defined by electrocardiographic characteristics (see later discussion), accepting the limitations of this analysis.[68]

Ventricular Premature Complexes

Ventricular premature complexes (VPCs) are characterized by premature, widened complexes, often followed by a compensatory pause. The pause occurs because the next sinus impulse is blocked by the refractory AV conduction system. If the sinus rate is slow or the ventricular premature complex is closely coupled to the preceding normal sinus beat, it may be interpolated between two normal beats. Ventricular ectopic impulses are characterized by QRS and T waves that are wide and often bizarre in appearance (Fig. 9.61; see also Fig. 9.60). The premature QRS bears no relationship to any preceding P waves, although the induction of ventricular ectopics may be dependent on underlying cardiac cycle length or HR. The morphology of the VPCs may be uniform (monomorphic) or

*References 57, 77-79, 80, 81, 82, 83, 97, 149, 150, 153, 161, 227, 263, 383, 400, 472, 475, 478, 479, 485, 502, 524, 575, 651, 653, 654, 662, 665, 667, 736, 864, 907, 917, 1028, 1029, 1103, 1116-1124.

multiform (polymorphic) as shown in Fig. 9.60E. The relationship of a ventricular extrasystole to the preceding sinus QRS-T is expressed by the "coupling interval" between them. Often the coupling interval is fixed, although it may vary minimally[1120] or markedly. A very short coupling interval may place the ectopic QRS on the preceding T wave, a phenomenon called "R-on-T" and related to increased ventricular vulnerability for fibrillation. Horses with repetitive ectopic activity with short coupling intervals are therefore considered at a higher risk of collapse or sudden cardiac death.[68]

Ventricular premature complexes are generally considered abnormal, except for possibly those occurring immediately after exercise (see Exercise Testing earlier in this chapter).* However, ventricular ectopy was identified in 14% of clinically normal horses during routine 24-hour continuous electrocardiographic monitoring.[400] The distributional pattern of ventricular ectopic beats may include haphazard distribution of single complexes, bigeminy (VPCs following normal beats in an alternating pattern), couplets (pairs of VPCs), triplets, or runs (four or more) of ventricular ectopic beats. Runs of accelerated idioventricular rhythm (see earlier discussion), usually developing at relatively slow HRs, are not uncommon. Runs of ventricular ectopic beats at a rapid rate—ventricular tachycardia—are more likely to be hemodynamically and electrically unstable, particularly when they develop at rates higher than 100 beats/min (see later discussion).

When premature beats are identified by auscultation, an ECG should be obtained, and a workup similar to that outlined in Box 9.4 should be undertaken. Occasional premature ventricular complexes, as with occasional supraventricular premature complexes, may be clinically irrelevant and do not require treatment. Occasional monomorphic VPCs overdriven with exercise or only detected in the immediate postexercise period are not usually a cause of poor performance, but the importance of these arrhythmias and the risk of sudden cardiac death requires further investigation.[68] VPCs occurring during exercise are more concerning, although their relationship with poor performance is also uncertain and requires further investigation. Generally, horses with occasional VPCs at rest or during exercise can be used with caution by an informed adult. However, because of concerns about underlying myocardial or electrical disease and increased risks of exercise-associated collapse and sudden cardiac death, these horses should not be used by a child or as a lesson horse.[68]

Rest is highly recommended for horses with frequent ventricular premature complexes. In the majority of horses with premature ventricular complexes occurring in the absence of significant structural heart disease, the arrhythmia seems to resolve spontaneously after 4 to 8 weeks of rest. The horse may then be able to return successfully to its prior performance level. Antiarrhythmic therapy (see Table 9.7) is usually successful in abolishing ventricular premature complexes, but most return when the antiarrhythmic agent is discontinued, unless the underlying problem has been resolved. Chronic administration is usually not practical. Dexamethasone and other antiinflammatory drugs have been used to treat ventricular arrhythmias, particularly in the presence of myocardial damage as demonstrated by increased plasma troponin concentrations, but their use is controversial and they should certainly not be administered to horses with a recent or current infection.[68]

* References 54, 77-80, 82, 90, 475, 478, 479.

Ventricular Tachycardia

Ventricular tachycardia (VT) is an ectopic ventricular rhythm characterized by three or more repetitive or linked VPCs either at a regular or irregular ventricular rate (see Figs. 9.60 and 9.61).[68] Ventricular tachycardia is recognized clinically by an increased HR, often exceeding 100 beats/min. Auscultation of VT is often characterized by a regular rhythm, with variable intensity and often booming heart sounds. Lack of discernible rhythm irregularity on auscultation may cause the possible existence of VT to be overlooked. Because of intermittent aortic valve opening, the rhythm can also sound irregular on auscultation, and an intermittent pulse deficit could be present. Arterial pulses are typically weak or variable in intensity. Multiform VT is characterized by an irregular rhythm (see Fig. 9.60E). With multiform ventricular tachycardia, heart sounds are more likely to vary in intensity, and arterial pulses are likely to be abnormal. Jugular pulsations may be detected due to AV dissociation. Syncope is associated with higher rates of ventricular tachycardia (180 beats/min or higher). Respiratory distress and pulmonary edema may develop from impaired ventricular function. Protracted junctional or ventricular tachycardias (greater than 120 beats/min) may lead to CHF due to reversible myocardial failure (tachycardia-induced cardiomyopathy).[149] Torsades de pointes represents a specific form of polymorphic ventricular tachycardia, characterized by progressive changes in QRS direction leading to a steady undulation in the QRS axis (see Fig. 9.61). Ventricular tachycardias can progress to ventricular flutter and ventricular fibrillation, characterized by chaotic ventricular activation patterns leading to uncoordinated undulations of the electrical baseline. These rhythms often represent terminal rhythms and usually result in cardiac arrest (see Fig. 9.61).

Ventricular tachycardia may be life threatening, and in general, the following are features of complex or potentially "malignant" ventricular arrhythmias: severe hemodynamic compromise (systemic hypotension, poor pulse quality, prolonged capillary refill time, weakness, collapse), sustained VT, rapid ventricular rate (greater than 120 beats/min), short coupling intervals (particularly with R-on-T complexes), and multiform or polymorphic QRS morphology (including torsades de pointes).[68] Immediate treatment of CV collapse may be required.[1027] Intravenous therapies for VT include lidocaine, procainamide, quinidine, magnesium salts, and amiodarone (see Table 9.7). Administration of antiarrhythmic drugs can be associated with adverse effects, including proarrhythmia, seizures, and sudden death. Lidocaine, if administered too fast or at doses exceeding the recommended therapeutic doses, causes central nervous system excitation, but boluses and infusions can be well tolerated in horses, and the drug causes minimal hemodynamic or electrophysiologic alterations at therapeutic, nontoxic doses.* Quinidine may be used for treatment of ventricular arrhythmias but is a myocardial depressant and vagolytic drug.[55,153,327,328,1082] Procainamide[55,327,328,1095] has been studied to a limited degree but is generally well tolerated when given in graded doses. It has similar electrophysiologic effects to quinidine but seems less vagolytic and possibly has fewer gastrointestinal side effects. Cumulative doses of up to 10 mg/kg are generally well tolerated; additional dosing should be guided by ECG, QRS duration, and arterial BP. Magnesium sulfate is an alternative antiarrhythmic that can be effective in both normomagnesemic

* References 55, 327, 328, 797, 798, 1126, 1127.

and hypomagnesemic horses and is generally well tolerated at therapeutic doses.[55,1128] Amiodarone has been evaluated in a small number of horses[1100,1101,1124] and can be considered as a second-line drug for serious, drug-resistant VT. Other antiarrhythmics, including propafenone,[1111] or phenytoin[916,1129-1131] may be tried in refractory ventricular arrhythmias. The use of steroids in horses with ventricular tachycardia is empiric and controversial but may be indicated if there is evidence of acute myocardial damage in the absence of a recent or current infection (see earlier discussion).[68]

A substantial number of horses suffering from VT can be successfully returned to performance after treatment with antiarrhythmics and rest, as long as there is not significant and untreatable structural heart disease evident. A period of rest (4–8 weeks) followed by echocardiographic and electrocardiographic reevaluation (including a 24-hour Holter ECG) once sinus rhythm is restored is indicated for horses with sustained VT.[68] An exercising ECG should be performed before the horse may be returned to normal work, if the preceding echocardiogram and the 24-hour Holter ECG are normal; another exercising ECG should be performed once the horse has returned to full work.[68] Horses diagnosed with a single episode of sustained monomorphic VT generally have a favorable prognosis, although recurrent VT is possible.[68] The safety of horses with a history of symptomatic or complex VA remains uncertain, and these horses should only be used by an informed adult. Strenuous athletic work is not recommended for horses that showed VA in the setting of moderate or severe structural heart disease, including myocardial fibrosis or scar and moderate to severe AR. These horses should only be ridden or driven by an informed adult because of the risk of possible recurrence of VT. These horses are not safe for use by a child or as a lesson horse. For horses with a history of VT that remain in work follow-up 24-hour and exercising ECGs should be performed at least annually.[68]

Conduction Disturbances

Once a cardiac electrical impulse is formed, it is conducted rapidly throughout the heart. The sequence of cardiac electric activation is usually dictated by the specialized conducting tissues in the atria, the AV node, the bundle of His, the bundle branches, and the Purkinje fiber system (see Fig. 9.3). This conduction system permits orderly activation of atrial and ventricular muscle and facilitates effective mechanical activity of the heart.[57,110,112-115,220] A variety of conduction disorders are recognized, including SA nodal exit block, atrial standstill (usually resulting from hyperkalemia), AV block, bundle branch block, and ill-defined ventricular conduction disturbances (Figs. 9.62 and 9.63).*

Rarely, accelerated conduction occurs in the heart, which involves a pathway around the normally slow-conducting AV node and results in early excitation of the ventricles.[1069,1147] These syndromes are termed *preexcitation* and have various associated labels related to similar human disorders (e.g., Wolff-Parkinson-White). A shortened P-R interval is typically found.

Atrioventricular Conduction Block

Delays in AV conduction are the most common conduction blocks. These delays are classified as first-, second-, and third-degree (or complete) block. *First-degree AV block* occurs when the PR (or PQ) interval exceeds a certain value (approximately 400–450 msec in large-breed horses and 250–350 msec in small breeds and ponies; see Fig. 9.16) while the atrial impulse still transmits through the AV conduction system and activates the ventricle, causing a QRS complex. Some P waves are not conducted to the ventricles during *second-degree AV block,* which results in occasional P waves not followed by a QRS-T complex (see Fig. 9.62A).* Second-degree AV block following progressive prolongation of the PQ interval is classified as *Mobitz type I (Wenckebach)* block. Conversely, the AV block is termed *Mobitz type II* if the PQ interval is constant. Occurrence of two or more consecutive second-degree AV blocks in the presence of a normal or slow sinoatrial rate is termed *high-grade (advanced) AV block.*[68, 1151] *Third-degree* or *complete AV block* is characterized by an absence of atrial to ventricular conduction. P waves are not followed by or related to QRS complexes, and to prevent ventricular asystole a junctional or ventricular escape rhythm must develop below the level of the AV block (see Fig. 9.62B).[1092,1138,1141]

First- and second-degree AV blocks are considered normal variations. These rhythms are most often associated with high vagal tone and may be seen with sinus bradycardia or sinus arrhythmia. Auscultation is characterized by an irregular rhythm with a repetitive pattern. In some horses, fourth (atrial) heart sounds that are not followed by first and second heart sounds are audible within the pauses, allowing a definitive diagnosis without ECG.[3,68,72,381,383,384,388] Second-degree AV block can usually be abolished with exercise, stress, or vagolytic drugs such as atropine or glycopyrrolate (though exercise is preferred and drugs are rarely needed).[1132,1152] Note that the AV blocks are likely to resume quickly after exercise.

High-grade (advanced) and third-degree AV blocks are considered abnormal, and high-grade AV block may progress into complete AV block over time. They are indicative of organic heart disease, severe drug toxicity, or abnormally high vagal activity. An exercising ECG should be performed if possible to evaluate the degree of contribution of high vagal tone. A continuous 24-hour Holter ECG with simultaneous video recording should be obtained when there is a history of collapse.[68] Horses with high-grade AV block that disappears with exercise should only be used by an informed adult, and the HR and heart rhythm should be frequently monitored. Horses with high-grade AVB that persists during exercise or after atropine administration should be rested and reevaluated; they are considered less safe to use than their age-matched peers. Horses with symptomatic bradyarrhythmias generally have a poor prognosis and are not safe to use.[68]

Sudden development of high-grade or complete AV block may require the administration of atropine or a catecholamine or temporary transvenous pacing. Chronic third-degree AV block requires treatment with a permanent pacemaker. Pacemakers have been successfully placed epicardially and transvenously in several horses and in a number of Miniature Donkeys with possible congenital complete AV block (Fig. 9.64; see also Fig. 9.62C).[198,1134,1153-1156]

Intraventricular Conduction Blocks

Intraventricular conduction blocks, such as bundle branch block, are less common and more difficult to diagnose. Widening of the QRS complex and axis deviation are typical

* References 43, 60, 198, 215, 220, 223, 224, 226, 240, 383, 488, 525, 1028, 1033, 1092, 1132-1146.

* References 43, 226, 487, 525, 541, 1028, 1133, 1135, 1137, 1140, 1148-1150.

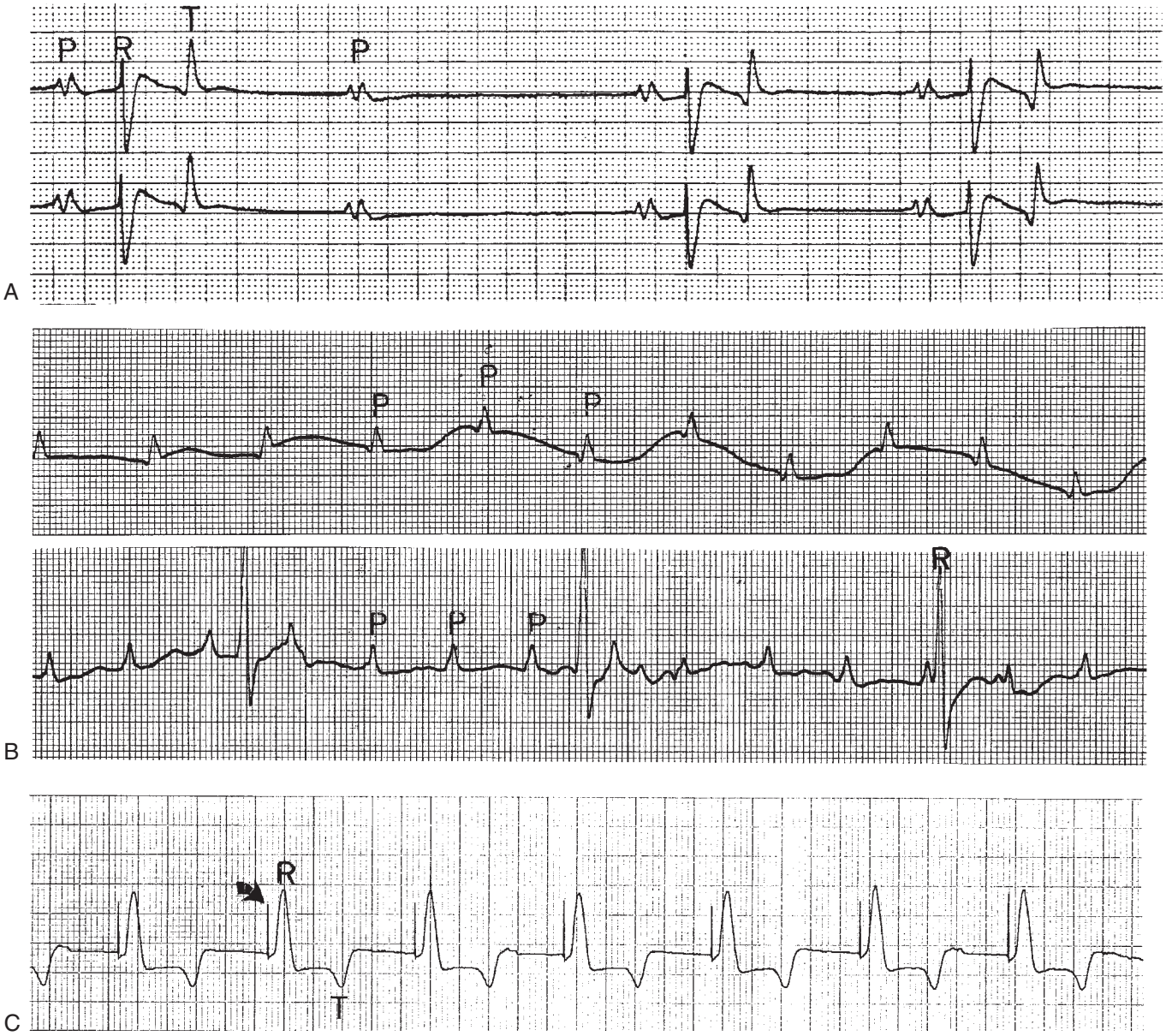


FIG. **9.62** Conduction disturbances. A, Second-degree atrioventricular block. Nonconducted P waves and varying P-R intervals are evident (base-apex lead recorded at 25 mm/sec). B, The tracings are from a horse with third-degree atrioventricular block. The upper strip demonstrates multiple blocked P waves. The lower tracing shows nonconducted P waves and ventricular escape complexes (base-apex lead recorded at 25 mm/sec). C, Permanent transvenous ventricular pacing in a Miniature Donkey with a complete atrioventricular block. A pacemaker spike *(arrow)* precedes each paced ventricular complex.

features (see Fig. 9.63B).[1133,1136] As opposed to ventricular premature beats, the aberrantly conducted QRS complex is preceded by a P wave. These abnormalities may also be found after atrial premature complexes, from overdosage with quinidine sulfate, from severe hyperkalemia, or secondary to supraventricular tachycardias with rapid ventricular response.

A recent study indicated a high prevalence of QRS broadening in horses with AF during exercise and suggested that QRS broadening may originate from ventricular ectopic foci or from aberrant intraventricular conduction due, for example, to bundle branch block.[472] In fact, intraventricular conduction blocks leading to aberrant ventricular conduction might explain the high number of complexes currently classified as VPCs recorded on exercise ECGs,[54,77-80,82,90,479] because differentiation between ventricular ectopic beats and aberrantly conducted beats originating from the sinus node is very difficult and often impossible at high HRs, when distinct P waves are not present and motion artifacts hamper interpretation of the ECG.

Preexcitation

Ventricular preexcitation, or accelerated AV conduction, in humans and in dogs is often due to an anomalous conducting pathway around the AV node, which serves as a path for reentrant supraventricular tachycardias. These rhythm

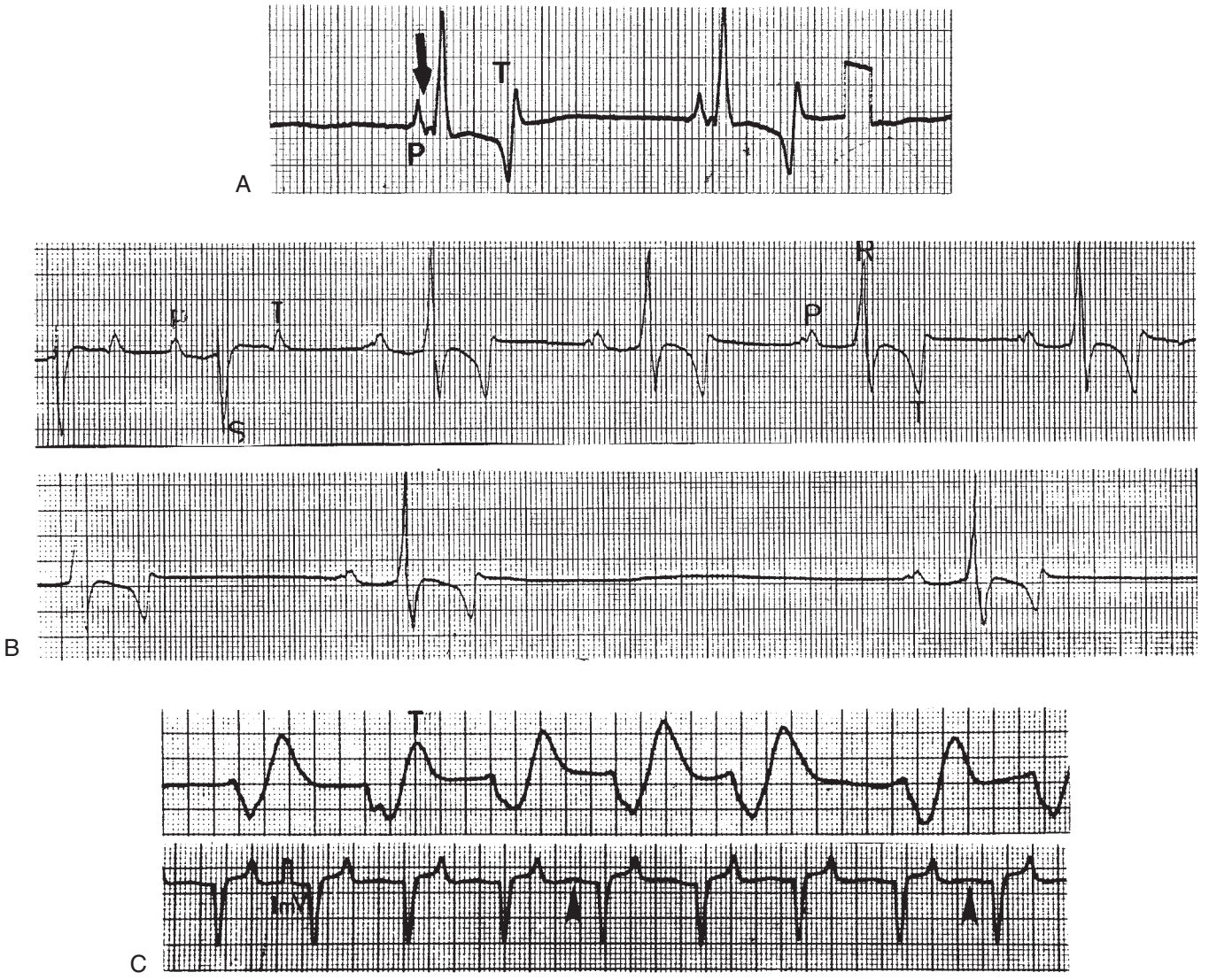


FIG. **9.63** Conduction disturbances. A, Ventricular preexcitation. The short P-R interval *(arrow)* and small deflection in the PR segment (delta wave) indicate an accessory pathway around the atrioventricular node with premature activation of a portion of the ventricle (delta wave and initial portion of the QRS). The large QRS and secondary T wave changes can be explained by the loss of normal cancellation of ventricular electrical forces. B, A sinus arrhythmia and intraventricular conduction disturbance. The sudden change and widening of the ventricular conduction pattern are notable, despite the consistent P-R interval and probably represent a bundle branch block, although this diagnosis is difficult to make in horses (base-apex lead recorded at 25 mm/sec). C, Hyperkalemia in a foal with a ruptured bladder. The top strip demonstrates atrial standstill (no P waves), significant widening of the QRS complexes, and large T waves with a shortened ST segment. The lower tracing shows the effects after initial medical therapy for hyperkalemia, which included treatment with saline and sodium bicarbonate. The normalization of the QRS complexes and the appearance of low-amplitude P waves *(arrows)* are notable. (Tracing courtesy of Ron Hilwig, DVM, PhD.)

disturbances may cause hypotension and syncope. Whether preexcitation syndromes are important in horses has yet to be determined. Nevertheless, ECG traces occasionally show evidence of ventricular preexcitation and are characterized by a P-QRS-T relationship but with an extremely short PR interval, early excitation of the ventricle characterized by a slurring of the initial QRS complex (a delta wave), and an overall widening of the QRS complex (see Fig. 9.63A).[1069,1147,1157]

Hyperkalemia

Hyperkalemia can cause significant depression of atrial, AV, and ventricular conduction and can shorten ventricular repolarization. Serum potassium is most likely to be markedly elevated in foals with ruptured bladder or ureter and uroperitoneum, following renal failure oliguria, during shock, with severe metabolic acidosis, or after excessive intravenous potassium replacement.[226,1143-1146] Hyperkalemia also occurs in Quarter Horses with hyperkalemic periodic paralysis.[1158,1159]



FIG. **9.64** A lateral radiograph of a permanent transvenous pacing system in a Miniature Donkey. The pacemaker is evident at the upper left of the radiograph. The thin transvenous pacing wire extends from the device through the jugular vein and vena cava and into the right ventricular (RV) apex.

Alterations in the ECG are usually evident at serum potassium concentrations greater than 6 mEq/L, with severe changes evident when serum concentrations are between 8 and 10 mEq/L. Broadening and flattening of the P wave are the most consistently observed change. Prolongation of the PQ interval and bradycardia develop, excitability decreases, and atrial standstill (sinoventricular rhythm) characterized by complete absence of P waves may be observed. Either inversion or enlargement (tenting) of the T waves is also likely. Marked widening of the QRS complex may be noted as near-lethal concentrations of potassium are approached (see Fig. 9.63C). Ventricular asystole or fibrillation can develop. The QT interval is not a reliable indicator of induced hyperkalemia, and other electrolyte and acid-base alterations, including serum calcium and sodium concentrations, influence the effect of hyperkalemia on the heart.

Therapy for hyperkalemia includes correction of the underlying problem; infusion of isotonic crystalloid solutions, dextrose, and insulin; and possibly administration of β_2 agonists, calcium salts, and sodium bicarbonate. Lactated Ringer's solution (LRS; 10–40 mL/kg/hr IV) can be used in patients with hyperkalemia. The small amount of potassium contained in LRS is generally not considered problematic, as the beneficial effects of volume replacement and treatment of metabolic acidosis largely outweigh the potential negative effects of additional potassium supply. Alternatively, NaCl or NaCl/dextrose solutions may be used, but maintenance solutions should not be used because of their relatively high potassium content. Dextrose (or glucose) can be administered as a bolus of 0.25 to 0.5 g/kg IV over 15 minutes. The administration of regular insulin (0.1 IU/kg) in conjunction with dextrose infusion (0.5–1 g/kg IV over 15 minutes) should be considered in cases of severe hyperkalemia and is considered most effective. β_2-Adrenoceptor agonists (e.g., clenbuterol, albuterol/salbutamol) administered intravenously or by inhalation might augment the effects of intravenous insulin and glucose by promoting potassium movement into cells,[1160,1161] but this has not been investigated in horses. Sodium bicarbonate may be used in cases of severe metabolic acidosis (provided that ventilation is adequate) at a dose of 1 to 2 mEq/kg IV over 15 minutes or depending on the base excess obtained by blood gas analyses (mEq Na bicarbonate = 0.3 [−0.5] × BE × kg of body weight). However, in the absence of severe acidosis, the use of sodium bicarbonate is not recommended because of the associated risks of sodium and fluid overload[1160,1161] and its effect to lower ionized calcium concentrations that might further destabilize the rhythm. In cases of severe arrhythmias with QRS broadening, 0.2 to 0.4 mL/kg calcium gluconate 23% can be administered over a 10-minute period to effect to stabilize the resting cardiac membrane potential.

REFERENCES

1. Detweiler DK. Electrocardiogram of the horse. *Fed Proc*. 1952;11:34.
2. Detweiler DK. Auricular fibrillation in horses. *J Am Vet Med Assoc*. 1955;126:47.
3. Patterson DF, Detweiler DK, Glendenning SA. Heart sounds and murmurs of the normal horse. *Ann N Y Acad Sci*. 1965;127:242.
4. Detweiler DK, Patterson DF. The cardiovascular system. In: Catcott EJ, Smithcors JF, eds. *Equine medicine and surgery*. Santa Barbara: American Veterinary Publications; 1972.
5. Bishop SP, Cole CR, Smetzer DL. Functional and morphologic pathology of equine aortic insufficiency. *Pathol Vet*. 1966;3:137.
6. Hamlin RL, Smetzer DL, Smith CR. Analysis of QRS complex recorded through a semiorthogonal lead system in the horse. *Am J Physiol*. 1964;207:325.
7. Hamlin RL, Smith CR. Categorization of common domestic mammels based on their ventricular activation process. *Ann N Y Acad Sci*. 1965;127:195.
8. Hamlin RL, Smetzer DL, Senta T, et al. Atrial activation paths and P waves in horses. *Am J Physiol*. 1970;219:306.
9. Hamlin RL, Klepinger WL, Gilpin KW, et al. Autonomic control of heart rate in the horse. *Am J Physiol*. 1972;222:976.
10. Hamlin RL, Levesque MJ, Kittleson MD. Intramyocardial pressure and distribution of coronary blood flow during systole and diastole in the horse. *Cardiovasc Res*. 1982;16:256.
11. Illera JC, Illera M, Hamlin RL. Unipolar thoracic electrocardiography that induces QRS complexes of relative uniformity from male horses. *Am J Vet Res*. 1987;48:1700.
12. McKeever KH, Hinchcliff KW, Reed SM, et al. Splenectomy alters blood pressure response to incremental treadmill exercise in horses. *Am J Physiol*. 1993;265:R409.
13. Pipers FS, Hamlin RL. Echocardiography in the horse. *J Am Vet Med Assoc*. 1977;170:815.
14. Pipers FS, Hamlin RL, Reef V. Echocardiographic detection of cardiovascular lesions in the horse. *J Equine Med Surg*. 1979;3:68.
15. Senta T, Smetzer DL, Smith CR. Effects of exercise on certain electrocardiographic parameters and cardiac arrhythmias in the horse. A radiotelemetric study. *Cornell Vet*. 1970;60:552.
16. Smetzer DL, Smith CR, Hamlin RL. The fourth heart sound in the equine. *Ann N Y Acad Sci*. 1965;127:306.
17. Smetzer DL, Smith CR. Diastolic heart sounds of horses. *J Am Vet Med Assoc*. 1965;146:937.
18. Smetzer DL, Bishop S, Smith CR. Diastolic murmur of equine aortic insufficiency. *Am Heart J*. 1966;72:488.
19. Smetzer DL, Hamlin RL, Smith CR. Cardiovascular sounds. In: Swenson MJ, ed. *Dukes' Physiology of Domestic Animals*. Ithaca: Comstock Publishing Company, Inc; 1970.
20. Smith CR, Hamlin RL. Regulation of the heart and blood vessels. In: Swenson MJ, ed. *Dukes' Physiology of Domestic Animals*. Ithaca: Comstock Publishers; 1970.
21. Brown CM, Holmes JR. Haemodynamics in the horse: 2. Intracardiac, pulmonary arterial and aortic pressures. *Equine Vet J*. 1978;10:207.

22. Brown CM, Holmes JR. Haemodynamics in the horse: 1. Pressure pulse contours. *Equine Vet J*. 1978;10:188.
23. Brown CM, Holmes JR. Phonocardiography in the horse. 1. The intracardiac phonocardiogram. *Equine Vet J*. 1979;11: 11.
24. Brown CM, Holmes JR. Assessment of myocardial function in the horse. 2. Experimental findings in resting horses. *Equine Vet J*. 1979;11:248.
25. Brown CM, Holmes JR. Phonocardiography in the horse: 2. The relationship of the external phonocardiogram to intracardiac pressure and sound. *Equine Vet J*. 1979;11:183.
26. Brown CM, Holmes JR. Assessment of myocardial function in the horse. 1. Theoretical and technical considerations. *Equine Vet J*. 1979;11:244.
27. Else RW, Holmes JR. Cardiac pathology in the horse. III: clinical correlations. *Equine Vet J*. 1972;4:195.
28. Else RW, Holmes JR. Cardiac pathology in the horse. 1. Gross pathology. *Equine Vet J*. 1972;4:1.
29. Else RW, Holmes JR. Cardiac pathology in the horse. II: microscopic pathology. *Equine Vet J*. 1972;4:57.
30. Gattland L, Holmes JR. ECG recording at rest and during exercise in the horse. *Equine Vet Educ*. 1990;2:28.
31. Hillyer MH, Mair TS, Holmes JR. Treatment of bacterial endocarditis in a Shire mare. *Equine Vet Educ*. 1990;2:5.
32. Holmes JR. The equine heart: problems and difficulties in assessing cardiac function on clinical examination. *Equine Vet J*. 1968;1:10.
33. Holmes JR, Darke PGG, Else RW. Atrial fibrillation in the horse. *Equine Vet J*. 1969;1:212.
34. Holmes JR, Rezakhani A, Else RW. Rupture of a dissecting aortic aneurysm into the left pulmonary artery in a horse. *Equine Vet J*. 1973;5:65.
35. Holmes JR, Rezakhani A. Observations on the T wave of the equine electrocardiogram. *Equine Vet J*. 1975;7:55.
36. Holmes JR. Prognosis of equine cardiac conditions. *Equine Vet J*. 1977;9:181.
37. Holmes JR. Sir Frederick Smith Memorial Lecture. A superb transport system—the circulation. *Equine Vet J*. 1982;14:267.
38. Holmes JR, Miller PJ. Three cases of ruptured mitral valve chordae in the horse. *Equine Vet J*. 1984;16:125.
39. Holmes JR, Henigan M, Williams RB, et al. Paroxysmal atrial fibrillation in racehorses. *Equine Vet J*. 1986;18:37.
40. Holmes JR. Electrocardiography in the diagnosis of common cardiac arrythmias in the horse. *Equine Vet Educ*. 1990;2:24.
41. Holmes JR. The development of clinical cardiology. *Equine Vet J Suppl*. 1995;2.
42. Miller PJ, Holmes JR. Effect of cardiac arrhythmia on left ventricular and aortic blood pressure parameters in the horse. *Res Vet Sci*. 1983;35:190.
43. Miller PJ, Holmes JR. Beat-to-beat variability in QRS potentials recorded with an orthogonal lead system in horses with second degree partial A-V block. *Res Vet Sci*. 1984;37:334.
44. Miller PJ, Holmes JR. Interrelationship of some electrocardiogram amplitudes, time intervals and respiration in the horse. *Res Vet Sci*. 1984;36:370.
45. Miller PJ, Holmes JR. Observations on structure and function of the equine mitral valve. *Equine Vet J*. 1984;16:457.
46. Miller PJ, Holmes JR. Relationships of left side systolic time intervals to beat-by-beat heart-rate and blood pressure variables in some cardiac arrhythmias of the horse. *Res Vet Sci*. 1984;37:18.
47. Miller PJ, Holmes JR. Computer processing of transaortic valve blood pressures in the horse using the first derivative of the left ventricular pressure trace. *Equine Vet J*. 1984;16:210.
48. Miller PJ, Holmes JR. Observations on seven cases of mitral insufficiency in the horse. *Equine Vet J*. 1985;17:181.
49. Voros K, Holmes JR, Gibbs C. Left ventricular volume determination in the horse by two- dimensional echocardiography: an in vitro study. *Equine Vet J*. 1990;22:398.
50. Voros K, Holmes JR, Gibbs C. Anatomical validation of two-dimensional echocardiography in the horse. *Equine Vet J*. 1990;22:392.
51. Voros K, Holmes JR, Gibbs C. Measurement of cardiac dimensions with two-dimensional echocardiography in the living horse. *Equine Vet J*. 1991;23:461.
52. Marr CM, Bowen M. *Cardiology of the Horse*. 2nd ed. Edinburgh: Saunders Elsevier; 2010.
53. Poole DC, Erickson HH. Heart and vessels: function during exercise and training adaptations. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. Edinburgh: Saunders Elsevier; 2014.
54. Young LE, van Loon G. Diseases of the heart and vessels. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. Edinburgh: W.B. Saunders; 2014.
55. Schwarzwald CC. Cardiovascular pharmacology. In: Robinson NE, Sprayberry KA, eds. *Current Therapy in Equine Medicine 6*. St. Louis: Saunders Elsevier; 2009.
56. Schwarzwald CC. Ultrasonography of the heart. In: Kidd JA, Lu KG, Frazer ML, eds. *Atlas of Equine Ultrasonography*. Chichester, UK: John Wiley & Sons, Ltd; 2014.
57. Schwarzwald CC, Bonagura JD, Muir III WW. The cardiovascular system. In: Muir III WW, Hubbell JAE, eds. *Equine Anesthesia: Monitoring and Emergency Therapy*. St. Louis: Saunders Elsevier; 2009.
58. Jesty SA. Cardiovascular system. In: Divers TJ, ed. *Equine Emergencies*. St. Louis: W.B. Saunders; 2014.
59. Bonagura JD. Congenital heart disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
60. Bowen IM. Cardiac dysrhythmias. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
61. Durando MM. Evaluation of cardiovascular function in the performance horse. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
62. Durando MM, Young LE. Cardiovascular examination and diagnostic techniques. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
63. Leroux A. Aortic root disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
64. Leroux A. Vascular diseases. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
65. Reef VB. Acquired valvular heart disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
66. Sleeper MM. Myocardial disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
67. Sleeper MM. Acquired pericardial disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. St. Louis: W.B. Saunders; 2003.
68. Reef VB, Bonagura J, Buhl R, et al. Recommendations for management of equine athletes with cardiovascular abnormalities. *J Vet Intern Med*. 2014;28:749.
69. Kriz NG, Hodgson DR, Rose RJ. Prevalence and clinical importance of heart murmurs in racehorses. *J Am Vet Med Assoc*. 2000;216:1441.
70. Lightfoot G, Jose-Cunilleras E, Rogers K, et al. An echocardiographic and auscultation study of right heart responses to training in young national hunt thoroughbred horses. *Equine Vet J Suppl*. 2006;153.
71. Marr CM, Reef VB. Physiological valvular regurgitation in clinically normal young racehorses: prevalence and two-dimensional colour flow Doppler echocardiographic characteristics. *Equine Vet J Suppl*. 1995;56.
72. Patteson MW, Cripps PJ. A survey of cardiac auscultatory findings in horses. *Equine Vet J*. 1993;25:409.

73. Reef VB. The significance of cardiac auscultatory findings in horses—insight into the age-old dilemma. *Equine Vet J*. 1993;25:393.
74. Young LE, Rogers K, Wood JL. Heart murmurs and valvular regurgitation in thoroughbred racehorses: epidemiology and associations with athletic performance. *J Vet Intern Med*. 2008;22:418.
75. Young LE, Wood JL. Effect of age and training on murmurs of atrioventricular valvular regurgitation in young thoroughbreds. *Equine Vet J*. 2000;32:195.
76. Zucca E, Ferrucci F, Stancari G, et al. The prevalence of cardiac murmurs among Standardbred racehorses presented with poor performance. *J Vet Med Sci*. 2010;72:781.
77. Barbesgaard L, Buhl R, Meldgaard C. Prevalence of exercise-associated arrhythmias in normal performing dressage horses. *Equine Vet J*. 2010;42:202.
78. Buhl R, Meldgaard C, Barbesgaard L. Cardiac arrhythmias in clinically healthy showjumping horses. *Equine Vet J*. 2010;42:196.
79. Buhl R, Petersen EE, Lindholm M, et al. Cardiac arrhythmias in Standardbreds during and after racing-possible association between heart size, valvular regurgitations, and arrhythmias. *J Equine Vet Sci*. 2013;33:590.
80. Jose-Cunilleras E, Young LE, Newton JR, et al. Cardiac arrhythmias during and after treadmill exercise in poorly performing thoroughbred racehorses. *Equine Vet J Suppl*. 2006;163.
81. Morgan RA, Raftery AG, Cripps P, et al. The prevalence and nature of cardiac arrhythmias in horses following general anaesthesia and surgery. *Acta Vet Scand*. 2011;53:62.
82. Slack J, Boston RC, Soma LR, et al. Occurrence of cardiac arrhythmias in Standardbred racehorses. *Equine Vet J*. 2015;47:398.
83. Leroux AA, Detilleux J, Sandersen CF, et al. Prevalence and risk factors for cardiac diseases in a hospital-based population of 3,434 horses (1994–2011). *J Vet Intern Med*. 2013;27:1563.
84. Deem DA, Fregin GF. Atrial fibrillation in horses: a review of 106 clinical cases, with consideration of prevalence, clinical signs, and prognosis. *J Am Vet Med Assoc*. 1982;180:261.
85. Baker JR, Ellis CE. A survey of post mortem findings in 480 horses 1958 to 1980: (1) causes of death. *Equine Vet J*. 1981;13:43.
86. Pipers FS. Applications of diagnostic ultrasound in veterinary medicine. *Equine Vet J*. 1982;14:341.
87. Morris EA, Seeherman HJ. Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. *Equine Vet J*. 1991;23:169.
88. Mitten LA. Cardiovascular causes of exercise intolerance. *Vet Clin North Am Equine Pract*. 1996;12:473.
89. Reef VB. Clinical approach to poor performance in horses. *Proc Am Coll Vet Internal Med San Diego*. 1989;566.
90. Martin Jr BB, Reef VB, Parente EJ, et al. Causes of poor performance of horses during training, racing, or showing: 348 cases (1992-1996). *J Am Vet Med Assoc*. 2000;216:554.
91. Cronin MTL, Leader GH. Coronary occlusion in a thoroughbred colt. *Vet Rec*. 1952;64:8.
92. Rooney JR, Prickett ME, Crowe MW. Aortic ring rupture in stallions. *Pathol Vet*. 1967;4:268.
93. Pascoe RR, O'Sullivan BM. Sudden death in a Thoroughbred stallion. *Equine Vet J*. 1980;12:211.
94. Platt H. Sudden and unexpected deaths in horses: a review of 69 cases. *Br Vet J*. 1982;138:417.
95. Hughes PE, Howard EB. Endocardial fibroelastosis as a cause of sudden death in the horse. *Equine Pract*. 1984;6:23.
96. Gelberg HB, Zachary JF, Everitt JI, et al. Sudden death in training and racing Thoroughbred horses. *J Am Vet Med Assoc*. 1985;187:1354.
97. Kiryu K, Nakamura T, Kaneko M, et al. Cardiopathology of sudden cardiac death in the race horse. *Heart Vessels Suppl*. 1987;2:40.
98. Lucke VM. Sudden death (editorial). *Equine Vet J*. 1987;19:85.
99. Brown CM, Kaneene JB, Taylor RF. Sudden and unexpected death in horses and ponies: an analysis of 200 cases. *Equine Vet J*. 1988;20:99.
100. Allen JR, Heidel JR, Hodgson DR, et al. Spontaneous rupture of the great coronary vein in a pony. *Equine Vet J*. 1987;19:145.
101. Leblond A, Villard I, Leblond L, et al. A retrospective evaluation of the causes of death of 448 insured French horses in 1995. *Vet Res Commun*. 2000;24:85.
102. Schiff P, Knottenbelt DC. Sudden death in a 11-year-old Thoroughbred stallion. *Equine Vet Educ*. 1990;2:8.
103. Boden LA, Charles JA, Slocombe RF, et al. Sudden death in racing Thoroughbreds in Victoria, Australia. *Equine Vet J*. 2005;37:269.
104. Navas de Solis C. Exercising arrhythmias and sudden cardiac death in horses: review of the literature and comparative aspects. *Equine Vet J*. 2016;48:406.
105. Lyle CH, Blissitt KJ, Kennedy RN, et al. Risk factors for race-associated sudden death in Thoroughbred racehorses in the UK (2000-2007). *Equine Vet J*. 2012;44:459.
106. Lyle CH, Uzal FA, McGorum BC, et al. Sudden death in racing Thoroughbred horses: an international multicentre study of post mortem findings. *Equine Vet J*. 2011;43:324.
107. Sisson S, Grossman JD. *Anatomy of the Domestic Animals*. 4th ed. Philadelphia: W.B. Saunders; 1953.
108. Geddes LA, Hoff HE, McCrady JD. Some aspects of the cardiovascular physiology of the horse. *[Baylor Univ College of Medicine] Cardiovascular Res Center Bull*. 1965;3:80.
109. Schummer A, Wilkens H, Vollmerhaus B, et al. The circulatory system, the skin, and the cutaneous organs of the domestic mammals, the anatomy of the domestic animals. In: Nickel R, Schummer A, Seiferle E, eds. vol. 3. Berlin: Verlag Paul Parey; 1981.
110. Berne RM, Levy MN. *Cardiovascular Physiology*. 8th ed. St. Louis: C.V. Mosby; 2001.
111. Dyce KM, Sack WO, Wensing CJG. The cardiovascular system. In: Dyce KM, Sack WO, Wensing CJG, eds. *Textbook of Veterinary Anatomy*. Philadelphia: Elsevier Saunders; 2002.
112. Opie LH. *Heart Physiology: From Cell to Circulation*. Philadelphia: Lippincott Williams & Wilkins; 2004.
113. Katz AM. *Physiology of the Heart*. 5th ed. Philadelphia: Wolters Kluwer; 2010.
114. Klabunde RE. *Cardiovascular Physiology Concepts*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins/Wolters Kluwer; 2012.
115. Hall JE. The heart. In: Hall JE, ed. *Guyton and Hall: Textbook of Medical Physiology*. Philadelphia: Saunders Elsevier; 2016.
116. Hall JE. The circulation. In: Hall JE, ed. *Guyton and Hall: Text-Book of Medical Physiology*. Philadelphia: Saunders Elsevier; 2016.
117. Lilly LS. *Pathyophysiology of Heart Disease*. 6th ed. Philadelphia, PA: Wolters Kluwer; 2016.
118. Muir WW, Hubbell JAE. Cardiopulmonary resuscitation. In: Muir WW, Hubbell JAE, eds. *Equine Anesthesia—Monitoring and Emergency Therapy*. St. Louis: Saunders Elsevier; 2009.
119. Rainey JW. A specific arthritis with pericarditis affecting young horses in Tasmania. *Aust Vet J*. 1944;20:204.
120. Ryan AF, Rainey JW. A specific arthritis with pericarditis affecting horses in Tasmania. *Aust Vet J*. 1945;21:146.
121. Wagner P, Miller R, Merritt F, et al. Constrictive pericarditis in the horse. *J Equine Med Surg*. 1977;1:242.
122. Reef VB. Advances in echocardiography. *Vet Clin North Am Equine Pract*. 1991;7:435.
123. Rantanen NW. Diseases of the heart. *Vet Clin North Am Equine Pract*. 1986;2:33.
124. Marr CM. Equine echocardiography—sound advice at the heart of the matter. *Br Vet J*. 1994;150:527.
125. Hardy J, Robertson JT, Reed SM. Constrictive pericarditis in a mare: attempted treatment by partial pericardiectomy. *Equine Vet J*. 1992;24:151.

126. Freestone JF, Thomas WP, Carlson GP, et al. Idiopathic effusive pericarditis with tamponade in the horse. *Equine Vet J*. 1987;19:38.
127. Dill SG, Simoncini DC, Bolton GR, et al. Fibrinous pericarditis in the horse. *J Am Vet Med Assoc*. 1982;180:266.
128. Carnine BL, Schneider G, Cook JE, et al. Pericardial mesothelioma in a horse. *Vet Pathol*. 1977;14:513.
129. Bonagura JD, Herring DS, Welker F. Echocardiography. *Vet Clin North Am Equine Pract*. 1985;1:311.
130. Bonagura JD, Blissitt KJ. Echocardiography. *Equine Vet J Suppl*. 1995;5.
131. Birks EK, Hultgren BD. Pericardial haemangiosarcoma in a horse. *J Comp Pathol*. 1988;99:105.
132. Wijnberg ID, Vink-Nooteboom M, Sloet van Oldruitenborgh-Oosterbaan MM. Idiopathic pericardial effusion with tamponade in a Friesian gelding. *Tijdschr Diergeneeskd*. 1997;122:216.
133. Worth LT, Reef VB. Pericarditis in horses: 18 cases (1986-1995). *J Am Vet Med Assoc*. 1998;212:248.
134. Bolin DC, Donahue JM, Vickers ML, et al. Microbiologic and pathologic findings in an epidemic of equine pericarditis. *J Vet Diagn Invest*. 2005;17:38.
135. Perkins SL, Magdesian KG, Thomas WP, et al. Pericarditis and pleuritis caused by *Corynebacterium pseudotuberculosis* in a horse. *J Am Vet Med Assoc*. 2004;224:1133.
136. Seahorn JL, Slovis NM, Reimer JM, et al. Case-control study of factors associated with fibrinous pericarditis among horses in central Kentucky during spring 2001. *J Am Vet Med Assoc*. 2003;223:832.
137. Armstrong SK, Raidal SL, Hughes KJ. Fibrinous pericarditis and pericardial effusion in three neonatal foals. *Aust Vet J*. 2014;92:392.
138. Baker D, Kreeger J. Infiltrative lipoma in the heart of a horse. *Cornell Vet*. 1987;77:258.
139. Dill SG, Moise NS, Meschter CL. Cardiac failure in a stallion secondary to metastasis of an anaplastic pulmonary carcinoma. *Equine Vet J*. 1986;18:414.
140. Byars TD, Dainis CM, Seltzer KL, et al. Cranial thoracic masses in the horse: a sequel to pleuropneumonia. *Equine Vet J*. 1991;23:22.
141. Evans DL. Cardiac respones to exercise and training. In: Marr CM, ed. *Cardiology of the Horse*. London: WB Saunders; 1999.
142. Buhl R, Ersboll AK, Eriksen L, et al. Changes over time in echocardiographic measurements in young Standardbred racehorses undergoing training and racing and association with racing performance. *J Am Vet Med Assoc*. 2005;226:1881.
143. Rugh KS, Garner HE, Sprouse RF, et al. Left ventricular hypertrophy in chronically hypertensive ponies. *Lab Anim Sci*. 1987;37:335.
144. Braunwald E, Bonow RO. *Braunwald's Heart Disease: A Text-Book of Cardiovascular Medicine*. 9th ed. Philadelphia: Saunders; 2012.
145. Cranley JJ, McCullagh KG. Ischaemic myocardial fibrosis and aortic strongylosis in the horse. *Equine Vet J*. 1981;13:35.
146. Dudan F, Rossi GL, Luginbuhl H. Cardiovascular study of the horse: relationships between vascular and tissue lesions in the myocardium. II. *Schweiz Arch Tierheilkd*. 1984;126:527.
147. Dudan F, Rossi GL, Luginbuhl H. Cardiovascular study in the horse: relationship between vascular and myocardial lesions. 3. *Schweiz Arch Tierheilkd*. 1985;127:319.
148. Reef VB, Bain FT, Spencer PA. Severe mitral regurgitation in horses: clinical, echocardiographic and pathological findings. *Equine Vet J*. 1998;30:18.
149. Traub-Dargatz JL, Schlipf Jr JW, Boon J, et al. Ventricular tachycardia and myocardial dysfunction in a horse. *J Am Vet Med Assoc*. 1994;205:1569.
150. Kiryu K, Machida N, Kashida Y, et al. Pathologic and electrocardiographic findings in sudden cardiac death in racehorses. *J Vet Med Sci*. 1999;61:921.
151. Fregin GF. The cardiovascular system. In: Mansmann RA, McCallister ES, Pratt PW, eds. *Equine Medicine and Surgery*. Santa Barbara: American Veterinary Publications Inc; 1982.
152. Bonagura JD. Equine heart disease. An overview. *Vet Clin North Am Equine Pract*. 1985;1:267.
153. Stern JA, Reina Doreste Y, Barnett S, et al. Resolution of sustained narrow complex ventricular tachycardia and tachycardia-induced cardiomyopathy in a Quarter Horse following quinidine therapy. *J Vet Cardiol*. 2012;14(3):445–451.
154. Doonan GR, Brown CM, Mullaney TP, et al. Monensin poisoning in horses—an international incident. *Can Vet J*. 1989;30:165.
155. Mollenhauer HH, Rowe LD, Witzel DA. Effect of monensin on the morphology of mitochondria in rodent and equine striated muscle. *Vet Hum Toxicol*. 1984;26:15.
156. Amend JF, Mallon FM, Wren WB, et al. Equine monensin toxicosis: some experimental clinicopathologic observations. *Compend Cont Educ Pract Vet*. 1980;2:S172.
157. Amend JF, Nichelson RL, Freeland LR, et al. Clinical toxicology of an antibiotic ionophore (monensin) in ponies and horses; diagnostic markers and therapeutic considerations. In: Miert ASJPAMV, Bogaert MG, Debackere M, eds. *Comparative Veterinary Pharmacology, Toxicology and Therapy*. Netherlands: Springer; 1986.
158. Bezerra PS, Driemeier D, Loretti AP, et al. Monensin poisoning in Brazilian horses. *Vet Hum Toxicol*. 1999;41:383.
159. Bila CG, Perreira CL, Gruys E. Accidental monensin toxicosis in horses in Mozambique. *J S Afr Vet Assoc*. 2001;72:163.
160. Hughes KJ, Hoffmann KL, Hodgson DR. Long-term assessment of horses and ponies post exposure to monensin sodium in commercial feed. *Equine Vet J*. 2009;41:47.
161. Decloedt A, Verheyen T, Sys S, et al. Tissue Doppler imaging and 2-dimensional speckle tracking of left ventricular function in horses exposed to lasalocid. *J Vet Intern Med*. 2012;26:1209.
162. Decloedt A, Verheyen T, De Clercq D, et al. Acute and long-term cardiomyopathy and delayed neurotoxicity after accidental lasalocid poisoning in horses. *J Vet Intern Med*. 2012;26:1005.
163. Divers TJ, Kraus MS, Jesty SA, et al. Clinical findings and serum cardiac troponin I concentrations in horses after intragastric administration of sodium monensin. *J Vet Diagn Invest*. 2009;21:338.
164. Schefer KD, Hagen R, Ringer SK, et al. Laboratory, electrocardiographic, and echocardiographic detection of myocardial damage and dysfunction in an Arabian mare with nutritional masseter myodegeneration. *J Vet Intern Med*. 2011;25:1171.
165. Barigye R, Dyer NW, Newell TK. Fatal myocardial degeneration in an adult Quarter Horse with vitamin E deficiency. *J Equine Vet Sci*. 2007;27:405.
166. Sweeney RW, Hamir AN, Fisher RR. Lymphosarcoma with urinary bladder infiltration in a horse. *J Am Vet Med Assoc*. 1991;199:1177.
167. Reef VB. Stress echocardiography and its role in performance assessment. *Vet Clin North Am Equine Pract*. 2001;17:179.
168. Schefer KD, Bitschnau C, Weishaupt MA, et al. Quantitative analysis of stress echocardiograms in healthy horses with 2-dimensional (2D) echocardiography, anatomical M-mode, tissue Doppler imaging, and 2D speckle tracking. *J Vet Intern Med*. 2010;24:918.
169. Bowen IM, Marr CM, Chester AH, et al. In-vitro contraction of the equine aortic valve. *J Heart Valve Dis*. 2004;13:593.
170. Rooney JR, Franks WC. Congenital cardiac anomalies in horses. *Vet Pathol*. 1964;1:454.
171. Bayly WM, Reed SM, Leathers CW, et al. Multiple congenital heart anomalies in five Arabian foals. *J Am Vet Med Assoc*. 1982;181:684.
172. Vitums A, Bayly WM. Pulmonary atresia with dextroposition of the aorta and ventricular septal defect in three Arabian foals. *Vet Pathol*. 1982;19:160.

173. McClure JJ, Gaber CE, Watters JW, et al. Complete transposition of the great arteries with ventricular septal defect and pulmonary stenosis in a Thoroughbred foal. *Equine Vet J*. 1983;15:377.
174. Tadmor A, Fischel R, Tov AS. A condition resembling hypoplastic left heart syndrome in a foal. *Equine Vet J*. 1983;15:175.
175. Musselman EE, LoGuidice RJ. Hypoplastic left ventricular syndrome in a foal. *J Am Vet Med Assoc*. 1984;185:542.
176. Crowe MW, Swerczek TW. Equine congenital defects. *Am J Vet Res*. 1985;46:353.
177. Physick-Sheard PW, Maxie MG, Palmer NC, et al. Atrial septal defect of the persistent ostium primum type with hypoplastic right ventricle in a Welsh pony foal. *Can J Comp Med*. 1985;49:429.
178. Reef VB. Cardiovascular disease in the equine neonate. *Vet Clin North Am Equine Pract*. 1985;1:117.
179. Clark ES, Reef VB, Sweeney CR, et al. Aortic valve insufficiency in a one-year-old colt. *J Am Vet Med Assoc*. 1987;191:841.
180. Reef VB, Mann P. Echocardiographic diagnosis of tricuspid atresia in two foals. *J Am Vet Med Assoc*. 1987;191:225.
181. Sojka JE. Persistent truncus arteriosus in a foal. *Equine Pract*. 1987;9:19.
182. Zamora CS, Vitums A, Nyrop KA, et al. Atresia of the right atrioventricular orifice with complete transposition of the great arteries in a horse. *Anat Histol Embryol*. 1989;18:177.
183. Cargile J, Lombard C, Wilson JH, et al. Tetralogy of Fallot and segmental uterine aplasia in a three-year-old Morgan filly. *Cornell Vet*. 1991;81:411.
184. Hinchcliff KW, Adams WM. Critical pulmonary stenosis in a newborn foal. *Equine Vet J*. 1991;23:318.
185. deGroot J, Sloet van Oldruitenborgh-Oosterbaan MM, van der Linde Sipman JS, et al. Heart diseases in foals. A literature review exemplified by 2 case reports. *Tijdschr Diergeneeskd*. 1996;121:382.
186. Meurs KM, Miller MW, Hanson C, et al. Tricuspid valve atresia with main pulmonary artery atresia in an Arabian foal. *Equine Vet J*. 1997;29:160.
187. Schober KE, Kaufhold J, Kipar A. Mitral valve dysplasia in a foal. *Equine Vet J*. 2000;32:170.
188. Gehlen H, Bubeck K, Stadler P. Valvular pulmonic stenosis with normal aortic root and intact ventricular and atrial septa in an Arabian horse. *Equine Vet Educ*. 2001;13:286.
189. Taylor SE, Else RW, Keen JA. Congenital aortic valve dysplasia in a Clydesdale foal. *Equine Vet Educ*. 2007;19:463.
190. Hall TL, Magdesian KG, Kittleson MD. Congenital cardiac defects in neonatal foals: 18 cases (1992-2007). *J Vet Intern Med*. 2010;24:206.
191. Brown CM. Acquired cardiovascular disease. *Vet Clin North Am Equine Pract*. 1985;1:371.
192. Sage AM. Cardiac disease in the geriatric horse. *Vet Clin North Am Equine Pract*. 2002;18:575.
193. Innes JR, Berger J, Francis J. Subacute bacterial endocarditis with pulmonary embolism in a horse associated with *Shigella equirulis*. *Br Vet J*. 1950;106:245.
194. Bonagura JD, Pipers FS. Echocardiographic features of aortic valve endocarditis in a dog, a cow, and a horse. *J Am Vet Med Assoc*. 1983;182:595.
195. Buergelt CD, Cooley AJ, Hines SA, et al. Endocarditis in six horses. *Vet Pathol*. 1985;22:333.
196. McCormick BS, Peet RL, Downes K. *Erysipelothrix rhusiopathiae* vegetative endocarditis in a horse. *Aust Vet J*. 1985;62:392.
197. Dedrick P, Reef VB, Sweeney RW, et al. Treatment of bacterial endocarditis in a horse. *J Am Vet Med Assoc*. 1988;193:339.
198. Hamir AN, Reef VB. Complications of a permanent transvenous pacing catheter in a horse. *J Comp Pathol*. 1989;101:317.
199. Collatos C, Clark ES, Reef VB, et al. Septicemia, atrial fibrillation, cardiomegaly, left atrial mass, and *Rhodococcus equi* septic osteoarthritis in a foal. *J Am Vet Med Assoc*. 1990;197:1039.
200. Nilsfors L, Lombard CW, Weckner D, et al. Diagnosis of pulmonary valve endocarditis in a horse. *Equine Vet J*. 1991;23:479.
201. Ewart S, Brown C, Derksen F, et al. *Serratia marcescens* endocarditis in a horse. *J Am Vet Med Assoc*. 1992;200:961.
202. Ball MA, Weldon AD. Vegetative endocarditis in an Appaloosa gelding. *Cornell Vet*. 1992;82:301.
203. Pace LW, Wirth NR, Foss RR, et al. Endocarditis and pulmonary aspergillosis in a horse. *J Vet Diagn Invest*. 1994;6:504.
204. Travers CW, van den Berg JS. *Pseudomonas* spp. associated vegetative endocarditis in two horses. *J S Afr Vet Assoc*. 1995;66:172.
205. Maxson AD, Reef VB. Bacterial endocarditis in horses: ten cases (1984-1995). *Equine Vet J*. 1997;29:394.
206. Church S, Harrigan KE, Irving AE, et al. Endocarditis caused by *Pasteurella caballi* in a horse. *Aust Vet J*. 1998;76:528.
207. Patteson MW, Blissitt KJ. Evaluation of cardiac murmurs in horses. 1. *Clinical Examination, In Pract*. 1996;18:367.
208. Buhl R, Ersboll AK, Eriksen L, et al. Use of color Doppler echocardiography to assess the development of valvular regurgitation in Standardbred trotters. *J Am Vet Med Assoc*. 2005;227:1630.
209. Buhl R, Ersboll AK. Effect of light exercise on valvular regurgitation in Standardbred trotters. *Equine Vet J Suppl*. 2006;178.
210. Young LE, Helwegen MM, Rogers K, et al. Associations between exercise-induced pulmonary haemorrhage, right ventricular dimensions and atrioventricular valve regurgitation in conditioned national hunt racehorses. *Equine Vet J Suppl*. 2006;193.
211. Buhl R, Ersboll AK. Echocardiographic evaluation of changes in left ventricular size and valvular regurgitation associated with physical training during and after maturity in Standardbred trotters. *J Am Vet Med Assoc*. 2012;240:205.
212. Brown CM, Bell TG, Paradis MR, et al. Rupture of mitral chordae tendineae in two horses. *J Am Vet Med Assoc*. 1983;182:281.
213. Reef VB. Mitral valvular insufficiency associated with ruptured chordae tendineae in three foals. *J Am Vet Med Assoc*. 1987;191:329.
214. Marr CM, Love S, Pirie HM, et al. Confirmation by Doppler echocardiography of valvular regurgitation in a horse with a ruptured chorda tendinea of the mitral valve. *Vet Rec*. 1990;127:376.
215. Kiryu K, Kaneko M, Kanemaru T, et al. Cardiopathology of sinoatrial block in horses. *Nihon Juigaku Zasshi*. 1985;47:45.
216. Matsui K, Sugano S, Amada A. Heart rate and ECG response to twitching in Thoroughbred foals and mares. *Nihon Juigaku Zasshi*. 1986;48:305.
217. Matsui K, Sugano S. Relation of intrinsic heart-rate and autonomic nervous tone to resting heart-rate in the young and the adult of various domestic-animals. *Jpn J Vet Sci*. 1989;51:29.
218. Moore EN, Spear JF. Electrophysiological studies on atrial fibrillation. *Heart Vessels Suppl*. 1987;2:32.
219. Meijler FL, Kroneman J, van der Tweel I, et al. Nonrandom ventricular rhythm in horses with atrial fibrillation and its significance for patients. *J Am Coll Cardiol*. 1984;4:316.
220. Meijler FL. Atrioventricular conduction versus heart size from mouse to whale. *J Am Coll Cardiol*. 1985;5:363.
221. Meijler FL, van der Tweel I. Comparative study of atrial fibrillation and AV conduction in mammals. *Heart Vessels Suppl*. 1987;2:24.
222. Raekallio M. Long term ECG recording with Holter monitoring in clinically healthy horses. *Acta Vet Scand*. 1992;33:71.
223. Mill J, Hanak J. Diagnosis of heart valve defects, arrhythmia and functional disorders of cardiac conduction in competition horses and racehorses. *Arch Exp Veterinarmed*. 1985;39:319.
224. Tschudi P. Electrocardiography in the horse. (2). Disorders of impulse formation and impulse conduction. *Tierarztl Prax*. 1985;13:529.

225. Reef VB. Heart murmurs irregularities and other cardiac abnormalities. In: Brown C, ed. *Problems in Equine Medicine*. New York: Lea & Febiger; 1992.
226. Bonagura JD, Miller MS. Common conduction disturbances. [ECG in the horse]. *J Equine Vet Sci*. 1986;6:23.
227. Reef VB. Twenty-four hour rhythm monitoring. In: Mayhew I, ed. *Equine Medicine and Surgery IV*. Santa Barbara: American Veterinary Publications; 1991.
228. Slinker BK, Campbell KB, Alexander JE, et al. Arterial baroreflex control of heart rate in the horse, pig, and calf. *Am J Vet Res*. 1926;43:1982.
229. Meijler FL. Atrial fibrillation—a new look at an old arrhythmia. *J Am Coll Cardiol*. 1983;2:391.
230. Horn J, Bailey S, Berhane Y, et al. Density and binding characteristics of beta-adrenoceptors in the normal and failing equine myocardium. *Equine Vet J*. 2002;34:411.
231. Badino P, Odore R, Re G. Are so many adrenergic receptor subtypes really present in domestic animal tissues? A pharmacological perspective. *Vet J*. 2005;170:163.
232. Evans DL, Rose RJ. Determination and repeatability of maximum oxygen uptake and other cardiorespiratory measurements in the exercising horse. *Equine Vet J*. 1988;20:94.
233. Evans DL, Rose RJ. Cardiovascular and respiratory responses in Thoroughbred horses during treadmill exercise. *J Exp Biol*. 1988;134:397.
234. Landgren GL, Gillespie JR, Fedde MR, et al. O_2 transport in the horse during rest and exercise. *Adv Exp Med Biol*. 1988;227:333.
235. Littlejohn A, Snow DH. Circulatory, respiratory and metabolic responses in Thoroughbred horses during the first 400 meters of exercise. *Eur J Appl Physiol Occup Physiol*. 1988;58:307.
236. Marsland WP. Heart rate response to submaximal exercise in the Standardbred horse. *J Appl Physiol*. 1968;24:98.
237. Donaldson LL. Retrospective assessment of dobutamine therapy for hypotension in anesthetized horses. *Vet Surg*. 1988;17:53.
238. Swanson CR, Muir 3rd WW, Bednarski RM, et al. Hemodynamic responses in halothane-anesthetized horses given infusions of dopamine or dobutamine. *Am J Vet Res*. 1985;46:365.
239. Trim CM, Moore JN, White NA. Cardiopulmonary effects of dopamine hydrochloride in anaesthetised horses. *Equine Vet J*. 1985;17:41.
240. Whitton DL, Trim CM. Use of dopamine hydrochloride during general anesthesia in the treatment of advanced atrioventricular heart block in four foals. *J Am Vet Med Assoc*. 1985;187:1357.
241. Young LE, Blissitt KJ, Clutton RE, et al. Temporal effects of an infusion of dobutamine hydrochloride in horses anesthetized with halothane. *Am J Vet Res*. 1998;59:1027.
242. Weil AB, Keegan RD, Greene SA. Effect of low-dose atropine administration on dobutamine dose requirement in horses anesthetized with detomidine and halothane. *Am J Vet Res*. 1997;58:1436.
243. Sandersen CF, Detilleux J, Delguste C, et al. Atropine reduces dobutamine-induced side effects in ponies undergoing a pharmacological stress protocol. *Equine Vet J*. 2005;37:128.
244. Sandersen CF, Detilleux J, de Moffarts B, et al. Effect of atropine-dobutamine stress test on left ventricular echocardiographic parameters in untrained warmblood horses. *J Vet Intern Med*. 2006;20:575.
245. Light GS, Hellyer PW. Effects of atropine on the arrhythmogenic dose of dobutamine in xylazine-thiamylal-halothane-anesthetized horses. *Am J Vet Res*. 1993;54:2099.
246. Hinchcliff KW, McKeever KH, Muir 3rd WW. Hemodynamic effects of atropine, dobutamine, nitroprusside, phenylephrine, and propranolol in conscious horses. *J Vet Intern Med*. 1991;5:80.
247. Frye MA, Bright JM, Dargatz DA, et al. A comparison of dobutamine infusion to exercise as a cardiac stress test in healthy horses. *J Vet Intern Med*. 2003;17:58.
248. Hardy J, Bednarski RM, Biller DS. Effect of phenylephrine on hemodynamics and splenic dimensions in horses. *Am J Vet Res*. 1994;55:1570.
249. Hollis AR, Ousey JC, Palmer L, et al. Effects of norepinephrine and a combined norepinephrine and dobutamine infusion on systemic hemodynamics and indices of renal function in normotensive neonatal thoroughbred foals. *J Vet Intern Med*. 2006;20:1437.
250. Valverde A, Giguere S, Sanchez LC, et al. Effects of dobutamine, norepinephrine, and vasopressin on cardiovascular function in anesthetized neonatal foals with induced hypotension. *Am J Vet Res*. 2006;67:1730.
251. Craig CA, Haskins SC, Hildebrand SV. The cardiopulmonary effects of dobutamine and norepinephrine in isoflurane-anesthetized foals. *Vet Anaesth Analg*. 2007;34:377.
252. Hollis AR, Ousey JC, Palmer L, et al. Effects of norepinephrine and combined norepinephrine and fenoldopam infusion on systemic hemodynamics and indices of renal function in normotensive neonatal foals. *J Vet Intern Med*. 2008;22:1210.
253. Ohta M, Kurimoto S, Ishikawa Y, et al. Cardiovascular effects of dobutamine and phenylephrine infusion in sevoflurane-anesthetized Thoroughbred horses. *J Vet Med Sci*. 2013;75:1443.
254. Fantoni DT, Marchioni GG, Ida KK, et al. Effect of ephedrine and phenylephrine on cardiopulmonary parameters in horses undergoing elective surgery. *Vet Anaesth Analg*. 2013;40:367.
255. Benamou AE, Marlin DJ, Lekeux P. Equine pulmonary and systemic haemodynamic responses to endothelin-1 and a selective ET(A) receptor antagonist. *Equine Vet J*. 2001;33:337.
256. Benamou AE, Marlin DJ, Lekeux P. Endothelin in the equine hypoxic pulmonary vasoconstrictive response to acute hypoxia. *Equine Vet J*. 2001;33:345.
257. Benamou AE, Marlin DJ, Callingham BC, et al. Spasmogenic action of endothelin-1 on isolated equine pulmonary artery and bronchus. *Equine Vet J*. 2003;35:190.
258. Gasthuys F, De Moor A, Parmentier D. Cardiovascular effects of low dose calcium chloride infusions during halothane anaesthesia in dorsally recumbent ventilated ponies. *Zentralbl Veterinarmed A*. 1991;38:728.
259. Schwarzwald CC, Bonagura JD, Luis-Fuentes V. Effects of diltiazem on hemodynamic variables and ventricular function in healthy horses. *J Vet Intern Med*. 2005;19:703.
260. Rooney JR. Internal hemorrhage related to gestation in the mare. *Cornell Vet*. 1964;54:11.
261. van der Linde-Sipman JS, Kroneman J, Meulenaar H, et al. Necrosis and rupture of the aorta and pulmonary trunk in four horses. *Vet Pathol*. 1985;22:51.
262. Roby KA, Reef VB, Shaw DP, et al. Rupture of an aortic sinus aneurysm in a 15-year-old broodmare. *J Am Vet Med Assoc*. 1986;189:305.
263. Marr CM, Reef VB, Brazil TJ, et al. Aorto-cardiac fistulas in seven horses. *Vet Radiol Ultrasound*. 1998;39:22.
264. Sleeper MM, Durando MM, Miller M, et al. Aortic root disease in four horses. *J Am Vet Med Assoc*. 2001;219:491.
265. Ploeg M, Saey V, de Bruijn CM, et al. Aortic rupture and aorto-pulmonary fistulation in the Friesian horse: characterisation of the clinical and gross post mortem findings in 24 cases. *Equine Vet J*. 2013;45:101.
266. Ploeg M, Saey V, Delesalle C, et al. Thoracic aortic rupture and aortopulmonary fistulation in the Friesian horse: histomorphologic characterization. *Vet Pathol*. 2015;52:152.
267. Maxie MG, Physick-Sheard PW. Aortic-iliac thrombosis in horses. *Vet Pathol*. 1985;22:238.
268. Reef VB, Roby KAW, Richardson DW, et al. Use of ultrasonography for the detection of aortic-iliac thrombosis in horses. *J Am Vet Med Assoc*. 1987;190:286.
269. Azzie MAJ. Clinical diagnosis of equine aortic iliac thrombosis and its histopathology as compared with that of the strongyle aneurysm. *Proc Am Assoc Equine Pract*. 1972. San Francisco.

270. Edwards GB, Allen WE. Aorto-iliac thrombosis in two horses: clinical course of the disease and use of real-time ultrasonography to confirm diagnosis. *Equine Vet J*. 1988;384:1988.
271. Azzie MAJ. Aortic/iliac thrombosis of Thoroughbred horses. *Equine Vet J*. 1969;1:113.
272. Physick-Sheard PW, Maxie MG. Aortoiliofemoral arteriosclerosis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 1*. Phildelphia: W.B. Saunders; 1983.
273. Reef VB. Vasculitis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 3*. Philadelphia: WB Saunders; 1992.
274. Patteson MW, Gibbs C, Wotton PR, et al. Effects of sedation with detomidine hydrochloride on echocardiographic measurements of cardiac dimensions and indices of cardiac function in horses. *Equine Vet J Suppl*. 1995;33.
275. Raisis AL, Young LE, Blissitt KJ, et al. A comparison of the haemodynamic effects of isoflurane and halothane anaesthesia in horses. *Equine Vet J*. 2000;32:318.
276. Young LE, Blissitt KJ, Clutton RE, et al. Feasibility of transoesophageal echocardiography for evaluation of left ventricular performance in anaesthetised horses. *Equine Vet J Suppl*. 1995;63.
277. Young LE, Blissitt KJ, Clutton RE, et al. Temporal effects of an infusion of dopexamine hydrochloride in horses anesthetized with halothane. *Am J Vet Res*. 1997;58:516.
278. Young LE, Blissitt KJ, Clutton RE, et al. Haemodynamic effects of a sixty minute infusion of dopamine hydrochloride in horses anaesthetised with halothane. *Equine Vet J*. 1998;30:310.
279. Lightowler C, Piccione G, Fazio F, et al. Systolic time intervals assessed by 2-D echocardiography and spectral Doppler in the horse. *Animal Science Journal*. 2003;74:505.
280. Reef VB, Lalezari K, De Boo J, et al. Pulsed-wave Doppler evaluation of intracardiac blood flow in 30 clinically normal Standardbred horses. *Am J Vet Res*. 1989;50:75.
281. Blissitt KJ, Bonagura JD. Pulsed wave Doppler echocardiography in normal horses. *Equine Vet J Suppl*. 1995;38.
282. Welker FH, Muir WW. An investigation of the second heart sound in the normal horse. *Equine Vet J*. 1990;22:403.
283. Smith BL, Jones JH, Pascoe JR, et al. Why are left atrial pressures high in exercising horses? *Physiologist*. 1992;35.
284. Jones JH, Smith BL, Birks EK, et al. Left atrial and pulmonary arterial pressures in exercising horses. *FASEB J*. 1992;6:A2020.
285. Long KJ. Doppler echocardiography in the horse. *Equine Vet Educ*. 1990;2:15.
286. Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of tissue Doppler imaging for assessment of left ventricular radial wall motion in horses. *J Vet Intern Med*. 2009;23:643.
287. Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of echocardiographic assessment of left atrial size and mechanical function in horses. *Am J Vet Res*. 2007;68:735.
288. Pagel PS, Kehl F, Gare M, et al. Mechanical function of the left atrium: new insights based on analysis of pressure-volume relations and Doppler echocardiography. *Anesthesiology*. 2003;98:975.
289. Schwarzwald CC, Schober KE, Bonagura JD. Echocardiographic evidence of left atrial mechanical dysfunction after conversion of atrial fibrillation to sinus rhythm in 5 horses. *J Vet Intern Med*. 2007;21:820.
290. DeClercq D, VanLoon G, Tavernier R, et al. Atrial and ventricular electrical and contractile remodeling and reverse remodeling owing to short-term pacing-induced atrial fibrillation in horses. *J Vet Intern Med*. 2008;22:1353.
291. Decloedt A, Schwarzwald CC, De Clercq D, et al. Risk factors for recurrence of atrial fibrillation in horses after cardioversion to sinus rhythm. *J Vet Intern Med*. 2015;29:946.
292. Muir WW, Hubbell JAE. *Equine Anesthesia—Monitoring and Emergency Therapy*. 2nd ed. St. Louis: Saunders Elsevier; 2009.
293. Hinchcliff KW, Kaneps AJ, Geor RJ. *Equine Sports Medicine and Surgery*. 2nd ed. Edinburgh: Saunders Elsevier; 2014.
294. Kutter AP, Bettschart-Wolfensberger R, Schwarzwald CC, et al. Evaluation of the non-calibrated pulse contour cardiac output monitor FloTrac/Vigileo against thermodilution in standing horses. *Vet Anaesth Analg*. 2016;43:153.
295. Shih AC, Queiroz P, Vigani A, et al. Comparison of cardiac output determined by an ultrasound velocity dilution cardiac output method and by the lithium dilution cardiac output method in juvenile horses with experimentally induced hypovolemia. *Am J Vet Res*. 2014;75:565.
296. Shih A. Cardiac output monitoring in horses. *Vet Clin North Am Equine Pract*. 2013;29:155.
297. McConachie E, Barton MH, Rapoport G, et al. Doppler and volumetric echocardiographic methods for cardiac output measurement in standing adult horses. *J Vet Intern Med*. 2013;27:324.
298. Ambrisko TD, Coppens P, Kabes R, et al. Lithium dilution, pulse power analysis, and continuous thermodilution cardiac output measurements compared with bolus thermodilution in anaesthetized ponies. *Br J Anaesth*. 2012;109:864.
299. Shih AC, Giguere S, Sanchez LC, et al. Determination of cardiac output in anesthetized neonatal foals by use of two pulse wave analysis methods. *Am J Vet Res*. 2009;70:334.
300. Shih A, Giguere S, Sanchez LC, et al. Determination of cardiac output in neonatal foals by ultrasound velocity dilution and its comparison to the lithium dilution method. *J Vet Emerg Critical Care (San Antonio, Tex : 2001)*. 2009;19:438.
301. Lepiz ML, Keegan RD, Bayly WM, et al. Comparison of Fick and thermodilution cardiac output determinations in standing horses. *Res Vet Sci*. 2008;85:307.
302. Durando MM, Corley KTT, Boston RC, et al. Cardiac output determination by use of lithium dilution during exercise in horses. *Am J Vet Res*. 2008;69:1054.
303. Valverde A, Giguere S, Morey TE, et al. Comparison of noninvasive cardiac output measured by use of partial carbon dioxide rebreathing or the lithium dilution method in anesthetized foals. *Am J Vet Res*. 2007;68:141.
304. Wilkins PA, Boston RC, Gleed RD, et al. Comparison of thermal dilution and electrical impedance dilution methods for measurement of cardiac output in standing and exercising horses. *Am J Vet Res*. 2005;66:878.
305. Hallowell GD, Corley KTT. Use of lithium dilution and pulse contour analysis cardiac output determination in anaesthetized horses: a clinical evaluation. *Vet Anaesth Analg*. 2005;32:201.
306. Giguere S, Bucki E, Adin DB, et al. Cardiac output measurement by partial carbon dioxide rebreathing, 2-dimensional echocardiography, and lithium-dilution method in anesthetized neonatal foals. *J Vet Intern Med*. 2005;19:737.
307. Corley KT, Donaldson LL, Durando MM, et al. Cardiac output technologies with special reference to the horse. *J Vet Intern Med*. 2003;17:262.
308. Corley KT, Donaldson LL, Furr MO. Comparison of lithium dilution and thermodilution cardiac output measurements in anaesthetised neonatal foals. *Equine Vet J*. 2002;34:598.
309. Linton RA, Young LE, Marlin DJ, et al. Cardiac output measured by lithium dilution, thermodilution, and transesophageal Doppler echocardiography in anesthetized horses. *Am J Vet Res*. 2000;61:731.
310. Pascoe JR, Hiraga A, Hobo S, et al. Cardiac output measurements using sonomicrometer crystals on the left ventricle at rest and exercise. *Equine Vet J Suppl*. 1999;30:148.
311. Blissitt KJ, Young LE, Jones RS, et al. Measurement of cardiac output in standing horses by Doppler echocardiography and thermodilution. *Equine Vet J*. 1997;29:18.
312. Young LE, Blissitt KJ, Bartram DH, et al. Measurement of cardiac output by transoesophageal Doppler echocardiography in anaesthetized horses: comparison with thermodilution. *Br J Anaesth*. 1996;77:773.

313. Mizuno Y, Aida H, Hara H, et al. Comparison of methods of cardiac output measurements determined by dye dilution, pulsed Doppler echocardiography and thermodilution in horses. *J Vet Med Sci*. 1994;56:1.
314. Dunlop CI, Hodgson DS, Chapman PL, et al. Thermodilution estimation of cardiac output at high flows in anesthetized horses. *Am J Vet Res*. 1991;52:1893.
315. Wetmore LA, Derksen FJ, Blaze CA, et al. Mixed venous oxygen tension as an estimate of cardiac output in anesthetized horses. *Am J Vet Res*. 1987;48:971.
316. Ward DS, Fessler JF, Bottoms GD. In vitro calibration and surgical implantation of electromagnetic blood flow transducers for measurement of left coronary blood flow and cardiac output in the pony. *Am J Vet Res*. 1987;48:1120.
317. Muir WW, Skarda RT, Milne DW. Estimation of cardiac output in the horse by thermodilution techniques. *Am J Vet Res*. 1976;37:697.
318. Hillidge CJ, Lees P. Cardiac output in the conscious and anaesthetised horse. *Equine Vet J*. 1975;7:16.
319. Brumbaugh GW, Thomas WP, Enos LR, et al. A pharmacokinetic study of digoxin in the horse. *J Vet Pharmacol Ther*. 1983;6:163.
320. Button C, Gross DR, Johnston JT, et al. Digoxin pharmacokinetics, bioavailability, efficacy, and dosage regimens in the horse. *Am J Vet Res*. 1980;41:1388.
321. Dyson DH, Pascoe PJ. Influence of preinduction methoxamine, lactated Ringer solution, or hypertonic saline solution infusion or postinduction dobutamine infusion on anesthetic-induced hypotension in horses. *Am J Vet Res*. 1990;51:17.
322. Francfort P, Schatzmann HJ. Pharmacological experiments as a basis for the administration of digoxin in the horse. *Res Vet Sci*. 1976;20:84.
323. Gasthuys F, De Moor A, Parmentier D. Influence of digoxin followed by dopamine on the cardiovascular depression during a standard halothane anaesthesia in dorsally recumbent, ventilated ponies. *Zentralbl Veterinarmed A*. 1991;38:585.
324. Grubb TL, Foreman JH, Benson GJ, et al. Hemodynamic effects of calcium gluconate administered to conscious horses. *J Vet Intern Med*. 1996;10:401.
325. Keen P. The use of drugs in the treatment of cardiac disease in the horse. *Equine Vet Educ*. 1990;2:81.
326. Meijler FL, van der Tweel I. Digitalis and atrial fibrillation in 1985. *Ned Tijdschr Geneeskd*. 1985;129:729.
327. Muir WW, Mcguirk SM. Pharmacology and pharmacokinetics of drugs used to treat cardiac disease in horses. *Vet Clin North Am Equine Pract*. 1985;1:335.
328. Muir WW, Mcguirk S. Cardiovascular drugs—their pharmacology and use in horses. *Vet Clin North Am Equine Pract*. 1987;3:37.
329. Muir 3rd WW. Cardiovascular effects of dopexamine HCl in conscious and halothane-anaesthetised horses. *Equine Vet J Suppl*. 1992;24:24.
330. Pedersoli WM, Belmonte AA, Purohit RC, et al. Pharmacokinetics of digoxin in the horse. *J Equine Med Surg*. 1978;2:384.
331. Pedersoli WM, Ravis WR, Belmonte AA, et al. Pharmacokinetics of a single, orally administered dose of digoxin in horses. *Am J Vet Res*. 1981;42:1412.
332. Staudacher G. Individual glycoside treatment by means of serum concentration determination in cardiac insufficiency in horses. *Berl Munch Tierarztl Wochenschr*. 1989;102:1.
333. Swanson CR, Muir III WW. Dobutamine-induced augmentation of cardiac output does not enhance respiratory gas exchange in anesthetized recumbent healthy horses. *Am J Vet Res*. 1986;47:1573.
334. Sweeney RW, Reef VB, Reimer JM. Pharmacokinetics of digoxin administered to horses with congestive heart failure. *Am J Vet Res*. 1993;54:1108.
335. Trim CM. Inotropic agents and vasopressors in equine anesthesia. *Comp Cont Educ Pract*. 1991;13:118.
336. Muir WW. The haemodynamic effects of milrinone HCl in halothane anaesthetised horses. *Equine Vet J Suppl*. 1995;108.
337. de Vries A, Brearley JC, Taylor PM. Effects of dobutamine on cardiac index and arterial blood pressure in isoflurane-anaesthetized horses under clinical conditions. *J Vet Pharmacol Ther*. 2009;32:353.
338. Vitale V, Sgorbini M, Briganti A, et al. Evaluation of echocardiographic parameters during increasing infusion rates of dobutamine in isoflurane-anesthetized horses. *J Equine Vet Sci*. 2013;33:1110.
339. Afonso T, Giguère S, Rapoport G, et al. Cardiovascular effects of pimobendan in healthy mature horses. *Equine Vet J*. 2016;48:352.
340. Long-Blissitt KJ, Bonagura JD, Darke PGG. Standardised imaging technique for guided M-mode and Doppler echocardiography in the horse. *Equine Vet J*. 1992;24:226.
341. Patteson MW, Gibbs C, Wotton PR, et al. Echocardiographic measurements of cardiac dimensions and indices of cardiac function in normal adult thoroughbred horses. *Equine Vet J Suppl*. 1995;18.
342. Slater JD, Herrtage ME. Echocardiographic measurements of cardiac dimensions in normal ponies and horses. *Equine Vet J Suppl*. 1995;28.
343. Bakos Z, Voros K, Jarvinen T, et al. Two-dimensional and M-mode echocardiographic measurements of cardiac dimensions in healthy standardbred trotters. *Acta Vet Hung*. 2002;50:273.
344. Boon JA. *Veterinary Echocardiography*. 2nd ed. Oxford: Wiley-Blackwell; 2011.
345. Grenacher PA, Schwarzwald CC. Assessment of left ventricular size and function in horses using anatomical M-mode echocardiography. *J Vet Cardiol*. 2010;12:111.
346. Schwarzwald CC, Schober KE, Berli AS, et al. Left ventricular radial and circumferential wall motion analysis in horses using strain, strain rate, and displacement by 2D speckle tracking. *J Vet Intern Med*. 2009;23:890.
347. Decloedt A, Verheyen T, Sys S, et al. Quantification of left ventricular longitudinal strain, strain rate, velocity, and displacement in healthy horses by 2-dimensional speckle tracking. *J Vet Intern Med*. 2011;25:330.
348. Decloedt A, Verheyen T, Sys S, et al. Two-dimensional speckle tracking for quantification of left ventricular circumferential and radial wall motion in horses. *Equine Vet J*. 2013;45:47.
349. Decloedt A, Verheyen T, Sys S, et al. Evaluation of tissue Doppler imaging for regional quantification of radial left ventricular wall motion in healthy horses. *Am J Vet Res*. 2013;74:53.
350. Kriz NG, Rose RJ. Repeatability of standard transthoracic echocardiographic measurements in horses. *Aust Vet J*. 2002;80:362.
351. Gehlen H, Marnette S, Rohn K, et al. Precision-controlled echocardiographic left ventricular function parameters by repeated measurement on three consecutive days in trained and untrained warmblood horses. *Dtsch Tierarztl Wochenschr*. 2005;112:48.
352. Nuytten J, Deprez P, Picavet T, et al. Heart-failure in horses—hemodynamic monitoring and determination of Ldh1 concentration. *J Equine Vet Sci*. 1988;8:214.
353. Fruhauf B, Stadler P, Deegen E. Evaluation of pulmonary wedge pressure in horses with and without left heart abnormalities detected by echocardiography. *Pferdeheilkunde*. 1996;12:544.
354. Gehlen H, Bubeck K, Stadler P. Pulmonary artery wedge pressure measurement in healthy warmblood horses and in warmblood horses with mitral valve insufficiencies of various degrees during standardised treadmill exercise. *Res Vet Sci*. 2004;77:257.
355. Trachsel DS, Schwarzwald CC, Bitschnau C, et al. Atrial natriuretic peptide and cardiac troponin I concentrations in healthy Warmblood horses and in Warmblood horses with mitral regurgitation at rest and after exercise. *J Vet Cardiol*. 2013;15:105.

356. Milne DW, Muir III WW, Skarda RT. Pulmonary artery wedge pressure blood gas tensions and pH in the resting horse. *Am J Vet Res*. 1975;36:1431.
357. Gehlen H, Bubeck K, Rohn K, et al. Pulmonary artery wedge pressure during treadmill exercise in warmblood horses with atrial fibrillation. *Res Vet Sci*. 2006;81:134.
358. Gehlen H, Groner U, Rohn K, et al. Day-to day variability of cardiac pressure values in horses measured with right heart catheterization on 3 consecutive days. *Berl Munch Tierarztl Wochenschr*. 2006;119:400.
359. Bertone JJ. Cardiovascular effects of hydralazine HCl administration in horses. *Am J Vet Res*. 1988;49:618.
360. Muir 3rd WW, Sams RA, Hubbell JA, et al. Effects of enalaprilat on cardiorespiratory, hemodynamic, and hematologic variables in exercising horses. *Am J Vet Res*. 2001;62:1008.
361. Guglielmini C, Giuliani A, Testoni S, et al. Use of an ACE inhibitor (ramipril) in a horse with congestive heart failure. *Equine Vet Educ*. 2002;14:297.
362. Gehlen H, Vieht JC, Stadler P. Effects of the ACE inhibitor quinapril on echocardiographic variables in horses with mitral valve insufficiency. *J Vet Med A Physiol Pathol Clin Med*. 2003;50:460.
363. Afonso T, Giguere S, Rapoport G, et al. Pharmacodynamic evaluation of 4 angiotensin-converting enzyme inhibitors in healthy adult horses. *J Vet Intern Med*. 2013;27:1185.
364. Rugh KS, Garner HE, Hatfield DG, et al. Ischaemia induced development of functional coronary collateral circulation in ponies. *Cardiovasc Res*. 1987;21:730.
365. Stadler P, Weinberger T, Deegen E. Pulsed Doppler-echocardiography in healthy Warm-blooded horses. *J Vet Med A*. 1993;40:757.
366. Koenig TR, Mitchell KM, Schwarzwald CC. Echocardiographic assessment of left ventricular function in healthy horses and in horses with heart disease using pulsed-wave tissue Doppler imaging (PW TDI). *J Vet Intern Med*. 2017 Mar-Apr; 31(2): 556–567.
367. Parks CM, Manohar M. Distribution of blood flow during moderate and strenuous exercise in ponies (*Equus caballus*). *Am J Vet Res*. 1861;44:1983.
368. Parks C, Manohar M, Lundeen G. Regional myocardial blood flow and coronary vascular reserve in unanesthetized ponies during pacing-induced ventricular tachycardia. *J Surg Res*. 1983;35:119.
369. Parks CM, Manohar M. Transmural coronary vasodilator reserve and flow distribution during severe exercise in ponies. *J Appl Physiol*. 1983;54:1641.
370. Manohar M. Transmural coronary vasodilator reserve and flow distribution during maximal exercise in normal and splenectomized ponies. *J Physiol*. 1987;387:425.
371. Parks CM, Manohar M. Transmural distribution of myocardial blood flow during graded treatmill exercise in ponies. In: Persson SGB, Rose RJ, eds. *Equine Exercise Physiology*. Cambridge, England: Granta; 1983.
372. Parks CM, Manohar M. Regional blood flow changes in response to near maximal exercise in ponies: a review. *Equine Vet J*. 1985;17:311.
373. Reddy VK, Kammula RG, Graham TC, et al. Regional coronary blood flow in ponies. *Am J Vet Res*. 1976;37:1261.
374. Davis JL, Gardner SY, Schwabenton B, et al. Congestive heart failure in horses: 14 cases (1984-2001). *J Am Vet Med Assoc*. 2002;220:1512.
375. Fraipont A, Van Erck E, Ramery E, et al. Subclinical diseases underlying poor performance in endurance horses: diagnostic methods and predictive tests. *Vet Rec*. 2011;169:154.
376. Kobluk CN, Gross GM. Exercise intolerance and poor performance in western performance and sprint horses. *Vet Clin North Am Equine Pract*. 1996;12:581.
377. Martin BB, Davidson EJ, Durando MM, et al. Clinical exercise testing: overview of causes of poor performance. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. Edinburgh: Saunders Elsevier; 2004.
378. Fregin GF. Medical evaluation of the cardiovascular system. *Vet Clin North Am Equine Pract*. 1992;8:329.
379. Gerring EL. Auscultation of the equine heart. *Equine Vet Educ*. 1990;2:22.
380. Littlejohn A, Button C. When is a murmur not a murmur. *J S Afr Vet Assoc*. 1982;53:130.
381. Littlewort MCG. The clinical auscultation of the equine heart. *Vet Rec*. 1962;74:1247.
382. Machida N, Yasuda J, Too K. Auscultatory and phonocardiographic studies on the cardiovascular system of the newborn thoroughbred foal. *Jpn J Vet Res*. 1987;35:235.
383. McGuirk SM, Muir WW. Diagnosis and treatment of cardiac arrhythmias. *Vet Clin North Am Equine Pract*. 1985;1:353.
384. Reef VB. Evaluation of the equine cardiovascular system. *Vet Clin North Am Equine Pract*. 1985;1:275.
385. Reimer J. Performing cardiac auscultation on horses. *Vet Med*. 1993;88:660.
386. Rossdale PD. Clinical studies on the newborn thoroughbred foal. II. Heart rate, auscultation and electrocardiogram. *Br Vet J*. 1967;123:521.
387. Vanselow B, McCarthy M, Gay CC. A phonocardiographic study of equine heart sounds. *Aust Vet J*. 1978;54:161.
388. Blissitt KJ. Auscultation. In: Marr CM, Bowen M, eds. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
389. Naylor JM, Wolker RE, Pharr JW. An assessment of the terminology used by diplomates and students to describe the character of equine mitral and aortic valve regurgitant murmurs: correlations with the physical properties of the sounds. *J Vet Intern Med*. 2003;17:332.
390. Reef VB. Heart murmurs in horses: determining their significance with echocardiography. *Equine Vet J Suppl*. 1995;71.
391. Blissitt KJ, Bonagura JD. Colour flow Doppler echocardiography in horses with cardiac murmurs. *Equine Vet J Suppl*. 1995;82.
392. Naylor JM, Yadernuk LM, Pharr JW, et al. An assessment of the ability of diplomates, practitioners, and students to describe and interpret recordings of heart murmurs and arrhythmia. *J Vet Intern Med*. 2001;15:507.
393. Abbott J. Auscultation: what type of practice makes perfect? *J Vet Intern Med*. 2001;15:505.
394. Kammerer H. Auscultation of the horse's heart, using a new stethoscope. *Dtsch Tierarztl Wochenschr*. 1983;90:521.
395. Bakos Z, Voros K. Comparative examination of percussional and echocardiographic determination of the cardiac dullness area in healthy horses. *Acta Vet Hung*. 2007;55:277.
396. Vörös K, Bonnevie A, Reiczigel J. Comparison of conventional and sensor-based electronic stethoscopes in detecting cardiac murmurs of dogs. *Tierärztliche Praxis Kleintiere*. 2012;40:103.
397. Blass KA, Schober KE, Bonagura JD, et al. Clinical evaluation of the 3M Littmann Electronic Stethoscope Model 3200 in 150 cats. *J Feline Med Surg*. 2013;15:893.
398. Hintz HF, Collyer C, Brant T. Resting heart rates in draft horses. *Equine Pract*. 1989;11:7.
399. Schwarzwald CC, Kedo M, Birkmann K, et al. Relationship of heart rate and electrocardiographic time intervals to body mass in horses and ponies. *J Vet Cardiol*. 2012;14:343.
400. Reef VB. Frequency of cardiac arrhythmias and their significance in normal horses. *ACVIM forum*. 1989. San Diego.
401. Bertone JJ. Atrial fibrillation in the horse: diagnosis, prognosis, treatment. *Equine Pract*. 1984;6:6.
402. Bertone JJ, Wingfield WE. Atrial fibrillation in horses. *Comp Cont Educ Pract*. 1987;9:763.
403. Gelzer ARM, Moise NS, Vaidya D, et al. Temporal organization of atrial activity and irregular ventricular rhythm during spontaneous atrial fibrillation: an in vivo study in the horse. *J Cardiovasc Electrophysiol*. 2000;11:773.
404. Li JK. Laminar and turbulent flow in the mammalian aorta: reynolds number. *J Theor Biol*. 1988;135:409.

405. Marr CM. Cardiac murmurs: congenital heart disease. In: Marr CM, ed. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
406. Marr CM. Cardiac Murmurs: Valvular regurgitation and insufficiency. In: Marr CM, ed. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
407. Stevens KB, Marr CM, Horn JNR, et al. Effect of left-sided valvular regurgitation on mortality and causes of death among a population of middle-aged and older horses. *Vet Rec*. 2009;164:6.
408. Machida N, Yasuda J, Too K, et al. A morphological study on the obliteration processes of the ductus arteriosus in the horse. *Equine Vet J*. 1988;20:249.
409. Thomas WP, Madigan JE, Backus KQ, et al. Systemic and pulmonary haemodynamics in normal neonatal foals. *J Reprod Fertil Suppl*. 1987;35:623.
410. Andersson B, Augustinsson O, Bademo E, et al. Systemic and centrally mediated angiotensin II effects in the horse. *Acta Physiol Scand*. 1987;129:143.
411. Bailey JE, Dunlop CI, Chapman PL, et al. Indirect Doppler ultrasonic measurement of arterial blood-pressure results in a large measurement error in dorsally recumbent anesthetized horses. *Equine Vet J*. 1994;26:70.
412. Branson KR. A clinical evaluation of an oscillometric blood pressure monitor on anesthetized horses. *J Equine Vet Sci*. 1997;17:537.
413. Ellis PM. The indirect measurement of arterial blood pressure in the horse. *Equine Vet J*. 1975;7:22.
414. Erickson BK, Erickson HH, Coffman JR. Pulmonary artery, aortic and oesophageal pressure changes during high intensity treadmill exercise in the horse: a possible relation to exercise-induced pulmonary haemorrhage. *Equine Vet J Suppl Supplement*. 1990;9:47.
415. Franco RM, Ousey JC, Cash RSG, et al. Study of arterial blood-pressure in newborn foals using an electronic sphygmomanometer. *Equine Vet J*. 1986;18:475.
416. Fritsch R, Bosler K. Monitoring circulation in the horse during sedation and anesthesia by indirect blood pressure measurement. *Berl Munch Tierarztl Wochenschr*. 1985;98:166.
417. Fritsch R, Hausmann R. Indirect blood pressure determination in the horse with the Dinamap 1255 research monitor. *Tierarztl Prax*. 1988;16:373.
418. Gasthuys F, De Moor A, Parmentier D. Haemodynamic changes during sedation in ponies. *Vet Res Commun*. 1990;14:309.
419. Will JA, Bisgard GE. Cardiac catheterization of unanesthetized large domestic animals. *J Appl Physiol*. 1972;33:400.
420. Gay CC, McCarthy M, Reynolds WT, et al. A method for indirect measurement of arterial blood pressure in the horse. *Aust Vet J*. 1977;53:163.
421. Geddes LA, Chaffee V, Whistler SJ, et al. Indirect mean blood pressure in the anesthetized pony. *Am J Vet Res*. 1977;38:2055.
422. Glen JB. Indirect blood pressure measurement in anesthetised animals. *Vet Rec*. 1970;87:349.
423. Johnson JH, Garner HE, Hutcheson DP. Ultrasonic measurment of arterial blood pressure in conditioned Thoroughbreds. *Equine Vet J*. 1976;8:55.
424. Kvart C. An ultrasonic method for indirect blood pressure measurement in the horse. *J Equine Med Surg*. 1979;3:16.
425. Latshaw H, Fessler JF, Whistler SJ, et al. Indirect measurement of mean blood pressure in the normotensive and hypotensive horses. *Equine Vet J*. 1979;11:191.
426. Lombard CW, Evans M, Martin L, et al. Blood pressure, electrocardiogram and echocardiogram measurements in the growing pony foal. *Equine Vet J*. 1984;16:342.
427. Parry BW, McCarthy MA, Anderson GA, et al. Correct occlusive bladder width for indirect blood pressure measurement in horses. *Am J Vet Res*. 1982;43:50.
428. Parry BW, Gay CC, McCarthy MA. Influence of head height on arterial blood pressure in standing horses. *Am J Vet Res*. 1980;41:1626.
429. Parry BW. Resting blood pressure values in various equine clinical cases. *J Equine Vet Sci*. 1984;4:49.
430. Parry BW, Anderson GA. Importance of uniform cuff application for equine blood pressure measurement. *Equine Vet J*. 1984;16:529.
431. Parry BW, McCarthy MA, Anderson GA. Survey of resting blood pressure values in clinically normal horses. *Equine Vet J*. 1984;16:53.
432. Taylor PM. Techniques and clinical application of arterial blood pressure measurement in the horse. *Equine Vet J*. 1981;13:271.
433. Wagner AE, Brodbelt DC. Arterial blood pressure monitoring in anesthetized animals. *J Am Vet Med Assoc*. 1997;210:1279.
434. Nout YS, Corley KTT, Donaldson LL, et al. Indirect oscillometric and direct blood pressure measurements in anesthetized and concious neonatal foals. *J Vet Emerg Crit Care*. 2002;12:75.
435. Giguere S, Knowles Jr HA, Valverde A, et al. Accuracy of indirect measurement of blood pressure in neonatal foals. *J Vet Intern Med*. 2005;19:571.
436. Heliczer N, Lorello O, Casoni D, et al. Accuracy and precision of noninvasive blood pressure in normo-, hyper-, and hypotensive standing and anesthetized adult horses. *J Vet Intern Med*. 2016;30:866.
437. Hatz LA, Hartnack S, Kummerle J, et al. A study of measurement of noninvasive blood pressure with the oscillometric device, Sentinel, in isoflurane-anaesthetized horses. *Vet Anaesth Analg*. 2015;42:369.
438. Tearney CC, Guedes AG, Brosnan RJ. Equivalence between invasive and oscillometric blood pressures at different anatomic locations in healthy normotensive anaesthetised horses. *Equine Vet J*. 2015.
439. Olsen E, Pedersen TLS, Robinson R, et al. Accuracy and precision of oscillometric blood pressure in standing conscious horses. *J Vet Emerg Crit Care*. 2016;26:85.
440. Tünsmeyer J, Hopster K, Feige K, et al. Agreement of high definition oscillometry with direct arterial blood pressure measurement at different blood pressure ranges in horses under general anaesthesia. *Vet Anaesth Analg*. 2015;42:286.
441. Geddes LA, Voelz M, Combs C, et al. Characterization of the oscillometric method for measuring indirect blood pressure. *Ann Biomed Eng*. 1982;10:271.
442. Kittleson MD, Olivier NB. Measurement of systemic arterial blood pressure. *Vet Clin North Am Small Anim Pract*. 1983;13:321.
443. Magdesian KG. Monitoring the critically ill equine patient. *Vet Clin North Am Equine Pract*. 2004;20:11.
444. Muir WW, Wade A, Grospitch B. Automatic noninvasive sphygmomanometry in horses. *J Am Vet Med Assoc*. 1983;182:1230.
445. Ramsey 3rd M. Blood pressure monitoring: automated oscillometric devices. *J Clin Monit*. 1991;7:56.
446. O'Rourke MF, Yaginuma T. Wave reflections and the arterial pulse. *Arch Intern Med*. 1984;144:366.
447. Bayly WM, Gabel AA, Barr SA. Cardiovascular effects of submaximal aerobic training on a treadmill in Standardbred horses, using a standardized exercise test. *Am J Vet Res*. 1983;44:544.
448. Physick-Sheard PW. Cardiovascular response to exercise and training in the horse. *Vet Clin North Am Equine Pract*. 1985;1:383.
449. Rugh KS, Garner HE, Miramonti JR, et al. Left ventricular function and haemodynamics in ponies during exercise and recovery. *Equine Vet J*. 1989;21:39.
450. Tillotson PJ, Kooper PH. Treatment of aortic thrombus in a horse. *J Am Vet Med Assoc*. 1966;149:766.
451. Tithof PK, Rebhun WC, Dietze AE. Ultrasonographic diagnosis of aorto-iliac thrombosis. *Cornell Vet*. 1985;75:540.
452. Mouchot E, Desbrosse F. Aorto-iliac thrombosis in a horse. Clinical examination and use of ultrasonography as a diagnostic aid. *Pratique Vétérinaire Équine*. 1994;26:147.

453. Moore LA, Johnson PJ, Bailey KL. Aorto-iliac thrombosis in a foal. *Vet Rec*. 1998;142:459.
454. Barrelet A. Aorto-iliac thrombosis in a breeding stallion and an eventer mare. *Equine Vet Educ*. 1993;5:86.
455. Trachsel D, Cohausz O, Scharf G, et al. Aorto-iliac thrombosis in a gelding treated with the anticoagulant phenprocoumon (Marcoumar (R). *Schweiz Arch Tierheilkd*. 2008;150:613.
456. Dickson LR, Badcoe LM, Burbidge H, et al. Jugular thrombophlebitis resulting from an anesthetic induction technique in the horse. *Equine Vet J*. 1990;22:177.
457. Traub-Dargatz JL, Dargatz DA. A retrospective study of vein thrombosis in horses treated with intravenous fluids in a veterinary teaching hospital. *J Vet Intern Med*. 1994;8:264.
458. Gardner S, Reef VB, Spencer PA. Ultrasonographic evaluation of horses with thrombophlebitis of the jugular vein: 46 cases (1985-1988). *J Am Vet Med Assoc*. 1991;199:370.
459. Durando M. Exercise and stress testing. In: Marr CM, ed. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
460. Franklin S, Allen K. Laboratory exercise testing. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. Edinburgh: Saunders Elsevier; 2014.
461. Couroucé-Malblanc A, van Erck-Westergren E. Exercise testing in the field. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. Edinburgh: Saunders Elsevier; 2014.
462. Verheyen T, Decloedt A, De Clercq D, et al. Electrocardiography in horses—part 1: how to make a good recording. *Vlaams Diergen Tijds*. 2010;79:331.
463. Rose R. Symposium on exercise physiology—foreword. *Vet Clin North Am Equine Pract*. 1985;1:437.
464. Poggenpoel DG. Measurements of heart rate and riding speed on a horse during a training programme for endurance rides. *Equine Vet J*. 1988;20:224.
465. Thornton JR. Exercise testing. *Vet Clin North Am Equine Pract*. 1985;1:573.
466. Evans DL, Rose RJ. Dynamics of cardiorespiratory function in Standardbred horses during different intensities of constant-load exercise. *J Comp Physiol B*. 1988;157:791.
467. Foreman JH, Bayly WM, Grant BD, et al. Standardized exercise test and daily heart rate responses of thoroughbreds undergoing conventional race training and detraining. *Am J Vet Res*. 1990;51:914.
468. Rose RJ, Hendrickson DK, Knight PK. Clinical exercise testing in the normal Thoroughbred racehorse. *Aust Vet J*. 1990;67:345.
469. Seeherman HJ, Morris EA. Comparison of yearling, two-year-old and adult Thoroughbreds using a standardised exercise test. *Equine Vet J*. 1991;23:175.
470. Reinhard HJ, Zichner M. Evaluation of telemetrically derived stress electrocardiograms of the horse using an electronic computer. *Dtsch Tierarztl Wochenschr*. 1970;77:211.
471. Physick-Sheard PW, Marlin DJ, Thornhill R, et al. Frequency domain analysis of heart rate variability in horses at rest and during exercise. *Equine Vet J*. 2000;32:253.
472. Verheyen T, Decloedt A, van der Vekens N, et al. Ventricular response during lungeing exercise in horses with lone atrial fibrillation. *Equine Vet J*. 2013;45:309.
473. Amada A, Kurita H. Five cases of paroxysmal atrial fibrillation in the racehorse. *Exp Rep Equine Health Lab*. 1975;12:89.
474. Hiraga A, Kubo K. Two cases of paroxysmal atrial fibrillation during exercise in horses. *Equine Vet Educ*. 1999;11:6.
475. Physick-Sheard PW, McGurrin MK. Ventricular arrhythmias during race recovery in Standardbred Racehorses and associations with autonomic activity. *J Vet Intern Med*. 2010;24:1158.
476. Hada T, Ohmura H, Mukai K, et al. Utilisation of the time constant calculated from heart rate recovery after exercise for evaluation of autonomic activity in horses. *Equine Vet J Suppl*. 2006;141.
477. Thayer JF, Hahn AW, Pearson MA, et al. Heart rate variability during exercise in the horse. *Biomed Sci Instrum*. 1997;34:246.
478. Physick-Sheard PW. Seek and ye shall find: cardiac arrhythmias in the horse. *Equine Vet J*. 2013;45:270.
479. Ryan N, Marr CM, McGladdery AJ. Survey of cardiac arrhythmias during submaximal and maximal exercise in Thoroughbred racehorses. *Equine Vet J*. 2005;37:265.
480. Reef VB. Ambulatory and exercise electrocardiography and post-exercise echocardiography. In: Marr CM, ed. *Cardiology of the Horse*. London: WB Saunders; 1999.
481. Sandersen C, Detilleux J, Art T, et al. Exercise and pharmacological stress echocardiography in healthy horses. *Equine Vet J Suppl*. 2006;159.
482. Durando M. Diagnosing cardiac disease in equine athletes: the role of stress testing. *Equine Vet J*. 2005;37:101.
483. Gehlen H, Marnette S, Rohn K, et al. Stress echocardiography in warmblood horses: comparison of dobutamine/atropine with treadmill exercise as cardiac stressors. *J Vet Intern Med*. 2006;20:562.
484. Durando MM, Slack J, Reef VB, et al. Right ventricular pressure dynamics and stress echocardiography in pharmacological and exercise stress testing. *Equine Vet J Suppl*. 2006;183–192.
485. Coudry V, Jean D, Desbois C, et al. Myocardial fibrosis in a horse with polymorphic ventricular tachycardia observed during general anesthesia. *Can Vet J*. 2007;48:623.
486. Finley MR, Li Y, Hua F, et al. Expression and coassociation of ERG1, KCNQ1, and KCNE1 potassium channel proteins in horse heart. *Am J Physiol Heart Circ Physiol*. 2002;283:H126.
487. Kuwahara M, Hiraga A, Kai M, et al. Influence of training on autonomic nervous function in horses: evaluation by power spectral analysis of heart rate variability. *Equine Vet J Suppl*. 1999;30:178.
488. Glomset DJ, Glomset ATA, Moines D. A morphologic study of the cardiac conduction system in ungulates, dog, and man. Part I: the sinoatrial node. *Am Heart J*. 1940;20:389.
489. Gross DR. Practical electrocardiography in the equine subject. *J Am Vet Med Assoc*. 1971;159:1335.
490. Grauerholz H, Jaeschke G. Construction of main and reference vectors from limb leads in the ECG of the horse. *Berl Munch Tierarztl Wochenschr*. 1988;101:376.
491. Grauerholz H, Jaeschke G. Training-induced changes of reference vectors in the QRS complex of the EKG of young trotting horses. *Berl Munch Tierarztl Wochenschr*. 1990;103:329.
492. Grauerholz H. Influence of respiration on the QRS complex of the ECG in clinically healthy horses and in horses with respiratory problems. *Berl Munch Tierarztl Wochenschr*. 1990;103:293.
493. Grauerholz H, Jaeschke G. Alterations induced by training in reference vectors of the electrocardiographic QRS complex of young trotting horses. *Berl Munch Tierarztl Wochenschr*. 1990;103:329.
494. Grauerholz H, Jaeschke G. The construction of the main and reference vectors from the limb circuits in the ECG of the horse. *Berl Munch Tierarztl Wochenschr*. 1988;101:376.
495. Hartley JW, Hahn AW, DeLorey M, et al. Digital processing of equine exercise electrocardiograms. *Biomed Sci Instrum*. 1990;26:11.
496. Hilwig RW. Cardiac Arrhythmias in the horse. *J Am Vet Med Assoc*. 1977;170:153.
497. Hanak J, Zert Z. Some ECG characters in Thoroughbred horses, common to parents and their offspring. *Vet Med (Praha)*. 1982;27:87.
498. Hanak J, Jagos P. Electrocardiographic lead system and its vector verification. *Acta Veterinaria Brno*. 1983;52:67.
499. Fregin GF. The equine electrocardiogram with standardized body and limb positions. *Cornell Vet*. 1982;72:304.
500. Fregin GF. Electrocardiography. *Vet Clin North Am Equine Pract*. 1985;1:419.
501. Bonagura JD, Miller MS. Electrocardiography. In: Jones WE, ed. *Equine Sports Medicine*. Philadelphia: Lea and Febiger; 1985.

502. Bonagura JD, Miller MS. Electrocardiography. What is your diagnosis? Junctional and ventricular arrhythmias. *J Equine Vet Sci*. 1985;5:347.
503. Clark DR, McCrady JD. Clinical use of the electrocardiogram in animals. I. Fundamentals of ECG examination. *Vet Med Small Anim Clin*. 1966;61:751.
504. Cornick JL, Seahorn TL. Cardiac arrhythmias identified in horses with duodenitis/proximal jejunitis: six cases (1985-1988). *J Am Vet Med Assoc*. 1990;197:1054.
505. Costa G, Illera M, Garcia-Sacristan A. Electrocardiographical values in non-trained horses. *Zentralbl Veterinarmed A*. 1985;32:196.
506. Illera JC, Illera M. Physiological electrocardiograms as the basis for diagnosis of heart diseases in horses. *Medicina Veterinaria*. 1986;3:239.
507. Illera JC, Illera M. Electrocardiography and heart score of horses competing in an endurance ride. *Aust Vet J*. 1987;64:88.
508. Illera JC, Illera M. Precordial heart score. *Aust Vet J*. 1988;65:355.
509. Irie T. A study of arrhythmias in Thoroughbred newborn foals immediately after birth. *Jpn J Vet Res*. 1990;38:57.
510. Kuwahara M, Hashimoto S, Ishii K, et al. Assessment of autonomic nervous function by power spectral analysis of heart rate variability in the horse. *J Auton Nerv Syst*. 1996;60:43.
511. Landgren S, Rutqvist L. Electrocardiogram of normal cold blooded horses after work. *Nord Vet Med*. 1953;5:905.
512. Lannek N, Rutqvist L. Normal area of variation for the electrocardiogram of horses. *Nord Vet Med*. 1951;3:1094.
513. Matsui K. Fetal and maternal heart rates in a case of twin pregnancy of the Thoroughbred horse. *Nihon Juigaku Zasshi*. 1985;47:817.
514. Miller MS, Bonagura JD. Genesis of the equine electrocardiogram and indications for electrocardiography in clinical practice. *J Equine Vet Sci*. 1985;5:23.
515. Polglaze K, Evans DL. The relationship between racing performance and electrocardiographic findings in the Standardbred racehorse. *Aust Equine Vet*. 1992;10:88.
516. Rose RJ, Davis PE. The use of electrocardiography in the diagnosis of poor racing performance in the horse. *Aust Vet J*. 1978;54:51.
517. Stewart JH, Rose RJ, Davis PE, et al. A comparison of electrocardiographic findings in racehorses presented either for routine examination or poor racing performance. In: Persson SGB, Rose RJ, eds. *Equine Exercise Physiology*. Cambridge, England: Granta; 1983.
518. Studzinski T, Czarnecki A. Relationship between the QRS duration (heart score) and ventricular weight in horses, Annales Universitatis Mariae Curie Sklodowska. *DD Medicina Veterinaria 35/36*. 1980;33.
519. Tovar P, Escabias MI, Santisteban R. Evolution of the ECG from Spanish bred foals during the post natal stage. *Res Vet Sci*. 1989;46:358.
520. Tschudi P. Electrocardiography in the horse (1). Principles and normal picture. *Tierarztl Prax*. 1985;13:181.
521. Tschudi P. Electrocardiography in the horse. (3). *Tierarztl Prax*. 1986;14:365.
522. White 2nd NA, Rhode EA. Correlation of electrocardiographic findings to clinical disease in the horse. *J Am Vet Med Assoc*. 1974;164:46.
523. Yamamoto K, Yasuda J, Too K. Electrocardiographic findings during parturition and blood gas tensions immediately after birth in Thoroughbred foals. *Jpn J Vet Res*. 1991;39:143.
524. Yamamoto K, Yasuda J, Too K. Arrhythmias in newborn thoroughbred foals. *Equine Vet J*. 1992;24:169.
525. Yamaya Y, Kubo K, Amada A, et al. Intrinsic atrioventricular conductive function in horses with a 2nd- degree atrioventricular-block. *J Vet Med Sci*. 1997;59:149.
526. Ayala I, Montes A, Benedito JL, et al. Modifications of the form and amplitude of the electrocardiographic QRS complex during growth in the Spanish-bred Horse. *Zentralbl Veterinarmed A*. 1998;45:309.
527. Verheyen T, Decloedt A, De Clercq D, et al. Oesophageal electrocardiography in healthy horses. *Equine Vet J*. 2012;44:640.
528. Verheyen T, Decloedt A, De Clercq D, et al. Electrocardiography in horses—part 2: how to read the equine ECG. *Vlaams Diergen Tijds*. 2010;79:337.
529. Vibe-Petersen G, Nielsen K. Electrocardiography in the horse (A report of findings in 138 horses). *Nord Vet Med*. 1980;32:105.
530. Gross DR. Practical electrocardiography in the horse. *J Am Vet Med Assoc*. 1972;160:672.
531. Banister EW, Purvis AD. Exercise electrocardiography in the horse by radiotelemetry. *J Am Vet Med Assoc*. 1968;152:1004.
532. Deegen E, Reinhard HJ. Electrocardiographic time patterns in the healthy Shetland pony. *Dtsch Tierarztl Wochenschr*. 1974;81:257.
533. Buss DD, Rwalings CA, Bisgard GE. The normal electrocardiogram of the domestic pony. *J Electrocardiol*. 1975;8:167.
534. Zucca E, Ferrucci F, Di Fabio V, et al. The use of electrocardiographic recording with Holter monitoring during treadmill exercise to evaluate cardiac arrhythmias in racehorses. *Vet Res Commun*. 2003;27:811.
535. Reef VB. Electrocardiography and echocardiography in the exercising horse. In: Robinson NE, ed. *Current Therapy in Equine Medicine 4*. Philadelphia: WB Saunders; 1997.
536. Pedersen PJ, Kanters JK, Buhl R, et al. Normal electrocardiographic QT interval in race-fit Standardbred horses at rest and its rate dependence during exercise. *J Vet Cardiol*. 2013.
537. Pedersen PJ, Karlsson M, Flethoj M, et al. Differences in the electrocardiographic QT interval of various breeds of athletic horses during rest and exercise. *J Vet Cardiol*. 2016.
538. Trachsel DS, Bitschnau C, Waldern N, et al. Observer agreement for detection of cardiac arrhythmias on telemetric ECG recordings obtained at rest, during and after exercise in 10 Warmblood horses. *Equine Vet J*. 2010;42:208.
539. Pedersen PJ, Moeller SB, Flethoj M, et al. Diurnal modulation and sources of variation affecting ventricular repolarization in Warmblood horses. *J Vet Cardiol*. 2014;16:265.
540. Hiraga A, Sugano S. History of research in Japan on electrocardiography in the racehorse. *J Equine Sci*. 2015;26:1.
541. Scheffer CW, Robben JH. Sloet van Oldruitenborgh-Oosterbaan MM: continuous monitoring of ECG in horses at rest and during exercise. *Vet Rec*. 1995;137:371.
542. Persson SGB, Forssbergy P. Exercise tolerance in Standardbred trotters with T-wave abnormalities. In: Gillespie JR, Robinson NE, eds. *Equine Exercise Physiology*. Davis, CA: ICEEP publications; 1987.
543. Matsui K, Sugano S. Species differences in the changes in heart rate and T-wave amplitude after autonomic blockade in Thoroughbred horses, ponies, cows, pigs, goats and chickens. *Nihon Juigaku Zasshi*. 1987;49:637.
544. Koblik PD, Hornof WJ. Diagnostic-radiology and nuclear cardiology—their use in assessment of equine cardiovascular-disease. *Vet Clin North Am Equine Pract*. 1985;1:289.
545. Carlsten J, Kvart C, Jeffcott LB. Method of selective and non-selective angiocardiography for the horse. *Equine Vet J*. 1984;16:47.
546. Lombard CW. Cardiovascular diseases. In: Koterba AM, ed. *Equine Clinical Neonatology*. Malvern, PA: Lea & Febiger; 1990.
547. Marr CM. Ancillary diagnostic aids in equine cardiology. *Equine Vet Educ*. 1990;2:18.
548. O'Brien RT, Biller DS. Field imaging of the respiratory tract. Radiology and ultrasonography. *Vet Clin North Am Equine Pract*. 1997;13:487.
549. Scott EA, Chaffee A, Eyster GE, et al. Interruption of aortic arch in two foals. *J Am Vet Med Assoc*. 1978;172:347.
550. Stewart JH, Rose RJ, Barko AM. Echocardiography in foals from birth to three months old. *Equine Vet J*. 1984;16:332.
551. O'Callaghan MW. Comparison of echocardiographic and autopsy measurements of cardiac dimensions in the horse. *Equine Vet J*. 1985;17:361.

552. Pipers FS, Reef V, Wilson J. Echocardiographic detection of ventricular septal defects in large animals. *J Am Vet Med Assoc*. 1985;187:810.
553. Bertone JJ, Paull KS, Wingfield WE, et al. M-mode echocardiographs of endurance horses in the recovery phase of long-distance competition. *Am J Vet Res*. 1987;48:1708.
554. Reef VB, Spencer P. Echocardiographic evaluation of equine aortic insufficiency. *Am J Vet Res*. 1987;48:904.
555. Reef VB. Echocardiographic examination in the horse—the basics. *Comp Cont Educ Pract*. 1990;12:1312.
556. Reef VB. Echocardiographic findings in horses with congenital cardiac disease. *Comp Cont Educ Pract*. 1991;13:109.
557. Stadler P, Weinberger T, Kinkel N, et al. B-mode-, M-mode- and Doppler sonographic findings in mitral valve insufficiency (MVI) in horses. *J Vet Med*. 1992;39:704.
558. Long KJ. Doppler echocardiography—clinical applications. *Equine Vet Educ*. 1993;5:161.
559. Bonagura JD. Echocardiography. *J Am Vet Med Assoc*. 1994; 204:516.
560. Blissitt KJ, Bonagura JD. Colour flow Doppler echocardiography in normal horses. *Equine Vet J Suppl*. 1995;47.
561. Tucker RL, Wickler SJ, London C, et al. Echocardiographic and right-sided cardiac pressure comparison of the mule and horse. *J Equine Vet Sci*. 1995;15:404.
562. Darke PGG, Bonagura JD, Kelly DF. *Colour Atlas of Veterinary Cardiology*. 1st ed. London: Mosby; 1996.
563. Reef VB. Evaluation of ventricular septal defects in horses using two-dimensional and Doppler echocardiography. *Equine Vet J Suppl*. 1995;86.
564. Lescure F, Tamzali Y. Reference values for echocardiography applied to sport horses (English Thoroughbreds and French riding horses). *Rev Med Vet (Toulouse)*. 1984;135:405.
565. Lord PF, Croft MA. Accuracy of formulae for calculating left ventricular volumes of the equine heart. *Equine Vet J Suppl Supplement*. 1990;9:53.
566. Young L. Transoesophageal echocardiography. In: Marr CM, ed. *Cardiology of the Horse*. London: WB Saunders; 1999.
567. Buhl R, Ersboll AK, Eriksen L, et al. Sources and magnitude of variation of echocardiographic measurements in normal standardbred horses. *Vet Radiol Ultrasound*. 2004;45:505.
568. Zucca E, Ferrucci F, Croci C, et al. Echocardiographic measurements of cardiac dimensions in normal Standardbred racehorses. *J Vet Cardiol*. 2008;10:45.
569. Al Haidar A, Farnir F, Deleuze S, et al. Comparison of the repeatability of echocardiographic measurements from different modes and views in horses of various breeds and sizes. *J Equine Vet Sci*. 2010;30:287.
570. Al Haidar A, Farnir F, Deleuze S, et al. Effect of breed, sex, age and body weight on echocardiographic measurements in the Equine species. *Res Vet Sci*. 2013;95:255.
571. Al Haidar A, Leroux A, Borde L, et al. Relationship between echocardiographic measurements and body size in horses. *J Equine Vet Sci*. 2013;33:107.
572. Decloedt A, de Clercq D, van der Vekens N, et al. Noninvasive determination of atrial fibrillation cycle length by atrial colour tissue Doppler imaging in horses. *Equine Vet J*. 2014;46:174.
573. Decloedt A, De Clercq D, Ven Sofie S, et al. Echocardiographic measurements of right heart size and function in healthy horses. *Equine Vet J*. 2016.
574. Decloedt A, Verheyen T, Sys S, et al. Influence of atrioventricular interaction on mitral valve closure and left ventricular isovolumic contraction measured by tissue Doppler imaging. *Circ Cardiovasc Imaging*. 2013;6:109.
575. Verheyen T, Decloedt A, De Clercq D, et al. Cardiac changes in horses with atypical myopathy. *J Vet Intern Med*. 2012;26:1019.
576. Huesler IM, Mitchell KJ, Schwarzwald CC. Echocardiographic assessment of left atrial size and function in Warmblood horses: reference intervals, allometric scaling, and agreement of different echocardiographic variables. *J Vet Intern Med*. 2016;30:1241.
577. Hallowell G, Bowen M. Reliability and identification of aortic valve prolapse in the horse. *BMC Vet Res*. 2013;9:9.
578. Slack J, Durando MM, Belcher CN, et al. Intraoperator, intraobserver and interoperator variability of echocardiographic measurements in healthy foals. *Equine Vet J*. 2012;44:69.
579. Underwood C, Norton JL, Nolen-Walston RD, et al. Echocardiographic changes in heart size in hypohydrated horses. *J Vet Intern Med*. 2011;25:563.
580. Collins NM, Palmer L, Marr CM. Two-dimensional and M-mode echocardiographic findings in healthy Thoroughbred foals. *Aust Vet J*. 2010;88:428.
581. Rovira S, Munoz A. Two-dimensional- and M-mode echocardiographic measurements and indices of cardiac function in Spanish colts and fillies of different age. *J Vet Med Sci*. 2009;71:957.
582. Rovira S, Muñoz A, Rodilla V. Allometric scaling of echocardiographic measurements in healthy Spanish foals with different body weight. *Res Vet Sci*. 2009;86:325.
583. Imhasly A, Tschudi PR, Lombard CW, et al. Clinical and echocardiographic features of mild mitral valve regurgitation in 108 horses. *Vet J*. 2010;183:166.
584. Menzies-Gow NJ. Effects of sedation with acepromazine on echocardiographic measurements in eight healthy thoroughbred horses. *Vet Rec*. 2008;163:21.
585. Durando MM, Reef VB, Birks EK. Right ventricular pressure dynamics during exercise: relationship to stress echocardiography. *Equine Vet J Suppl*. 2002;472.
586. Helwegen MM, Young LE, Rogers K, et al. Measurements of right ventricular internal dimensions and their relationships to severity of tricuspid valve regurgitation in national hunt Thoroughbreds. *Equine Vet J Suppl*. 2006;171.
587. Buhl R, Ersboll AK, Larsen NH, et al. The effects of detomidine, romifidine or acepromazine on echocardiographic measurements and cardiac function in normal horses. *Vet Anaesth Analg*. 2007;34:1.
588. Young LE, Rogers K, Wood JL. Left ventricular size and systolic function in Thoroughbred racehorses and their relationships to race performance. *J Appl Physiol*. 2005;99:1278.
589. Brown DJ, Rush JE, MacGregor J, et al. M-mode echocardiographic ratio indices in normal dogs, cats, and horses: a novel quantitative method. *J Vet Intern Med*. 2003;17:653.
590. Marr CM, Patteson M. Echocardiography. In: Marr CM, ed. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
591. Reef VB. Cardiovascular ultrasonography. In: Reef VB, ed. *Equine Diagnostic Ultrasound*. Philadelphia: W.B. Saunders; 1998.
592. Young LE, Scott GR. Measurement of cardiac function by transthoracic echocardiography: day to day variability and repeatability in normal Thoroughbred horses. *Equine Vet J*. 1998;30:117.
593. Sampson SN, Jacobson RL, Sande RD, et al. Reproducibility and repeatability of M-mode echocardiographic measurements collected from 25 normal horses. *J Equine Vet Sci*. 1999;19:51.
594. Otto CM. *Textbook of Clinical Echocardiography*. 5th ed. Philadelphia: Elsevier Saunders; 2013.
595. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2015;28:1.
596. Gehlen H, Kroker K, Deegen E, et al. Influence of detomidine on cardiac function and hemodynamic in horses with and without heart murmur. *Schweiz Arch Tierheilkd*. 2004;146:119.
597. Underwood C, Schwarzwald CC. *Equine Echocardiography: Do We All Know What We All Do? Results of a Recent Survey (unpublished data)*. Switzerland: University of Queensland, Australia and University of Zurich, Switzerland.; 2013.
598. Rantanen NW. Diseases of the thorax. *Vet Clin North Am Equine Pract*. 1986;2:49.
599. Bonagura JD, Pipers FS. Diagnosis of cardiac lesions by contrast echocardiography. *J Am Vet Med Assoc*. 1983;182:396.

600. Young LE, Long KJ, Clutton RE, et al. The use of two dimensional and Doppler echocardiography for haemodynamic monitoring during general anaesthesia: preliminary findings in halothane-anaesthetised horses. *Vet Anaesth Analg*. 1993;20:42.
601. Guglielmini C, Pietra M, Bernardini D, et al. Colour-coded Doppler echocardiography in the horse. Part II: abnormal findings. *Ippologia*. 2001;12:15.
602. Ven S, Decloedt A, Van Der Vekens N, et al. Assessing aortic regurgitation severity from 2D, M-mode and pulsed wave Doppler echocardiographic measurements in horses. *Vet J*. 2016;210:34.
603. Stoylen A. *Strain Rate Imaging: Cardiac Deformation Imaging by Ultrasound/Echocardiography—Tissue Doppler and Speckle Tracking*; 2016. Available from: http://folk.ntnu.no/stoylen/strainrate/.
604. Sepulveda MF, Perkins JD, Bowen IM, et al. Demonstration of regional differences in equine ventricular myocardial velocity in normal 2-year-old Thoroughbreds with Doppler tissue imaging. *Equine Vet J*. 2005;37:222.
605. Atkins CE, Snyder PS. Systolic time intervals and their derivatives for evaluation of cardiac function. *J Vet Intern Med*. 1992;6:55.
606. Amend JF, Garner HE, Rosborough JP, et al. Hemodynamic studies in conscious domestic ponies. *J Surg Res*. 1975;19:107.
607. Bove AA. Effects of strenuous exercise on myocardial blood flow. *Med Sci Sports Exerc*. 1985;17:517.
608. Davis JL, Manohar M. Effect of splenectomy on exercise-induced pulmonary and systemic hypertension in ponies. *Am J Vet Res*. 1988;49:1169.
609. Drummond WH, Sanchez IR, Kosch PC, et al. Pulmonary vascular reactivity of the newborn pony foal. *Equine Vet J*. 1989;21:181.
610. Erickson BK, Erickson HH, Coffman JR. Pulmonary artery and aortic pressure changes during high intensity treadmill exercise in the horse: effect of frusemide and phentolamine. *Equine Vet J*. 1992;24:215.
611. Gasthuys F, Muylle E, de Moor A, et al. Influence of premedication and body position during halothane anaesthesia on intracardial pressures in the horse. *Zentralbl Veterinarmed A*. 1988;35:729.
612. Hall LW, Nigam JM. Papers and articles measurement of central venous pressure in horses. *Vet Rec*. 1975;97:66.
613. Hall LW. Cardiovascular and pulmonary effects of recumbency in two conscious ponies. *Equine Vet J*. 1984;16:89.
614. Hellyer PW, Dodam JR, Light GS. Dynamic baroreflex sensitivity in anesthetized horses, maintained at 1.25 to 1.3 minimal alveolar concentration of halothane. *Am J Vet Res*. 1991;52:1672.
615. Hillidge CJ, Lees P. Influence of general anaesthesia on peripheral resistance in the horse. *Br Vet J*. 1977;133:225.
616. Hinchcliff KW, McKeever KH, Schmall LM, et al. Renal and systemic hemodynamic responses to sustained submaximal exertion in horses. *Am J Physiol*. 1990;258:R1177.
617. Manohar M. Right heart pressures and blood-gas tensions in ponies during exercise and laryngeal hemiplegia. *Am J Physiol*. 1986;251:H121.
618. Manohar M, Goetz TE, Hutchens E, et al. Atrial and ventricular myocardial blood flows in horses at rest and during exercise. *Am J Vet Res*. 1994;55:1464.
619. Manohar M. Pulmonary vascular pressures of strenuously exercising Thoroughbreds after administration of flunixin meglumine and furosemide. *Am J Vet Res*. 1994;55:1308.
620. Manohar M, Goetz TE, Hutchens E, et al. Effects of graded-exercise on pulmonary and systemic hemodynamics in horses. *Equine Pract*. 1995;17:17.
621. Muir WW, McGuirk SM. Hemodynamics before and after conversion of atrial fibrillation to normal sinus rhythm in horses. *J Am Vet Med Assoc*. 1984;184:965.
622. Schatzmann U, Battier B. Factors influencing central venous pressure in horses. *Dtsch Tierarztl Wochenschr*. 1987;94:147.
623. Sheridan V, Zeller R, Deegen E. Central venous pressure (C.V.P.) measurements during halothane anesthesia in horse. *Vet Rec*. 1972;90:149.
624. Sinha AK, Gleed RD, Hakim TS, et al. Pulmonary capillary pressure during exercise in horses. *J Appl Physiol*. 1996;80:1792.
625. Spörri H, Denac M. The ventricular pressure increase velocity as a parameter of myocardial strength development. *Schweiz Arch Tierheilkd*. 1969;111:239.
626. Staddon GE, Weaver BM, Webb AI. Distribution of cardiac output in anaesthetised horses. *Res Vet Sci*. 1979;27:38.
627. Stadler P, Kinkel N, Deegen E. Evaluation of systolic heart function of the horse with PW- Doppler-echocardiography compared with thermodilution. *Dtsch Tierarztl Wochenschr*. 1994;101:312.
628. Weber JM, Dobson GP, Parkhouse WS, et al. Cardiac output and oxygen consumption in exercising Thoroughbred horses. *Am J Physiol*. 1987;253:R890.
629. West JB, Mathieucostello O. Stress failure of pulmonary capillaries as a mechanism for exercise-induced pulmonary hemorrhage in the horse. *Equine Vet J*. 1994;26:441.
630. Wetmore LA, Derksen FJ, Blaze CA, et al. Mixed venous oxygen-tension as an estimate of cardiac-output in anesthetized horses. *Am J Vet Res*. 1987;48:971.
631. Ringer SK, Schwarzwald CC, Portier KG, et al. Effects on cardiopulmonary function and oxygen delivery of doses of romifidine and xylazine followed by constant rate infusions in standing horses. *Vet J*. 2013;195:228.
632. Nollet H, Van Loon G, Deprez P, et al. Use of right ventricular pressure increase rate to evaluate cardiac contractility in horses. *Am J Vet Res*. 1999;60:1508.
633. Moscucci M. *Grossman & Baim's Cardiac Catheterization, Angiography, and Intervention*. 8th ed. Philadelphia, PA: Wolters Kluwer; 2014.
634. Button K. Application of percutaneous technique to equine vascular catheterization. *J Equine Med Surg*. 1979;3:320.
635. Manohar M. Left ventricular oxygen extraction during submaximal and maximal exertion in ponies. *J Physiol*. 1988; 404:547.
636. Butler HC. Subcutaneous relocation of the carotid artery for experimental purposes. *Am J Vet Res*. 1962;23:165.
637. Manohar M. Pulmonary artery wedge pressure increases with high-intensity exercise in horses. *Am J Vet Res*. 1993;54:142.
638. Constable P, Muir 3rd W, Sisson D. Clinical assessment of left ventricular relaxation. *J Vet Intern Med*. 1999;13:5.
639. Stadler P, Fruhauf B, Deegen E. Echocardiographic determination of diastolic heart function and measurement of pulmonary wedge pressure in horses. *Pferdeheilkunde*. 1995;11:109.
640. Klein L, Sherman J. Effects of preanesthetic medication, anesthesia, and position of recumbency on central venous pressure in horses. *J Am Vet Med Assoc*. 1977;170:216.
641. Bergsten G. Blood pressure, cardiac output, and blood-gas tension in the horse at rest and during exercise. *Acta Vet Scand Suppl*. 1974;48:1.
642. Grubb TL, Constable PD, Benson GJ, et al. Techniques for evaluation of right ventricular relaxation rate in horses and effects of inhalant anesthetics with and without intravenous administration of calcium gluconate. *Am J Vet Res*. 1999;60:872.
643. Goetz TE, Manohar M. Pressures in the right side of the heart and esophagus (Pleura) in ponies during exercise before and after furosemide administration. *Am J Vet Res*. 1986;47:270.
644. Art T, Bayly W. Lower airway function: response to exercise and training. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sports Medicine and Surgery*. W.B. Saunders; 2014.
645. Edwards WD. Pathology of pulmonary hypertension. *Cardiovasc Clin*. 1988;18:321.
646. Bisgard GE, Orr JA, Will JA. Hypoxic pulmonary hypertension in the pony. *Am J Vet Res*. 1975;36:49.

647. Gelberg HB, Smetzer DL, Foreman JH. Pulmonary hypertension as a cause of atrial fibrillation in young horses: four cases (1980-1989). *J Am Vet Med Assoc*. 1991;198:679.
648. Long KJ, Young LE, Utting JE, et al. Determination of cardiac output in the standing horse by Doppler echocardiography and thermodilution. *Proc 30th British Equine Vet Assoc Congress 30*. 1991.
649. O'Brien PJ, Dameron GW, Beck ML, et al. Differential reactivity of cardiac and skeletal muscle from various species in two generations of cardiac troponin-T immunoassays. *Res Vet Sci*. 1998;65:135.
650. Rossi TM, Pyle WG, Maxie MG, et al. Troponin assays in the assessment of the equine myocardium. *Equine Vet J*. 2014;46:270.
651. Schwarzwald CC, Hardy J, Buccellato M. High cardiac troponin I serum concentration in a horse with multiform ventricular tachycardia and myocardial necrosis. *J Vet Intern Med*. 2003;17:364.
652. Slack JA, McGuirk SM, Erb HN, et al. Biochemical markers of cardiac injury in normal, surviving septic, or nonsurviving septic neonatal foals. *J Vet Intern Med*. 2005;19:577.
653. Cornelisse CJ, Schott 2nd HC, Olivier NB, et al. Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. *J Am Vet Med Assoc*. 2000;217:231.
654. Diana A, Guglielmini C, Candini D, et al. Cardiac arrhythmias associated with piroplasmosis in the horse: a case report. *Vet J*. 2007;174:193.
655. Jesty SA, Kraus MS, Gelzer AR, et al. Effect of transvenous electrical cardioversion on plasma cardiac troponin I concentrations in horses with atrial fibrillation. *J Vet Intern Med*. 2009;23:1103.
656. Kraus MS, Jesty SA, Gelzer AR, et al. Measurement of plasma cardiac troponin I concentration by use of a point-of-care analyzer in clinically normal horses and horses with experimentally induced cardiac disease. *Am J Vet Res*. 2010;71:55.
657. Serra M, Papakonstantinou S, Adamcova M, et al. Veterinary and toxicological applications for the detection of cardiac injury using cardiac troponin. *Vet J*. 2010;185:50.
658. Durando MM, Birks EK, Hussey SB, et al. Cardiac troponin I concentrations in ponies challenged with equine influenza virus. *J Vet Intern Med*. 2011;25:339.
659. Gilliam LL, Holbrook TC, Ownby CL, et al. Cardiotoxicity, inflammation, and immune response after rattlesnake envenomation in the horse. *J Vet Intern Med*. 2012;26:1457.
660. Nath LC, Anderson GA, Hinchcliff KW, et al. Clinicopathologic evidence of myocardial injury in horses with acute abdominal disease. *J Am Vet Med Assoc*. 2012;241:1202.
661. Nath LC, Anderson GA, Hinchcliff KW, et al. Serum cardiac troponin I concentrations in horses with cardiac disease. *Aust Vet J*. 2012;90:351.
662. Nostell K, Brojer J, Hoglund K, et al. Cardiac troponin I and the occurrence of cardiac arrhythmias in horses with experimentally induced endotoxaemia. *Vet J*. 2012;192:171.
663. Radcliffe RM, Divers TJ, Fletcher DJ, et al. Evaluation of L-lactate and cardiac troponin I in horses undergoing emergency abdominal surgery. *J Vet Emerg crit care (San Antonio, TX: 2001)*. 2012;(22):313.
664. van der Vekens N, Decloedt A, De Clercq D, et al. The use of cardiac biomarkers in veterinary medicine: the equine perspective. *Vlaams Diergen Tijds*. 2012;81:319.
665. Peters ST, Hopkins A, Stewart S, et al. Myocardial contusion and rib fracture repair in an adult horse. *J Vet Emerg Crit Care*. 2013;23:663.
666. Seco Diaz OM, Durando MM, Birks EK, et al. Cardiac troponin I concentrations in horses with colic. *J Am Vet Med Assoc*. 2014;245:118.
667. Navas de Solis C, Dallap Schaer BL, Boston R, et al. Myocardial insult and arrhythmias after acute hemorrhage in horses. *J Vet Emerg Crit Care*. 2015;25:248.
668. Raftery AG, Garcia NC, Thompson H, et al. Arrhythmogenic right ventricular cardiomyopathy secondary to adipose infiltration as a cause of episodic collapse in a horse. *Ir Vet J*. 2015;68:1.
669. Durando MM, Reef VB, Kline K, et al. Acute effects of short duration, maximal exercise on cardiac troponin I in healthy horses. *Equine Comp Exerc Physiol*. 2006;3:217.
670. Holbrook TC, Birks EK, Sleeper MM, et al. Endurance exercise is associated with increased plasma cardiac troponin I in horses. *Equine Vet J Suppl*. 2006;27.
671. Nostell K, Haggstrom J. Resting concentrations of cardiac troponin I in fit horses and effect of racing. *J Vet Cardiol*. 2008;10:105.
672. Slack J, Boston RC, Soma L, et al. Cardiac troponin I in racing standardbreds. *J Vet Intern Med*. 2012;26:1202.
673. Flethoj M, Kanters JK, Haugaard MM, et al. Changes in heart rate, arrhythmia frequency, and cardiac biomarker values in horses during recovery after a long-distance endurance ride. *J Am Vet Med Assoc*. 2016;248:1034.
674. Van Der Vekens N, Decloedt A, Ven S, et al. Cardiac troponin I as compared to troponin T for the detection of myocardial damage in horses. *J Vet Intern Med*. 2015;29:348.
675. Van Der Vekens N, van Dievoet MA, De Puydt H, et al. Analytical validation of a high-sensitivity cardiac troponin T assay in horses. *J Vet Diagn Invest*. 2015;27:504.
676. Van Der Vekens N, Decloedt A, Sys S, et al. Evaluation of assays for troponin I in healthy horses and horses with cardiac disease. *Vet J*. 2015;203:97.
677. Shields E, Seiden-Long I, Massie S, et al. Analytical validation and establishment of reference intervals for a 'high-sensitivity' cardiac troponin-T assay in horses. *BMC Vet Res*. 2016;12:104.
678. Begg LM, Hoffmann KL, Begg AP. Serum and plasma cardiac troponin I concentrations in clinically normal Thoroughbreds in training in Australia. *Aust Vet J*. 2006;84:336.
679. Phillips W, Giguere S, Franklin RP, et al. Cardiac troponin I in pastured and race-training Thoroughbred horses. *J Vet Intern Med*. 2003;17:597.
680. Kraus MS, Kaufer BB, Damiani A, et al. Elimination half-life of intravenously administered equine cardiac troponin I in healthy ponies. *Equine Vet J*. 2013;45:56.
681. Mifune H, Richter R, Forssmann WG. Detection of immunoreactive atrial and brain natriuretic peptides in the equine atrium. *Anat Embryol (Berl)*. 1995;192:117.
682. McKeever KH, Hinchcliff KW, Cooley JL, et al. Arterial-venous difference in atrial natriuretic peptide concentration during exercise in horses. *Am J Vet Res*. 1992;53:2174.
683. Kokkonen UM, Hackzell M, Rasanen LA. Plasma atrial natriuretic peptide in standardbred and Finnhorse trotters during and after exercise. *Acta Physiol Scand*. 1995;154:51.
684. McKeever KH, Hinchcliff KW. Neuroendocrine control of blood volume, blood pressure and cardiovascular function in horses. *Equine Vet J*. 1995;27:77.
685. Nyman S, Kokkonen UM, Dahlborn K. Changes in plasma atrial natriuretic peptide concentration in exercising horses in relation to hydration status and exercise intensity. *Am J Vet Res*. 1998;59:489.
686. Kokkonen UM, Hyyppa S, Poso AR. Plasma atrial natriuretic peptide during and after repeated exercise under heat exposure. *Equine Vet J Suppl*. 1999;30:184.
687. McKeever KH, Malinowski K. Endocrine response to exercise in young and old horses. *Equine Vet J Suppl*. 1999;561.
688. Trachsel DS, Grenacher B, Weishaupt MA, et al. Plasma atrial natriuretic peptide concentrations in horses with heart disease: a pilot study. *Vet J*. 2012;192:166.
689. Trachsel DS, Grenacher B, Schwarzwald CC. Plasma atrial/A-type natriuretic peptide (ANP) concentration in horses with various heart diseases. *J Vet Cardiol*. 2015.
690. Leroux AA, Al Haidar A, Remy B, et al. Atrial natriuretic peptide as an indicator of the severity of valvular regurgitation and heart failure in horses. *J Equine Vet Sci*. 2014;34:1226.

691. Trachsel DS, Schwarzwald CC, Grenacher B, et al. Analytic validation and comparison of three commercial immunoassays for measurement of plasma atrial/A-type natriuretic peptide concentration in horses. *Res Vet Sci*. 2014;96:180.
692. Oh JK, Seward JB, Tajik AJ. Pulmonary hypertension. In: Oh JK, Seward JaB, Tajik AJ, eds. *The Echo Manual*. Philadelphia: Lippincott Williams & Wilkins; 1999.
693. Rosenkranz S. Pulmonary hypertension 2015: current definitions, terminology, and novel treatment options. *Clin Res Cardiol*. 2015;104:197.
694. Simonneau G, Gatzoulis MA, Adatia I, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 2013;62:D34.
695. Schwarzwald CC, Stewart AJ, Morrison CD, et al. Cor pulmonale in a horse with granulomatous pneumonia. *Equine Vet Educ*. 2006;18:182.
696. Johansson AM, Gardner SY, Atkins CE, et al. Cardiovascular effects of acute pulmonary obstruction in horses with recurrent airway obstruction. *J Vet Intern Med*. 2007;21:302.
697. Dixon PM, Nicholls JR, McPherson EA, et al. Chronic obstructive pulmonary disease anatomical cardiac studies. *Equine Vet J*. 1982;14:80.
698. Littlejohn A, Bowles F. Studies on the physiopathology of chronic obstructive pulmonary disease in the horse. II. Right heart haemodynamics. *Onderstepoort J Vet Res*. 1980;47:187.
699. Dixon PM. Pulmonary artery pressures in normal horses and in horses affected with chronic obstructive pulmonary disease. *Equine Vet J*. 1978;10:195.
700. Atkins CE. The role of noncardiac disease in the development and precipitation of heart failure. *Vet Clin North Am Small Anim Pract*. 1991;21:1035.
701. Rhodes J. Comparative physiology of hypoxic pulmonary hypertension: historical clues from brisket disease. *J Appl Physiol*. 2005;98:1092.
702. Wilkins PA. Lower respiratory problems of the neonate. *Vet Clin North Am Equine Pract*. 2003;19:19.
703. Drummond WH. Neonatal pulmonary hypertension. *Equine Vet J*. 1987;19:169.
704. Schoster A, Anderson ME. Caudal vena cava thrombosis-like syndrome in a horse. *Can Vet J*. 2010;51:891.
705. Norman TE, Chaffin MK, Perris EE, et al. Massive pulmonary thromboembolism in six horses. *Equine Vet J*. 2008;40:514.
706. Long PH, Payne JW. Red maple-associated pulmonary thrombosis in a horse. *J Am Vet Med Assoc*. 1984;184:977.
707. Jones RS, Payne-Johnson CE, Seymour CJ. Pulmonary microembolism following orthopaedic surgery in a Thoroughbred gelding. *Equine Vet J*. 1988;20:382.
708. Bryan J, Puggioni A, McAllister H, et al. Thrombosis of the pulmonary artery in a yearling Thoroughbred colt. *J Vet Intern Med*. 2009;23:215.
709. Carr EA, Carlson GP, Wilson WD, et al. Acute hemorrhagic pulmonary infarction and necrotizing pneumonia in horses: 21 cases (1967-1993), *J Am Vet Med Assoc*. 1997;210:1774.
710. Wagner PD, Gillespie JR, Landgren GL, et al. Mechanism of exercise-induced hypoxemia in horses. *J Appl Physiol*. 1989;66:1227.
711. Manohar M, Coney E, Hutchens E. Pulmonary vascular pressure in near-maximally exercised horses. *Equine Pract*. 1993;15:16.
712. Manohar M, Goetz TE. Pulmonary vascular pressures of exercising Thoroughbred horses with and without endoscopic evidence of EIPH. *J Appl Physiol*. 1996;81:1589.
713. Fedde MR, Erickson HH. Increase in blood viscosity in the sprinting horse: can it account for the high pulmonary arterial pressure? *Equine Vet J*. 1998;30:329.
714. Fedde MR, Wood SC. Rheological characteristics of horse blood: significance during exercise. *Respir Physiol*. 1993;94:323.
715. Kindig CA, Gallatin LL, Erickson HH, et al. Cardiorespiratory impact of the nitric oxide synthase inhibitor L-NAME in the exercising horse. *Respir Physiol*. 2000;120:151.
716. Kindig CA, Erickson HH, Poole DC. Dissociation of exercise-induced pulmonary hemorrhage and pulmonary artery pressure via nitric oxide synthase inhibition. *J Equine Vet Sci*. 2000;20:715.
717. Langsetmo I, Meyer MR, Erickson HH. Relationship of pulmonary arterial pressure to pulmonary haemorrhage in exercising horses. *Equine Vet J*. 2000;32:379.
718. Spörri H, Schlatter C. Blutdruckerhöhungen im Lungenkreislauf. *Schweiz Arch Tierheilkd*. 1959;101:525.
719. Slater J. Cor pulmonale and respiratory disease: unique features of the equine cardiopulmonary system. *Equine Vet Educ*. 2006;18:188.
720. Sage AM, Valberg S, Hayden DW, et al. Echocardiography in a horse with cor pulmonale from recurrent airway obstruction. *J Vet Intern Med*. 2006;20:694.
721. Hanka J, van den Hoven R, Schwarz B. Paroxysmal atrial fibrillation and clinically reversible cor pulmonale in a horse with complicated recurrent airway obstruction. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2015;43:109.
722. Lester GD, DeMarco VG, Norman WM. Effect of inhaled nitric oxide on experimentally induced pulmonary hypertension in neonatal foals. *Am J Vet Res*. 1999;60:1207.
723. Gehlen H, Sundermann T, Rohn K, et al. Aldosterone plasma concentration in horses with heart valve insufficiencies. *Res Vet Sci*. 2008;85:340.
724. Marr CM. Heart failure. In: Marr CM, Bowen M, eds. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
725. Belgrave JOS. A case of atrial fibrillation with congestive heart failure. *Equine Vet Educ*. 1990;2:2.
726. Bonagura JD. Clinical evaluation and management of heart disease. *Equine Vet Educ*. 1990;2:31.
727. Brumbaugh GW, Thomas WP, Hodge TG. Medical management of congestive heart failure in a horse. *J Am Vet Med Assoc*. 1982;180:878.
728. Glazier DB. Congestive heart failure and congenital cardiac defects in horses. *Equine Pract*. 1986;8:20.
729. Reef VB, Levitan CW, Spencer PA. Factors affecting prognosis and conversion in equine atrial fibrillation. *J Vet Intern Med*. 1988;2:1.
730. Reimer JM, Reef VB, Sommer M. Echocardiographic detection of pulmonic valve rupture in a horse with right-sided heart failure. *J Am Vet Med Assoc*. 1991;198:880.
731. Reimer JM, Marr CM, Reef VB, et al. Aortic origin of the right pulmonary artery and patent ductus arteriosus in a pony foal with pulmonary hypertension and right-sided heart failure. *Equine Vet J*. 1993;25:466.
732. Seahorn TL, Hormanski CE. Ventricular septal defect and atrial fibrillation in an adult horse—a case report. *J Equine Vet Sci*. 1993;13:36.
733. Taylor FG, Wotton PR, Hillyer MH, et al. Atrial septal defect and atrial fibrillation in a foal. *Vet Rec*. 1991;128:80.
734. Wijnberg ID, van der Kolk JH, van Garderen E, et al. Atrial fibrillation associated with central nervous symptoms and colic in a horse: a case of equine cardiomyopathy. *Vet Q*. 1998;20:73.
735. Nout YS, Hinchcliff KW, Bonagura JD, et al. Cardiac amyloidosis in a horse. *J Vet Intern Med*. 2003;17:588.
736. Bonagura JD. Diagnosis of cardiac arrhythmias. In: Robinson NE, ed. *Current Therapy in Equine Medicine 4*. Philadelphia: W.B. Saunders; 1997.
737. Garber JL, Reef VB, Reimer JM. Sonographic findings in horses with mediastinal lymphosarcoma: 13 cases (1985-1992). *J Am Vet Med Assoc*. 1994;205:1432.
738. Reef VB, Gentile DG, Freeman DE. Successful treatment of pericarditis in a horse. *J Am Vet Med Assoc*. 1984;185:94.
739. Bernard W, Reef VB, Clark ES, et al. Pericarditis in horses: six cases (1982-1986). *J Am Vet Med Assoc*. 1990;196:468.
740. Malalana F, Bardell D, McKane S. Idiopathic aseptic pericardial effusion with cardiac tamponade in a horse. *Equine Vet Educ*. 2011;23:64.

741. Voros K, Felkai C, Szilagyi Z, et al. Two-dimensional echocardiographically guided pericardiocentesis in a horse with traumatic pericarditis. *J Am Vet Med Assoc*. 1991;198:1953.
742. Edem E, Kahyaoglu B, Cakar MA. Acute effusive pericarditis due to horse chestnut consumption. *Am J Case Rep*. 2016;17:305.
743. Opie LH, Gersh BJ. *Drugs for the Heart*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2013.
744. Hinchcliff KW, Muir 3rd WW. Pharmacology of furosemide in the horse: a review. *J Vet Intern Med*. 1991;5:211.
745. Johansson AM, Gardner SY, Levine JF, et al. Pharmacokinetics and pharmacodynamics of furosemide after oral administration to horses. *J Vet Intern Med*. 2004;18:739.
746. Rivas LJ, Hinchcliff KW. Effect of furosemide and subsequent intravenous fluid administration on right atrial pressure of splenectomized horses. *Am J Vet Res*. 1997;58:632.
747. Johansson AM, Gardner SY, Levine JF, et al. Furosemide continuous rate infusion in the horse: evaluation of enhanced efficacy and reduced side effects. *J Vet Intern Med*. 2003;17:887.
748. Abbott LM, Kovacic J. The pharmacologic spectrum of furosemide. *J Vet Emerg Crit Care*. 2008;18:26.
749. Baggot JD. The pharmacological basis of cardiac drug selection for use in horses. *Equine Vet J Suppl*. 1995;97.
750. Pearson EG, Ayres JW, Wood GL, et al. Digoxin toxicity in a horse. *Comp Cont Educ Pract*. 1987;9:958.
751. Marr CM. Treatment of cardiac arrhythmias and cardiac failure. In: Robinson NE, ed. *Current Therapy in Equine Medicine 4*. Philadelphia: WB Saunders; 1997.
752. Parraga ME, Kittleson MD, Drake CM. Quinidine administration increases steady state serum digoxin concentration in horses. *Equine Vet J Suppl*. 1995;114.
753. Manohar M. Effects of glyceryl trinitrate (nitroglycerin) on pulmonary vascular pressures in standing thoroughbred horses. *Equine Vet J*. 1995;27:275.
754. Gardner SY, Atkins CE, Sams RA, et al. Characterization of the pharmacokinetic and pharmacodynamic properties of the angiotensin-converting enzyme inhibitor, enalapril, in horses. *J Vet Intern Med*. 2004;18:231.
755. Sleeper MM, McDonnell SM, Ely JJ, et al. Chronic oral therapy with enalapril in normal ponies. *J Vet Cardiol*. 2008;10:111.
756. Muñoz A, Esgueva M, Gómez-Díez M, et al. Modulation of acute transient exercise-induced hypertension after oral administration of four angiotensin-converting enzyme inhibitors in normotensive horses. *Vet J*. 2016;208:33.
757. Gomez-Diez M, Munoz A, Caballero JM, et al. Pharmacokinetics and pharmacodynamics of enalapril and its active metabolite, enalaprilat, at four different doses in healthy horses. *Res Vet Sci*. 2014;97:105.
758. Luciani A, Civitella C, Santori D, et al. Haemodynamic effects in healthy horses treated with an ACE-inhibitor (Ramipril). *Vet Res Commun*. 2007;31(suppl 1):297.
759. Serrano-Rodriguez JM, Gomez-Diez M, Esgueva M, et al. Pharmacokinetics and pharmacodynamics of ramipril and ramiprilat after intravenous and oral doses of ramipril in healthy horses. *Vet J*. 2016;208:38.
760. Davis JL, Kruger K, LaFevers DH, et al. Effects of quinapril on angiotensin converting enzyme and plasma renin activity as well as pharmacokinetic parameters of quinapril and its active metabolite, quinaprilat, after intravenous and oral administration to mature horses. *Equine Vet J*. 2014;46:729.
761. Afonso T, Giguere S, Rapoport G, et al. Attenuation of the blood pressure response to exogenous angiotensin I after oral administration of benazepril to healthy adult horses. *Equine Vet J*. 2016.
762. Fuentes VL. Use of pimobendan in the management of heart failure. *Vet Clin North Am Small Anim Pract*. 2004;34:1145.
763. Macgregor JM, Rush JE, Laste NJ, et al. Use of pimobendan in 170 cats (2006-2010). *J Vet Cardiol*. 2011;13:251.
764. Reina-Doreste Y, Stern JA, Keene BW, et al. Case-control study of the effects of pimobendan on survival time in cats with hypertrophic cardiomyopathy and congestive heart failure. *J Am Vet Med Assoc*. 2014;245:534.
765. Vollmar AC, Fox PR. Long-term outcome of Irish wolfhound dogs with preclinical cardiomyopathy, atrial fibrillation, or both treated with pimobendan, benazepril hydrochloride, or methyldigoxin monotherapy. *J Vet Intern Med*. 2016; 30:553.
766. Haggstrom J, Lord PF, Hoglund K, et al. Short-term hemodynamic and neuroendocrine effects of pimobendan and benazapril in dogs with myxomatous mitral valve disease and congestive heart failure. *J Vet Intern Med*. 2013;27:1452.
767. Haggstrom J, Boswood A, O'Grady M, et al. Longitudinal analysis of quality of life, clinical, radiographic, echocardiographic, and laboratory variables in dogs with myxomatous mitral valve disease receiving pimobendan or benazepril: the QUEST study. *J Vet Intern Med*. 2013;27:1441.
768. Buergelt CD, Carmichael JA, Tashjian RJ, et al. Spontaneous rupture of the left pulmonary artery in a horse with patent ductus arteriosus. *J Am Vet Med Assoc*. 1970;157:313.
769. Chaffin MK, Miller MW, Morris EL. Double outlet right ventricle and other associated congenital cardiac anomalies in an American miniature horse foal. *Equine Vet J*. 1992;24:402.
770. Cottrill CM, Rossdale PD. A comparison of congenital heart disease in horses and man. *Equine Vet J*. 1992;24:338.
771. Cox VS, Weber AF, de Lima A. Left cranial vena cava in a horse. *Anat Histol Embryol*. 1991;20:37.
772. Critchley KL. An interventricular septal defect, pulmonary stenosis and bicuspid pulmonary valve in a Welsh pony foal. *Equine Vet J*. 1976;8:176.
773. Ecke P, Malik R, Kannegieter NJ. Common atrioventricular canal in a foal. *N Z Vet J*. 1991;39:97.
774. Glazier DB, Farrelly BT, Neylon JF. Patent ductus arteriosus in an eight-month-old foal. *Irish Vet J*. 1974;28:12.
775. Glazier DB, Farrelly BT, O'Connor J. Ventricular septal defect in a 7-year-old gelding. *J Am Vet Med Assoc*. 1975;167:49.
776. Greene HJ, Wray DD, Greenway GA. Two equine congenital cardiac anomalies. *Irish Vet J*. 1975;29:115.
777. Guarda F, Rattazzi C, Appina S. Pathology of cardiac aneurysms in horses. *Pferdeheilkunde*. 1992;8:241.
778. Huston R, Saperstein G, Leipold HW. Congenital-defects in foals. *J Equine Med Surg*. 1977;1:146.
779. Johnson JW, DeBowes RM, Cox JH, et al. Diaphragmatic hernia with a concurrent cardiac defect in an Arabian foal. *J Equine Vet Sci*. 1984;4:225.
780. King JM, Flint TJ, Anderson WI. Incomplete subaortic stenotic rings in domestic animals—a newly described congenital anomaly. *Cornell Vet*. 1988;78:263.
781. Kvart C, Carlsten J, Jeffcott LB, et al. Diagnostic value of contrast echocardiography in the horse. *Equine Vet J*. 1985;17:357.
782. Lombard CW, Scarratt WK, Buergelt CD. Ventricular septal defects in the horse. *J Am Vet Med Assoc*. 1983;183:562.
783. Reef VB. Equine pediatric ultrasonography. *Comp Cont Educ Pract*. 1991;13:1277.
784. Reppas GP, Canfield PJ, Hartley WJ, et al. Multiple congenital cardiac anomalies and idiopathic thoracic aortitis in a horse. *Vet Rec*. 1996;138:14.
785. Steyn PF, Holland P, Hoffman J. The angiocardiographic diagnosis of a persistent truncus arteriosus in a foal. *J S Afr Vet Assoc*. 1989;60:106.
786. Wilson RB, Haffner JC. Right atrioventricular atresia and ventricular septal defect in a foal. *Cornell Vet*. 1987;77:187.
787. Zamora CS, Vitums A, Foreman JH, et al. Common ventricle with separate pulmonary outflow chamber in a horse. *J Am Vet Med Assoc*. 1985;186:1210.
788. Zamora CS, Vitums A, Sande RD, et al. Multiple cardiac malformation in a horse. *Anat Histol Embryol*. 1988;17:95.

789. Patterson-Kane JC, Harrison LR. Giant right atrial diverticulum in a foal. *J Vet Diagn Invest*. 2002;14:335.
790. Anderson RH. The pathological spectrum of pulmonary atresia. *Equine Vet Educ*. 1997;9:128.
791. Young LE, Blunden AS, Bartram DH, et al. Pulmonary atresia with an intact ventricular septum in a Thoroughbred foal. *Equine Vet Educ*. 1997;9:123.
792. Stephen JO, Abbott J, Middleton DM, et al. Persistent truncus arteriosus in a Bashkir Curly foal. *Equine Vet Educ*. 2000;12:251.
793. McGurrin MK, Physick-Sheard PW, Southorn E. Parachute left atrioventricular valve causing stenosis and regurgitation in a Thoroughbred foal. *J Vet Intern Med*. 2003;17:579.
794. Michlik KM, Biazik AK, Henklewski RZ, et al. Quadricuspid aortic valve and a ventricular septal defect in a horse. *BMC Vet Res*. 2014;10:142.
795. Spiro I. Hematuria and a complex congenital heart defect in a newborn foal. *Can Vet J*. 2002;43:375.
796. Tabaran AF, Nagy AL, Catoi C, et al. Congenital diaphragmatic hernia with concurrent aplasia of the pericardium in a foal. *BMC Vet Res*. 2015;11:309.
797. Anderson R. Nomenclature and classification: sequential segmental analysis. In: Moller JH, HJ, eds. *Pediatric Cardiovascular Medicine*. New York: Churchill Livingstone; 2003.
798. Van Praagh R. Nomenclature and classification: morphologic and segmental approach to diagnosis. In: Moller JH, Hoffman JIE, eds. *Pediatric Cardiovascular Medicine*. New York: Churchill Livingstone; 2003.
799. Shinebourne EA, Macartney FJ, Anderson RH. Sequential chamber localization—logical approach to diagnosis in congenital heart disease. *Br Heart J*. 1976;38:327.
800. Schwarzwald CC. Sequential segmental analysis—a systematic approach to the diagnosis of congenital cardiac defects. *Equine Vet Educ*. 2008;20:305.
801. Tynan MJ, Becker AE, Macartney FJ, et al. Nomenclature and classification of congenital heart disease. *Br Heart J*. 1979;41:544.
802. Ezon D, Goldberg J, Kyle W. *Atlas of Congenital Heart Disease Nomenclature: An Illustrated Guide to the Van Praagh and Anderson Approaches to Describing Congenital Cardiac Pathology*. Houston, TX: CreateSpace Independent Publishing Platform; 2015.
803. Fennell LC, Church S, Tyrell D, et al. Double-outlet right ventricle in a 10-month-old Friesian filly. *Aust Vet J*. 2009;87:204.
804. Vitums A. Origin of the aorta and pulmonary trunk from the right ventricle in a horse. *Pathol Vet*. 1970;7:482.
805. Vitums A, Grant BD, Stone EC, et al. Transposition of the aorta and atresia of the pulmonary trunk in a horse. *Cornell Vet*. 1973;63:41.
806. Sleeper MM, Palmer JE. Echocardiographic diagnosis of transposition of the great arteries in a neonatal foal. *Vet Radiol Ultrasound*. 2005;46:259.
807. Petrick SW, Roos CJ, van Niekerk J. Persistent right aortic arch in a horse. *J S Afr Vet Assoc*. 1978;49:355.
808. Butt TD, MacDonald DG, Crawford WH, et al. Persistent right aortic arch in a yearling horse. *Can Vet J*. 1998;39:714.
809. van der Linde-Sipman JS, Goedegebuure SA, Kroneman J. Persistent right aortic arch associated with a persistent left ductus arteriosus and an interventricular septal defect in a horse. *Tijdschr Diergeneeskd*. 1979;104(suppl).
810. Valdes-Martinez A, Eades SC, Strickland KN, et al. Echocardiographic evidence of an aortico-pulmonary septal defect in a 4-day-old thoroughbred foal. *Vet Radiol Ultrasound*. 2006;47:87.
811. Cottrill CM, O'Connor WN, Cudd T, et al. Persistence of foetal circulatory pathways in a newborn foal. *Equine Vet J*. 1987;19:252.
812. Coumbe KM. Cardiac disease: endocardial fibroelastosis. *Equine Vet Educ*. 2002;14:81.
813. Seco DO, Desrochers A, Hoffmann V, et al. Total anomalous pulmonary venous connection in a foal. *Vet Radiol Ultrasound*. 2005;46:83.
814. Sedacca CD, Bright JM, Boon J. Doppler echocardiographic description of double-inlet left ventricle in an Arabian horse. *J Vet Cardiol*. 2010;12:147.
815. Cottrill CM, Ho SY, O'Connor WN. Embryological development of the equine heart. *Equine Vet J Suppl*. 1997;14.
816. Rudolph A. *Congenital Diseases of the Heart: Clinical-Physiological Considerations*. New York: Blackwell; 2011.
817. Edwards JE, Edwards BS, Jesse E. *Edwards' Synopsis of Congenital Heart Disease*. New York: Futura Publishing Company; 2000.
818. Michaëlsson M, Ho SY. *Congenital Heart Malformations in Mammals: An Illustrated Text*. London: Imperial College Press; 2000.
819. Ho SY, Rigby ML, Anderson RH. *Echocardiography in Congenital Heart Disease Made Simple*. London: Imperial College Press; 2005.
820. MacDonald AA, Fowden AL, Silver M, et al. The foramen ovale of the foetal and neonatal foal. *Equine Vet J*. 1988;20:255.
821. Rang H, Hurtienne H. Persistent truncus arteriosus in a 2-year old horse. *Tierarztl Prax*. 1976;4:55.
822. Jesty SA, Wilkins PA, Palmer JE, et al. Persistent truncus arteriosus in two Standardbred foals. *Equine Vet Educ*. 2007;19:307.
823. Koblik PD, Hornof WJ. Use of first-pass nuclear angiocardiography to detect left-to- right cardiac shunts in the horse. *Vet Radiology*. 1987;28:177.
824. Froehlich W, Wlaschitz S, Riedelberger K, et al. Tricuspid valve endocarditis in a horse with a ventricular septal defect. *Equine Vet Educ*. 2006;18:172.
825. Gehlen H, Stadler P. Advantages and disadvantages of invasive and non-invasive examination techniques in 11 horses with ventricular septal defects. *Tierarztl Prax Ausg Grosstiere Nutztiere*. 2001;29:234.
826. Short DM, Seco OM, Jesty SA, et al. Spontaneous closure of a ventricular septal defect in a horse. *J Vet Intern Med*. 2010;24:1515.
827. Hughes KJ. Diagnostic challenge: lethargy and weakness in an Arabian foal with cardiac murmurs. Ventricular septal defect (VSD). *Aust Vet J*. 2006;84:209.
828. Button C, Gross DR, Allert JA, et al. Tricuspid atresia in a foal. *J Am Vet Med Assoc*. 1978;172:825.
829. Kutasi O, Voros K, Biksi I, et al. Common atrioventricular canal in a newborn foal—Case report and review of the literature. *Acta Vet Hung*. 2007;55:51.
830. Kraus MS, Pariaut R, Alcaraz A, et al. Complete atrioventricular canal defect in a foal: clinical and pathological features. *J Vet Cardiol*. 2005;7:59.
831. Steininger Berli, Jud, et al. Echocardiography in Saanen-goats: normal findings, reference intervals in awake goats, and the effect of general anesthesia. *Schweiz Arch Tierheilkd*. 2011;153:553.
832. Krüger MU, Wünschmann A, Ward C, et al. Pulmonary atresia with intact ventricular septum and hypoplastic right ventricle in an Arabian foal. *J Vet Cardiol*. 2016.
833. Prickett ME, Reeves JT, Zent WW. Tetralogy of fallot in a Thoroughbred foal. *J Am Vet Med Assoc*. 1973;162:552.
834. Reynolds DJ, Nicholl TK. Tetralogy of Fallot and cranial mesenteric arteritis in a foal. *Equine Vet J*. 1978;10:185.
835. Borst GH. Tetralogy of Fallot in a Belgian foal (author's transl). *Tijdschr Diergeneeskd*. 1978;103:968.
836. Keith Jr JC. Tetralogy of Fallot in a quarter horse foal. *Vet Med Small Anim Clin*. 1981;76:889.
837. Schmitz RR, Klaus C, Grabner A. Detailed echocardiographic findings in a newborn foal with tetralogy of Fallot. *Equine Vet Educ*. 2008;20:298.
838. Tschudi PR, Staufenbiel B, Ueltschi G. Echocardiographical documentation of a truncus arteriosus communis in a colt. *Pferdeheilkunde*. 1997;13:387.

839. Honnas CM, Puckett MJ, Schumacher J. Tricuspid-atresia in a quarter horse foal. *Southwest Vet*. 1987;38:17.
840. van Nie CJ, van der Kamp JS. Congenital tricuspid atresia in a premature foal. *Tijdschr Diergeneeskd*. 1979;104:411.
841. van der Linde-Sipman JS, van den Ingh TS. Tricuspid atresia in a foal and a lamb. *Zentralbl Veterinarmed A*. 1979;26A:239.
842. Gumbrell RC. Atresia of the tricuspid valve in a foal. *N Z Vet J*. 1970;18:253.
843. Marr CM. Cardiac and respiratory disease in aged horses. *Vet Clin North Am Equine Pract*. 2016;32:283.
844. Cipone M, Pietra M, Guglielmini C, et al. Aortic insufficiency in horses. Results of electrophonocardiography, ultrasonic cardiography and carotid pulsed-wave Doppler echocardiography in two cases. *Obiettivi e Documenti Veterinari*. 1995;16:37.
845. Deprez P, Sustronck B, Vanroy M, et al. A case of mitral-valve insufficiency due to a ruptured chorda tendinea in a horse. *Vlaams Diergen Tijds*. 1993;62:180.
846. Littlewort MC. Cardiological problems in equine medicine. *Equine Vet J*. 1977;9:173.
847. Yamaga Y, Shibui I, Yasuda J, et al. Echocardiographic and ultrasonographic observations in a horse with mitral regurgitation and "intrahepatic cholangiocellular fibroadenomatosis." *Advances in Animal Cardiology*. 1985;18:65.
848. Buergelt CD. Equine cardiovascular pathology: an overview. *Anim Health Res Rev*. 2003;4:109.
849. Patteson MW. Echocardiographic evaluation of horses with aortic regurgitation. *Equine Vet Educ*. 1994;6:159.
850. Young L. Equine aortic valve regurgitation: a disease worthy of further consideration. *Equine Vet Educ*. 2007;19:469.
851. Shaftoe S, Mcguirk SM. Valvular insufficiency in a horse with atrial fibrillation. *Comp Cont Educ Pract*. 1987;9:203.
852. Frohlich W, Wlaschitz S, Riedelberger K, et al. Case report: aortic valve endocarditis in a horse. *Dtsch Tierarztl Wochenschr*. 2004;111:370.
853. Afonso T, Verheyen T, Saey V, et al. Severe aortic regurgitation due to endocarditis in a horse. *Vlaams Diergen Tijds*. 2011;80:49.
854. Muñoz A, Riber C, Trigo P, et al. Bacterial endocarditis in two Spanish foals after neonatal septicemia. *J Equine Vet Sci*. 2012;32:760.
855. Porter SR, Saegerman C, Galen Gv, et al. Vegetative endocarditis in equids. *J Vet Intern Med*. 2008;22:1411.
856. Roussel AJ, Kasari TR. Bacterial endocarditis in large animals. Part II. Incidence, causes, clinical signs, and pathologic findings. *Comp Cont Educ Pract*. 1989;11:769.
857. Sponseller BT, Ware WA. Successful treatment of staphylococcal endocarditis in a horse. *Equine Vet Educ*. 2001;13:289.
858. Verdegaal EJMM, de Heer N, Meertens NM, et al. A right-sided bacterial endocarditis of dental origin in a horse. *Equine Vet Educ*. 2006;18:191.
859. Verdegaal EJMM. Sloet van Oldruitenborgh-Oosterbaan MM. Clinical commentary: endocarditis in the horse. *Equine Vet Educ*. 2006;18:196.
860. Aalbaek B, Ostergaard S, Buhl R, et al. *Actinobacillus equuli* subsp. *equuli* associated with equine valvular endocarditis. *Acta Pathol Microbiol Scand*. 2007;115:1437.
861. Blissitt KJ, Patteson MW. Evaluation of cardiac murmurs in horses. 2. *Echocardiography, In Pract*. 1996;18:416.
862. Gehlen H, Goltz A, Rohn K, et al. A survey of the frequency and development of heart disease in riding horses—Part 2: Clinical and echocardiographic follow-up examination. *Pferdeheilkunde*. 2007;23:378.
863. Spörri H, Leemann W. Pathophysiology of aortic valve insufficiency in horses. *Berl Munch Tierarztl Wochenschr*. 1972;85:441.
864. Lester GD, Lombard CW, Ackerman N. Echocardiographic detection of a dissecting aortic root aneurysm in a Thoroughbred stallion. *Vet Radiol Ultrasound*. 1992;33:202.
865. Reef VB, Klumpp S, Maxson AD, et al. Echocardiographic detection of an intact aneurysm in a horse. *J Am Vet Med Assoc*. 1990;197:752.
866. Shirai W, Momotani E, Sato T, et al. Dissecting aortic aneurysm in a horse. *J Comp Pathol*. 1999;120:307.
867. Stadler P, Wohlsein P, Gratopp M, et al. Echocardiographic and radiographic imaging of aortic root and aortic arch aneurysm in the horse. *Pferdeheilkunde*. 1996;12:91.
868. Spörri H. Two clinical types of aortic insufficiency in horses. *Ann N Y Acad Sci*. 1965;127:358.
869. Stadler P, Hoch M, Fruhauf B, et al. Echocardiography in horses with and without heart murmurs in aortic regurgitation. *Pferdeheilkunde*. 1995;11:373.
870. Jesty SA, Reef VB. Septicemia and cardiovascular infections in horses. *Vet Clin North Am Equine Pract*. 2006;22:481.
871. Guidi EE, Thomas A, Cadore JL, et al. *Citrobacter freundii* induced endocarditis in a yearling colt. *Can Vet J*. 2016;57:767.
872. Marr CM. Cardiovascular infections. In: Sellon DC, Long MT, eds. *Equine Infectious Diseases*. St. Louis: Saunders Elsevier; 2014.
873. Haldar SM, O'Gara PT. Infective endocarditis: diagnosis and management. *Nat Clin Pract Cardiovasc Med*. 2006;3:310.
874. Bertone JJ, Dill SG. Traumatic gastropericarditis in a horse. *J Am Vet Med Assoc*. 1985;187:742.
875. Wagner PC. Pericarditis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 1*. Philadelphia: W.B. Saunders; 1983.
876. Robinson JA, Marr CM, Reef VB, et al. Idiopathic, aseptic, effusive, fibrinous, nonconstrictive pericarditis with tamponade in a standardbred filly. *J Am Vet Med Assoc*. 1992;201:1593.
877. Bradfield T. Traumatic pericarditis in a horse. *Southwestern Vet*. 1970;23:145.
878. Orsini JA, Koch C, Stewart B. Peritoneopericardial hernia in a horse. *J Am Vet Med Assoc*. 1981;179:907.
879. Buergelt CD, Wilson JH, Lombard CW. Pericarditis in horses. *Comp Cont Educ Pract*. 1990;12:872.
880. Morley PS, ChirinoTrejo M, Petrie L, et al. Pericarditis and pleuritis caused by *Mycoplasma felis* in a horse. *Equine Vet J*. 1996;28:237.
881. May KA, Cheramie HS, Howard RD, et al. Purulent pericarditis as a sequela to clostridial myositis in a horse. *Equine Vet J*. 2002;34:636.
882. Guarda F, Rattazzi C, Appino S. Ventricular myocardium and pericardium disease in horses. *Ippologia*. 1993;4:43.
883. Wilson JH, Olson EJ, Haugen EW, et al. Systemic blastomycosis in a horse. *J Vet Diagn Invest*. 2006;18:615.
884. Sebastian M, Gantz MG, Tobin T, et al. The mare reproductive loss syndrome and the eastern tent caterpillar: a toxicokinetic/statistical analysis with clinical, epidemiologic, and mechanistic implications. *Vet Ther*. 2003;4:324.
885. Sebastian MM, Bernard WV, Riddle TW, et al. Review paper: mare reproductive loss syndrome. *Vet Pathol*. 2008;45:710.
886. Donahue JM, Sells SF, Bolin DC. Classification of *Actinobacillus* spp isolates from horses involved in mare reproductive loss syndrome. *Am J Vet Res*. 2006;67:1426.
887. Marcus LC, Ross Jr JN. Microscopic lesions in the hearts of aged horses and mules. *Pathol Vet*. 1967;4:162.
888. King JM, Roth L, Haschek WM. Myocardial necrosis secondary to neural lesions in domestic animals. *J Am Vet Med Assoc*. 1982;180:144.
889. Shawley RV, Rolf Jr LL. Experimental cantharidiasis in the horse. *Am J Vet Res*. 1984;45:2261.
890. Hulland TJ. Leptomeric fibrils in the myocardial fibers of a foal. *Vet Pathol*. 1988;25:175.
891. Schmitz DG. Cantharidin toxicosis in horses. *J Vet Intern Med*. 1989;3:208.
892. Freestone JF, Williams MM, Norwood G. Thoracic haemangiosarcoma in a 3-year-old horse. *Aust Vet J*. 1990;67:269.
893. Guarda F, Rattazzi C. Pathology of cardiac ventricular aneurysms in the horse. *Schweiz Arch Tierheilkd*. 1994;136:76.

894. Reef VB. Myocardial disease. In: Robinson NE, ed. *Current Therapy in Equine Medicine 3*. Philadelphia: W.B. Saunders; 1992.
895. Weldon AD, Step DL, Moise NS. Lymphosarcoma with myocardial infiltration in a mare. *Vet Med*. 1992;87:595.
896. Ferrari JJF, Leme MCM, Macruz R. Occurrence of non-physiological cardiac hypertrophy in a racehorse. *Arquivos do Instituto Biologico Sao Paulo*. 1989;56:54.
897. Galey FD, Holstege DM, Plumlee KH, et al. Diagnosis of oleander poisoning in livestock. *J Vet Diagn Invest*. 1996;8:358.
898. Tiwary AK, Puschner B, Kinde H, et al. Diagnosis of Taxus (yew) poisoning in a horse. *J Vet Diagn Invest*. 2005;17:252.
899. Freel KM, Morrison LR, Thompson H, et al. Arrhythmogenic right ventricular cardiomyopathy as a cause of unexpected cardiac death in two horses. *Vet Rec*. 2010;166:718.
900. Hoffman A, Levi O, Orgad U, et al. Myocarditis following envenoming with *Vipera palaestinae* in two horses. *Toxicon*. 1993;31:1623.
901. Peek SF, Marques FD, Morgan J, et al. Atypical acute monensin toxicosis and delayed cardiomyopathy in Belgian draft horses. *J Vet Intern Med*. 2004;18:761.
902. Van Vleet JF, Ferrans VJ. Myocardial diseases of animals. *Am J Pathol*. 1986;124:98.
903. Navas de Solis C, Slack J, Boston RC, et al. Hypertensive cardiomyopathy in horses: 5 cases (1995–2011). *J Am Vet Med Assoc*. 2013;243:126.
904. Bauquier J, Stent A, Gibney J, et al. Evidence for marshmallow (*Malva parviflora*) toxicosis causing myocardial disease and myopathy in four horses. *Equine Vet J*. 2016.
905. Headley SA, de Carvalho PH, Cunha Filho LF, et al. Equine pulmonary aspergillosis with encephalitic, myocardial, and renal dissemination. *Mycopathologia*. 2014;177:129.
906. Haaland MA, Davidson JP. Spontaneous left atrial rupture with associated chronic fibrotic myocarditis in a stallion. *Vet Med Small Anim Clin*. 1983;78.
907. Delesalle C, van Loon G, Nollet H, et al. Tumor-induced ventricular arrhythmia in a horse. *J Vet Intern Med*. 2002; 16:612.
908. Reef VB, Dyson SS, Beech J. Lymphosarcoma and associated immune-mediated hemolytic anemia and thrombocytopenia in horses. *J Am Vet Med Assoc*. 1984;184:313.
909. Southwood LL, Schott 2nd HC, Henry CJ, et al. Disseminated hemangiosarcoma in the horse: 35 cases. *J Vet Intern Med*. 2000;14:105.
910. Hamir AN, Habecker P, Tulleners E. Equine intrapericardial lipoma. *Vet Rec*. 1994;135:235.
911. Penrose LC, Brower A, Kirk G, et al. Primary cardiac lymphoma in a 10-year-old equine gelding. *Vet Rec*. 2012;171:20.
912. Renier AC, Kass PH, Magdesian KG, et al. Oleander toxicosis in equids: 30 cases (1995–2010). *J Am Vet Med Assoc*. 2013;242:540.
913. Decloedt A, de Clercq D, Ven S, et al. Right atrial and right ventricular ultrasound-guided biopsy technique in standing horses. *Equine Vet J*. 2016;48:346.
914. Peet RL, McDermott J, Williams JM, et al. Fungal myocarditis and nephritis in a horse. *Aust Vet J*. 1981;57:439.
915. Aleman M, Magdesian KG, Peterson TS, et al. Salinomycin toxicosis in horses. *J Am Vet Med Assoc*. 2007;230:1822.
916. Wijnberg ID, van der Kolk JH, Hiddink EG. Use of phenytoin to treat digitalis-induced cardiac arrhythmias in a miniature Shetland pony. *Vet Rec*. 1999;144:259.
917. Hughes KJ, Dart AJ, Hodgson DR. Suspected *Nerium oleander* (Oleander) poisoning in a horse. *Aust Vet J*. 2002;80:412.
918. Smith PA, Aldridge BM, Kittleson MD. Oleander toxicosis in a donkey. *J Vet Intern Med*. 2003;17:111.
919. Cortinovis C, Caloni F. Epidemiology of intoxication of domestic animals by plants in Europe. *Vet J*. 2013;197:163.
920. Woods LW, Filigenzi MS, Booth MC, et al. Summer pheasant's eye (*Adonis aestivalis*) poisoning in three horses. *Vet Pathol Online*. 2004;41:215.
921. Beasley VR, Wolf GA, Fischer DC, et al. Cantharidin toxicosis in horses. *J Am Vet Med Assoc*. 1983;182:283.
922. Schoeb TR, Panciera RJ. Blister beetle poisoning in horses. *J Am Vet Med Assoc*. 1978;173:75.
923. Schoeb TR, Panciera RJ. Pathology of blister beetle (*Epicauta*) poisoning in horses. *Vet Pathol*. 1979;16:18.
924. Ray AC, Post LO, Hurst JM, et al. Evaluation of an analytical method for the diagnosis of cantharidin toxicosis due to ingestion of blister beetles (*Epicauta lemniscata*) by horses and sheep. *Am J Vet Res*. 1980;41:932.
925. Ray AC, Kyle AL, Murphy MJ, et al. Etiologic agents, incidence, and improved diagnostic methods of cantharidin toxicosis in horses. *Am J Vet Res*. 1989;50:187.
926. Helman RG, Edwards WC. Clinical features of blister beetle poisoning in equids: 70 cases (1983-1996). *J Am Vet Med Assoc*. 1997;211:1018.
927. Edwards WC, Edwards RM, Ogden L, et al. Cantharidin content of two species of Oklahoma blister beetles associated with toxicosis in horses. *Vet Hum Toxicol*. 1989;31:442.
928. Whitlock RH. Feed additives and contaminants as a cause of equine disease. *Vet Clin North Am Equine Pract*. 1990;6:467.
929. Cassart D, Baise E, Cherel Y, et al. Morphological alterations in oxidative muscles and mitochondrial structure associated with equine atypical myopathy. *Equine Vet J*. 2007;39:26.
930. McKenzie RK, Hill FI, Habyarimana JA, et al. Detection of hypoglycin A in the seeds of sycamore (*Acer pseudoplatanus*) and box elder (*A. negundo*) in New Zealand; the toxin associated with cases of equine atypical myopathy. *N Z Vet J*. 2016;64:182.
931. Westermann CM, van Leeuwen R, van Raamsdonk LW, et al. Hypoglycin a concentrations in maple tree species in the Netherlands and the occurrence of atypical myopathy in horses. *J Vet Intern Med*. 2016;30:880.
932. Baise E, Habyarimana JA, Amory H, et al. Samaras and seedlings of *Acer pseudoplatanus* are potential sources of hypoglycin A intoxication in atypical myopathy without necessarily inducing clinical signs. *Equine Vet J*. 2016;48:414.
933. Zuraw A, Dietert K, Kuhnel S, et al. Equine atypical myopathy caused by hypoglycin A intoxication associated with ingestion of sycamore maple tree seeds. *Equine Vet J*. 2016;48:418.
934. Gonzalez-Medina S. Update on the cause of equine atypical myopathy. *Vet Rec*. 2015;176:143.
935. Unger L, Nicholson A, Jewitt EM, et al. Hypoglycin A concentrations in seeds of *Acer pseudoplatanus* trees growing on atypical myopathy-affected and control pastures. *J Vet Intern Med*. 2014;28:1289.
936. Valberg SJ, Sponseller BT, Hegeman AD, et al. Seasonal pasture myopathy/atypical myopathy in North America associated with ingestion of hypoglycin A within seeds of the box elder tree. *Equine Vet J*. 2013;45:419.
937. Westermann CM, Dorland L, Votion DM, et al. Acquired multiple Acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy. *Neuromuscul Disord*. 2008;18:355.
938. Sponseller BT, Valberg SJ, Schultz NE, et al. Equine multiple acyl-CoA dehydrogenase deficiency (MADD) associated with seasonal pasture myopathy in the midwestern United States. *J Vet Intern Med*. 2012;26:1012.
939. Dickinson CE, Traub-Dargatz JL, Dargatz DA, et al. Rattlesnake venom poisoning in horses: 32 cases (1973-1993). *J Am Vet Med Assoc*. 1996;208:1866.
940. Bautista AC, Tahara J, Mete A, et al. Diagnostic value of tissue monensin concentrations in horses following toxicosis. *J Vet Diagn Invest*. 2014;26:423.
941. Rollinson J, Taylor FG, Chesney J. Salinomycin poisoning in horses. *Vet Rec*. 1987;121:126.
942. Nicpon J, Czerw P, Harps O, et al. Salinomycin poisoning in a Polish stud horse. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 1997;25:438.
943. Kronfeld DS. Lasalocid toxicosis is inadequately quantified for horses. *Vet Hum Toxicol*. 2002;44:245.

944. Novilla MN. The veterinary importance of the toxic syndrome induced by ionophores. *Vet Hum Toxicol*. 1992;34:66.
945. Oehme FW, Pickrell JA. An analysis of the chronic oral toxicity of polyether ionophore antibiotics in animals. *Vet Hum Toxicol*. 1999;41:251.
946. Kamphues J, Meyer H, Liebler EM, et al. Animal nutrition for veterinarians—recent cases of clinical disorders in horses after intake of ionophore-containing feed. *Dtsch Tierarztl Wochenschr*. 1990;97:537.
947. Cranley JJ. Focal medial calcification of the pulmonary artery: a survey of 1066 horses. *Equine Vet J*. 1983;15:278.
948. Harrington DD, Page EH. Acute vitamin D3 toxicosis in horses: case reports and experimental studies of the comparative toxicity of vitamins D2 and D3. *J Am Vet Med Assoc*. 1983;182:1358.
949. Lombardo de Barros CS. Aortic body adenoma in a horse. *Aust Vet J*. 1983;60:61.
950. Knezevic PF, Fessl L. Thrombectomy of the descending aorta in the horse. *Tierarztl Prax Suppl*. 1985;1:94.
951. Spier S. Arterial thrombosis as the cause of lameness in a foal. *J Am Vet Med Assoc*. 1985;187:164.
952. Platt H. Vascular malformations and angiomatous lesions in horses: a review of 10 cases. *Equine Vet J*. 1987;19:500.
953. Parks AH, Guy BL, Rawlings CA, et al. Lameness in a mare with signs of arteriovenous fistula. *J Am Vet Med Assoc*. 1989;194:379.
954. Wallace KD, Selcer BA, Tyler DE, et al. In vitro ultrasonographic appearance of the normal and verminous equine aorta, cranial mesenteric artery, and its branches. *Am J Vet Res*. 1989;50:1774.
955. McDonnell SM, Love CC, Martin BB, et al. Ejaculatory failure associated with aortic-iliac thrombosis in two stallions. *J Am Vet Med Assoc*. 1992;200:954.
956. Welch RD, Dean PW, Miller MW. Pulsed spectral Doppler evaluation of a peripheral arteriovenous fistula in a horse. *J Am Vet Med Assoc*. 1992;200:1360.
957. Dias DP, de Lacerda Neto JC. Jugular thrombophlebitis in horses: a review of fibrinolysis, thrombus formation, and clinical management. *Can Vet J*. 2013;54:65.
958. Dolente BA, Beech J, Lindborg S, et al. Evaluation of risk factors for development of catheter-associated jugular thrombophlebitis in horses: 50 cases (1993-1998). *J Am Vet Med Assoc*. 2005;227:1134.
959. Lankveld DP, Ensink JM, van Dijk P, et al. Factors influencing the occurrence of thrombophlebitis after post-surgical long-term intravenous catheterization of colic horses: a study of 38 cases. *J Vet Med A Physiol Pathol Clin Med*. 2001;48:545.
960. Matsuda K, Suzuki H, Tsunoda N, et al. Jugular thrombophlebitis developed from buccal ulcer in a thoroughbred horse. *J Vet Med Sci*. 2010;72:913.
961. Moreau P, Lavoie JP. Evaluation of athletic performance in horses with jugular vein thrombophlebitis: 91 cases (1988-2005). *J Am Vet Med Assoc*. 2009;235:1073.
962. Ryu SH, Kim JG, Bak UB, et al. A hematogenic pleuropneumonia caused by postoperative septic thrombophlebitis in a Thoroughbred gelding. *J Vet Sci*. 2004;5:75.
963. Hoskinson JJ, Wooten P, Evans R. Nonsurgical removal of a catheter embolus from the heart of a foal. *J Am Vet Med Assoc*. 1991;199:233.
964. Lees MJ, Read RA, Klein KT, et al. Surgical retrieval of a broken jugular catheter from the right ventricle of a foal. *Equine Vet J*. 1989;21:384.
965. Little D, Keene BW, Bruton C, et al. Percutaneous retrieval of a jugular catheter fragment from the pulmonary artery of a foal. *J Am Vet Med Assoc*. 2002;220:212.
966. Scarratt WK, Moll HD, Pleasant RS. Fragmentation of intravenous catheters in three horses. *J Equine Vet Sci*. 1997;17:608.
967. Barakzai S, Chandler K. Use of indwelling intravenous catheters in the horse. *In Pract*. 2003;25:264.
968. Hilbert BJ, Rendano VT. Venous aneurysm in a horse. *J Am Vet Med Assoc*. 1975;167:394.
969. Guglielmini C, Bernardini D. Echo-Doppler findings of a carotid-jugular fistula in a foal. *Vet Radiol Ultrasound*. 2003;44:310.
970. Jabara AG, Hazard GH, O'Shea JD. A congenital vascular naevus in a foal. *Aust Vet J*. 1984;61:286.
971. Miller LM, Reed SM, Gallina AM, et al. Ataxia and weakness associated with fourth ventricle vascular anomalies in two horses. *J Am Vet Med Assoc*. 1985;186:601.
972. Schott 2nd HC, Barbee DD, Hines MT, et al. Clinical vignette. Renal arteriovenous malformation in a quarter horse foal. *J Vet Intern Med*. 1996;10:204.
973. Trope GD, Steel CM, Bowers JR, et al. Distensible superficial venous orbital malformations involving the lower eyelid in two horses. *J Am Vet Med Assoc*. 2010;237:943.
974. Campbell-Beggs CL, Johnson PJ, Wilson DA, et al. Chyloabdomen in a neonatal foal. *Vet Rec*. 1995;137:96.
975. Rooney JR. Rupture of the aorta. *Mod Vet Pract*. 1979;60:391.
976. Saey V, Ploeg M, Delesalle C, et al. Morphometric properties of the thoracic aorta of Warmblood and Friesian horses with and without aortic rupture. *J Comp Pathol*. 2016;154:225.
977. Saey V, Famaey N, Smoljkic M, et al. Biomechanical and biochemical properties of the thoracic aorta in warmblood horses, Friesian horses, and Friesians with aortic rupture. *BMC Vet Res*. 2015;11:285.
978. Ueno T, Nambo Y, Tajima Y, et al. Pathology of lethal peripartum broad ligament haematoma in 31 Thoroughbred mares. *Equine Vet J*. 2010;42:529.
979. Pascoe RR. Rupture of the utero-ovarian or middle uterine artery in the mare at or near parturition. *Vet Rec*. 1979;104:77.
980. Dechant JE, Nieto JE, Le Jeune SS. Hemoperitoneum in horses: 67 cases (1989-2004). *J Am Vet Med Assoc*. 2006;229:253.
981. Pusterla N, Fecteau ME, Madigan JE, et al. Acute hemoperitoneum in horses: a review of 19 cases (1992-2003). *J Vet Intern Med*. 2005;19:344.
982. Orsini JA, Divers TJ. *Equine Emergencies: Treatment and Procedures*. 4th ed. St. Louis: W.B. Saunders; 2014. ed. Divers TJ.
983. Lepage OM, Piccot-Crezollet C. Transarterial coil embolisation in 31 horses (1999-2002) with guttural pouch mycosis: a 2-year follow-up. *Equine Vet J*. 2005;37:430.
984. Freeman DE. Long-term follow-up on a large number of horses that underwent transarterial coil embolisation (TCE) for guttural pouch mycosis (GPM). *Equine Vet J*. 2006;38:271. author reply 271.
985. Dobesova O, Schwarz B, Velde K, et al. Guttural pouch mycosis in horses: a retrospective study of 28 cases. *Vet Rec*. 2012;171:561.
986. Fales-Williams A, Sponseller B, Flaherty H. Idiopathic arterial medial calcification of the thoracic arteries in an adult horse. *J Vet Diagn Invest*. 2008;20:692.
987. Martinez J, Montgomery DL, Uzal FA. Vascular mineralization in the brain of horses. *J Vet Diagn Invest*. 2012;24:612.
988. Imaizumi K, Nakamura T, Kiryu K, et al. Morphological changes of the aorta and pulmonary artery in Thoroughbred racehorses. *J Comp Pathol*. 1989;101:1.
989. Arroyo LG, Hayes MA, Delay J, et al. Arterial calcification in race horses. *Vet Pathol*. 2008;45:617.
990. Guglick MA, MacAllister CG, Ewing PJ, et al. Thrombosis resulting in rectal perforation in a horse. *J Am Vet Med Assoc*. 1996;209:1125.
991. Diaz OS, Sleeper MM, Reef VB, et al. Aortitis in a Paint gelding. *Equine Vet J*. 2000;32:354.
992. Mahony C, Rantanen NW, DeMichael JA, et al. Spontaneous echocardiographic contrast in the Thoroughbred: high prevalence in racehorses and a characteristic abnormality in bleeders. *Equine Vet J*. 1992;24:129.
993. Rantanen NW, Byars TD, Hauser ML, et al. Spontaneous contrast and mass lesions in the hearts of race horses: ultrasound diagnosis-preliminary data. *J Equine Vet Sci*. 1984;4:220.

994. Russell TM, Kearney C, Pollock PJ. Surgical treatment of septic jugular thrombophlebitis in nine horses. *Vet Surg*. 2010;39:627.
995. Rijkenhuizen AB, van Swieten HA. Reconstruction of the jugular vein in horses with post thrombophlebitis stenosis using saphenous vein graft. *Equine Vet J*. 1998;30:236.
996. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis*. 2011;52:e162.
997. Spurlock SL, Spurlock GH, Parker G, et al. Long-term jugular vein catheterization in horses. *J Am Vet Med Assoc*. 1990;196:425.
998. Divers TJ. Prevention and treatment of thrombosis, phlebitis, and laminitis in horses with gastrointestinal diseases. *Vet Clin North Am Equine Pract*. 2003;19:779.
999. Tan RHH, Dart AJ, Dowling BA. Catheters: a review of the selection, utilisation and complications of catheters for peripheral venous access. *Aust Vet J*. 2003;81:136.
1000. Herschl MA, Trim CM, Mahaffey EA. Effects of 5% and 10% guaifenesin infusion on equine vascular endothelium. *Vet Surg*. 1992;21:494.
1001. Geraghty TE, Love S, Taylor DJ, et al. Assessment of subclinical venous catheter-related diseases in horses and associated risk factors. *Vet Rec*. 2009;164:227.
1002. Schwarzwald CC, Feige K, Wunderli-Allenspach H, et al. Comparison of pharmacokinetic variables for two low-molecular-weight heparins after subcutaneous administration of a single dose to horses. *Am J Vet Res*. 2002;63:868.
1003. Feige K, Schwarzwald CC, Bombeli T. Comparison of unfractioned and low molecular weight heparin for prophylaxis of coagulopathies in 52 horses with colic: a randomised double-blind clinical trial. *Equine Vet J*. 2003;35:506.
1004. Tomlinson JE, Byrne E, Pusterla N, et al. The use of recombinant tissue plasminogen activator (rTPA) in the treatment of fibrinous pleuropneumonia in horses: 25 cases (2007-2012). *J Vet Intern Med*. 2015;29:1403.
1005. Baumer W, Herrling GM, Feige K. Pharmacokinetics and thrombolytic effects of the recombinant tissue-type plasminogen activator in horses. *BMC Vet Res*. 2013;9:158.
1006. Whelan MF, O'Toole TE. The use of thrombolytic agents. *Compendium*. 2007;476.
1007. Spurlock SL, Spurlock GH. Risk factors of catheter-related complications. *Compend Contin Educ Pract Vet*. 1990;12:214.
1008. Bayly WM, Vale BH. Intravenous catheterization and associated problems in the horse. *Compend Contin Educ Pract Vet*. 1982;4:S227.
1009. Wiemer P, Gruys E, van Hoeck B. A study of seven different types of grafts for jugular vein transplantation in the horse. *Res Vet Sci*. 2005;79:211.
1010. Dyson SJ, Worth L. Aortoiliacofemoral thrombosis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 4*. Philadelphia: WB Saunders; 1997.
1011. Duggan VE, Holbrook TC, Dechant JE, et al. Diagnosis of aorto-iliac thrombosis in a quarter horse foal using Doppler ultrasound and nuclear scintigraphy. *J Vet Intern Med*. 2004;18:753.
1012. Hilton H, Aleman M, Textor J, et al. Ultrasound-guided balloon thrombectomy for treatment of aorto-iliac-femoral thrombosis in a horse. *J Vet Intern Med*. 2008;22:679.
1013. Brama PAJ, Rijkenhuizen ABM, vanSwieten HA, et al. Thrombosis of the aorta and the caudal arteries in the horse; additional diagnostics and a new surgical treatment. *Vet Q*. 1996;18:S85.
1014. Mayhew IG, Kryger MD. Aortic-iliac-femoral thrombosis in a horse. *Vet Med Small Anim Clin*. 1975;70:1281.
1015. Moffett FS, Vaden P. Diagnosis and treatment of thrombosis of the posterior aorta or iliac arteries in the horse. *Vet Med Small Anim Clin*. 1978;73:184.
1016. Schmidt AR. Transrectal ultrasonography of the caudal portion of abdominal and pelvic cavities in horses. *J Am Vet Med Assoc*. 1989;194:365.
1017. Swanson TD. Aortic-iliac thrombosis in horses. *Compend Contin Educ Vet*. 2011;33:E1.
1018. Ross MW, Maxson AD, Stacy VS, et al. First-pass radionuclide angiography in the diagnosis of aortoiliac thromboembolism in a horse. *Vet Radiol Ultrasound*. 1997;38:226.
1019. Warmerdam EP. Ultrasonography of the femoral artery in six normal horses and three horses with thrombosis. *Vet Radiol Ultrasound*. 1998;39:137.
1020. Rijkenhuizen AB, Sinclair D, Jahn W. Surgical thrombectomy in horses with aortoiliac thrombosis: 17 cases. *Equine Vet J*. 2009;41:754.
1021. Voortman O, Binkhorst GJ, Walvoort HC. An unusual complication in a case of verminous aneurysm (author's transl). *Tijdschr Diergeneeskd*. 1980;105:87.
1022. Fio L. Sudden death on the racetrack—major causes revealed by racehorse necropsy program. *ERL Update*. 1994;1.
1023. Halper J. Connective tissue disorders in domestic animals. *Adv Exp Med Biol*. 2014;802:231.
1024. Stadler P. Echocardiographic findings in ruptured sinus of Valsalva aneurysm. *Tierarztl Prax Suppl*. 1993;29.
1025. van Loon G, De Clercq D, de Bruijn CM, et al. *Clinical Findings and Diagnosis of Aortopulmonary Fistula in Four Friesian Horses*. Birmingham, United Kingdom: British Equine Veterinary Association Congress; 2010.
1026. de Bruijn M, van Loon G, Ploeg M, et al. *Use of Transoesophageal Ultrasound to Visualise the Aortopulmonary Region in Two Normal Friesian Horses and Three Friesians With Aortic Rupture or Aortopulmonary Fistulation*. Manchester, United Kingdom: British Equine Veterinary Association Congress; 2013.
1027. Reef VB. Dysrhythmias: assessment and medical management. In: Marr CM, ed. *Cardiology of the Horse*. Edinburgh: Saunders Elsevier; 2010.
1028. Gabriel F, Lekeux P. Cardiac arrhythmias encountered in 159 Belgian riding horses. *Ann Med Vet*. 1986;130:205.
1029. Hesselkilde EZ, Almind ME, Petersen J, et al. Cardiac arrhythmias and electrolyte disturbances in colic horses. *Acta Vet Scand*. 2014;56:58.
1030. Broux B, De Clercq D, Decloedt A, et al. Pharmacokinetics of intravenously and orally administered sotalol hydrochloride in horses and effects on surface electrocardiogram and left ventricular systolic function. *Vet J*. 2016;208:60.
1031. Schwarzwald CC, Hamlin RL, Bonagura JD, et al. Atrial, SA nodal, and AV nodal electrophysiology in standing horses: normal findings and electrophysiologic effects of quinidine and diltiazem. *J Vet Intern Med*. 2007;21:166.
1032. Schwarzwald CC, Sams RA. Determination of plasma protein binding of diltiazem in horses by ultrafiltration. *J Vet Pharmacol Ther*. 2006;29:579.
1033. Schwarzwald CC, Sams RA, Bonagura JD. Pharmacokinetics of the calcium-channel blocker diltiazem after a single intravenous dose in horses. *J Vet Pharmacol Ther*. 2006;29:165.
1034. van Loon G, Jordaens L, Muylle E, et al. Intracardiac overdrive pacing as a treatment of atrial flutter in a horse. *Vet Rec*. 1998;142:301.
1035. Amada A, Kiryu K. Atrial fibrillation in the race horse. *Heart Vessels Suppl*. 1987;2:2.
1036. Belloli C, Zizzadoro C. Atrial fibrillation in horses: difficult diagnosis for a therapeutic orphan. *Vet J*. 2006;172:8.
1037. Bentz BG, Erkert RS, Blaik MA. Evaluation of atrial fibrillation in horses. *Comp Cont Educ Pract*. 2002;24:734.
1038. Decloedt A, Verheyen T, Van Der Vekens N, et al. Long-term follow-up of atrial function after cardioversion of atrial fibrillation in horses. *Vet J*. 2013;197:583.
1039. Dukes-Mcewan J. Atrial fibrillation: onset and perpetuation. *Vet J*. 2002;164:87.
1040. Else RW, Holmes JR. Pathological changes in atrial fibrillation in the horse. *Equine Vet J*. 1971;3:56.
1041. Fenton FH, Cherry EM, Kornreich BG. Termination of equine atrial fibrillation by quinidine: an optical mapping study. *J Vet Cardiol*. 2008;10:87.

1042. Glazier DB, Nicholson JA, Kelly WR. Atrial fibrillation in the horse. *Irish Vet J*. 1959;13:47.
1043. Kiryu K, Amada A, Kaneko M, et al. Atrial fibrillation in the horse: clinical and histopathological studies of two cases. II. Formal pathogenesis. *Exp Rep Equine Health Lab*. 1974;11:70.
1044. Kiryu K, Kaneko M, Oikawa M, et al. Histopathogenesis of atrial fibrillation in the horse: cardiopathology of an additional case. *Exp Rep Equine Health Lab*. 1977;14:54.
1045. Kubo K, Senta T, Sugimoto O. Changes in cardiac output with experimentally induced atrial fibrillation in the horse. *Exp Rep Equine Health Lab*. 1975;12:101.
1046. Kuwahara M, Hiraga A, Nishimura T, et al. Power spectral analysis of heart rate variability in a horse with atrial fibrillation. *J Vet Med Sci*. 1998;60:111.
1047. Manohar M, Smetzer DL. Atrial fibrillation. *Comp Cont Educ Pract*. 1992;14:1327.
1048. Marr CM, Reef VB, Reimer JM, et al. An echocardiographic study of atrial fibrillation in horses: before and after conversion to sinus rhythm. *J Vet Intern Med*. 1995;9:336.
1049. Morris DD, Fregin GF. Atrial fibrillation in horses: factors associated with response to quinidine sulfate in 77 clinical cases. *Cornell Vet*. 1982;72:339.
1050. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol*. 2014;63:2335.
1051. Ohmura H, Hiraga A, Takahashi T, et al. Risk factors for atrial fibrillation during racing in slow-finishing horses. *J Am Vet Med Assoc*. 2003;223:84.
1052. Physick-Sheard P, Kraus M, Basrur P, et al. Breed predisposition and heritability of atrial fibrillation in the Standardbred horse: a retrospective case-control study. *J Vet Cardiol*. 2014;16:173.
1053. Senta T, Kubo K. Experimental induction of atrial fibrillation by electrical stimulation in the horse. *Exp Rep Equine Health Lab*. 1978;15:37.
1054. Senta T, Kubo K, Sugimoto O, et al. Induction of atrial fibrillation by electrical stimulation in the horse. *Exp Rep Equine Health Lab*. 1975;12:109.
1055. navasvan Loon G. *Atrial Pacing and Experimental Atrial Fibrillation in Equines, in Department of Large Animal Internal Medicine*. Gent: Faculty of Veterinary Medicine, University of Gent; 2001:268.
1056. Van Loon G, Duytschaever M, Tavernier R, et al. An equine model of chronic atrial fibrillation: methodology. *Vet J*. 2002;164:142.
1057. Van Loon G, Tavernier R, Duytschaever M, et al. Pacing induced sustained atrial fibrillation in a pony. *Can J Vet Res*. 2000;64:254.
1058. Wachtell K, Greve AM, Structural, Cardiac Functional. Changes are target organ damage that increases risk of atrial fibrillation. *J Am Coll Cardiol*. 2014;63:2014.
1059. Wyse DG, Van Gelder IC, Ellinor PT, et al. Lone atrial fibrillation: does it exist? *J Am Coll Cardiol*. 2014;63:1715.
1060. Navas de Solis C, Reef VB, Slack J, et al. Evaluation of coagulation and fibrinolysis in horses with atrial fibrillatio. *J Am Vet Med Assoc*. 2016;248:201.
1061. Asano K, Suzuki K, Chiba M, et al. Relationship between trace elements status in mane hair and atrial fibrillation in horse. *J Vet Med Sci*. 2006;68:769.
1062. De Clercq D, Decloedt A, Sys SU, et al. Atrial fibrillation cycle length and atrial size in horses with and without recurrence of atrial fibrillation after electrical cardioversion. *J Vet Intern Med*. 2014;28:624.
1063. Decloedt A, de Clercq D, van der Vekens N, et al. Influence of detomidine on atrial fibrillation cycle length measured by intracardiac electrogram recording and by colour tissue Doppler imaging in horses. *Equine Vet J*. 2016;48:21.
1064. Mullen KR, Kraus MS, Divers TJ. ECG of the month. Atrial fibrillation due to hypokalemia in a horse. *J Am Vet Med Assoc*. 2014;244:657.
1065. Machida N, Yasuda J, Too K. Three cases of paroxysmal atrial fibrillation in the Thoroughbred newborn foal. *Equine Vet J*. 1989;21:66.
1066. Gehlen H, Stadler P. Comparison of systolic cardiac function before and after treatment of atrial fibrillation in horses with and without additional cardiac valve insufficiencies. *Vet Res Commun*. 2004;28:317.
1067. Nattel S. New ideas about atrial fibrillation 50 years on. *Nature*. 2002;415:219.
1068. Haissaguerre M, Jais P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659.
1069. Jesty SA, Kraus MS, Johnson AL, et al. An accessory bypass tract masked by the presence of atrial fibrillation in a horse. *J Vet Cardiol*. 2011;13:79.
1070. Reef VB, Reimer JM, Spencer PA. Treatment of atrial fibrillation in horses: new perspectives. *J Vet Intern Med*. 1995;9:57.
1071. Stadler P, Deegen E, Kroker K. Echocardiography and therapy of atrial fibrillation in horses. *Dtsch Tierarztl Wochenschr*. 1994;101:190.
1072. Bentz BG, Erkert RS, Blaik MA. Atrial fibrillation in horses: treatment and prognosis. *Comp Cont Educ Pract*. 2002;24:817.
1073. Bertone JJ, Traub-Dargatz JL, Wingfield WE. Atrial fibrillation in a pregnant mare: treatment with quinidine sulfate. *J Am Vet Med Assoc*. 1987;190:1565.
1074. Blissitt KJ. Diagnosis and treatment of atrial fibrillation. *Equine Vet Educ*. 1999;11:11.
1075. Gerber H, Chuit P, Schatzmann HJ. Treatment of atrial fibrillation in the horse with intravenous dihydroquinidine gluconate. *Equine Vet J*. 1971;3:110.
1076. Gerber H, Chuit P, Schatzmann HJ, et al. Intravenous treatment of atrial fibrillation in the horse. *Schweiz Arch Tierheilkd*. 1972;114:57.
1077. Glendinning SA. The use of quinidine sulfate for the treatment of atrial fibrillation in horses. *Vet Rec*. 1965;77:951.
1078. Matsuda H. Treatment of atrial fibrillation in horses. *Jpn J Vet Res*. 1992;40:44.
1079. Muir 3rd WW, Reed SM, McGuirk SM. Treatment of atrial fibrillation in horses by intravenous administration of quinidine. *J Am Vet Med Assoc*. 1990;197:1607.
1080. Rose RJ, Davis PE. Treatment of atrial fibrillation in three racehorses. *Equine Vet J*. 1977;9:68.
1081. Young L, van Loon G. Editorial: atrial fibrillation in horses: new treatment choices for the new millennium? *J Vet Intern Med*. 2005;19:631.
1082. McGuirk SM, Muir WW, Sams RA. Pharmacokinetic analysis of intravenously and orally administered quinidine in horses. *Am J Vet Res*. 1981;42:938.
1083. Cipone M, Venturoli M. Atrial fibrillation in five horses. Changes in the ECG during quinidine therapy. *Summa*. 1986;3:53.
1084. Frye MA, Selders CG, Mama KR, et al. Use of biphasic electrical cardioversion for treatment of idiopathic atrial fibrillation in two horses. *J Am Vet Med Assoc*. 2002;220:1039.
1085. McGurrin MK, Physick-Sheard PW, Kenney DG, et al. Transvenous electrical cardioversion in equine atrial fibrillation: technique and successful treatment of 3 horses. *J Vet Intern Med*. 2003;17:715.
1086. McGurrin MK, Physick-Sheard PW, Kenney DG. How to perform transvenous electrical cardioversion in horses with atrial fibrillation. *J Vet Cardiol*. 2005;7:109.
1087. McGurrin MK, Physick-Sheard PW, Kenney DG, et al. Transvenous electrical cardioversion of equine atrial fibrillation: technical considerations. *J Vet Intern Med*. 2005;19:695.
1088. Bellei MH, Kerr C, McGurrin MK, et al. Management and complications of anesthesia for transvenous electrical cardioversion of atrial fibrillation in horses: 62 cases (2002-2006). *J Am Vet Med Assoc*. 2007;231:1225.

1089. McGurrin MK, Physick-Sheard PW, Kenney DG. Transvenous electrical cardioversion of equine atrial fibrillation: patient factors and clinical results in 72 treatment episodes. *J Vet Intern Med*. 2008;22:609.
1090. DeClercq D, VanLoon G, Schauvliege S, et al. Transvenous electrical cardioversion of atrial fibrillation in six horses using custom made cardioversion catheters. *Vet J*. 2008;177:198.
1091. Schauvliege S, van Loon G, De Clercq D, et al. Cardiovascular responses to transvenous electrical cardioversion of atrial fibrillation in anaesthetized horses. *Vet Anaesth Analg*. 2009;36:341.
1092. VanLoon G, DeClercq D, Tavernier R, et al. Transient complete atrioventricular block following transvenous electrical cardioversion of atrial fibrillation in a horse. *Vet J*. 2005;170:124.
1093. Marly-Voquer C, Schwarzwald CC, Bettschart-Wolfensberger R. The use of dexmedetomidine continuous rate infusion for horses undergoing transvenous electrical cardioversion—A case series. *Can Vet J*. 2016;57:70.
1094. Preiss EE, Kenney DG, McGurrin MK, et al. Influence of electrode position on cardioversion energy requirements during transvenous electrical cardioversion in horses. *Am J Vet Res*. 2011;72:1193.
1095. Ellis EJ, Ravis WR, Malloy M, et al. The pharmacokinetics and pharmacodynamics of procainamide in horses after intravenous administration. *J Vet Pharmacol Ther*. 1994;17:265.
1096. Moïse NS, Pariaut R, Gelzer ARM, et al. Cardioversion with lidocaine of vagally associated atrial fibrillation in two dogs. *J Vet Cardiol*. 2005;7:143.
1097. Pariaut R, Moise NS, Koetje BD, et al. Lidocaine converts acute vagally associated atrial fibrillation to sinus rhythm in German Shepherd dogs with inherited arrhythmias. *J Vet Intern Med*. 2008;22:1274.
1098. DeClercq D, VanLoon G, Baert K, et al. Intravenous amiodarone treatment in horses with chronic atrial fibrillation. *Vet J*. 2006;172:129.
1099. DeClercq D, VanLoon G, Baert K, et al. Effects of an adapted intravenous amiodarone treatment protocol in horses with atrial fibrillation. *Equine Vet J*. 2007;39:344.
1100. Trachsel D, Tschudi P, Portier CJ, et al. Pharmacokinetics and pharmacodynamic effects of amiodarone in plasma of ponies after single intravenous administration. *Toxicol Appl Pharmacol*. 2004;195:113.
1101. De Clercq D, Baert K, Croubels S, et al. Evaluation of the pharmacokinetics and bioavailability of intravenously and orally administered amiodarone in horses. *Am J Vet Res*. 2006;67:448.
1102. Birettoni F, Porciello F, Rishniw M, et al. Treatment of chronic atrial fibrillation in the horse with flecainide: personal observation. *Vet Res Commun*. 2007;31(suppl 1):273.
1103. Dembek KA, Hurcombe SD, Schober KE, et al. Sudden death of a horse with supraventricular tachycardia following oral administration of flecainide acetate. *J Vet Emerg Crit Care*. 2014;24:759.
1104. Haugaard MM, Pehrson S, Carstensen H, et al. Antiarrhythmic and electrophysiologic effects of flecainide on acutely induced atrial fibrillation in healthy horses. *J Vet Intern Med*. 2015;29:339.
1105. Ohmura H, Hiraga A, Aida H, et al. Influence of quinidine and flecainide on autonomic nervous activity in Thoroughbred horses. *Vet Rec*. 2003;152:114.
1106. Ohmura H, Hiraga A, Aida H, et al. Determination of oral dosage and pharmacokinetic analysis of flecainide in horses. *J Vet Med Sci*. 2001;63:511.
1107. Ohmura H, Nukada T, Mizuno Y, et al. Safe and efficacious dosage of flecainide acetate for treating equine atrial fibrillation. *J Vet Med Sci*. 2000;62:711.
1108. Risberg AI, McGuirk SM. Successful conversion of equine atrial fibrillation using oral flecainide. *J Vet Intern Med*. 2006;20:207.
1109. van Loon G, Blissitt KJ, Keen JA, et al. Use of intravenous flecainide in horses with naturally-occurring atrial fibrillation. *Equine Vet J*. 2004;36:609.
1110. DeClercq D, VanLoon G, Tavernier R, et al. Use of propafenone for conversion of chronic atrial fibrillation in horses. *Am J Vet Res*. 2009;70:223.
1111. Puigdemont A, Riu JL, Guitart R, et al. Propafenone kinetics in the horse. Comparative analysis of compartmental and noncompartmental models. *J Pharmacol Methods*. 1990;23:79.
1112. Haugaard MM, Hesselkilde EZ, Pehrson S, et al. Pharmacologic inhibition of small-conductance calcium-activated potassium (SK) channels by NS8593 reveals atrial antiarrhythmic potential in horses. *Heart Rhythm*. 2015;12:825.
1113. Hancox JC, James AF, Marrion NV, et al. Novel ion channel targets in atrial fibrillation. *Expert Opin Ther Targets*. 2016;20:947.
1114. Estrada AH, Pariaut R, Moise NS. Avoiding medical error during electrical cardioversion of atrial fibrillation: prevention of unsynchronized shock delivery. *J Vet Cardiol*. 2009;11:137.
1115. Dicken M, Gordon SJ, Mayhew IG. The use of phenytoin in two horses following conversion from atrial fibrillation. *N Z Vet J*. 2012;60:210.
1116. Cornick JL, Hartsfield SM, Miller M. ECG of the month. Premature ventricular complexes in an anesthetized colt. *J Am Vet Med Assoc*. 1990;196:420.
1117. Marr CM, Reef VB. ECG of the month. [Multifocal ventricular tachycardia in a horse]. *J Am Vet Med Assoc*. 1991;198:1533.
1118. Miller PJ, Rose RJ, Hoffman K, et al. Idioventricular tachycardia in a horse. *Aust Vet J*. 1987;64:55.
1119. Nielsen IL. Ventricular tachycardia in a Thoroughbred racehorse. *Aust Vet J*. 1990;67:140.
1120. Reimer JM, Reef VB, Sweeney RW. Ventricular arrhythmias in horses: 21 cases (1984-1989). *J Am Vet Med Assoc*. 1992;201:1237.
1121. Vrins A, Doucet M, DeRoth L. Paroxysmal ventricular tachycardia in a horse. *Medecin Veterinaire du Quebec*. 1989;19:79.
1122. Pfister R, Seifertalioth C, Beglinger R. Location of the ectopic focus of ventricular extrasystoles in the horse. *Schweiz Arch Tierheilkd*. 1984;126:165.
1123. Johnson AL, Jesty SA, Gelzer AR, et al. ECG of the month. Ventricular tachycardia in a horse. *J Am Vet Med Assoc*. 2007;231:706.
1124. DeClercq D, VanLoon G, Baert K, et al. Treatment with amiodarone of refractory ventricular tachycardia in a horse. *J Vet Intern Med*. 2007;21:878.
1125. Garcia-Lopez JM, Provost PJ, Rush JE, et al. Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. *Am J Vet Res*. 2001;62:7.
1126. Meyer GA, Lin HC, Hanson RR, et al. Effects of intravenous lidocaine overdose on cardiac electrical activity and blood pressure in the horse. *Equine Vet J*. 2001;33:434.
1127. Mullen KR, Gelzer AR, Kraus MS, et al. ECG of the month. Cardiac arrhythmias in a horse after lidocaine administration. *J Am Vet Med Assoc*. 2009;235:1156.
1128. Stewart AJ. Magnesium disorders in horses. *Vet Clin North Am Equine Pract*. 2011;27:149.
1129. Kowalczyk DF, Beech J. Pharmacokinetics of phenytoin (diphenylhydantoin) in horses. *J Vet Pharmacol Ther*. 1983;6:133.
1130. Soma LR, Uboh CE, Guan F, et al. Disposition, elimination, and bioavailability of phenytoin and its major metabolite in horses. *Am J Vet Res*. 2001;62:483.
1131. Wijnberg ID, Ververs FF. Phenytoin sodium as a treatment for ventricular dysrhythmia in horses. *J Vet Intern Med*. 2004;18:350.
1132. Gasthuys F, Parmentier D, Goossens L, et al. A preliminary study on the effects of atropine sulphate on bradycardia and heart blocks during romifidine sedation in the horse. *Vet Res Commun*. 1990;14:489.

1133. Kroneman J. Heart arrhythmias in horses caused by delays of conduction in the atrioventricular nodes and in the bundle of His. *Berl Munch Tierarztl Wochenschr*. 1991;104:351.
1134. Reef VB, Clark ES, Oliver JA, et al. Implantation of a permanent transvenous pacing catheter in a horse with complete heart block and syncope. *J Am Vet Med Assoc*. 1986;189:449.
1135. Matsui K, Amada A, Sawazaki H. Second-degree atrioventricular block observed in a Thoroughbred foal on 2.5 months of age. *Nihon Juigaku Zasshi*. 1985;47:175.
1136. Glazier DB, Littledike ET, Cook HM. The electrocardiographic changes in experimentally induced bundle-branch block in the equine heart. *Ir Vet J*. 1983;37:71.
1137. Suzuki K, Yamaya Y, Asano K, et al. Relationship between hair elements and severity of atrioventricular block in horses. *Biol Trace Elem Res*. 2007;115:255.
1138. Lawler JB, Frye MA, Bera MM, et al. Third-degree atrioventricular block in a horse secondary to rattlesnake envenomation. *J Vet Intern Med*. 2008;22:486.
1139. Meijler FL. Comparative pathophysiology of the atrioventricular node. *Verh K Acad Geneeskd Belg*. 1990;52:127.
1140. Rezakhani A, Godarzi M. Tabatabei Naeini I: a combination of atrioventricular block and sinoatrial block in a horse. *Acta Vet Scand*. 2005;46:173.
1141. Sugiyama A, Takeuchi T, Morita T, et al. Mediastinal lymphoma with complete atrioventricular block in a horse. *J Vet Med Sci*. 2008;70:1101.
1142. Yamashita K, Tsubakishita S, Futaok S, et al. Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *J Vet Med Sci*. 2000;62:1025.
1143. Hardy J. ECG of the month. Hyperkalemia in a mare. *J Am Vet Med Assoc*. 1989;194:356.
1144. Epstein V. Relationship between potassium administration, hyperkalaemia and the electrocardiogram: an experimental study. *Equine Vet J*. 1984;16:453.
1145. Castex AM, Bertone JJ. ECG of the month. Sinus tachycardia and hyperkalemia in a horse. *J Am Vet Med Assoc*. 1989;194:654.
1146. Glazier DB, Littledike ET, Evans RD. Electrocardiographic changes in induced hyperkalemia in ponies. *Am J Vet Res*. 1934;43:1982.
1147. Muir WW, McGuirk SM. Ventricular preexcitation in two horses. *J Am Vet Med Assoc*. 1983;183:573.
1148. Asano K, Suzuki K, Chiba M, et al. Correlation between 25 element contents in mane hair in riding horses and atrioventricular block. *Biol Trace Elem Res*. 2005;108:127.
1149. Smetzer DL, Smith CR, Senta T. Second-degree atrioventricular block in the horse. *Am J Vet Res*. 1969;30:933.
1150. Kiryu K, Kaneko M, Satoh H. Cardiopathological observations on histopathogenesis of incomplete atrioventricular block in horses. *Nihon Juigaku Zasshi*. 1977;39:425.
1151. Smetzer DL, Senta T, Smith CR, et al. High-grade second-degree atrioventricular block in a horse. *Am J Vet Res*. 1969; 30:337.
1152. Ohmura H, Hiraga A, Aida H, et al. Effects of repeated atropine injection on heart rate variability in Thoroughbred horses. *J Vet Med Sci*. 2001;63:1359.
1153. Nihouannen JC, Sevestre J, Dorso Y, et al. Implantation of a cardiac pacemaker into horses. I. Equipment and techniques. *Rev Med Vet (Toulouse)*. 1984;135:91.
1154. Nihouannen JC, Sevestre J, Dorso Y, et al. Implantation of a cardiac pacemaker into horses. II. Postoperative monitoring of a pacemaker with epicardial and myocardial electrodes in a pony. *Rev Med Vet (Toulouse)*. 1984;135:165.
1155. van Loon G, Laevens H, Deprez P. Temporary transvenous atrial pacing in horses: threshold determination. *Equine Vet J*. 2001;33:290.
1156. van Loon G, Fonteyne W, Rottiers H, et al. Dual-chamber pacemaker implantation via the cephalic vein in healthy equids. *J Vet Intern Med*. 2001;15:564.
1157. Kaese S, Frommeyer G, Verheule S, et al. The ECG in cardiovascular-relevant animal models of electrophysiology. *Herzschrittmacherther Elektrophysiol*. 2013;24:84.
1158. Naylor JM. Hyperkalemic periodic paralysis. *Vet Clin North Am Equine Pract*. 1997;13:129.
1159. Meyer TS, Fedde MR, Cox JH, et al. Hyperkalaemic periodic paralysis in horses: a review. *Equine Vet J*. 1999;31:362.
1160. Batterink J, Cessford TA, Taylor RAI, et al. Pharmacological interventions for the acute management of hyperkalaemia in adults. *Cochrane Database of Systematic Reviews Art*. 2015; No.: CD010344.
1161. Maxwell AP, Linden K, O'Donnell S, et al. Management of hyperkalaemia, *J R Coll Physicians Edinb*. 43:246.

CHAPTER 10

Disorders of the Musculoskeletal System

Stephanie J. Valberg

MUSCLE FORM AND FUNCTION

Skeletal muscle is the most plastic tissue in the body, constantly remodeling in response to exercise, diet, and the hormonal milieu. The primary components of muscle are water (80%), protein (10%–15%), and glycogen (1%–2%), with the proteins largely comprising contractile proteins. The type of contractile protein, more specifically the isoforms of myosin heavy chains, determines the speed with which the fiber contracts and the muscle fiber type. The speed of contraction varies from slowest to fastest through type 1, type 2a, and type 2x fibers.

Most equine locomotor muscles are comprised of a mosaic of type 1 and type 2 muscle fibers, but the exact composition depends on the muscle's function and use. For example, digital flexor muscles that support the fetlock joint consist of 50% type 1 and 50% type 2a muscle fibers, whereas proximal locomotor muscles that power the hind end consist of less than 25% type 1 and 75% or more type 2a and type 2x fibers in proportions that vary among individuals, breeds, and state of training (Fig. 10.1).[1] The contractile fiber types have distinctive metabolic properties that also vary with the state of training. Type 1 fibers generally have lower glycogen and glycolytic capacity and higher lipid and oxidative capacity than type 2 fibers. Type 2a fibers have intermediate properties whereas type 2x fibers have higher glycogen, higher glycolytic, and lower lipid and oxidative capacity than type 1 and 2a fibers.

A motor unit consists of a subset of myofibers supplied by the branches of one nerve. Tonic motor neurons with a slow discharge rate supply type 1 fibers and fast discharging phasic motor neurons supplying type 2a and 2x fibers. At slow exercise intensities, type 1 and a small number of type 2a muscle fibers are activated, and as the speed or duration of exercise increases, more muscle fibers will be recruited in the order type 1 to type 2a to type 2x.

Peripheral neuropathies usually affect both fast- and slow-twitch motor units, causing weakness and muscle atrophy. Specific myopathies often affect one particular fiber type and a specific cellular pathway within that myofiber. The disruption in that cellular pathway often produces typical clinical signs for that disease. Wherever possible the goal of internists and researchers is to define the pathway that is dysfunctional in order to provide the most accurate diagnosis and targeted treatments.

Diagnostic Approach

History and physical examination remain the cornerstones of the diagnostic approach. Goals are to (1) determine whether the clinical signs represent a primary or secondary muscle disorder; (2) assess the contributions of fasciculations, weakness, atrophy, muscle pain, or exercise intolerance to the primary presenting complaint; (3) generate a logical list of differential diagnoses; and (4) select the most appropriate diagnostic tests to further characterize and identify the cause of the muscle disorder. The requisite systematic approach begins with the history, is followed by inspection and palpation of every possible aspect of the musculature, and includes assessment of movement using both neurologic and lameness examinations.

History

A thorough history is particularly important with myopathies because many disorders are intermittent in nature and are not necessarily apparent on initial physical examination. In addition, many myopathies are triggered by certain environmental stimuli, and identifying those stimuli can help both to diagnose the disorder and to formulate a management plan to prevent further episodes in the future. In particular, it is important to determine from the history whether clinical signs are associated with exercise. Horses, unlike many other animals, are athletes, and forced exercise is routine. Riders readily note subtle clinical signs of pain, weakness, or exercise intolerance under saddle, and careful note of the rider's complaints aids in the identification of specific exertional myopathies. Temperament, diet, supplements, current medications, exercise regimens, level of performance, changes in exercise tolerance, vaccinations, other animals affected, and any relationships among those animals are documented. Details of the onset, progression of clinical signs, frequency, severity, and duration are recorded. Every effort is made to characterize possible eliciting factors such as recent alterations in diet, tack, exercise schedule, level of fitness, type and duration of exercise, or behavior. The environment, pasture access and quality, size of turnout facilities, footing, and general horsemanship are noted.

Inspection

Before beginning the physical examination, it is important to assess muscle mass, symmetry, stance, and behavior. Inspection is best done at a distance with the horse quietly standing

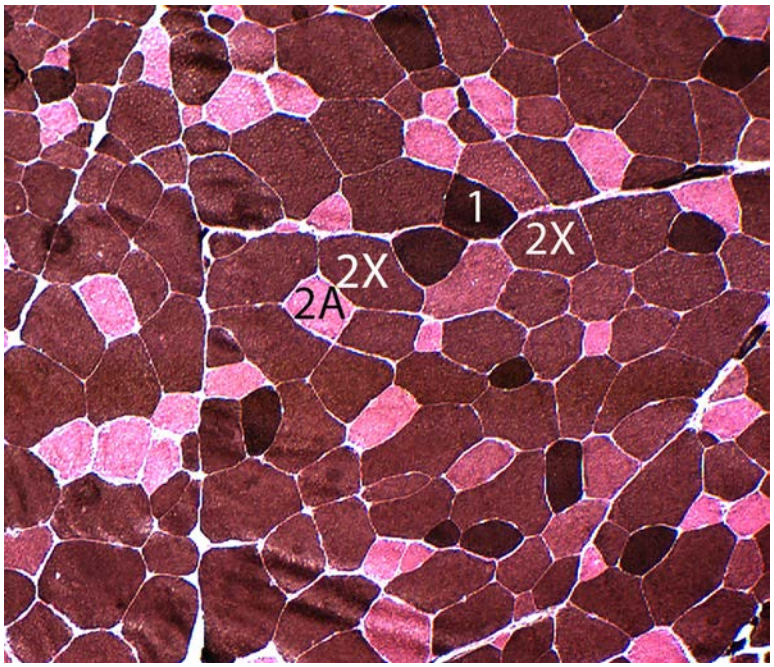


FIG. **10.1** Cross section of equine gluteus medius muscle stained with myosin ATPase (pH 4.6) showing a normal mosaic pattern of type 1 (slow-twitch), type 2A (fast-twitch oxidative), and type 2X (fast-twitch glycolytic) fibers. (20×)

square. If signs of anxiety are present the horse may need to be reevaluated in calm surroundings. The horse is viewed from all sides, comparing left to right, and the tail is moved to assess adductor muscles. Each athletic discipline results in hypertrophy of specific muscle groups. The veterinarian performing the examination should be familiar with the ideal muscle mass present in a horse of that discipline and fitness and should compare the patient at that level of training with the ideal to determine regions of underdevelopment (Fig. 10.2). Notes are made regarding tropic changes, atrophy, alterations in symmetry of particular muscle groups, and any observed spontaneous muscle activity.

Physical Examination

Following a standard general physical examination, each muscle should be palpated, beginning with the temporal and masseter muscles and working caudally to the tail. Where possible, left and right sides are palpated simultaneously and compared. Impressions of muscle tone, consistency, swelling, atrophy, and heat are compared between left and right sides and documented. Sensitivity to superficial followed by deep palpation of each muscle group is noted. The temperament of the horse and level of comfort with the examiner needs to be considered when interpreting results of palpation. Some animals are tense and demonstrate apparent evidence of myalgia when palpation is first performed or when performed by certain individuals. However, given time and patience or another examiner, some horses will relax and muscles or muscle groups that at first appeared to be very sensitive or hypertonic may in reality be normal. Palpation is repeated to ensure the response is consistent. A blunt instrument such as a needle cap or a pen can be run over the lumbar and gluteal muscles to ensure that the horse shows extension (swayback) followed by flexion (hogback) of the spine and sacroiliac joints. Guarding against movement may reflect abnormalities in the pelvic or thoracolumbar muscles or pain associated with the thoracolumbar spine or sacroiliac joints.

In animals with spontaneous muscle activity, muscle groups should also be percussed with a percussion hammer. The triceps, pectoral, and semitendinosus muscles are easily accessed for percussion. A positive percussion sign occurs when the muscle holds a contracture for several seconds, creating a dimpled appearance below the contracted muscle (percussion myotonia). This occurs as the result of abnormal mechanical irritability and sustained contraction of the percussed fibers.

Locomotor and Neurologic Examination

A locomotor examination is performed to characterize the impact of a primary neuromuscular disorder on gait and determine whether muscle pain is a secondary consequence of orthopedic lameness. Horses are observed for evidence of lameness, pain, asymmetry, decreased range of joint motion, stiffness, or muscle cramping at a walk and trot and flexion tests performed where appropriate. If necessary to elicit the abnormality noted by the owner, the horse can be observed under saddle. For neurologic assessment horses are observed at a walk on a straight line, serpentines, and spiraled circles, as well as when backing. A tail pull examination while the horse is standing still and walking is used to assess stability and hindlimb weakness. Any atrophy, spasticity, weakness, or ataxia should trigger a full neurologic examination to determine whether clinical signs are associated with the central nervous system, peripheral nerves, or muscle. Severe weakness can be difficult to differentiate from ataxia. In such cases, horses are observed for other signs of muscle/peripheral nerve weakness such as muscle fasciculations, knuckling at the walk, frequent recumbency with difficulty rising, and shifting of weight because of an inability to fix the stifles.



FIG. **10.2** Rear view of two age-matched Quarter Horses. The horse on the right demonstrates normal hindquarter muscle development. The horse on the left has a generalized decrease in muscle mass particularly affecting the biceps femoris and semitendinosus muscles. The horse on the left was diagnosed with myotonia dystrophica by electromyography.

Classification of Muscle Disorders

Findings from the muscle evaluation should be analyzed to determine the following: (1) whether the complaint is a primary or secondary muscle disorder; (2) whether the complaint is focal or generalized; (3) whether it is exercise associated or not exercise associated; (4) if rhabdomyolysis is present; and (5) if clinical signs are primarily associated with pain, atrophy, weakness, abnormal electrical conductivity, or exercise intolerance. A classification scheme for muscle disorders with differential diagnoses is provided in Box 10.1. The first principal divisions are based on the presence of an exertional or nonexertional disorder and whether the condition is characterized by rhabdomyolysis, atrophy, or muscle fasciculations.

Pathophysiology of Rhabdomyolysis

A variety of extrinsic (trauma, toxins, nutritional deficiency) and intrinsic (errors of metabolism, excitation-contraction coupling) insults can cause rhabdomyolysis; however, they often share a final common pathway that leads to cell death—aberrant calcium cycling.[2] If the energy pathways that generate adenosine triphosphate for the calcium pump are impaired, if the calcium pump or channels are not functioning properly, or if the cell membrane is damaged, excessive calcium accumulates in the sarcoplasm. Mitochondria sequester excessive calcium but quickly become overloaded, disrupting oxidative metabolism, generating oxygen free radicals, activating proteases and phospholipases, and generating inflammatory cytokines, all of which combine to disrupt cellular architecture and function within the damaged segment of the myofiber (Fig. 10.3). Fortunately, muscle is a highly plastic tissue and capable of complete repair as long as the basal lamina of the cell is left intact and damaged

BOX 10.1 Exertional Myopathy

1. Rhabdomyolysis
   a. Extrinsic (sporadic) tying-up
      - Dietary imbalances, vitamins, minerals, electrolytes
      - Exercise in excess of training
      - Exhaustion syndrome
   b. Intrinsic (chronic) tying-up
      - Recurrent exertional rhabdomyolysis
      - Malignant hyperthermia
      - Type 1 polysaccharide storage myopathy
      - Type 2 polysaccharide storage myopathy
      - Idiopathic chronic exertional rhabdomyolysis
2. Normal creatine kinase
   a. Focal muscle strain
   b. Type 2 polysaccharide storage myopathy
   c. Vitamin E–deficient myopathy
   d. Mitochondrial myopathy

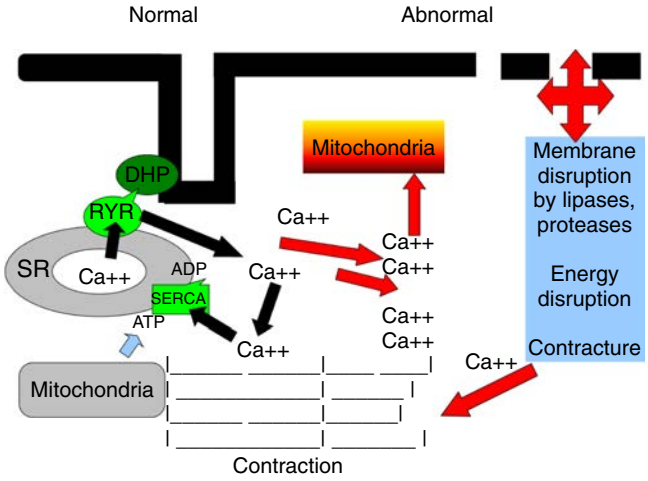


FIG. **10.3** (A) The normal course of excitation-contraction coupling where depolarization of the cell membrane activates the dihydropyridine receptor (DHPR), which triggers release of calcium ions from the terminal cisternae into the sarcoplasm by opening the ryanodine receptor (RYR) in the terminal cisternae of the SR membrane is depicted on the left. This raises myoplasmic calcium concentrations and causes contraction of the myofilaments. Calcium, is actively resequestered in the SR through the calcium pump (SERCA), which utilizes ATP and causes relaxation of the contraction. Excessive release of calcium via the RYR1 can raise myoplasmic calcium, resulting in uptake by mitochondria. Past a threshold, excessive calcium release can alter energy metabolism and cause muscle contracture without relaxation and activation of lipases and proteases, which destroy muscle cell membranes.

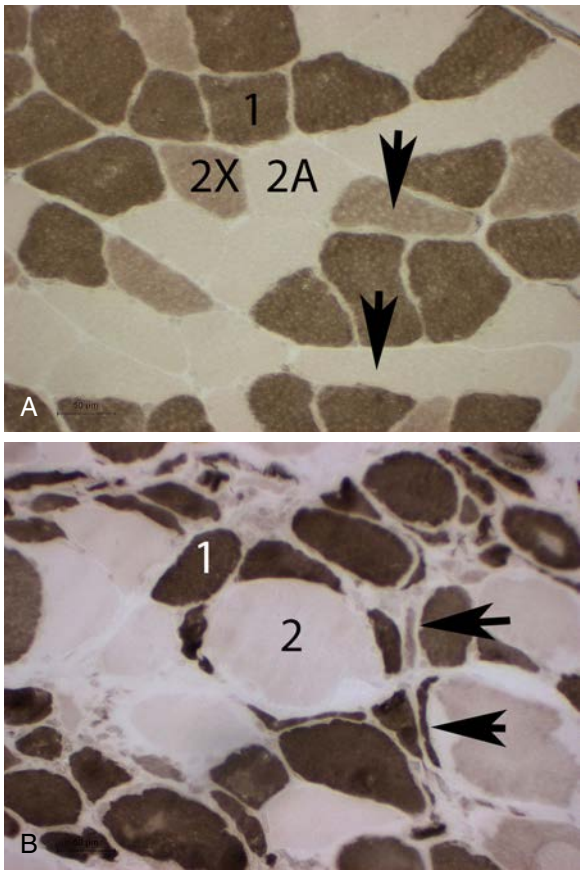


FIG. **10.4** (A) Cross section of equine gluteus medius muscle stained with myosin ATPase (pH 4.6) showing myogenic atrophy in which type 2A or type 2B fibers are smaller in cross-sectional area than type 1 muscle fibers and have lost their normal rounded shape and have an anguloid appearance *(arrow)*. (40×) (B) Cross section of equine sacrocaudalis dorsalis medialis muscle stained with myosin ATPase (pH 4.6) showing neurogenic atrophy defined by the presence of angular atrophy of both type 1 and type 2 muscle fibers *(arrows)*. (40×)

segments can locate their corresponding end. Both ends of the damaged muscle fiber segment are sealed off within hours of damage, and macrophage infiltration and phagocytosis of necrotic debris begin within 16 to 48 hours of the muscle injury. Progenitor cells called *satellite cells* migrate along the remaining basal lamina and form regenerative myotubes within 3 to 4 days of injury, connecting damaged ends, and mature muscle fibers are observed within a month of the original damage.

Pathophysiology of Atrophy

Atrophy can be the result of overt muscle cell damage or activation of ubiquitin-proteasome or autophagy-lysosome machinery within a muscle cell, so-called myogenic atrophy. Atrophy can also be due to damage to the motor nerve supplying that muscle, so-called neurogenic atrophy. Myogenic atrophy usually produces anguloid atrophy of fast-twitch muscle fibers (Fig. 10.4A) whereas neurogenic atrophy usually involves both type 1 and type 2 muscle fibers and is characterized by small angular atrophied fibers (Fig. 10.4B).

Pathophysiology of Fasciculations

Muscle fasciculations can be due to abnormal recruitment of motor nerves causing cramping and fasciculations. This occurs with nerve root irritation, electrolyte abnormalities, weakness, fatigue, anxiety, cold, and many other causes. Less often, fasciculations can be due to ion channel defects within the sarcolemma that cause abnormal propagation of muscle cell depolarization.

Diagnostic Tools

Muscle disorders characterized by rhabdomyolysis disrupt cellular homeostasis to the point that the muscle cell membrane (sarcolemma) ruptures, resulting in leakage of cytoplasmic contents from the muscle cell into the bloodstream and urine. The activity of muscle enzymes within the serum and the presence of myoglobin in urine are used as diagnostic indicators of rhabdomyolysis.

Serum Creatine Kinase and Aspartate Transaminase Activities

Serum activities of the skeletal muscle enzymes creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are commonly used to detect rhabdomyolysis.[3] Optimal information can be obtained by not only assessing the activity of the enzymes in serum but also by evaluating the respective activities of the enzymes relative to the onset of muscle disease. Note that it is the activity of the enzyme in serum expressed in units per liter (U/L) that is assessed rather than the concentration of proteins.

Serum CK is both sensitive and specific for skeletal and cardiac muscle damage. Serum CK activity increases within

hours of a muscle insult, peaks within 4 to 6 hours after injury, and usually declines to the normal range within 48 hours of an insult (longer if activities were greater than 100,000 U/L). Persistently elevated CK activity indicates ongoing muscle degeneration. Normal ranges vary among laboratories but are generally less than 430 U/L. Elevations from several thousand to hundreds of thousands of U/L occur with moderate to severe rhabdomyolysis. Minor elevations in CK activity can occur with training, transport, and strenuous exercise likely due to increased sarcolemmal permeability rather than muscle cell damage. Endurance rides or the cross-country phase of a 3-day event can result in CK activity greater than 1000 U/L but usually less than 4000 U/L with CK rapidly returning to baseline.[4] CK activity can increase by in general less than 3000 U/L as a result of prolonged recumbency without an underlying myopathy.

Serum AST activity is not specific for skeletal and cardiac muscle damage because it is also present in liver, red blood cells, and other tissues. Elevated AST can result from hemolysis, muscle, liver, or other organ damage, and measures of hemolysis and liver necrosis, as well as CK activity, are used to assess the significance of elevated serum AST. One of the main utilities of assessing AST in muscle disease is that it persists in serum following muscle damage longer than CK and is therefore a good means to assess the chronicity of muscle cell necrosis. AST activity rises more slowly following damage to muscle cell membranes than CK, often peaking 24 hours after the insult, and the half-life of AST is much longer than that of CK. By comparing serial activities of CK and AST the progression and chronicity of muscle degeneration can be assessed. Concurrent elevations in CK and AST reflect relatively recent or active degeneration. If AST activity is elevated accompanied by decreasing or normal CK, activite muscle degeneration is not ongoing.

Elevations in serum LDH activity are not specific to skeletal muscle and can occur with rhabdomyolysis, myocardial necrosis, and/or hepatic necrosis. Because it is not specific for skeletal muscle and because it has a half-life intermediate to CK and AST, it is not used as often as CK and AST for monitoring muscle damage.

Serum CK Exercise Response Test

If serum CK and AST activities are normal at rest but exertional rhabdomyolysis (ER) is suspected based on history, an exercise response test can be a useful adjunct to diagnose an underlying predisposition to ER.[5] The goal of an exercise challenge test is to provoke subclinical rather than clinical ER. Quantifying the extent of subclinical rhabdomyolysis during mild exercise assists in determining the intensity and rapidity with which to put a horse back into training. Blood samples are taken before exercise and about 4 to 6 hours after exercise to evaluate peak changes in serum CK. Serum CK activity measured immediately after exercise will not reflect the amount of damage occurring during the exercise test. Small fluctuations in serum CK activity may occur with exercise due to enhanced muscle membrane permeability, particularly if exercise is prolonged or strenuous and the horse is untrained. A submaximal exercise test provides more consistent evidence of subclinical rhabdomyolysis than maximal exercise tests.[6,7] With unfit horses alternating 2-minute intervals of walking and trotting for a maximum of 15 minutes is often sufficient to induce abnormal elevations in serum CK activity in horses susceptible to chronic ER. In fit horses 3 minutes of walking followed by 12 minutes of trotting should not normally induce elevations in serum CK. If signs of stiffness develop during the test, exercise should be concluded. A normal response would be less than a threefold increase from basal CK; however, most healthy horses show no change in CK activity with this exercise test.

Electrolytes

In horses with muscle fasciculations or moderate to severe rhabdomyolysis serum sodium, potassium, calcium, chloride, and phosphorus concentrations and potentially measures of blood pH are essential parts of the diagnostic profile. Hypocalcemia and hypophosphatemia, especially in alkalotic horses, can contribute to marked muscle fasciculations or synchronous diaphragmatic flutter. With severe rhabdomyolysis, damage to muscle cell membranes results in movement of electrolytes and water from areas of high concentration to lower concentration. Following damage to muscle cell membranes as occurs with rhabdomyolysis, plasma concentration of extracellular electrolytes (sodium, chloride, and calcium) may be lower than normal as ions move out of blood and into muscle. Conversely, plasma concentrations of electrolytes that are typically higher intracellularly (e.g., potassium and phosphorus) may be increased.[8]

Renal Parameter

In horses with myoglobinuria and marked elevations in serum CK and AST, blood urea nitrogen (BUN) and creatinine should be monitored because myoglobinuria is associated with renal tubular necrosis and renal failure.

Urinalysis

Urinalysis can be the fastest means to detect severe rhabdomyolysis and myoglobinuria. Myoglobinuria can be detected by visual inspection of urine color and a positive urine Hemastix test in the absence of hemolysis or hematuria. Urine pH can be a valuable first screen for hypoglycin A myopathy as equine urine is normally alkaline but is acidic with this condition. Urine can be screened for organic acids to diagnose hypoglycin A myopathy.[9]

Selenium and Vitamin E

Selenium should be assessed in foals with severe rhabdomyolysis and α-tocopherol assessed in horses with generalized muscle atrophy. Selenium concentrations are best measured in whole blood (anticoagulated with ethylenediaminetetraacetic acid, [EDTA]) because selenium is incorporated into red blood cell glutathione peroxidase. Serum or plasma for assessment of α-tocopherol concentrations should be chilled as soon as possible after obtaining a sample and protected from light by wrapping in aluminum foil. If analysis is delayed, serum/plasma samples should be stored frozen (−21°F [−70°C]) because α-tocopherol deteriorates rapidly.

Genetic Testing

Genetic testing of hair roots or EDTA blood is currently available for definitive diagnosis of muscle disorders, including hyperkalemic periodic paralysis (HYPP), glycogen branching enzyme deficiency (GBED), malignant hyperthermia (MH), and type 1 polysaccharide storage myopathy (PSSM1). The decision to perform testing should be based on the prevalence

of the mutation within a breed and whether clinical signs fit with the genetic disease. Type 1 PSSM, for example, has such a low prevalence in Thoroughbred horses that genetic testing for PSSM1 is unlikely to yield valuable information.[10] In contrast, PSSM1 is present in over 50% of Percherons, and therefore the significance of a positive PSSM1 test result must be correlated with clinical signs of a myopathy.[10] It is important to note that commercially available genetic tests determine if one specific mutation previously associated with the disease is present in the sample submitted. Commercial tests do not detect any other alterations in DNA sequence within that disease gene, and such a question would require sequencing of the gene of interest.

Muscle Biopsy

Muscle histopathology can reveal the underlying basis for disorders characterized by weakness, atrophy, rhabdomyolysis, and exercise intolerance. The underlying basis for myopathies primarily characterized by muscle fasciculations is often not apparent in muscle biopsies unless there are underlying dystrophic changes. It is imperative that the most appropriate muscle is selected for biopsy based on the differential diagnoses; that an adequate amount of muscle is obtained; that the specimen is handled appropriately; that the proper fixation is used; and that the most appropriate tinctorial, histochemical, and immunohistochemical stains are used to obtain a diagnosis.

In general, open biopsies of the semimembranosus muscle (Fig. 10.5A) or percutaneous 6-mm needle biopsies of the middle gluteal muscle (Fig. 10.5B) are optimal for exertional myopathies. For Quarter Horses with suspected immune-mediated myositis, a gluteal or lumbar muscle biopsy obtained temporal to the onset of atrophy is best whereas for focal atrophy a sample of that specific muscle group is indicated.[11] Equine motor neuron disease or vitamin E–deficient myopathy is optimally diagnosed through biopsy of the sacrocaudalis dorsalis medialis muscle.[12,13] Samples of the diaphragm or deep postural muscles are most likely to reveal excessive lipid storage in hypoglycin A myopathy.[14] Deep pelvic muscles such as the iliopsoas should be obtained postmortem when examining a horse with recumbency from severe rhabdomyolysis.

A limited amount of information restricted to cellular infiltrates and fiber sizes is obtained from standard hematoxylin and eosin stains of formalin fixed paraffin embedded skeletal muscle. Fresh samples frozen in a particular fashion in isopentane suspended in liquid nitrogen can be stained with a battery of tinctorial and histochemical stains to fully characterize a neuromuscular disorder. Such stains will highlight muscle fiber sizes, shapes, fiber type distribution, mitochondrial distribution, polysaccharide and lipid staining pattern, vacuolar content, neuromuscular junctions, nerve branches, connective tissue, and blood vessels.[15] In addition, frozen samples can be used for immunohistochemistry and biochemical analysis of substrate concentrations and enzyme activities, as well as DNA isolation. Fresh samples can be shipped in a watertight hard container on ice packs to specialized laboratories. Samples for electron microscopy (EM) require appropriate fixation in glutaraldehyde. Ideally, thin sections of muscle for EM should be clamped in vivo to maintain fibers at a resting length before they are excised. However, if pathology other than the alignment of thick and thin myofilaments is to be investigated, small muscle pieces can be excised and placed directly in appropriate EM fixative.

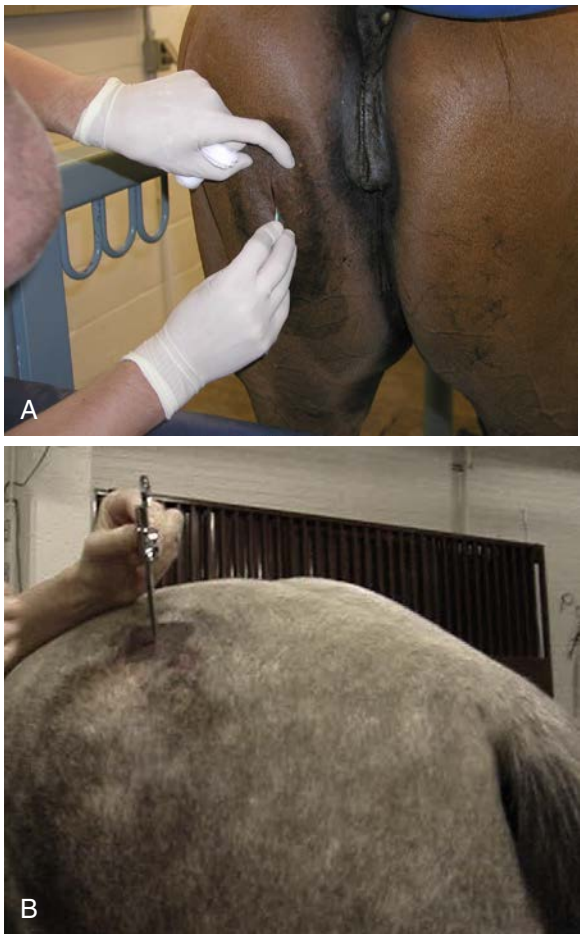


FIG. **10.5** (A) Site of the open surgical biopsy of the semimembranosus muscle. (B) Site of the percutaneous needle biopsy of the middle gluteal muscle.

Electromyography

Fine-needle electromyography (EMG) is very useful for differentiating myogenic and neurogenic atrophy and identifying electrical disturbances of sarcolemmal sodium and chloride channels.[16,17] EMG in horses is usually done under sedation to avoid painful reactions to insertion of the fine EMG needles. EMG of normal skeletal muscle shows temporary insertional activity—a brief burst of electrical activity—when the needle is inserted in muscle and then quiescence. If motor units are recruited (motor unit action potentials) in the muscle examined or the needle is very close to a motor end plate (miniature end plate potentials), normal regular wave patterns are briefly observed. Horses with denervation of motor units have abnormal spontaneous electrical activity in the form of fibrillation potentials and positive sharp waves. Horses with abnormalities in the electrical conduction system of muscle demonstrate forms of complex repetitive discharges or myotonic discharges in numerous muscles. A more detailed EMG analysis evaluates amplitude, duration, phase, and number of phases of motor unit action potentials to identify myopathic features, as well as neuropathic features.[16] Myopathic changes include a decrease in duration and amplitude of motor unit action potentials.

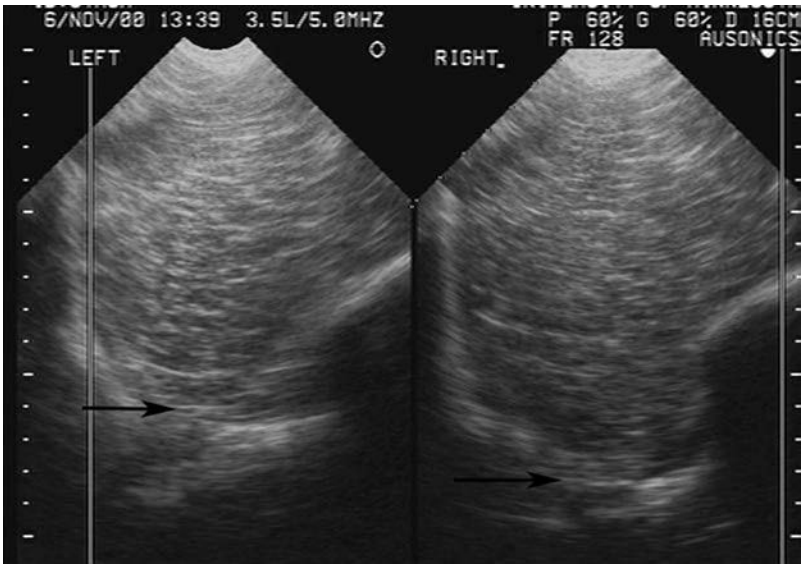


FIG. **10.6** Ultrasonographic appearance of a horse with an acute onset of a stiff short-strided gait on the right hind gait. The depth of the semimembranosus on the right side is much greater than at the corresponding site on the left side *(arrow),* indicating swelling of the right semimembranosus muscles. There is no evidence of tearing or hemorrhage in the muscle.

Imaging

Ultrasonography. Diagnostic ultrasonography can be of value to map the extent of muscle trauma, atrophy, and fibrosis. The typical striated echogenic pattern of skeletal muscle varies among muscle groups, and ultrasonographic evaluation requires that comparisons are made between similar sites in contralateral muscles in both transverse and longitudinal views. It is important that the horse is standing squarely and bearing weight evenly as these can alter the ultrasonographic image. Muscle fiber disruption with an acute injury appears as relatively hypoechoic areas and loss of the normal muscle fiber striation. The jagged edge of the margin of the torn muscle may be increased in echogenicity. Minor trauma may only be apparent as an increase in the depth of the muscle relative to the muscle on the other side (Fig. 10.6). Relatively hyperechoic regions can represent fibrous scarring, whereas hyperechoic regions causing shadowing artifacts can reflect mineralization.[18]

Nuclear Scintigraphy. Nuclear scintigraphy is useful for identification of forms of muscle damage that may not be apparent on physical examination because of depth or location and to assess horses with persistent gait abnormalities and poor performance. Technetium-99m methylene diphosphonate (MDP) is taken up in damaged muscle fibers in the horse and is best seen in the bone phase images (i.e., 3 hours after injection).[19] The mechanism of MDP binding is unknown, but the exposure of calcium binding sites on protein macromolecules in the damaged muscle may be responsible.

EXERTIONAL MYOPATHIES

There are numerous reasons for horses to develop muscle pain with exercise. In many cases mild to moderate muscle soreness can be secondary to extrinsic factors such as orthopedic disease, trauma, ill-fitting saddles, and suboptimal training. A detailed history, physical examination, and lameness examination will often identify the underlying primary disorder and, on correction, exertional muscle pain resolves. When muscle pain and cramping with exercise are associated with elevations in serum CK and AST activity, the muscle disorder is classified as exertional rhabdomyolysis (ER). Overt ER can arise as a sporadic event due to extrinsic factors such as exercise in excess of training, exercise during illness, or nutritional imbalances. ER can also occur as a chronic disease due to intrinsic abnormalities in muscle function.

Exertional myopathies can present with exercise intolerance or exercise-induced pain and poor performance without elevations in serum CK and AST activities or very infrequent elevations. These myopathies are more difficult to accurately diagnose than ER. Evaluation of these cases requires that other orthopedic, respiratory, cardiovascular, and behavioral causes of decreased performance be critically assessed relative to histopathologic abnormalities in muscle biopsies.

The critical steps necessary to diagnose the basis for exertional myopathies include a detailed history, thorough lameness evaluation, measurement of serum CK and AST activities, genetic testing, and muscle biopsy. An exercise challenge test and 4-hour postexercise serum CK activity can be of value in diagnosing ER if not immediately apparent on first evaluation. These procedures are described in detail previously in this chapter.

Sporadic Exertional Rhabdomyolysis

Sporadic forms of ER develop because of an extrinsic event or recurring extrinsic events that induce muscle damage with exercise. Causes of sporadic ER include focal or generalized trauma to muscle, dietary imbalances that affect muscle function, and exercise performed beyond any training adaptation or to the point of exhaustion.

Signalment, History, and Diagnosis

Horses with sporadic ER may be of any age, breed, or sex; may be involved in a wide variety of athletic disciplines; and may have a history of adequate performance before onset of ER.[20]

A diagnosis is made on the basis of history, clinical signs, and elevations of serum muscle enzymes. A reasonable period of rest, a balanced diet, and a gradual introduction of a training program matched with performance will result in resolution of clinical signs and a successful return to performance. If over time ER recurs despite reasonable management, a diagnosis of chronic ER becomes more likely, warranting further diagnostic testing.

Focal or Generalized Trauma

Horses that strain a particular muscle may be very reluctant to move and show signs resembling more generalized ER. This is particularly true when lumbar, gluteal, psoas, adductor, semimembranosus, or semitendinosus muscles are acutely involved.[18] Further, horses that struggle when cast, are caught in a fence, or are thrashed by another horse may have acute signs of rhabdomyolysis caused by severe exertion or trauma. A diagnosis of focal trauma is established by history, physical examination, and serum muscle enzymes. Rectal examination of deep pelvic muscles and ultrasonography may help to localize the extent of muscle damage for focal lesions. On occasion scintigraphy may help to identify specific muscle involvement.[21]

Overexertion

A training imbalance as a cause of ER is usually suspected based on a history of an increase in work intensity without a foundation of consistent training for this level of exercise. Signs of muscle stiffness and gait changes range from mild to severe, and severity is reflected by the magnitude of elevations of serum CK activity. Overexertion is a well-described cause of ER in polo horses with 81% of cases of ER attributed to overexertion and 30% of cases occurring after a day of rest.[22] The incidence of ER is as high as 9% in U.S. polo horses, with most of these horses only having one episode of ER early in the polo season.[22]

Pathologic changes are often not evident in light microscopic evaluation of muscle biopsies from individuals performing unaccustomed exercise, but electron microscopy shows significant disruption of the alignment of muscle contractile proteins within muscle fibers. In more severe cases overt segmental damage to myofibers may be apparent in muscle biopsy. Repetitive overuse of muscles, such as occurs with overtraining, may result in exercise intolerance and is associated with pathologic changes such as increased muscle fiber size variation and centrally displaced myonuclei in muscle biopsies.[23]

Exhaustion

Exhaustion occurs most commonly in endurance horses or racehorses exercising in hot, humid weather. Signs of heat exhaustion include weakness, ataxia, rapid breathing, muscle fasciculations, sweating, and in severe cases collapse. The body temperature may be elevated to 105° to 108°F. Muscles are frequently not firm on palpation, although serum CK activity can be markedly elevated and myoglobinuria may be noted.[24,25]

Dietary Imbalances

Diets with a high nonstructural carbohydrate (NSC) content and low forage content,[26,27] deficient in electrolytes, or inadequate in selenium and vitamin E may exacerbate episodes of ER.

Electrolyte Imbalances

It is important to ensure that horses receive sodium chloride in the diet, especially horses that compete in hot, humid weather. Between 30 and 50 g per day of sodium chloride combined with 15 to 25 g of light salt containing potassium chloride are recommended for horses exercising in hot, humid conditions and sweating extensively. In addition, the entire diet should be adequately balanced with calcium and phosphorus (ratio of 2:1 Ca:P is ideal). Assessing total body electrolyte balance is difficult to accurately perform. One suggested means is to measure urinary fractional excretion of electrolytes[28]; however, marked variation can occur in electrolyte excretion depending on the diet, exercise, and sampling technique, as well as between and within individuals from day to day.[29] Furthermore, the high calcium crystal concentration of alkaline equine urine requires acidification to accurately assess calcium and magnesium content.

Vitamin E and Selenium Concentrations

Whole blood selenium concentrations measured in EDTA or heparin tubes are of value in assessing selenium status in animals housed in areas where soil and therefore diet is deficient in selenium. In cases where selenium has been administered before blood collection, glutathione peroxidase activity can be used to assess potential selenium deficiency. Vitamin E concentration should be measured in serum samples kept chilled and protected from light; however, variability in serum levels can be quite large, and repeat sampling is recommended if marginal levels are identified in the first sample tested. Horses with ER are infrequently deficient in selenium and vitamin E, and alone it may not be responsible for ER[30]; however, anecdotal reports suggest that in some cases supplementation may help to further prevent episodes of ER.

Treatment of Acute Rhabdomyolysis

Hand walking horses with acute rhabdomyolysis is contraindicated as it exacerbates muscle damage. If ER occurs at a distance from home, a trailer should be brought to the horse and a tranquilizer administered to assist the horse in moving to the trailer and loading. During a competition, the horse is treated for rhabdomyolysis on site, and transport home over a long distance should be delayed for at least 24 to 48 hours.

The objectives of treatment are to relieve anxiety and muscle pain, as well as to correct fluid and acid-base deficits.[31] Acepromazine (0.04–0.07 mg/kg), an α-adrenergic antagonist, is helpful in relieving anxiety and may increase muscle blood flow. Dehydrated horses may become hypotensive and recumbent if given acepromazine. Alternatively, xylazine (0.4–0.8 mg/kg) may provide short-term relief from anxiety. In horses in more pain, detomidine (0.02–0.04 μg/kg) combined with butorphanol (0.01–0.04 mg/kg) provides excellent sedation and analgesia. Nonsteroidal antiinflammatory drugs (NSAIDs) such as ketoprofen (2.2 mg/kg), phenylbutazone (2.2–4.4 mg/kg), or flunixin meglumine (1.1 mg/kg) provide additional pain relief. Analgesic treatment is continued to effect, but most horses are relatively pain free within 18 to 24 hours. For horses with extreme pain and distress a constant rate infusion of detomidine (0.22 μg/kg intravenously followed by 0.1 μg/kg/min IV) or lidocaine (1.3 mg/kg intravenously followed by 0.05 mg/kg/min intravenously) or butorphanol (13 μg/kg/hr) can make the difference between adequate time for recovery and euthanasia.

Intravenous (IV) or intragastric dimethyl sulfoxide (as a less than 20% solution) can be used as an antioxidant, antiinflammatory, and osmotic diuretic in severely affected horses.

Some veterinarians administer corticosteroids in the acute stage. If the horse is recumbent methylprednisolone succinate (2–4 mg/kg intravenously) can be given once. Muscle relaxants such as methocarbamol (5–22 mg/kg, slow IV administration) seem to produce variable results possibly depending on the dosage used. Dantrolene sodium (Dantrium) (2–4 mg/kg orally) is administered to decrease release of calcium from the sarcoplasmic reticulum and further aggravation of muscle contractures and necrosis. This can be repeated in 4 to 6 hours. Caution is advised when administering Dantrium to horses with HYPP as it can elevate serum K concentrations and precipitate an episode.

Severe rhabdomyolysis can lead to renal compromise due to ischemia and the combined nephrotoxic effects of myoglobinuria, dehydration, and NSAIDs. The first priority in horses with hemoconcentration, or myoglobinuria, is to reestablish fluid balance and induce diuresis. In mild cases administration of fluids via a nasogastric tube may be adequate, but generally fluids are better given intravenously. Balanced polyionic electrolyte solutions are best. If severe rhabdomyolysis is present, isotonic saline or 2.5% dextrose in 0.45% saline may be necessary because horses often have hyponatremia, hypochloremia, and hyperkalemia.[8] If hypocalcemia is present, supplementing IV fluids with 100 to 200 mL of 24% calcium borogluconate is recommended, but serum calcium should not exceed a low normal range. Affected animals are usually alkalotic, making bicarbonate therapy inappropriate.

The total fluid replacement is based on an estimation of the degree of dehydration and the clinical response: 10 L of fluids may be given rapidly. If the adult horse is 5% dehydrated an additional 15 L may be given over the next 4 hours; if dehydration is severe (20%) an additional 50 L of polyionic fluids may be required at 4 L per hour. Ideally, the need for further IV fluids is guided by reassessment of the packed cell volume, total plasma protein, and serum electrolytes after the initial period of therapy. In severely affected horses, regular monitoring of blood urea nitrogen and/or serum creatinine is advised to assess the extent of renal damage. Diuretics are usually contraindicated unless the horse is in oliguric renal failure.

Good nursing care is important with recumbent animals. This involves deep bedding, prevention or minimization of trauma around the eyes, and appropriate care of decubital sores. Recumbent animals require frequent turning to allow reperfusion of compressed muscle. The use of slings to assist recumbent animals to rise is highly beneficial.

Following treatment of an acute ER episode, after stiffness resolves, rest with regular access to a paddock and weekly monitoring of serum CK activity are recommended. Horses are much more susceptible to a second episode of ER in the 2 weeks following an acute episode, and allowing horses the ability to calmly determine their own exercise through turnout often avoids exacerbating rhabdomyolysis. If that is not feasible, hand walking needs to be performed with caution and limited to no more than a few minutes initially. While the horse is rested, the diet can be assessed to ensure that the ration is fed in an amount recommended by the manufacturer for the corresponding level of exercise. This will ensure that the proper balance of vitamins and minerals is provided. If excessive calories are provided when fed according to the manufacturer's recommendations, the diet should be switched to a less calorie-dense vitamin/mineral-balanced diet. A salt block or 30 to 50 g (1–3 tablespoons) of salt per day will provide the necessary additional sodium chloride, with the amount fed depending on the heat, humidity, and intensity of exercise. Once serum CK returns to normal, training can be resumed gradually. A regular exercise schedule, beginning with 20 minutes or less of exercise per day, is gradually increased to eventually match the expected amount of daily exercise to the underlying state of conditioning.

Chronic Exertional Myopathies

Several inherent disorders of equine skeletal muscle can cause repeated episodes of ER or exertional myopathy: recurrent exertional rhabdomyolysis, malignant hyperthermia, type 1 polysaccharide storage myopathy, type 2 polysaccharide storage myopathy, idiopathic chronic exertional rhabdomyolysis, and mitochondrial myopathy. There are likely many other causes yet to be identified. In these forms of chronic exertional myopathies, there are specific environmental stimuli that are necessary to trigger muscle pain in susceptible horses. Horses cannot be cured of their susceptibility to this condition, but if the specific form of exertional myopathy is identified, changes in management may help to minimize episodes of muscle pain.

Recurrent Exertional Rhabdomyolysis

The term *recurrent exertional rhabdomyolysis* (RER) describes a subset of ER that is believed to be due to an abnormality in the regulation of muscle contraction and relaxation.[5,32-34] Research into RER has primarily been performed in Thoroughbreds and, to a lesser extent, Standardbred horses.[32,35-37] Scientific studies of RER have used a small number of horses that share common clinical signs and have abnormal muscle contracture tests.[32,34,38] Broader epidemiologic and genetic studies have assumed that the same pathophysiologic basis for ER exists in the majority of Thoroughbred and Standardbred horses with similar clinical signs. Whether this is true or not will require more research. There are reports of some Arabian and Warmblood horses[39] with ER that may also suffer from RER based on the overlapping histories, clinical signs, muscle biopsy findings, and response to management.

Rhabdomyolysis is triggered suddenly during exercise in RER horses resulting in a sharp increase in serum myoglobin and CK activity.[6] Clinically, the triggering event is often associated with excitement in a horse that already has an underlying nervous temperament.[35,40] Segmental necrosis in single fibers or small clusters of necrotic fibers scattered throughout fascicles may be apparent in fewer than 5% of type 2a and 2x muscle fibers in a gluteus medius muscle biopsy.[36,37] Serum cortisol concentrations are higher in RER horses than normal horses before exercise and increase during an episode of ER.[6] Serum concentrations of epinephrine and norepinephrine are normal before an episode but increase dramatically in horses with marked elevations in serum CK activity.[6]

Exercise. In Standardbreds, ER commonly begins after 15 to 30 minutes of jogging at 5 m/sec, although clinical signs may not be apparent until after exercise.[36] In Thoroughbreds exercising on the treadmill, ER most commonly develops with intervals of walk, trot, and canter and is less common if horses are allowed to gallop.[41,42] At the racetrack, ER occurs commonly when RER horses are held back to a paced gallop.[35] During eventing, RER commonly occurs after the excitement of the steeplechase or early in the cross-country phase when horses are held to a predetermined speed.[43] ER rarely occurs

when horses are allowed to achieve maximal exercise speeds such as racing. A day or more of rest before this type of exercise results in higher postexercise serum CK activity.

Lactic Acid. There is no basis for an association between RER and lactic acidosis. Thoroughbreds and Standardbreds rarely develop ER during near-maximal exercise[42,44] when muscle lactates reach 240 mmol/kg[45] and plasma lactates are as high as 25 mM. Standardbred horses experience ER during jogging when muscle lactate concentrations are less than 24 mmol/kg.[46] Similarly, plasma lactate concentrations in RER Thoroughbred horses are less than 1.5 mM when performing the walk, trot, and canter treadmill exercise that induces high serum CK activity.[41] Thus rhabdomyolysis in RER horses is not due to lactic acidosis.

Intracellular Calcium Regulation. Research suggests that horses with RER may have an inherent abnormality in intramuscular calcium regulation that is intermittently manifested during exercise.[34,47] Myoplasmic calcium concentrations are tightly controlled by channels and pumps in the sarcoplasmic reticulum and usually are unaffected by normal serum calcium concentrations. Basal muscle intracellular calcium concentrations in RER horses are similar to healthy horses based on assays in muscle cell cultures.[47] Higher intracellular calcium concentrations have been measured in horses of unspecified breeds during an episode of ER; however, this also could be secondary to any insult that impairs energy generation or cell membrane integrity.[48] To specifically study shuttling of calcium between the sarcoplasmic reticulum and myoplasm within the muscle cell, technically complex procedures such as muscle contracture testing, calcium imaging in muscle cell cultures, and calcium release by isolated muscle membrane preparations have been evaluated. Results are reported as the threshold at which a significant increase in muscle tension occurs above baseline levels. Caffeine and halothane are most frequently used to trigger the release of calcium from the sarcoplasmic reticulum to the myoplasm via the ryanodine receptor. This test remains an experimental procedure because of the difficulty in obtaining accurate and timely results. Early studies by Hildebrand et al[38] suggested that some but not all horses with ER showed a lower threshold for caffeine- and halothane-induced muscle contractures than healthy horses. Waldron-Mease et al[49] and Beech et al[32] found that caffeine contracture thresholds were lower in semimembranosus muscle from Thoroughbreds and Standardbreds susceptible to RER versus breed-matched controls. Lentz et al[34,50] studied intact external intercostal muscle fibers and found that Thoroughbreds with RER had a lower contracture threshold for triggering agents such as potassium, caffeine, and halothane but not 4 chloro-m-cresol compared with control horses. An abnormality in intracellular calcium regulation was also supported by a muscle cell culture study of RER Thoroughbred horses.[47] Thus all of these studies support an abnormality in intracellular calcium regulation in most of the Thoroughbred or Standardbred horses studied. Isolated sarcoplasmic reticulum membrane studies, however, have not conclusively identified any abnormalities in intracellular calcium regulation, indicating that there are distinct differences between RER and disorders such as malignant hyperthermia.[32,51] Genetic linkage analysis has failed to associate RER with the genes *RYR1* (ryanodine receptor), *SERCA* (SR ATPase), or *DHPR1* (dihydropyridine receptor), all of which are involved in intramuscular calcium regulation. Thus RER could be a novel defect in muscle excitation-contraction coupling, calcium regulation, or contractility whose basis is yet to be defined. Alternatively, the increased sensitivity of RER horses in contracture testing may be a nonspecific indicator of an abnormality in other pathways governing muscle contraction.

Prevalence and Risk Factors. Assuming that the majority of cases of ER at racetracks are due to RER, the prevalence in Thoroughbred racehorses is remarkably similar around the world, with estimates of 4.9% in the United States,[35] 5.4% in Australia,[21] and 6.7% in the United Kingdom.[40,43] The annual incidence of ER in Standardbred racehorses in Sweden is 6.4%.[52] Exercise obviously increases the prevalence of RER in horses, and episodes are observed more frequently once horses achieve a level of fitness.

In Standardbreds and Thoroughbreds, mares more commonly have RER than males; however, no general correlation has been observed between episodes of rhabdomyolysis and stages of the estrous cycle.[53] There is an interaction between age and gender in RER Thoroughbred horses such that the proportion of affected females to males is much higher in young horses compared with older age groups.[35] Swedish Standardbreds with RER, which do not begin racing until 3 years of age, did not show the same age association with RER as Thoroughbreds.[53] Temperament has a strong effect on the expression of RER in both Standardbreds and Thoroughbreds, with nervous horses, particularly fillies, having a higher incidence of rhabdomyolysis than calm horses.[35,43,52] Young fillies are more likely to have a nervous temperament than mares or male horses. Diet also has an impact, with Thoroughbred horses, fed more than 2.5 kg of grain being more likely to show signs of RER.[41] One study also found a higher prevalence of rhabdomyolysis among Thoroughbred horses with lameness.[35] A few days of rest before exercise increases the degree of elevation on serum CK activity with the next exercise session and in practice predisposes horses to an episode of ER.[42]

Although there is a familial tendency for RER, its genetic basis has not yet been defined. A genetic susceptibility appears to exist in Thoroughbred horses, and RER-afflicted Thoroughbreds may pass the trait to their offspring.[54,55] The difficulty in establishing a specific phenotype, the variable penetrance of the disease, and the possible existence of potential modifying genes have made this hard to firmly establish. Of note is the finding that Standardbred trotters with a history of RER have faster racing times from a standstill start than those without a history of RER, suggesting that the trait may have beneficial as well as deleterious effects.[52]

Diagnosis. A presumptive diagnosis of RER is based on clinical signs of muscle pain and the presence of risk factors commonly associated with RER. Serum CK and AST activities serve as the basis for detecting muscle degeneration, and they often show intermittent abnormal elevations that return to normal relatively quickly during the course of training. If CK elevations are mild there are a number of factors to consider. Timing of the sample with regard to the onset of ER and exercise is important. Reliability is improved by obtaining blood samples at a standardized time after exercise, preferably 4 to 6 hours when CK peaks, and consistently with regard to exercise on the preceding day, because serum CK activity is higher on exercise days that are preceded by a day or more of rest.[56] In addition, normal values need to be adjusted for the age and sex of horses. Two-year-old fillies generally have greater fluctuations in serum CK activity during race training than 3-year-old fillies or geldings.[56]

Muscle histopathology is nonspecific in RER horses; either no abnormalities or evidence of centrally located nuclei in mature fibers and potentially waves of myofiber degeneration or regeneration are found.[5] There is a notable absence of abnormal amylase-resistant polysaccharide, although increased subsarcolemmal amylase-sensitive glycogen may be present.[57] The value of a muscle biopsy as a diagnostic tool in horses with suspected RER is largely confined to particularly recurrent unmanageable cases where a need arises to rule in or out other forms of ER.

Management. Prevention of rhabdomyolysis in horses susceptible to RER is complex, and multiple factors need to be changed to decrease episodes. These factors include the environment, the exercise regimen, and diet. Medication may be needed at times to prevent further episodes of muscle damage.

ENVIRONMENT. Because excitement and nervousness often trigger rhabdomyolysis, stressful situations in the environment that can be modified need to be identified. This may involve a change to a smaller barn with fewer horses and fewer handlers, compatible companions, and a more consistent daily routine. Many stressed RER-susceptible horses respond to a regular routine including feeding before other horses and training before other horses, especially if the horse becomes impatient while waiting. Other horses respond to being housed in a quiet area of the barn next to calm companionable horses. The use of hot walkers, exercise machines, and swimming pools should be evaluated on an individual basis, as some horses develop rhabdomyolysis when using this type of equipment. Horses that develop rhabdomyolysis at specific events, such as horse shows, may need to be reconditioned to decrease the stress level associated with such events or tranquilized as part of the accommodation process. Providing daily turnout with other horses seems to be very beneficial for RER horses and may decrease anxiety and thereby the likelihood of rhabdomyolysis.

EXERCISE. Although for sporadic forms of ER a period of rest is recommended until serum CK normalizes, it is not recommended for horses with RER.[18] Daily exercise is important in preventing episodes of rhabdomyolysis, and when serum CK is less than about 3000 U/L horses should be returned to regular daily exercise. Horses with more severe episodes of rhabdomyolysis may require additional time off in a paddock before gradually resuming exercise. Once back in training, some form of daily exercise is recommended. Avoiding fighting to hold racehorses to slower speeds during galloping may decrease rhabdomyolysis in RER-susceptible Thoroughbreds. Interval training and reduction of jog miles to no more than 15 minutes per session will benefit Standardbreds. For horses under saddle, a relaxed warm-up with intermittent periods of long low stretching may be of benefit, and continued breaks to stretch between episodes of collection are recommended. Event horses may require training that incorporates calm exposure to speeds achieved during the steeplechase or cross-country phase especially when used in an interval training program.

DIET. A nutritionally balanced diet with appropriate caloric intake and adequate vitamin and mineral intake are the core elements of managing RER. For RER Thoroughbreds and Standardbreds in training, the challenge is usually supplying enough calories in a highly palatable form to meet their daily energy demands. This is in part because they often require greater than 30 MCal of digestible energy (DE) a day (5 kg sweet feed, 1.5% of body weight in hay) and because of their nervous temperament they may be more discriminating in their eating habits or prone to gastric ulcers. Of the total daily calories required by RER horses, research suggests that less than 20% DE should be supplied by nonstructural carbohydrates (NSC = starch and sugar), and at least 20% of DE should be supplied by fat.[41,42]

SELECTION OF FORAGE. Thoroughbred horses do not appear to show the same significant increase in serum insulin concentrations in response to consuming hay with an NSC of 17% as seen in Quarter Horses (Borgia L, 2010, PhD thesis, University of Minnesota). This fact combined with the high caloric requirements of racehorses may mean that it is not as important to select hay with very low NSC content in RER Thoroughbreds as it is in PSSM horses. Anecdotally some trainers find that horses with RER have more frequent episodes on alfalfa hay. The nervous disposition of some RER horses may predispose them to gastric ulcers, and thus frequent provision of hay with a moderate NSC and mixed alfalfa content may be indicated.

LOW-STARCH, HIGH-FAT CONCENTRATES. When RER Thoroughbreds are fed a moderate caloric intake (24 MCal/day) in the form of high-starch concentrates (2.5 kg of corn/oats/wheat middlings/molasses), they show very little elevation in serum CK activity with exercise.[41] Most Thoroughbred racehorses, however, are fed at least 5 kg of high-starch concentrate per day at 30 MCal or more per horse per day, and at this level of feeding, postexercise serum CK activity rises significantly.[35] The discovery that substitution of fat for starch in a high-calorie ration significantly reduces muscle damage in exercising RER horses was a major advance in nutritional management of RER.[42] Practically, however, it was difficult to achieve the desired caloric intake of racehorses because the maximum amount of fat that finicky Thoroughbreds will happily consume is often limited to 600 mL for vegetable oil or 5 lb/day of balanced rice bran. Management of RER horses was significantly improved when a palatable means to provide the amount of fat required by fit finicky RER Thoroughbreds was developed. A controlled trial using a specialized feed developed for RER (Re-Leve 13% fat by weight and 9% NSC) determined that NSC should be no greater than 20% of daily DE, and 20% to 25% of daily DE should be provided by fat for optimal management of RER horses fed 30 MCal or more per day.[42] No beneficial effect on serum CK activity occurred when sodium bicarbonate (4.2% of the pellet) was added to a high-starch pellet feed.[42] The amount of fat in the diet of an RER horse depends on its caloric requirements. If an affected horse being fed hay at 1.5% to 2% of body weight and 2.5 kg of grain daily requires additional caloric intake to maintain body weight, additional calories should be supplied by adding a source of fat to the diet.

The benefit of a fat-supplemented diet does not appear to be a change in muscle metabolism. Preexercise and postexercise muscle glycogen and lactate concentrations are the same in RER horses fed a low-starch, high-fat diet compared to a high-starch diet.[44] Low-starch, high-fat diets in RER horses may decrease muscle damage by assuaging anxiety and excitability, which are tightly linked to developing rhabdomyolysis in susceptible horses. High-fat, low-starch diets fed to fit RER horses produce lower glucose, insulin, and cortisol responses and lead to a calmer demeanor and lower preexercise heart rates. Neurohormonal changes may develop in response to high serum glucose, insulin, and cortisol concentrations, resulting in an anxious demeanor.[58] Although a calm

demeanor is desired during training, some racehorse trainers feeding low-starch, high-fat feeds prefer to supplement with a titrated amount of high-starch grain 3 days before a race to potentially increase liver glycogen and increase a horse's energy during the race.

Studies in RER horses show that significant reductions or normalization of postexercise serum CK activity occurs within a week of commencing a low-starch, fat-supplemented diet.[42] Days off training in a stall are discouraged because postexercise CK activity is higher following 2 days of rest compared to values taken when performing consecutive days of the same amount of submaximal exercise.[42,59]

SUPPLEMENTS. Horses require daily dietary supplementation with sodium and chloride either in the form of loose salt (30–50 g/day) or a salt block. Additional electrolyte supplementation is indicated in hot, humid conditions. A myriad of supplements are sold that are purported to decrease lactic acid buildup in skeletal muscle of RER horses. These include sodium bicarbonate, B vitamins, branched chain amino acids, and dimethylglycine. Given that lactic acidosis is no longer implicated as a cause for rhabdomyolysis, it is difficult to find a rationale for their use.

MEDICATIONS. Low doses of tranquilizers, such as acepromazine, before exercise have been used in RER horses prone to excitement.[60] A dose of 7 mg intravenously 20 minutes before exercise is reported to make horses more relaxed and manageable. Reserpine and fluphenazine, which have a longer duration of effect, have also been used for this purpose. Horses given fluphenazine may occasionally exhibit extrapyramidal adverse effects with prolonged bizarre behavior. Use of tranquilizers may only be necessary when horses are in their initial phase of training and accommodation to a new environment. Horses obviously cannot compete on these medications, and withdrawal times must be observed.

Dantrolene sodium (Dantrium) acts to decrease release of calcium from the ryanodine receptor in skeletal muscle and is used to treat malignant hyperthermia. Studies of intact equine intercostal muscle from ER horses show that Dantrium lowers already elevated myoplasmic calcium concentrations.[48] Controlled and field studies have also shown that oral Dantrium can significantly decrease signs of rhabdomyolysis in RER horses.[59,61] One study indicated that horses need to be fasted to achieve measurable blood levels of Dantrium,[59] but more recent controlled trials suggest that absorption of Dantrium is superior in fed versus fasted horses.[62] Administration of 4 mg/kg of Dantrium PO 1 hour before exercise to RER-susceptible horses fed high-grain diets resulted in significantly lower serum CK activity 4 hours after exercise compared with placebo.[59] A dose of 800 mg of Dantrium was given to Thoroughbred horses in the United Kingdom 1 hour before exercise and resulted in significantly lower postexercise CK activity than a placebo.[61]

Phenytoin (1.4–2.7 mg/kg PO b.i.d.) has been reported to be effective in preventing rhabdomyolysis in horses with RER.[33,63] Phenytoin acts on a number of ion channels within muscle and nerves, including sodium and calcium channels. Therapeutic levels vary, so oral doses are adjusted by monitoring serum levels to achieve 8 µg/mL and not to exceed 12 µg/mL.[33,63] Drowsiness and ataxia are evidence that the dose of phenytoin is too high and the dose should be decreased by half. Initial dosages start at 6 to 8 mg/kg orally twice a day for 3 to 5 days. If the horse is still experiencing rhabdomyolysis but is not drowsy, the dose can be increased by 1-mg/kg increments every 3 to 4 days. Phenytoin is a monoaminoxidase activator and can affect plasma concentrations of other medications. Unfortunately, long-term treatment with Dantrium or phenytoin is expensive, and these drugs must be withdrawn before competition.

HORMONE THERAPY. Various hormones, ranging from thyroxine to progesterone and testosterone, have been given to horses with RER. Initial studies linking low T_3 and T_4 concentrations in horses with ER have not been substantiated.[64,65] Some mares appear to exhibit signs of rhabdomyolysis during estrus, and it may well be of benefit in these horses to suppress estrous behavior using progesterone injections. Testosterone and anabolic steroids have been used at racetracks to prevent signs of RER, but their use is no longer permitted.

ADJUNCT THERAPIES. Massage, myofascial release, mesotherapy, stretching, aqua-treadmill therapy, and hot/cold therapy performed by experienced therapists may be of benefit in individual horses.[66] Their use may promote relaxation, promote normal muscle tension, and build muscle strength.

Malignant Hyperthermia

Pathogenesis

Malignant hyperthermia (MH) occurs in Quarter Horses and Paint Horses as a result of an autosomal dominant mutation in exon 46 of the skeletal muscle ryanodine receptor 1 gene *(RYR1)*.[67,68] The mutation lowers the activation and heightens the deactivation threshold of the ryanodine receptor, which intermittently can result in a dramatic efflux of calcium from the sarcoplasmic reticulum, increasing myoplasmic calcium and producing a contracture.[69] Anaerobic glycogen metabolism is activated, lactate is produced, excessive heat is generated, and massive muscle necrosis ensues.

Prevalence and Risk Factors

MH affects less than 1% of Quarter Horses and Paint Horses, particularly affecting halter and pleasure horse lines. Rhabdomyolysis with MH can be induced by exercise or anesthesia and is exacerbated by the presence of the *GYS1* mutation for type 1 PSSM. In many horses, clinical signs are completely unapparent or very intermittent in nature.[68]

Clinical Signs

Clinical signs of hyperthermia (104.9°F [40.5°C]), hypercapnia (P_aCO_2 of 274 mm Hg), and acidosis (pH of 6.72) were reported in an experimental protocol approximately 60 minutes after anesthesia was induced by delivering halothane via a face mask without premedication.[70] Death occurred from cardiopulmonary arrest and was preceded by profound rigor mortis. Hematologic changes present 2 minutes after death included hemoconcentration, hyperkalemia, hypercalcemia, hyperphosphatemia, hyperglycemia, and elevated creatinine. Serum CK activity was mildly increased at 843 U/L, and myoglobin was 10 times higher than the reference range in this horse.

Horses with the *RYR1* mutation may intermittently show signs of ER and high body temperatures.[68] Some MH-affected horses have died suddenly after an episode of ER. Horses with the MH mutation may also have the *GYS1* mutation associated with type 1 PSSM.[71] Such horses have more severe episodes of ER, higher serum CK activity after exercise, and a more moderated response to the diet and exercise regimens recommended for type 1 PSSM.[71]

Diagnosis

Genetic testing is recommended in Quarter Horses and Paint Horses with severe ER or ER associated with elevated body temperature. Muscle biopsy is not particularly useful for diagnosing MH. Biopsies may contain mild myopathic changes, including increased variation in fiber sizes, centrally located nuclei, fiber necrosis, glycogen depletion, and ringbinden fibers.

Treatment

Horses with suspected MH should be treated with oral Dantrium (4 mg/kg) 30 to 60 minutes before anesthesia.[70] The exception to this rule may be horses with MH and hyperkalemic periodic paralysis because Dantrium can cause an excessive rise in serum potassium that can trigger an episode of muscle paralysis.[72] There is no cost-effective means to deliver Dantrium to horses intravenously once an episode of MH has begun. The nature of ER with horses positive for MH is so intermittent that it is hard to justify premedication with Dantrium before exercise.

Polysaccharide Storage Myopathy

Polysaccharide storage myopathy (PSSM) was first recognized as a specific myopathy in Quarter Horse–related breeds in 1992,[73] but reports of abnormal polysaccharide inclusions in equine muscle of individual horses date back to 1979.[74] The remarkable feature of the first horses reported to have PSSM was twofold higher muscle glycogen concentrations than normal horses and abnormal granular amylase-resistant inclusions in histologic sections.[73] Similar biopsy findings were reported in Belgian and Percheron draft horses.[74]

Terminology

PSSM has also been referred to as *equine polysaccharide myopathy* (EPSM or EPSSM). [27,75] Considerable controversy existed as to whether these acronyms encompassed one muscle condition.[7,27,76] In 2008 a mutation in the glycogen synthase 1 gene *(GYS1)* was found to be highly associated with the presence of amylase-resistant polysaccharide in skeletal muscle.[77] Genetic testing of hundreds of horses previously diagnosed with PSSM by muscle biopsy revealed that the majority of cases of PSSM characterized by amylase-resistant polysaccharide in skeletal muscle had the *GYS1* genetic mutation. However, some cases previously diagnosed with PSSM by muscle biopsy, particularly those with excessive amylase-sensitive glycogen, did not possess the genetic mutation. This suggested that there are at least two forms of PSSM.[77,78] For clarity, the form of PSSM caused by an H309G *GYS1* mutation is now termed *type 1 (PSSM1)* whereas the form or forms of PSSM not caused by the *GYS1* mutation and whose origins are yet unknown are termed *type 2 (PSSM2)*.[78] PSSM1 is likely to be the same disorder that was called "azoturia" or "Monday morning disease" in work horses in the nineteenth and twentieth centuries.

Type 1 PSSM

Genetics. PSSM1 is caused by an autosomal dominant missense mutation in *GYS1* that results in an arginine substitution for histidine at codon 309 in glycogen synthase.[77] The effect of this amino acid substitution is a higher than normal activity of glycogen synthase both at basal states and when activated by glucose-6-phosphate.

Prevalence. PSSM1 is estimated to have emerged approximately 1600 years ago when the great horse was being developed from European draft and light horse breeds to carry knights with heavy armor into battle.[77] The highest prevalence of PSSM1 appears to occur in draft horses derived from Continental European breeds.[79] In fact, many Continental European drafts are homozygous for the dominant PSSM1 trait (90% prevalence of PSSM in Trekpaards with 40% of tested Belgian Trekpaards homozygous).[79] North American Belgians and Percherons also have a high prevalence of PSSM1 at 36% and 54% of horses affected.[10] In contrast, the prevalence of PSSM is very low in UK-derived breeds such as Shires and Clydesdales, but PSSM1 is present in other UK horse breeds such as Irish Drafts, Cob, and Connemara.[10,79] Horses of more than 20 other breeds may have the *GYS1* mutation responsible for PSSM1.[80-82] Prevalence estimates of PSSM1 in Quarter Horses range from 6% to 10% of the breed and 6% to 8% for American Paint and Appaloosa horses.[83] The highest frequency of PSSM1 occurs in halter Quarter Horses (28% affected) and the lowest frequency in racing Quarter Horses.[83] The *GYS1* mutation has been identified in approximately 72% of Quarter Horses diagnosed with PSSM by muscle biopsy and in 18% of Warmbloods of a variety of types diagnosed with PSSM by muscle biopsy.[84] The prevalence of PSSM1 is very low to nonexistent in light horse breeds such as Arabians, Standardbreds, and Thoroughbreds.[10]

Risk Factors. Some horses with PSSM1 are asymptomatic, whereas other horses routinely show stiffness with exercise. In a family of Warmbloods sired by a stallion with the *GYS1* mutation, the mutation resulted in a sevenfold higher risk of developing ER, with owners reporting that 66% of horses with the *GYS1* mutation had clinical signs of ER.[85] The reason for variability in clinical signs of PSSM1 probably lies in the influence of diet, exercise, other environmental factors, and other genes on the expression of the disease. There is no significant temperament, body type, or gender predilection for PSSM1.[86,87] The most common trigger for clinical signs of PSSM1 is less than 20 minutes of exercise. Clinical signs occur most commonly at a walk and trot, particularly if the horse has been rested for several days before exercise or is unfit.[86] Diets high in NSC also increase the risk of muscle pain and stiffness in PSSM1 horses.[88] About 40% of owners feel there is a seasonal incidence to the development of clinical signs, which some have attributed to quality of grass available at the time.[82] Although exercise is by far the most common trigger for PSSM, Quarter Horse and Paint Horse foals and weanlings may develop rhabdomyolysis in conjunction with a systemic illness.[89]

Diagnosis. Persistent elevations in serum CK and AST activities are common in symptomatic horses with PSSM1 (Fig. 10.7). If horses have normal serum muscle enzymes at rest, an exercise test consisting of a maximum of 15 minutes lunging at a walk and trot may help to determine if subclinical ER is present.[5] Supportive evidence would include a minimum of a threefold elevation in CK activity 4 hours after exercise.

The gold standard for diagnosis of PSSM1 is genetic testing for the *GYS1* mutation performed on whole blood or hair root samples. It is important to note that in draft horses of continental European origin, the very high prevalence of PSSM in essence means that there is a high chance that any clinical sign could be falsely associated with the disease PSSM1.[79,87] Clinical judgment is required to determine whether the clinical signs of the animal could reasonably be associated with a myopathic process. Muscle biopsy provides a means to diagnose PSSM; however, to classify PSSM as type 1 a genetic test is required. The distinctive features of PSSM1 in muscle biopsy

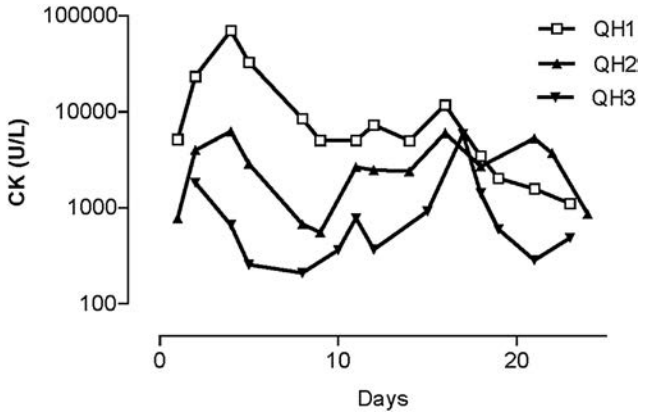


FIG. **10.7** Serum creatine kinase (CK) activity obtained 4 hours after exercise in three Quarter Horses with type 1 polysaccharide storage myopathy (PSSM1) over a 3-week period of training for 20 minutes or less each day. Note that these severely affected PSSM horses have persistent elevations in serum CK activity that do not return to normal reference range.

samples are numerous subsarcolemmal vacuoles and dense, crystalline periodic acid–Schiff (PAS)–positive and amylase-resistant inclusions in fast-twitch fibers (Fig. 10.8). A diagnosis can be made irrespective of diet and proximity of sampling to recent episodes of rhabdomyolysis. A false-negative diagnosis of type 1 PSSM by muscle biopsy may occur if biopsy samples are small or if horses are less than 1 year of age.[90]

Acute Clinical Signs. Horses with the *GYS1* mutation begin to show clinical signs of muscle disease between 1 and 14 years of age, with an average age at onset of signs of 5 years. Acute clinical signs occur when horses are calm and include tucking up of the abdomen, fasciculations in the flank, muscle stiffness, sweating, reluctance to move forward, and overt firm muscle contractures (Fig. 10.9).[86] The hindquarters are frequently most affected, but back, abdominal, and forelimb muscles may also be involved. Signs of pain can last for more than 2 hours, and about 10% of cases become recumbent.[86] Less common signs of PSSM in Quarter Horses include gait abnormalities, mild colic, and muscle wasting. During an acute episode of ER, horses with PSSM1 often have markedly elevated serum CK activity of greater than 35,000 U/L, and myoglobinuria may be present. Severe colic-like pain after exercise and myoglobinuric renal failure are less common presenting complaints.[91,92]

Chronic Clinical Signs

LIGHT BREEDS. Chronic signs of PSSM1 in riding horses include a lack of energy when under saddle, reluctance to move forward, stopping and stretching out as if to urinate, and a sour attitude toward exercise.[39,86] Horses may have a combination of low-grade reluctance to exercise, poor performance, and repeated episodes of ER.[81] PSSM1 may also present in dressage and show jumpers as chronic back pain, failure to round over fences, and fasciculations or pain on palpation of lumbar muscles.[57] The range of severity of clinical signs of PSSM1 can be wide with complete incapacitation in rare cases. Serum CK activities are often elevated in unmanaged horses, even when horses are rested.[92] The median CK and AST activity for all PSSM1 Quarter Horses with muscle biopsies submitted to the University of Minnesota was 2809 and 1792 U/L, respectively.[93]

A small number of Quarter Horses and Paint Horses have both the *GYS1* mutation and the genetic mutation for MH.[71] This results in particularly severe signs of ER and a limited response to diet and exercise changes. During an episode of ER, excessively high body temperatures may develop and sudden death can occur in horses with the *RYR1* mutation. This combination occurs most often in halter and pleasure horses.

DRAFT HORSES AND DRAFT CROSSES. The average age of draft horses diagnosed with PSSM is about 6 years.[87] Many draft horses with PSSM are asymptomatic. Draft horses homozygous for the PSSM mutation have higher serum muscle enzymes than healthy or heterozygous horses.[94] Signs of severe rhabdomyolysis and myoglobinuria may occur in horses fed high-grain diets, exercised irregularly with little turnout, or horses that undergo general anesthesia.[91,95] Rhabdomyolysis can be so severe that it leads to recumbency and death.[91,95] Other signs of PSSM in draft horses include progressive weakness and muscle loss resulting in difficulty rising in horses with normal serum CK activity. Pronounced weakness is more prevalent in horses homozygous for the *GYS1* mutation. Gait abnormalities, such as excessive limb flexion, fasciculations, and trembling, are also reported in draft horses. Although the condition "shivers" was previously attributed to PSSM, studies found no causal association between these two conditions.[87] There is no evidence of impaired myocardial function in horses with PSSM1.[96] In Belgian draft horses mean (± standard deviation [SD]) AST activity was 502 ± 116 U/L for PSSM1 homozygotes, 357 ± 92 U/L for heterozygotes, and 311 ± 64 U/L for controls, with significant differences among groups.[94] The median serum CK and AST activities in draft horses from which biopsies were sent to the University of Minnesota were 459 and 537 U/L, respectively.[93]

Pathophysiology. The *GYS1* mutation causes a gain of function in glycogen synthase both in basal states and when activated by insulin and glucose-6-phosphate, and horses with PSSM1 have on average 1.8-fold higher muscle glycogen concentrations.[77] The abnormal polysaccharide in PSSM1 is less branched than normal glycogen as a result of chronically enhanced glycogen synthase enzyme activity in the presence of elevated glucose-6-phosphate and insulin without the same relative activation of branching enzyme.[97] Foals and yearlings with the *GYS1* mutation rarely have abnormal polysaccharide in skeletal muscle yet they have rhabdomyolysis.[09] Thus abnormal polysaccharide itself is an indicator of PSSM1 but not the direct cause for rhabdomyolysis.

The vast majority of horses that develop rhabdomyolysis with PSSM1 do so with aerobic exercise when blood lactate concentrations are unchanged from preexercise samples at 1.0 mmol/L.[7] Although elevated serum CK activity occurs with near-maximal exercise on occasion, the degree of elevation is often less than with submaximal exercise.[7] A deficit in energy metabolism represented by high myofiber inosine monophosphate (IMP) occurs after light exercise in individual muscle fibers of horses with PSSM1 in association with high serum CK activity. [99]

A high-NSC diet increases the propensity to develop muscle pain with aerobic exercise in PSSM1 horses.[86] A high-NSC versus a low-NSC, high-fat diet results in higher muscle lactate and citrate concentrations, lower glucose-6-phosphate, and similar muscle pyruvate concentrations with aerobic exercise in PSSM1 horses.[98] This suggests that the enhanced glycogen synthase activity in PSSM horses may impair oxidative metabolism of substrates such as pyruvate and fatty acids. Although the exact mechanism for this is not known, it may involve a link between glycogen synthase or glycogen concentrations

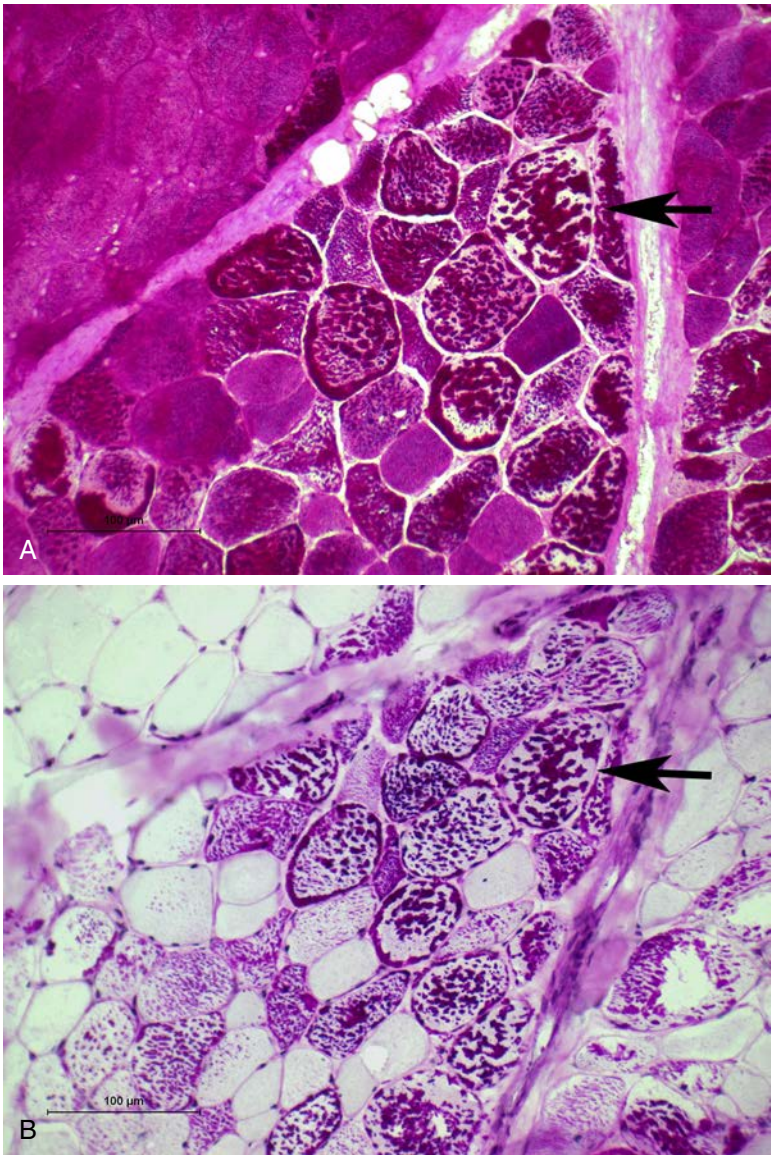


FIG. **10.8** (A) Cross section of semimembranosus muscle showing accumulation of granular periodic acid–Schiff (PAS) inclusions *(arrow)* in a horse with PSSM1. (B) Serial section of the same muscle preincubated in amylase before PAS staining. Note that the abnormal granular material is amylase resistant. (20×)

and metabolic switches and nutrient sensors. One of the key sensors and regulators of substrate flux in skeletal muscles is AMP kinase.[100,101] When energy supply is deemed to be insufficient (↓ [ATP]/[ADP]), activation of AMP kinase leads to the increased transcription of genes involved in oxidative metabolism, stimulation of fatty acid oxidation, and activation of pyruvate dehydrogenase, which increases carbohydrate oxidation.[100-102] Nutrient sensors in the cell could interpret excessive stimulation of glycogen synthesis (arising from the *GYS1* mutation and stimulated by insulin) as an indication not to activate glycogenolysis and lipolysis. PSSM horses fed high-NSC diets may be unable to generate sufficient acetyl CoA from either carbohydrate or fat metabolism to fuel muscle contraction during submaximal exercise. The common occurrence of clinical signs during the first 20 minutes of exercise may be because at this stage of exercise, muscles rely heavily on glycogen/glucose for energy. Low dietary starch and fat supplementation decrease muscle citrate concentrations and increase plasma free fatty acid concentrations, which may facilitate muscle fat metabolism in PSSM1 horses.[98,103]

Type 2 PSSM

Much less is known about type 2 PSSM, because previous controlled research trials on PSSM have largely involved horses with PSSM1 (Valberg, unpublished data). The term *type 2 PSSM* refers to those horses that have altered staining for muscle glycogen that do not possess the *GYS1* mutation.[78] As such it should not be interpreted to necessarily indicate one specific disease. Furthermore, because PSSM2 is not consistently defined by the presence of amylase-resistant polysaccharide but rather includes abnormal appearance of amylase-sensitive glycogen, there is considerable room for overlap of a diagnosis with RER or for false-positive diagnoses in trained animals.[75,76,80]

Much of the current knowledge of managing PSSM2 is based on extrapolation of what works for PSSM1, retrospective



FIG. **10.9** Horse with PSSM1 showing acute clinical signs of rhabdomyolysis that include anxiety, sweating, muscle stiffness, and reluctance to move.

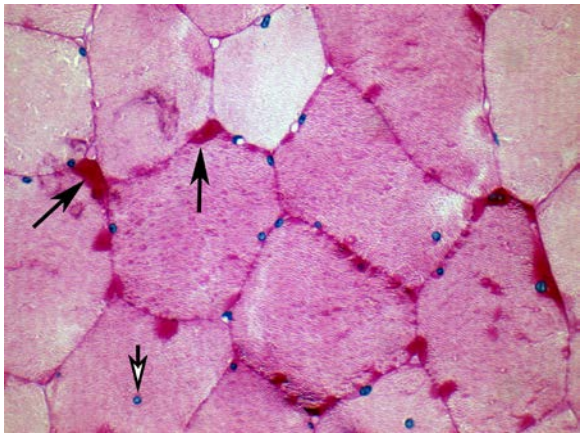


FIG. **10.10** Cross section of semimembranosus muscle (20×) from a horse showing increased accumulation of subsarcolemmal periodic acid–Schiff (PAS) material *(arrows)*. The horse was negative for the *GYS1* mutation, establishing a diagnosis of PSSM2. Note the myopathic finding of centrally displaced myonuclei in some fibers.

evaluation of cases diagnosed with PSSM2 by muscle biopsy,[39] and a few years of prospective clinical data.

Prevalence. Approximately 28% of Quarter Horses with PSSM diagnosed by muscle biopsy do not have the *GYS1* mutation and therefore have PSSM2.[77,93] PSSM2 seems to be common in both high-performance Quarter Horse types such as barrel racing, reining, and cutting horses as well as pleasure and halter horses. In the United Kingdom approximately 35% of PSSM cases diagnosed by muscle biopsy have PSSM2.[80] PSSM2 is also documented in Paint, Appaloosa, and Morgan horses. About 80% of Warmblood horses diagnosed with PSSM by muscle biopsy have PSSM2.[84] Other breeds diagnosed with PSSM2 include Dutch Warmbloods, Swedish Warmbloods, Selle Francais, Hanoverians, Friesians, Westfalians, Canadian Warmbloods, Irish Sport Horses, Gerdlanders, Hussiens, Standardbreds, Thoroughbreds, and Arabians.[77]

Pathogenesis. The cause or causes of PSSM2 are currently unknown. Muscle glycogen concentrations are not as high as those found in horses with PSSM1, and glycogenolytic enzyme defects have not been identified in the few cases where enzymes were measured (Valberg, unpublished data). To date, the only reported abnormality in muscle ultrastructure is an increase in normal β-glycogen particles.[78] It may be that there are a group of conditions currently captured under the term *type 2 PSSM* that have separate etiologies but share common findings of focal glycogen accumulation and poor performance.

Clinical Signs. In adult Quarter Horses, ER is the predominant clinical sign of PSSM2. In Quarter Horses less than 1 year of age an inability to rise or a stiff hindlimb gait is reported with PSSM2.[78] In Warmblood Horses, signs of PSSM2 are usually related to an exertional myopathy with few or rare occasions of a postexercise increase in serum CK activity. Clinical complaints usually relate to poor performance, an undiagnosed lameness, sore muscles, and decreased energy level. Warmbloods with PSSM2 may also have painful firm back and hindquarter muscles, reluctance to collect and engage the hindquarters, poor rounding over fences, poor canter departs, and slow onset of atrophy, particularly if taken out of work. The mean age of onset of clinical signs in Warmbloods is between 8 and 11 years of age, with median CK and AST activities of 323 and 331 U/L, respectively.

Diagnosis. PSSM2 is diagnosed by muscle biopsy where increased or abnormal PAS-positive material that is usually amylase-sensitive is apparent (Fig. 10.10).[78] Determination of what constitutes an abnormal amount of amylase-sensitive glycogen is subjective, the specificity of the diagnosis is low, and false-positive diagnoses occur. Highly trained horses have increased glycogen storages as a normal response to training, and this should be considered when histopathologic interpretations are made. Glycogen is commonly found near capillaries in normal horses, and PAS-positive sarcoplasmic masses can be found in 60% of healthy horses, so the presence of these structures should not be diagnostic criteria for PSSM2.[104] Type of fixation is also a consideration as formalin-fixed sections show a greater deposition of subsarcolemmal glycogen and precipitation of glycogen in one area of a fiber even in healthy horses, and these should not be considered diagnostic criteria for PSSM2.[105] Other histopathologic features that may be present with PSSM2 include centrally located nuclei, subsarcolemmal vacuoles, muscle necrosis, macrophage infiltration of myofibers, regenerative fibers, and anguloid myofiber atrophy.[78] Some laboratories grade polysaccharide accumulation as mild, moderate, and severe where mild accumulation represents a category that has a higher chance of being a false-positive diagnosis. Mild PSSM cases in particular should receive a full physical and lameness examination to ensure that there are no other underlying causes for poor performance.

Management of Type 1 and Type 2 PSSM

Horses diagnosed with PSSM will always have an underlying tendency for muscle soreness. Affected horses should

be managed appropriately to minimize clinical signs. With adherence to both diet and exercise recommendations provided below, at least 70% of horses with PSSM show notable improvement in clinical signs, and many return to acceptable levels of performance.[27,39,86] There is, however, a wide range in the severity of clinical signs, shown by horses with PSSM; those horses with severe or recurrent clinical signs will require more stringent adherence to diet and exercise recommendations in order to regain muscle function.

Rest. PSSM horses that are confined for days to weeks following an episode of rhabdomyolysis often have persistently elevated serum CK activity. In contrast, PSSM horses kept on pasture with little grain supplementation often show few clinical signs of rhabdomyolysis and have normal serum CK activity. After an episode of rhabdomyolysis, horses with PSSM should be confined to a stall for only a brief time (less than 48 hours) followed by turnout in paddocks of gradually increasing size. Providing horses with as much free exercise as possible on pasture appears to be beneficial in the long term. If pasture is lush a grazing muzzle may be needed.[81] Following an acute episode, excitable horses may require tranquilization before turnout to avoid excessive galloping. Hand walking horses for more than 5 to 10 minutes at a time when initially recovering from an episode of PSSM may trigger another episode of rhabdomyolysis.

Exercise. The beneficial response to low-starch, fat-supplemented diets only occurs if it is instituted in conjunction with a regular incremental exercise program.[88] Regular daily exercise in PSSM horses over a 3-week period results in a dramatic decrease in serum CK responses to exercise, whereas stall confinement is associated with elevations in serum CK activity after exercise.[88,98] One common adaptation to daily training is an increase in oxidative capacity in skeletal muscle. The oxidative capacity of skeletal muscle in PSSM Quarter Horses was very low based on markers such as citrate synthase activity and β–fatty acid oxidative marker HAD.[88] The activity of these enzymes, however, was equally low in control Quarter Horses. Whether the beneficial effect of daily exercise on PSSM horses is a result of improved oxidative enzyme capacities or enhanced substrate flux or both has not been elucidated.

EXERCISE REGIMENS. Important principles to follow when starting exercise programs in PSSM horses include the following: (1) provide adequate time for adaptation to a new diet before commencing exercise; (2) recognize that the duration of exercise, not the intensity, is of primary importance; (3) ensure that the program is gradually introduced and consistently performed; and (4) minimize any days without some form of exercise. If horses have experienced a moderate to marked episode of rhabdomyolysis recently, 2 weeks of turnout and diet change are often beneficial before recommencing exercise. Exercise should be very relaxed, and the horse should achieve a long, low frame without collection. For many horses this is most readily done in a round pen or on a lunge-line but can be done under saddle if needed. Successive daily addition of 2-minute intervals of walk and trot beginning with only 4 minutes of exercise and working up to 30 minutes after 3 weeks is often recommended.[18,92] If horses had minor elevations in 4-hour postexercise CK with the 15-minute exercise test, horses may begin at 15 minutes of exercise. Owners often do not recognize that walking the horse for 10 minutes or more initially can trigger muscle soreness in PSSM horses. Advancing the horse too quickly often results in an episode of rhabdomyolysis and repeated frustration for the owner. Work can usually begin under saddle after 3 weeks of ground work and can gradually be increased by adding 2-minute intervals of collection or canter to the initial relaxed warm-up period at a walk and trot. Unless a horse shows an episode of overt rhabdomyolysis during the initial first 4 weeks of exercise, reevaluating serum CK activity is not helpful for the first month. This is because it is very common to have subclinical elevations in CK activity when exercise is reintroduced, and a return to normal levels often requires 4 to 6 weeks of gradual exercise.[92] Keeping horses with PSSM fit seems the best prevention against further episodes of rhabdomyolysis.

Diets for PSSM. The basis for designing PSSM diets is the belief that lowering the daily starch and sugar intake and increasing dietary fat content will decrease the glucose load, increase the availability of nonesterified free fatty acids for muscle metabolism, and lower serum insulin concentrations. It is important to note that the dietary recommendations provided here were tested in horses with PSSM1, and there are no controlled trials to indicate whether these are beneficial for PSSM2 horses. Quarter Horses naturally have very little lipid stored within muscle fibers. The end result would thus be decreased uptake of glucose into muscles cells, less substrate available for—and stimulation of—glycogen synthesis, and normalization of substrate flux. Owners report that this type of diet improves clinical signs of muscle pain, stiffness, and exercise tolerance in draft horses, Warmbloods, Quarter Horses, and other breeds.[27,39,81,86,88] Dietary change appears to have less impact on alleviating gait changes such as shivers.[39]

CALORIC BALANCE. The horse's caloric requirements at an ideal body weight are the most important considerations in designing the diet for PSSM. This is because many horses with PSSM are easy keepers and may be overweight at the time of diagnosis. Adding excessive calories in the form of fat to an obese horse may result in equine metabolic syndrome and is contraindicated. If necessary, caloric intake should be reduced by using a grazing muzzle during turnout, feeding hay with a low NSC content at 1% to 1.5% of body weight, providing a low-calorie ration balancer, and gradually introducing daily exercise. Rather than provide dietary fat to an overweight horse, fasting for 6 hours before exercise can be used to elevate plasma free fatty acids before exercise and alleviate any restrictions in energy metabolism in muscle.

SELECTION OF FORAGE. Quarter Horses develop a significant increase in serum insulin concentrations in response to consuming hay with an NSC of 17%, whereas insulin concentrations are fairly stable when hay with 12% or 4% NSC content is fed.[106] Because insulin stimulates the already overactive enzyme glycogen synthase in the muscle of PSSM1 horses, selecting hay with 12% or less NSC is advisable. The degree to which the NSC content of hay should be restricted below 12% NSC depends on the caloric requirements of the horse. Feeding a low-NSC hay of 4% provides room to add an adequate amount of fat to the diet of easy keepers without exceeding the daily caloric requirement and inducing excessive weight gain. For example, a 500-kg horse on a routine of light exercise generally requires 18 MCal per day of digestible energy (DE). Fed at 2% of body weight, a 12% NSC mixed grass hay almost meets their daily caloric requirement by providing 17.4 MCal per day. Thus with a 12%-NSC hay there is only room for 0.6 MCal of fat per day (72 mL of vegetable oil) to achieve 18 MCal of energy.

In contrast, a 4%-NSC blue grama hay would provide 13.5 MCal per day, which would allow a reasonable addition of 4.5 MCal of fat per day (538 mL of vegetable oil).

SELECTION OF FAT SOURCE. High-fat diets increase plasma free fatty acid concentrations and thus the availability of fats for oxidation in skeletal muscle.[98] The major sources of dietary fat for horses are vegetable-based fat, including vegetable oils and rice bran, or animal-based fat (tallow, lard, fish oil). Vegetable oils are highly unsaturated, very digestible (90%–100%), and very energy dense. Suitable forms include soybean, corn, safflower, canola, flaxseed, linseed, peanut, and coconut. Controlled research studies in exercising PSSM horses have shown a decrease in muscle pain and serum CK in response to the addition of corn oil and also a product containing soy hulls, soybean oil, and rice bran (RE•LEVE®).[86,98] The amount of oil added in these trials constituted at least 13% of daily DE. Some veterinarians have advocated as much as 25% of DE be fed in the form of fat to PSSM horses.[27] As discussed, the principal consideration here should be whether this provides excessive calories and additional weight gain because feeding 13% DE as fat may well be effective in reducing muscle pain.

Limited research has been performed on the form of oil to feed PSSM horses. An odd carbon 7 chain fat (triheptanoin) fed to PSSM horses had a detrimental effect on muscle pain, exercise tolerance, and serum CK activity whereas long-chain fats fed in the form of corn oil or a rice bran/soy oil–supplemented feed had a beneficial effect on lowering serum CK activity.[98] Whether there is any direct beneficial effect on skeletal muscle of providing energy in the form of omega-3 versus omega-6 fatty acids has yet to be determined. Corn oil, sunflower oil, and safflower oil are high in omega-6 and lower in omega-3, whereas soybean and canola oils are moderately high in omega-6 and omega-3. Flax seed, linseed, and fish oils contain more omega-3 than omega-6. It is usually cost-prohibitive to provide sufficient energy to a PSSM horse each day in the form of dense omega-3 fat supplements. Soybean and canola oils provide a relatively affordable alternative with moderately high omega-6 or a mix of these oils and flax or fish oil can be provided. Due to the potential additional oxidant stress of fats, vitamin E (1000–5000 U/day) is recommended for horses receiving oil-supplemented diets.

LOW-STARCH, HIGH-FAT CONCENTRATES. Although oils are energy dense and inexpensive, they have the disadvantages of being messy, unpalatable to some horses, prone to rancidity in warm temperatures, and difficult to feed in large amounts. As such a number of concentrates have been developed that contain fats. The important principle to be met by such feeds is that the starch and sugar components are low enough and fat supplementation high enough to ensure that in the total diet, the calories supplied by NSC comprise no more than 10% to 15% of the daily DE and the calories supplied by fat comprise about 12% to 15% of daily DE. Common fat sources used in such concentrates include, in addition to the oils mentioned above, stabilized rice bran or animal fats. Rice bran and its products are palatable to most horses, have a moderate NSC content (about 25% by weight), contain approximately 20% fat by weight, and are excellent sources of vitamin E and phosphorus. The NSC component of rice bran can vary if the manufacturing process is not careful to exclude the white rice grain. Commercial rice bran products occur as powder or an extruded pellet and are considerably more stable in warm temperatures compared to animal fat and vegetable oils. A number of well-balanced, low-starch, high-fat commercial diets are suitable for horses with PSSM. Some commercial feeds meet the recommended nutritional needs of PSSM horses in one pelleted ration. These feeds typically contain at least 10% to 15% fat by weight and less than 20% NSC by weight. Some feed companies offer similar nutritional content by blending two or more manufactured feeds or by supplementing with oils or rice bran. At present, the NSC content of equine feed products is not consistently listed on the feed tag, and consultation with the feed manufacturer is necessary to obtain this information. Nutritional support is available through most feed manufacturers in designing an appropriate diet. There is a great deal of variation in individual tolerance to dietary starch; however, horses with more severe clinical signs of PSSM appear to require the greatest restriction in starch intake.

EXPECTATIONS. The beneficial effect of low-starch, high-fat diets requires that horses are trained daily to enhance enzymes involved in fat and glucose metabolism. With adherence to both the diet and exercise recommendations 70% to 75% of Quarter Horses and Warmbloods show notable improvement in clinical signs, and many return to acceptable levels of performance.[28,39,81,90] There is, however, a wide range in the severity of clinical signs shown by horses with PSSM; those horses with severe or recurrent clinical signs will require more stringent adherence to diet and exercise recommendations in order to regain muscle function. PSSM horses that also have the mutation for MH do not respond as well to diet and exercise recommendations and may continue to develop ER with the possibility of a fatal episode.[93]

SUPPLEMENTS. There are no specific supplements that have been shown to benefit horses with PSSM. Chromium, which is reported to increase glucose absorption, may be contraindicated in PSSM horses. Carnitine is a dietary supplement that may replenish muscle carnitine stores if depleted and assist in transport of fat into the mitochondria. A deficiency of plasma carnitine has not been identified in PSSM1 horses. Plasma free carnitine concentrations ranged from 10.24 to 27.11 μM in PSSM1-affected horses and from 13.20 to 25.63 μM in normal horses (Valberg, unpublished data). Plasma carnitine does not always reflect muscle carnitine concentrations.

Myofibrillar Myopathy

One recent addition to potential causes of exertional myopathies includes a suspected myofibrillar myopathy (MFM) that was identified in Arabian endurance horses with a history of intermittent elevations in serum CK activity (greater than 10,000 U/L after endurance rides).[107] The Arabian horses did not necessarily show the same degree of clinical signs of reluctance to move, muscle stiffness, and pain that is frequently seen with cases of ER. Muscle biopsies resembled those of PSSM2 with increased aggregates of cytoplasmic glycogen, amylase-resistant polysaccharide in a few myofibers of some horses, and centrally located myonuclei in mature myofibers. Muscle glycogen concentrations in horses with MFM were similar to controls. The distinguishing feature of muscle biopsies in these horses was disrupted myofibrillar alignment and large desmin- and αβ crystallin-positive cytoplasmic aggregates found in horses without a recent episode of ER. The ectopic accumulation of cytoskeletal proteins and Z-disc degeneration identified on electron microscopy had features of a myofibrillar myopathy. Many of these horses were previously diagnosed with type 2 PSSM likely because pools of glycogen forming within disrupted myofibrils appeared to give

the false appearance of a glycogen storage disorder. MFM may represent a specific myopathy formerly grouped under the diagnosis of type 2 PSSM.

Mitochondrial Myopathy

A deficiency of mitochondrial respiratory chain function was identified in a 3-year-old Arabian filly that presented with profound exercise intolerance and normal serum CK activity 4 hours after exercise.[108] Over the course of years the filly developed significant muscle atrophy. During exercise at a trot, the horse would be unable to continue after 7 minutes, becoming stiff and sweating profusely. Venous pH and bicarbonate concentrations dropped abnormally after 6 minutes of trotting, lactic acidosis was present (20 mM), and the horse had dramatically reduced maximum oxygen consumption (maximal VO_2 0.5 mL/kg/sec; normal 2.5 mL/kg/sec). A deficiency of complex I (nicotinamide adenine dinucleotide [NADH]:ubiquinone reductase) in the respiratory chain was identified in muscle samples.

Idiopathic Chronic Exertional Myopathies

Many horses develop chronic ER for which no known cause is apparent. More research needs to be done to further define causes of exertional myopathies in horses.

Fibrotic Myopathy

Fibrotic myopathy affects semimembranosus/semitendinosus muscles and is usually a consequence of trauma to these muscles. Horses have a classic gait abnormality where the anterior phase of the stride ceases abruptly, causing the leg to be pulled back before the foot suddenly slaps the ground rather than continuing its forward motion. In acute cases these muscles may feel warm and painful on deep palpation. Chronically, hardened areas within the muscle may represent fibrosis and ossification.

The extent of tearing can be determined by ultrasonography, providing the muscle is within the depth of penetration of the ultrasound machine. Acute injuries are treated with cold therapy and stretching once the limb is no longer acutely painful. Deep heating ultrasound may be of help in promoting healing. For more subtle strain, intermittent electrical stimulation of focal areas within a muscle may be beneficial. The strength of stimulus necessary to induce contraction can be incrementally assessed and compared between left and right sides. Damaged muscle tends to respond with a greater degree of pain and contraction at lower stimulus strength. Underwater treadmill exercises to strengthen the muscles once healing is apparent on ultrasound can help restore normal limb function. Horses with a fibrotic band within the muscle or ossification of the muscle have a much poorer prognosis. Surgical resection of fibrotic muscle is attempted, but full restoration of function is unlikely to occur.[18]

NONEXERTIONAL MYOPATHIES

Specific causes of nonexertional rhabdomyolysis are listed in Box 10.2.

BOX 10.2 Nonexertional Rhabdomyolysis

1. Metabolic myopathy
   a. Glycogen branching enzyme deficiency in Quarter Horses
   b. Type 1 and type 2 polysaccharide storage myopathy
   c. Malignant hyperthermia
2. Inflammatory myopathies
   a. Immune mediated
      - Infarctive hemorrhagic purpura
      - Immune-mediated myopathy
3. Infectious
   a. Clostridial myositis
   b. *S. equi* myositis
   c. *Anaplasma* myositis
   d. Viral myositis
   e. *Sarcocystis* myositis
4. Nutritional myopathy
   a. Vitamin E and selenium deficiency
5. Toxic myopathy
   a. Ionophore toxicity
   b. Pasture myopathies
      - Hypoglycin A myopathy
      - Rayless goldenrod/white snakeroot
      - *Cassia occidentalis*
6. Traumatic myopathy
   a. Compressive anesthetic myopathy
   b. Trauma

Glycogen Branching Enzyme Deficiency

Pathogenesis

Glycogen branching enzyme deficiency (GBED) occurs in foals of Quarter Horse–related breeds caused by a premature stop codon in exon 1 of the *GBE1* gene.[109] Branching enzyme activity is minimal in skeletal and cardiac muscle as well as liver.

Prevalence

Nine percent of the breed is a carrier of this autosomal recessive mutation, and 3% of abortions are attributed to this disease in Quarter Horses.[110] The prevalence of carriers is particularly high in pleasure horses, with 26% carrying the *GBE1* mutation.[83]

Clinical Signs

Clinical signs of GBED include abortion in second or third trimester, stillbirth, hypothermia at birth, mild flexural deformities, intermittent seizures, skeletal and respiratory muscle weakness, and sudden death (Fig. 10.11).[109-111] Most foals diagnosed with GBED have presented with transient flexural contractures at 1 day of age. Persistent leukopenia, intermittent hypoglycemia, and moderately high serum CK (1000–15,000 U/L), AST, and γ-glutamyltransferase (GGT) activities are usually present in affected foals.

Diagnosis

A diagnosis is best obtained by confirming the presence of the *GBE1* genetic mutation in blood, hair, or tissue samples or by identifying typical PAS-positive inclusions in muscle or cardiac samples.[109-111] Gross postmortem changes are not evident, and routine hematoxylin and eosin stains of tissues may be normal or show basophilic inclusions in skeletal muscle and cardiac tissues. Frozen sections of muscle, heart, and liver show a notable lack of normal PAS staining for glycogen, as well as abnormal PAS-positive globular or crystalline intracellular inclusions (see Fig. 10.11).



FIG. **10.11** Foal with glycogen branching enzyme deficiency (GBED). Foals with GBED are often born with slight forelimb flexural limb deformities and appear mentally less alert and less active than healthy foals. (Photo courtesy Dr. Alicia Foley.)

Treatment

Although foals initially respond to treatment in neonatal intensive care, all foals with GBED have died. Death occurs as sudden collapse, with the longest survivor being 18 weeks of age.

Nutritional Myodegeneration

Nutritional myodegeneration (NMD) (white muscle disease, nutritional muscular dystrophy) is an acute degenerative disease affecting cardiac and skeletal muscle of primarily young foals.

Pathogenesis

NMD is caused by a dietary deficiency of selenium and/or vitamin E (α-tocopherol) in young, rapidly growing foals born to dams that consumed selenium-deficient diets during gestation.[112,113] Selenium and vitamin E appear to be synergistic in preventing NMD; however, selenium deficiency appears to be the most important contributor to oxidant damage of muscle cell membranes. Selenium is a key component of glutathione peroxidase (GSH-Px) that acts to destroy hydrogen peroxide and lipoperoxides that are generated during normal muscle metabolism. Vitamin E acts within the cell membrane as an antioxidant that scavenges free radicals that otherwise might react with unsaturated fatty acids to form lipid hydroperoxides. The precise interrelationships among selenium, vitamin E, other metabolic factors, and triggering mechanisms in NMD are not fully understood because many foals deficient in selenium and/or vitamin E have no evidence of muscle disease. In certain situations, deficiencies of both selenium and vitamin E are necessary for disease to occur. In other animals NMD can occur when a deficiency of only one of the nutrients is present and the other is normal in blood and tissues.

Clinical Signs

Some foals present with a cardiorespiratory syndrome characterized by dyspnea, a rapid irregular heartbeat, profound weakness, recumbency, and sudden death.[8,112] Other foals have primary skeletal muscle involvement with signs of weakness, trembling, stiffness, and inability to stand for more than short periods. Affected muscle groups are firm and painful to palpation. Dysphagia due to necrosis in the tongue may be the only sign in some affected foals and is frequently accompanied by aspiration pneumonia. The tongue, gastrocnemius, semitendinosus, semimembranosus, biceps femoris, lumbar, gluteal, and neck musculature are often affected.

Clinical Pathology

Serum CK and AST activities are usually in the thousands to hundreds of thousands of U/L. Myoglobinuria is common. Hyperkalemia, hyperphosphatemia, hyponatremia, hypochloremia, and hypocalcemia can occur with severe rhabdomyolysis when the normal distinction between extracellular and intracellular compartments is destroyed by massive tissue necrosis.[8] Neutrophilia is common due to the high incidence of aspiration pneumonia.

Diagnosis

Whole blood selenium (normal range 0.07 to greater than 0.1 ppm) or plasma α-tocopherol (normal range 1.1–2 ppm) concentrations below the normal range in conjunction with appropriate clinical signs are considered diagnostic. Selenium-dependent GSH-Px formed in the red cells during erythropoiesis could also provide an index of body selenium status. Adequate GSH-Px activities are greater than 20 to 50 U/mg of hemoglobin per minute in horses. However, GSH-Px reference values are only specific to the laboratory where the analysis is performed and must be validated by comparison with blood selenium concentration. Plasma samples for α-tocopherol analysis need to be put on ice immediately, protected from the light by wrapping in aluminum foil, and stored frozen (−21°F [−70°C]) if analysis is to be delayed. Tissue specimens can be assayed for selenium content. Normal liver concentrations of selenium are 1.05 to 3.5 ppm on a dry matter (DM) basis (fresh liver approximately 30% DM).

Pathology

Skeletal muscle degeneration is characterized by pale streaks of discoloration and a dry appearance of affected muscle, calcification, and intramuscular edema in bilaterally symmetric muscle groups.[112] Affected muscle bundles are often adjacent to apparently normal or minimally affected muscle. Histologically, affected muscle fibers are often hypercontracted and fragmented, with some mineralization of muscle fibers, and in later stages macrophage infiltration is notable.[114]

Treatment and Prognosis

The cardiorespiratory form of NMD carries a poor prognosis. In contrast, the skeletal form of NMD is more amenable to treatment if secondary complications such as pneumonia can be managed. Progressively decreasing CK activity and ability to stand are good prognostic indicators for NMD. The label

dose for selenium is 0.055 to 0.067 mg/kg (2.5–3 mg/45 kg) body weight given intramuscularly. Injections can be irritating so dilution in sterile saline and administration in muscles such as pectorals is recommended. Dosage should not be greatly increased above the label dose to prevent an inadvertent selenium toxicosis. Absorption and distribution of injectable selenium occurs rapidly and may account for the rapid improvement in clinical signs seen in reversible cases. Injectable selenium products contain a minimal amount of α-tocopherol (50 mg/mL [68 IU]), and additional supplementation with water-dispersible RRR-α-tocopherol is recommended (10 IU/kg).

Supportive therapy may include administration of antibiotics to help combat secondary pneumonia and infected decubital lesions that are common in recumbent patients. When dysphagia is present feeding via nasogastric tube, provision of adequate energy intake, and attention to the fluid and electrolyte balance are of critical importance if recovery is to be successful. Hyperkalemia may be life threatening in affected foals. Mineralocorticoids, alkalinizing fluids, and dextrose and insulin may be used to reduce circulating potassium concentrations.[8]

Foals with primary muscular NMD often show improvement after a few days of rest and treatment with selenium. They can often stand and walk within 3 to 5 days.

Prevention and Control

In regions where selenium deficiency exists, diets of pregnant mares need to be supplemented with selenium either in their daily concentrate or through additional oral supplementation to provide 1 mg of selenium per day.[115,116] Only limited selenium may cross the placenta; however, supplementation during lactation increases the levels of selenium in milk and thus provides a potential means of selenium supplementation in foals. In deficient areas, this increased level of selenium in milk may not meet a young animal's nutrient requirements.[117] In such cases injectable selenium products used every 30 to 45 days might be needed in areas where NMD is a particular problem.[118] Periodic blood sampling of foals in high-risk areas is recommended. On the basis of these assessments, adjustments to the rate or extent of selenium supplementation can be made. Access to high-quality green forage should ensure adequate vitamin E intake.

Masseter Muscle Myodegeneration

Pathogenesis

Masseter muscle swelling and degeneration occur in adult horses and can be followed by masseter muscle atrophy and trismus (Fig. 10.12). An inflammatory myopathy such as that seen in dogs with canine masseter muscle myositis has not been identified (Valberg, unpublished data). An association has been made between selenium deficiency and masseter muscle myodegeneration in some cases.[119-121] Histopathologic evidence of bilateral symmetric lesions in masseter muscles, as well as muscles of the hind and thoracic limbs, has been observed in such cases.[120]

Clinical Signs

Unilateral or bilateral swelling of the masseter and temporal muscles is the predominant acute clinical sign accompanied by pain or difficulty opening the jaw. Exophthalmos can be present. If tachycardia and dysrhythmias are present myocardial damage should be suspected. Chronic clinical signs include masseter muscle atrophy, trismus, dysphagia, and weight loss. Horses usually have normal postural and locomotor muscles on clinical examination, although stiffness has been reported. Accompanying clinical pathologic findings include pigmenturia, elevated serum AST and CK activity, and potentially elevated cardiac troponin concentrations. Ultrasonography is useful to determine the extent of masseter muscle swelling or atrophy and to assess myocardial dysfunction.



FIG. **10.12** Pronounced atrophy of the masseter muscle in a Quarter Horse resulting in prominence of the facial crest.

Diagnosis

Elevated serum CK and AST activities accompanied by low whole blood selenium concentrations (less than 1.1–2 ppm) support selenium deficiency as a cause of masseter muscle atrophy.

Treatment

Analgesics, intramuscular selenium (0.055–0.067 mg/kg), oral α-tocopherol (2000–5000 IU/day RRR-α-tocopherol), nutritional and fluid support if needed via stomach tube, and IV fluids are recommended. A fasciotomy to relieve pressure in masseter muscles has been advocated, but results are not reported. Progression to bilateral muscle atrophy can occur, and depending on the severity of myofiber and nerve damage this can resolve after a period of months. Prognosis is guarded, but there are individual reports of successful treatment. Without early treatment, permanent trismus can occur due to muscle atrophy and fibrosis.

Vitamin E–Deficient Myopathy

Pathogenesis

Vitamin E–deficient myopathy may be an entity unto itself or a predecessor to development of equine motor neuron disease (EMND). Clinical signs of EMND occur in the absence of the

hallmark of EMND, neurogenic atrophy in sacrocaudalis dorsalis medialis muscle biopsies.[13] Unlike EMND, horses with a vitamin E–deficient myopathy have characteristic histochemical alterations in mitochondrial distribution that appears to be a reversible manifestation of skeletal muscle mitochondrial oxidative stress.[13]

Clinical Signs

Horses can present with a gradual onset of reduced performance and muscle loss, but usually owners report a sudden onset of muscle weakness. Horses show signs of symmetric generalized muscle atrophy, trembling, muscle fasciculations, inability to fix the stifles with weight shifting, and frequent attempts to lie down (Fig. 10.13A). Differential diagnoses for horses with muscle atrophy are provided in Box 10.3.

Diagnosis

Low muscle α-tocopherol concentrations have been identified in affected horses, and serum α-tocopherol concentrations can be low or within the normal range. The distinction between vitamin E–deficient myopathy and EMND is the presence of myopathic rather than neuropathic features in sacrocaudalis dorsalis muscle biopsies and the remarkable response to treatment with vitamin E.[13,122] The characteristic finding in muscle biopsy is the presence of a "moth-eaten" staining pattern in mitochondrial stains of frozen sections of the sacrocaudalis dorsalis muscle. Mitochondrial stains of muscles such as the gluteus medius are normal.





FIG. **10.13** (A) Belgian Warmblood gelding with generalized symmetric muscle loss, decreased performance, and low serum and muscle vitamin E concentrations. Muscle biopsy of the sacrocaudalis dorsalis muscle revealed numerous moth-eaten mitochondria establishing a diagnosis of vitamin E–deficient myopathy. (B) The same horse after 8 weeks of vitamin E treatment and an exercise program. In addition to weight gain, increased muscle mass in the hindquarters is apparent.

Treatment

Vitamin E–deficient myopathy is highly responsive to treatment with natural liquid RRR-α-tocopherol (5000 IU/day for a 500-kg horse) and a very gradual reintroduction to exercise once strength returns (Fig. 10.13B).[13] Powdered formulations of α-tocopherol acetate are not recommended as an acute treatment because they do not restore serum α-tocopherol concentrations at the same rapid rate as liquid α-tocopherol.

INFLAMMATORY MYOPATHIES

Viral-Associated Myopathies

Infrequently, skeletal muscle degeneration and stiffness occur in association with equine influenza A2 and equine herpesvirus 1 in association with typical clinical signs of viral disease.[123,124]

Sarcocystosis

Pathogenesis

Equine skeletal muscle, especially esophageal muscle, commonly contains a few dormant cysts of the sporozoan parasite *Sarcocystis fayeri,* for which the dog is the definitive host. Cysts usually pose no problem, but when ponies and horses were experimentally infected with large doses of *S. fayer* dysphagia, severe myositis and stiff gait occurred. There are also a few clinical cases of *S. fayeri* myositis reported in horses.[125]

Clinical Signs

Clinical signs include fever, malaise, chronic muscle atrophy, stiffness, weakness, and muscle fasciculations. Clinicopathology is typified by normochromic normocytic anemia and elevations in CK and AST activities.[125,126]

BOX 10.3 Atrophy

1. Myogenic
   a. Disuse
   b. Cachexia
   c. Cushing's disease
   d. Immune-mediated myositis (rapid atrophy)
   e. Vitamin E–deficient myopathy
   f. Type 2 PSSM
   g. Severe rhabdomyolysis
2. Neurogenic atrophy
   a. Focal nerve damage
      - Trauma
      - Tumor
      - Infectious (equine protozoal myelitis)
      - Idiopathic peripheral neuropathy
   b. Generalized nerve damage
      - Equine motor neuron disease

Diagnosis

Diagnosis of sarcocystosis requires history, clinical signs, laboratory and serologic evaluation, and the demonstration of immature cysts in muscle biopsies, ideally in conjunction with inflammatory infiltrates.

Treatment and Control

Successful treatment of sarcocystosis is reported using phenylbutazone, trimethoprim-sulfamethoxazole, and pyrimethamine.[125] Current antiparasitic medications such as diclazuril should also be effective. Control involves preventing gross contamination of equine feeds with carnivore feces.

Bacterial-Associated Myositis

Clostridial Myonecrosis

Clostridial myonecrosis is caused by infection with one of a variety of clostridial bacteria, most commonly *Clostridium perfringens.* Sporadic cases have been attributed to *C. septicum, C. chauvoei, C. novyi,* and other related species. Clostridial organisms are gram-positive, rod-shaped, spore-forming obligate anaerobes with the potential to produce a variety of exotoxins that can cause extensive tissue damage and necrosis with secondary life-threatening toxemia, multiple-organ failure, and death.

Pathogenesis. Clostridia gain access to the body through the alimentary tract and are present in liver and muscle in the dormant spore form.[127] Most reports of clostridial myonecrosis in horses implicate intramuscular injection sites or, less commonly, puncture wounds or other traumatic injuries in creating a suitable environment for the development of clostridial myonecrosis.[128,129] Intramuscular injections of irritating drugs such as NSAIDs, prostaglandins, anthelmintics, and antihistamines are commonly implicated as initiating factors for clostridial myonecrosis in horses. When local tissue is devitalized and the appropriate anaerobic condition is created, spores may vegetate and begin exponential growth, releasing powerful exotoxins that act locally and systemically to create widespread organ dysfunction. The specific toxins vary depending on the clostridial species involved. With *C. perfringens* infection, the most important toxin implicated in clinical disease is alpha toxin, a dermonecrotic toxin with phospholipase C and sphingomyelinase activity. Theta toxin (perfringolysin), kappa toxin (collagenase), and mu toxin (hyaluronidase) may also play important roles in the development of clinical disease. *Clostridium septicum, C. chauvoei, C. sporogenes,* and mixed infections are associated with a high fatality rate, whereas *C. perfringens* type A has a mortality rate of 20% in horses that receive early and aggressive treatment.[129]

Clinical Signs. Horses initially have clinical signs related to their wound or disease that required an intramuscular injection. Depending on the injection site, lameness or a sore neck may be the initial clinical sign. Within 48 hours of the inciting event horses develop swelling and variable crepitus at the wound/injection site accompanied by depression, fever, toxemia, and tachypnea. Clostridial infection can migrate along fascial planes to affect extensive areas of the body with resultant spread of crepitus (Fig. 10.14). Severe toxemia, multiorgan failure, coma, and death can occur within the next 12 to 24 hours in severely affected horses. An intravascular hemolytic crisis may develop late in the disease process in some affected horses. It is unclear whether hemolysis is the result of a bacterial exotoxin or another mechanism.

Diagnosis. Hematology and serum biochemical analyses usually reflect a generalized state of toxemia with hemoconcentration and a stress/toxic leukogram. In severely affected horses with rapid disease progress, there is usually evidence of multiorgan failure and disseminated intravascular coagulation. Moderate increases in the activities of serum CK and serum AST usually occur; however, they often do not reflect the clinical toxicity of clostridial myonecrosis. Characteristic hyperechoic gas accumulation and hypoechoic fluid are usually observed with ultrasonography of the affected site. Fasciotomy or aspiration of the affected area usually reveals the presence of malodorous, serosanguineous fluid with an odor of rancid butter. Aspirates of affected tissues examined via direct smears or fluorescent antibody staining should show characteristic gram-positive, rod-shaped bacteria. Anaerobic bacterial culture of freshly acquired samples may also be of value. At postmortem, swelling, crepitus, and autolysis are rapid, and bloodstained fluid is often observed discharging from body orifices.

Treatment. Wound fenestration and aggressive surgical debridement over the entire affected area are required for successful treatment.[129] Sufficient fenestrations should be made to establish drainage and aeration over the entire affected area. The horse should be frequently reevaluated to assess spread of cellulitis and repeat fenestration procedures may be required. Fenestrations should be made in parallel to muscle fiber bundles to prevent excessive scarring and future impairment of muscle function. Additional treatment includes high doses of IV potassium penicillin (up to 88,000 IU/kg) every 2 to 4 hours until the animal is stable (1–5 days) in combination with oral metronidazole. Aggressive fluid, electrolyte, and cardiovascular support is often required. Severe hypotension, diminished cardiac output, impaired renal function, and high levels of myoglobinemia place the patient at risk for pigment nephropathy and acute renal failure. Extensive skin sloughing over the affected area is common in surviving horses. The prognosis for life in all horses with clostridial myonecrosis is guarded, and the owner should be aware from the start of treatment that extensive skin sloughing can occur and euthanasia could become a consideration at a later stage.

Anaplasma-Associated Rhabdomyolysis

There is a report of a Quarter Horse with clinical signs of stiffness, pyrexia, tachycardia, and marked elevations of serum CK activity in association with *Anaplasma phagocytophilum* infection.[130]

Streptococcus equi Subsp. *equi*–Associated Myopathy

Acute Rhabdomyolysis

Pathogenesis. Quarter Horses generally less than 7 years of age can develop severe, generalized rhabdomyolysis in association with an active *Streptococcus equi* subsp. *equi* infection (referred to as *S. equi* for the remainder of this chapter).[131,132] Two etiologies have been proposed: (1) a toxic shock–like reaction arising from profound nonspecific T-cell stimulation by streptococcal superantigens with the release of high levels of inflammatory cytokines and (2) bacteremia with local multiplication and production of exotoxins or proteases within skeletal muscle. *S. equi* virulence factors that may account for muscle necrosis include an unidentified cytotoxic protein, several proteases, streptokinase, and streptolysin S.[133] Although *S. equi* has not been cultured in skeletal muscle from horses



FIG. **10.14** *Clostridium perfringens* myositis in the cervical muscles following an intramuscular injection. The low head posture was due to pain and depression. (A) Malodorous fluid exudes from multiple fenestrations made over areas of crepitus. (B) Granulation tissue and minor sloughing of skin is apparent at wound edges. (C) Subsequent massive sloughing of skin. (D) Healing wound after many months of treatment.

with rhabdomyolysis, *S. equi* bacteria have been identified in affected muscle using immunofluorescent stains for both Lancefield group C carbohydrate and *S. equi* M protein.[131] There is currently no evidence that the *S. equi* involved is an atypical genetic strain of *S. equi*.[134]

Clinical Signs. Affected horses have evidence of enlarged submandibular lymph nodes and/or guttural pouch empyema. Horses develop a stiff gait that progresses rapidly to markedly firm, swollen, painful epaxial and gluteal muscles. The majority of reported cases become recumbent, are unable to rise, and develop unrelenting pain necessitating euthanasia within 24 to 48 hours of hospitalization.

Diagnosis. Hematologic abnormalities include mature neutrophilia, hyperfibrinogenemia, normal albumin, and marked increases in serum CK (115,000–587,000 U/L) and AST activities (600–14,500 U/L). Titers to the M protein of *S. equi* are low in affected horses, unless horses have been recently vaccinated for strangles. Titers to another protein called *myosin binding protein* have been high in a small number of horses that were tested.[131] Endoscopy of the guttural pouches reveals pools of pus that are positive in culture and polymerase chain reaction (PCR) for *S. equi*.

At postmortem examination, large pale areas of necrotic muscle are evident in hindlimb and lumbar muscles. The histopathologic lesions are characterized by severe acute myonecrosis with a degree of macrophage infiltration. Sublumbar muscles often show the most severe and chronic necrosis as indicated by greater macrophage infiltration of myofibers.

Treatment. A high mortality rate has been reported in horses receiving IV penicillin therapy once clinical signs of strangles and myopathy were well established. It is possible that early recognition of the signs of muscle stiffness in horses with *S. equi* infections and prompt aggressive treatment may be required for a successful outcome. Although streptococcal species are exquisitely susceptible to β-lactam antibiotics, a mortality rate of 85% has been reported in human group A streptococcal myositis despite penicillin treatment.[135] An antimicrobial that inhibits protein synthesis, such as rifampin, combined with IV penicillin, might enhance survival rates in horses with *S. equi* rhabdomyolysis. In addition, flushing infected guttural pouches and draining abscessed lymph nodes will diminish the bacterial load. NSAIDs and possibly high doses of short-acting corticosteroids may assist in diminishing the inflammatory response. Control of unrelenting pain is a major challenge in horses with severe rhabdomyolysis. Constant rate infusion of lidocaine, detomidine, or ketamine may provide better anxiety and pain relief than periodic injections of tranquilizers. Horses should be placed in a deeply bedded stall and moved from side to side every 4 hours if they are unable to rise. Some horses may benefit from a sling if they will bear weight on their hindlimbs when assisted to stand.



FIG. **10.15** Infarctive hemorrhagic purpura resulting in firm painful swelling within the muscles of the antebrachium on the right forelimb. (Photo courtesy Dr. Raphaela Texeira.)



FIG. **10.16** Extensive infarction of the large colon in a horse with infarctive hemorrhagic purpura.

Immune-Mediated Myopathies

Infarctive Purpura Hemorrhagica

Pathogenesis. Infarctive purpura hemorrhagica (IPH) is a severe form of purpura with a high fatality rate. Infarctions initially occur in skeletal muscles that are compressed during recumbency and progress to involve multiple organs, including the gastrointestinal tract and the mucous membranes.[136] In one study prevalence of IPH was 3 of 53 cases of purpura.[137] Exposure to *S. equi* within 3 weeks of presentation, vaccination for *S. equi,* or a concurrent *Salmonella infantum* infection are reported inciting causes. IPH resembles Henoch-Schönlein purpura in humans, which is characterized by infarctive vasculitis of the skin, kidneys, and gastrointestinal tract resulting from immunoglobulin A (IgA) immune complex deposition. Immune complexes present in the sera of horses with IPH appear to be composed primarily of IgM or IgA and streptococcal M protein.[138] Deposition of complement near immune complexes in vessel walls may result in cell membrane destruction, cell death, and vascular occlusion.

Clinical Signs. The primary presenting complaint is often painful lameness with limb swelling, muscle stiffness, and/or colic (Fig. 10.15). Careful physical examination reveals classic signs of IPH such as petechiae, oral infarctions resembling ulcers, and well-demarcated moderate limb edema. Horses with IPH will have focal firm intramuscular swellings in abdominal, pectoral, adductor, and tarsocrural muscles. Horses with evidence of colic may have markedly decreased borborygmi and hemorrhagic gastric reflux.

Diagnosis. Hematologic abnormalities include a leukocytosis characterized by neutrophilia with a left shift and toxic change, hyperproteinemia, hypoalbuminemia, and marked elevations in CK (47,000–280,000 U/L) and AST (960–7000 U/L) activities.[132,139] Serum titers for enzyme-linked immunosorbent assay (ELISA) M protein are usually markedly elevated.[139] Peritoneal fluid obtained by abdominocentesis may be normal or may have an increased total protein and nucleated and red blood cell counts if gastrointestinal infarction is present. Ultrasonographic examination of swollen muscle reveals focal hypoechoic lesions within muscle tissue. Biopsies of abnormal muscle show diffuse acute coagulative necrosis, whereas samples from palpably normal muscle tissue show no pathologic abnormalities.

Postmortem findings of horses with IPH include infarction of the skeletal musculature, skin, gastrointestinal tract, pancreas, and lungs and *S. equi* abscessation of a lymph node (Fig. 10.16). Definitive histopathologic findings include leukocytoclastic vasculitis and acute coagulative necrosis resembling infarction in numerous tissues.[139]

Treatment. Early recognition of signs and aggressive antibiotic and corticosteroid treatment are essential to combat the high fatality rate with IHP. Treatment of Henoch-Schönlein purpura in humans, including cases with intestinal infarctions, involves high-dose IV pulse therapy with methylprednisolone followed by oral corticosteroids plus immunosuppressive agents such as cyclophosphamide and azathioprine. One horse with IPH was successfully treated with penicillin, NSAIDs, and 3 weeks of dexamethasone (0.1–0.07 mg/kg) followed by a 10-week tapering course of oral prednisolone (2 mg/kg initially).[139]

Immune-Mediated Myositis in Quarter Horse–Related Breeds

Pathogenesis. Malaise, rapid onset of severe atrophy particularly affecting epaxial and gluteal muscles, and high serum CK and AST activities occur in equine immune-mediated myositis (IMM) as a result of lymphocytic destruction of myofibers by CD4+ and to a lesser extent CD8+ cells.[11] For yet unknown reasons a loss of self-tolerance to antigens expressed by muscle cells may occur after rhabdomyolysis, infection with *S. equi,* or vaccination. Myoblasts and myotubes do not normally express detectable human leukocyte antigen (HLA) class II molecules and therefore do not display potentially immunogenic peptides to cells of the immune system. Loss of self-tolerance could occur with IMM as a result of activation of otherwise inactivated autoreactive T cells by either (1) shared epitopes with an infectious agent causing antigenic mimicry; (2) microbial superantigens produced by infectious agents; (3) high concentrations of local cytokines such as interleukin-2 (IL2) or IL4; or (4) expression of an abnormal protein on sarcolemmal membranes. There is strong evidence of a genetic susceptibility to IMM in the Quarter Horse breed.[11]

History and Clinical Signs. Generalized IMM usually affects horses of Quarter Horse–related breeds that are either younger than 8 years of age or older than 16 years of age without gender predilection.[11] A history of exposure to a



FIG. **10.17** Pronounced atrophy of the gluteal muscles of a young Paint Horse with immune-mediated myositis. Atrophy is more severe on the right side, which creates shadowing over the area where a biopsy was previously obtained. (Photo courtesy Dr. Carrie Finno.)

triggering factor exists in approximately one third of cases; the known triggering factor is usually exposure to *S. equi,* another respiratory pathogen, or possibly vaccination. The most prominent clinical sign is dramatic muscle atrophy, particularly of the epaxial and gluteal muscles, accompanied by stiffness and general malaise (Fig. 10.17). Muscle atrophy is usually rapid and may progress to involve 40% of the horse's muscle mass within a week. Horses can develop generalized weakness leading to frequent periods of recumbency. Muscle atrophy often persists for months before a gradual increase in muscle mass occurs.

Diagnosis. Hematologic abnormalities apart from elevations in CK and AST activities are uncommon with IMM unless there is a concurrent infectious process. During the acute phase of muscle atrophy serum CK and AST activities are moderately to markedly elevated, but during the later phase of chronic atrophy serum CK and AST activities can be normal. The most useful diagnostic test during the acute phase of the disease is a muscle biopsy of the epaxial and/or gluteal muscles.[11] Several formalin-fixed Trucut samples from the epaxial or gluteal muscles are often sufficient to establish a clinical diagnosis. Histologic abnormalities include infiltration of the myofibers by lymphocytes, lymphocytic vasculitis, anguloid atrophy of the myofibers, fiber necrosis, and multinucleated giant cells. Biopsies taken several weeks after the onset of atrophy may reveal only nonspecific findings of myogenic atrophy without evidence of the hallmark feature of lymphocytic infiltrates. Biopsies of the semitendinosus or semimembranosus muscles may not be helpful in establishing a diagnosis because they are often normal or have evidence of atrophy and mild inflammatory cell infiltration.

Treatment and Prevention. Appropriate antimicrobials should be selected if there is evidence of concurrent infection. Corticosteroids are the mainstay of treatment, and, unlike immune-mediated disorders in humans and dogs, antiinflammatory rather than immunosuppressive doses are effective. Recommended dosages are dexamethasone (0.05 mg/kg intravenously) for 3 days, followed by prednisolone (1 mg/kg PO) for 7 to 10 days tapered by 100 mg per week over 1 month.[11] Prevention is challenging because only a small proportion of affected horses have a history of a triggering factor. Suggested strategies include preventing exposure to respiratory infections where possible; spacing out vaccines to prevent a surge of inflammatory cytokines; avoiding immune stimulants; and monitoring horses closely to institute treatment early if atrophy recurs. Malaise often resolves rapidly following treatment with corticosteroids and muscle atrophy quickly ceases. Serum CK activity often returns to normal after 7 to 10 days of corticosteroid treatment. Recovery of the muscle mass is more gradual and usually takes 2 to 3 months, and some focal residual muscle atrophy can remain.[11] Horses that are not treated with corticosteroids may develop extensive muscle atrophy, but in many cases muscle mass will gradually recover. Atrophy recurs in approximately 40% of susceptible horses, and these horses may require repeated bouts of corticosteroid treatment.

Systemic Calcinosis

Pathogenesis. Systemic calcinosis occurs in humans, particularly those on dialysis because of renal failure. In human medicine a product of Ca multipled by P of greater than 65 to 70 is used as an indicator of a potential risk of dystrophic calcification.[142] A Ca × P product greater than 66 was present in horses that developed systemic dystrophic calcification. The reason for the elevated Ca × P in horses with systemic calcinosis is not known, but there is a strong suspicion that a predisposing inflammatory process could be a trigger. Hyperphosphatemia can induce dystrophic calcification through (1) passive calcium phosphate deposition from phosphate supersaturation in the blood; (2) an active process promoting the conversion of smooth muscle cells to osteogenic cell types; (3) directly increasing parathyroid hormone secretion and transcription; or (4) interference with renal production of 1,25-(OH)2D levels, which has been associated with increased coronary artery calcification in humans.[143]

A novel fatal syndrome of systemic dystrophic calcification called *systemic calcinosis* or *calciphylaxis* occurs in horses that share a similar initial presentation to generalized IMM that is followed by diverse organ failure.[140,141] Systemic calcification appears to be triggered when the serum calcium concentration multiplied by serum phosphorus concentration exceeds 65.

Clinical Signs. All horses reported with systemic calcinosis have been of Quarter Horse–related breeds and under 9 years of age.[140,141] Initial clinical signs include a mild fever, malaise, stiffness, and loss of muscle mass, particularly over the lumbar and gluteal area (Fig. 10.18). Many horses have evidence of cough, tachypnea, and mild ventral edema. As the disease progresses progressive weakness, inability to remain standing, respiratory distress, laminitis, or gastrointestinal inflammation may become evident. Whether these signs are a direct result of systemic dystrophic calcification or an underlying precipitating inflammatory disorder is unclear.

Diagnosis. A wide range of hematologic abnormalities may be observed depending on the underlying disease process that precedes dystrophic calcification. The most consistent alterations in the hemogram are hyperfibrinogenemia and mild leukocytosis.



FIG. **10.18** Severe muscle wasting of the topline and hindquarter muscles in a horse with systemic calcinosis.

In the biochemistry profile the product of calcium multiplied by phosphorus is greater than 65, and serum CK activities are at least threefold higher than the normal range.[140]

Systemic calcinosis should be suspected if a horse presents with muscle atrophy, malaise, respiratory distress, or renal insufficiency with evidence of hyperfibrinogenemia and an elevated Ca × P product. The histopathologic findings in muscle biopsies resemble those of IMM; however, multinucleated giant cells are consistently present, and dystrophic calcification of muscle fibers is also evident.[140] At postmortem, dystrophic calcification is also evident in one or all of the following tissues in addition to skeletal muscle, the alveoli in pulmonary tissue, cardiac myofibers, renal tubules, and tunica intima of blood vessels.[140] In one case, dramatic lesions of arterial medial calcification of the aorta and coronary and pulmonary arteries were reported.[141]

Treatment and Prognosis. This condition is often associated with a fatal outcome. Horses are usually euthanized because of progressive weakness, inability to remain standing, respiratory distress, laminitis, or other underlying disease. Treatment is largely directed toward the underlying inflammatory condition. In human medicine, corticosteroid treatment has been implicated as a triggering mechanism for systemic calcinosis. In horses, however, the clinical signs that precede systemic calcinosis so closely resemble IMM, a corticosteroid-responsive disease, that corticosteroid treatment is often instituted when signs of rapid muscle atrophy develop.

Idiopathic Immune-Mediated/Inflammatory Myopathies

Individual cases of myositis occur outside the Quarter Horse breed that likely have an inflammatory or immune-mediated basis. Focal atrophy of neck muscles was reported in a 16-year-old pony with normal serum CK and AST activities and interstitial aggregates of lymphocytes, plasma cells, histiocytes, and eosinophils.[144] Muscle mass returned to normal following treatment with dexamethasone. Within the Neuromuscular Diagnostic Laboratory database at Michigan State University a small number of horses of breeds other than Quarter Horses have also been diagnosed with a suspected inflammatory or immune-mediated myositis. These included an Anglo-Arabian, a mixed breed pony, a Percheron, a Warmblood, and two Thoroughbreds. The common clinical presentation was muscle atrophy, and the suspicion of an inflammatory/immune process was based on the presence of lymphocytic infiltrates in muscle biopsy specimens.

TOXIC CAUSES OF RHABDOMYOLYSIS

Chemical

Horses can periodically gain access to ionophores when equine grains are mixed in a feedmill that did not properly clean machinery after producing cattle feeds containing ionophores. The primary clinical signs of ionophore toxicity depend on the type of ionophore but usually suggest cardiomyopathy or neurologic disease rather than overt rhabdomyolysis. Experimental studies have indicated that LD_{50} values for monensin are 2 to 3 mg/kg body weight for horses versus 21 to 36 mg/kg for cattle.

Several miniature horses being fed a complete feed containing tetrachlorovinphos, an oral fly control agent, were reported to develop chronic myonecrosis involving masseter, tongue, neck, respiratory, and postural muscles and occasionally cardiac muscle.[145] Affected horses showed signs of lethargy, dysphagia, fasciculations, tachypnea, and tachycardia. Muscle tissue showed evidence of chronic myonecrosis and lipid accumulation. Myonecrosis was attributed to acetylcholine accumulation at muscarinic and nicotinic sites producing oxidant stress. Low selenium concentrations may contribute to the toxicosis.

Blister beetle toxicosis can cause elevations in serum CK in some horses.[146]

Plants

Cassia obtusifolia (sicklepod) is prevalent in the southeastern United States, and ingestion of seeds can cause degenerative myopathy and cardiomyopathy with evidence of myofiber atrophy, segmental necrosis, and mitochondrial disruption.[147] Tremetone-containing plants can cause a fatal cardiomyopathy and severe skeletal muscle degeneration in horses when ingested at 0.5% to 2% of body weight.[148,149] Both white snakeroot *(Eupatorium rugosum),* which grows in shaded areas of the eastern and central United States, and rayless goldenrod *(Isocoma wrightii),* which is common in the Southwest on open pastures, contain tremetone. Tremetone remains active in hay and in the stalks of the dead plants on pasture, so both the fresh and the dried forms of the plants should be kept from horses.[150] Microsomal activation of the toxin in the liver may be necessary for toxic effects.[148]

Hypoglycin a Myopathy

Pathogenesis. Seeds or young shoots of *Acer* species trees such as the box elder *(Acer negundo)* and the European sycamore maple *(Acer pseudoplatanus)* contain highly variable amounts of the toxic nonproteogenic amino acid hypoglycin A (Fig. 10.19).[151,152] Hypoglycin A is metabolized in the liver to methylenecyclopropylacetic acid (MCPA), and MCPA CoA irreversibly binds to multiple acyl CoA dehydrogenases, enzymes that are essential for metabolism of short- and medium-chain fatty acids and branched chain amino acids.[9,153] Accumulation of fat esters damages muscle cell membranes, and an energy deficiency arises from an inability to metabolize fat.

Prevalence. Cases of hypoglycin A myopathy have been reported in northern and central Europe, the United Kingdom, New Zealand, and central and northeastern North America.



FIG. **10.19** (A) Seeds from box elder trees. (B) Sycamore maple seed. When either seeds are ingested by horses they can cause hypoglycin A myopathy.

The distribution of cases overlaps with the distribution of sycamore maple trees (UK and Europe) or box elder trees (North America).[14,154-157] Before identification of the toxin, hypoglycin A myopathy was termed *atypical myopathy* or *seasonal pasture myopathy* because it occurred in pastured horses in the fall and less often the spring and often preceded by wet, windy weather. Affected horses are usually young or new to affected pastures and kept on wooded pastures for more than 12 hours a day without additional feed provided.[158,159] Selenium deficiency appears to enhance toxic effects.[160]

Clinical Signs. Clinical signs develop acutely within 3 to 5 days of exposure to toxic doses and include muscular weakness, sweating, fasciculations, stiffness, tachycardia, tachypnea, recumbency, and, when urine is observed, myoglobinuria. A rapid rise in respiratory rate is usually followed by collapse and death from respiratory or cardiac failure. Pasture-mates can be subclinically affected.

Diagnosis. A high index of suspicion of hypoglycin A myopathy occurs in horses with rhabdomyolysis and an acidic urine pH. The most notable change in serum biochemistry is a marked increase in serum CK and AST activity, which is often accompanied by hyperglycemia, lactic acidemia, and in some cases elevated serum troponin I concentrations.[161] A definitive diagnosis can be made by identifying a specific pattern of accumulation of serum acylcarnitines and urine organic acids and glycine conjugates typical of a deficiency in multiple acyl CoA dehydrogenases, as well as isolation of the conjugated toxic metabolite methylenecyclopropyl acetic acid (MCPA) or hypoglycin A in blood or urine.[9,151,153,154] Postmortem findings include extensive necrosis in deep postural and respiratory muscles and the myocardium in 50% or more of cases. Frozen sections of myocardium, intercostal, diaphragm, or deep postural muscles show marked intracellular lipid accumulation in oxidative fibers (oil red O stain).[14,156]

Treatment and Prognosis. The mortality rate for horses with overt clinical signs is high at 75%; however, early recognition may improve the prognosis.[159] Early aggressive fluid therapy with antioxidant and antiinflammatory treatment, including dimethylsulfoxide (DMSO), vitamin E, vitamin C, and NSAIDs, is indicated.[14] Cograzing horses with elevated serum CK activity and no clinical signs may be successfully treated if removed from the source of seeds immediately.

Although many older horses are asymptomatic on pastures with *Acer* species trees, young horses or new horses should not be pastured with box elder or European sycamore maple trees in the fall or early spring. Provision of additional feed or reducing grazing time during these seasons may also decrease the incidence of hypoglycin A myopathy.

POSTANESTHETIC MYOPATHY

Postanesthetic myopathies occur in several forms: focal myopathy-neuropathy, generalized postoperative rhabdomyolysis, and fulminant malignant hyperthermia.

Focal Myopathy

Pathogenesis

Focal myopathy and potentially neuropathy occurs in muscles that are in contact with a hard surface during anesthesia or in muscles that have positional occlusion of arterial blood supply. If mean arterial pressure is allowed to fall below 70 mm Hg for several hours during inhalation anesthesia the incidence of postanesthetic myopathy increases substantially.[162,163] Muscles with dense fascia are most susceptible to necrosis as arterial blood flow and oxygen supply decrease further with the increased pressure within a compartmentalized swollen muscle. Commonly affected muscles include triceps, deltoid, masseter, hindlimb extensors, or, if the horse has been in dorsal recumbency, the hindlimb adductor and gluteal muscles.[164-166] Injury can also occur to nerves in the compressed swollen muscles, resulting in temporary radial, peroneal, or femoral nerve paralysis.

Clinical Signs

Clinical signs become apparent on recovery or 30 to 60 minutes after recovery from anesthesia. Affected muscles are swollen, hot, and painful on deep palpation, and the horse is often reluctant to bear weight on the affected limb. Weakness of affected muscles is common, particularly with peripheral nerve involvement. In some horses this condition may limit the animal's ability to stand for some time following anesthesia. The loss of muscle strength, particularly when involving adductor muscles, can contribute to orthopedic injury during repeated attempts to rise. Many horses with mild to moderate muscle injury recover over a period of hours to days even if untreated.[164]

Diagnosis

Diagnosis is based on a history of anesthesia or prolonged recumbency, clinical signs, and marked elevations in serum CK activity (thousands to tens of thousands of IU/L) within 4 to 6 hours after anesthesia.

Treatment

Antiinflammatory drugs, dimethylsulfoxide, and dantrolene sodium 2 to 4 mg/kg PO often are sufficient in mild

to moderate cases.[59] Significant muscle atrophy may develop over the ensuing 3 to 4 weeks but usually will resolve within 2 to 3 months unless substantial nerve damage has occurred. Treatment of more severe cases is outlined previously in this chapter (see section on treatment of acute rhabdomyolysis).

Prevention

Correct positioning and judicious use of padding and water- or air-filled mattresses can reduce dependent muscle pressure up to 50%, thereby aiding in the reduction of this disorder. In addition, by elevating the upper limb during anesthesia, the pressure on the lower limbs is significantly reduced. Pulling the lower forelimb forward also markedly reduces pressure in the dependent triceps muscle. When the horse is in dorsal recumbency, padding under the shoulders and hips is absolutely imperative.

Maintaining anesthesia at the lightest plane possible for a specific surgical procedure is beneficial in prophylaxis. Similarly, if possible, maintaining systemic mean arterial blood pressure above 80 to 85 mm Hg during anesthesia is advisable. The use of ionotropic agents such as dobutamine during anesthesia has been useful in reducing the occurrence of anesthetic myopathies. Administration of dantrolene sodium (2 to 4 mg/kg PO) 1 hour before induction of anesthesia may result in a reduction in the incidence of this myopathy; however, decreased cardiac output can occur in conjunction with higher Dantrium doses.[72]

Generalized Postanesthetic Myopathy

Pathogenesis

The development of generalized rhabdomyolysis and weakness in horses can occur during recovery from anesthesia or hours after recovery. In some cases, systemic hypotension and hypoxemia may create local ischemic lesions, with the pathologic changes becoming more generalized as a result of the stress of anesthesia and the sensitivity of muscle cells to anesthetic agents or muscle relaxants.[162,164] In other cases, an underlying myopathy such as hyperkalemic periodic paralysis, recurrent exertional rhabdomyolysis, or PSSM may increase the risk of a generalized postanesthetic myopathy.[34,38,95,167]

Clinical Signs

Anxiety, hyperthermia, tachycardia, tachypnea, profuse sweating, and myoglobinuria with firm painful swelling of multiple muscle groups is an occasional serious complication of anesthesia.[165,166] Horses may not be able to rise and may struggle violently, resulting in prolonged, traumatic recoveries.

Diagnosis

Diagnosis is based on clinical signs and assessment of serum CK activity taken at least 4 hours after anesthesia to allow levels to peak.

Treatment

Aims of therapy include pain relief, correction of fluid and electrolyte deficits, prevention of ongoing necrosis, and nursing care as outlined previously in this chapter (see section on treatment of acute rhabdomyolysis).

Prevention

The principles described for localized myoneuropathies apply to the prevention of generalized anesthetic-related myopathies. Horses with HYPP should be premedicated with acetazolamide. Dantrolene sodium at 2 to 4 mg/kg PO 1 to 2 hours before anesthesia may be beneficial in reducing the incidence of postanesthetic myopathies in problematic horses; however, 6 mg/kg has caused decreased cardiac output and hypotension, as well as elevated serum potassium, which would be detrimental to horses with HYPP.[72,168]

Malignant Hyperthermia

Pathogenesis

Fulminant cases of malignant hyperthermia (MH) are documented in horses of many breeds. In Quarter Horses a mutation in the ryanodine receptor 1 *(RYR1)* gene, the same gene involved in MH of swine, has been linked to fulminant anesthetic reactions caused by excessive calcium release from the sarcoplasmic reticulum.[67-72] In other breeds, a specific cause of MH reactions has not been identified. Excessive myoplasmic calcium concentrations result in muscle contractures, anaerobic metabolism of glycogen, hyperthermia, lactic acidosis, and hypercapnia.

Clinical Signs

Marked muscle rigidity, hyperthermia, hyperkalemia, hypercapnia, and metabolic and respiratory acidosis develop during anesthesia.[38,70,169,170] Horses usually die within a matter of hours. In some cases, shock and pigmenturia may lead to renal failure.

Treatment and Prevention

Genetic screening for MH in Quarter Horses and Paints may be warranted before elective procedures, although the prevalence of the MH mutation is less than 1% in these breeds. If hyperthermia and contracture develop during anesthesia, anesthesia should be immediately discontinued. Attempts to cool the animal with alcohol or cold-water baths are indicated. IV administration of sodium bicarbonate at dosages calculated from acid-base measurements is most appropriate. If not possible, a dosage of 1 to 2 mEq/kg slowly is recommended. Administration of a large amount of soluble, lyophilized dantrolene sodium for IV administration may alleviate clinical signs in these horses. However, availability and expense of the agent in this form highly restrict its use. A dosage rate of 1 mg/kg intravenously may be appropriate, although more controlled studies are required.[164]

MYOTONIC DISORDERS

Differential diagnoses for muscle fasciculations in horses are listed in Box 10.4.

Myotonia

Pathogenesis

Myotonic muscle disorders are a heterogeneous group of acquired or congenital diseases that share the feature of delayed relaxation of muscle after mechanical stimulation or voluntary contraction caused by abnormal muscle membrane excitability. Congenital or inherited causes include defects in the skeletal muscle chloride channel (myotonia congenita) or sodium channel (hyperkalemic periodic paralysis). Acquired causes include infestation with the spinous ear tick *Otobius megnini.* The cause of many cases of myotonia in horses, especially dystrophic forms, remains unknown.

BOX 10.4 Muscle Fasciculations

1. Pain, anxiety
2. Weakness
   a. Botulism
   b. Vitamin E–deficient myopathy
   c. Equine motor neuron disease
3. Increased motor neuron firing
   a. Electrolyte abnormalities
   b. Focal nerve irritation
   c. *Otobius megnini* ear tick infestation
   d. Stiff horse syndrome
   e. Shivers
   f. Neuromyotonia
4. Abnormal sarcolemmal conductivity
   a. Hyperkalemic periodic paralysis
   b. Myotonia congenita
   c. Myotonia dystrophica



FIG. **10.20** Middle gluteal muscle hypertrophy and superficial gluteal muscle atrophy in a horse with myotonia dystrophica.

Clinical Signs

Myotonia Congenita. Myotonia congenita is usually detected in the first year of life.[171-174] Affected foals have conspicuously well-developed musculature with mild to moderate pelvic limb stiffness that is most pronounced when exercise begins and diminishing as exercise continues. Bilateral bulging (dimpling) of the thigh and rump muscles is often obvious, and percussion dimpling occurs with tactile stimulation over the muscle. Percussed muscles can remain contracted for up to a minute or more with subsequent slow relaxation.[174,175] Progression of clinical signs does not usually occur beyond 6 to 12 months of age.[175] An autosomal recessive mutation of the *CLCN1* gene was identified in one New Forest Pony with myotonia congenita.[176]

Myotonia Dystrophica. Myotonia dystrophica resembles myotonia congenita in early phases; however, weanlings have progressive multifocal muscle atrophy, hypertrophy, and persistent stiffness that worsen with exercise (Fig. 10.20). It has been described in a variety of breeds, including Quarter Horses, Appaloosa, and Italian-bred foals.[175,177,178] In some foals retinal dysplasia, lenticular opacities, and gonadal hypoplasia have been documented.[178] The basis for myotonia dystrophica is unknown.



FIG. **10.21** Severe muscle cramping *(arrow)* associated with ear ticks that is so painful to horses it results in colic-like signs and recumbency.

Diagnosis

A tentative diagnosis of myotonia can be made on the basis of age, clinical signs of stiff gait, muscle bulging, and myotonic dimpling following percussion. Definitive diagnosis requires electromyographic (EMG) examination. Affected muscles manifest pathognomonic high-frequency repetitive discharges that wax and wane, creating the sound of "dive bombers" resulting from abnormal repetitive firing of affected muscle fibers.[49] Muscle biopsies from foals with myotonia congenita can have normal histopathology or may demonstrate extremely variable muscle fiber diameters up to twice those of normal age-matched controls.[174] Type 1 fiber hypertrophy or hypotrophy is reported. Muscle pathology is more dramatic in myotonia dystrophica, including ringed fibers, centrally displaced myonuclei, sarcoplasmic masses, and an increase in endomysial and perimysial connective tissue.[175,177-179] Fiber type grouping and atrophy of both type 1 and type 2 muscle fibers are present.

Treatment and Prognosis

There is no efficacious treatment for myotonia in horses. Prognosis appears to be variable and dependent on the severity of clinical signs. Mildly affected animals with myotonia congenita can show improvement in clinical signs with increasing age. The reason(s) for regression of signs is unknown. Other more severely affected horses, particularly those with myotonia dystrophica, have progression of signs, including atrophy and fibrosis or pseudohypertrophy to the point at which the animal is no longer able to move without great difficulty. Euthanasia of such animals is often warranted. Conclusive evidence regarding the genetic basis of myotonia dystrophia is lacking.

Ear-Tick Associated Myotonia

Otobius megnini is commonly found in the southwestern United States. Intermittent painful nonexertional muscle contraction and mild to moderate rhabdomyolysis occur in some horses in conjunction with *O. megnini* infestations.[180] Intermittent contraction of pectoral, triceps, abdominal, or semitendinosus/semimembranosus muscles, lasting from minutes to a few hours, can be so painful that it results in recumbency often resembling colic (Fig. 10.21). Contraction with myotonic dimpling of a muscle can be induced by tactile stimulation of the muscle with a percussion hammer. Horses may fall over with pain when stimulated. Between muscle cramps horses appear

to be normal. Horses have elevated serum CK activities ranging from 4000 to 170,000 IU/L. Numerous ear ticks are present in the external ear canal of affected horses. Without treatment for ear ticks, the spasms continue; however, local treatment of the ear ticks using pyrethrins and piperonal butoxide results in recovery within 12 to 36 hours. Acepromazine may be helpful to relieve painful cramping.

HYPERKALEMIC PERIODIC PARALYSIS

Sharon J. Spier

Pathogenesis

Hyperkalemic periodic paralysis (HYPP) is an autosomal codominant form of myotonia in Quarter Horses, American Paint Horses, and Appaloosas that are descendants of the popular Quarter Horse stallion Impressive. HYPP is due to a mutation in the gene encoding the voltage-dependent skeletal muscle sodium channel.[181-185] Horses with HYPP have resting sarcolemmal membrane potentials that are closer to firing. When muscle cells begin to depolarize during an episode of HYPP, a subpopulation of sodium channels fail to inactivate, producing an excessive inward flux of sodium and outward flux of potassium resulting in persistent depolarization of muscle cells, general muscle fasciculations, and weakness.

Prevalence

Approximately 1.5% of the Quarter Horse breed and 4.5% of the Paint Horse breed are affected.[83] Notably, however, 58% of halter horses of these breeds have the HYPP mutation likely because the disorder is linked to large muscle mass and success in the halter horse industry.[83] Horses homozygous for the HYPP mutation can no longer be registered with the American Quarter Horse Association.

Clinical Signs

Clinical signs are intermittent and highly variable in nature beginning by 2 to 3 years of age.[182,183] Some heterozygous horses are completely asymptomatic, whereas other heterozygous horses have daily muscle fasciculations and weakness. Episodes often begin with a brief period of facial myotonia and potentially prolapse of the third eyelid. Sweating and muscle fasciculations progress to involve the flanks, neck, and shoulders. Attempts to move the horse can exacerbate muscle tremors. Overt myotonic cramping of muscle groups can occur in some horses (Fig. 10.22). Heart and respiratory rates can be elevated. During mild attacks horses remain standing, but episodes can evolve within a few minutes to encompass severe weakness, swaying, staggering, dog sitting, and recumbency. Paralysis of upper respiratory muscles during episodes can result in respiratory distress and sudden death if a tracheostomy is not performed. Horses remain conscious even when recumbent and often have an anxious alert eye responding to noise and painful stimuli. Episodes usually last from 15 to 60 minutes. After the episode subsides, horses regain their feet and appear normal with no apparent or minimal gait abnormalities.

Foals homozygous for the HYPP mutation can present with dysphagia, respiratory stridor, and periodic obstruction of the upper respiratory tract (Fig. 10.23). Endoscopic findings include pharyngeal collapse and edema, laryngopalatal dislocation, and laryngeal paralysis.[186]

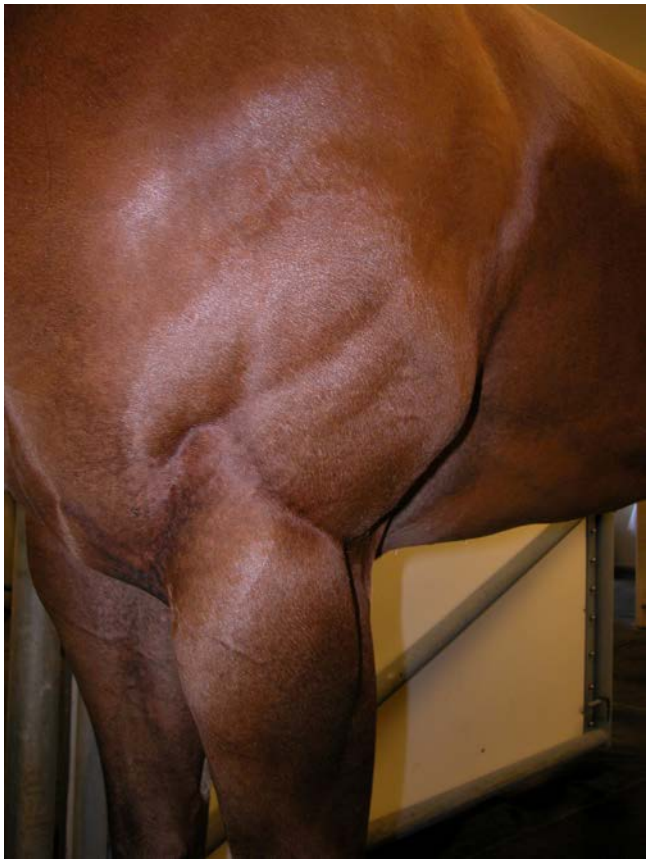


FIG. **10.22** Heavily muscled phenotype and myotonic cramping in a Quarter Horse suffering from HYPP. (Photo courtesy Dr. Monica Aleman.)

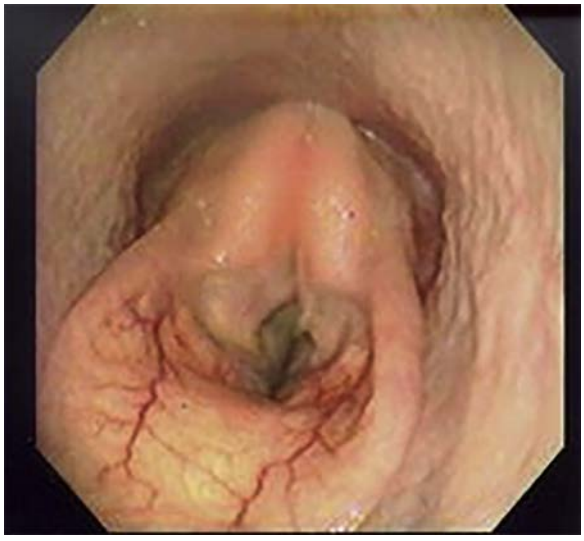


FIG. **10.23** Hypertrophy and closure of the vocal folds in a foal homozygous for HYPP that resulted in severe dyspnea.

Risk Factors

The factor that precipitates an episode of HYPP in genetically susceptible horses is often not obvious. Diets high in potassium (greater than 1.1%), such as those containing alfalfa hay, molasses, electrolyte supplements, and kelp-based supplements, can precipitate an episode.[187,188] Other potential

triggering factors include sudden dietary changes, fasting, anesthesia or heavy sedation, trailer rides, stress, cold, pregnancy, and concurrent disease. HYPP is not triggered by exercise.

Clinical Pathology

In most cases, hyperkalemia (6–9 mEq/L), hemoconcentration, and mild hyponatremia occur during periods of muscle fasciculations with normal acid-base balance.[183] Serum potassium concentration returns to normal following the abatement of clinical signs. Some affected horses may have normal serum potassium concentrations during minor episodes of muscle fasciculations.[182] Serum CK activity shows no change or only mild increases during episodic fasciculations and weakness.

Diagnosis

A definitive diagnosis of HYPP is made through genetic testing for the F1416L mutation in the *SCN4A* gene encoding the alpha subunit of the sodium channel.[184] Submission of mane or tail hair with roots should be made to a licensed laboratory such as the Veterinary Genetics Laboratory at the University of California, Davis, www.vgl.ucdavis.edu. Electromyographic examination of affected horses has also been used for diagnostic purposes. Affected horses have abnormal fibrillation potentials, complex repetitive discharges, occasional myotonic potentials, and trains of doublets even between episodes of HYPP.[183,189]

Treatment

Many horses experience spontaneous recovery from episodes of paralysis and appear normal by the time a veterinarian arrives. If horses are just beginning to exhibit clinical signs, owners may be advised to provide mild exercise or to feed grain or corn syrup before the veterinarian's arrival. Grain and high-fructose corn syrup (e.g., Karo syrup) lower serum potassium by stimulating insulin-mediated movement of potassium across cell membranes. Other options for treatment during an acute episode of hyperkalemia include epinephrine (3 mL of 1:1000/500 kg administered intramuscularly), acetazolamide (3 mg/kg PO every 8–12 hours), or calcium gluconate (0.2–0.4 mL/kg of a 23% IV solution, diluted in 1 L of 5% dextrose). An increase in extracellular calcium concentration raises the muscle membrane threshold potential, which decreases membrane hyperexcitability. Other options include IV dextrose (6 mL/kg of a 5% solution) alone or combined with sodium bicarbonate (1–2 mEq/kg) to enhance intracellular movement of potassium. With severe respiratory obstruction, a tracheostomy may be necessary.

Control

Ideally, horses with recurrent episodes of HYPP should be fed a balanced diet containing between 0.6% and 1.1% total potassium by weight and meals containing less than 33 g of potassium.[187,188,190] The principal source of potassium in the equine diet is forage. In general, early cuts of alfalfa, orchard grass, and brome hay have higher potassium concentrations, and later cuts of timothy or Bermuda grass hay have lower potassium concentrations, but this is not always the case. Horses having frequent episodes should have forages analyzed for potassium concentrations.[190] Pasture works well for horses with HYPP because the high water content of pasture grass makes it unlikely that horses will consume large amounts of potassium in a short period of time. Feeds containing soybean meal, sugar molasses, or beet molasses can have higher potassium concentrations. Oats, corn, wheat, barley, and beet pulp should be fed in small meals several times a day. Horses will adapt to diets higher in potassium over a period of 2 weeks and will experience fewer fluctuations in serum potassium with subsequent decreased frequency of clinical signs.[188] Regular exercise and/or frequent access to a large paddock or yard are also beneficial. Supplementation with salt and balanced vitamins and minerals is indicated to meet nutritional requirements. Commercially available complete feeds with a guaranteed K^+ content may be more convenient for some HYPP horses, especially for owners with few horses.

For horses with recurrent episodes of muscle fasciculations even with dietary modification, acetazolamide (2–3 mg/kg orally, every 8–12 hours) or hydrochlorothiazide (0.5–1 mg/kg orally, every 12 hours) may be indicated. Although these agents exert their effects through different mechanisms they both increase renal potassium excretion. Acetazolamide also stabilizes blood glucose and potassium by stimulating insulin secretion. Breed registries and other associations may have restrictions on the use of these drugs during competitions as diuretics may mask prohibited substances.

Prognosis

HYPP is usually a manageable disorder, although recurrent bouts may occur and severe episodes can be fatal. As this is a dominant trait, breeding an affected horse to a normal horse results in a 50% chance of producing a foal with HYPP. All affected horses share the same mutation regardless of whether or not owners have witnessed symptoms in their horses.[191] Owners of affected horses should advise veterinarians of HYPP status before anesthesia or procedures requiring heavy sedation, as these circumstances could precipitate an episode of paralysis.[192]

ELECTROLYTE IMBALANCES

Muscle Cramping

Severe electrolyte imbalances cause muscle cramping. Muscle cramps are a painful condition that arises from hyperactivity of motor units caused by repetitive firing of the peripheral and/or central nervous system. The origin of the cramp in most cases is believed to be the intramuscular portion of the motor nerve terminals.[193,194] Most muscle cramps are also accompanied by fasciculations in the same muscle. Muscle cramps can be induced by forceful contraction of a shortened muscle, by changes in the electrolyte composition of extracellular fluid, and by ear tick infestations in horses.[180,193] In contrast, muscle contractures are painful muscle spasms that represent a state of muscle contracture unaccompanied by depolarization of the muscle membrane.[192] Muscle contractures occur with MH and some forms of exertional myopathies and are invariably accompanied by markedly increased serum CK activity.

Hypocalcemic Tetany

Pathogenesis. Hypocalcemia is a rare occurrence in horses and is usually associated with parturition, heavily lactating mares, transportation, prolonged endurance rides, or grazing lush pastures.[195] Idiopathic fatal hypocalcemia has also been reported in foals.[196]

Clinical Signs. Clinical signs are similar to those seen with tetanus and include a stiff, stilted gait, muscle fasciculations (especially temporal, masseter, and triceps muscles),

trismus, dysphagia, salivation, anxiety, profuse sweating, tachycardia, elevated body temperature, cardiac arrhythmias, synchronous diaphragmatic flutter, convulsions, coma, and death.[195] Subclinical evidence of abnormal neuromuscular conductivity has been documented by EMG in horses with experimentally induced hypocalcemia.[197] In lactating mares this disorder can be progressive over a 24- to 48-hour period and potentially fatal. Clinical signs are related to the magnitude of decline in serum calcium concentration. Increased excitability is usually the only sign when values are below normal but above 8 mg/dL total calcium. Values of 5 to 8 mg/dL usually produce tetanic spasms and incoordination. Concentrations below 5 mg/dL usually result in recumbency and stupor.

Diagnosis. Diagnosis is usually based on clinical signs, a history of lactation, previous prolonged exercise, or transport. Along with hypocalcemia, metabolic alkalosis, hypomagnesemia/hypermagnesemia, and hyperphosphatemia/hypophosphatemia are frequently present. These alterations may need correction before tetanus and fasciculation abate.

Treatment. IV administration of calcium solutions such as 20% calcium borogluconate or those recommended for the treatment of parturient paresis in cattle is required. These preparations should be administered slowly in conjunction with close monitoring of the cardiovascular response. Dilution at the rate of 250 to 500 mL/500 kg diluted 1:4 with saline or dextrose before infusion decreases the chance of cardiotoxicity. Normally there is a positive inotropic effect in response to calcium administration; however, alterations in rate or rhythm provide evidence to suspend the infusion. If no response to an initial infusion occurs, a second dose may be given 15 to 30 minutes later. Most cases respond to this form of therapy, although in some cases in which signs persist, repeated treatments may be necessary.

Synchronous Diaphragmatic Flutter

Pathogenesis. Synchronous diaphragmatic flutter (SDF), or "thumps," arises when fluid and electrolyte imbalances disrupt the membrane potential of the phrenic nerve. The phrenic nerve begins to discharge in concert with atrial depolarization with subsequent contraction of the diaphragm.[198] The most consistent metabolic derangement reported in horses with SDF is low serum ionized calcium concentrations usually associated with hypochloremic metabolic alkalosis.[198,199] Metabolic alkalosis may alter the ratio of free to bound calcium (increasing calcium binding to protein and decreasing ionized calcium), which possibly induces SDF.

Risk Factors. Inciting causes include hypocalcemia arising from lactation, transport, prolonged endurance exercise, primary hypoparathyroidism, digestive disturbances, administration of furosemide, and possibly other medications.[198-200] Ingestion of blister beetles (cantharidin toxicosis) can also cause hypocalcemia and SDF.[146]

Clinical Signs. A thumping or twitch in the flank region (unilateral or bilateral) is seen when the diaphragm contracts synchronously with the heart. In severe cases this twitch may produce an audible thumping sound. Signs of dehydration and volume depletion can be present depending on the etiology of the calcium imbalance. SDF is a component of the exhausted horse syndrome in endurance horses and often accompanies signs of dehydration, inappropriate sweating responses, persistently elevated body temperature, depression, anorexia, and aperistalsis.[25] In some horses SDF may be a chronic, recurring problem, particularly if they have received prolonged administration of high-calcium supplements.[199]

Treatment. In most cases SDF is a transient event, usually abating when the underlying cause resolves, either spontaneously or in response to treatment.[199] Most horses undergo rapid remission of signs when given calcium solutions intravenously as described earlier for treatment of hypocalcemic tetany. Although hypomagnesemia is often present with SDF, horses do not respond to magnesium supplementation unless calcium is administered concurrently. Response to therapy is also reflected by improved mental status, return of appetite, and gut motility.[198]

Control. Electrolyte supplementation and some dietary manipulations may help reduce the incidence of SDF in some endurance horses suffering recurrent bouts. Provision of chloride, potassium, and sodium during prolonged exercise may help reduce fluid losses and the metabolic alkalosis that commonly accompanies this form of exercise and frequently occurs in association with SDF. Metabolic alkalosis decreases the amount of free calcium available. Supplementation of calcium and magnesium during endurance rides has been suggested to be helpful in horses prone to SDF.

Alternative approaches involve reduction of dietary calcium in horses prone to SDF for a few days before an endurance ride. It is postulated that this reduction in dietary calcium stimulates the endocrine homeostatic mechanisms and increases osteoclastic activity. In the short term the horse depends less on dietary calcium and is able to mobilize substantial amounts of calcium in response to the demands imposed by exercise; calcium losses in sweat are overcome by the release of calcium from endogenous storage pools (bone).[199] Horses routinely fed alfalfa hay, which has a relatively high calcium concentration, may be more prone to development of SDF. Limitation of this feedstuff may be indicated in chronically affected horses.

CONFLICT OF INTEREST STATEMENT

Dr. Valberg is co-owner of the license for PSSM testing and receives sales income from its use. Her financial and business interests are managed by Michigan State University in accordance with its conflict of interest policies. Dr. Valberg receives royalties from Kentucky Equine Research for the sale of Re-Leve.

REFERENCES

1. Firshman AM, Borgia LA, Valberg SJ. Effects of training at a walk on conventional and underwater treadmills on fiber properties and metabolic responses of superficial digital flexor and gluteal muscles to high-speed exercise in horses. *Am J Vet Res*. 2015;76:1058–1065.
2. Cheng AJ, Andersson DC, Lanner JT. Can't live with or without it: calcium and its role in Duchenne muscular dystrophy-induced muscle weakness. Focus on SERCA1 overexpression minimizes skeletal muscle damage in dystrophic mouse models. *Am J Physiol Cell Physiol*. 2015;308:C697–C698.
3. Cardinet GH, Littrell JF, Freedland RA. Comparative investigations of serum creatine phosphokinase and glutamic-oxaloacetic transaminase activities in equine paralytic myoglobinuria. *Res Vet Sci*. 1967;8:219–226.
4. Wilberger MS, McKenzie EC, Payton ME, et al. Prevalence of exertional rhabdomyolysis in endurance horses in the Pacific Northwestern United States. *Equine Vet J*. 2015;47:165–170.

5. Valberg SJ, Mickelson JR, Gallant EM, et al. Exertional rhabdomyolysis in quarter horses and thoroughbreds: one syndrome, multiple aetiologies. *Equine Vet J Suppl*. 1999;30:533–538.
6. Valberg S, Haggendal J, Lindholm A. Blood chemistry and skeletal muscle metabolic responses to exercise in horses with recurrent exertional rhabdomyolysis. *Equine Vet J*. 1993;25:17–22.
7. Valberg SJ, MacLeay JM, Billstrom JA, et al. Skeletal muscle metabolic response to exercise in horses with "tying-up" due to polysaccharide storage myopathy. *Equine Vet J*. 1999;31:43–47.
8. Perkins G, Valberg SJ, Madigan JM, et al. Electrolyte disturbances in foals with severe rhabdomyolysis. *J Vet Intern Med*. 1998;12:173–177.
9. Westermann CM, Dorland L, Votion DM, et al. Acquired multiple Acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy. *Neuromuscul Disord*. 2008;18:355–364.
10. McCue ME, Anderson SM, Valberg SJ, et al. Estimated prevalence of the type 1 polysaccharide storage myopathy mutation in selected North American and European breeds. *Anim Genet*. 2010;41(Suppl 2):145–149.
11. Lewis SS, Valberg SJ, Nielsen IL. Suspected immune-mediated myositis in horses. *J Vet Intern Med*. 2007;21:495–503.
12. Valentine BA, de Lahunta A, George C, et al. Acquired equine motor neuron disease. *Vet Pathol*. 1994;31:130–138.
13. Bedford HE, Valberg SJ, Firshman AM, et al. Histopathologic findings in the sacrocaudalis dorsalis medialis muscle of horses with vitamin E–responsive muscle atrophy and weakness. *J Am Vet Med Assoc*. 2013;242:1127–1137.
14. Finno CJ, Valberg SJ, Wunschmann A, et al. Seasonal pasture myopathy in horses in the midwestern United States: 14 cases (1998-2005). *J Am Vet Med Assoc*. 2006;229:1134–1141.
15. Cardinet 3rd GH, Holliday TA. Neuromuscular diseases of domestic animals: a summary of muscle biopsies from 159 cases. *Ann N Y Acad Sci*. 1979;317:290–313.
16. Wijnberg ID, Franssen H. The potential and limitations of quantitative electromyography in equine medicine. *Vet J*. 2016;209: 23–31.
17. Robinson JA, Naylor JM, Crichlow EC. Use of electromyography for the diagnosis of equine hyperkalemic periodic paresis. *Can J Vet Res*. 1990;54:495–500.
18. Valberg SJ, Dyson S. Skeletal muscle and lameness. In: Ross M, Dyson S, eds. *Lameness in the horse*. Philadelphia: Saunders; 2003:723–743.
19. Morris E, Seeherman HJ, O'Callaghan MW, et al. Scintigraphic identification of skeletal muscle damage in horses 24 hours after strenuous exercise. *Equine Vet J*. 1991;23:347–352.
20. Cole FL, Mellor DJ, Hodgson DR, et al. Prevalence and demographic characteristics of exertional rhabdomyolysis in horses in Australia. *Vet Rec*. 2004;155:625–630.
21. Steckel RR. The role of scintigraphy in the lameness evaluation. *Vet Clin North Am Equine Pract*. 1991;7:207–239.
22. McGowan CM, Posner RE, Christley RM. Incidence of exertional rhabdomyolysis in polo horses in the USA and the United Kingdom in the 1999/2000 season. *Vet Rec*. 2002;150:535–537.
23. Grobler LA, Collins M, Lambert MI, et al. Skeletal muscle pathology in endurance athletes with acquired training intolerance. *Br J Sports Med*. 2004;38:697–703.
24. Carlson GP: Response to oral and intravenous fluid. In Proceedings of the Fifth Annual Scientific Meeting of the Association for Equine Sports Medicine, 1985, pp 82–90.
25. Foreman JH. The exhausted horse syndrome. *Vet Clin North Am Equine Pract*. 1998;14:205–219.
26. Valentine BA, Van Saun RJ, Thompson KN, et al. Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy. *J Am Vet Med Assoc*. 2001;219:1537–1544.
27. Valentine BA, Hintz HF, Freels KM, et al. Dietary control of exertional rhabdomyolysis in horses. *J Am Vet Med Assoc*. 1998;212:1588–1593.
28. Harris P, Colles C. The use of creatinine clearance ratios in the prevention of equine rhabdomyolysis: a report of four cases. *Equine Vet J*. 1988;20:459–463.
29. McKenzie EC, Valberg SJ, Godden SM, et al. Comparison of volumetric urine collection versus single-sample urine collection in horses consuming diets varying in cation-anion balance. *Am J Vet Res*. 2003;64:284–291.
30. Roneus B, Hakkarainen J. Vitamin E in serum and skeletal muscle tissue and blood glutathione peroxidase activity from horses with the azoturia-tying-up syndrome. *Acta Vet Scand*. 1985;26:425–427.
31. Beech J. Treating and preventing chronic intermittent rhabdomyolysis. *Vet Med May*. 1994:458–461.
32. Beech J, Lindborg S, Fletcher JE, et al. Caffeine contractures, twitch characteristics and the threshold for Ca^{2+}-induced Ca^{2+} release in skeletal muscle from horses with chronic intermittent rhabdomyolysis. *Res Vet Sci*. 1993;54:110–117.
33. Beech J. Chronic exertional rhabdomyolysis. *Vet Clin North Am Equine Pract*. 1997;13:145–168.
34. Lentz LR, Valberg SJ, Balog EM, et al. Abnormal regulation of muscle contraction in horses with recurrent exertional rhabdomyolysis. *Am J Vet Res*. 1999;60:992–999.
35. MacLeay JM, Sorum SA, Valberg SJ, et al. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in Thoroughbreds. *Am J Vet Res*. 1999;60:1562–1566.
36. Valberg S, Jonsson L, Lindholm A, et al. Muscle histopathology and plasma aspartate aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent exertional rhabdomyolysis. *Equine Vet J*. 1993;25:11–16.
37. Lindholm A, Johansson HE, Kjaersgaard P. Acute rhabdomyolysis ("tying-up") in standardbred horses. A morphological and biochemical study. *Acta Vet Scand*. 1974;15:325–339.
38. Hildebrand SV, Arpin D, Cardinet III G. Contracture test and histologic and histochemical analyses of muscle biopsy specimens from horses with exertional rhabdomyolysis. *J Am Vet Med Assoc*. 1990;196:1077–1083.
39. Hunt LM, Valberg SJ, Steffenhagen K, et al. An epidemiological study of myopathies in Warmblood horses. *Equine Vet J*. 2008;40:171–177.
40. McGowan CM, Fordham T, Christley RM. Incidence and risk factors for exertional rhabdomyolysis in thoroughbred racehorses in the United Kingdom. *Vet Rec*. 2002;151:623–626.
41. MacLeay JM, Valberg SJ, Pagan JD, et al. Effect of ration and exercise on plasma creatine kinase activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis. *Am J Vet Res*. 2000;61:1390–1395.
42. McKenzie EC, Valberg SJ, Godden SM, et al. Effect of dietary starch, fat, and bicarbonate content on exercise responses and serum creatine kinase activity in equine recurrent exertional rhabdomyolysis. *J Vet Intern Med*. 2003;17:693–701.
43. Upjohn MM, Archer RM, Christley RM, et al. Incidence and risk factors associated with exertional rhabdomyolysis syndrome in National Hunt racehorses in Great Britain. *Vet Rec*. 2005;156:763–766.
44. MacLeay JM, Valberg SJ, Sorum SA, et al. Heritability of recurrent exertional rhabdomyolysis in Thoroughbred racehorses. *Am J Vet Res*. 1999;60:250–256.
45. Valberg SJ. Metabolic response to racing and fiber properties of skeletal muscle in Standardbred and Thoroughbred horses. *J Equine Vet Sci*. 1987;7:7.
46. Valberg S, Gustavsson BE, Lindholm A, et al. Blood chemistry and skeletal muscle metabolic responses during and after different speeds and durations of trotting. *Equine Vet J*. 1989;21: 91–95.
47. Lentz LR, Valberg SJ, Herold LV, et al. Myoplasmic calcium regulation in myotubes from horses with recurrent exertional rhabdomyolysis. *Am J Vet Res*. 2002;63:1724–1731.
48. Lopez JR, Linares N, Cordovez G, et al. Elevated myoplasmic calcium in exercise-induced equine rhabdomyolysis. *Pflugers Arch*. 1995;430:293–295.
49. Waldron-Mease E, Klein LV, Rosenberg H, et al. Malignant hyperthermia in a halothane-anesthetized horse. *J Am Vet Med Assoc*. 1981;179:896–898.

50. Lentz LR, Valberg SJ, Mickelson JR, et al. In vitro contractile responses and contracture testing of skeletal muscle from Quarter Horses with exertional rhabdomyolysis. *Am J Vet Res*. 1999;60:684–688.
51. Ward TL, Valberg SJ, Gallant EM, et al. Calcium regulation by skeletal muscle membranes of horses with recurrent exertional rhabdomyolysis. *Am J Vet Res*. 2000;61:242–247.
52. Isgren CM, Upjohn MM, Fernandez-Fuente M, et al. Epidemiology of exertional rhabdomyolysis susceptibility in Standardbred horses reveals associated risk factors and underlying enhanced performance. *PLoS One*. 2010;5:e11594.
53. Fraunfelder HC, Rossdale PD, Rickets SW. Changes in serum muscle enzyme levels in associated with training schedules and stages of oestrus cycle in Thoroughbred racehorses. *Equine Vet J*. 1986;18:371–374.
54. Dranchak PK, Valberg SJ, Onan GW, et al. Inheritance of recurrent exertional rhabdomyolysis in Thoroughbreds. *J Am Vet Med Assoc*. 2005;227:762–767.
55. MacLeay JM, Valberg SJ, Sorum SA, et al. Heritability of recurrent exertional rhabdomyolysis in Thoroughbred racehorses. *Am J Vet Res*. 1999;60:250–256.
56. Harris PA, Snow DH, Greet TR, et al. Some factors influencing plasma AST/CK activities in Thoroughbred racehorses. *Equine Vet J Suppl*. 1990:66–71.
57. Quiroz-Rothe E, Novales M, Guilera-Tejero E, et al. Polysaccharide storage myopathy in the m. longissimus lumborum of showjumpers and dressage horses with back pain. *Equine Vet J*. 2002;34:171–176.
58. Finno CJ, McKenzie E, Valberg SJ, et al. Effect of fitness on glucose, insulin and cortisol responses to diets varying in starch and fat content in Thoroughbred horses with recurrent exertional rhabdomyolysis. *Equine Vet J Suppl*. 2010:323–328.
59. McKenzie EC, Valberg SJ, Godden SM, et al. Effect of oral administration of dantrolene sodium on serum creatine kinase activity after exercise in horses with recurrent exertional rhabdomyolysis. *Am J Vet Res*. 2004;65:74–79.
60. Freestone JF, Wolfsheimer KJ, Kamerling SG, et al. Exercise induced hormonal and metabolic changes in Thoroughbred horses: effects of conditioning and acepromazine. *Equine Vet J*. 1991;23:219–223.
61. Edwards JG, Newtont JR, Ramzan PH, et al. The efficacy of dantrolene sodium in controlling exertional rhabdomyolysis in the Thoroughbred racehorse. *Equine Vet J*. 2003;35:707–711.
62. Beech J, Fletcher JE, Lizzo F, et al. Effect of phenytoin on the clinical signs and in vitro muscle twitch characteristics in horses with chronic intermittent rhabdomyolysis and myotonia. *Am J Vet Res*. 1988;49:2130–2133.
63. Beech J. Treating and preventing chronic intermittent rhabdomyolysis. *Vet Med*. 1994;*May*:458–461. 41.
64. Vischer CM, Foreman JH, Constable PD, et al. Hemodynamic effects of thyroidectomy in sedentary horses. *Am J Vet Res*. 1999;60:14–21.
65. Waldron-Mease E. Hypothyroidism and myopathy in racing Thoroughbreds and Standardbreds. *J Equine Vet Sci*. 1979;3:124–128.
66. Tiidus PM. Manual massage and recovery of muscle function following exercise: a literature review. *J Orthop Sports Phys Ther*. 1997;25:107–112.
67. Aleman M, Riehl J, Aldridge BM, et al. Association of a mutation in the ryanodine receptor 1 gene with equine malignant hyperthermia. *Muscle Nerve*. 2004;30:356–365.
68. Aleman M, Nieto JE, Magdesian KG. Malignant hyperthermia associated with ryanodine receptor 1 (C7360G) mutation in Quarter Horses. *J Vet Intern Med*. 2009;23:329–334.
69. Mickelson JR, Louis CF. Malignant hyperthermia: excitation-contraction coupling, Ca^{2+} release channel, and cell Ca^{2+} regulation defects. *Physiol Rev*. 1996;76:537–592.
70. Aleman M, Brosnan RJ, Williams DC, et al. Malignant hyperthermia in a horse anesthetized with halothane. *J Vet Intern Med*. 2005;19:363–366.
71. McCue ME, Valberg SJ, Jackson M, et al. Polysaccharide storage myopathy phenotype in Quarter Horse–related breeds is modified by the presence of an RYR1 mutation. *Neuromuscul Disord*. 2009;19:37–43.
72. McKenzie EC, Di Concetto S, Payton M, et al. Effect of dantrolene premedication on various cardiovascular and biochemical variables and the recovery in healthy isoflurane-anesthetized horses. *Am J Vet Res*. 2015;76:293–301.
73. Valberg SJ, Cardinet III GH, Carlson GP, et al. Polysaccharide storage myopathy associated with recurrent exertional rhabdomyolysis in horses. *Neuromuscul Disord*. 1992;2:351–359.
74. Valentine BA, Credille KM, Lavoie JP, et al. Severe polysaccharide storage myopathy in Belgian and Percheron draught horses. *Equine Vet J*. 1997;29:220–225.
75. Valentine BA, McDonough SP, Chang YF, et al. Polysaccharide storage myopathy in Morgan, Arabian, and Standardbred related horses and Welsh-cross ponies. *Vet Pathol*. 2000;37:193–196.
76. Valentine BA, Cooper BJ. Incidence of polysaccharide storage myopathy: necropsy study of 225 horses. *Vet Pathol*. 2005;42:823–827.
77. McCue ME, Valberg SJ, Miller MB, et al. Glycogen synthase (*GYS1*) mutation causes a novel skeletal muscle glycogenosis. *Genomics*. 2008;91:458–466.
78. McCue ME, Armien AG, Lucio M, et al. Comparative skeletal muscle histopathologic and ultrastructural features in two forms of polysaccharide storage myopathy in horses. *Vet Pathol*. 2009;46:1281–1291.
79. Baird JD, Valberg SJ, Anderson SM, et al. Presence of the glycogen synthase 1 (*GYS1*) mutation causing type 1 polysaccharide storage myopathy in continental European draught horse breeds. *Vet Rec*. 2010;167:781–784.
80. Stanley RL, McCue ME, Valberg SJ, et al. A glycogen synthase 1 mutation associated with equine polysaccharide storage myopathy and exertional rhabdomyolysis occurs in a variety of UK breeds. *Equine Vet J*. 2009;41:597–601.
81. McGowan CM, Menzies-Gow NJ, McDiarmid AM, et al. Four cases of equine polysaccharide storage myopathy in the United Kingdom. *Vet Rec*. 2003;152:109–112.
82. McGowan CM, McGowan TW, Patterson-Kane JC. Prevalence of equine polysaccharide storage myopathy and other myopathies in two equine populations in the United Kingdom. *Vet J*. 2009;180:330–336.
83. Tryon RC, Penedo MC, McCue ME, et al. Evaluation of allele frequencies of inherited disease genes in subgroups of American Quarter Horses. *J Am Vet Med Assoc*. 2009;234:120–125.
84. McCue ME, Ribeiro WP, Valberg SJ. Prevalence of polysaccharide storage myopathy in horses with neuromuscular disorders. *Equine Vet J Suppl*. 2006:340–344.
85. Johlig L, Valberg SJ, Mickelson JR, et al. Epidemiological and genetic study of exertional rhabdomyolysis in a Warmblood horse family in Switzerland. *Equine Vet J*. 2011;43:240–245.
86. Firshman AM, Valberg SJ, Bender JB, et al. Epidemiologic characteristics and management of polysaccharide storage myopathy in Quarter Horses. *Am J Vet Res*. 2003;64:1319–1327.
87. Firshman AM, Baird JD, Valberg SJ. Prevalences and clinical signs of polysaccharide storage myopathy and shivers in Belgian draft horses. *J Am Vet Med Assoc*. 2005;227:1958–1964.
88. Ribeiro WP, Valberg SJ, Pagan JD, et al. The effect of varying dietary starch and fat content on serum creatine kinase activity and substrate availability in equine polysaccharide storage myopathy. *J Vet Intern Med*. 2004;18:887–894.
89. Byrne E, Jones SL, Valberg SJ, et al. Rhabdomyolysis in two foals with polysaccharide storage myopathy and concurrent pneumonia. *Comp Cont Educ Pract Vet*. 2000;22:503–507.
90. De La Corte FD, Valberg SJ, MacLeay JM, et al. Developmental onset of polysaccharide storage myopathy in 4 Quarter Horse foals. *J Vet Intern Med*. 2002;16:581–587.
91. Sprayberry KA, Madigan J, Lecouteur RA, et al. Renal failure, laminitis, and colitis following severe rhabdomyolysis in a draft horse-cross with polysaccharide storage myopathy. *Can Vet J*. 1998;39:500–503.

92. Valberg SJ, MacLeay JM, Mickelson JR. Polysaccharide storage myopathy associated with exertional rhabdomyolysis in horses. *Comp Cont Educ Pract Vet*. 1997;19(9):1077–1086.
93. Valberg SJ. Milne Lecture: Muscling in on the cause of tying-up. *Proceedings American Assoc Equine Pract*. 2012;58:85–123.
94. Rosie J. Naylor, RJ, Livesey L, Schumacher J, et al., Allele copy number and underlying pathology are associated with subclinical severity in equine type 1 polysaccharide storage myopathy (PSSM1) PLoS One. 2012; 7(7): e42317.
95. Bloom BA, Valentine BA, Gleed RD, et al. Postanaesthetic recumbency in a Belgian filly with polysaccharide storage myopathy. *Vet Rec*. 1999;144:73–75.
96. Naylor RJ, Luis-Fuentes V, Livesey L, et al. Evaluation of cardiac phenotype in horses with type 1 polysaccharide storage myopathy. *J Vet Intern Med*. 2012;26:1464–1469.
97. Raben N, Danon M, Lu N, et al. Surprises of genetic engineering: a possible model of polyglucosan body disease. *Neurology*. 2001;56:1739–1745.
98. Borgia LA, Valberg SJ, McCue ME, et al. Effect of dietary fats with odd or even numbers of carbon atoms on metabolic response and muscle damage with exercise in Quarter Horse–type horses with type 1 polysaccharide storage myopathy. *Am J Vet Res*. 2010;71:326–336.
99. Annandale EJ, Valberg SJ, Essen Gustavsson B. The effect of submaximal exercise on adenine nucleotide concentrations in skeletal muscle fibers of horses with polysaccharide storage. *Myopathy Am J Vet Res*. 2005;66:839–845.
100. Hardie DG, Schaffer BE, Brunet A. AMPK: an energy-sensing pathway with multiple inputs and outputs. *Trends Cell Biol*. 2016;26:190–201.
101. Hardie DG. AMP-activated protein kinase: a key system mediating metabolic responses to exercise. *Med Sci Sports Exerc*. 2004;36:28–34.
102. Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. *J Physiol*. 2005;565:537–546.
103. Valberg SJ, McCue ME, Mickelson JR. The interplay of genetics, exercise and nutrition in polysaccharide storage myopathy. *Equine Vet Sci*. 2011;31:5.
104. Aleman M, Lecouteur RA, Nieto JE, et al. Sarcoplasmic masses in equine skeletal muscle. *Neuromuscul Disord*. 2005;15:147–153.
105. Firshman AM, Valberg SJ, Bender JB, et al. Comparison of histopathologic criteria and skeletal muscle fixation techniques for the diagnosis of polysaccharide storage myopathy in horses. *Vet Pathol*. 2006;43:257–269.
106. Borgia L, Valberg S, McCue M, et al. Glycaemic and insulinaemic responses to feeding hay with different non-structural carbohydrate content in control and polysaccharide storage myopathy-affected horses. *J Anim Physiol Anim Nutr (Berl)*. 2011;95:798–807.
107. Valberg SJ, McKenzie EC, Eyrich LV, et al. Suspected myofibrillar myopathy in Arabian horses with a history of exertional rhabdomyolysis. *Equine Vet J*. 2016;48(5):548–56.
108. Valberg SJ, Carlson GP, Cardinet III GH, et al. Skeletal muscle mitochondrial myopathy as a cause of exercise intolerance in a horse. *Muscle Nerve*. 1994;17:305–312.
109. Ward TL, Valberg SJ, Adelson DL, et al. Glycogen branching enzyme (GBE1) mutation causing equine glycogen storage disease IV. *Mamm Genome*. 2004;15:570–577.
110. Wagner ML, Valberg SJ, Ames EG, et al. Allele frequency and likely impact of the glycogen branching enzyme deficiency gene in Quarter Horse and Paint Horse populations. *J Vet Intern Med*. 2006;20:1207–1211.
111. Valberg SJ, Ward TL, Rush B, et al. Glycogen branching enzyme deficiency in Quarter Horse foals. *J Vet Intern Med*. 2001;15:572–580.
112. Dill SG, Rebhun WC. White muscle disease in foals. *Comp Cont Educ Pract Vet*. 1985;7:S627–S636.
113. Maylin GA, Rubin DS, Lein DH. Selenium and vitamin E in horses. *Cornell Vet*. 1980;70:272–289.
114. Roneus B, Jonsson L. Muscular dystrophy in foals. *Zentralbl Veterinarmed A*. 1984;31:441–453.
115. Roneus B, Lindholm A. Glutathione peroxidase activity in the blood of healthy horses given different selenium supplementation. *Nord Vet Med*. 1983;35:337–345.
116. Roneus B. Glutathione peroxidase and selenium in the blood of healthy horses and foals affected by muscular dystrophy. *Nord Vet Med*. 1982;34:350–353.
117. Koller LD, Whitbeck GA, South PJ. Transplacental transfer and colostral concentrations of selenium in beef cattle. *Am J Vet Res*. 1984;45:2507–2510.
118. Maas J, Peauroi JR, Tonjes T, et al. Intramuscular selenium administration in selenium-deficient cattle. *J Vet Intern Med*. 1993;7:342–348.
119. Aharonson-Raz K, Milgram J, Chai O, et al. Fibrosis of the masseter leading to trismus and dysphagia in a mare. *Vet Rec*. 2009;164:597–598.
120. Pearson EG, Snyder SP, Saulez MN. Masseter myodegeneration as a cause of trismus or dysphagia in adult horses. *Vet Rec*. 2005;156:642–646.
121. Step DL, Divers TJ, Cooper B, et al. Severe masseter myonecrosis in a horse. *J Am Vet Med Assoc*. 1991;198:117–119.
122. Divers TJ, Mohammed HO, Cummings JF, et al. Equine motor neuron disease: findings in 28 horses and proposal of a pathophysiological mechanism for the disease. *Equine Vet J*. 1994;26:409–415.
123. Harris PA. An outbreak of the equine rhabdomyolysis syndrome in a racing yard. *Vet Rec*. 1990;127:468–470.
124. Freestone JF, Carlson GR. Muscle disorders in the horse: a retrospective study. *Equine Vet J*. 1991;23:86–90.
125. Traub-Dargatz JL, Schlipf Jr JW, Granstrom DE, et al. Multifocal myositis associated with *Sarcocystis* sp in a horse. *J Am Vet Med Assoc*. 1994;205:1574–1576.
126. Fayer R, Dubey JP. Development of *Sarcocystis fayeri* in the equine. *J Parasitol*. 1982;68:856–860.
127. Vengust M, Arroyo LG, Weese JS, et al. Preliminary evidence for dormant clostridial spores in equine skeletal muscle. *Equine Vet J*. 2003;35:514–516.
128. Rebhun WC, Shin SJ, King JM, et al. Malignant edema in horses. *J Am Vet Med Assoc*. 1985;187:732–736.
129. Peek SF, Semrad SD, Perkins GA. Clostridial myonecrosis in horses (37 cases 1985–2000). *Equine Vet J*. 2003;35:86–92.
130. Hilton H, Madigan JE, Aleman M. Rhabdomyolysis associated with *Anaplasma phagocytophilum* infection in a horse. *J Vet Intern Med*. 2008;22:1061–1064.
131. Sponseller BT, Valberg SJ, Tennent-Brown BS, et al. Severe acute rhabdomyolysis associated with *Streptococcus equi* infection in four horses. *J Am Vet Med Assoc*. 2005;227:1800–1804.
132. Valberg SJ, Bullock P, Hogetvedt W, et al. Myopathies associated with *Streptococcus equi* infections in horses. *Proceedings Am Assoc Equine Pract*. 1996;42:292–293.
133. Sweeney CR, Timoney JF, Newton JR, et al. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med*. 2005;19:123–134.
134. Al-Ghamdi GM, Kapur V, Ames TR, et al. Use of repetitive sequence-based polymerase chain reaction for molecular epidemiologic analysis of *Streptococcus equi* subspecies *equi*. *Am J Vet Res*. 2000;61:699–705.
135. Adams EM, Gudmundsson S, Yocum DE, et al. Streptococcal myositis. *Arch Intern Med*. 1985;145:1020–1023.
136. Kaese HJ, Valberg SJ, Hayden DW, et al. Infarctive purpura hemorrhagica in five horses. *J Am Vet Med Assoc*. 2005;226(11):1893–8, 1845.
137. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118–121.

138. Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. *J Immunol*. 1985;135:3134–3137.
139. Kaese HJ, Valberg SJ, Hayden DW, et al: Infarctive purpura hemorrhagica in five horses. *J Am Vet Med Assoc*. 2005;226:1845, 1893–1898.
140. Tan JY, Valberg SJ, Sebastian MM, et al. Suspected systemic calcinosis and calciphylaxis in 5 horses. *Can Vet J*. 2010;51:993–999.
141. Fales-Williams A, Sponseller B, Flaherty H. Idiopathic arterial medial calcification of the thoracic arteries in an adult horse. *J Vet Diagn Invest*. 2008;20:692–697.
142. Carter T, Ratnam S. Calciphylaxis: a devastating complication of derangements of calcium-phosphorus metabolism—a case report and review of the literature. *Nephrol Nurs J*. 2013;40:431–435; quiz 436.
143. Llach F. Hyperphosphatemia in end-stage renal disease patients: pathophysiological consequences. *Kidney Int Suppl*. 1999;73:S31–37.
144. Barrott MJB, H.W, McGowan C.M. Suspected immune-mediated myositis in a pony. *Equine Vet Educ*. 2004;16:4.
145. Myers CJ, Aleman M, Heidmann R, et al. Myopathy in American miniature horses. *Equine Vet J*. 2006;38:272–276.
146. Helman RG, Edwards WC. Clinical features of blister beetle poisoning in equids: 70 cases (1983–1996). *J Am Vet Med Assoc*. 1997;211:1018–1021.
147. Putnam MR, Boosinger T, Spano J, et al. Evaluation of *Cassia obtusifolia* (sicklepod) seed consumption in Holstein calves. *Vet Hum Toxicol*. 1988;30:316–318.
148. Beier RC, Norman JO, Irvin TR, et al. Microsomal activation of constituents of white snakeroot (*Eupatorium rugosum* Houtt) to form toxic products. *Am J Vet Res*. 1987;48:583–585.
149. Beier RC, Norman JO. The toxic factor in white snakeroot: identity, analysis and prevention. *Vet Hum Toxicol*. 1990;32(suppl):81–88.
150. Thompson LJ. Depression and choke in a horse: probable white snakeroot toxicosis. *Vet Hum Toxicol*. 1989;31:321–322.
151. Valberg SJ, Sponseller BT, Hegeman AD, et al. Seasonal pasture myopathy/atypical myopathy in North America associated with ingestion of hypoglycin A within seeds of the box elder tree. *Equine Vet J*. 2013;45:419–426.
152. Unger L, Nicholson A, Jewitt EM, et al. Hypoglycin A concentrations in seeds of *Acer pseudoplatanus* trees growing on atypical myopathy-affected and control pastures. *J Vet Intern Med*. 2014;28:1289–1293.
153. Sponseller BT, Valberg SJ, Schultz NE, et al. Equine multiple acyl-CoA dehydrogenase deficiency (MADD) associated with seasonal pasture myopathy in the midwestern United States. *J Vet Intern Med*. 2012;26:1012–1018.
154. Votion D-M, van Galen G, Sweetman L, et al. Identification of methylenecyclopropyl acetic acid in serum of European horses with atypical myopathy. *Equine Vet J*. 2014;46:146–149.
155. Whitwell KE, Harris P, Farrington PG. Atypical myoglobinuria: an acute myopathy in grazing horses. *Equine Vet J*. 1988;20:357–363.
156. Cassart D, Baise E, Cherel Y, et al. Morphological alterations in oxidative muscles and mitochondrial structure associated with equine atypical myopathy. *Equine Vet J*. 2007;39:26–32.
157. Gerber V, Straub R, Frey J. Equine botulism and acute pasture myodystrophy: new soil-borne emerging diseases in Switzerland? *Schweiz Arch Tierheilkd*. 2006;148:553–559.
158. van Galen G, Marcillaud PC, Saegerman C, et al. European outbreaks of atypical myopathy in grazing equids (2006–2009): spatiotemporal distribution, history and clinical features. *Equine Vet J*. 2012;44:614–620.
159. van Galen G, Saegerman C, Marcillaud PC, et al. European outbreaks of atypical myopathy in grazing horses (2006–2009): determination of indicators for risk and prognostic factors. *Equine Vet J*. 2012;44:621–625.
160. Gomez DE, Valberg SJ, Magdesian KG, et al. Acquired multiple acyl-CoA dehydrogenase deficiency and marked selenium deficiency causing severe rhabdomyolysis in a horse. *Can Vet J*. 2015;56:1166–1171.
161. van Galen G, Cerri S, Porter S, et al. Traditional and quantitative assessment of acid-base and shock variables in horses with atypical myopathy. *J Vet Intern Med*. 2013;27(1):186–93.
162. Grandy JL, Steffey EP, Hodgson DS, et al. Arterial hypotension and the development of postanesthetic myopathy in halothane-anesthetized horses. *Am J Vet Res*. 1987;48:192–197.
163. Duke T, Filzek U, Read MR, et al. Clinical observations surrounding an increased incidence of postanesthetic myopathy in halothane-anesthetized horses. *Vet Anaesth Analg*. 2006;33:122–127.
164. White NAI. Postanesthetic recumbency myopathy in horses. *Compend Cont Educ*. 1982;4:544–550.
165. Hennig GE, Court MH. Equine postanesthetic myopathy: an update. *Compend Cont Educ*. 1991;13:1709–1715.
166. Norman WM, Williams R, Dodman NH, et al. Postanesthetic compartmental syndrome in a horse. *J Am Vet Med Assoc*. 1989;195:502–504.
167. Klein L, Ailes N, Fackelman GE, et al. Postanesthetic equine myopathy suggestive of malignant hyperthermia. A case report. *Vet Surg*. 1989;18:479–482.
168. Valverde A, Boyd CJ, Dyson DH, et al. Prophylactic use of dantrolene associated with prolonged postanesthetic recumbency in a horse. *J Am Vet Med Assoc*. 1990;197:1051–1053.
169. Hildebrand SV, Howitt GA. Succinylcholine infusion associated with hyperthermia in ponies anesthetized with halothane. *Am J Vet Res*. 1983;44:2280–2284.
170. Manley SV, Kelly AB, Hodgson D. Malignant hyperthermia-like reactions in three anesthetized horses. *J Am Vet Med Assoc*. 1983;183:85–89.
171. Lehmann-Horn F, Jurkat-Rott K, Rudel R. Periodic paralysis: understanding channelopathies. *Curr Neurol Neurosci Rep*. 2002;2:61–69.
172. Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. *Physiol Rev*. 1999;79:1317–1372.
173. Bryant SH, Lipicky RJ, Herzog WH. Variability of myotonic signs in myotonic goats. *Am J Vet Res*. 1968;29:2371–2381.
174. Schooley EK, MacLeay JM, Cuddon P, et al. Myotonia congenita in a foal. *J Equine Vet Science*. 2004;24:483–488.
175. Jamison JM, Baird JD, Smith-Maxie LL, et al. A congenital form of myotonia with dystrophic changes in a Quarter Horse. *Equine Vet J*. 1987;19:353–358.
176. Wijnberg ID, Owczarek-Lipska M, Sacchetto R, et al. A missense mutation in the skeletal muscle chloride channel 1 (CLCN1) as candidate causal mutation for congenital myotonia in a New Forest pony. *Neuromuscul Disord*. 2012;22:361–367.
177. Montagna P, Liguori R, Monari L, et al. Equine muscular dystrophy with myotonia. *Clin Neurophysiol*. 2001;112:294–299.
178. Reed SM, Hegreberg GA, Bayly WM, et al. Progressive myotonia in foals resembling human dystrophia myotonica. *Muscle Nerve*. 1988;11:291–296.
179. Hegreberg GA, Reed SM. Skeletal muscle changes associated with equine myotonic dystrophy. *Acta Neuropathol (Berl)*. 1990;80:426–431.
180. Madigan JE, Valberg SJ, Ragle C, et al. Muscle spasms associated with ear tick *(Otobius megnini)* infestations in five horses. *J Am Vet Med Assoc*. 1995;207:74–76.
181. Meyer TS, Fedde MR, Cox JH, et al. Hyperkalaemic periodic paralysis in horses: a review. *Equine Vet J*. 1999;31:362–367.
182. Naylor JM. Equine hyperkalemic periodic paralysis: review and implications. *Can Vet J*. 1994;35:279–285.
183. Spier SJ, Carlson GP, Holliday TA, et al. Hyperkalemic periodic paralysis in horses. *J Am Vet Med Assoc*. 1990;197:1009–1017.
184. Rudolph JA, Spier SJ, Byrns G, et al. Linkage of hyperkalaemic periodic paralysis in quarter horses to the horse adult skeletal muscle sodium channel gene. *Anim Genet*. 1992;23:241–250.

185. Cox JH. An episodic weakness in four horses associated with intermittent serum hyperkalemia and the similarity of the disease to hyperkalemic periodic paralysis in man. *Proc Am Assoc Equine Practitioners*. 1985;21:383–391.
186. Carr EA, Spier SJ, Kortz GD, et al. Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. *J Am Vet Med Assoc*. 1996;209:798–803.
187. Reynolds AJ. Equine hyperkalemic periodic paralysis (HYPP): overview and management strategies. http://www.admani.com/AllianceEquine/TechBulletins/HYPP.htm. Accessed February 2017.
188. Reynolds JA, Potter GD, Greene LW. Genetic-diet interactions in the hyperkalemic periodic paralysis syndrome in Quarter Horses fed varying amounts of potassium: III. The relationship between plasma potassium concentration and HYPP symptoms. *J Equine Vet Sci*. 1998;18:731–735.
189. Naylor JM. Hyperkalemic periodic paralysis. *Vet Clin North Am Equine Pract*. 1997;13:129–144.
190. Ott EA. *National Research Council: Nutrient Requirements of Horses, 2007*. Washington, DC: National Academies Press; 1989.
191. Zhou J, Spier SJ, Beech J, et al. Pathophysiology of sodium channelopathies: correlation of normal/mutant mRNA ratios with clinical phenotype in dominantly inherited periodic paralysis. *Hum Mol Genet*. 1994;3:1599–1603.
192. DS (1) Pang, Panizzi L, Paterson JM. Successful treatment of hyperkalaemic periodic paralysis in a horse during isoflurane anaesthesia. *Vet Anaesth Analg*. 2011;38(2):113–120.
193. Sieb JP, Penn AS. Myoglobinuria. In: Engel AG, Franzini-Armstrong C, eds. *Myology*. 3rd ed.Vol. New York: McGraw-Hill; 2004:1677–1692.
194. Layzer RB. Muscle pain, cramps and fatigue. In: Engel AG, Franzini-Armstrong C, eds. *Myology*. 2nd ed. Vol. New York: McGraw-Hill; 1994:1754–1768.
195. Baird JD. Lactation tetany (eclampsia) in a Shetland pony mare. *Aust Vet J*. 1971;47:402–404.
196. Beyer MJ, Freestone JF, Reimer JM, et al. Idiopathic hypocalcemia in foals. *J Vet Intern Med*. 1997;11:356–360.
197. Wijnberg ID, van der Kolk JH, Franssen H, et al. Electromyographic changes of motor unit activity in horses with induced hypocalcemia and hypomagnesemia. *Am J Vet Res*. 2002;63:849–856.
198. Mansmann RA, Carlson GP, White NA, et al. Synchronous diaphragmatic flutter in horses. *J Am Vet Med Assoc*. 1974;165:265–270.
199. Carlson GP. Synchronous diaphragmatic flutter. In: Robinson NE, ed. *Current therapy in equine medicine*. 2nd ed. Philadelphia: WB Saunders; 1987:485–486.
200. Hudson NP, Church DB, Trevena J, et al. Primary hypoparathyroidism in two horses. *Aust Vet J*. 1999;77:504–508.

CHAPTER 11

Disorders of the Neurologic System

Monica Aleman, Yvette S. Nout-Lomas, and Stephen M. Reed*

NEUROLOGIC EXAMINATION

Assessment of the central nervous system (CNS) in horses may seem a difficult task; however, with careful examination using a craniocaudal approach, assessment is not difficult. A craniocaudal approach is the most logical and efficient. The goal of the examination is to determine the neuroanatomic localization of the lesion or lesions and should be completed as part of the physical examination. Subtle neurologic deficits may be hidden by musculoskeletal disease or missed because of lack of knowledge or understanding of these disorders. To accomplish a complete and accurate neurologic examination, the veterinarian must feel comfortable with the format chosen for evaluating the nervous system and must have knowledge of which musculoskeletal disorders commonly are associated with neurologic disease. Problems such as osteochondrosis of the stifle, hock, and shoulder joints often occur concurrently in horses with cervical vertebral stenotic myelopathy.

Examples of typical histories in horses presented for neurologic examination, especially for signs of spinal ataxia, include previous medial patellar desmotomy, bilateral bog spavin in early life, osteochondrosis of the distal tibia or femur, and contracted tendons. All of these problems associated with developmental orthopedic diseases are examples of conditions that may occur simultaneously. Bilateral bog spavin often is associated with osteochondrosis of the distal tibia or other sites in the tibiotarsal joint. Patellar desmotomy to correct upward fixation of the patella may be necessary because of a conformational problem of the stifle joint or as a result of abnormal joint proprioception or quadriceps weakness after neurologic disease. The foregoing problem is more commonly associated with neurologic disease than many veterinarians realize, and the gait deficits caused by these lamenesses often mimic neurologic disease. Horses with bilateral osteochondritis dissecans (OCD) lesions of the stifle joints will often "bunny-hop" while running, which may be interpreted as a sign of neurologic disease rather than as a result of an underlying musculoskeletal disease.

The goals of a neurologic examination are to establish whether a neurologic problem is present and to determine the anatomic localization of the problem. Being able to account for all clinical signs with a single lesion is ideal; however, if this is not possible, one should consider the possibility of multifocal disease or multiple diseases. In addition to anatomic localization of a lesion, it is important to record the findings of the examination as a baseline for comparison of future evaluations. After anatomic localization, one must decide what additional testing is necessary to determine the underlying cause of the clinical signs. Ancillary imaging, cerebrospinal fluid (CSF) analysis, and electrodiagnostic testing may be useful in locating and determining the cause of the lesions.

The neurologic examination should be considered part of the physical examination. The author prefers to begin the examination at the head and proceed caudally to the tail. The examiner should proceed in a consistent fashion and should record findings in an orderly manner to avoid any part of the examination being omitted. Fig. 11.1 shows a sample format. One may use the craniocaudal approach for all animals, whether ambulatory or recumbent.

The equine neurologic examination procedure has been described in detail elsewhere.[1-4] The author follows the format developed by Mayhew,[4] which divides the examination into five categories: (1) the head and mental status, (2) gait and posture, (3) neck and forelimbs, (4) trunk and hindlimbs, and (5) tail and anus. The functional divisions of the nervous system include the sensory, integration, and motor systems.

Before starting the examination, the age, gender, breed, and use of the horse should be determined, although these are not essential. The owner should be questioned about any unusual behavior the horse has exhibited and the date of onset of the behavior. Age is helpful because problems such as cervical vertebral stenotic myelopathy and cerebellar abiotrophy begin at a young age, usually less than 1 year. These problems occur more often in certain breeds. For example, cervical vertebral stenotic myelopathy is most common in Thoroughbreds, and cerebellar abiotrophy most often is observed in Arabians.

Examination

The evaluation of the head should include observation of the horse at rest and during motion; palpation, postural reactions, cranial nerve function, and cervicofacial reflexes; and evaluation of sensation. At the start of the examination the horse should be observed for alertness, mentation, attitude, and behavior (remembering that the animal's environment may influence its degree of excitement). Evaluation of the head

*The editors and authors acknowledge and appreciate the contributions of Frank M. Andrews, Veronique A. Lacombe, Bonnie R. Rush, Barbara A. Byrne, Caroline N. Hahn, Martin Furr, W. David Wilson, Nicola Pusterla, William J. Saville, Joseph Bertone, Maureen T. Long, Cartla S. Sommerdahl, Peter R. Morresey, Ramiro E. Toribio, Robert H. Mealey, and Eduard Jose-Cunillerasas previous contributors to this chapter. Some of their original work has been incorporated into this edition.

includes observation of the behavior and mental status of the horse. This portion of the examination can be completed by careful observation even before handling the horse. A close and careful examination is necessary to evaluate the head and neck posture and coordination and to identify abnormalities of the cranial nerves. Initial consideration includes the environmental awareness of the horse. A normal horse appears bright and alert and responds appropriately to external stimuli. While the horse is being caught, the examiner can look for unusual behaviors such as yawning, abnormal or aimless wandering, seizures, or circling or head tilt and can begin to assess the vision and hearing of the horse. This is also a good time to observe how the horse is standing and whether it has the limbs positioned appropriately under its body. The veterinarian should note any

NEUROLOGIC EXAMINATION

Date: _____
Sire _____
Dam _____
Dam's sire _____

History _____

General observations _____

CRANIAL NERVE EXAMINATION:

Menace (2, 7)		Facial symmetry:	
Pupil size (2, 3, sym.)		Temporal/masseter (5)	
Pupil symmetry (3, sym.)		Expressive muscles (7)	
PLR (2, 3)		Palpebral reflex (5, 7)	
Doll's eye (8, 3, 4, 6)		Retractor oculi (5, 6)	
Ocular position (8, 3, 4, 6)		Gag reflex (9, 10)	
Pathologic nystagmus		Tongue (12)	

Symmetry of neck/body (muscle mass, scoliosis, etc.) _____

Manipulation of the neck L/R _____
up/down _____

Spontaneous involuntary movements (tremor, myoclonus, myotonia, etc.) _____

Description of gait (at walk and trot) _____

Circling	large L		
	R		
	small L		
	R		
Backing			
Up/down an incline			
Elevation of head			
Proprioception	LF	RF	
	LR	RR	
Sway reaction	fore		
	rear		
	tail pull		

Grading System—write in grading according to deLahunta
0 = No gait deficits
1 = Deficits barely perceptible—worsened with head elevation
2 = Deficits noted at a walk
3 = Deficits noted at rest, walking; nearly falls with head elevation
4 = Falls or nearly falls at normal gaits
5 = Recumbent patient

FIG. **11.1** Example of a neurologic examination form. *PLR,* Pupillary light response.

Continued

Gait and posture (graded 0 to +4)

	Motor		Sensory
	Weakness	Spasticity	Ataxia
LF			
RF			
LR			
RR			

Reflexes
Anal
Patellar
Triceps
Other

Nociceptive (withdrawal)

Tail tone

Autonomic
Urinary
Rectal
Sweating

Cutaneous sensation

Assessment/comments

Lesion localization

Tentative DX

CSF

	Cells	Protein	Culture	Cytology
L/S				
A/O				

Radiographs

Myelogram

Comments

FIG. **11.1, cont'd**

behavioral abnormalities, such as head pressing (a clear sign of cerebral disease) and aggressiveness (noted in animals that are not feeling well, as well as animals with CNS disease). If the examiner suspects rabies, precautions should be taken to avoid unnecessary and potentially dangerous exposure.

Lesions of the reticular activating system or the cerebral hemispheres could result in coma or obtundation of consciousness. Horses demonstrating compulsive walking may have a diffuse cerebral disease, whereas a horse walking in large circles may have an asymmetric forebrain lesion. Horses that have a serious systemic illness also may appear depressed or stuporous. The level of consciousness is recorded as *alert, depressed, stuporous,* or *comatose.* An animal that is depressed may react to its environment in an inappropriate or unresponsive fashion. Stuporous horses may appear to be asleep unless stimulated with pain, light, or noise and often demonstrate impaired reactivity. Comatose animals are completely unresponsive and cannot be aroused.

Head posture and coordination are controlled by the cerebellar and vestibular regions of the brain and brainstem in response to sensory input from receptors in the head, neck, limbs, and trunk. Examining the head and neck posture with the horse at rest, while eating, and while moving is helpful. In horses with normal head carriage, the head should be held straight and upright when viewed from the front. Careful examination of the vestibular region is important because many horses develop a head tilt resulting from head trauma, temporohyoid osteoarthropathy (THO), or guttural pouch disease. A head tilt is characterized by the poll deviated about the muzzle and must be distinguished from abnormal or unusual turning of the head or lateral deviation of the head as may occur with damage to the forebrain or injury to the cervical vertebrae. Additional signs that may accompany vestibular disease or injury include nystagmus, ipsilateral weakness, and facial nerve paralysis.

Postural abnormalities of the head and neck sometimes can be difficult to distinguish from head tilt. Horses with torticollis of the head and neck may have a congenital abnormality of the vertebrae or may have injured the muscles of the neck region. Damage to the dorsal gray columns can result in abnormal head and neck posture (acquired scoliosis) that has been described as a result of parasitic migration such as *Paralaphostrongylus tenuis.*[5] Careful examination, including palpation, should help identify fractures of the cervical vertebrae or painful muscles caused by trauma or an injection reaction. Neck pain may manifest as a reluctance to move the head and neck through a full range of motion. In the absence of pain or mechanical limitations to movement, normal horses should be able to turn their head to retrieve feed offered at the lateral thorax on each side, elevated to a level above the withers, or lowered to a point between the front legs just beneath the pectoral muscles. In some horses, radiographs of the cervical vertebrae may be useful to confirm a fracture or osteomyelitis. Blindness sometimes can lead to an abnormal head or neck posture.

The cerebellum helps regulate rate and range of motion. With damage to this area a horse often shows fine resting tremors of the head that worsen with intentional movements. One may normally observe this tremorous movement of the head and neck in the newborn or young foal, but it is not normal in older foals and adult horses. In young Arabians and a few other breeds, a condition of cerebellar abiotrophy has been reported. Horses with this condition show a hypermetric gait, failure to blink when exposed to bright light, and absence of a menace response.

After evaluating the alertness, mental attitude, head and neck posture, and coordination of the horse, the examiner should examine the face and cranial nerves closely. Systematic examination of the first to twelfth cranial nerves helps to ensure that a subtle lesion along the brainstem is less likely to be missed, although in fact the examiner assesses many of the cranial nerves simultaneously on approach to the horse. The examiner evaluates facial symmetry, facial sensation (including sensation inside the nares), head posture, eyes, nose, mouth, jaw tone, pharynx, and larynx. To determine whether an animal can detect scent is usually not as important, but determining whether it can see, hear, breathe, and swallow is critical.

Examination of the eyes should include evaluation of the blink or menace response, the ability of the horse to negotiate in a strange environment, pupillary light response, and in some cases a funduscopic examination. Excellent descriptions of the equine neuroophthalmic examination are available elsewhere.[6,7] If a horse has a lesion of the eye or optic nerve, the lesion will result in complete or partial ipsilateral blindness. To develop contralateral blindness, the horse would have to have a lesion in the optic tract or the lateral geniculate nucleus. Pupil size and symmetry are under control of the autonomic nervous system and are affected by environmental light and level of fear or excitement. To test the pupillary response, one should direct a light in each eye and note the constriction of the pupil. Identifying a consensual response in the horse is often difficult when one works alone. A swinging light test has been described. In the performance of this test, the light is shone alternately into each eye, and the more powerful direct pupillary light response is observed. A unilateral lesion affecting the afferent tracts in the eye, optic nerve, optic chiasm, or optic tract to the level of the midbrain will result in ipsilateral dilation when the light reaches the affected eye. Moving the light from side to side takes advantage of the ipsilateral light response being stronger than the consensual response. The examiner can perform this procedure alone. These reflexes are in the brainstem and are not affected by lesions in the visual cortex.

One possible cause of asymmetric pupils in a horse is Horner syndrome. Horner syndrome is the collection of clinical signs associated with dysfunction of the sympathetic nerve supply to the head and neck. The sympathetic nerve supply to the head includes upper motor neurons in the caudal hypothalamus, parts of the midbrain and pons, and parts of the medulla oblongata. Axons from these centers descend in the cervical spinal cord to the preganglionic neurons in the cranial thoracic spinal cord. From here, preganglionic axons leave the cord and join the paravertebral sympathetic trunk, where they ascend up the neck to synapse in the cranial cervical ganglion in the guttural pouch. This syndrome includes ptosis of the upper eyelid, miosis of the pupil, and protrusion of the third eyelid (nictitating membrane). In addition, the horse has unilateral facial sweating, increased facial temperature, and hyperemia of the nasal and conjunctival membranes. These signs should alert the examiner to the possibility of a previous perivascular injection, guttural pouch disease, or damage to the sympathetic nerves in the vagosympathetic trunk, which courses from the cranial thoracic spinal cord through the thoracic inlet and upward to the orbit. Loss of sympathetic innervation to the head results in the triad of clinical signs described, with the most prominent initial sign being increased sweating caused by disruption of sympathetic fibers to the blood vessels and sweat glands of the head and neck. This results in cutaneous vasodilation with subsequent more local circulating adrenaline.

Loss of symmetric positioning of the eyes or the presence of abnormal deviation (i.e., strabismus) occurs when a horse has injured the third (oculomotor), fourth (trochlear), or sixth (abducens) cranial nerve or the connections between the eighth (vestibulocochlear) cranial nerve and these nuclei in the brainstem along the medial longitudinal fasciculus. Deviations of the eyes may occur with head trauma or midbrain lesions or can be normal variations in newborn foals. When the head is elevated dorsally, the eyes should move ventrally to maintain a normal horizontal gaze. When the head is moved from side to side, the eyes should move slowly opposite to the direction of the head movement, move quickly in the direction of the head movement; this is referred to as *normal vestibular nystagmus.* Spontaneous or positional nystagmus is always abnormal.

The nuclei along the fifth (trigeminal) cranial nerve are among the largest nuclei along the brainstem of the horse. The fifth cranial nerve contains motor and sensory branches that supply innervation to the muscles of mastication and sensation to the skin and mucous membranes of the head. Injury to this nerve leads to dropped jaw and ipsilateral loss of, or decreased sensation to, the side of the face and the inside of the nares.

Damage to the seventh (facial) and eighth cranial nerves is common in horses. Injury to the seventh cranial nerve results in unilateral facial paralysis. This nerve contains branches that supply the ears, eyelids, and nares; therefore injury to this nerve may affect all or only part of these structures. The most easily recognized sign is deviation of the nares toward the unaffected side coupled with drooping of the eyelid and ear on the affected side. Because this nerve also innervates the salivary and lacrimal glands, loss of or damage to this nerve may cause dry eye and decreased salivation.

Eighth cranial nerve deficits are easy to recognize, because unilateral injury to this nerve results in a head tilt toward the affected side. The eighth cranial nerve is important to hearing and control of balance. Projections from this nerve pass to the medulla and cerebellum. A horse that has damage to this nerve often appears disoriented and has a head tilt toward the side of the lesion along with abnormal position of the limbs and body and horizontal nystagmus. If the lesion involves the peripheral portion of the vestibular system, the fast phase of the nystagmus is directed away from the side of the lesion. If the lesion involves the central portion of the vestibular system, the nystagmus may appear vertical, rotary, or horizontal and may not always appear the same, depending on head position.

Horses that have peripheral damage to the vestibular system usually compensate for the deficits in a short time by use of visual and proprioceptive input. Therefore avoiding the use of a blindfold in cases of suspected vestibular disease is judicious. Using a blindfold hampers the ability of the horse to compensate, and the horse may become dangerous. However, blindfolding a horse with a suspected vestibular disease but no longer showing an obvious head tilt may be helpful to localize the lesion.

Within the medial compartment of the guttural pouch, along the caudodorsal and lateral walls, are the ninth (glossopharyngeal) cranial nerve and a branch of the vagus nerve. When a horse develops guttural pouch disease, these nerves may be damaged, resulting in loss of innervation to the pharyngeal muscles. The clinical signs include dysphagia on the same side as the damaged nerve. If the disease is severe enough to involve the internal carotid nerve, which contains postganglionic sympathetic fibers to the structures of the head and eye, Horner syndrome results. As mentioned, the signs include ptosis of the upper eyelid, enophthalmos resulting in prolapse of the third eyelid, miosis of the pupil, and sweating along the side of the face.

Other diseases one should consider when Horner syndrome is evident during a neurologic examination include injury or infarction to the cranial thoracic spinal cord, avulsion of the brachial plexus, a hematoma, or tumor invading the sympathetic trunk in the region of the caudal, cervical, or cranial thoracic sympathetic trunk. Mycosis of the guttural pouch can cause damage to the internal carotid nerve or the cranial cervical ganglion along the caudodorsal wall of the guttural pouch. Finally, an injury to or neoplasia of the structures within or just behind the orbit also may cause Horner syndrome. Focal sweating in a horse indicates involvement of the peripheral preganglionic or postganglionic sympathetic neurons. Identification of this problem also can aid in the anatomic localization of a neurologic lesion.

Intact innervation to the larynx and pharynx is important, especially if the horse is to be used as an athlete. The easiest means to evaluate this region is by endoscopic examination of the pharyngeal and laryngeal regions. One can perform throat latch palpation and a laryngeal adductory slap test. Endoscopic examination is helpful and important to a complete evaluation. The innervation of the pharynx and larynx is via the ninth, tenth (vagus), and eleventh (accessory) cranial nerves and the connections of these nerves in the caudal medulla oblongata.

The twelfth cranial nerve, the hypoglossal nerve, has cell bodies in the hypoglossal nucleus of the caudal medulla oblongata and provides motor innervations to the muscle of the tongue. Normal horses provide a strong resistance to withdrawing the tongue from the mouth; horses with unilateral cranial nerve XII dysfunction have unilateral atrophy with weak retraction. Bilateral dysfunction interferes with prehension and swallowing, the tongue often protrudes from the mouth, and the horse has difficulty or is unable to withdraw the tongue. Severe cerebral lesions may also interfere with tongue retraction because of interference with voluntary control pathways.

After careful examination of the mental status and behavior of the horse and of the head, neck, and cranial nerves, the examiner should look for asymmetry of the muscles of the trunk, pelvic region, tail, and anus. Identification of focal sweating, focal muscle atrophy, or increased or decreased pain responses is helpful in localizing signs. In addition, the horse has two cervical reflexes. The cervicofacial reflexes result in a local twitch and drawing back of the lips (smile reflex) when one pricks the skin along the side of the neck down to the region of the second cervical vertebra. Below the region of the second cervical vertebra one should observe a local response.[8]

To evaluate the tail and anus, the tail carriage is first observed at rest and with the horse in motion. The normal tail carriage is straight down but with free movement in all directions. Some normal horses allow the tail to be lifted, giving little resistance, whereas other horses strongly resist and clamp the tail. The usual response to anal stimulation is to clamp the tail and squat down, although with prolonged stimulation the horse may relax and eventually raise its tail.

Before evaluation of gait, evaluation of postural reactions and possibly evaluation of spinal reflexes should take place. Spinal reflexes can be evaluated in foals or small horses, as well as in recumbent horses. Evaluation of spinal reflexes can be very helpful in lesion localization, although interpretation of responses is sometimes difficult in adult recumbent horses.[9] The author nearly always places the feet and limbs in an unusual position to observe the response of the horse; however, because postural reactions are sometimes difficult to interpret in horses, it is essential to use gait evaluation to help localize a lesion. Additional tests to assess proprioceptive function include a standing sway test in which one applies pressure to the shoulder. The horse initially should press into the examiner and then lean away, and finally it should step away with the offside limb. Also, the examiner might lift one thoracic limb and force the horse to hop on the opposite limb in a modified postural reaction test. The important centers for posture and coordination in the brainstem and spinal cord are

located in the regions of the sixth cervical to second thoracic (T2) and fourth lumbar to second sacral (S2) vertebrae in the spinal cord, along with coordination centers in the brainstem. Horses that demonstrate a wide base stance at rest often have a lesion of the cerebellum or vestibular system or may have conscious proprioceptive abnormalities.

Evaluation of Gait

Foals are ambulatory within hours after birth, making it possible to evaluate gait at a young age; however, newborns may appear weak and ataxic. Gait abnormalities that are observed commonly in horses with neurologic disease include ataxia, dysmetria, and weakness or paresis. Evaluation of gait is critical because subtle neurologic gait deficits often go unrecognized or sometimes may incorrectly be considered insignificant.

The horse should be observed at a walk and trot in a straight line and while turning. At a walk the examiner can walk alongside, in step with first the pelvic limbs and then the thoracic limbs. This allows the examiner to more easily determine the stride length and foot placement. A weak limb often has a low arc and longer stride length. In some horses, observing the horse negotiate over small obstacles such as a curb is helpful. It can also be helpful to evaluate the horse on different surfaces (hard ground vs. arena) at different gaits. Blindfolding the horse can also be helpful during a neurologic evaluation. Observing a horse during the backing process is important. When a normal horse is backing, it should lift each leg and place it in a coordinated and appropriate location. Horses with neurologic abnormalities often place the limbs in wide-based positions or lean back and are reluctant or refuse to move. The horse also may step on the feet of a pelvic limb with the front feet. The horse should be observed closely while being turned in a tight circle to identify abnormal wide outward excursions of the pelvic limb (circumduction). When possible, the examiner should observe the horse turned free and walking up and down an incline. Elevation of the head and walking on a slope may exaggerate a subtle deficit and make it more noticeable. These procedures provide a degree of "challenge" to the horse and may help to demonstrate if the horse is showing persistent irregular movements with its limbs. In some circumstances horses may need to be evaluated under saddle or in tack. Although observing the horse running free in a paddock or round pen and while being ridden can sometimes be helpful, this is not always possible. One must consider safety for the horse and rider, as well as certain legal ramifications, before asking a person to ride a horse during the examination.

The examiner should pay attention to which limbs show abnormal posture or abnormal movements and must be able to determine whether the horse has a painful or mechanical musculoskeletal problem or a neurologic gait deficit. Horses with a musculoskeletal problem have been described as having a regularly irregular gait, whereas a horse with neurologic gait deficits shows less consistency placing its limbs (an irregularly irregular gait). Differentiating between subtle lameness and subtle neurologic disease can be very difficult. One should reexamine horses with an obscure or unusual gait that might be the result of lameness after the use of local anesthetics (to block selected peripheral nerves) or intraarticular medications. The use of nonsteroidal antiinflammatory drugs (NSAIDs) for a period of 1 to 2 days or longer also may alleviate pain and help one distinguish between lameness and a neurologic gait deficit.

Following a neurologic examination, the examiner must identify the presence of weakness, ataxia, and dysmetria in each limb. One can evaluate the strength and ability of the horse to correct body positions by performing the sway test at a walk, in addition to the standing sway test. One applies lateral pressure at the shoulder, hip, and tail while the horse is walking. One should apply pressure several times while the horse is walking to catch the limb in various stages of weight bearing. The examiner should observe the movements of each limb carefully to determine whether a deficit is present and should assign a grade to the deficit. The author uses a system modified from the grades described by de Lahunta and colleagues[1] and Mayhew.[4] The severity is graded between 0 and 5. Grade 0 means no gait deficits. A grade 1 deficit requires careful observation to be certain the gait abnormality is caused by a neurologic dysfunction. Grade 2 deficits are mild to moderate but obvious to most observers as soon as the horse begins to move. Grade 3 deficits are obvious and are exaggerated during the negotiation of a slope or with head elevation. Grade 4 gait deficits may cause a horse to fall or nearly fall. When attempting to walk, an animal with these severe deficits often displays abnormal positioning while standing in its stall. Grade 5 horses are recumbent. However, clinicians should be cautious making decisions about horses based on this subjective assessment of gait, especially when signs are subtle. A recent investigation showed only poor to fair agreement between skilled and experienced observers of gait abnormalities in horses using this scale.[10]

Weakness or paresis is a deficiency of voluntary movement as a result of loss of muscle power because of either damage to upper motor neurons, lower motor neurons, or the muscle. It can be described as knuckling, stumbling, or buckling and sometimes can be characterized by toe-dragging while walking. Weakness may be associated with an upper motor neuron or lower motor neuron lesion. In the case of a lower motor neuron or peripheral nerve injury or illness, the horse shows muscle atrophy and sensory loss. A spastic movement of the limbs often accompanies the weakness that results from loss of upper motor neurons. Ataxia is typified by abnormal foot placement and wide swaying of the foot and limb, especially while turning. Ataxia appears as a lack of coordination of motor movements. It can arise from damage to the vestibular, cerebellar, or sensory portions of the nervous system. Ataxia in horses is most often a result of loss of sensory input through injury, damage, or disease in the spinal cord blocking normal input to the cerebellum. Cerebellar ataxia is seen most often in young horses, typically Arabians with a condition known as *cerebellar abiotrophy*. Vestibular ataxia is often accompanied by a head tilt and other cranial nerve deficits.

Horses may also demonstrate dysmetria, which is characterized as exaggerated hypermetric or hypometric movements of the limbs. Horses with hypometric gaits often appear to have a straight leg or "tin soldier" way of moving (sometimes referred to as *spasticity*). A spastic gait is the result of increased muscle tone and is generally associated with upper motor neuron disease resulting from reduced inhibition of extensor motor neurons.

The gait abnormalities, along with the findings from other parts of the neurologic examination, allow the examiner to

determine the neuroanatomic site of the disease. The severity of the clinical signs also helps one evaluate the extent or severity of the condition.

One must realize that many horses that have minimal (grade 1 or mild grade 2) deficits often can race or perform other athletic activities.[11] The examining veterinarian has the responsibility of separating a neurologic from a musculoskeletal gait deficit and helping the owner determine the usefulness of the horse. For example, a horse with gait deficits up to grade 3 may be useful as a broodmare or breeding stallion if the condition is not considered genetic and if the horse is handled by careful, knowledgeable persons who understand the risks and have the facilities to accommodate a horse in need of special management. Stallions with this degree of impairment may require assistance when mounting and dismounting mares. If the breed association allows artificial insemination, the stallion may be easier to manage.

Localization of the Lesion

At the conclusion of the examination, the veterinarian needs to determine whether a neurologic abnormality exists and where it is located. If the horse shows no evidence of abnormal behavior, seizures, or abnormal mental status and shows no cranial nerve deficits, the lesion is most likely caudal to the foramen magnum. The most difficult lesions to localize are in the brainstem, unless the signs include cranial nerve deficits or depression. Horses with brainstem lesions often show signs of weakness and ataxia similar to horses with a lesion in the cervical spinal cord. Two of the most common brainstem lesions in horses involve the seventh and eighth cranial nerves. Vestibular disease or injury may be a sequela of head trauma or an inner ear infection. Facial nerve paralysis may result from trauma to the nerve root origin in the brainstem or where the nerves course along the neck and face to the ears, eyelids, and nares. Specific cranial nerve involvement, such as head tilt, facial nerve paralysis, or loss of facial sensation, can result from trauma, guttural pouch infection, THO, or equine protozoal myeloencephalitis. In addition, cranial nerve deficits may occur with polyneuritis equi and equine motor neuron disease. Cerebellar lesions are characterized by a failure to blink to bright light, lack of a menace response, and a head tremor that worsens with intentional movements. The most common form of cerebellar disease in horses is abiotrophy, which occurs most frequently in Arabian horses.

Cervical spinal cord disease includes gait and proprioceptive deficits in all four limbs with no signs of brain, brainstem, or cranial nerve deficits. One should note that horses with cervical vertebral stenotic myelopathy might have mild pelvic limb deficits with minimal or barely detectable signs in the thoracic limbs. Horses with signs of neurologic gait deficits confined to the pelvic limbs have a neuroanatomic localization caudal to T2. When examining a horse with a lesion caudal to T2, carefully checking the tail and anus for involvement of the peripheral nerves or spinal cord segments in this region is important.

Horses that have peripheral nerve injury (Table 11.1), equine motor neuron disease, or polyneuritis equi show evidence of weakness, muscle atrophy, and in some cases selected areas of sensory loss. Primary muscle diseases such as exertional rhabdomyolysis, myotonia (congenita or dystrophica), and hyperkalemic periodic paralysis are covered elsewhere in this book (see Chapter 10). These conditions sometimes can mimic a neurologic problem.

Description of Normal and Abnormal Gaits

A walk is a natural four-beat gait in the horse. At this gait the normal horse has three feet on the ground at all times. Therefore the walk is a stable gait. The trot is a two-beat symmetric gait in which the diagonal limbs are in contact with the ground at the same time. If one examines the horse on hard pavement, the trot is the most helpful gait to distinguish lameness from a neurologic gait deficit. The pace is a two-beat symmetric gait in which the legs on the same side of the body strike the ground simultaneously, resulting in significant truncal sway. In horses that have neurologic disease, identifying ataxia with truncal sway, which is often accompanied by pacing, is common. In horses with subtle ataxia, one observes pacing when horses walk with the head held in an extended position. Whenever one observes a horse pacing, the pacing may indicate an underlying neurologic disorder; however, many normal horses will also pace when their head is elevated.

The gallop is a high-speed four-beat gait that often seems to be easier to perform than walking in a tight circle or moving at a slow trot. Therefore some horses with a neurologic gait deficit may perform better at high speed. As the horse accelerates or slows down, one may detect abnormalities, and one must observe the horse carefully at this time when an ataxic horse is most unsafe.

Abnormal gaits associated with stringhalt, upward fixation of the patella, and fibrotic myopathy deserve careful attention. Horses that show these gait abnormalities have a mechanical lameness, although the exact cause is unknown and may sometimes be associated with underlying neurologic disease. Stringhalt often begins as an abrupt onset of excessive flexion of one or both rear limbs. In some horses the condition may worsen and result in frequent episodes of the foot hitting the abdomen. The condition has been reported for a long time and in some areas of the world may occur as an outbreak. The clinical syndrome is similar to the movement of a horse with a tibial neurectomy with unopposed flexion of the hock and extension of the digit. In the case of Australian stringhalt, the forelimbs and neck muscles may be involved. Stringhalt deserves mention in this chapter because when the examiner observes this gait, the examiner should be certain no other signs of neurologic gait deficits exist. The primary condition usually can be corrected by a tenectomy of the lateral digital extensor tendon, including a portion of the muscle belly. The underlying cause of the disease may be a sensory neuropathy, a myopathy, or primary spinal cord disease. The defect likely affects the neuromuscular spindle, as well as the efferent and afferent pathways controlling muscle tone. Fibrotic myopathy results from scar tissue formation after injury to the semitendinosus and semimembranosus muscles. One may confuse the characteristic foot placement coupled with the abrupt rearward movement of the affected limb with a spastic gait caused by spinal cord injury or disease. With careful examination, a horse suffering from fibrotic myopathy likely will not go undiagnosed. Upward fixation of the patella in horses with neurologic disease may result from weakness in the quadriceps muscle group. This weakness is thought to occur because of a lack of use of these muscles or may be caused

TABLE **11.1 Localization of Peripheral Nerve Injuries in Horses**

Nerve	Clinical Signs	Common Causes of Injury
Suprascapular	Injury is termed *sweeney.* Eventual atrophy of supraspinatus and infraspinatus muscles. Lateral subluxation (popping) of the shoulder on weight bearing. This may be caused by lack of lateral collateral support (suprascapular muscles) or by additional involvement of the pectoral nerve, subscapular nerve, caudal cervical nerve roots, or other muscular and tendinous supporting structures of the shoulder.	Collision of shoulder with objects.
Radial	Unable to bear weight on affected limb because of a lack of elbow extension. The shoulder is rested in an extended position, and the limb rests with the dorsum of the pastern on the ground. The limb may be moved forward by the action of the pectoral girdle muscles while the horse walks.	Often damaged in conjunction with humeral fracture; occasionally observed after recovery from general anesthesia.
Brachial plexus	Signs of radial nerve paralysis often predominate. Evidence of suprascapular involvement not uncommon. Involvement of other nerves and nerve roots may be confirmed with EMG. Possible long-term atrophy of triceps. May have diffuse hypalgesia of lower limb.	Compression of the brachial plexus and radial nerve roots between the scapula and the ribs.
Musculocutaneous	Paralysis not common. Gait not markedly altered. Elbow may be overextended. Hypalgesia of medial forearm. Ultimately biceps and brachial muscles atrophy.	Uncommon.
Median and ulnar	Stiff, goose-stepping gait with hyperextension of the carpal, fetlock, and pastern joints.	Uncommon.
Femoral	Unable to support weight as a result of lack of stifle extension. At a walk, the limb is advanced only with difficulty, and the stride is markedly shortened. The limb buckles due to stifle, hock, and fetlock flexion if the horse attempts to bear weight. Quadriceps muscle will atrophy after 10–14 days. Patellar reflex is absent.	External blow to the limb; occasionally observed after recovery from general anesthesia.
Sciatic	Poor limb flexion with stifle and hock extended and fetlock flexed when the horse is not bearing weight. Weight can be supported if the foot is extended; otherwise weight is supported on the dorsal surface of the foot. Limb hypalgesia from stifle downward with the exception of the medial surface between stifle and hock.	Variety of injuries may be causal. Occasionally occurs secondary to intramuscular injections, especially in foals; occasionally observed after recovery from general anesthesia.
Tibial	Hypermetric or stringhalt-like gait with flexion of the hock (dropped hock) at rest. Hypalgesia of areas of caudal limb distal to the hock including most of the caudal and medial coronary band area.	Uncommon.
Peroneal	Frequently a component of sciatic nerve injury. Inability to flex the hock and extend the digits. Acutely there is hyperextension of the hock and hyperflexion of the fetlock and interphalangeal joints causing the horse to drag the fetlock along the ground. Short protraction phase to the stride. If the foot is placed in a normal position, the limb may bear weight. Hypalgesia of the craniolateral portion of the limb from the level of the hock to the fetlock.	Accessible to injury as it passes across the lateral surface of the tibia. Injury seen in horses after recovery from general anesthesia or secondary to a kick or blow to the lateral side of the pelvic limb.

by abnormal transmission of proprioceptive information to and from the muscles and joint capsule because of damage to the spinal cord.

Horses with profound ataxia may pace when they walk, which often is accompanied by circumduction of the outside rear limb while turning. These signs suggest general proprioceptive deficits. If the horse has these deficits while walking, the examiner should observe the horse walking with its head elevated and on a slope (these maneuvers often exaggerate a subtle problem). If the horse shows signs only in the trunk and pelvic limbs, the neuroanatomic localization of the lesion is between T2 and S2 or involves the nerves and muscles of the pelvic limbs. However, horses with cervical spinal cord lesions often demonstrate signs in the pelvic limbs that are one grade worse than those observed in the thoracic limbs. Therefore a mild cervical spinal cord or brainstem lesion could show minimal or no thoracic limb signs with grade 1 or subtle signs in the pelvic limbs.

Palpating the horse over its back, rump, and muscles of the rear limbs while being careful to detect any muscle atrophy is helpful.

The author routinely stimulates the horse over the side of its body and observes for any twitching of the cutaneous trunci muscles. Such twitching usually is accompanied by a cerebral response and requires a fairly severe lesion to detect areas of analgesia.

A sway reaction performed while the horse is standing and walking is also necessary to assess the pelvic limb strength and proprioceptive functions. In addition, one may evaluate strength by slow but deliberate and forceful pressure along the back and sacral muscles. A normal horse should reflexively arch its back upward, whereas a horse with rear limb weakness may be unable to withstand this pressure, and its rear limbs may even buckle.

To complete the neurologic examination, one must examine the tail and anus to determine whether damage has occurred to the sacrococcygeal nerve and muscle segments. A normal perineal reflex results in contraction of the anus and clamping of the tail in response to light stimulation of the skin in this region. Cauda equina neuritis or polyneuritis equi, trauma, and iatrogenic injury resulting from an alcohol tail block are some disorders that may affect this area.

The evaluation of the major peripheral nerves in the horse is also an important part of the neurologic examination (see Table 11.1). The important points to remember are that damage to a peripheral nerve can result in sensory and motor deficits in the area supplied by the nerve and that focal muscle atrophy follows within a short time after damage to one of these nerves. The examiner is referred to other portions of this book for a more detailed anatomic description of these nerves in the horse; however, a dropped elbow joint with radial nerve paralysis, inability to fix the stifle with femoral nerve paralysis, and atrophy of the supraspinatus and infraspinatus muscles (sweeney) with damage to the suprascapular nerve are classic examples of what to expect with peripheral nerve injuries.

CONCLUSION

For a horse to be a good athlete, it must gather information from muscles, tendons, and nerves; process this information in the brain, brainstem, and spinal cord; and relay this information to the musculoskeletal system. The horse must accomplish all of this in a short time to perform complex maneuvers at a high rate of speed. When performing in a setting involving many other horses or when a rider or driver is involved, the horse needs to be able to control these movements in a coordinated fashion to protect itself, other horses, and especially those persons handling, riding, or driving the horse.

Beyond this, veterinarians must recognize which musculoskeletal and neurologic conditions occur together to assist prospective buyers with horse-purchasing decisions. Recognizing and understanding these things helps the veterinarian determine whether a particular horse with subtle neurologic gait deficits caused by trauma, infection, or compression may still be a safe and useful athlete.

CEREBROSPINAL FLUID EVALUATION

Yvette S. Nout-Lomas

Cerebrospinal fluid evaluation has diagnostic importance regarding neurologic disease in horses. Collection and analysis of CSF is indicated to make or confirm a diagnosis of neurologic disease, but this is not without limitations. CSF values may be normal in an animal with severe neurologic deficits because the lesion is extradural, collection occurred early or late in the course of disease, collection occurred too far from the lesion, or the ventral roots and peripheral nerve have been affected by the disease. Even with its limitations, CSF provides valuable information about the CNS. However, one must emphasize that CSF evaluation is another piece of the diagnostic puzzle and together with the history, physical examination, neurologic examination, and other ancillary procedures may help in the diagnosis and prognosis of neurologic disease in horses.

Formation, Flow, and Function of Cerebrospinal Fluid

CSF is produced as an ultrafiltrate of plasma and is actively secreted by ependymal cells of the ventricles and choroid plexus. The CSF is located in the ventricles of the brain and subarachnoid space of the spinal canal (Fig. 11.2) and bathes the CNS. CSF flows from the ventricular system up and over the cerebral hemispheres and through the subarachnoid space surrounding the spinal cord. Pulsation of blood in the choroid plexuses forces the CSF in a caudal direction. The rate of CSF production is constant, varies among species, and is independent of vascular hydrostatic pressure. The rate of CSF production for horses has not been determined but in humans is estimated to be approximately 0.34 mL/min.[12] Hypertonic solutions such as mannitol, when added to blood, decrease CSF production and decrease CSF pressure and edema.[1]

Collections of arachnoid villi (arachnoid granulations) are located in the venous sinus or the cerebral vein and absorb CSF. CSF absorption is related directly to the pressure gradient between the CSF and venous sinus. When CSF pressure exceeds venous pressure, these villi act as one-way ball valves, forcing CSF flow to the venous sinus.

CSF functions to protect the brain from trauma and to maintain a consistent extracellular environment for the CNS.

Collection of Cerebrospinal Fluid

Techniques for collecting CSF in horses have been described in detail elsewhere.[1,3,4,13] CSF may be collected from the lumbosacral site in a standing sedated horse. Alternatively, it can be collected from the atlantooccipital site of an anesthetized horse. In foals and recumbent adult horses, one can collect CSF from the atlantooccipital site while the animal is recumbent, restrained, and heavily sedated. The lumbosacral collection of CSF is traditionally performed based on anatomic landmarks, but one can also perform the procedure using ultrasound guidance.[14] Similarly, atlantooccipital collection of CSF is typically performed in the recumbent horse using anatomic landmarks. Two techniques have been described for upper cervical puncture in the standing sedated horse using ultrasound guidance.[15,16] Pease and colleagues[16] describe fluid collection between C1 and C2 using a lateral approach in the standing sedated horse under ultrasound guidance, and Depecker and colleagues[15] describe fluid collection from the spinal cistern at the atlantooccipital site using parasagittal ultrasound guidance. If the lesion is localized to an area above the foramen magnum (at least cranial to the second cervical vertebra), CSF collected from the atlantooccipital site may be more diagnostic. If the lesion is localized to an area below the

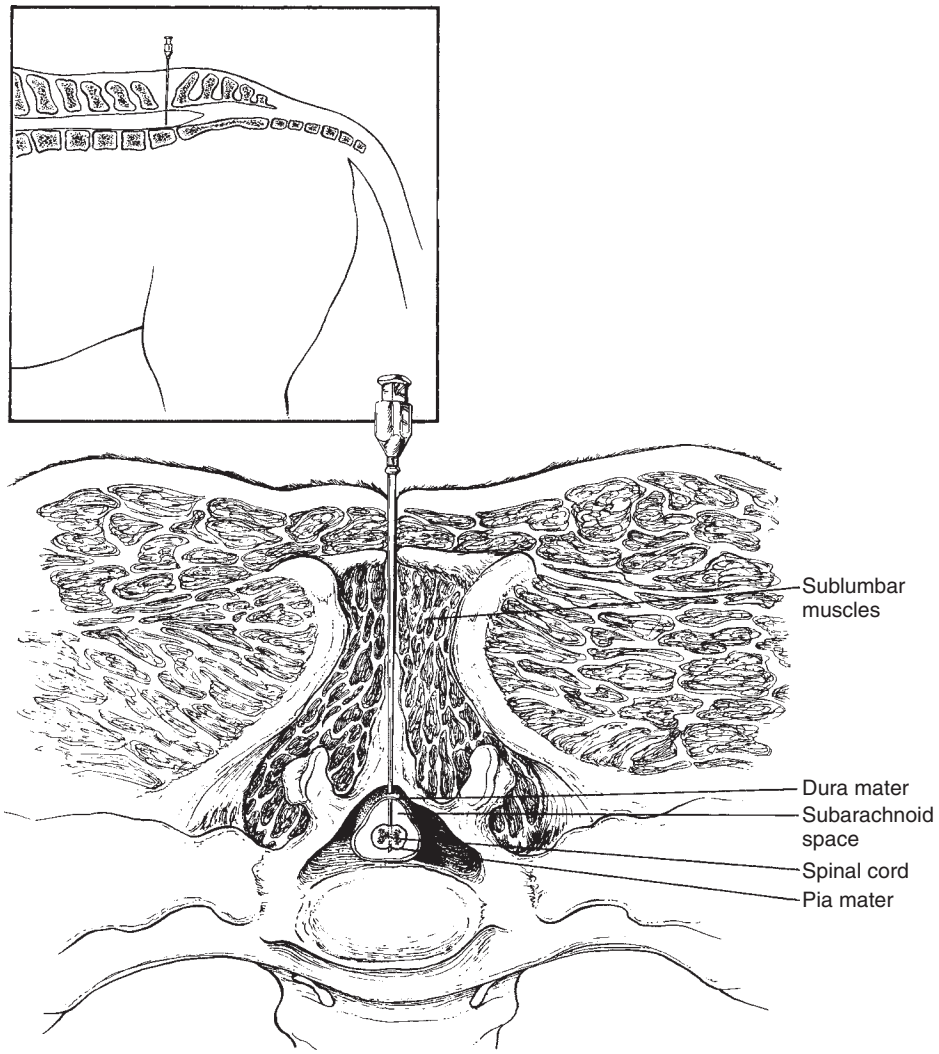


FIG. **11.2** Lumbosacral spinal fluid collection from a horse showing the various tissue layers that the spinal needle must pass through to obtain a sample. The spinal fluid is collected ventral to the spinal cord in the subarachnoid space. *Inset,* Lateral view of spinal needle placement in the lumbosacral space for collection of cerebrospinal fluid (CSF). (From deLahunta A, Glass EN: *Veterinary neuroanatomy and clinical neurology,* ed 3, St. Louis, 2009, WB Saunders.)

foramen magnum (caudal to the second cervical vertebra), CSF collected from the lumbosacral site will be more diagnostic. These differences result from the craniocaudal circulation of CSF. Collecting CSF from both sites at the same time and comparing the findings may be helpful in cases in which neuroanatomic localization of the lesion is difficult.[4]

Examination of Cerebrospinal Fluid

Reference values for CSF in foals, horses, and other equids have been reported,[3,17-23] but each laboratory should determine its own reference ranges. CSF determinations that are helpful in evaluating horses with neurologic diseases include pressure, appearance, cellular content, protein concentration (total protein, albumin, immunoglobulin G [IgG]), enzyme activity (creatine kinase [CK], aspartate aminotransferase [AST], lactate dehydrogenase), and lactic acid concentration.

Pressure

One can measure opening CSF pressure before withdrawal of CSF by attaching a manometer tube with a three-way stopcock to a properly placed spinal needle, allowing the CSF to rise within it.[21] Because the cranial and vertebral cavities are enclosed in a rigid bony compartment, changes in blood pressure or volume can cause a concomitant increase in CSF pressure. Thus increased CSF pressure may occur from venous compression or jugular occlusion. Venous compression causes increased blood volume in the cranial cavity and compression of the CSF space, leading to increased CSF pressure. One can use jugular occlusion clinically to increase CSF pressure and facilitate collection of CSF fluid. The jugular compression maneuver, or Queckenstedt's phenomenon, can help one diagnose compressive lesions, neoplastic lesions, or an abscess along the spinal cord. With compression and obliteration of the subarachnoid space by a compressive lesion in the cervical or thoracic spinal cord, jugular vein compression does not cause a CSF pressure increase in the lumbosacral site.[1]

Increased CSF pressure may occur after injury, after systemic changes in blood pressure, and in the presence of an intracranial space-occupying mass such as a tumor, abscess, hemorrhage, or edema. One must provide an adequate airway

after injury and during surgery to prevent hypoxia-mediated cytotoxic cerebral edema and vasogenic cerebral edema. Cytotoxic edema is caused by inadequate cerebral oxygenation and leads to neuronal, glial, and endothelial cell swelling. Such reactions are especially important during long surgical procedures or recumbency in which respiratory hypoxia and poor alveolar ventilation (hypercapnia) may occur. Hypercapnia increases cerebral blood flow in the cranial cavity, and CSF pressure and may worsen existing cerebral edema.

Increased CSF volume may occur in hydrocephalus, which is defined as an increased volume of CSF and can be classified as *compensatory* or *obstructive*. Compensatory hydrocephalus is an accumulation of CSF in areas in which brain tissue has been destroyed and may occur from brain injury or inflammation. Hydranencephaly is destruction of brain tissue from a viral or other infectious agent and results in severe accumulation of CSF. CSF pressure in compensatory hydrocephalus usually does not increase.

Obstructive hydrocephalus is an accumulation of CSF in the ventricles from an obstruction to CSF outflow or absorption. Cerebral aqueduct malformation may lead to obstruction of ventricular CSF outflow. Inflammatory lesions, especially of the arachnoid villi, result in decreased absorption of CSF and increased CSF pressure. The white matter is affected more severely than the gray matter in obstructive hydrocephalus, but the cerebral cortex usually is spared. CSF pressure in obstructive hydrocephalus usually increases. The presence of an abnormally high opening pressure that drops by 25% to 50% after removing 1 to 2 mL of fluid suggests a space-occupying intracranial mass or spinal cord compression cranial to the site of collection. The removal of more fluid would risk causing tentorial herniation.[21]

Appearance

One can evaluate the appearance of CSF immediately after collection. Normal CSF is clear and colorless and does not clot, and newsprint is visible through it. CSF may be red tinged from blood contamination after a traumatic tap or from preexisting trauma to the CNS. In the case of a traumatic tap, the CSF usually will clear if allowed to flow for several seconds (about 0.5–1.0 mL). With preexisting trauma and secondary hemorrhage, the supernatant of CSF after centrifugation is xanthochromic.

Other causes of CSF xanthochromia include increased protein concentration (150 mg/dL) and direct bilirubin leakage from serum in horses with high serum bilirubin concentration. In addition, indirect bilirubin may leak across a damaged blood-brain barrier. Clots in the CSF are abnormal and may be caused by increased amounts of fibrinogen resulting from inflammation.

Turbid CSF may indicate an increased number of white blood cells (greater than 200/μL), an increased number of red blood cells (RBCs) (greater than 400/μL), epidural fat, bacteria, fungal elements, or amebic organisms. Cytologic evaluation and cultures can help differentiate causes of turbidity.

Cytologic Evaluation

One can use a standard hemocytometer to obtain a complete blood count (CBC). In addition, a sedimentation chamber requiring 0.5 to 1.0 mL of CSF is a rapid method for cytologic evaluation. One must perform cell counts and cytologic evaluation within 30 minutes to avoid degeneration. If cell counts or cytologic evaluation cannot be performed immediately, one can mix a portion of the sample with an equal volume of 50% ethanol to preserve cellular characteristics.[21] CSF from normal horses and foals usually contains fewer than 7 white blood cells per microliter.[3] Most studies show no differences in white blood cell counts in normal CSF samples obtained from the atlantooccipital space compared with samples obtained from the lumbosacral space.

Small mononuclear cells (70%–90%) and large mononuclear cells (10%–30%) predominate in equine CSF. Rarely, one may see neutrophils in horse CSF. Increased CSF large mononuclear phagocytes are visible in diseases of axonal degeneration.[18] One may see increased CSF neutrophil numbers in encephalomyelitis, bacterial meningitis, parasitism, and diseases with extensive inflammation. Occasionally, in severe inflammatory diseases of the neurologic system or parasitism, eosinophils may be visible.[18,24] In some cases, cytologic evaluation of CSF may reveal specific agents causing neurologic disease such as fungal organisms, bacteria, or tumor cells. Although CSF cytologic examination may support a diagnosis of neurologic disease, it may not yield a specific causative diagnosis. Analysis of cytokine gene expression in the nucleated cells of equine CSF suggests that there are distinct gene signatures expressed by nucleated cells in the CSF of horses without neurologic signs versus horses with inflammatory or traumatic neurologic disorders.[25]

Protein Concentration and Composition

Normal total protein values range from 20 to 124 mg/dL, depending on the measuring method used (Table 11.2). Total protein concentration is higher in lumbosacral CSF compared with atlantooccipital CSF.[26] A difference of 25 mg/dL of protein between the atlantooccipital and lumbosacral spaces may suggest a lesion closer to the space with greater spinal fluid protein.[22] Proteins in the CSF are derived from the peripheral blood and include albumin, IgG, and possibly other globulins. Increased CSF albumin and IgG concentrations may occur with damage to the blood-brain barrier or increased intrathecal production of IgG. One can determine CSF albumin and IgG concentrations by electrophoresis and radial immunodiffusion, respectively, and can compare these with serum concentrations. One can calculate the albumin quotient (AQ) ([Albc]/[Albs] × 100) and IgG index ([IgGc]/[IgGs] × [Albs]/[Albc]) to determine blood-brain barrier permeability and intrathecal IgG production. Increased intrathecal IgG production (increased IgG index) may occur in inflammatory spinal cord disease such as equine protozoal myeloencephalitis (EPM), equine neuroborreliosis, bacterial meningitis, some tumors, and equine motor neuron disease. Determining blood-brain barrier integrity is also important in planning therapy. If the blood-brain barrier is damaged, pharmacologic agents such as penicillin that do not normally penetrate the blood-brain barrier will penetrate a disrupted blood-brain barrier and attain bactericidal CSF concentration.

Enzyme Determination

CSF enzyme activity may be increased in neurologic disease. CK and AST activity may be increased in diseases with myelin degeneration and neuronal cell damage such as EPM, polyneuritis equi, equine degenerative myelopathy, and equine motor neuron disease. Increased CK activity also may occur in conditions that alter blood-brain barrier permeability, such as equine herpesvirus–1. In diseases in which the blood-brain barrier is damaged, serum CK can leak into the CSF and increase CSF CK activity. This increased CK activity is not associated with damaged myelin.

TABLE **11.2 Cerebrospinal Fluid Values from Atlantooccipital and Lumbosacral Spaces of Normal Healthy Adult Horses**

	Atlantooccipital Space (Mean ± SD [Range])	Lumbosacral Space (Mean ± SD [Range])
Red blood cell (RBC) count (per μL)	51.0 ± 160 (0–558)	36.8 ± 59.7 (0–167)
White blood cell count (per μL)	0.33 ± 0.49 (0–1)	0.83 ± 1.11 (0–3)
Total protein (mg/dL)[a]	87.0 ± 17.0 (53 ± 11.6) (59–118) (35–74)	93.0 ± 16.0 (58.0 ± 11.0) (65–124) (39–78)
Albumin (mg/dL)	35.8 ± 9.7 (24–51)	37.8 ± 11.2 (24–56)
Albumin quotient (AQ)	1.4 ± 0.4 (1.0–2.0)	1.5 ± 0.4 (1.0–2.0)
Immunoglobulin G (IgG) (mg/dL)	5.6 ± 1.4 (3–8)	6.0 ± 2.1 (3–10)
IgG index	0.19 ± 0.046 (0.12–0.27)	0.19 ± 0.5 (0.12–0.26)
Creatine kinase (CK) (IU/L)	0–8	0–8
Lactate dehydrogenase (IU/L)	0–8	0–8
Aspartate aminotransferase (AST) (IU/L)	4–16	0–16
Glucose (mg/dL)	35%–70% of blood glucose	
Lactate (mg/dL)	1.92 ± 0.12	2.30 ± 0.21
Sodium (mEq/L)	140–150	140–150
Potassium (mEq/L)	2.5–3.5	2.5–3.5

[a]Total protein concentration next to the mean ± SD in parentheses is the value expected using a total protein standard.

Increased CK activity also may suggest other diseases of the CNS. In one study, CK activity (greater than 1 IU/L) most often was associated with EPM in horses and may be helpful in differentiating compressive spinal cord disease from EPM. Furthermore, persistently increased CSF CK activity may be associated with a poor prognosis in horses with EPM.[27] Lactate dehydrogenase activity may be increased in spinal lymphosarcoma.

Lactic Acid Concentration

CSF lactic acid concentration may be an indicator of neurologic disease (see Table 11.2). CSF lactic acid concentration increases in eastern equine encephalomyelitis (4.10 ± 0.6 mg/dL), head trauma (5.40 ± 0.9 mg/dL), and brain abscess (4.53 mg/dL). Lactic acid concentration may be the only CSF parameter increased in horses with brain abscess.[28]

Caffeine

CSF concentrations of caffeine and its metabolites were evaluated using a novel microdialysis system that allowed simultaneous monitoring of locomotor activity and collection of CSF and blood samples for pharmacokinetic analysis.[29] Investigators found that 3 mg/kg caffeine increased spontaneous locomotor activity, which was correlated to increased blood and CSF caffeine concentrations.

Summary

Normal CSF findings do not always rule out the presence of neurologic disease. CSF values may be normal with lesions outside the CNS that are not bathed in the CSF, such as extradural, ventral root, and peripheral nerve lesions. Normal CSF values also may occur early or late in the disease process and in CSF samples obtained from a site distant from the lesion. Acute neurologic disease, especially if multifocal, may not have sufficient time to cause significant damage to the blood-brain barrier and alter CSF constituents, whereas in chronic CNS disease the blood-brain barrier may be repaired and functional but with nervous tissue replaced by fibrous tissue. Fibrosis of nervous tissue may result in significant neurologic gait deficits and normal CSF constituents. CSF taken away from the site of the lesion shows normal findings despite significant neurologic gait deficits. For example, CSF in a horse with a cervical spinal cord abscess may show a suppurative inflammation in the lumbosacral CSF and a normal atlantooccipital CSF. This discrepancy is caused by the caudad flow of CSF.

CSF may be helpful in supporting the diagnosis of neurologic disease in horses and is part of the diagnostic workup. Because CSF evaluation is an ancillary diagnostic test, one should use it with, and not as a substitute for, a thorough history, physical examination, neurologic examination, and other diagnostic tests.

NEUROELECTRODIAGNOSTICS

M. Aleman

Neuroelectrodiagnostic testing is a useful modality that comprises various diagnostic modalities to evaluate brain, brainstem, nerve, neuromuscular, and muscle electrical activity, among others. Neuroelectrodiagnostics should be used as an extension of the neurologic examination that might aid in further localization of lesions in certain challenging cases. Depending on the specific diagnostic aid, horses might require sedation (i.e., electromyography, electroretinography, brainstem auditory evoked potential, otoacoustic emissions, visual evoked potentials, electroencephalography) or general anesthesia (i.e., nerve conduction studies, repetitive nerve stimulation).[30]

Electromyography

Electromyography (EMG) refers to the study of the electrical activity of the muscle.[31-34] The distribution of EMG abnormalities can assist in the localization of lesions to spinal cord segments, nerves, and/or muscles.[31-34] Quantitative EMG can further assist in the determination of disorders as myopathic,

TABLE **11.3** **Muscles, Nerves, and Nerve Roots Evaluated during Routine Electromyographic Examination of Horses**

Muscles	Peripheral Nerve	Spinal Nerve Root
REAR LIMB		
Long digital extensor	Peroneal nerve	L6–S1
Gastrocnemius	Tibial nerve	S1–S2
Deep digital flexor	Tibial nerve	S1–S2
Semimembranosus	Ischiatic nerve	L5–S2
Vastus lateralis	Femoral nerve	L3–L5
Biceps femoris	Caudal gluteal, ischiatic, and peroneal nerves	L6–S2
Middle gluteal	Cranial and caudal gluteal nerves	L5–S2
PARAVERTEBRAL		
Paravertebral muscles (segmentally)	Dorsal branches of ventral spinal nerves (L6–C1)	L6–C1
THORACIC LIMB		
Subclavius	Pectoral nerve	C6–C7, T1
Supraspinatus	Suprascapular nerve	C6–C8
Infraspinatus		
Deltoideus	Axillary nerve	C6–C8
Biceps brachii	Musculocutaneous nerve	C6–C8
Triceps	Radial nerve	C7–T1
Extensor carpi radials	Radial nerve	C7–T1
Superficial digital flexor	Ulnar nerve	C8–T2
Deep digital flexor	Ulnar and median nerve	C7–T1, T2

neurogenic, or both.[31-35] EMG might not provide a definitive diagnosis but will aid to support or refute a suspected clinical diagnosis and further our understanding of disease processes. EMG can be performed in the unsedated horse; however, for safety reasons and duration of the study, sedation is recommended. Sedation can be achieved with xylazine hydrochloride at 0.2 to 0.5 mg/kg IV or detomidine hydrochloride at 0.005 to 0.01 mg/kg IV.[30] Examination of the awake horse aids in evaluating motor unit action potentials (MUAPs).[30] The waveforms of EMG are derived from the action potentials of the muscle fibers that are firing singly or in groups near the electrode.[32]

In the needle EMG examination, one should thrust the exploring electrode briskly into the muscle and hold it until the animal completely relaxes. To enable relaxation, pressure can be applied to the animal to bear weight on the opposite limb. Once relaxation has occurred, resting activity or any postinsertional activity of the muscle can be evaluated. EMG records insertional and spontaneous activities (normal and pathologic). Evaluation of multiple areas (at least four to six) and depth within skeletal muscles under study should be performed. Table 11.3 provides examples of muscles, nerves, and spinal cord segments that can be tested. A thorough and systematic evaluation must be performed to avoid missing a lesion.

Normal Electromyographic Potentials

Motor Unit Action Potentials. MUAPs result from voluntary or reflex muscle contractions observed after insertion of the needle electrode. They represent the sum of a number of muscle fiber potentials. MUAPs are characterized by its appearance, duration, amplitude, number of turns, number of phases, area, and rise time.[32] MUAPs normally have a triphasic configuration but could be monophasic, biphasic, or polyphasic (Fig. 11.3).[32] A few polyphasic potentials (greater than four phases) might occur in normal muscle but usually do not exceed 5% to 15% of the population of MUAPs observed.[31-33] MUAPs have an amplitude ranging from 500 to 3000 μV and a duration ranging from 1 to 15 ms. Examination of the conscious horse enhances the evaluation of the amplitude and number of phases of MUAPs in the muscle. MUAPs can be observed when the horse bears weight on or retracts a limb, resulting in contraction of that explored muscle. As muscle contraction becomes more intense, more motor units are recruited and the greater frequency of MUAPs is observed.

Resting Activity. Resting activity (electrical silence appears as a flat line) is observed in relaxed muscle.[31-33] When the needle comes to rest near a nerve twig or end plate, the needle might irritate small intramuscular nerve terminals. However, end plate noise and end plate spikes might be observed. End plate noise produces a rippling of the baseline and a low-pitched continuous noise (Fig. 11.4).[31,33] End plate spikes, on the other hand, are high-amplitude intermittent spikes and make a popping sound. End plate noise and spikes can occur alone or together. The origin of end plate noise is due to extracellularly recorded miniature end plate potentials,[31,33] whereas end plate spikes are due to single muscle fiber contractions after needle electrode irritation of the nerve terminals.[31,33] In human beings, these potentials are associated with dull pain; repositioning the needle often eliminates their activity.

Insertional Activity. Insertional activity is that EMG activity caused by mechanical stimulation of muscle fibers on needle insertion and usually does not go beyond 1 to 2 seconds after insertion. Spontaneous activity might be found at the end plate

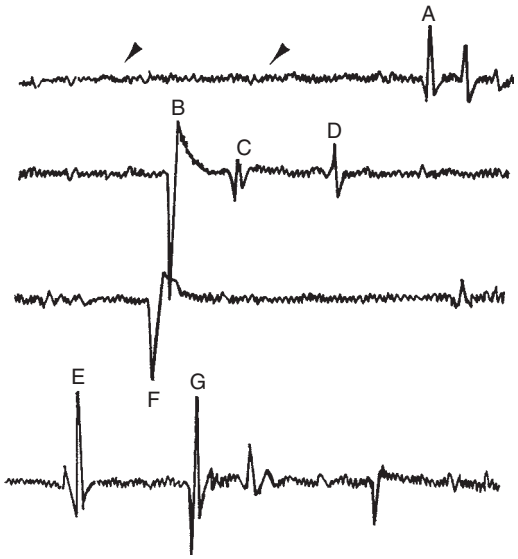


FIG. **11.3** Electromyograph of the middle gluteal muscle showing normal resting activity *(arrowheads),* fasciculation potentials ***(A, E, G),*** fibrillation potentials ***(C),*** positive sharp waves ***(B, F),*** and a small motor and action potential ***(D).***

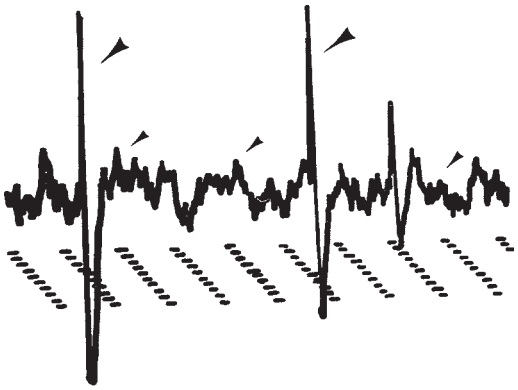


FIG. **11.4** Electromyograph from triceps brachii muscle showing end plate spikes *(large arrowheads)* and end plate noise *(small arrowheads).* Gain: 0.500 mV/stair step; time: 10 ms/division.

or motor unit region.[31-33] Alterations of insertional activity include absent, decreased, increased, or prolonged activities.[31-33] Insertional activity consists of short bursts of high-amplitude, moderate- to high-frequency (less than 200 Hz) electrical activity during and shortly after the insertion or movement of the exploring electrode in the muscle (Fig. 11.5). Prolonged ongoing electrical activity after cessation of needle insertion is considered abnormal.

Prolonged Insertional Activity. Prolonged electric activity continuing 1 to 10 ms after needle insertion and placement in the muscle is considered abnormal and caused by hyperirritability and instability of the muscle fiber membrane.[31-34] Increased or prolonged insertional activity usually precedes the onset of other denervation potentials (fibrillation potentials and positive sharp waves) and might suggest early denervation atrophy.[31-34] However, prolonged insertional activity can also be observed in myotonic disorders and myositis.[31-34]

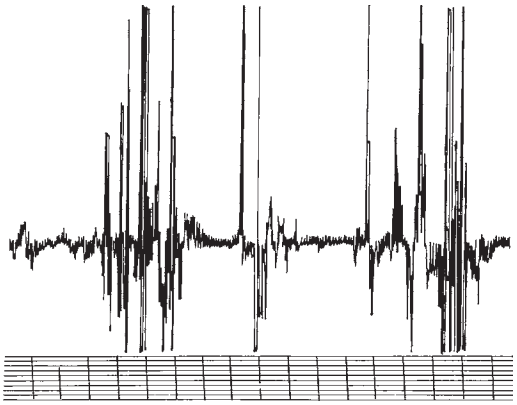


FIG. **11.5** Normal insertional activity in the infraspinatus muscle. Gain: 0.500 mV/division; time: 10 ms/division.

Decreased Insertional Activity. Decreased insertional activity (decreased amplitude, duration, or both) is associated with a decreased number of functioning muscle fibers and can be seen with neuropathies and myopathies. Infiltration of connective tissue and fat in the muscle leads to a decreased number of muscle fibers, which decreases insertional activity. Complete fibrosis of the muscle results in loss of insertional activity. Insertional activity also might be absent when muscle fibers are functionally inexcitable, as occurs during attacks of familial periodic paralysis,[31-34] or if a faulty needle electrode is used.

Abnormal Electromyographic Potentials

Spontaneous activity in a relaxed muscle after cessation of needle movement is considered abnormal. Abnormal spontaneous activities include fibrillation potentials, positive sharp waves, complex repetitive discharges, myotonic discharges, pseudomyotonic discharges, and neuromyotonic discharges.[31-34] In acute neuropathic disorders, EMG abnormalities might not be evident immediately.[31-34] It might take about 2 weeks to detect EMG alterations.[31-34]

Polyphasic Motor Unit Action Potentials. Polyphasic MUAPs (myopathic potentials) have increased frequency (greater than four phases) and decreased amplitude and duration (Fig. 11.6) observed during submaximal muscle contraction and that result from an increased number of action potentials for a given strength of contraction. Myopathic potentials result from a diffuse loss of muscle fibers and indicate the need for extra motor units to perform the work normally done by fewer motor units.[31-33] Myopathic potentials are polyphasic and most often occur in primary myopathies such as myotonia-like syndromes, periodic paralysis, myositis, botulism, and myasthenia gravis–like syndromes. These potentials also have been reported in steroid-induced myopathies, pars intermedia pituitary dysfunction, and membrane defect myopathies.[36] MUAPs of neurogenic origin can have a decreased frequency and longer duration than myopathic potentials and might occur during minimal and maximal muscle contraction (see Fig. 11.6). Thus one observes fewer MUAPs of increased amplitude than expected for the strength of contraction, which is more noticeable during maximal contraction and produces a sputtering or motorboat sound. Abnormal MUAPs of neurogenic origin can be caused by a decreased

EMG FINDINGS

	Lesion / EMG steps	Normal	Neurogenic lesion		Myogenic lesion		
			Lower motor	Upper motor	Myopathy	Myotonia	Polymyositis
1	Insertional activity	Normal	Increased	Normal	Normal	Myotonic discharge	Increased
2	Spontaneous activity		Fibrillation Positive wave				Fibrillation Positive wave
3	Motor unit potential	0.5-3.0 mV 5-10 ms	Large unit Limited recruitment	Normal	Small unit Early recruitment	Myotonic discharge	Small unit Early recruitment
4	Interference pattern	Full	Reduced Fast firing rate	Reduced Slow firing rate	Full Low amplitude	Full Low amplitude	Full Low amplitude

FIG. **11.6** Differential electromyographic findings in neurogenic and myogenic conditions. (Modified from Kimura J: *Electrodiagnosis in diseases of nerve and muscle: principles and practice*, Philadelphia, 1984, FA Davis.)

number of functioning axons firing during maximal muscle contraction. These potentials are most often present in primary neuropathies in which collateral reinnervation has occurred.[31-33]

Fibrillation Potentials. These spontaneous discharges (see Fig. 11.3) sound like frying eggs, crinkling cellophane, or frying bacon and have an initial positive deflection of 100 to 300 μV in amplitude and 2 to 4 ms in duration.[31-33] They are diphasic or triphasic in waveform. Fibrillation potentials strongly suggest denervation but have been observed in polymyositis, muscular dystrophy, and botulism.[37] Fibrillation potentials could result from spontaneous discharges from acetylcholine-hypersensitive denervated muscle fibers or muscle inflammation and focal muscle degeneration.[31-33] A few fibrillation potentials have been observed in normal healthy muscle, but they are usually not reproducible in other areas of the muscle.

The onset of fibrillation potentials after denervation depends on the size of the animal. The larger the animal, the later the onset of fibrillation potentials; they have been reported between days 5 and 16 after denervation in dogs and human beings.[31-33,37,38] The authors have observed fibrillation potentials 7 to 10 days after nerve injury in horses. Fibrillation potentials often occurred along with positive sharp waves; they increase and then decrease in amplitude as the muscle atrophies, with activity ceasing on complete muscle atrophy. Fibrillation potentials occurring alone denote a more severe disease process than the presence of positive sharp wave potentials alone. Fibrillation potentials are helpful in evaluating the length of time muscle denervation has been present and are important in diagnosing denervation before clinical muscle atrophy. Fibrillation potentials can be used to monitor progression of disease over time. In addition, a decrease in fibrillation

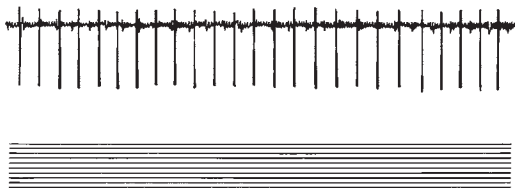


FIG. **11.7** Electromyograph showing trains of positive sharp waves. Gain: 500 V/stair step.

potentials followed by the recording of MUAPs might indicate reinnervation and might suggest a favorable prognosis.[31-33]

Positive Sharp Waves. Positive sharp waves are potentials in which the primary deflection is downward, followed by a lower amplitude, longer duration negative deflection (see Fig. 11.3). This waveform has been described as resembling a sawtooth.[31-33] Positive sharp waves occur with muscle denervation and muscular diseases such as myositis, exertional rhabdomyolysis (tying-up syndrome), and spinal shock. Positive sharp waves might occur in denervated muscle after chronic exertional rhabdomyolysis, myotonia, EPM, laryngeal hemiplegia, suprascapular nerve injury (sweeney), and compressive myelopathies.[39-42] Positive sharp waves often precede or appear along with fibrillation potentials in denervated muscle. These potentials can be observed singly or in trains (Fig. 11.7) and sound like a machine gun.[31-33]

Fasciculation Potentials. Fasciculation potentials are spontaneous discharges from a group of muscle fibers representing the whole or part of a motor unit (see Fig. 11.3).[32] Fasciculation potentials occur in diseases of anterior horn cells and irritative-type lesions of root or peripheral nerve, such as radiculopathies and nerve entrapments in human beings.[31-33]

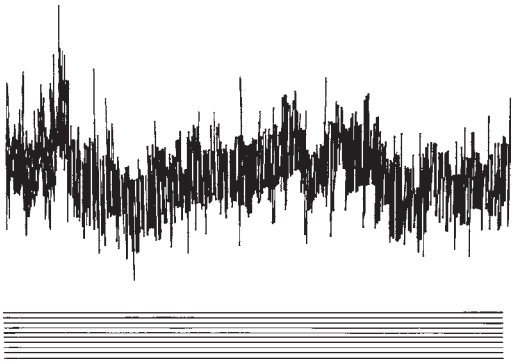


FIG. **11.8** Electromyograph showing waxing and waning myotonic potentials. Gain: 500 V/stair step.

Fasciculation potentials in the presence of fibrillation potentials or positive sharp waves might indicate lower motor neuron disease[43] and might occur in suprascapular nerve entrapment (sweeney) in horses.

Complex Repetitive Discharges and Myotonic Potentials. These potentials are repetitive MUAPs induced by insertion of the needle electrode or percussion of muscle.[31-33] Bizarre high-frequency potentials tend to be shorter in duration and end abruptly compared with myotonic discharges. Bizarre high-frequency potentials sound like a machine gun. However, myotonic potentials wax and wane in amplitude, last 4 to 5 seconds, and sound like a dive-bomber, hence the nickname dive-bomber potential (Fig. 11.8).[31-33] Myotonic and bizarre high-frequency potentials are associated with hyperexcitability of the muscle cell membrane.[31-33] Bizarre high-frequency potentials might occur in diseases of the lower motor unit such as muscular dystrophy, steroid-induced myopathy, polymyositis, chronic denervation, and hyperkalemic periodic paralysis.[31-33,44,45] Myotonic potentials occur in myotonia congenita and myotonia dystrophica and might occur in hyperkalemic periodic paralysis in human beings, which reflects abnormal muscle chloride or potassium conductance.[31-33] Complex repetitive discharges are seen in horses with hyperkalemic periodic paralysis.[45]

Nerve Conduction Studies

Nerve conduction velocity (NCV) studies assess the motor and sensory evoked responses to stimulation of nerves.[33] The evaluation of nerve conduction velocities requires knowledge of the topographic anatomy of nerves and muscles. In horses, this diagnostic modality could be technically challenging, is time consuming, and requires general anesthesia because electrical stimulation is applied.[30] NCV studies aid in the localization of nerve, neuromuscular junction, and muscle disease. Furthermore, it aids in the distinction between axonal degeneration and demyelination.[33] Nerve conduction velocity studies of the maxillary, facial, radial, median, ulnar, lateral and medial palmar and plantar, sural, superficial, and deep peroneal nerves have been reported in horses and ponies.[46-56]

Stimulation of a specific motor nerve consists of inserting a needle near the nerve that records a compound motor action potential from an innervated muscle (Figs. 11.9 and 11.10). The examiner might see or palpate the contraction of appropriate muscles on stimulation. The unaffected limb can be used as a control. In horses, radial nerve recordings can be obtained from the *extensor carpi radialis* and *abductor digitorum longus* muscles (Fig. 11.10) and for the median nerve from the humeral and radial heads of the deep digital flexor muscle[50,51] (Fig. 11.9). Facial nerve recordings can be obtained from the *levator nasolabialis* muscle by stimulating the buccal branch of the facial nerve just ventral to the facial crest.[47] Sensory nerve action potentials (SNAPs) can also be evaluated in the anesthetized horse.

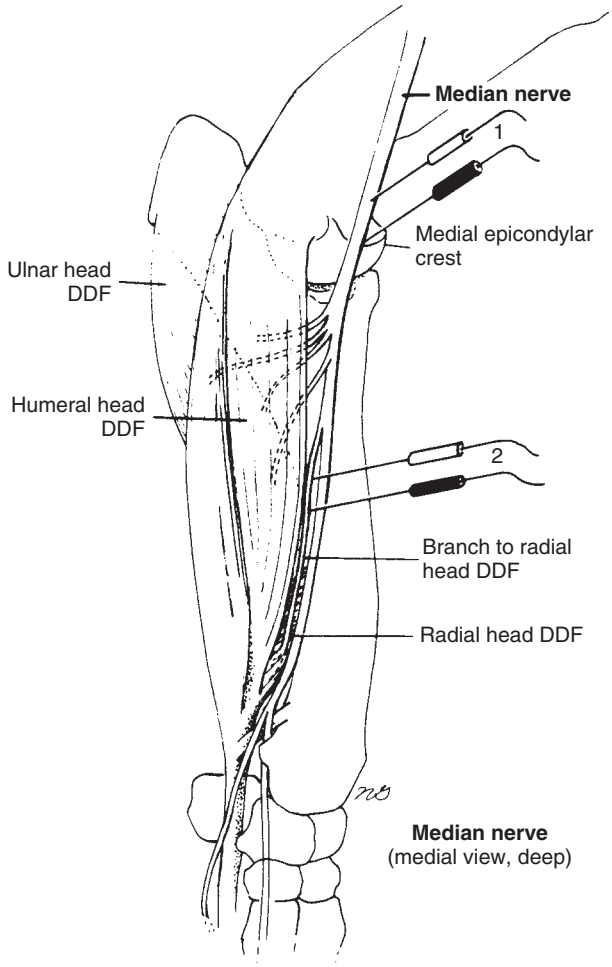


FIG. **11.9** Illustration of anatomy and electrode placement used in median nerve conduction velocities. *DDF,* Deep digital flexor. (From Henry RW, Diesem CD, Wiechers MD: Evaluation of equine radial and median nerve conduction velocities, *Am J Vet Res.* 40:1406-1410, 1979.)

Diseases Affecting the Motor Unit and Nerves

Focal and Multifocal Myelopathies

Compressive cervical myelopathies, cervical stenotic myelopathy, and EPM are common causes of neurologic disease in the horse. These conditions can affect sensory and motor pathways. Physical examination might reveal muscle atrophy and sweating over affected muscles. Needle EMG of the cervical axial musculature in horses with truncal, thoracic, and pelvic limb ataxia might reveal increased insertional activity, fibrillation potentials, and positive sharp waves supporting the conclusion that there is a lesion of the ventral horn cells or ventral roots. Abnormal postinsertional activity at the level of the

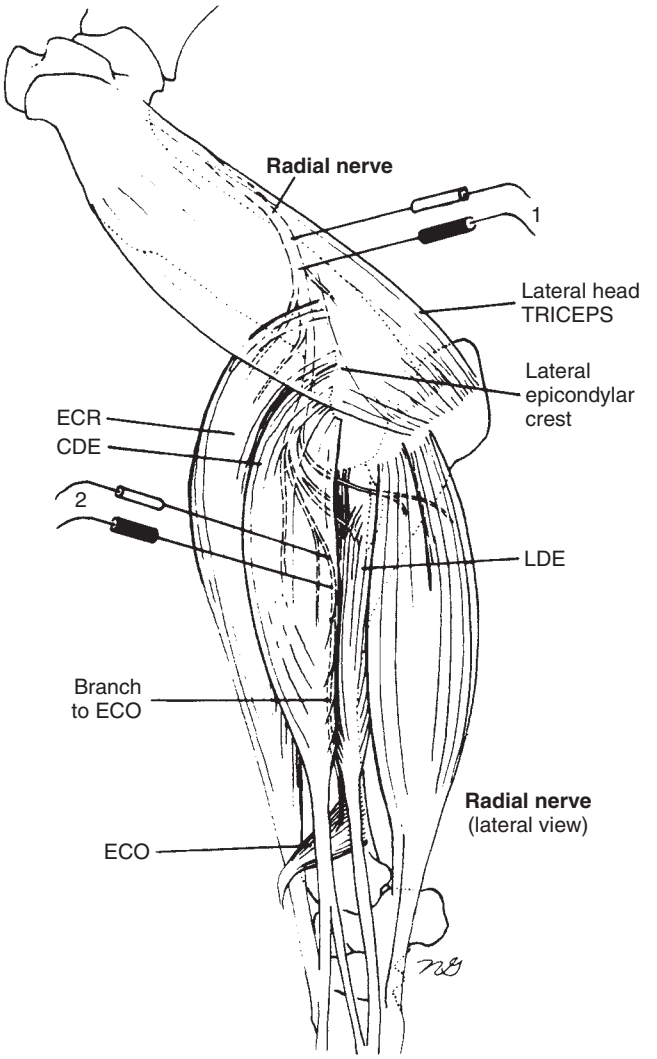


FIG. **11.10** Illustration of anatomy and electrode placement used in radial nerve conduction velocities. *ECR,* Extensor carpi radialis; *CDE,* common digital extensor; *LDE,* long digital extensor; *ECO,* extensor carpi obliquus. (From Henry RW, Diesem CD, Wiechers MD: Evaluation of equine radial and median nerve conduction velocities, *Am J Vet Res.* 40:1406-1410, 1979.)



FIG. **11.11** Horse with gluteal muscle atrophy due to EPM showing distribution of abnormal electromyographic potentials.

spinal cord compression might allow the clinician to focus the radiographic examination.

In cases of EPM, needle EMG might reveal fibrillation potentials, positive sharp waves, and abnormal insertional activity in affected limb muscles in horses presented for obscure lameness. One also can examine horses with muscle asymmetry (Fig. 11.11) via needle EMG to determine the extent of muscle involvement. Serial needle EMG examination also might be helpful in monitoring the response to treatment and prognosis. For more in-depth information on electrodiagnostics in the horse, the reader is referred to multiple publications by Wijnberg.[34]

Radial and Suprascapular Nerve Injury

Damage to nerves leads to muscle atrophy of the innervated muscle. Damage to the radial and suprascapular nerves can occur with trauma to the cranial aspect of the shoulder. Needle EMG and nerve conduction studies are helpful in evaluating the extent of damage to these and other nerves and might be able to differentiate lost or reduced limb function caused by nerve damage from painful conditions. Muscle groups that have atrophied because of disuse (disuse atrophy) caused by a painful condition show no postinsertional activity on needle EMG examination.

Positive sharp waves and fibrillation potentials in the *triceps brachii* and *extensor carpi radialis* muscles might suggest radial nerve injury. Positive sharp waves and fibrillation potentials in the *supraspinatus* and *infraspinatus* muscles suggest injury to those muscles or suprascapular nerve injury (Fig. 11.12). Postinsertional activity in these muscle groups and the lateral head of the triceps suggests damage to the brachial plexus (Fig. 11.13). Thus needle EMG might be helpful in differentiating suprascapular nerve injury from brachial plexus injury. Visible evidence of muscle atrophy might not be present until several days to weeks after injury. To confirm a radial nerve injury, nerve conduction velocity studies are recommended (Fig. 11.14). Nerve conduction velocity less than 60 m/sec or significantly less than the opposite limb supports radial nerve dysfunction. In addition to reduced nerve conduction velocity, alterations in amplitude and duration of the action potential support abnormalities. Needle EMG and nerve conduction studies might be helpful in determining success of nerve decompression surgery and evaluating whether permanent nerve damage has occurred and the extent of return of nerve function.

Laryngeal Hemiplegia

Laryngeal hemiplegia has been described extensively in the literature as denervation of the intrinsic laryngeal musculature, specifically the recurrent laryngeal nerve.[57] Recognizing

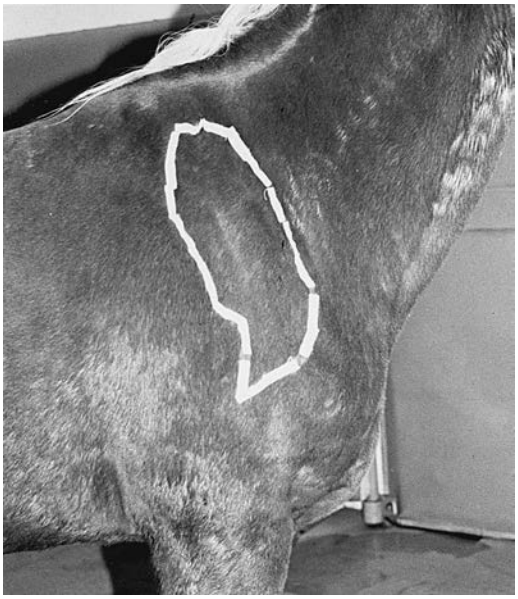


FIG. **11.12** Horse with suprascapular nerve injury with characteristic electromyographic distribution of fibrillation potentials and positive sharp waves in supraspinatus and infraspinatus muscles.



FIG. **11.13** Horse with brachial plexus injury showing characteristic distribution of electromyographic charges, including fibrillations and positive sharp waves. The involvement of the supraspinatus, infraspinatus, triceps brachii, extensor carpi radialis, and pectoral muscles is notable.

the disorder before the onset of clinical signs could be challenging. However, fibrillation potentials and positive sharp waves in the dorsal cricoarytenoid muscle on needle EMG examination might appear before clinical signs.[58] Thus needle EMG might be useful in the early detection of laryngeal hemiplegia in horses. Spontaneous EMG activity, including fibrillation potentials, positive sharp waves, or bizarre high-frequency discharges, have been reported in the dorsal cricoarytenoid muscle of horses affected with laryngeal hemiplegia. Fibrillation potentials and positive sharp waves are the most common abnormalities observed in horses with laryngeal hemiplegia. Decreased insertional activity and bizarre high-frequency discharges were reported at a lesser frequency.[58]

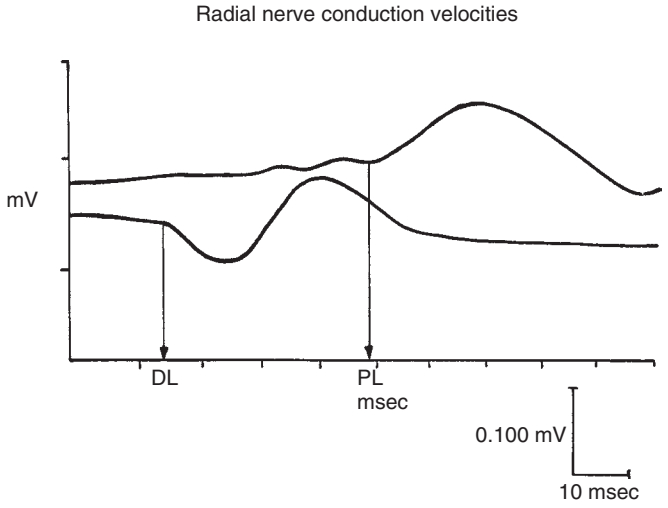


FIG. **11.14** Evoked muscle action potential from the extensor carpi radialis muscle showing decreased amplitude and decreased nerve conduction velocity, suggesting radial nerve injury. *DL,* Distal latency; *PL,* proximal latency.

Brainstem Auditory Evoked Response

Brainstem auditory evoked response (BAER) testing evaluates the integrity of the auditory pathway from its peripheral part (cochlear nerve) to the brainstem.[59] It is useful in the detection of unilateral and bilateral deafness, as well as differentiation between conductive and sensorineural hearing loss. Reference values for BAERs in adult horses and ponies[60-73] and neonatal foals are available in the literature.[61,72] Headphones or earphones are used to transmit sound through the external ear canal to the middle ear to the inner ear to evoke a response. The BAERs are evoked potentials, or waves, arising within the first 10 ms after delivery of an acoustic stimulus (clicks; Fig. 11.15). In human beings, BAERs are recognized as consisting of from five to seven waves, generally designated I through VII. Of these, waves I through V are the most common. In dogs, cats, and horses waves I to V are easily identified if normal (Fig. 11.16).[60] In human beings and animals, a correspondence exists between these waves and certain anatomic generator sites.[59,60] Wave I corresponds to cochlear nerve, II to cochlear nucleus, III to olivary nucleus, IV to lateral lemniscus, and V to caudal colliculus. Two derivations (vertex to mastoid and vertex to C2), depending on recording electrode placement, have been used in the horse to better identify the waves.

BAERs vary with age, gender, breed, head size, and temperature in other species. No differences in BAER latencies are apparent between neonatal foals and adult horses.[60,61] However, older horses might suffer from partial hearing loss as they age. Auditory brainstem response (ABR) testing is a method of assessing not only auditory function but also a variety of neurologic disorders involving the brainstem. ABR testing is unaffected by the state of arousal of the test subject, and responses are not degraded by sedation or general anesthesia.[61] Examination can be done in the awake horse, with or without mild sedation, or anesthetized. If a conscious awake horse is tested, one stimulates each ear and records the resultant waveforms independently. If the patient is anesthetized, one examines the uppermost ear first, turns the horse, and examines the lower ear.

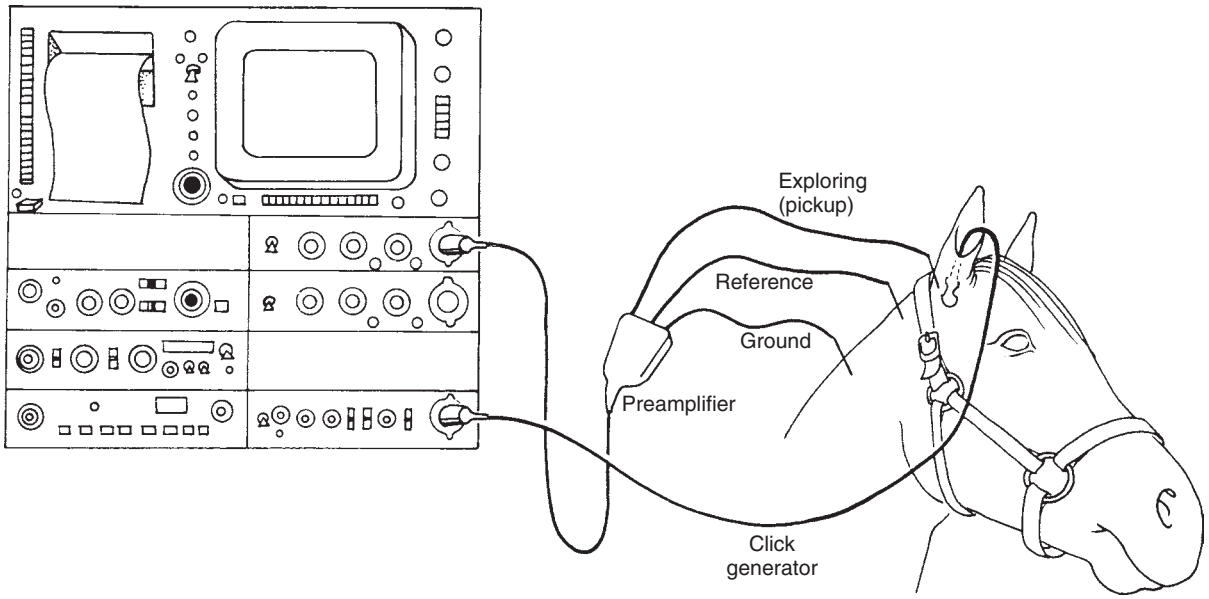


FIG. **11.15** System used for auditory brainstem response (ABR) testing in the horse. (From Rolf SL, Reed SM, Melnick W, et al.: Auditory brainstem response testing in anesthetized horses, *Am J Vet Res.* 48:910-914, 1987.)

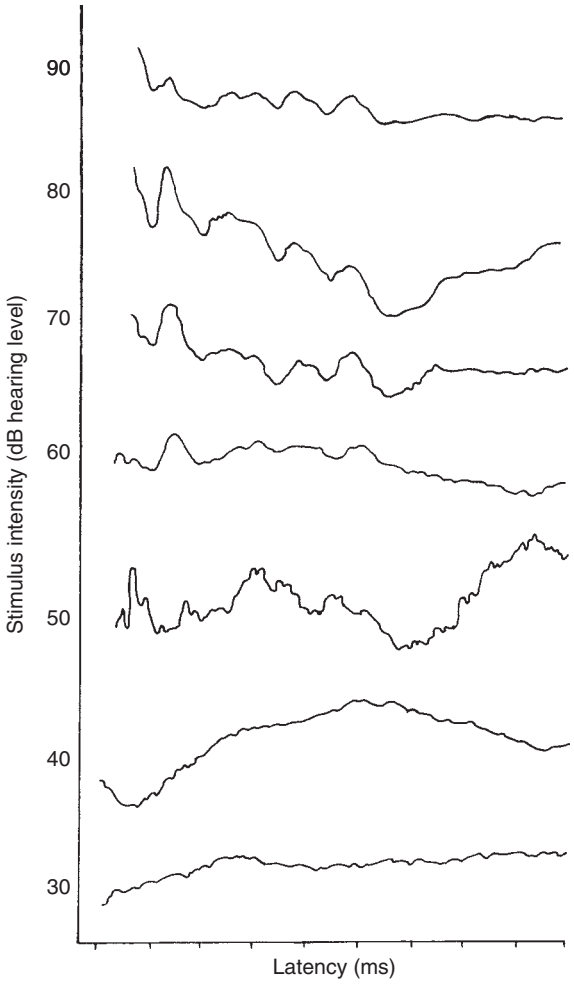


FIG. **11.16** Mean latency waves at 136 dB, sound pressure level (SPL) through 87 dB SPL. Five waves are present, except at 40 of 80 dB SPL. (From Rolf SL, Reed SM, Melnick W, et al.: Auditory brainstem response testing in anesthetized horses, *Am J Vet Res.* 48:910-914, 1987.)

The most common causes of BAER abnormalities in adult horses include temporohyoid osteoarthropathy, congenital sensorineural deafness in Paint Horses, multifocal brain disease, and otitis media/interna. BAER can be used in horses with vestibular disease (Fig. 11.17) to investigate if hearing is also affected as is the case in horses with temporohyoid osteoarthropathy. BAER testing might be helpful in the diagnosis and prognosis of traumatic, infectious, or inflammatory brainstem lesions such as vascular infarcts or anomalies, ischemic fibrocartilaginous emboli, basisphenoid bone fracture, and protozoal encephalomyelitis. Reported causes of hearing loss in neonatal foals include congenital sensorineural hearing loss, congenital anomalies (skull deformation), sepsis, neonatal encephalopathy, neonatal isoerythrolysis, and prematurity.[61,72]

Electroencephalography

Electroencephalography (EEG) is the graphic representation of the difference in voltage in microvolts between two different locations within the cerebral cortex plotted over time. EEG recordings represent spontaneous electrical activity primarily synaptic, excitatory postsynaptic potentials, and inhibitory postsynaptic potentials from cortical neurons. Pyramidal cells are the most important neuronal source for the EEG. It is estimated that a minimum of 108 neurons or an area of 6 cm^2 is required to produce a detectable change in voltage. Thalamic oscillations are transmitted to the cortex via projection neurons and are thought to be responsible for the slow waves and spindles associated with slow wave sleep (SWS). This electrical activity might be modified by deeper structures such as the brainstem, reticular activating system, and thalamus. EEG is a valuable tool for the investigation of brain disease, particularly seizures and sleep disorders, localization of abnormal activity, and extent (focal or diffuse). EEG has been used extensively in other species and more limited in horses.[74-88] In horses, EEG has been used to study sleep, seizures, nociception, effects of drugs on cerebral activity, and euthanasia.[74-88]

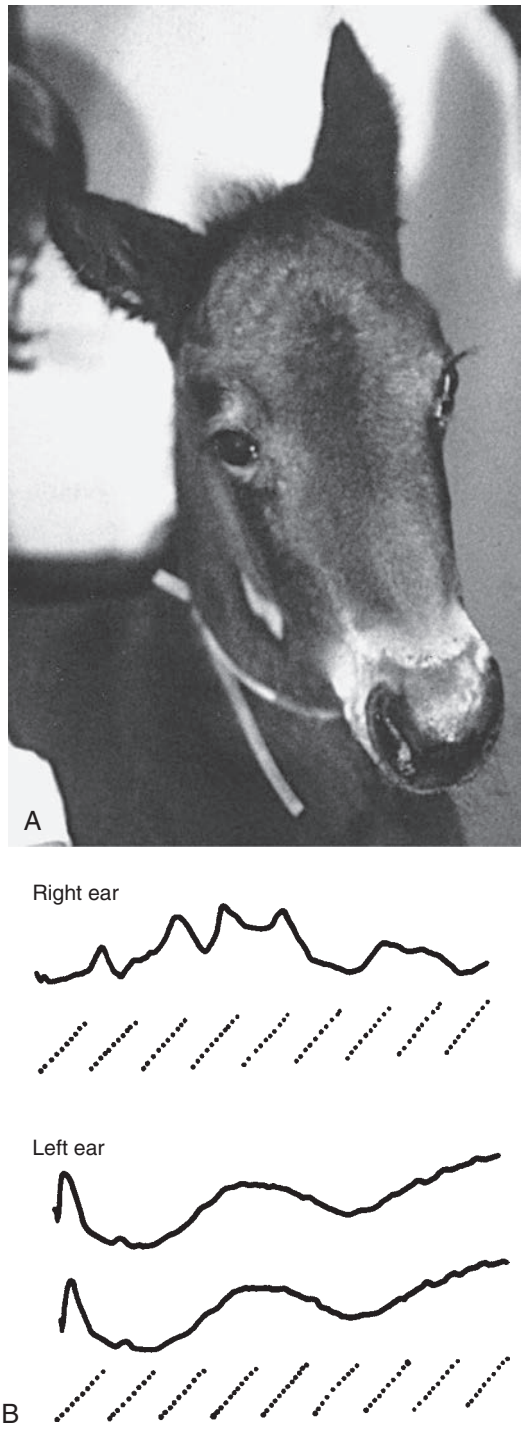


FIG. **11.17** A, Photograph of foal with a right head tilt and drooping of the right ear. B, Auditory brainstem response (ABR) (see Fig. 11.18) shows a hearing loss in the left ear, the presumed cause of the head tilt. The right ear was normal.

Two EEG montages have been described: bipolar and referential. Electrode placement could be rostral to caudal or transverse in a bipolar montage (Fig. 11.18). EEG recordings are evaluated for symmetry, waveform, morphology, frequency, and amplitude. There is a wide range of frequencies but confined to a relatively low range (0.5–50 Hz) with most below 30 Hz. Amplitude varies from a few to several hundred microvolts but usually remaining below 100 μV. Waveform nomenclature

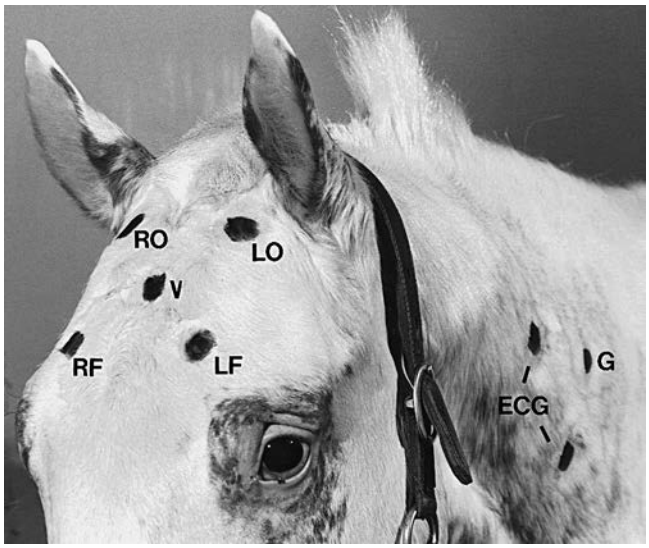


FIG. **11.18** Photograph of horse illustrating bipolar montage for electroencephalography (EEG). *RO,* Right occipital; *LO,* left occipital; *RF,* right frontal; *LF,* left frontal; *V,* vertex; *G,* ground; *ECG,* electrocardiogram position.

is as follows: delta 0 to 3 Hz, theta 4 to 7 Hz, alpha 8 to 12 Hz, beta 13 to 29 Hz, gamma ≥30 Hz.

Whenever possible EEG should be done in the nonanesthetized horse because the effects of drugs on EEG recordings such as suppression of EEG activity, transient paroxysmal discharges, and the lack of observation of all states of vigilance. The major disadvantage of performing EEGs in the sedated horse is movement artifacts. However, interpretable recordings are possible. Further, the states of vigilance can be evaluated. States of vigilance in the horse as determined through EEG consist of wakefulness, drowsiness, slow wave sleep, and rapid eye movement (REM) sleep.[86]

Transcranial magnetic stimulation testing: Although not entirely new to equine neurology, transcranial magnetic stimulation testing has not be used a lot in evaluation of neurological disease in horses. In human medicine, evoked potentials are widely used to quantify the function of both descending and ascending spinal cord tracts. The descending tracts, which carry motor information from the brain to spinal cord neurons, can be evaluated using transcranial magnetic motor evoked potentials (TMMEPs). These evoked potentials are non-painful and produced by electromagnetic stimulation of the brain in the conscious human or animal with simultaneous electrical recordings made from needle electrodes in relevant muscle groups.[265]

MAGNETIC RESONANCE IMAGING

Katherine S. Garrett

Although magnetic resonance imaging (MRI) has been used for diagnosis of neurologic disease in human beings for decades, it has only come into clinical use for horses within the past 15 years.[89-102] Imaging of the brain and spinal cord is essentially impossible without MRI or computed tomography (CT), so this represents an exciting new imaging modality to aid in the diagnosis of neurologic disease in horses.

Principles

Magnetic field strength is measured in Tesla (T). The strength of the magnetic field varies among types of magnets but is typically between 0.25 T and 1.5 T for most magnets currently in routine equine clinical use, although 3.0-T magnets will likely become more widely available in the near future.

Most MRI applications use hydrogen nuclei (protons) as the molecule of interest, but other nuclei can be used. Application of the strong magnetic field causes the magnetic moments of the protons within tissues to orient themselves parallel to the main magnetic field. A radiofrequency pulse at a specific frequency is then applied, which causes a change in the cumulative magnetic moment of the protons and synchronizes the phase of the protons' precessions (rotation about an axis). After the radiofrequency pulse is discontinued, the protons will return to the resting state governed by the main magnetic field alone, and the precessional phase synchrony will degrade. Different tissues return to the resting state at different times and in different ways. In a basic sense, the time for the magnetic moment of a tissue to return to the direction of the main magnetic field is a description of the T1 time of the tissue, and the time for the phase coherence to degrade is a description of the T2 time of the tissue. These are inherent properties, and the differences between tissues form the basis of MRI.

Different pulse sequences produce different types of contrast, based on each tissue's hydrogen content and its response. Proton density sequences have contrast determined by the proton content of each tissue. Cortical bone, tendons, ligaments, and air have low proton density, so they appear hypointense (dark) on all sequences, as well as on proton density–weighted sequences. T1-weighted sequences have contrast dominated by the T1 relaxation times of the tissues. Fat is hyperintense (bright) and fluid is hypointense. T2-weighted sequences have contrast dominated by the T2 relaxation time of the tissues. Fat is hypointense and fluid is hyperintense. Fluid attenuation inversion recovery (FLAIR) sequences have the signal from CSF specifically nulled using an inversion pulse. This allows abnormal fluid within the brain or spinal cord parenchyma to be more apparent. Intravenous contrast can also be used in MRI. Gadolinium compounds are typically used, which allow assessment of abnormal patterns of blood flow or delineation of anatomic structures, similar to the use of contrast in CT imaging.

Two major families of pulse sequences exist. Spin echo sequences use multiple radiofrequency pulses to generate a signal. Turbo or fast spin echo sequences are a subset of spin echo sequences that are faster than traditional spin echo sequences. Image contrast can be altered slightly; for example, fat on T2-weighted turbo spin echo sequences is hyperintense instead of hypointense. Gradient echo sequences use a changing magnetic field gradient to generate a signal. Gradient echo sequences can be acquired more quickly than spin echo sequences but are more susceptible to magnetic field inhomogeneities.

For each series of slices, the operator must choose the orientation of the slices, as well as the type of pulse sequence used. A typical MRI examination will consist of many series, each with a different combination of orientation and pulse sequence. Unlike CT imaging, the orientation of the images obtained during each pulse sequence can be selected by the operator. These are typically 1- to 5-mm-thick slices. A complete examination produces hundreds of individual images to be reviewed, and it usually takes between 1 and 2 hours to complete the acquisition of images. Some series are acquired in a way that permits reconstruction in multiple planes or in three dimensions.

Equipment

The main magnetic field can be generated in a variety of ways. Permanent magnets are used in lower field systems (less than 0.4 T). These magnetic fields are generated by using ferromagnetic materials of the same type as in everyday magnets. Stronger magnetic fields are produced using superconducting electromagnets. These magnets use liquid helium to cool the electromagnetic coils and reduce the resistance, allowing much stronger magnetic fields to be generated. Liquid helium is expensive and requires periodic replenishment, but it is necessary to efficiently generate the stronger magnetic fields in clinical use. Regardless of the type of magnet, the room must be shielded to prevent any external radiofrequency pulses (e.g., radio signals, cellular phone transmission) from entering the room and introducing artifacts.

Two types of MRI systems are used for horses. One type consists of systems designed for use in human beings. These tend to be high field magnets with strengths ≥1.0 T of closed bore construction and require the horse to be under general anesthesia. The horse is positioned either in lateral or dorsal recumbency on a nonferromagnetic padded table with the body part of interest positioned into the bore. Foals can be imaged using the human patient table in any recumbency.

The other group consists of magnets that have been specifically designed for veterinary use. These magnets are generally low field magnets with a strength in the 0.25-T range. Many have an open bore design and can be positioned about a body part; some require general anesthesia and some only sedation. The images produced with these weaker magnets require longer scan times to obtain and are of lower resolution. Motion artifact in studies obtained on standing horses can significantly reduce image quality.

Examinations are limited to regions that can fit into the bore of the magnet to be positioned at or near the isocenter (center of the magnetic field). Depending on the type of magnet, this requires that either the horse be pushed into the center of the magnet bore or that the magnet be positioned around the region of interest. These limits will vary among magnets based on their construction.

Safety

Most safety hazards associated with MRI are related to the introduction of ferromagnetic objects into a strong magnetic field, as opposed to the existence of the magnetic field itself. No known adverse effects have been reported for MRI at field strengths currently used in equine clinical imaging. Any object that is magnetic (scissors, oxygen tanks, scalpel blades) will be attracted to the magnet; thus all the equipment in the room must be nonmagnetic or MRI compatible, including the anesthesia equipment. The strength of the magnetic field increases exponentially as the distance to the magnet decreases. In practical terms, this means that the pull of the magnet on any ferromagnetic object becomes quickly stronger as the object approaches the magnet (to prevent injury this is especially important to bear in mind when considering stronger magnets).

The strong magnetic field can also affect internal implants and is especially dangerous for people with cardiac pacemakers, who should not enter an MRI room under any circumstances because of the potential for serious cardiac arrhythmias resulting from the effect of the magnetic field on the pacemaker. Metal shards in the cornea can also be affected by the magnet and can move within the cornea, causing ocular damage. The changing of the magnetic gradients can be quite loud. Any personnel who remain in the scan room during the examination should wear protective equipment to prevent hearing damage.

Patient Preparation

Horseshoes should be removed before the examination to eliminate the risk of potential magnetic attraction. Appropriate preparations should be carried out for images requiring general anesthesia. After induction to anesthesia but before entering the scan room, any metal objects (e.g., halters) should be removed from the horse. A specialized, nonferromagnetic table must be used to support the horse during the examination. After the horse is positioned in the bore of the magnet, it must remain completely motionless during image acquisition to prevent blurring of the image. Because MRI examinations typically take between 1 and 2 hours, urinary catheterization or some other means of urine collection is recommended to protect the equipment from contamination with fluid. Because an MRI examination can be lengthy, it is extremely important that the primary region of interest be localized as specifically as possible. MRI has proven particularly useful in areas that are not amenable to examination using other diagnostic imaging modalities.

Use of Magnetic Resonance Imaging in Equine Neurologic Disease

In adult horses, examination is limited to the brain and cranial cervical spine because of limitations of current magnet design. However, the trunk of foals up to approximately 500 lb may fit inside the bore of some magnets, enabling imaging of any part of the spinal column. Few reports exist of clinical use of MRI in neurologic disease.[89-102] Some examples of neurologic disease include nigropallidal encephalomalacia, head trauma, brain abscess, pituitary mass, cholesterol granuloma, congenital hydrocephalus, trigeminal neuritis, stylohyoid bone fracture, EPM, and cervical vertebral compressive myelopathy.[89-91,93,95,97,98,101,102] Diagnoses of equine protozoal myelitis, neoplasia, inflammatory disease, encephalomyelitis, meningitis, otitis media, hydrocephalus, and ischemic injury have been made at the author's clinic. In addition, substantial information has been gained regarding the extent of damage after traumatic injury. Although this clinic's experience with MRI of the nervous system is limited, the possibilities of this technique are exciting.

Pituitary Adenoma

A 13-year-old Thoroughbred mare had decreased mentation, poor appetite, weight loss, and absence of normal estrous cycles. The decreased mentation had progressed to prolonged periods of somnolence, slow circling behavior, and an apparent decrease in vision. The mare had decreased menace and pupillary light responses and demonstrated slight weakness on a tail pull test. An anatomic diagnosis of a CNS lesion rostral to the foramen magnum was made. Endogenous adrenocorticotropic hormone, triiodothyronine (T_3), and thyroxine (T_4) levels were normal. An MRI examination was undertaken in an attempt to find the cause of the neurologic signs and to further investigate a possible pituitary lesion.

A mass was found on the median plane ventral to the diencephalon, with the ventral aspect of the mass within the sella turcica (Fig. 11.19). A portion of the pituitary distinct from the mass can be identified in part of the sella turcica. The mass compresses the thalamus, hypothalamus, and optic chiasm. Portions of the mass appear hemorrhagic, and regions of the thalamus and hypothalamus surrounding the mass are edematous.

This horse was treated with pergolide and cyproheptadine in an attempt to ameliorate the signs of pituitary pars intermedia dysfunction, and improvement was seen for a brief period of time. Its condition then deteriorated after 6 weeks with progressive anorexia, weight loss, and depression despite aggressive supportive care. The mare was euthanized, and necropsy results showed a large pituitary mass with histologic features consistent with a pituitary macroadenoma of the pars intermedia.

Equine Protozoal Myeloencephalitis

An 18-year-old Thoroughbred gelding became ataxic 9 months before presentation. Two months before presentation, the horse started to exhibit weight loss, dysphagia, and facial muscle wasting. The animal had been treated for equine protozoal myelitis twice before presentation and exhibited grade 3/4 ataxia in all limbs. In addition, the horse had atrophy of the left facial muscles, as well as the

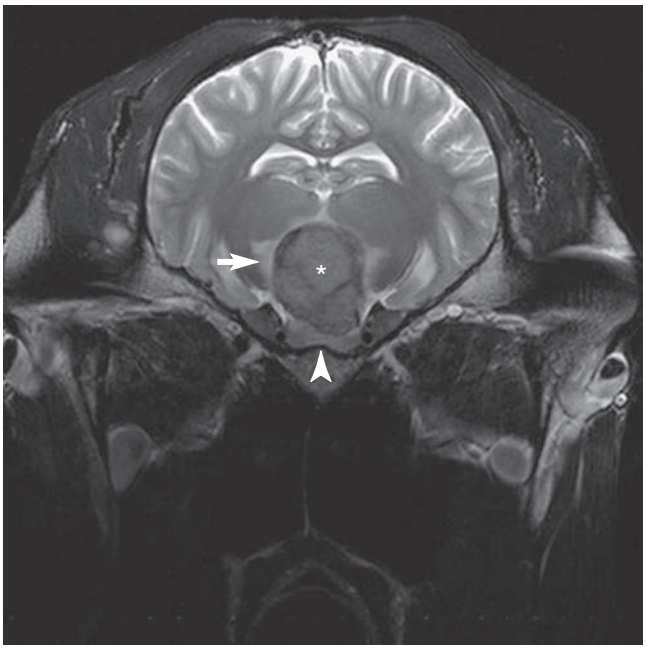


FIG. **11.19** Transverse T2-weighted fast spin echo image of the brain at the level of the sella turcica. The *asterisk* indicates a large mass of heterogeneous signal intensity displacing the diencephalon laterally. The *arrowhead* indicates normal pituitary tissue compressed by the mass. The *arrow* indicates high T2 signal (edema) within the diencephalon adjacent to the mass.

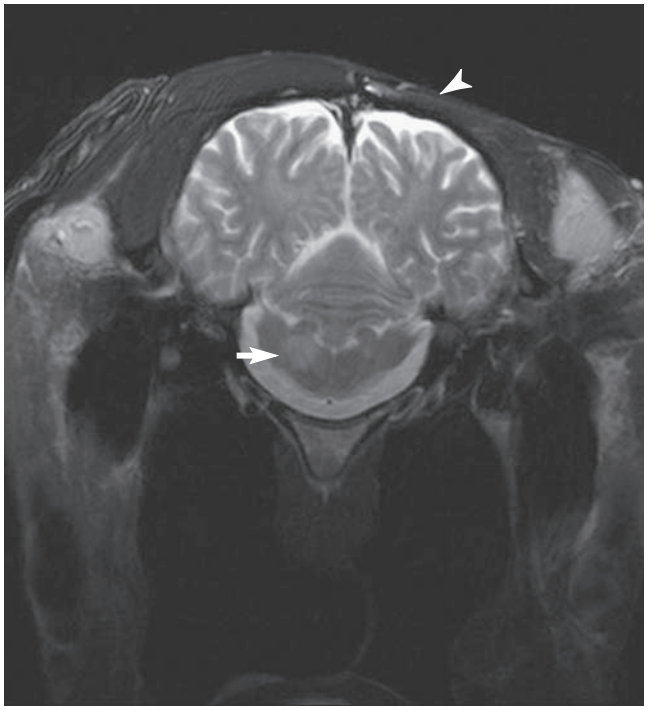


FIG. **11.20** Transverse T2-weighted fast spin echo image of the brain at the level of the pons. The arrow indicates an area of T2 hyperintensity in the region of the nuclei of the seventh and eighth cranial nerves. The arrowhead indicates temporalis muscle atrophy.

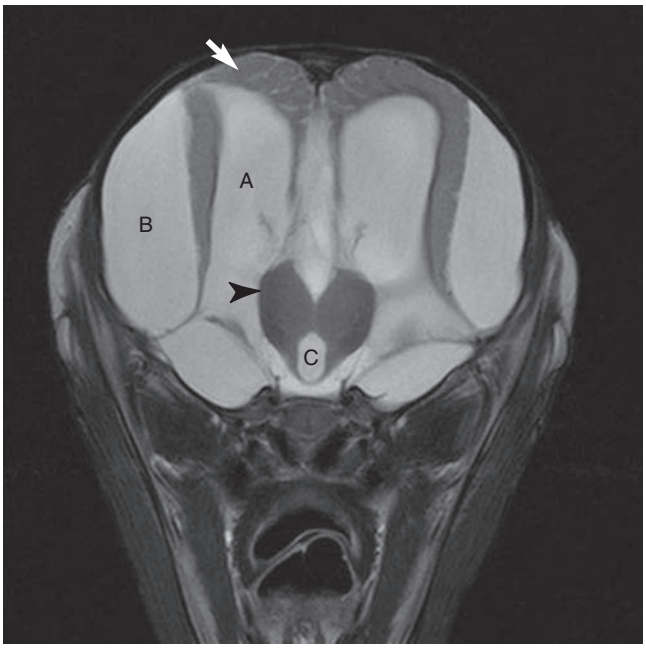


FIG. **11.21** Transverse T2-weighted turbo spin echo image of the brain at the level of the thalamus. The lateral ***(A)*** and third ***(C)*** ventricles are massively dilated with cerebrospinal fluid (CSF) present outside of the brain ***(B)***. The *arrow* indicates the cerebral cortex, and the arrowhead indicates the thalamus.

left temporalis and masseter muscles, muzzle deviation to the right side, and difficulty moving food to the pharynx with normal tongue tone, prehension, and ability to swallow. These neurologic signs suggested multifocal disease with involvement of cranial nerves V, VII, IX, and X, as well as the cervical spinal cord and brainstem. Equine protozoal myelitis Western blot was weakly positive on the CSF and positive on serum.

MRI findings consistent with inflammation and edema were seen throughout the right side of the medulla and caudal pons in areas including the nuclei of cranial nerves V, VII, VIII, and XII (Fig. 11.20). These findings are consistent with those seen in a previously reported case of EPM.[95]

Nitazoxanide, vitamin E, and flunixin meglumine were administered. Supportive care, including intravenous fluid therapy and enteral feeding via nasogastric tube, was also performed. The horse became progressively more depressed and developed pleuropneumonia 1 week after admission, likely secondary to aspiration because of dysphagia. The next day, the horse became acutely more ataxic, was unable to rise, and died. On postmortem examination, neuronal degeneration, inflammation, and protozoal organisms consistent with *Sarcocystis neurona* were identified in the nuclei and nerves of cranial nerves V, VII, and VIII.

Hydrocephalus

A 1-month-old Quarter Horse filly was noticed by the owners to have a dome-shaped head, blindness, and obtundation. A congenital brain lesion was suspected.

MRI images showed severe communicating hydrocephalus with a specific location of obstruction not identified (Fig. 11.21). The ventricles were enlarged and filled with CSF. The parenchyma of the cerebral hemispheres was thinned, and the cerebellum was compressed and herniated through the foramen magnum. A meningocele and an open fontanelle were noted at the junction of the left and right frontal and parietal bones.

Treatment was not pursued, although the possibility of placement of a ventriculoperitoneal shunt was discussed with the owners. The foal remained alive and was weaned successfully.

Spinal Cord Compression

A 4-month-old Thoroughbred colt had a 2-day history of pyrexia and "knuckling over" of the left hind fetlock. Ultrasound examination of the lumbar, sacral, and gluteal regions showed an abscess within the musculature. The extent of the abscess could not be determined but was suspected to involve the pelvis, so MRI examination was undertaken.

Severe compression and inflammation of the spinal cord in the sacral region was seen on MRI (Fig. 11.22). The left side of the sacrum had heterogeneous signal intensity suggestive of osteomyelitis.

The foal was treated with antiinflammatory and antimicrobial medication and establishment of drainage of the intramuscular portion of the abscess. The foal's condition improved, and it was discharged on long-term antimicrobial therapy. During follow-up MRI examination 2 months later, the compression of the spinal cord had been relieved, and the intramuscular abscess had decreased in size. The heterogeneous signal intensity within the sacrum had improved but not resolved completely.

Conclusion

Although MRI of equine neurologic disease remains in its infancy, veterinarians are increasingly able to use this

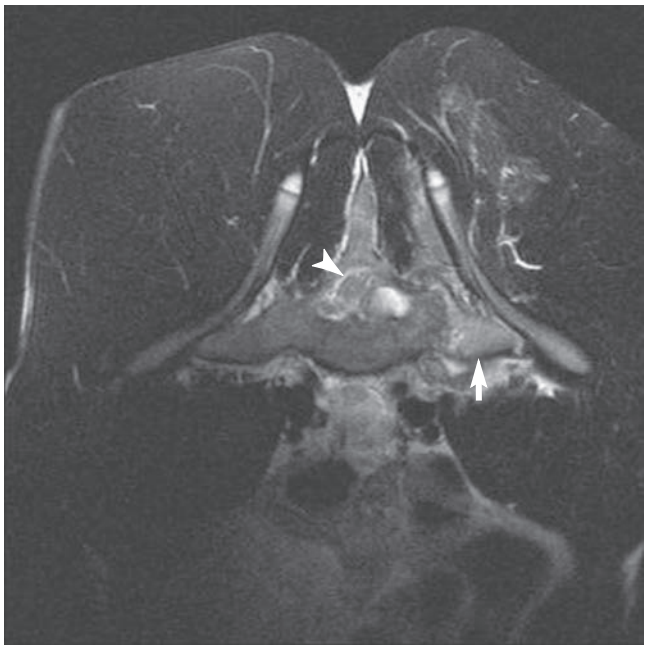


FIG. **11.22** Transverse T2-weighted turbo spin echo image of the spinal canal at the level of the sacroiliac joint. The *arrowhead* indicates the laterally compressed spinal cord, and the *arrow* indicates a region of edema and proteinaceous fluid accumulation within and around the sacrum (osteomyelitis).

technology to make more specific and timely diagnoses. As the knowledge base regarding MRI progresses, practitioners will be able to advance their care and treatment of equine patients to new levels.

SEIZURES AND SLEEP DISORDERS

Seizures

Monica Aleman

Terminology

Seizures (from the Latin *sacire,* "to take possession of") have been referred as fits, attacks, strokes, convulsions, or epilepsy and were first described around 500 to 700 B.C. Seizures are a common problem in both human and veterinary medicine but are less common in horses compared with other species. Seizures are clinical manifestations of rapid excessive and/or hypersynchronous abnormal neuronal activity from the cerebral cortex that result in involuntary alterations of motor activity, consciousness, autonomic functions, or sensation.[103-105] Seizures can occur as single or recurrent events. Recurrent seizures are termed *epilepsy.* There has been a great deal of confusion about the definition and classification of *seizures* and *epilepsy,* terms that have been used mistakenly as the same. The International League Against Epilepsy (ILAE) has defined *epileptic seizure* as "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain" and *epilepsy* as "a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, congnitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure." *Status epilepticus* (SE) is defined as "a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally prolonged seizures (after time point t1). It is a condition [that] can have long-term consequences (after time point t2), including neuronal death, neuronal injury, and alteration of neuronal networks, depending on the type and duration of seizures." In the case of tonic-clonic convulsions, the time points are 5 minutes for t1 and 30 minutes for t2. Emergency treatment of SE in human medicine is instituted if epileptic seizure is greater than 5 minutes.

Epileptic seizure has a specific neural origin and can occur as a single event or be recurrent. Epileptic seizures can be further classified as primary (i.e., idiopathic), secondary (as the result of structural cerebral abnormalities), or reactive (reaction of the healthy brain to transient systemic insult). Epilepsy specifically refers to reoccurring epileptic seizures from a chronic brain disorder, which may be genetic (primary) or acquired (secondary). Epilepsy has been rarely documented in horses, except for juvenile idiopathic epilepsy in Arabian foals.[75] Finally, convulsions refer to seizures accompanied by tonic-clonic muscle activity and loss of consciousness. Classification of epilepsy has been done by the ILAE in human medicine. Previously, epilepsy was classified as idiopathic (primary) and symptomatic or cryptogenic (secondary). Idiopathic epilepsies included disorders with suspected genetic etiology with no brain abnormalities. Symptomatic epilepsies have a known cause that involves brain pathology, whereas cryptogenic epilepsies were those with unknown etiology. Classification of epilepsy has evolved to genetic, structural, and unknown. In small animal medicine, the International Veterinary Epilepsy Task Force reported a consensus on epilepsy definition, classification, and terminology.[103,105]

Similar to standardized classifications described in small animals and humans, seizures in horses can be classified as being partial (simple and complex), generalized (primary or secondary), or status epilepticus.[106,107] Seizures that involve a discrete area of the cerebral cortex are classified as simple partial seizures when consciousness is not impaired and complex partial seizures when consciousness is impaired. Partial seizures result in localized clinical signs, such as facial or limb twitching, compulsive running in a circle, or self-mutilation. A partial seizure might be observed following a procedure such as collection of CSF, cervical myelography, anesthesia, or cranial trauma that might spread throughout the cerebral cortex and produce a secondary generalized seizure. A generalized seizure involves the entire cerebral cortex and results in generalized tonic-clonic muscle activity over the whole body, with loss of consciousness. Seizures generalized from the onset are defined as primary generalized seizures. Secondary generalized seizures could originate from both types of partial seizures. The most common types of seizures observed in an equine referral center were partial seizures (simple or complex, 30%) and secondary generalized seizures (30%).[106] This is in agreement with data in dogs, for which partial seizures with or without secondary generalization appear to be the most common types of seizures observed. Complex partial seizures are commonly observed in neonatal foals and are referred as "chewing-gum fits," jaw chomping, and lip smacking.[4]

TABLE **11.4 Known and Suspected Causes of Seizures in Horses Less Than 1 Year of Age**

	Differential Diagnosis		
Classification	**Extracranial**	**Intracranial**	**Diagnostic Aids[a]**
Anomalies (congenital)		Hydrocephalus	
		Hydranencephaly	1–6, 8
		Benign epilepsy	1, 10
Metabolic	Hypoxia, hyponatremia, hypoglycemia, hyperkalemia		2–6
Toxic	Organophosphates	Moldy corn	2–6
	Strychnine	Locoweed	9–11
	Metaldehyde		
Traumatic		Brain trauma	2–6, 8
		Lightning	2–5
Vascular		Neonatal maladjustment syndrome (vascular accidents)	2–6
Infectious	Septicemia	Bacterial meningitis	2–6
	Hyperthermia	Cerebral abscesses	5, 6, 8
		Rabies	2, 3, 11
		Viral encephalitis	6, 7

[a]Key: 1, Breed; 2, onset; 3, clinical course; 4, physical examination; 5, neurologic examination; 6, clinical pathology (e.g., cerebrospinal fluid analysis); 7, serologic testing; 8, radiology (computed tomography scan, ultrasound, radiographs); 9, toxicologic testing; 10, electrodiagnostics (electroencephalography, electromyelography); 11, pathologic examination.

Etiology/Pathogenesis

Etiologies of seizures in horses include intracranial and extracranial diseases (for which the healthy brain is reactive to an insult) (Tables 11.4 and 11.5). The most common causes of seizures in foals under 2 weeks of age are neonatal maladjustment syndrome (both hypoxic-ischemic and nonhypoxic encephalopathy), trauma, and bacterial meningitis.[108-111] The most common causes of seizures in foals less than 1 year of age are shown in Table 11.4. The most common causes of seizures in adult horses older than 1 year of age are brain trauma, hepatoencephalopathy, and toxicity.[105] Masses such as melanoma, pituitary adenoma, cholesteatoma, and rarely glioma can cause seizures in mature to older horses.

Irrespective of the etiology, the pathogenesis of epileptic seizure is caused by an abnormal hypersynchronous electrical activity of neurons, caused by an imbalance between excitation and inhibition in the brain.[104,112-116] If the balance shifts toward excessive excitation (theory of excitatory postsynaptic potentials), a prolonged depolarization of neuronal aggregate will occur, a phenomenon known as "paroxysmal depolarization shift," which might generate an epileptic seizure. Several mechanisms are thought to cause paroxysmal depolarization shifts and include increased excitatory neural transmitters (e.g., glutamate), decreased inhibitory neural transmitters (e.g., γ-aminobutyric acid), alteration in neural transmitter receptor sites, or a derangement in the internal cellular metabolism of the neuron. Notably, much research has focused on glutamate, the principal excitatory neurotransmitter in the brain, and its receptor complex, the *N*-methyl-D-aspartate (NMDA) receptor. Glutamate binds to NMDA receptors, which open sodium and calcium channels, leading to entry of these ions in the neuron and to postsynaptic depolarizations.[112-116] In addition, NMDA receptors have been implicated in the pathogenesis of seizures in infants and foals because if exacerbated (e.g., during perinatal asphyxia), intracellular calcium overload causes neuronal necrosis by activation of lytic enzyme systems and nitric oxide synthase with generation of free radicals.[113,114] Conditions leading to the summation of excitatory postsynaptic potentials include excessive excitations and also loss of inhibitions without normal regulatory feedbacks. In response to the sudden depolarization shift of a neuronal aggregate, local surround inhibitory zones are established to try to prevent the spread of the epileptogenic activity. γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the CNS. GABAergic inhibition can be presynaptic (release of GABA from the GABAergic nerve terminal into presynaptic nerve terminals causing a reduction of neural transmitter release) or postsynaptic (caused by the interaction of GABA with specific postsynaptic receptors).[115] In GABA-system dysfunction, an abnormal neuronal hypersynchronous activity will spread, resulting in depolarization of more neurons.[115] Once a "critical mass" of neurons has fired, uncontrolled spread of electric activity may occur over the cerebral cortex, which may precipitate an epileptic seizure. Thus these seizures can be blocked by use of agents that potentiate inhibitory neural transmitters, such as phenobarbital. In brief, seizures may develop because of a summation of synchronous excitatory postsynaptic potentials in large groups of neurons that may be precipitated by an increase in excitatory neurons, a decrease in inhibitory neurons, a decrease in inhibitory neurotransmitters, or any combination of these mechanisms.

Alterations in the neuronal cell microenvironment might also lead to seizure generation. Systemic and local neuronal electrolyte abnormalities might disturb excitatory neuron homeostasis and lead to spontaneous and excessive action potentials.[115] Intracellular potassium released during neuronal activity may reach sufficient concentration to move the resting membrane potential toward the threshold and

TABLE **11.5 Known and Suspected Causes of Seizures in Horses More Than 1 Year of Age**

	Differential Diagnosis		
Classification	**Extracranial**	**Intracranial**	**Diagnostic Aids[a]**
Metabolic	Hepatoencephalopathy, hypocalcemia, uremia, hyperlipidemia		2–6, 8
Toxic	Organophosphates	Moldy corn	2–6
	Strychnine	Locoweed	9, 11
	Metaldehyde	Bracken fern	
		Lead, arsenic, mercury	
		Rye grass	
Traumatic		Brain trauma	2–6, 8
Vascular		*Strongylus vulgaris*	2–5
		Cerebral thromboembolism	5, 6, 11
		Intracarotid injection	2, 3
Tumor		Neoplasia	2–5, 8, 10, 11
		Hemarthroma	6, 8, 10, 11
		Cholesterol granuloma	8, 11
Infectious		Cerebral abscess	5, 6, 8
		Rabies	2, 3, 11
		Arbovirus encephalitides	6, 7
		Mycotic cryptococcosis	5, 6
		Equine protozoal myeloencephalitis	6, 7

[a]Key: 1, Breed; 2, onset; 3, clinical course; 4, physical examination; 5, neurologic examination; 6, clinical pathology (e.g., cerebrospinal fluid analysis); 7, serologic testing; 8, radiology (computed tomography scan, ultrasound, radiographs); 9, toxicologic testing; 10, electrodiagnostics (electroencephalography, electromyelography); 11, pathologic examination.

generate a seizure focus. Spontaneous action potentials generated by alteration in intracellular potassium concentration may spread to other parts of the cerebral cortex, causing a generalized seizure. Alterations in one intracellular neuronal electrolyte may alter the homeostasis of other intracellular electrolytes. Increased intracellular potassium concentration and decreased intracellular calcium, magnesium, and chloride concentrations are observed during interictal epileptogenesis and the transition from interictal to ictal activities.

Alterations in sodium conductance have also been implicated in the onset of seizures. Rapid influxes of sodium into the neuron may lead to hyperexcitability of the neuron and rapid firing. Phenytoin, a hydantoin derivative, blocks these rapid influxes of sodium into neurons and suppresses repetitive firing by hyperexcitable neurons.[116] Thus a complex interaction may occur between these electrolytes and the internal cell homeostasis that precipitates seizure formation.

Clinical Signs and Diagnosis

Seizure activity is manifested clinically in a variety of ways depending on the area and the extent of the cerebral cortex involved. In partial seizures, asymmetric twitching of a limb, facial twitching, excessive chewing, compulsive behavior, or self-mutilation might occur. A localized seizure might develop into a generalized seizure. Three distinct clinical periods might be observed in a generalized seizure. Just before the seizure (aura), horses might exhibit signs of anxiety and uneasiness. During the seizure (ictus), horses might become recumbent, unconscious, and have symmetric clonic muscle contractions (contractions and relaxations of muscles occurring in rapid succession), followed by symmetric tonic muscle contractions (continuous unremitting muscle contractions).[4,75,106,107] Horses also might show deviation of eyeballs, dilated pupils, ptyalism, trismus or jaw clamping, opisthotonos, lordosis or kyphosis, violent paddling movements of the limbs, uncontrolled urination and defecation, and excessive sweating. After the seizure (postictus), horses might appear obtunded, disoriented, and blind for a few minutes, hours, or days.[4,75,106,107]

Diagnosis of seizure is based on history, clinical signs, and ancillary diagnostic tests to determine an underlying cause, whenever possible (see Tables 11.4 and 11.5). Diagnosing seizures in horses can be challenging. Because many horses are unobserved for long periods of the day, the presence of a seizure might be suspected after recognition of unexplained trauma to the head or limbs and disruption of the stall or outside paddock. At other times, the caretaker might identify a change in attitude or behavior of the horse such as restlessness or a "far-off" appearance, which might be a prodromal sign (aura) of an impending seizure. Careful and thorough questioning of the caretaker should be performed to determine the duration and frequency of the episodes, date(s), the time of day, the relationship to feeding, any event(s) associated with the episode (e.g., injection in the carotid and traumatic event), any unusual environmental circumstances (stimuli such as thunderstorms, fireworks, changes in housing), recent trauma, febrile episodes, exposure to drugs or toxins, recent behavioral changes, and the seizure history of the dam, sire, and other siblings. The veterinarian should always consider rabies in the list of differential diagnoses, even if the horse has been vaccinated and especially in endemic areas.

A complete physical examination should be performed to rule out noncerebral clinical conditions that might mimic seizures, including painful conditions (acute severe pain, tremors and fasciculations, episodes of hyperkalemic periodic

paralysis, sleep disorders). In these conditions, horses do not lose consciousness but remain bright and alert. Horses with HYPP might show prolapse of the nictitating membranes of the eye and muscle fasciculations but remain anxious, alert, and have normal pain perception. Cardiac disorders might precipitate syncopal episodes, which could be confused with seizures. Auscultation of the heart might help identify the presence of murmurs, and electrocardiography may help determine the presence of arrhythmias.

A complete blood cell count (CBC) and serum biochemistry analysis should elucidate the presence of systemic extracranial disease, which may predispose to seizures. CBC and fibrinogen may suggest inflammation (leukocytosis and hyperfibrinogenemia) or viral infection. A complete serum biochemistry profile should be performed to rule out metabolic abnormalities that might trigger seizures. Hypoglycemia could be a cause of seizures in both adults and foals. An arterial blood gas evaluation is indicated to rule out hypoxia. Liver function tests, liver biopsy, and determination of serum ammonia concentration should be performed if hepatic encephalopathy is suspected.

CSF evaluation might be helpful in approaching a diagnosis but commonly does not provide a definitive diagnosis. A normal cytology and biochemistry profile does not rule out disease. The site of choice is the atlantooccipital cistern, but this may be contraindicated if the horse is showing signs of increased CSF pressure (such as mydriasis or papilledema) or if general anesthesia is not appropriate. Alternatively, CSF fluid collection from the lumbosacral space could be performed, providing that it can be safely done in the standing and sedated animal. Interpretation of equine CSF abnormalities is described previously in this chapter.

EEG, the graphic recording of the rhythmic bioelectrical activity arising predominantly from the cerebral cortex, is the most important clinical test in humans suffering from epilepsy. Similarly, this diagnostic test has been useful in horses with seizures.[80,85-88,117] Paroxysmal activity includes spikes, sharp waves, spike-and-wave discharges, and other transient events.[80,85-88,117] Focal, multifocal, or generalized epileptiform activity can be recorded on EEGs of epileptic patients, depending on the localization of the discharges and the extent of the cerebral cortex involved.[80,85-88,117] The lack of recording of epileptical activity does not rule out seizures.[88] The proximity of a seizure to the EEG examination might influence the amount of epileptiform activity present in the EEG. Therefore consideration of performing more than one EEG examination is recommended.[80,85-88,117] Horses suffering from extracranial diseases might also have an abnormal EEG recording such as during metabolic diseases.[80,85-88,117]

The best imaging modality specifically for the brain is MRI. Other modalities might aid in identifying abnormalities of the skull, including trauma that might be associated with or causing seizures. Those modalities include skull radiographs and CT.

Treatment

The goals of treatment are to stop ongoing seizures, address underlying diseases, and prevent future seizure activity.

Initial Therapy: Controlling Seizures. Prompt control of seizures is a priority as prolonged or recurring seizures might result in neuronal injury, self-trauma, and trauma to caregivers. Short- and long-term anticonvulsants have been used in horses.[75,118-121] Short-term treatment implies controlling seizures at the time they are occurring. Long-term therapy should be considered if recurrent seizures occur. However, long-term therapy can be expensive, and expectations must be discussed with the owner because antiseizure medication is not a curative measurement and the horse might still have seizures while on medication. For short-term management, drugs such as benzodiazepines (diazepam, midazolam) or intravenous barbiturates (phenobarbital) can be used.[114]

Benzodiazepines hyperpolarize neuronal cells by binding to GABA receptors, resulting in change of the chloride conductance pathways and making cells resistant to depolarization. The overall result is a decrease in the electrical activity of the seizure focus and an increase in the seizure threshold.[114] Although quite effective, the half-life of diazepam is short, 10 to 15 minutes, making repeated doses sometimes necessary. Caution is warranted as prolonged usage might cause respiratory depression/arrest and drug accumulation in foals as its clearance is slower in this age group.[120] In patients that do not respond to the initial bolus doses of diazepam, a constant-rate infusion of diazepam can be implemented at an initial rate of 0.1 mg/kg/hr.[75,122] Midazolam is a potent short-acting benzodiazepine that can be used in foals at 0.05 to 0.1 mg/kg intravenously or intramuscularly or as a continuous-rate infusion.[122]

Intravenous phenobarbital rapidly (within 20 minutes) provides a high serum concentration and will reduce cerebral metabolic rate. The mechanisms of action of phenobarbital include (1) facilitation of inhibitory neurotransmission via GABA receptors; (2) inhibition of postsynaptic potentials produced by glutamate; and (3) inhibition of voltage-gated calcium channels at excitatory nerve terminals. Caution must be practiced in neonatal foals, and lower loading dosages compared with adult horses can be used such as 3 to 6 mg/kg IV diluted in saline and infused over 30 minutes (unpublished data, Aleman). Start with the lowest dosage and increase if needed. Older foals and adults can be loaded with 6 mg/kg IV diluted in saline (500 mL) and given over 30 minutes. Maintenance dosage is 3 to 4 mg/kg PO q 12 h. If necessary, higher dosages or frequency of administration can be used (q 8 h instead of q 12 h). Other authors have reported higher loading dosages for foals of 20 m/kg IV followed by maintenance dosages of 9 mg/kg IV q 8 h.[118] In adult horses, a parenteral loading dosage of 12 mg/kg IV followed by 6 mg/kg IV (20-minute infusion) q 12 h has been recommended.[119] The therapeutic dosage for horses is unknown. Maintenance therapy is administered by the oral route at dosages of 3 to 6 mg/kg.

Sodium pentobarbital could be used for the short-term management (1–3 days) of seizures in neonatal foals because of its anesthetic effects. Although this anesthetic agent has little use in the management of seizures in adult horses, general anesthesia with continuous infusion of pentobarbital (for up to 24 hours) might be required for horses suffering from status epilepticus, which might not be controlled effectively with regular anticonvulsant therapy. Other agents such as propofol and ketamine might be considered for the management of status epilepticus.[123]

Primidone and phenytoin might be considered as an alternative therapy if previous drugs are ineffective. Primidone is metabolized to phenobarbital (main active metabolite) and to a smaller extent to phenylethylmalonamide (a metabolite that might potentiate the anticonvulsant effects of phenobarbital). Primidone has been reported anecdotally for treating foals with seizures.[124] Phenytoin inactivates voltage-dependent neuronal sodium channels, preventing depolarization of the

presynaptic neuronal membrane at the excitatory nerve terminal, and thus reducing release of glutamate. Adverse effects of phenytoin include prolonged depression in foals, as well as mild atrioventricular block and decrease in blood pressure in adults.[114]

Drugs such as acetylpromazine, xylazine, and ketamine should be used with caution for emergency seizure management if emergency antiseizure medication is not available. There have been reports of acetylpromazine lowering seizure threshold; however, the editor has used acepromazine safely in the past in small and large animals with seizure disorders without triggering seizures. Ketamine might exacerbate seizures because it increases cerebral blood flow, oxygen consumption, and intracranial pressure. However, ketamine also antagonizes NMDA receptors and thus appears helpful in a rodent model of status epilepticus, as well as in some neonatal foals with seizures, although further studies are required.[122]

Maintenance Therapy: Preventing Seizures. The goal of long-term anticonvulsant therapy is to reduce the frequency, duration, and severity of seizures with no or minimal adverse effects associated with such therapy. Therefore the initiation of long-acting anticonvulsant therapy depends on consideration of the underlying cause, frequency, and severity of the episodes. Client factors such as owner preference and compliance and the long-term expense of medication should also be considered. The owners should be aware that therapy for the control of seizures, particularly in adult horses, might continue for months or years. In addition, the initiation of treatment does not guarantee elimination of seizures. It is paramount to recommend against riding a horse with seizures or on antiseizure medication.

With identified intracranial diseases, the therapy should be initiated at the onset of seizures. When no etiology has been determined, the decision to place the horse on long-term maintenance therapy is based on recognition of either a cluster of seizures (greater than 3–4 times per year or 2 seizures per month) or severe generalized seizures that result in injury to the horse and danger to those in the environment regardless of frequency. Foals with idiopathic epilepsy that have several seizures over 1 to 3 days also might require long-term (months) anticonvulsant therapy.[105,108]

One should select a single anticonvulsant medication for maintenance; the choices include phenobarbital, bromide, phenytoin, and primidone. Phenobarbital is the drug of choice for long-term maintenance in horses. Phenobarbital is well absorbed following oral administration and is metabolized in the liver. It induces hepatic cytochrome P450 enzyme complex with resultant increase in its own rate of metabolism, often resulting in the need to adjust the dose over time.[125] Therapy must be tailored to fit the individual needs, using the lowest concentration effective at controlling seizures, in conjunction with monitoring of serum drug concentration. Once the seizure activity is under control, phenobarbital monitoring approximately every 60 days is usually adequate. Although phenobarbital is well tolerated in horses, potential adverse effects include excessive sedation, respiratory depression, bradycardia, hypotension, and hypothermia in neonatal foals. Veterinarians should avoid using other drugs with known interactions, such as ivermectin, a GABA agonist. Tetracyclines and chloramphenicol inhibit hepatic microsomal enzymes, thereby prolonging the effects of phenobarbital. If the adverse effects are unacceptable and seizures are not controlled with one anticonvulsant therapy, one could consider reducing the dose by 20% or to nontoxic level and beginning a second anticonvulsant therapy, such as potassium bromide.[124]

Bromide (sodium or potassium) appears to compete with chloride to hyperpolarize neuronal membranes, as well as enhancing GABA-activated chloride conductance. Because the elimination half-life of this drug in horses is 3 to 5 days, it often takes a few weeks to reach a steady-state level in horses. Because of the long elimination half-life, potassium bromide should not be used alone to treat ongoing seizures but in combination with phenobarbital, with an initial dose of 25 to 40 mg/kg/day PO.[126,127] When used as monotherapy, a loading dose of 120 mg/kg daily for 5 days followed by a daily oral dose of 40 mg/kg achieved a serum level of 1 mg/mL. The therapeutic level for horses is not known. Adverse effects appear to be rare in horses.

Newer antiepileptic drugs have been introduced in veterinary medicine, with improved therapeutic indices (i.e., effective dose compared with toxic dose) with a reduction of the sedative and organ-toxic adverse reactions. These drugs include felbamate, gabapentin, clorozepate, toparimate, levetiracetam, and zonisamide and can be used as monotherapy or in combination.[128,129] Gabapentin, a structural analog of GABA, has been used as adjunctive therapy in humans and dogs for the treatment of partial seizures refractory to phenobarbital and bromide therapy. Gabapentin[128] at 5 to 10 mg/kg orally 2 to 3 times a day has been used anecdotally to aid in seizure management. However, the clinical efficacy of gabapentin and other new anticonvulsant drugs is currently unknown in horses. Based on pharmacokinetic studies on levetiracetam in horses using 20 mg/kg IV or 30 mg/kg intragastric immediate and extended release, an intravenous or oral dosage of 32 mg/kg q 12 h was proposed.[129] Bioavailability in this study was determined to be excellent, and the mean terminal half-life was 6 to 7 hours.[129]

It is difficult to provide recommendation for when to terminate anticonvulsant therapy. If no adverse effects are seen, the dose should not be altered for most horses until the patient has been seizure free for at least 6 months in the author's opinion (Aleman). If one needs to discontinue anticonvulsant therapy, this should be done slowly because sudden withdrawal of drugs might precipitate seizures. In neonates, the dose can be decreased by one-fourth to one-half each day for a minimum of 3 days and then discontinued. In an adult that has been on long-term anticonvulsant maintenance therapy, a longer weaning period is suggested. Anecdotal reports of decreasing dosages by 20% every other day for 1 week before stopping the anticonvulsant have been proposed. The editor prefers a more gradual decrease of the dosage by 20% every 2 to 4 weeks. Serial EEGs might provide valuable information on the progression or resolution of the disease in horses and might help the clinician in determining whether continued maintenance anticonvulsant therapy is necessary.

Sleep

Monica Aleman

Sleep in the horse is essential for its overall health.[130] Lack of sleep and sleep disorders can compromise the horse's health, performance, and quality of life. Limited information and lack

of understanding of normal sleep in the horse can result in erroneous interpretation of normal and abnormal sleep, inaccurate diagnosis of presumed sleep disorders, and management failure. Studies of sleep in the horse have mainly relied on behavioral observations, which is the first essential step in the evaluation of sleep. In addition to observation, the use of simultaneous EEG has redefined sleep medicine in humans through the determination of EEG characteristics of sleep and its various stages. This is important because the definition of various sleep disorders in people depends on identifying alterations in the sequence, duration, and pattern of stages of sleep. Sleep staging is based on specific features recorded in the EEG, electrooculogram (EOG), EMG, electrocardiogram (ECG), and respiratory patterns. Collectively, these studies and their interpretation are known as polysomnography. Polysomnography is emerging in veterinary medicine.

Horses sleep an average of 3 to 4 hours per day with multiple phases of rest and sleep (polyphasic sleepers) throughout a 24-hour period.[130] Most of their sleep occurs at night. Approximately 6 phases of rest and sleep during nighttime have been seen in horses based on observational studies. Duration of lateral recumbency during nighttime has been reported to be 2% to 9% and 5% to 15% in wild and stabled horses, respectively. In the wild, horses take turns for rest and sleep, where a few horses remain on alert while others rest or sleep. Horses on alert stand on higher areas where visualization of possible predators is better. Foals and especially neonatal foals sleep more time per day than adults. Their periods of rest and sleep are more in number and frequency and of longer duration than those of adults. The need for rest and sleep in the foal declines as the foal matures. From observational studies in wild horses and horses housed in pastures, it has been noted that a shift in the amount of sleep substantially declines in foals by 3 months of age. Differences in breed and gender have been reported, with draft breeds resting more than light breeds and fillies resting more than colts. There are many factors that can influence sleep in horses such as the environment, safety, companions, hierarchy, physiologic state and age, exercise, diet, and disease (see causes of sleep deprivation). Naturally, horses are herd animals, and through domestication we have altered sleep in horses.

Stages of Sleep

Sleep staging in horses is not nearly as sophisticated as it is in humans because of the unease of performing EEG in the natural setting.[86] However, the recent introduction of telemetric EEG units in veterinary medicine has opened a major opportunity to study sleep in the horse. Challenges presented in performing EEG include distance of recording, maintaining EEG electrodes, and movement artifacts. In horses, four states of vigilance can be determined through observation and simultaneous video and EEG recording. The electrophysiologic evaluation includes EEG, EMG, EOG, ECG, and respiratory monitoring for the definition of the stages of sleep. The stages include wakefulness, drowsiness, slow wave sleep, and REM sleep.

In wakefulness, the horse is bright and alert, standing with all limbs unless moving. Movement artifacts (eyes, ears, jaw, head) and EMG activity are common. Heart and respiratory rates are within reference values. Horses can adopt a stance characterized by weight bearing of both thoracic limbs and one pelvic limb with the other pelvic limb primed (ready to kick predators?) during drowsiness. Occasional vertex sharp waves (V waves) and transient benign variants that may appear epileptiform have been observed in healthy horses. These transient discharges are similar to those observed in healthy humans during this stage of sleep. These benign variants have been observed in sleep-deprived horses. During slow wave sleep, horses can sleep standing with the head held low or in sternal recumbency if safe and comfortable. Many horses in this stage display a second atrioventricular block (not present during other states of vigilance).

REM sleep is similar among species in which low voltage and mixed frequencies with episodic fast eye movements and loss of EMG activity are noted. Horses appear to devote about 15% of their total sleep time to this state. Horses can have REM sleep while in sternal recumbency while supporting their head on the ground or on an object, or more commonly in lateral recumbency if safe and comfortable. During REM sleep there is loss of muscle tone. In addition to rapid eye movements, twitching, blinking, flaring nostrils, and even limb stretching might be observed. These movements have been wrongly interpreted as seizures. Brief standing REM sleep has been documented, but the accompanying loss of muscle tone precludes extended periods because of resulting collapse. Lack of REM sleep can result in sleep deprivation.

Sleep Deprivation

Sleep deprivation is the result of lack of adequate sleep.[130,131] What is considered adequate or enough sleep in the horse? This should be considered on an individual basis, and variations among healthy individuals can be expected. Sleep deprivation can be manifested in the horse as excessive daytime sleepiness and collapsing episodes. Horses commonly present with abrasions in their dorsal carpi and fetlocks that suggest collapsing episodes. Sleep deprivation must be considered as a possible cause of collapse, but other causes of collapse must be ruled out. Complete history, presence of concurrent medical problems, and information about the environment where the horse sleeps are essential in the investigation of alterations of sleep. Horses could be subjected to a variety of factors that can interfere with normal sleep. These factors are listed in Table 11.6.

OTHER SLEEP DISORDERS

Sleep disorders in horses are poorly understood. A few disorders have been reported or suspected in equine medicine. These include hypersomnia, narcolepsy with cataplexy, altered arousal, and REM disorders. Before considering a sleep disorder, first rule out sleep deprivation.[132,133] Hypersomnia means excessive sleepiness. However, these horses appear to have "apparently normal" cycles of sleep but may lack periods of REM sleep despite being observed lying down for considerable time. A common complaint in these horses besides excessive sleep is decreased performance. This disorder might be secondary to other diseases such as endocrinopathies (suspected hypothalamic dysfunction, pituitary pars intermedia dysfunction),[134] neurologic disease (encephalitis, brain trauma, EPM, West Nile virus observed by author), and others yet to be identified. Specific mechanism of disease is unknown.

TABLE **11.6 Causes of Sleep Deprivation**

Environmental	Nonneurologic Medical Problems	Neurologic Problems

Wild horses
• Predators
• Rank in the herd
  • Dominant horses (on constant alert)
  • Bottom of herd (constant harassment by others)
  • Establishing ranking order in the pack
• Constant search for food
• Extreme temperatures, natural disasters (e.g., fire)
• Physiologic state
  • Pregnant mares not lying down enough
  • Lactating mares on constant alert to protect their foal
• Domesticated horses
• Housing (indoors: stall vs. outdoors: pasture)
• Predators in the area
• Rank in the herd
• Lack of companion(s)
• Bedding (lack of or uncomfortable)
• Traveling and shows
• Quarantine areas
• Hospitalization (more common in intensive care units)
	Pain (specially chronic)
	• Orthopedic, musculoskeletal disease
	• Gastrointestinal (e.g., enteroliths)
	• Pleuropneumonia
		Inability or unsafe to lie down
		Recumbency
		Neuropathic pain

A history of collapse or presence of abrasions or fibrosis in the dorsal carpi and fetlocks should alert the clinician of a possible sleep disorder. Other causes of collapse must be ruled out (cardiovascular, respiratory, neurologic conditions, electrolyte derangements). Video surveillance for 24 hours for a minimum of 7 days will provide useful information about the sleep behavior of the affected horse. Ideally, this video should be recorded in the environment where the horse usually sleeps. Hospital video monitoring is useful but can greatly alter sleep patterns (stress, noise, unfamiliar territory). However, it is recommended. A questionnaire with specific questions regarding the environment, presence of wildlife, companions, diet, traveling, shows, medical or painful conditions, and medication should be included. Ask if other horses suffer from similar problems. Before considering a sleep disorder, rule out sleep deprivation as the cause of abnormal sleep behavior because this might be corrected if the primary cause is corrected. Without simultaneous video and electrodiagnostic testing, it is not possible to determine whether all stages of sleep occur or if paroxysmal activity may be occurring.

Narcolepsy

Narcolepsy is a nonprogressive sleep disorder of the central nervous system characterized by excessive daytime sleepiness with manifestations of REM sleep. These manifestations also include uncontrolled episodes of loss of muscle tone (cataplexy), although narcolepsy might also occur without cataplexy.[132]

Narcolepsy has been recognized in humans and suspected in a variety of domestic animals, affecting 0.02% of adults worldwide and over 17 breeds of dogs.[132,133,135] Although its prevalence is not known in horses, narcolepsy appears to be rare and is breed specific. Both familial and sporadic cases of narcolepsy have been presumed observed in horses.[136-140] The disease has been reported in Suffolk and Shetland foals (the fainting disease),[137] Welsh ponies, Miniature Horses, Thoroughbreds, Quarter Horses, Morgans, Appaloosas, Standardbreds, and Lippizaners.[136-140] A familial occurrence is thought to exist in affected Suffolk and Shetland pony foals, American Miniature Horses, and Lippizaners. In general, narcolepsy is identified in two distinct groups: in foals (for which the onset occurs within a few months of life) and in adult horses.

A condition of excessive daytime sleepiness with episodes of collapse or near-collapse is described in horses that have been deprived of REM sleep or not enough slow wave sleep for a period of time.[130,131] This might be the result of medical conditions inhibiting recumbency (pain, altered neurologic status), housing and environmental factors (weather, wildlife), or social and behavioral factors. This apparently excessive sleepiness has been commonly confused as narcolepsy, but this clinical manifestation in many instances is due to lack of adequate sleep as defined for the particular individual. However, this lack of sleep can have serious consequences in the overall health and performance of the horse. Therefore every effort to determine and address the primary cause must be made.[130]

Pathophysiology. Four components of narcolepsy are apparent in human beings: excessive daytime sleepiness associated with short periods of REM sleep, cataplexy, hypnagogic hallucinations, and sleep paralysis.[133] Cataplexy or sudden collapse with complete inhibition of skeletal muscle tone occurs in horses with narcolepsy.[137-140] Respiratory and cardiac muscles are spared.

With narcolepsy and cataplexy, a biochemical abnormality of the brainstem or sleep-wake center might be present. In people, evidence suggests that narcolepsy with cataplexy is related to an underlying deficiency in the hypothalamic orexin/hypocretin system.[132,133,135] Although their physiologic roles are not fully understood, hypocretins are hypothalamic-specific neuropeptides, which are involved in various functions such as sleep, feeding, energy homeostasis, and neuroendocrine and autonomic nerve functions. A mutation in the hypocretin receptor 2 gene has been identified in Dobermans and Labrador Retrievers, with secondary impairment in postsynaptic hypocretin neurotransmission. In acquired canine narcolepsy, the concentration of hypocretin-1 in the brain and CSF was undetectably low, suggesting a loss of production of hypocretin peptides, similar to the pathophysiologic mechanisms reported in humans.[132,133] Although the role of hypocretins in equine narcolepsy is unknown, horses with pituitary pars intermedia dysfunction and narcolepsy-like episodes had low concentration of hypocretin-1 in the CSF.[134]

Clinical Signs. Cataplectic episodes are triggered by strong emotions (usually positive), such as laughing or anger in humans and excitement associated with play or the presentation of food in dogs. In horses, the initiation of eating or drinking, petting or stroking of the head and neck, hosing with cold water after exercise, and leading out of a stall might precipitate a cataplectic episode. The episodes have also been reported during riding. Adult horses might drop their heads, buckle at the knees, and stumble. If forced to walk, the horse might appear ataxic. Pony breeds are more likely to become recumbent. Affected horses are neurologically normal between attacks.

Diagnosis. Diagnosis of narcolepsy is based on history, clinical signs, pharmacologic testing (not always reliable), and the absence of other diseases. Careful questioning of the caretaker should be performed to determine any event(s) associated with the episode, and video documentation of the episode(s) is optimal. Physical examination may reveal the presence of superficial trauma, particularly over the dorsal aspect of the fetlocks and carpi in most, but not all, cases. A CBC and serum biochemical profile are normal in affected horses and may help to exclude underlying systemic and metabolic abnormalities. CSF cytology is usually normal, and EEG might reveal REM sleep during an episode.

A presumed provocative test with variable results to diagnose narcolepsy or cataplexy in horses is the use of physostigmine salicylate. Physostigmine is an anticholinesterase drug given at 0.05 to 0.1 mg/kg slowly intravenously that might precipitate a cataplectic attack within 3 to 10 minutes after administration in affected horses. This compound crosses the blood-brain barrier. Careful monitoring of the horse after physostigmine administration is necessary because of untoward effects such as colic and cholinergic stimulation. In addition, lack of positive response to physostigmine does not rule out a diagnosis of narcolepsy because individual response to the drug is quite variable and some affected horses failed to respond to this provocative testing.

Atropine sulfate, a muscarinic blocker, given at 0.04 to 0.08 mg/kg intravenously, reduces the severity of cataplectic attacks minutes after administration and can prevent their reoccurrence for 12 to 30 hours following administration. Response to atropine sulfate supports a possible cholinergic mechanism for causing cataplexy. One must monitor horses given atropine sulfate for ileus and colic.

Differential Diagnosis. Cataplexy, which is commonly seen with narcolepsy, is the best diagnostic marker of this disease and should be distinguished from other causes of episodic weakness or collapse. Ruling out collapse secondary to prolonged sleep deprivation is important as it might be common in horses. This disorder might be caused by chronic pain, environmental factors, or social/behavioral factors. In these cases, administrating NSAIDs to animals with chronic pain and/or providing the horse with a comfortable environment (to encourage the horse to lie down) might resolve the clinical signs.[130] Acute collapse without premonitory signs is characteristic of cardiovascular collapse or syncope, and unlike narcolepsy is not preceded by gradual lowering of the head and drowsiness. Atrial fibrillation, ruptured chordae tendinae, myocardial infarction, myocardial fibrosis, aortic endocarditis, and pericarditis have been associated with syncope in horses. Cerebral hypoxia might occur in these conditions and might lead to coma, with or without signs of cardiac failure. One should differentiate seizures from narcolepsy/cataplexy, botulism, myasthenia-like syndrome, hypocalcemia, and hyperkalemic periodic paralysis, among others.

Treatment and Prognosis. In general, drugs that stimulate monoamine systems (e.g., dopamine) in the brain are effective suppressors of narcolepsy, whereas drugs that stimulate cholinergic activity in the brain exacerbate narcolepsy. Imipramine, a tricyclic antidepressant drug, has been recommended to control narcolepsy and cataplexy.[138] The drug blocks the uptake of serotonin and norepinephrine and decreases REM sleep. Oral administration (250–750 mg) produces inconsistent results.[138] Potentially serious adverse effects including muscle fasciculations, tachycardia, hyperresponsiveness to sound, and hemolysis may be seen in horses receiving intravenous dosages of imipramine that exceed 2 mg/kg.[138] Adverse effects are not seen when the drug is administered orally, but that is likely because of very poor bioavailability. The disorder might persist for life.

CENTRAL NERVOUS SYSTEM TRAUMA

Yvette S. Nout-Lomas

Trauma to the CNS is the most common cause of neurologic disease in horses, accounting for 22% to 24% of neurologic disorders.[141,142] Both of these studies reported trauma to the spinal cord to be more prevalent than brain injury. Traumatic brain injury (TBI) was reported in 23% to 44% of cases, and spinal cord injury (SCI) was reported in 56% to 77% of cases. A more recent investigation into exercise-associated sudden deaths in previously apparently healthy Thoroughbred racehorses from six racing jurisdictions around the world showed that 13% (18 of 143) of deaths were attributed to CNS trauma.[143] Of these, 60% (11 of 18) had SCI associated with cervical vertebral fracture, 17% (3 of 18) had CNS hemorrhage, 11% (2 of 18) had TBI associated with skull fracture, 6% (1 of 18) had CNS injury associated with both skull and cervical vertebral fractures, and 6% (1 of 18) of cases had SCI associated with cervical vertebral instability.

Although mechanisms of CNS cell damage, to a certain degree, are similar after TBI and SCI, a few different processes exist with regard to cause and pathophysiology. Clinical syndromes as a result of traumatic injury to the CNS can vary tremendously, but the most common are coma, vestibular disease, tetraparesis, paraparesis, tetraplegia, and cauda equina syndrome. Treatment regimens for CNS injury are aimed toward reducing inflammation and swelling, halting secondary injury mechanisms, and promoting regeneration and recovery of function. Prognosis depends primarily on severity of primary injury and on the extent and neuroanatomic location of CNS damage.

Traumatic Brain Injury

Pathophysiology

Head trauma is typically caused by incidents such as collision with an immovable object such as a fencepost or another horse, falling down, flipping over backward, being kicked by another horse, and, although rare, impact by a projectile (e.g., gunshot). A retrospective study involving 34 horses with TBI showed that the most common cause (44%) of injury was rearing and falling backward, resulting in poll injury.[144] Another type of head injury that may lead to CNS damage is fracture of the petrous temporal bone associated with THO.[145]

Although traumatic injury to the head is reported to be common in horses, subsequent TBI occurs only in 25% to 50% of cases.[146,147] The remaining horses typically present for fractures of the orbit, periorbital rim, and zygomatic, mandibular, or maxillary bones. TBI may occur with or without fracture of the calvarium; however, some of the most severe injuries to the brain occur when the injury is contained within the closed calvarium (i.e., no skull fractures).[148] Within the closed calvarium, the sum of the intracranial volumes of the brain, blood, and CSF are constant; volume or pressure changes within one of these compartments will affect volume (anatomy) or pressure of the other compartments.

Consequences of impact sustained to the poll in horses that flip over backward include fracture of the bones on the side and base of the calvarium such as the petrous temporal, squamous temporal, and parietal bones. However, more commonly these bones remain intact, and more serious injury occurs to the basilar bones as a result of strong traction forces from the rectus capitis ventralis muscles (Fig. 11.23).[149,150] In addition, adjacent large vessels are lacerated, and hemorrhage into the retropharyngeal spaces or guttural pouches can occur. In severe cases, transverse fracture of the basilar bones at the level of the basioccipital-basisphenoidal suture can occur. Young horses may be more susceptible to this type of injury because the joint between the basilar bones fuses by 5 years of age.[150] In most cases the fracture site is stable, and minimal displacement occurs. The cerebellum is seldom severely damaged after poll impact,[145] but the cerebral parenchyma is more commonly injured after being subjected to rapid acceleration-deceleration forces. Moreover, optic nerves and other attachments may be torn from the cerebral hemispheres.[151] Impact to the dorsal surface of the head may result in damage to the frontal or parietal bones, with subsequent cerebral cortical injury or, more commonly, damage to the cervical vertebrae with subsequent SCI. In addition, cranial nerve XII may be injured as it exits the hypoglossal foramen. Furthermore, occipital cortical injury may occur and, as described for poll impact injuries, with frontal injuries the optic nerves may be stretched.

Pneumocephalus was diagnosed by radiographs or CT in horses after trauma to the head with suspected or confirmed fractures to the sinuses[152] and after sinus surgery.[153] *Pneumocephalus,* also known as intracerebral airocele or pneumatocele, is defined as the presence of gas within any intracranial compartment (intraventricular, intraparenchymal, subarachnoid, subdural, and epidural). In the six horses described in these reports, there were no neurologic sequelae noticed that could be attributable specifically to the pneumocephalus. However, in humans clinical signs include headache, nausea and vomiting, seizures, dizziness, and depressed neurologic status. Although pneumocephalus can be an incidental finding, it is a possible life-threatening complication that can occur after head trauma in horses.

After trauma, the most severe damage generally takes place at the place of impact (coup), opposite to the side of impact (contrecoup), or in both locations. Additionally, the brain is subjected to other forces after trauma, such as rotational and shock wave forces. TBI is a result of both direct, immediate mechanical disruption of brain tissue (primary injury) and indirect, delayed (secondary) injury mechanisms. The primary damage is a result of the biomechanical effects of the injury and is characterized by immediate and often irreversible damage to neuronal cell bodies, dendritic arborizations, axons, glial cells, and brain vasculature. This initial brain injury may be focal, multifocal, or diffuse. Secondary injury is a complex cascade of molecular, cellular, and biochemical events that can occur for days to months after the initial insult, resulting in delayed tissue damage. In addition, systemic alterations further contribute to the tissue damage. Hypoxia, ischemia, brain swelling, alterations in intracranial pressure, hydrocephalus, infection, breakdown of blood-brain barrier, impaired energy metabolism, altered ionic homeostasis, changes in gene expression, inflammation, and activation and release of autodestructive molecules occur and exacerbate the initial injury. Recently the pathology of TBI after blunt nonmissile and penetrating missile head injuries in veterinary species was reviewed.[154]

Principles of raised intracranial pressure are described in the Monro-Kellie doctrine, which states that once the fontanelles and sutures of the skull are closed, the brain is enclosed in a nonexpandable case of bone, the brain parenchyma is nearly incompressible, the blood volume in the cranial cavity is therefore nearly constant, and a continuous outflow of venous blood from the cranial cavity is required to make room for continuous incoming arterial blood.[155] Blood flow to the brain is controlled by changes in diameter of resistance blood vessels, and cerebral blood flow is controlled by autoregulation whereby the perfusion pressure is maintained within a range of approximately 50 to 150 mm Hg. Beyond these limits, cerebral blood flow decreases at pressures below the lower limit and increases at pressures above the higher limit. Cerebral perfusion pressure (mean arterial blood pressure minus intracranial pressure) is the stimulus to which the autoregulatory response of the vasculature occurs. Cerebral autoregulation is altered unpredictably after TBI, and it appears that the minimum acceptable cerebral perfusion pressure is higher than normal after trauma. Increased intracranial pressure leads to a decreased cerebral perfusion pressure and subsequently

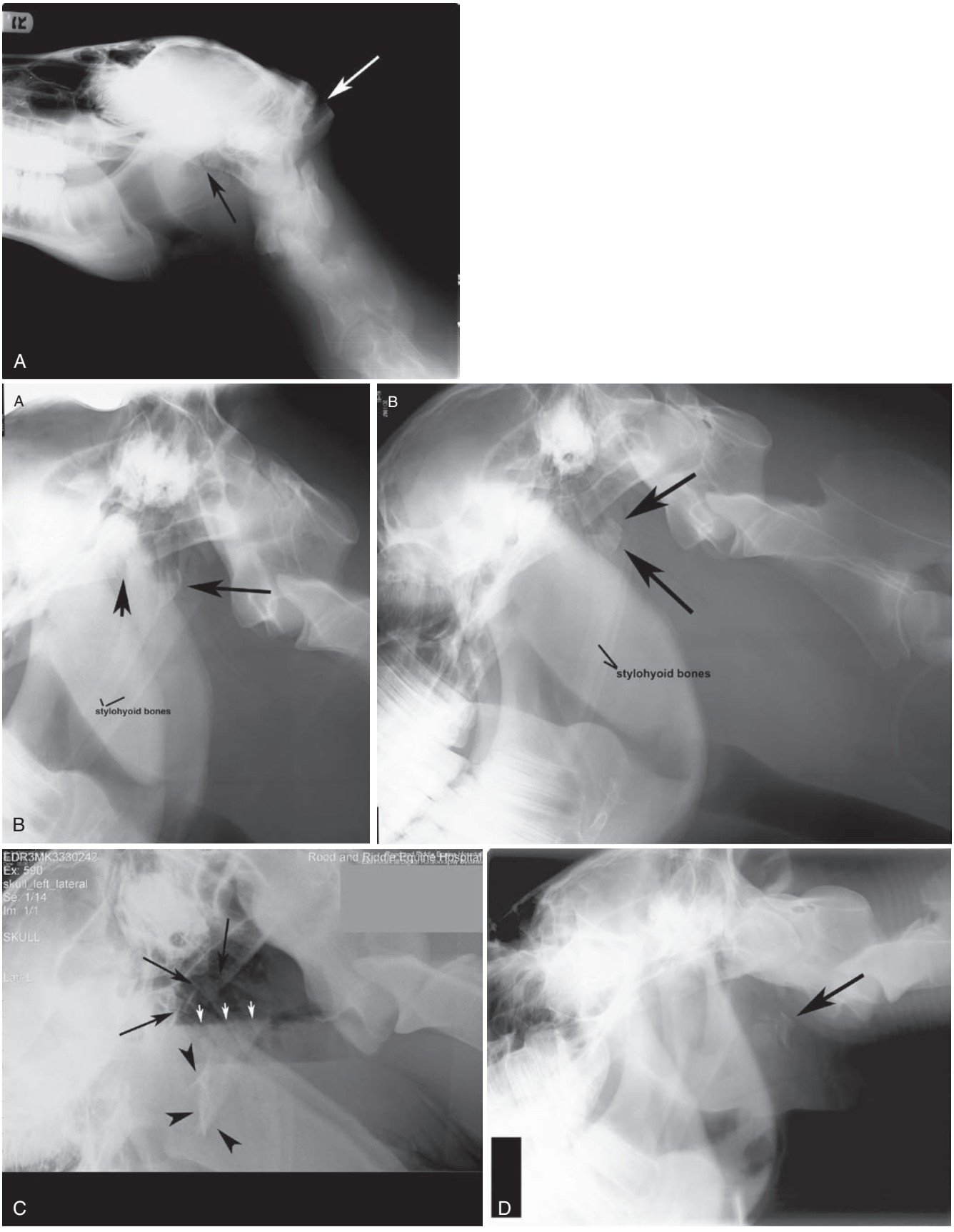


FIG. **11.23** A, Radiograph from a 3-year-old Thoroughbred colt that flipped while playing on the end of the lead shank. The horse hit its head on a small board along the side of the walkway and was unconscious for 3 to 5 minutes. The horse had blood coming from the nostrils, paralysis of the right eyelid, a corneal ulcer on the right eye, and weakness in all four limbs. The radiograph shows a fluid line in the guttural pouch *(small white arrows)* and a basisphenoid fracture *(large black arrows)* with a large fragment *(arrowheads)* visible in the guttural pouch area. B, This is a radiograph taken from a horse after head injury and subsequent rupture of the longus capitis muscle and an avulsion fracture from the basisphenoid bone. This is a radiograph image C taken from a horse that flipped while playing on the end of the lead shank. This is a radiograph image D taken from a horse with head injury that resulted in rupture of the longus capitus. The *arrow* is pointed at the avulsed bone located behind the guttural pouch. Image from The Ohio State University. (A, Courtesy Stephen Reed, DVM, Rood and Riddle Equine Hospital. B, Courtesy The Ohio State University.)

reduces cerebral blood flow. Reduced cerebral blood flow results in areas of ischemia and subsequent restriction of delivery of substrates such as oxygen and glucose to the brain. Brain swelling after TBI because of edema formation and hematoma formation within the skull are the most common causes of increased intracranial pressure. Vascular damage that occurs after head injury can be epidural (between dura mater and the skull), subdural (between the dura mater and arachnoid mater), subarachnoid (between arachnoid mater and pia mater), superficial (vessels immediately under the pia mater), intraparenchymal, and intraventricular.

The cascade of secondary injury that results in necrotic and apoptotic cell death is described in more detail in the section on pathophysiology of SCI. Uncontrolled glutamate release and failure of energy systems in neuronal and supporting tissues lead to elevated intracellular calcium concentrations and subsequent cell death. Hemorrhage, ischemia, and the primary tissue damage lead to sequestration of vasoactive and inflammatory mediators at the injury site and are thus involved in the secondary injury cascade. Inflammation and endothelial damage cause derangements in normal cerebrovascular reactivity and contribute to a mismatch of oxygen delivery to tissue demand, resulting in local or diffuse ischemia.

A major consequence of ischemia is reduced delivery of oxygen and glucose. Blood flow interruption is responsible for disruption in ion homeostasis (especially calcium, sodium, and potassium) and a switch to anaerobic glycolysis resulting in lactic acid production and acidosis. Cell membrane lipid peroxidation with subsequent prostaglandin and thromboxane synthesis, formation of reactive oxygen species (ROS), nitric oxide, and energy failure also ensue. Because of the high metabolic rate and oxygen demand of the brain, disruption of blood flow rapidly compromises the energy-supplying processes and leads to impaired nerve cell function and even cell death. Impaired mitochondrial function with subsequent energy depletion leads to a loss in maintenance of membrane potentials resulting in depolarization of neurons and glia.[156] Cytotoxic edema develops through the failure of the sodium-potassium ATPase-dependent pump in the presence of hypoxia and the subsequent influx of water that passively follows sodium and chloride. In addition to cytotoxic edema, vasogenic edema develops as a result of disruption of the blood-brain barrier, which includes damage of endothelial cells, degeneration of pericytes, and loss of astrocytes.[157] Extravasation of blood components and water occurs resulting in increased extracellular fluid accumulation. Understanding the complex pathophysiologic events that take place after TBI is important for the development of effective monitoring and treatment strategies.

Neurologic Evaluation

Clinical signs associated with TBI range from inapparent to recumbency with unconsciousness or death (Fig. 11.24). A complete physical examination is very important in head trauma cases, because fractures and other concurrent injuries are not uncommon and require identification and treatment. Physical examination findings as a result of head trauma can include fractures; hemorrhage from the nostrils, mouth, and ears; CSF draining from the ear (Fig. 11.25); respiratory distress; cardiac arrhythmias; and hypotension or hypertension.

Hemorrhage from the nose typically presents as dark-colored (venous) blood and originates from a paranasal sinus, ethmoid turbinates, or nasal cavity. Occasionally, blood is bright red and then usually originates from larger vessels in the guttural pouches. Respiratory distress can occur associated with significant throat swelling after profuse hemorrhage into the guttural pouches. Furthermore, neurogenic pulmonary edema has been reported to occur. Acute elevation of intracranial pressure may result in Cushing's reflex, which is a hypothalamic response to brain ischemia and is characterized by hypertension and secondary baroreceptor-mediated bradycardia. Continued elevation of intracranial pressure and reduction of cerebral blood flow results in increased sympathetic discharge (catecholamines), with subsequent myocardial ischemia and development of cardiac arrhythmias. This is referred to as the *brain-heart syndrome*.

Life-threatening injuries should be attended to first, and then a complete neurologic examination should be performed. Horses may be recumbent, intractable, or both after a traumatic incident; examination and management of these horses can be difficult and dangerous.[9] Signs of focal brain injury are listed in Table 11.7. Sedation may be necessary for examination. Although α_2-agonists may transiently cause hypertension, which may potentiate intracranial hemorrhage, xylazine has been found to cause a minor decrease in cerebrospinal pressure in normal, conscious horses[158] and is considered a safe sedative to use in horses with head trauma if the head is not allowed to drop too low that it could affect physiologic changes in intracranial pressure.

The complete neurologic examination should include an assessment of the horse's mentation, cranial nerve function, posture, and ability to coordinate movements, as well as its ability to regulate rate and range of motion. In addition, reflex and nociceptive testing should be performed to investigate any concurrent SCI. Serial neurologic examinations, particularly during the first hours, are important for diagnostic purposes and to allow prediction of a more accurate prognosis. Furthermore, serial examinations are important to assess response to therapy. Pupil size, symmetry, and response to light should be assessed in all horses and followed carefully, particularly in recumbent horses. A change from bilateral pupillary constriction to bilateral dilation with no response to light is a poor prognostic indicator.

The most common neurologic syndromes after head trauma are a result of hemorrhage into the middle and inner ear cavities. Signs are those of central or peripheral vestibular disease and facial nerve damage and include recumbency, head tilt, neck turn, body lean, and circling, all toward the side of the lesion. The ipsilateral eye may be rotated ventrally and laterally, and there may be horizontal or rotary nystagmus with the fast phase away from the lesion. Facial paralysis on the same side as the lesion is seen with cranial nerve VII damage. Central vestibular disease is suspected when signs of brainstem disease or other cranial nerve deficits are present. Central vestibular lesions can result in a paradoxic vestibular syndrome, in which the lesion is located on the side opposite to that which is expected from the clinical signs. More serious trauma can lead to alterations of mentation, behavior, or both.

The level of consciousness is affected by the degree of damage to the cerebrum and reticular activating system in the brainstem. Immediately after cerebral injury, a period of concussion occurs, with unconsciousness for various periods of time, or even coma. Usually a horse recovers from this in minutes to hours. Comatose horses may have an irregular breathing pattern with periods of either Cheyne-Stokes breathing or hyperventilation. In some horses, seizures can

occur after initial concussion; these are typically generalized seizures. Level of mentation and responsiveness to reflexes should be assessed and recorded. Injury to the occipital cortex can result in impairment of vision and menace response of the eye contralateral to the lesion. Pupillary light reflex, however, should remain intact. Injury to the parietal cortex can result in decreased facial sensation on the contralateral side. Another sign of cerebral damage is dementia, or altered behavior, such as walking in circles (toward the side of the lesion), head pressing, hyperexcitability, or aggression.

Severe rostral brainstem injuries (mesencephalon) can be associated with coma and depression because of damage to the reticular activating system. Strabismus, asymmetric pupil size, and loss of pupillary light response can be present because of damage to cranial nerve III. Apneustic or erratic breathing reflects a poor prognosis, and bilaterally dilated and unresponsive pupils indicate an irreversible brainstem lesion. These lesions can occur immediately after injury, secondary to herniation of components of the cerebrum or cerebellum, or after hemorrhage. Severe brainstem injuries may result in a decorticate posture, characterized by rigid extension of neck, back, and limbs. Injury to caudal parts of the brainstem (pons and medulla) result in dysfunction of multiple cranial nerves in addition to depression and limb ataxia and weakness. To make a distinction between a cranial cervical spinal cord and caudal brainstem lesion, careful assessment of the

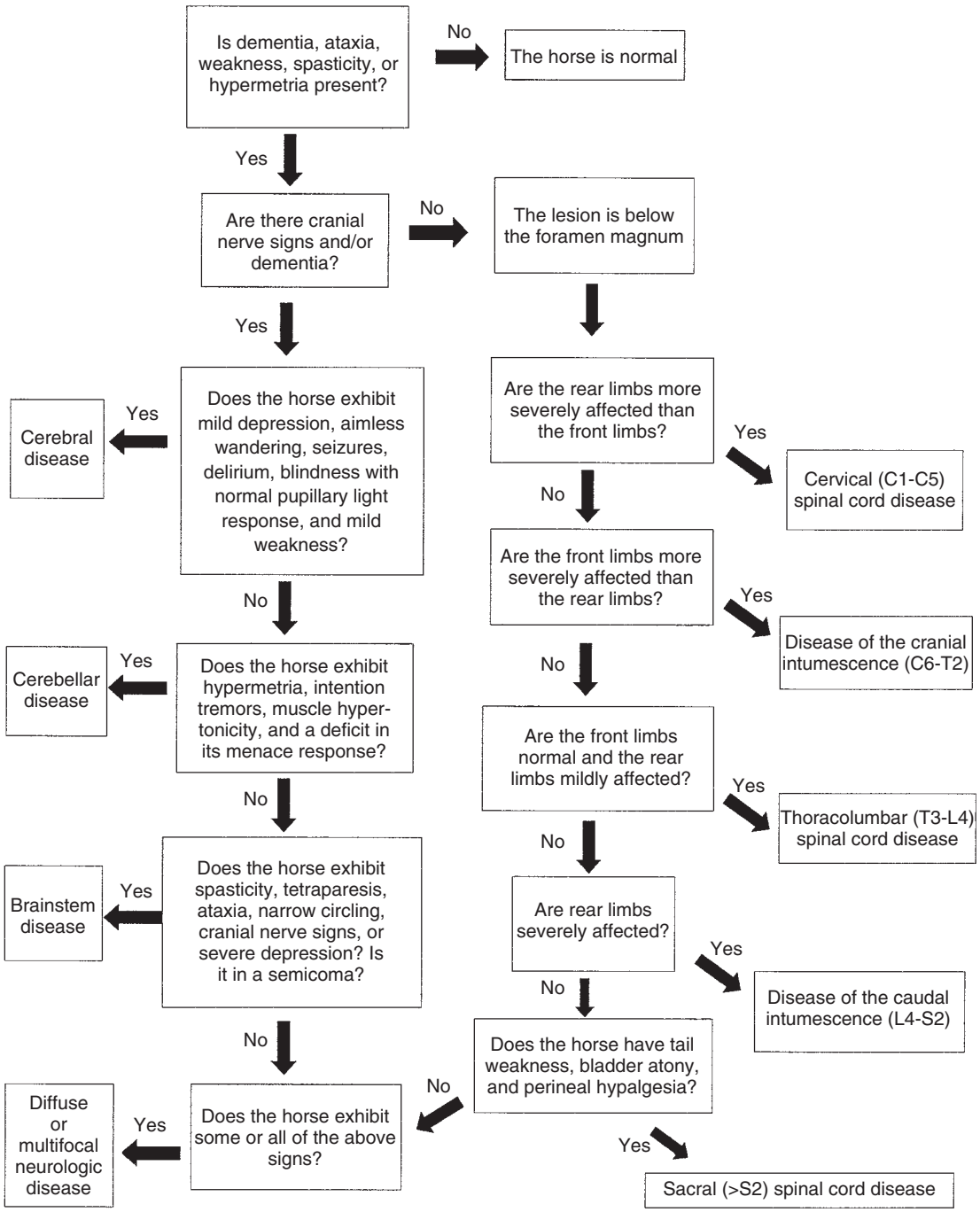


FIG. **11.24** Flow chart for localizing a lesion in the nervous system in a recumbent horse.

horse's mentation and function of cranial nerves X and XII is important.

Signs of cerebellar injury occur infrequently and include intention tremor, broad-based stance, spastic limb movements, and absent menace response with normal vision. If multiple areas of the brain are damaged, this will be reflected in the different clinical signs. Multifocal damage or progression of disease through hemorrhage, secondary injury mechanisms, or both is suggested when clinical signs become more widespread.

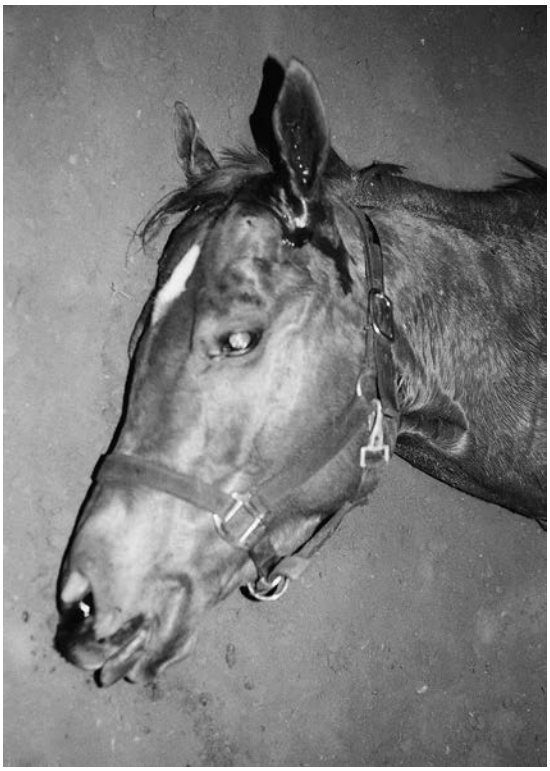


FIG. **11.25** Blood and cerebrospinal fluid (CSF) draining from the ear after a basilar skull fracture.

Diagnostic tools that are helpful in further defining cranial trauma include radiography, CT, MRI, endoscopy, electrodiagnostics, transcranial magnetic stimulation, and CSF analysis. Although radiographs typically are the first-line diagnostic tool to use for determination of the presence and severity of fractures, hemorrhage in cavities, or thickening of stylohyoid bone or bulla (or both), one study showed that only 50% of bony fractures of the calvarium were confirmed radiographically.[144] CT has been shown to be an excellent neurodiagnostic tool in identifying skull fractures, intracranial space-occupying lesions (e.g., neoplasia), and acute hemorrhage and allows one to rule in intracranial disorders.[159] However, this same report showed that CT has limited sensitivity in identifying inflammatory disorders and small parenchymal lesions in the equine brain. Soft tissue changes that can be seen on CT after TBI include change in the size, shape, and position of the ventricles, deviation of the falx cerebri, and focal changes in brain opacity. Enhancement of areas of injury or hemorrhage is sometimes possible with iodinated contrast agents. MRI offers a higher sensitivity for examination of soft tissue structures and has the ability to acquire images in all planes. However, the use of MRI is not yet possible for all equine clinicians. Upper respiratory endoscopy is an important diagnostic procedure for evaluation of cranial nerve function, stylohyoid bones, retropharyngeal area, and appearance of guttural pouches. Transcranial magnetic stimulation and measurement of the magnetic motor-evoked potentials in the thoracic and pelvic limbs has been shown to be helpful to assess the integrity of the descending motor pathways, to confirm or exclude a descending motor tract lesion as the cause of the recumbency.[160] Electroencephalograms are used for assessment of seizure activity, BAER is used for examination of vestibular function, and

TABLE **11.7** **Signs Characteristic of Focal Brain Injury**

Levels	Consciousness	Motor Function	Pupils	Other Sign
Cerebrum	Behavior change, depression, coma	Circling	Normal	Blindness
Cerebellum		Ataxia and hypermetria, intention tremor		Menace response deficit without blindness
Diencephalon (thalamus)	Depression to stupor	Normal to mild tetraparesis, "aversive syndrome"[a]	Bilateral, nonreactive pupils with visual deficit	None
Midbrain	Stupor to coma	Hemiparesis, tetraparesis or tetraplegia	Nonreactive pupils, mydriasis, anisocoria	Ventrolateral strabismus
Pons	Depression	Ataxia and tetraparesis, tetraplegia	Normal	Head tilt, abnormal nystagmus, facial paralysis, medial strabismus
Rostral medulla oblongata (including inner ear)	Depression	Ataxia or hemiparesis to tetraplegia	Normal	Same
Caudal medulla oblongata	Depression	Ataxia, hemiparesis to tetraparesis, abnormal respiratory patterns	Normal	Dysphagia, flaccid tongue

From Robinson NE, editor: *Current therapy in equine medicine,* ed 6, St. Louis, 2009, WB Saunders.
[a]Deviation of the head and eyes with circling toward the side of a unilateral lesion.

visual function is examined with visual-evoked potential in combination with electroretinography. CSF analysis may not always be indicated after acute trauma, but it may be useful for excluding other diseases. Cisternal CSF collection is contraindicated if increased intracranial pressure is suspected because of the possibility of brain herniation through the foramen magnum. Lumbosacral collection is a safer alternative but can be normal despite a traumatic episode, especially in the acute phase, and because if the sample is not obtained closest to the lesion it may not reflect the changes that have occurred.

In human medicine, development of advanced bedside neuromonitoring devices has been an important focus in neurocritical care. The four most important parameters to be determined are (1) intracranial pressure, (2) cerebral blood flow, (3) information on brain metabolism, and (4) functional outcome. For example, catheters can be placed into the brain tissue to determine glucose, glycerol, and glutamate concentrations, indicators of secondary ischemia, by microdialysis, and probes can be placed to determine brain tissue oxygen tension. Methods to measure and monitor intracranial and cerebral perfusion pressures have been described for use in foals[161] and adult horses[162]; however, these techniques have thus far not been scientifically evaluated in clinical cases. One study demonstrated the use of near-infrared spectroscopy to measure regional cerebral oxygen saturation in horses.[163]

Treatment and Prognosis

Based on the pathophysiology of events that occur after TBI, it is likely that single-drug intervention would not be effective. Treatment of TBI is aimed at optimizing delivery of oxygen and substrates to brain tissue to salvage brain tissue that is undamaged or reversibly damaged. This requires optimizing cerebral blood flow (i.e., optimizing mean arterial blood pressure and hemoglobin concentration; ensuring intracranial pressure is not elevated). Emergency surgical treatment (although not commonly performed) is warranted in open cranial fractures and in the face of deterioration despite medical therapy. In Fig. 11.26 a flow chart is shown that can serve as a guide to the management of cranial trauma in horses.

Methods to reduce intracranial pressure include hyperventilation, CSF drainage, treatment with hyperosmolar agents or barbiturates, head elevation, and decompressive surgery. Hyperventilation reduces the partial pressure of carbon dioxide in blood and subsequently leads to cerebral vasoconstriction. Reduced cerebral blood volume reduces intracranial pressure. However, cerebral vasoconstriction may lead to reduction of cerebral blood flow to ischemic levels. Hyperventilation could be considered in cases of increased intracranial pressure in horses. Proper hyperventilation requires monitoring of arterial blood gases and may require use of neuromuscular blockers if the horse is not comatose and is resisting the ventilator. CSF drainage is commonly used in people to reduce intracranial pressure; however, it is therapeutic only if CSF outflow obstruction is noted. If these methods are ineffective, repeat imaging is pursued to investigate the presence of mass lesions before medical treatments are commenced such as administration of hyperosmolar substances and induction of barbiturate coma. Hyperosmolar treatment is commonly used in horses with neurologic signs attributable to TBI. This is discussed later in this section.

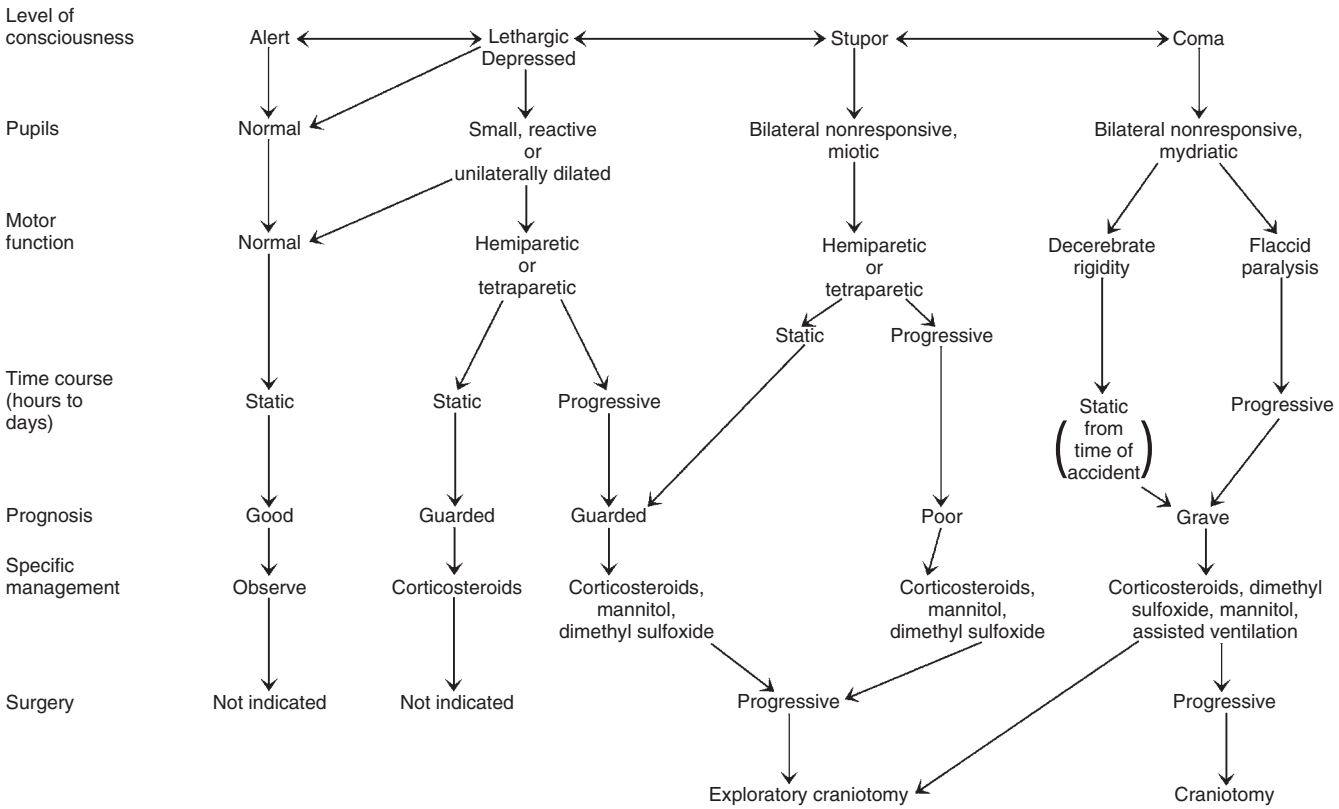


FIG. **11.26** Flow chart for management of cranial trauma. (From Robinson NE, editor: *Current therapy in equine medicine,* ed 6, St. Louis, 2009, WB Saunders; modified from Kirk KW, editor: *Current veterinary therapy VII,* Philadelphia, 1980, WB Saunders.)

Mean arterial blood pressure should be maintained within normal limits. Blood transfusion may be indicated in cases of severe hemorrhage. Crystalloid fluids are recommended as fluid therapy of choice, particularly in light of findings of the serum versus albumin fluid evaluation (SAFE) study[164] that determined no difference in outcomes between administering albumin versus normal saline in the intensive care unit. Furthermore a post hoc follow-up study demonstrated a higher mortality rate in TBI patients that were treated with albumin compared with those treated with saline.[165] There remain questions concerning optimal composition and volume of fluid therapy for TBI. There is a physiologic rationale why excessive fluid administration and positive fluid balance may increase brain swelling and intracranial pressure, and current recommendations include refined usage of intravenous fluids.[166]

It has been well established that maintaining blood glucose concentrations at 80 to 110 mg/dL through intensive insulin therapy reduces morbidity and mortality in human critical care patients.[167] However, neurointensivists have shown that intensive insulin therapy increases markers of cellular distress in the brain and suggest that systemic glucose concentrations of 80 to 110 mg/dL are too low in TBI and may lead to cerebral hypoglycemia. Recommendations now are to maintain blood glucose concentrations at 120 to 140 mg/dL in TBI.

Treatment with antiinflammatories is likely the most commonly used treatment in equine TBI. Indications for use of antiinflammatory treatment are to combat the inflammatory pathways of secondary injury mechanisms (cytokine release, free radicals), improve comfort level, and reduce fever. Fever is extremely common after TBI, and it has been well documented in animal models and in human beings to negatively affect outcome after TBI (e.g., by augmenting secondary injury mechanisms).[168,169] Researchers have proposed that a proactive approach should be taken toward reducing fever. In fact, hypothermia has been shown to be neuroprotective. Hypothermia results in decreased cellular metabolism, and in humans therapeutic hypothermia and targeted temperature management have been proven efficacious as a treatment for TBI in experimental and clinical settings; however, large multicenter trials have so far failed to demonstrate clinical benefits.[170] Therapeutics with antiinflammatory properties that are common to equine practice include corticosteroids, NSAIDs, dimethyl sulfoxide (DMSO), and vitamin E. These drugs are discussed in more detail in the section pertaining to SCI. Corticosteroids are no longer recommended for use in TBI following results of studies showing no benefit of these drugs and results of the CRASH trial that showed increased mortality in adults who received methylprednisolone after TBI.[171]

Controlling seizure activity is very important after TBI, because seizure activity increases cerebral metabolic rate and is detrimental to secondary injury. It is not unusual for horses sustaining cranial trauma to develop seizures. Diazepam, midazolam, phenobarbital, and pentobarbital are drugs that can be used to control seizures. Intractable seizures may necessitate general anesthesia. Agents useful for general anesthesia include guaifenesin, chloral hydrate, barbiturates, and gas anesthesia. Ketamine is not recommended as part of a balanced anesthesia regimen because it increases cerebral blood flow and intracranial pressure.

Barbiturate treatment or coma may decrease cerebral metabolism, thereby providing a protective effect against cerebral ischemia. Barbiturates may also limit lipid peroxidation. However, the actual benefits of barbiturate use on the neurologic outcome remain controversial. The effects of barbiturates on lowering intracranial pressure are enhanced by concurrent hyperventilation. An exact dosage regimen for barbiturate treatment in horses has not been investigated, but 5 to 10 mg/kg IV to effect is reported to be useful. The major adverse effect of barbiturates is hypotension, especially if mannitol and furosemide have been administered, so they must be used with caution and adequate blood pressure monitoring. Barbiturates should be reserved for those cases in which elevated intracranial pressure is refractory to other treatments. Other methods to lower intracranial pressure include elevation of the head by 30 degrees if no cervical fractures are present and decompressive craniectomy.

Hyperosmolar therapy, which includes mannitol or hypertonic saline, is frequently used in human patients to reduce intracranial pressure. Both of these treatments appear effective at reducing intracranial pressure, and there does not appear to be a clinically significant difference between the two with regard to mortality or neurologic outcomes.[172] Hypertonic saline has a number of beneficial effects in TBI. The permeability of the blood-brain barrier to sodium is low. Hypertonic saline produces an osmotic gradient between the intravascular and the interstitial-intracellular compartments, leading to shrinkage of brain tissue and subsequent reduction of intracranial pressure. It augments volume resuscitation and increases circulating blood volume, mean arterial blood pressure, and cerebral perfusion pressure. Other beneficial effects include restoration of neuronal membrane potential, maintenance of blood-brain barrier integrity, and modulation of the inflammatory response by reducing adhesion of leukocytes to endothelium. In horses, hypertonic saline can be administered intravenously to head trauma patients in shock as 5% or 7% sodium chloride (NaCl) solutions (4–6 mL/kg) over 15 minutes. Isotonic fluids can then be used for maintenance if needed. Contraindications to the use of hypertonic saline include dehydration, ongoing intracerebral hemorrhage, hypernatremia, renal failure, hyperkalemic periodic paralysis, and hypothermia. Systemic side effects include coagulopathies, excessive intravascular volume, and electrolyte abnormalities. Monitoring central venous pressure and maintaining it within normal limits (5–7 cm H_2O) is therefore important, as well as monitoring serum sodium and potassium concentrations if hypertonic saline is used frequently. Mannitol induces changes in blood rheology and increases cardiac output, leading to improved cerebral perfusion pressure and cerebral oxygenation. Improved cerebral oxygenation induces cerebral artery vasoconstriction and subsequent reduction in cerebral blood volume and intracranial pressure. Mild dehydration after osmotherapy is desirable and may improve cerebral edema; however, severe dehydration can lead to hyperosmolality and renal failure. In horses, 20% mannitol can be administered at 0.25 to 2.0 mg/kg IV over 20 minutes. Horses receiving osmotic diuretics should be adequately hydrated. The use of osmotic substances is warranted in any horse with worsening mental status, abnormal pupillary size or inequality indicating transtentorial herniation, or development of paresis. Although mannitol administration is very effective in reducing intracranial pressure, several limitations to its use exist. Hyperosmolality can be associated with renal and CNS effects. Furthermore, administration of multiple doses of mannitol may lead to intravascular dehydration, hypotension, and reduction of cerebral blood flow. Furosemide has experimentally been found effective in

decreasing intracranial pressure. Normal hydration status is required before furosemide is administered. Furosemide may also be used concurrently with mannitol to increase the duration of intracranial pressure reduction provided by mannitol and to diminish the potential for rebound intracranial pressure elevation. Antibiotic treatment is usually warranted in cases of head trauma, especially when fractures are involved. The presence of hemorrhage increases the possibility of septic meningitis. Antibiotic choice should be based on culture and sensitivity testing. Good empiric choices for broad-spectrum coverage include trimethoprim-sulfamethoxazole and penicillin in combination with gentamicin. Appropriate monitoring for aminoglycoside toxicity should be undertaken with their use. Owing to disruption of the blood-brain barrier, other antimicrobials probably penetrate into the CNS, and therefore their use may also be efficacious.

Nutritional support plays a role in the outcome after neurologic injury. In human beings, neurologic recovery from head injury occurs faster in patients receiving early adequate nutritional support.[173] If the horse is able to eat and the gastrointestinal tract is functioning normally, water and good-quality hay should be available at all times. Small amounts of grain should be fed three to four times a day to boost caloric intake. The amount of grain fed should be based on the horse's condition and ability to tolerate grain feeding. Horses in which enteral feeding is not possible are candidates for total parenteral nutrition. Thiamine may be of benefit in treating head injuries because thiamine aids in metabolism of lactic acid and is a necessary coenzyme in brain energy pathways.

The prognosis for cranial trauma is dependent on severity of insult and early treatment, and it is gauged by response to treatment. An early prognosis based on initial findings is important to establish for owners and thus can influence clinical decisions. However, in a study performed in human TBI patients, even with sophisticated clinical and radiologic technologies, it was not possible to predict outcome on the first day after the accident with sufficient accuracy to guide early management.[174] In general, recumbency of more than 4 hours' duration and the presence of basilar fractures and severe brainstem injuries carry a grave prognosis.[144] Time, good nursing care, and adequate nutritional support, especially in the recumbent horse, are vital for a positive outcome.

Spinal Cord and Vertebral Injury

Pathophysiology

Trauma to the vertebral column is typically caused by incidents such as collision with an immovable object or falling down. Recently there have been a number of reports on exercise-associated vertebral trauma with or without secondary SCI as a cause of sudden death in Thoroughbred and Quarter Horse racehorses.[143,175,176] Injury to the vertebral column can occur at all sites, but trauma to or fractures of the cervical vertebrae are the most common.[141,142] Foals appear to be more susceptible to vertebral trauma than adults and frequently suffer injury to the cranial cervical (C1–C3) and caudal thoracic (T15–T18) regions.[148] In fact, fracture of the axial dens with atlantoaxial subluxation is most commonly seen in foals less than 6 months of age and involves disruption of the physis of the dens and separation of the odontoid process.[177] Vertebral fracture with subsequent SCI, not as a result of trauma but secondary to osteomyelitis, has been reported in a foal.[178] Predilection sites for vertebral trauma in adult horses are the occipital-atlantoaxial region, the caudal cervical region (C5–T1), and the caudal thoracic region. Reports also exist of injuries at the lumbosacral[143,175,176] and coccygeal[179] regions.

Typically, the more severe the insult is, the more damage occurs to the vertebral column (and the more severe clinical signs are due to soft tissue damage and osseous fragments compressing the spinal cord; Table 11.8). With very severe injury, soft tissue structures supporting the vertebral column may be disrupted, resulting in dislocation of vertebrae. Both subluxation and luxation of vertebrae have been reported in horses.[180] An increased incidence of luxations, subluxations, and epiphyseal separations is seen in young horses, which is likely because cervical vertebral growth plate closure does not occur until 4 to 5 years of age. Compression injuries are

TABLE **11.8 Common Types of Vertebral Trauma**

Level of Injury	Age	Type of Vertebral Trauma	Common Traumatic Injury	Syndrome
Cervical	Foal to yearling	Fracture of dens, luxation C1–C2	Hyperflexion (e.g., somersault)	Tetraparesis, respiratory depression, death
Cervical	Young adult	Epiphyseal fracture	Hyperextension	Tetraparesis to tetraplegia
Cervical	Adult	Compression fracture	Head-on collision	Tetraparesis to tetraplegia
Cranial thoracic	Usually young	Fracture of dorsal spinous process	Flipping over backward	Often none
T2–S1	Any	Transverse fracture of vertebral arch, with dislocation	Somersaulting or falls	Paraparesis
Sacroiliac subluxation	Adult	Subluxation	Falls or slipping on ice	None
Sacral fracture	Any	Compression	Fall over backward or dog sitting when backed	Urinary and fecal incontinence with or without posterior paresis, paralysis of the tail and anus

From Robinson NE, editor: *Current therapy in equine medicine,* ed 6, St. Louis, 2009, WB Saunders.

associated with shortening of the vertebral body and result from a head-on collision with an immovable object.

SCI secondary to trauma is a dynamic process of which the severity is related to the velocity, degree, and duration of the impact. Cord concussion with transient neurologic deficits is a result of local axonal depolarization and transient dysfunction, whereas permanent paralysis is a result of primary tissue injury followed by spreading of secondary damage that expands from the injury epicenter.

Primary injury is the initial mechanical damage to the components of the spinal cord that follows acute insult. Blood vessels are broken, axons are disrupted, and neuron and glial cell membranes are damaged. Ensuing pathophysiologic processes involving ischemia, release of chemicals from injured cells, and electrolyte shifts alter the metabolic milieu at the level of the lesion and trigger a secondary injury cascade that substantially compounds initial mechanical damage. These secondary injury processes do not necessarily coincide with the clinical picture, because pathologic changes may progress in severity for weeks to months, even in the face of clinical improvement.

Secondary injury involves both necrotic and programmed cell death, and although mechanisms involved in this are not fully understood, some aspects of this process are well described. Disruption of cellular and subcellular membranes of glia, neurons, and vascular endothelial cells is believed to be the initiator of this autodestructive cascade of events, and it is likely that multiple mechanisms are involved such as ischemia, inflammation, free radical–induced cell death, excitotoxicity, cytoskeletal degradation, and induction of apoptotic pathways. The consequence of secondary injury is enlargement of the area of cell death. The phase of secondary injury is widely studied because this process progresses from minutes to months after injury and is thus considered to be a target for therapeutic interventions. Minimizing secondary injury through protection of neural elements that initially survived the mechanical injury would increase the quantity of spared tissue and could lead to reduced functional impairment.

Acute injury results in immediate hemorrhage and cell destruction within the central gray matter. Loss of microcirculation and cord swelling within minutes of injury occur mainly because of hemorrhage and development of edema. The initial hemorrhage, edema, and hypoperfusion of the gray matter extend centripetally within minutes to hours of injury and result in central necrosis, white matter edema, and, eventually, demyelination of axons through secondary injury processes. Spinal cord ischemia develops over several hours after injury and is considered one of the most important contributors to secondary injury.[181,182] Mechanical disruption of the microvasculature, vasospasm of intact vessels, and edema together lead to profound local hypoperfusion and ischemia. Cord swelling that exceeds venous blood pressure results in secondary ischemia, and ischemia is further exacerbated by cessation of autoregulation of spinal cord blood flow and systemic hypotension.

During the ischemic hypoxic state, cell metabolism is altered such that a shift occurs from aerobic to anaerobic metabolism, which is a less efficient method of energy production. Anaerobic metabolism results in lactic acid accumulation, causing acidosis in nervous tissue, thus decreasing glucose and oxygen consumption. Furthermore, lactic acid stimulates prostaglandin production, adenosine diphosphate release, platelet aggregation, thromboxane A_2 release, vasospasm, vasoconstriction, and the inhibition of neurotransmitter release. In addition, in hypoxic states, the sodium-potassium ATPase-dependent cell pump is inhibited or damaged, resulting in the cell's inability to maintain its electric polarity. Damage to this pump allows for accumulation of potassium extracellularly and sodium intracellularly, which contributes to the development of edema.[183]

Free radicals can cause progressive oxidation of fatty acids in cellular membranes (lipid peroxidation) through reactions with their unpaired electrons. Furthermore, oxidative stress can disable key mitochondrial respiratory chain enzymes, alter DNA/RNA and their associated proteins, and inhibit sodium-potassium ATPase. These changes can induce metabolic collapse and necrotic or apoptotic cell death and are considered important during the initial period of hypoperfusion (and perhaps even more important during the period of reperfusion). In addition to oxidative stress and membrane damage, nitric oxide production and excitatory amino acid–induced calcium entry are considered important mediators of necrotic and apoptotic cell death.[184] Apoptosis, or programmed cell death, is a slowly spreading form of cell death induced by the injury. It is characterized by apoptotic neurons at the lesion margins and, even later, apoptosis of oligodendrocytes in areas with degenerating axons that were injured at the original lesion site.[184,185] Apoptosis can thus occur at quite remote distances from the point of impact. Oligodendrocytes appear vulnerable to apoptosis, and death of these cells can result in demyelination of otherwise spared axons, thus contributing to the loss of distal neurologic function.

Excitotoxicity refers to the deleterious cellular effects of excess glutamate and aspartate stimulation of ionotropic and metabotropic receptors. Extracellular concentrations of both of these excitatory amino acids are increased after acute SCI, which occurs through release from damaged neurons, decreased uptake by damaged astrocytes, and through depolarization-induced release. Ionotropic receptors include the NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainite receptors through which extracellular calcium and sodium can pass down a massive concentration gradient into the cell or, when activated, can result in release of calcium from intracellular stores. Metabotropic glutamate receptors are coupled to G proteins that act as secondary intracellular messengers to mediate a wide spectrum of cellular functions. Furthermore, elevation of intracellular calcium concentration can occur through direct membrane damage and voltage-gated calcium channels triggered by membrane depolarization. Elevated intracellular calcium concentrations can trigger a multitude of calcium-dependent processes that can lethally alter cellular metabolism, such as activation of lytic enzymes (calpains, phospholipase A_2, proteases, and lipoxygenase), generation of free radicals, impairment of mitochondrial function, spasm of vascular smooth muscle, and binding of phosphates with subsequent depletion of cell energy sources. Sodium dysregulation is thought to be important in the pathophysiology of damage to axonal and glial components in the white matter through similar mechanisms that lead to elevated intracellular calcium concentrations.

Controversy exists surrounding the role of inflammation in acute SCI, mainly because the effects of inflammatory cells can be both cytotoxic and protective. After SCI, the injury site is rapidly infiltrated by blood-borne neutrophils, which can secrete lytic enzymes and cytokines. Later, blood-borne macrophages and monocytes are recruited, as well as locally activated resident microglia, both of which subsequently invade to

phagocytize the injured tissue. These and other reactive cells produce cytokines, such as tumor necrosis factor–α (TNF-α), interleukins (ILs), and interferons, that mediate the inflammatory response and can further damage local tissue and recruit other inflammatory cells. Among the cytokines involved in secondary SCI, TNF-α is perhaps the most extensively studied. It is produced by a range of different cell populations, including neutrophils, macrophages and microglia, astrocytes, and T cells and has been shown to accumulate quickly at the site of SCI. It has been suggested that the early inflammatory phases are deleterious in nature, whereas the later inflammatory events appear to be protective.[182]

Neurologic Evaluation

Clinical signs seen as a result of SCI reflect the extent and location of the injury. Neurologic signs are usually observed immediately after the accident but may occur weeks to months after the initial insult because of delayed damage to the spinal cord caused by instability, arthritis, or bony callus formation at the site of impact. Clinical signs depend on the neuroanatomic location of injury and range between inapparent to severe incapacitating tetraparesis or tetraplegia (see Fig. 11.24). Lesions causing recumbency are mostly found in the caudal cervical or thoracic spinal cord, whereas lesions of nonrecumbent horses are mostly found farther cranial in the cervical spinal cord or in the lumbosacral cord.[142]

Initial evaluation of the patient should be directed toward stabilization and correction of any life-threatening problems such as airway obstruction, hemorrhage, cardiovascular collapse, and pneumothorax. In addition, major long-bone fractures must be identified, because these may be the limiting factor for survival of the horse. All affected horses may be nervous or agitated as a result of pain and the inability to stand. A systematic neurologic evaluation should then be performed to localize the site of injury.[3] In recumbent horses the use of a sling to assist standing may be a valuable diagnostic tool for localizing the site of injury and for assessing progression of disease and prognosis.[9]

In animals, SCI usually occurs as a solitary lesion and the level of the lesion can be diagnosed by neurologic examination.[186] Depression or loss of a segmental spinal reflex implies damage to either the afferent, efferent, or connecting pathways of the reflex arc. However, after acute SCI a phase of spinal shock can occur in which profound depression is noted in segmental spinal reflexes caudal to the level of the lesion, even though reflex arcs are physically intact. Spinal shock occurs in all species; however, it appears to be of much shorter duration in dogs, cats, and rabbits compared with primates.[1,186] Another syndrome that occurs infrequently and is short-lived in the horse is Schiff-Sherrington syndrome, in which extensor hypertonus is present in otherwise normal thoracic limbs in patients with severe cranial thoracic lesions.[1]

Cord injury typically results in damage that is worse in the large myelinated motor and proprioceptive fibers compared with the smaller or nonmyelinated nociceptive fibers. Therefore ataxia and loss of proprioception and motor function will occur before the loss of deep pain. Flaccid paralysis with hyporeflexia or areflexia, muscular hypotonia, and neurogenic muscle atrophy are characteristic of a lower motor neuron lesion. Signs resulting from an upper motor neuron spinal cord lesion include loss of voluntary motor function, whereas muscle tone may be increased and spinal reflexes may be normal to hyperactive.

In horses, lesions in the C1 to T2 region are most common and result in varying degrees of dysfunction ranging from tetraparesis to recumbency. Thoracolumbar SCI can result in paraparesis to recumbency, and horses may dog sit. Sacral cord damage can result in fecal and urinary incontinence, loss of use of tail and anus, muscle atrophy, and mild deficits of pelvic limb function. Sacrococcygeal cord injury can produce hypalgesia; hypotonia and hyporeflexia of the perineum, tail, and anus; or total analgesia and paralysis of those structures. In addition to these clinical signs, loss of sensation can occur distal to the level of SCI. Furthermore, diffuse sweating can be seen as a result of loss of supraspinal input to the preganglionic cell bodies of the sympathetic system in the thoracolumbar intermediate gray matter. Patchy sweating can be seen with damage to specific preganglionic or postganglionic nerve fibers.

Ancillary diagnostics that may aid in diagnosing or localizing SCI include radiography, myelography, CT, MRI, nuclear scintigraphy, CSF analysis, nerve conduction velocities, EMG, and transcranial magnetic stimulation. Radiography may demonstrate fractures, luxations, subluxations, and vertebral compression. As presented previously, CT and radiography are the diagnostic aids of choice to evaluate skeletal injury, whereas MRI is more sensitive for evaluation of soft tissue structures such as the spinal cord and ligaments. With respect to imaging the vertebral column with computed tomography and MRI devices, however, the size of the horse, the size of the equipment aperture, and the cost of the equipment aperture limit its use to investigations of the cervical and cranial thoracic spinal cord. Myelography may be required to confirm spinal cord compression and can be used at the level of the cervical, cranial thoracic, and sacral-coccygeal spinal cord. Nuclear scintigraphy can be useful in diagnosing nondisplaced or occult fractures and soft tissue lesions. Common CSF abnormalities after SCI include xanthochromia and mild to moderate increased total protein concentrations. CSF analysis may be normal, especially in very acute or chronic cases. NCV and EMG studies evaluate the lower motor neuron and aid in lesion localization. Electromyographic changes, however, may not develop until 1 to 4 weeks after nerve damage. Transcranial magnetic stimulation allows detection of functional lesions in descending motor tracts through recording of magnetic motor-evoked potentials. This method has been validated and used to distinguish motor tract disorders from other causes of recumbency in clinical cases.[160]

Treatment and Prognosis

Spinal cord injury is currently incurable, and treatment is limited to minimizing secondary complications and maximizing residual function by rehabilitation. It is the assortment of pathophysiologic processes that occur during the period of secondary injury that are considered the target for pharmaceutical intervention. The first 48 hours after acute SCI are dominated by the vascular and biochemical changes that occur within the spinal cord. Then follows a period characterized by the effects of inflammatory cells that occurs within hours of injury, peaking around 4 days after injury. Last there is a period that starts approximately 1 week after injury and is characterized by axonal regeneration and lesion repair. The goals of treatment are to stop the cascade of cellular events initiated by the traumatic insult, to protect spared neural tissue, and to promote regeneration. Studies of spinal cord repair can be categorized by clinical target and have been reviewed

elsewhere.[183,187] Rescue therapies encompass early interventions that prevent the spread of damage beyond the initial site of injury. Examples include surgical decompression, cooling or hypothermia, and interventions targeting a specific biologic event such as inflammation. Reactivate therapies refer to how spared systems, which are nearly always present in clinical SCI, can be exploited through rehabilitation, pharmacologic or electrical stimulation of spinal networks, or remyelination of denuded axons. Rewiring interventions encompass treatments aimed at regrowth of injured axons or repurposing of spared ones, with strategies aimed at increasing the intrinsic growth capacity of injured neurons or reducing inhibitors of axon growth.

Surgical intervention is warranted when the need exists for stabilization or fracture repair, as well as when a compressive lesion is evident; however, this is uncommonly done in equine medicine.[177] Conservative management is often effective, but there may be advantages of performing stabilization surgery before clinical signs deteriorate.[188] Furthermore, it is possible that future neurologic disease progression occurs as a result of a "domino effect" involving the vertebral bodies cranial and/or caudal to an (incomplete) fused articulation.[189] This process may take months to years to occur. The use of medical treatment to stabilize the patient should always be instituted before surgery is performed.

Acute SCI often results in impaired cardiopulmonary function such as impaired ventilation, bradycardia, and hypotension. This is particularly the case in lesions cranial to C5 (respiratory center affected) and cranial to T2 (origin of sympathetic outflow = thoracolumbar spinal cord). Systemic hypotension may exacerbate spinal cord hypoperfusion and ischemia, and maintaining systemic blood pressure has been shown to improve spinal cord perfusion. Volume resuscitation is clearly indicated in shock and for restitution of tissue perfusion. The current recommendation is to maintain euvolemic normotension; because of sympathetic outflow disruption after cranial SCI, pressor therapy is commonly indicated in the treatment regimen. Maintaining normal mean arterial blood pressure is also important to consider during stabilization of the acutely injured horse, and it is particularly important when horses are placed under general anesthesia for various diagnostic and therapeutic procedures.

Similar to TBI, SCI has a complex multifactorial pathophysiology and likely requires a combinational treatment intervention for successful outcome. Many agents, which could target different aspects of the secondary injury mechanisms, have been investigated for use in SCI and have been reviewed.[187] After the initial trauma of SCI, cell death and tissue loss continue for several weeks, a period during which one could intervene with neuroprotective strategies. Historically, three approaches have been taken to restrict secondary damage: surgical decompression, therapeutic hypothermia, and drugs targeting inflammation or excitotoxicity.[187] Methylprednisolone sodium succinate (MPSS) offers little benefit and might actually have detrimental effects, but although it has been formally denounced as a standard of care for human SCI in most countries, the drug is still used in some clinical practices. In horses, corticosteroids, alone or in combination with other drugs, are likely the most commonly used drugs for acute CNS trauma. Reported dosages of dexamethasone for horses range from 0.1 to 0.25 mg/kg intravenously every 6 to 24 hours for 24 to 48 hours. A favorable response is expected within 4 to 8 hours after administration. Horses on corticosteroid therapy should be monitored closely for the development of laminitis or *Aspergillus* spp. pneumonia. If improvement in clinical signs is observed, the horse may be placed on oral prednisolone therapy (0.5–1.0 mg/kg tapered over 3–5 days) to decrease the chance of laminitis. The neuroprotective effect of corticosteroids is thought primarily to be mediated by free radical scavenging but may include decreased catecholamines and glutamate, as well as decreased apoptosis-related cell death.[190] Other potential beneficial effects of corticosteroids include reduction in the spread of morphologic damage, prevention of the loss of axonal conduction and reflex activity, preservation of vascular membrane integrity, and stabilization of white matter neuronal cell membranes in the presence of central hemorrhagic lesions.[183] Furthermore, their antiinflammatory properties may be useful in reducing edema and fibrin deposition, as well as their ability to reverse sodium and potassium imbalance because of edema and necrosis. Another beneficial effect of corticosteroids is maintenance of normal blood glucose concentrations while maintaining electrolyte balance.[183]

Promising studies that target rescue investigate (1) the macrophage response after SCI; (2) peroxisome proliferator-activated receptors, which are a family of ligand-activated transcription factors that regulate many aspects of neuroinflammation; (3) GM6001, a broad-spectrum matrix metalloprotease inhibitor; and (4) riluzole, a sodium channel blocker and glutamate antagonist. As mentioned for TBI, the use of NSAIDs such as flunixin meglumine and phenylbutazone may decrease the inflammation in horses associated with a traumatic episode. In addition, there is likely a benefit to maintaining a normal rectal temperature. These compounds work by inhibiting cyclooxygenase, which converts arachidonic acid to inflammatory mediators (endoperoxides). In addition, the potential beneficial properties of DMSO, 1 g/kg intravenously as a 10% solution for 3 consecutive days followed by three treatments every other day, likely warrant inclusion of this drug in the treatment of CNS trauma.[147] Reported benefits of DMSO include increased brain and spinal cord blood flow, decreased brain and spinal cord edema, increased vasodilating prostaglandin E_1 (PGE_1), decreased platelet aggregation, decreased prostaglandin E_2 (PGE_2) and prostaglandin F_2 (PGF_2), protection of cell membranes, and trapping of hydroxyl radicals.[183] The exact mechanism of DMSO remains unknown, and this treatment remains controversial because some researchers have found no positive effects on neurologic outcome from the use of DMSO. Although the free radical scavengers vitamin E and selenium have been shown to be beneficial in SCI, these antioxidants do not appear useful in the acute management because of the length of time required to achieve therapeutic concentrations in the CNS.

Similar as is stated for TBI, antibiotics are not always necessary in the treatment of vertebral or spinal cord trauma; however, they are indicated in treating open fractures and secondary complications associated with a recumbent horse, such as pneumonia and decubital sores.

Physical therapy is important in the rehabilitative process in spine-injured horses. The field of equine rehabilitation is growing rapidly, and novel techniques and ideas have recently been reviewed.[191-194] Controlled exercise allows the unaffected parts of the nervous system to compensate for the affected parts by increasing strength and conscious proprioception. Exercise is especially helpful in improving weakness, ataxia, spasticity, and hypermetria. In recumbent horses, massage,

therapeutic ultrasound, and hydrotherapy of affected muscle groups for 10 to 15 minutes at least twice a day are important. These measures help combat necrosis and muscle atrophy of the horse's dependent muscle groups. Passive flexion and extension of all limbs are helpful in maintaining full range of motion in recumbent horses. Furthermore, experimental studies have shown that exercise enhances functional recovery after SCI.[195]

Prognosis is based on response to therapy and is directly related to the time from injury to the institution of treatment. Horses that show rapid neurologic improvement have a fair to good prognosis. Recumbent horses or horses suffering from fractures or luxations have a guarded to poor prognosis. Horses that have lost deep pain sensation have a functional or anatomic spinal cord transection and have a grave prognosis. The longer the time from loss of deep pain to treatment, the poorer the prognosis. Partial or complete recovery of horses with spinal cord trauma may take weeks to months, so time and nursing care are required.

VESTIBULAR DISEASE

Monica Aleman

The vestibular system is a special proprioceptive system responsible for maintenance of balance and reflex orientation to gravitational forces. This system functions to maintain appropriate eye, head, trunk, and limb position during rest and movement.[1,4,196]

Vestibular System

The vestibular pathway, from neural receptor to cerebral cortex and tracts in the spinal cord, is comprised of peripheral and central components.[196] The peripheral part of the vestibular system is housed in the petrous part of the temporal bone. Neuron 1 (first-order neuron) is located in the vestibular ganglion, which receives impulses from five sites: the crista of the ampulla of each of three semicircular canals, and the macula of the utricle and macula of the saccule. The cristae record movement of the head, and the maculae record position of the head. Movement and gravity act on neuroepithelial cells that transform physical forces into electrical impulses. The axon of neuron 1 projects into the vestibular nuclei of the same side. Neuron 2 is located in the vestibular nuclei, which lie near the cochlear nuclei in the medulla oblongata. Some of the axons from these neurons ascend on the same side, and others decussate. Their axons project to one of multiple areas[196]:

1. Cerebral cortex:
   a. Axons of the medial geniculate nucleus (neuron 3) within the medial geniculate body project to the cerebral cortex.
2. Brainstem:
   a. Motor nuclei of cranial nerves III, IV, and VI—influencing eye movement (nystagmus) when the head moves. This normal movement is called physiologic nystagmus or vestibular-ocular nystagmus.
   b. Ascending and descending reticular formation (involved in cardiovascular and vomiting reactions [people] with vestibular disturbances)
3. Cerebellum:
   a. Flocculonodular lobe of cerebellum (when altered: paradoxic vestibular disease)
4. Spinal cord:
   a. Lateral vestibulospinal tract
   b. Medial vestibulospinal tract

A more detailed explanation follows. Axons from the medial vestibular nucleus project through the medial longitudinal fasciculus. Vestibular signals travel through to the contralateral medial geniculate nuclei of the thalamus to the cerebral cortex. In addition to proprioceptive information from other parts of the body, the cerebral cortex facilitates conscious perception of orientation.[1]

The ascending portions of the medial longitudinal fasciculus course to the motor nuclei of the third, fourth, and sixth cranial nerves (oculomotor, trochlear, and abducens).[1,4,196] These fibers coordinate conjugate eye movement with changes in head position. This pathway, along with cerebellar input, controls physiologic (vestibular) nystagmus.[1,196] Nystagmus is characterized by involuntary, conjugate, rhythmic eyeball oscillations with a fast and slow phase. The direction of nystagmus is defined by the direction of the fast phase and is induced by movements of the head. Rapid dorsiflexion of the neck results in vertical nystagmus, whereas side-to-side movement of the head induces horizontal nystagmus. Turning the head to the left results in a horizontal nystagmus with the fast phase to the left. The accompanying slow phase is in a direction opposite to body motion and allows the eyes to fix on a stationary image. The fast phase is initiated when the eyeball reaches the lateral limit of ocular movement and allows the eyeballs to jump forward and focus on a new image.[1] The slow phase is controlled by vestibular input, and the fast phase is a function of the brainstem.[1] Physiologic nystagmus induced by rapid manipulation of the head is called the *oculocephalic reflex*. This reflex occurs independent of vision.[1,196] Descending portions of the medial longitudinal fasciculus travel in the ventral funiculus of the cervical and cranial thoracic spinal cord segments and control the position and activity of the limbs and trunk in coordination with head position.[1,196]

Fibers from the vestibular nuclei that project to the reticular formation provide afferent nerves to the vomit center, which is the pathway for the development of motion sickness. Reticulospinal tracts also aid in the maintenance of extensor tone to support the body against gravity.[1,196]

Vestibular impulses enter the cerebellum via the caudal cerebellar peduncles. Fibers of the vestibulocerebellar tracts terminate primarily in the flocculonodular lobe and fastigial nucleus.[1,196] The cerebellum functions to coordinate protagonistic, antagonistic, and synergistic muscle groups for controlled responses to gravity. The vestibular apparatus provides information to the cerebellum, dictating the relative degree of contraction necessary to maintain equilibrium.[1,196]

The vestibulospinal tract courses in the ipsilateral ventral funiculus and terminates in interneurons of the ventral gray column. Stimulation of the vestibulospinal tract is facilitatory to α- and γ-motor neurons of the ipsilateral extensor muscles, inhibitory to the α-motor neurons of the ipsilateral flexor muscles, and inhibitory to contralateral extensor muscles.[1,196] The net result is ipsilateral extensor tonus and contralateral flexor tonus that act as an adaptive mechanism against gravity by catching the body and preventing a fall in the direction of vestibular stimulation.[196]

The vestibular system is capable only of detecting movement and orientation of the head in relationship to the rest of the body. Afferent pathways from the neck allow the head

to be cognizant of the orientation of the rest of the body.[1,196] Exteroceptor receptors of the skin and proprioceptive receptors in other joints also are integrated in the cerebellum and reticular formation to aid in the maintenance of equilibrium. These signals allow the vestibular system to know if the body remains in an appropriate position with respect to gravity while the head is bent. Visual images can help to maintain balance by visual detection of the upright stance. In addition, slight linear or angular movement of the head shifts the image on the retina, which relays directional information to equilibrium centers. Visual compensation might be capable of maintaining balance in the face of vestibular dysfunction, if the eyes are open and motions are performed slowly.[196]

The seventh cranial nerve (the facial nerve) emerges from the lateral medulla ventral to the vestibulocochlear nerve at the level of the trapezoid body. The two nerves are associated closely with the petrous temporal bone and enter the internal auditory meatus together.[1,196-198] Within the internal auditory meatus, the facial nerve separates from the vestibular nerve and courses through the facial canal of the petrosal bone.[1,196-198] The facial nerve exits the cranium from the stylomastoid foramen located immediately caudal to the external auditory meatus. Because of the proximity of the facial and vestibular nerves, a single disease process commonly affects both nerves simultaneously.[197,199]

Sympathetic innervation to the eye also is associated anatomically with the petrous temporal bone. Damage to this nerve (Horner syndrome), along with vestibular and facial nerve deficits, frequently occurs with petrous temporal bone trauma and otitis media in small animals.[4] The association of Horner syndrome, facial nerve paralysis, and vestibular disease rarely is documented in the horse.[1]

Clinical Signs of Vestibular Dysfunction

Knowledge of the anatomy and function of structures related to the peripheral and central vestibular system aids in neuroanatomic localization of the lesion.[1] Differentiation of central versus peripheral vestibular disease is important for establishing a list of differential diagnoses, initiating therapy, and formulating a prognosis. A thorough physical and neurologic examination is essential. Historical information, including duration of condition, rate of onset, and disease progression, also may aid in differentiation of central from peripheral vestibular disease.

Signs of acute peripheral vestibular system dysfunction include head tilt (Fig. 11.27), nystagmus, falling, circling, reluctance to move, and asymmetric ataxia with preservation of strength. Horses affected with peracute vestibular disease are often violent because of disorientation.[4] Head tilt is a consistent sign of vestibular disease and is characterized by ventral deviation of the poll of the head toward the affected side (Fig. 11.28).[1,4,197,199,200] The horse prefers to lie on the side of the lesion and might lean on the wall toward the affected side when standing. When forced to move, the horse takes short, uncoordinated steps in a circle toward the direction of the lesion. The body may be flexed laterally with a concavity toward the lesion.[198,201] Extensor hypotonia ipsilateral to the lesion and mild hypertonia and hyperreflexia of the extensor muscles of the contralateral side result in asymmetric ataxia.[200] Extensor hypotonia occurs from loss of facilitatory neurons of the vestibulospinal tract to ipsilateral extensor muscles.



FIG. **11.27** Horse with peripheral vestibular disease. Note head tilt and turn to the left; horse is bright and alert.

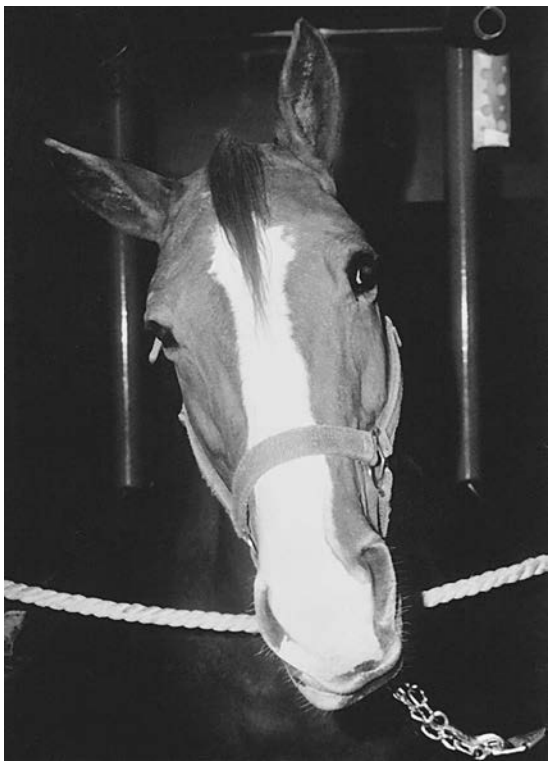


FIG. **11.28** Thoroughbred with right-sided peripheral vestibular disease caused by a temporohyoid osteoarthropathy (THO) and acute fracture. The head tilt, dropped pinna, and eyelid droop occur on the affected side, whereas the muzzle is pulled toward the contralateral side.

Contralateral extensor hypertonia occurs from loss of inhibitory neurons and unopposed extensor tone of the contralateral vestibulospinal tract.[1] Central vestibular disease has similar clinical signs, but proprioceptive deficits are a distinguishing sign from peripheral vestibular disease. Other signs of central vestibular disease include involvement of other cranial nerves, alterations in mentation, cerebellar disease, and dysconjugate and/or positional nystagmus.[1,202] The presence of vertical nystagmus is only observed with central vestibular disease.[1]

Pathologic nystagmus, rhythmic oscillations of the eyes occurring while the head is in a normal position, is involuntary and indicates a lesion in the vestibular system or cerebellum.[202] As in physiologic nystagmus, one can identify a fast and a slow phase. The direction of nystagmus is defined by the direction of the fast phase.[1,198,203] Pathologic nystagmus might be spontaneous, occurring with the head in the resting position, or positional, which is induced by elevation or lateral flexion of the head.[1] Nystagmus usually appears with the onset of other peripheral vestibular signs but might last only 2 to 3 days because of central compensation.[200] Concomitant blinking of the eyelid might hinder detection of nystagmus.[1]

Peripheral vestibular dysfunction might result in horizontal or rotary nystagmus. The fast phase of nystagmus is directed away from the lesion and does not change with changing head position.[1,201] The direction of rotary nystagmus is defined by the direction the limbus moves from the 12 o'clock position during the fast phase.[1] Horizontal, rotary, or vertical nystagmus can result from a central vestibular lesion. In addition, the type of nystagmus observed might change with changing head position in a patient with central vestibular disease.[4,198] In central vestibular disease, the fast phase is away from the lesion except in cerebellar-vestibular disease (paradoxic vestibular disease).[4,204]

In the healthy animal a constant stream of electric stimulation arises in each vestibular end organ and transmits signals that control ocular position via the medial longitudinal fasciculus. These signals normally drive the eyes toward the opposite direction. The eyes are maintained centrally, however, because vestibuloocular pathways are opposed in an equal and opposite manner. Unilateral vestibular disease upsets this balance, resulting in slow deviation of both eyes toward the lesion. Individuals that are blind at birth or have been blind for an extended time might exhibit irregular eyeball oscillation with no slow or fast component.[1] Loss of hearing is a common finding with peripheral vestibular disease because of the proximity of the cochlea to the vestibular receptor organs.[60-62,205]

Signs of vestibular disease along with obtundation and proprioceptive deficits are signs of central vestibular disease (Fig. 11.29).[1] With a central lesion, abnormal proprioception occurs because of damage within the brainstem of the descending upper motor neuron tracts to the limbs.[4] Damage to the spinocerebellar tracts or caudal cerebellar peduncles results in abnormal unconscious proprioception and hypermetria.[4] The nuclei of the trigeminal (fifth cranial nerve) and the abducens nerves (sixth cranial nerve) are in anatomic proximity to the vestibular nuclei and are damaged readily in a common disease process. Trigeminal nerve paralysis creates a loss of sensation to the head and atrophy of the muscles of mastication. Trochlear nerve damage results in medial strabismus.[197]

Destructive space-occupying lesions in the cerebellopontine angle or flocculonodular lobe may result in paradoxic central vestibular disease.[1,4,202] This syndrome is manifested by ataxia, head tilt contralateral to the side, and postural reaction deficits.[1,4,202]



FIG. **11.29** Horse with central vestibular disease. Note mild head tilt and turn to the right; horse is obtunded.

Central or peripheral vestibular disease might produce strabismus. One observes ventrolateral strabismus ipsilateral to the vestibular lesion with elevation of the head and extension of the neck.[1,4,202] One observes mild ventral deviation of the eyes in normal horses when the head is elevated, but the finding is symmetric. Ventrolateral strabismus of vestibular disease is not a sign of a cranial nerve deficit of the extraocular muscles[1] but is a reflection of abnormal upper motor neuron influences on the oculomotor nucleus from the ipsilateral vestibular nucleus via the medial longitudinal fasciculus. If the strabismus is purely vestibular in origin, normal ocular mobility is visible with manipulation of the head.[197]

Signs of vestibular disease might improve rapidly 2 to 3 weeks after onset because of visual and central accommodation.[1,197] Central vestibular lesions are slower to compensate than peripheral vestibular lesions; signs might even progress if the central lesion is an expanding space-occupying mass. Blindfolding a horse with compensated disease results in ataxia and a head tilt (Romberg's test). Blindfolding eliminates visual and limb proprioceptive orientation; the body is forced to rely on the impaired vestibular system for equilibrium.[1,197,199] This test is unreliable for localizing the side of the lesion.[196] Horses might decompensate dramatically when the blindfold is placed over the eyes, resulting in anxiety, disorientation, and falling.[1] One should perform the test with caution on a padded surface with good footing.

Horses affected with bilateral peripheral disease demonstrate no head tilt, circling, or pathologic nystagmus, and one cannot induce physiologic nystagmus by rapid manipulation of the head (the oculocephalic reflex) or by caloric testing. The head might sway with wide excursions from side to side.[203] As with all peripheral vestibular disease, strength is preserved.[1] Clinically, horses affected with bilateral vestibular disease exhibit more symmetric ataxia similar to generalized cerebellar disease.[4]

Facial nerve (seventh cranial nerve) paralysis frequently occurs concurrently with peripheral vestibular disease because of its proximity to the vestibular nerve within the petrous temporal bone. Facial nerve paralysis worsens the long-term prognosis and complicates the management of vestibular disease

patients. The facial nerve innervates the muscles of facial expression, and damage to this nerve results in muzzle deviation away from the affected side, lack of menace and palpebral response, ear droop, decreased nostril flare impeding air flow, and buccal impaction of feed.[197,198] Keratitis and corneal ulceration are common because of the inability to blink and decreased tear production.[62,198] Decreased tear production results from damage to parasympathetic fibers to the lacrimal gland. Preganglionic fibers travel with the facial nerve through the internal auditory meatus and separate in the facial canal proximal to the geniculate nucleus.[198] The fibers split from the facial nerve to join the superior petrosal nerve, which carries fibers to the sphenopalatine ganglion. Postganglionic fibers join the sympathetic fibers to the eye and travel with the vasculature to the lacrimal gland.[1,198] Corneal ulcerations occur in the inferior portion of the cornea and are slow to heal because of ongoing exposure.[62,197] Lack of tear production aids in localization of the lesion, indicating the damage is within the petrous temporal bone, proximal to the geniculate nucleus.[1,62,198] Clinical signs of facial nerve paralysis might not appear for several days after the onset of vestibular disease, because damage to the nerve might result from hematoma, callus, or an extension of inflammation and secondary neuritis.[203] Because of the proximity of the nuclei of the facial and vestibular nerves, extensive lesions of the medulla might involve both nerves. If no improvement of facial nerve deficits occurs within 3 to 4 months after the onset of disease, the prognosis is poor for recovery. If one notes even mild improvement in the first 4 months, facial nerve function might return.[203] Horses might learn to retract the globe, allowing the eyelid and nictitating membrane to slide across the surface of the cornea, distributing lubrication and protecting the eye from trauma.[197] Careful observation is necessary to differentiate this adaptation from improvement of lid function.

Peripheral Vestibular Disease

Acute onset of peripheral vestibular disease and facial nerve paralysis is not a rare occurrence in the horse. Damage to the temporal bone is the most likely anatomic location when these nerves are affected concurrently. THO and traumatic skull fractures are the most common causes of these signs in large animals.[1] THO is the most common cause of peripheral vestibular disease in horses and should be the number one rule out for horses with acute onset of vestibular dysfunction, especially if accompanied by facial nerve palsy.[62,205-207]

Temporohyoid Osteoarthropathy

Temporohyoid osteoarthropathy is one of the most common causes of acute-onset peripheral vestibular disease, facial nerve paralysis, or both in horses.[60,62,205-207] Varying causes for the disorder have been suggested, including extension of inflammation and infection from otitis media-interna[208] or guttural pouch infection, repetitive trauma, and nonseptic osteoarthritis. Much of the older veterinary literature refers to this disorder as *otitis media-interna,* whereas newer references use the designation *THO* because of the uncertaintly concerning underlying cause in most horses. The earlier presumption of underlying otitis media-interna hypothesized that rather than rupturing the tympanic membrane, the inflammatory process might extend ventrally to involve the bones of the tympanic bulla and temporohyoid joint. However, this theory remains speculative, and relatively few horses with THO have evidence of otitis or guttural pouch infection at the time of diagnosis or historically. Some or all cases of THO are quite likely the result of primary degenerative joint disease rather than an extension of a bacterial ear or guttural pouch infection.

Regardless of initial cause, inflammation induces bony proliferation at the articulation of the stylohyoid bone with the petrous temporal bone, resulting in loss of the joint space and fusion of the temporohyoid joint. The hyoid apparatus is attached to the tongue and larynx; fusion of the temporohyoid joint results in impaired flexibility of the unit. A stress fracture of the petrous temporal bone, the stylohyoid bone, or both might result from eating, vocalization, or any activity associated with normal tongue movement.[201,202] Occasionally, fracture and acute onset of severe neurologic signs (collapse and seizure) may be associated with passage of a nasogastric tube or other veterinary procedure.[207] The fracture line may extend into the cranial vault at the level of the internal auditory meatus, resulting in direct neural tissue trauma and hemorrhage into the middle and inner ear.[4,206]

Neurologic signs are rarely apparent during the formation of proliferative osteitis and temporohyoid joint fusion. The onset of neurologic signs corresponds with the occurrence of the stress fracture. Early signs, before fracture, might include headshaking, resistance to the bit, ear rubbing, resentment of manipulation of the head or ears, or pain on pressure at the base of the ear. Occasionally more overt signs of otitis externa or otitis media-interna might be present, including evidence of exudate from the external ear canal. Most horses exhibit acute onset of cranial nerve VII or VIII abnormalities (or both) at the time of fracture. Fractures can tear or stretch the facial or vestibulocochlear nerves as they exit the skull or within the skull, respectively. Some fractures extend into the cranial vault with resultant seizures, bacterial meningitis, or death.[206,207] Vestibular signs can vary between mild head tilt to circling with severe loss of balance and horizontal nystagmus or recumbency. Auditory abnormalities are evident in most affected horses when assessed by brainstem auditory-evoked responses.[60,62,205] If the facial nerve is damaged, ptosis, muzzle deviation away from the side of the lesion, a drooping lower lip and ear on the side of the lesion, and accumulation of feed within the cheek occur. Preganglionic parasympathetic fibers in the facial nerve that innervate the lacrimal gland may also be damaged with resultant keratoconjunctivitis sicca. Many affected horses have corneal ulceration varying from mild to severe. Corneal disease is secondary to decreased tear production and facial nerve dysfunction that diminishes the horse's ability to effectively close the eyelids and spread the tear film across the eye. Rarely, horses may have intermittent acute episodes of vestibular disease with intervening periods of apparent normal neurologic function. Occasionally, the fracture extends to the foramen lacerum, caudal to the petrous temporal bone, where the glossopharyngeal and vagus nerves exit the skull. Trauma to these nerves might result in dysphagia.[197]

A diagnosis of THO may be confirmed by imaging of the stylohyoid bone or petrous temporal bone by endoscopy, radiography, nuclear scintigraphy, CT, or MRI.[207,209] Although most affected horses have acute onset of unilateral neurologic signs, it is not uncommon for imaging to identify bilateral THO and bony proliferation.

CT of the skull followed by endoscopy of the pharynx and guttural pouch are the most sensitive diagnostic tests for THO in horses.[207,209] One should perform this noninvasive procedure on every horse suspected of having proliferative osteitis,

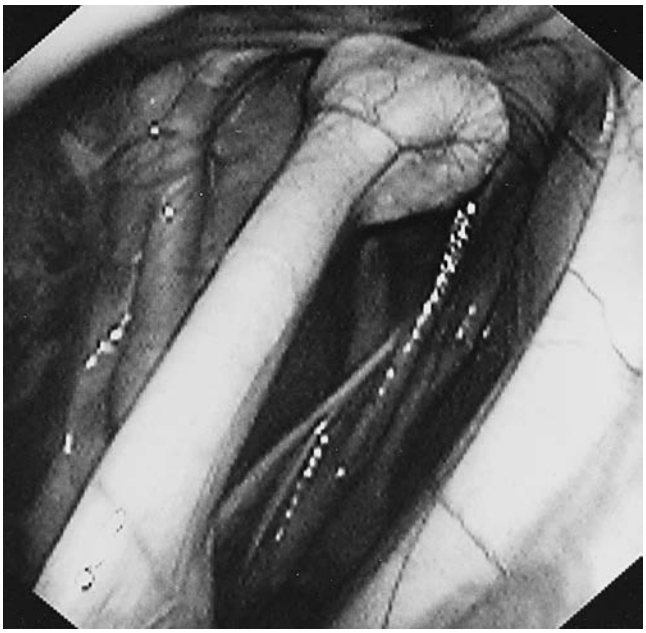


FIG. **11.30** Endoscopic view of the right guttural pouch of a horse with acute onset of right-sided vestibular disease and facial nerve paralysis. Bony proliferation of the proximal stylohyoid bone is consistent with chronic THO.

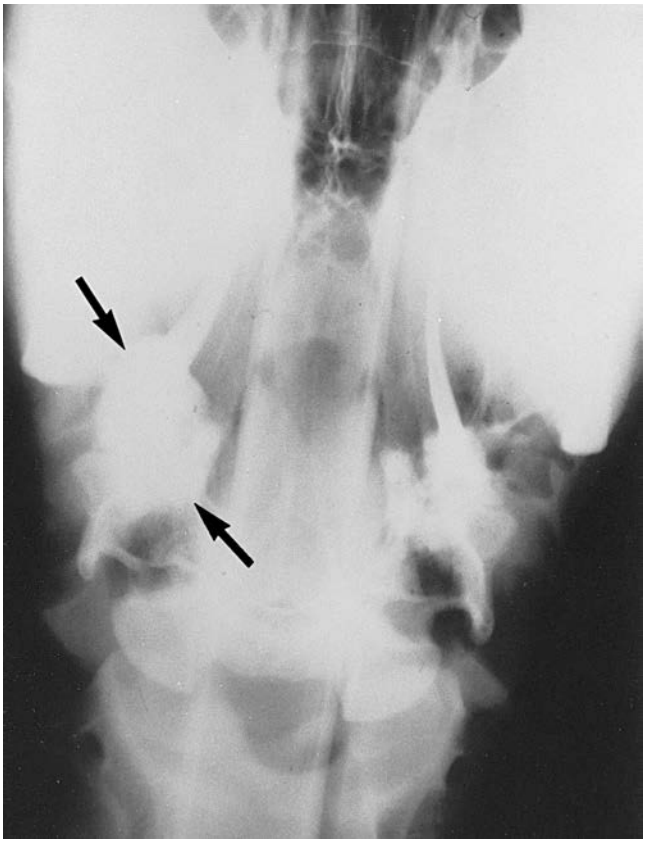


FIG. **11.31** Ventrodorsal skull radiograph demonstrating bony proliferation of the proximal left stylohyoid and petrous temporal bones *(arrows)* associated with THO.

because one often can identify bony proliferation of the proximal stylohyoid bone within the guttural pouch (Fig. 11.30). Many horses also have evidence of hyperemia, bruising, or hematoma formation in the dorsal mucosa of the guttural pouch, near the head of the stylohyoid bone.

Dorsoventral radiographs of the caudal skull should reveal the characteristic periosteal proliferation and sclerosis of the stylohyoid bone and petrous temporal bone (Fig. 11.31). The fracture line is often difficult to identify because of minimal displacement of the fracture fragments. Lateral oblique radiographs obtained at varying angles might aid in localization of a fracture of the basisphenoid, occipital, or petrous temporal bones. Moderate bony proliferation might not occur for several weeks and must be present to diagnose the condition radiographically.[205]

Bone scintigraphy is a noninvasive technique that may allow for an immediate identification of early lesions of the petrous temporal bone. Radiography can identify only structural abnormalities of bone. Bone scintigraphy is capable of detecting dynamic characteristics of bone. Increased metabolic activity and blood supply to the bone, caused by infection or fracture, results in increased uptake of the radiolabeled compound (technetium-99m–labeled phosphate) before radiographic evidence of bony proliferation.[210,211]

When available, CT and MRI are extremely sensitive diagnostic tests for identification of inflammation, bony proliferation, and fracture.[212-214] However, CT and MRI of the skull of horses require general anesthesia. The risks associated with recovery from general anesthesia, when a horse has compromised neurologic function and possible skull fracture, should be considered seriously before recommendation of these procedures.

One might consider analysis of CSF in affected horses. Cytologic evaluation, culture, and sensitivity may help to reveal the presence of secondary bacterial meningitis and can be used to direct the selection of an appropriate antimicrobial treatment plan.[200]

Treatment of horses with acute onset of neurologic dysfunction secondary to THO should focus on four areas:

1. Stabilization of the horse and decreasing inflammation near the fracture site
2. Treatment with broad-spectrum antimicrobials for either extension of a possible otitis interna-media or secondary infection in the hemorrhage that follows the fracture
3. Treatment of exposure keratitis and keratoconjunctivitis sicca
4. Surgical procedures to remove pressure on the temporohyoid articulation to decrease pain and to decrease the likelihood of repeated petrous temporal bone fracture

Medical therapy usually includes antiinflammatory medications as described elsewhere in this text for treatment of acute head trauma. Trimethoprim-sulfonamide antimicrobials for 30 days are often recommended to treat potential bacterial infection. If culture and sensitivity of CSF or other diagnostic sample are available, those results may direct alternative antimicrobial choices. Treatment of corneal ulceration and keratoconjunctivitis sicca is described elsewhere in this text.

Surgical treatment of THO might take the form of either a partial stylohyoid ostectomy or a ceratohyoidectomy.[205-207,215-217] Surgical removal of a 2- to 3-cm segment of the midbody of the stylohyoid bone results in a fibrous nonunion of the stylohyoid bone that should interrupt the transmission of hyoid forces to the temporohyoid joint. Reported

complications include transection of the lingual artery, injury to the hypoglossal or facial nerve, and regrowth of the stylohyoid ostectomy site.[215,216] The goal of ceratohyoidectomy is also to remove mechanical stress on the fused temporohyoid joint, but the surgery is technically easier to accomplish without the risk of many of the more serious complications that may be seen with partial stylohyoidectomy.[216,217]

Diagnosis of THO before the acute onset of neurologic signs is difficult because most horses exhibit few, if any, clinical signs before fracture. Some horses may demonstrate ear rubbing, head tossing or shaking, chomping movements, and sensitivity or pain on palpation at the base of the ear.

Prognosis for life in horses with THO is fair to good if the horse survives the immediate fracture episode.[205,207] However, it might take up to 2 years for maximum neurologic improvement after fracture, and the majority of affected horses have long-term, probably permanent, deficits. Some degree of facial nerve paralysis is common, and persistent or recurrent corneal ulceration may result. However, many horses eventually learn to retract the globe to assist in eyelid closure to spread the tear film across the eye, lessening the severity and frequency of corneal ulceration. Many horses return to some level of athletic function after THO.[207] However, owners of horses with THO should be cautioned regarding the potential for acute onset of severe neurologic signs with no warning as a result of refracture. Therefore affected horses pose a risk to human beings in the environment, and recognition of this risk is important. Surgical treatment may possibly diminish the risk for subsequent fracture[217]; however, currently no long-term follow-up studies of large numbers of affected horses exist to quantify or compare risk with or without surgery.

Head Trauma

Traumatic fractures of the petrous temporal bone result in damage to the vestibular and facial nerves. Profuse aural hemorrhage or loss of CSF from the external ear canal frequently is observed. Bleeding from the nose occurs if the fracture extends to the cribriform plate.[203] Clinical signs usually appear immediately after trauma and include vestibular disease, facial nerve paralysis, recumbency, or coma. Damage to nervous tissue might be caused by hematoma, callus formation, or displacement of fracture fragments resulting in delayed onset of clinical signs. Signs from brainstem contusion or concussion might be more severe than vestibular dysfunction. If blindness is present, the prognosis worsens because of the loss of visual compensation of vestibular disease in the future. If one cannot elicit the oculocephalic reflex (physiologic nystagmus), the examiner should suspect damage to the medial longitudinal fasciculus, indicating extensive brainstem damage; a poor prognosis is indicated.[1] One might recall that bilateral peripheral vestibular disease also results in loss of the oculocephalic reflex; however, this is an unlikely scenario for trauma.

Fractures of the basioccipital and basisphenoid bones occur most frequently in horses that rear over backward and strike the poll of the head. This fracture does not result from referred impact from the poll but is thought to be an avulsion fracture from the pull of the powerful ventral straight muscle of the neck (rectus capitis ventralis) on its insertion on the basioccipital bone.[218] Basioccipital fractures result in neurologic signs associated with damage to the brainstem; signs of vestibular disease are common. Petrous temporal bone fractures are difficult to identify radiographically; tympanosclerosis appears as early as 20 days after trauma and obscures the fracture line.[203] One often can identify basioccipital fractures easily but can confuse them with the suture lines in the base of the skull.[218] Treatment of vestibular signs after head trauma is similar to that of any acute head trauma as described previously in this chapter.

Drug Toxicities

Drug toxicities can result in unilateral or bilateral peripheral vestibular disease and deafness. Degeneration of the hair cells within the peripheral receptor organs of the auditory and vestibular system occurs with prolonged administration of aminoglycoside antibiotics. Severely affected animals also develop neural degeneration. A more common manifestation of aminoglycoside toxicity is renal failure. As renal clearance of the aminoglycoside decreases, the ototoxic effects of the antibiotics are potentiated.[4,199] Clinical signs of vestibular disease appear before deafness. Early vestibular disease may be reversible or centrally compensated, but loss of auditory function is permanent.[1,199] Streptomycin preferentially affects the vestibular system, whereas dihydrostreptomycin, kanamycin, gentamicin, neomycin, and vancomycin are more toxic to the auditory system.[1,202] Vincristine, a vinca alkaloid, can cause bilateral cochlear nerve damage in human beings. Auditory function improves several months after discontinuation of the drug. This antimitotic drug is a common component of multiagent chemotherapy protocols in the treatment of lymphoma in the horse. Vincristine also is used for immunosuppression and stimulation of platelet function in refractory cases of immune-mediated thrombocytopenia. One should carefully monitor auditory function when using this drug.

Sudden loud noises can result in degeneration and necrosis of the sensory hair cells of the inner ear. A lightning strike, although usually fatal, is reported to cause acute onset of unilateral vestibular disease in the horse. Facial nerve paralysis may or may not accompany the vestibular signs. Documentation of histopathologic findings in one case revealed hemorrhage and necrosis of the temporal bone, vestibular nerve, and adjacent tissue. Whether the mechanism of damage is electrocution or noise trauma is unknown.[4]

Central Vestibular Disease

Any inflammatory disease or space-occupying mass of the CNS may damage the vestibular nuclei and related tracts. Clinical signs vary with the type and extent of the disease process. One should suspect a CNS disease if abnormal mentation, seizures, blindness, or multiple cranial nerve abnormalities are seen with general proprioceptive deficits. One should perform an EEG to detect the location and extent of the CNS lesion. The EEG can detect only cerebral damage and cannot identify lesions of the brainstem. Inflammatory, parasitic, and neoplastic diseases have been implicated in central vestibular disease of the horse.

Inflammatory disease affecting the CNS includes bacterial abscess, equine protozoal myelitis, and viral encephalitis. One should perform CSF analysis to identify the inflammatory process. In the case of brain abscessation, a culture of the CSF might identify the causative organism; *Streptococcus equi* subsp. *equi* is a common causative agent.[210,211] EPM is a common neurologic disease in America and should be suspected if multifocal disease is present.[4] For polyneuritis equi to occur with vestibular dysfunction

is common, but the signs of cauda equina neuritis predominate.[1] Rabies might present as an encephalitis or spinal cord disease and should be considered in the differential diagnosis of any horse with neurologic disease. Spinal ataxia is the primary neurologic deficit observed in horses affected with equine herpesvirus (EHV) myelitis, but the presence of concurrent vestibular disease is reported.[219] The major clinical signs observed with a togavirus (eastern, western, and Venezuelan encephalitis) infection are depression and seizure, although cranial nerve deficits are observed.[4,211] Horses with West Nile virus (WNV) infection may display cranial nerve signs, including vestibular disease. Concurrent behavioral and mentation changes, ataxia, fever, and muscle fasciculations are common

Aberrant parasite migration of the CNS in horses results in acute onset of neurologic signs. Clinical signs vary, but progression of clinical signs occurs in most instances. Neurologic signs are generally asymmetric because of the random nature of migration. Neurologic disease secondary to parasite migration is discussed in detail later in this chapter. Fungal granulomata caused by *Aspergillus* spp. and *Cryptococcus neoformans* have been reported as space-occupying masses within the cranium of a horse.[4,220] Cholesteatoma (cholesterol granuloma) could involve the vestibular system by extending from the choroid plexus of the fourth ventricle of the brain.[4] Neoplastic diseases of the CNS are rare in the horse. Any tumor affecting the cerebellomedullary angle could result in vestibular signs.[1] Lymphoma, ependymoma, meningeal melanoma, and melanotic hamartoma have been reported to affect the CNS of the horse.[1,221]

Ancillary Diagnostic Tests

Caloric Testing

The caloric test is a diagnostic aid that might be helpful in differentiating central from peripheral vestibular disease. The test is able to assess each peripheral vestibular sensory organ separately. In the normal animal, irrigation of ice-cold water (12°C) into the external auditory canal for 3 to 5 minutes induces a horizontal nystagmus with the fast phase away from the tested labyrinth.[1,201] The water cools endolymph closest to the tympanic membrane, increasing its density. A density gradient is created within the semicircular canal, and the cooled endolymph sinks, causing displacement of the hair cells. Warm water (45°C) irrigation of the external auditory canal results in horizontal nystagmus with a fast phase toward the tested labyrinth. The warm-water test is less reliable.[199] The test does not induce nystagmus in a nonfunctional labyrinth. Animals may resist the procedure, making the test difficult to interpret, and in some animals, one cannot induce nystagmus. If an asymmetric response is obtained, the depressed reaction indicates the abnormal labyrinth.[1] The test is difficult to perform and not entirely reliable, although it might be a helpful diagnostic aid in the anesthetized or comatose horse.[201]

Brainstem Auditory Evoked Response

The cochlea is damaged by trauma or inflammation of the peripheral vestibular receptor organs, and detection of hearing loss may help to differentiate central from peripheral vestibular disease. Unilateral hearing loss is difficult to assess subjectively in the horse. BAER is a method of objective assessment of auditory function in the horse. This noninvasive, electrodiagnostic test stimulates the auditory system with a series of clicks. Far-field potentials of the brainstem auditory components are recorded via cutaneous electrodes and a signal-averaging system.[60-62,67,71,205] The response is a series of evoked potentials occurring within 10 ms after the stimulus. In the horse the evoked potentials appear on the oscilloscope as a series of five waveforms.[33] Abnormalities of the specific waveforms can identify a lesion of the corresponding neurologic structure. In the horse, functional loss of the cochlea or eighth cranial nerve results in the loss of the entire waveform on the side of injury, and the presence or absence of the waveform can differentiate a central from a peripheral vestibular lesion. General anesthesia is not necessary to perform the test, but sedation is recommended.[60-62,71,205]

DISEASES OF THE CEREBELLUM

Cerebellar abnormalities reported in horses consist primarily of neurodegenerative disorders confined primarily to a small number of breeds, including Arabians and Gotland pony foals. The cerebellum is essential for the coordination of movement. Afferent information arises from the general and special (vestibular) proprioceptive systems and the special somatic (auditory and visual) systems. The cerebellum is responsible for regulation of the rate, range, and strength of movement, as well as integration and coordination for balance and posture. Cerebellar abnormalities in horses are unusual; however, when present, they can have a profound effect on gait and posture.

Structure and Function

Knowledge of the structure and development of the cerebellum is important for understanding cerebellar function in health and disease. The cerebellum is located in the metencephalon dorsal to the pons and is attached to the pons via three cerebellar peduncles (Fig. 11.32). The caudal peduncle is composed primarily of afferent fibers arising from the medulla, the vestibular nuclei via the vestibulocerebellar tracts, and the spinal cord via the spinocerebellar tracts. The middle cerebellar peduncle contains only afferent fibers to the cerebellum that arise from the transverse fibers of the pons. The rostral cerebellar peduncle is the primary connection to the mesencephalon and carries the majority of cerebellar efferent fibers, although a few afferent fibers arise from the spinocerebellar tracts. The cerebellum consists of two hemispheres and a central region known as the *vermis*.[1] The extensive convolutions of the cerebellar cortex are termed *folia*. The cortex covers the surface of the cerebellum. On cut section, the cerebellar medulla is a central region of white matter with multiple projections called *arbor vitae*. These branches extend to the cerebellar cortex and form the white matter portion adjacent to the cerebellar cortex.

The cerebellar medulla has three nuclei: (1) the fastigial, (2) the interpositus, and (3) the lateral nuclei from medial to lateral on each side of the cerebellum. The cerebellum also can be divided into three bilateral longitudinal regions in association with these nuclei.[1,222] The medial zone, containing the vermis and the fastigial nucleus, primarily regulates the tone, posture, and equilibrium of the body in general. The intermediate zones contain the interpositus nucleus and cortex adjacent to the vermis and adjust the orientation of limbs in space, maintaining balance, posture, and muscle tone during

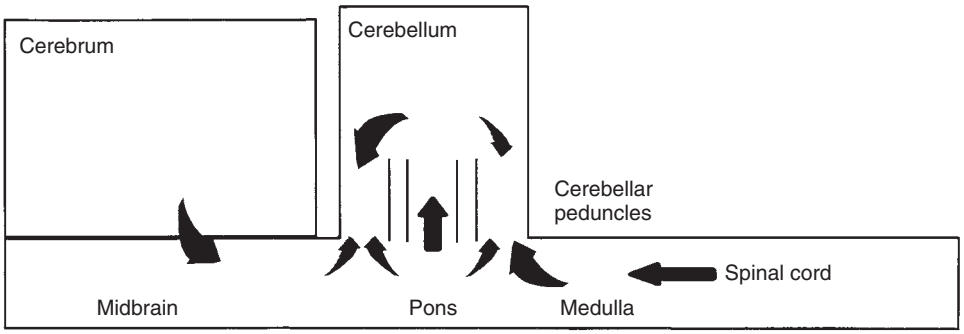


FIG. **11.32** Schematic diagram of cerebellar efferent and afferent information pathways via the cerebellar peduncles. The arrow size reflects the relative contribution of each pathway (see text for details).

complex movements. The lateral zones, consisting of the lateral nuclei and lateral portions of the cerebral hemispheres, have a similar function but do not influence posture or muscle tone directly.[222]

The cerebellum arises from the alar plate region of the metencephalon and originates initially as a proliferation of cells in the rhombic lip that extend dorsally and medially to form the dorsal portion of the metencephalon. Germinal cells proliferating in the rhombic lip eventually migrate into the cerebellum and differentiate to form the specialized neurons of the cerebellar cortex. The cerebellar cortex has three layers: (1) the outer molecular layer, (2) the middle Purkinje layer, and (3) the inner granular layer (Fig. 11.33). The molecular layer is acellular and consists primarily of the dendritic zones of the Purkinje cells and axons of the granular cells.[1] The Purkinje layer is only one cell thick and consists of Purkinje neurons. The granular layer is densely cellular with granular neurons. All layers must be present and aligned in proper orientation for normal function.

Organization of the specialized structure of the cerebellar cortex allows integration and coordination of movement. The cerebellum primarily provides regulation of skeletal movement, allowing coordinated movement; it does not initiate muscular activity. Afferent information regarding movement and balance arising from the mesencephalon, the brainstem, and the spinal cord enters the cerebellum via the cerebellar peduncles, and regulation of movement is coordinated by the inhibitory influence of Purkinje neurons on the cerebellar nuclei. Information enters the cerebellum via the cerebellar peduncles and is carried on two major afferent nerves termed *Mossy fibers* and *climbing fibers*.[1] Mossy fibers originate from the brainstem and spinal cord. Mossy fibers send collateral fibers to synapse with the cerebellar nuclei; they terminate by synapsing with granular neurons in the cerebellar cortex. These fibers are facilitatory at these synapses. The axons that granular neurons send to the molecular layer course transversely through this layer to synapse with the dendritic zone of multiple Purkinje cells and also provide facilitatory influence at these synapses. Climbing fibers originate in the olivary nucleus, which provides most of the extrapyramidal projections to the cerebellum. Similar to mossy fibers, climbing fibers send collaterals to synapse on neurons in the cerebellar nuclei; however, the axon continues through the cerebellar cortex to synapse with the dendritic zone of the Purkinje neurons in the molecular layer. As with mossy fibers, climbing fibers provide a facilitative influence at the synapses.

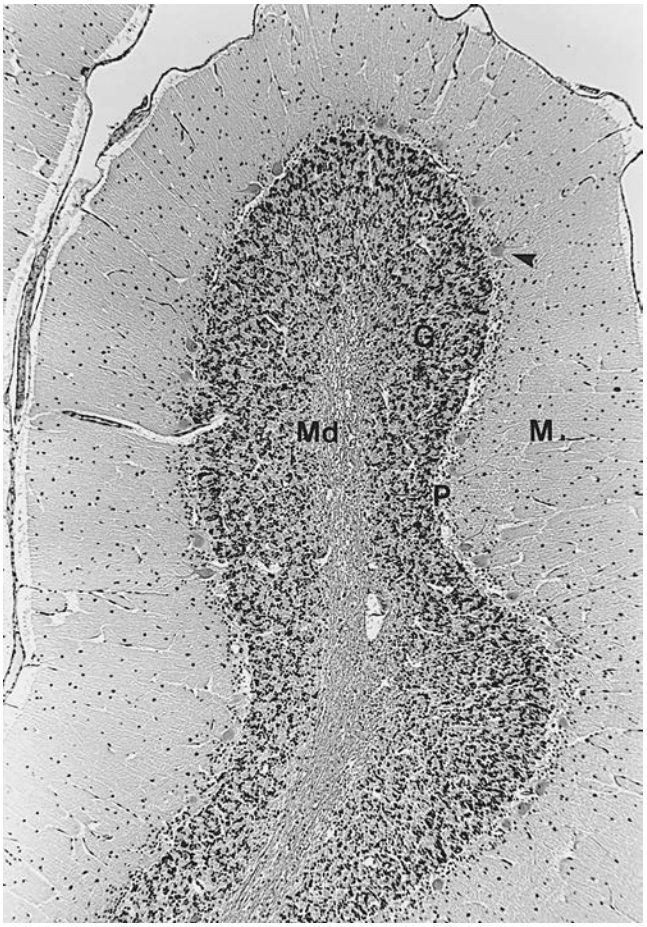


FIG. **11.33** Photomicrograph of a normal cerebellum. *M,* Molecular layer; *P,* Purkinje layer; *G,* granular layer; *Md,* medulla; *arrowhead,* a Purkinje neuron. (Hematoxylin-eosin stain; ×55.) (Courtesy the Washington Animal Disease Diagnostic Laboratory, Pullman, WA.)

Purkinje neurons provide the sole efferent fibers from the cerebellar cortex. The majority of Purkinje cell axons terminate on neurons in the cerebellar nuclei, although direct projections from these neurons to the vestibular nuclei occur via the caudal cerebellar peduncle. Purkinje neurons are inhibitory and use the neurotransmitter GABA.[1] Efferent nerves from the cerebellum are primarily from the cerebellar nuclei that facilitate activity of upper motor neurons originating in the brainstem.

Thus much of the influence of the cerebellum on skeletal muscle activity is to modulate the upper motor neuron. Information regarding movement and balance enters the cerebellar cortex via the cerebellar peduncles. This afferent activity stimulates inhibitory Purkinje neurons by climbing or mossy fibers. Purkinje neurons in turn modulate the activity of the cerebellar nuclei to regulate movement and muscular tone. Purkinje neurons also provide direct inhibitory input to the vestibular nuclei.

Clinical Signs

Clinical signs associated with cerebellar disease generally reflect the loss of coordination. Mentation is normal in horses with cerebellar disease, provided that other regions of the brain are unaffected and metabolic disturbances such as septicemia or endotoxemia are not present. Cerebellar disturbances result in ataxia and inability to regulate the rate, range, and force of movement.[1] *Dysmetria* refers to alterations in the range of gait. *Hypermetria* is an exaggerated range of movement. When moving, the limb has a higher or longer flight compared with normal. Initiation of movement might be jerky and awkward, and the trunk may sway from side to side when the horse moves. Spasticity is caused by hypertonia and results in a jerky stiff gait. Diffuse cerebellar disease results in bilateral signs. In general, unilateral lesions result in signs ipsilateral to the lesion. Intention tremor is another prominent sign of diffuse severe cerebellar disease. Tremor is most obvious as vertical or horizontal head motions and can be observed readily as a horse approaches feed or attempts to nurse. The tremor is present only when a movement is initiated and tends to become more exaggerated as the horse approaches an object.

Cerebellar disease also might cause loss of menace response and vestibular abnormalities. Disruption of the flocculonodular lobe, located in the ventral cerebellum, or the fastigial nucleus might result in vestibular signs characterized by disequilibrium; a variable nystagmus, which may be positional; and positioning difficulties.[1] Unilateral lesions might result in a head and body tilt toward the side of the lesion and nystagmus with the fast phase away from the lesion. Paradoxic vestibular syndrome, characterized by a head tilt away from the lesion and nystagmus with the fast phase toward the lesion, is apparent with unilateral lesions involving the cerebellar peduncle.[1,222]

Diseases of the Cerebellum

Cerebellar Abiotrophy and Degeneration

Cerebellar abiotrophy is the most commonly reported cerebellar disease in horses.[202,223-229] Abiotrophy in the nervous system refers to premature degeneration of neurons caused by some intrinsic abnormality in their structure or metabolism.[230] Cerebellar abiotrophy has been reported in Arabian, Gotland pony, and Oldenburg horse breeds. Degenerative cerebellar lesions have been observed in one Thoroughbred and two Paso Fino newborn foals.[231] The disorder has been commonly reported in Arabian and Arabian-related breeds.[202,223-225,227,229] The disorder is inherited as an autosomal recessive trait and is associated with a single-nucleotide polymorphism (SNP) on equine chromosome 2 (13074277G>A), located in the fourth exon of TOE1 and in proximity to MUTYH on the antisense strand.[232] The incidence in some Arabian horse herds has been reported to be as high as 8%. In one report, colts were affected more frequently than fillies, although subsequent reports have not substantiated this finding.[225] The cerebellar abiotrophy that occurs in Oldenburg horses is progressive and fatal, with atypical histologic lesions compared with the syndrome that occurs in Arabian foals, in which the degeneration is characterized by apoptosis of Purkinje cells.[233] Cerebellar abiotrophy generally affects foals less than 1 year of age and occurs most frequently in 1- to 6-month-old foals. Adult-onset cerebellar abiotrophy has been reported in other species such as the dog and has been observed in two horses.[1] Many foals are born with no abnormalities and later develop disease; however, occasionally they are affected at or shortly after birth.[202,225,228]

Clinical signs associated with cerebellar abiotrophy include intention tremors of the head, ataxia, wide-based stance and gait, dysmetria, and spasticity.[202,223-229] The most frequently reported initial signs noted by owners are an intention tremor of the head, vertical or horizontal, or a hypermetric forelimb gait.[223,228] The neurologic examination reveals no change in mentation. One almost never observes nystagmus, which has been reported in only one case of abiotrophy in a Gotland pony.[226] A menace reflex frequently is absent or diminished.[202,228] One must interpret this finding with caution because normal foals may lack or have a depressed menace reflex until at least 2 weeks of age.[234]

Stance and gait abnormalities seen with cerebellar abiotrophy generally consist of a wide-based stance or gait and ataxia.[202,227,228] The foal may move stiffly and have a high goose-stepping gait. The horse may protract the limb when walking, resulting in slamming of the foot to the ground. Movement may be spastic with circumduction. Walking on an incline, asking the foal to step over obstacles, and blindfolding the foal exacerbate gait abnormalities. Generally, gait abnormalities are symmetric, although in a Welsh Cob and Arabian cross foal the initial signs were characterized by a stiff motion in the left front limb. Signs in this foal progressed to severe ataxia.[224] Foals affected at birth may have difficulty rising.[202,225,226] Segmental reflexes are normal. Some affected animals fall when startled or when raising their heads. Signs are generally progressive for several months after diagnosis. Once the animal has reached maturity, the condition becomes static, although mild improvement has been observed.[229]

Ancillary testing is of limited value in diagnosing cerebellar abiotrophy but can be helpful to rule out other causes of ataxia. The CBC and serum biochemistry profile are normal in affected foals. Usually, there are no abnormalities in CSF and, if present, are not specific. In one study, three of four foals had an elevated CSF CK activity. Values in affected foals ranged from 6.6 to 62 IU/μL (normal range, 0–8 IU/μL).[228] CSF CK elevations generally are associated with neural necrosis or degeneration, although they are not associated specifically with a particular disease.[27,235,236]

In addition, CSF total protein might be elevated. In the study cited previously, three foals had elevated total protein with an average of 226 mg/dL (normal, 0–100 mg/dL) in all foals with cerebellar abiotrophy. As with CK, total protein elevations are not specific for abiotrophy and might occur with disruption of the blood-brain barrier or with CNS inflammation or degeneration. Many foals with cerebellar abiotrophy have normal CSF analysis. EEG abnormalities, including increased synchrony and increased number of abrupt frequency changes, also may be detectable in affected foals.[228] In this study, these abnormalities were not observed in normal foals anesthetized under similar conditions. Skull and cervical radiographs are

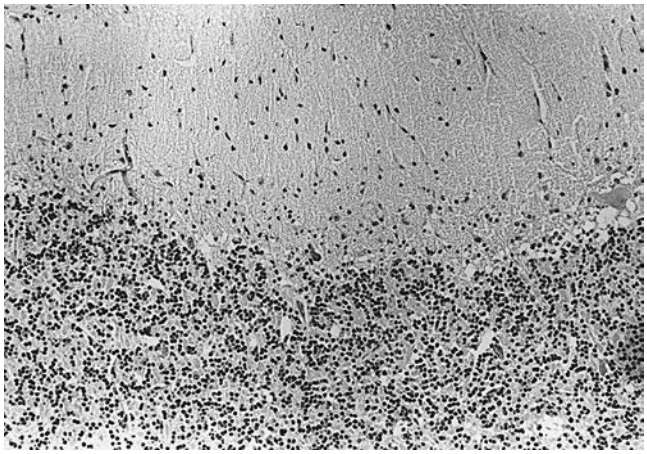


FIG. **11.34** Photomicrograph of the cerebellum from a 9-month-old foal with cerebellar abiotrophy. The decreased number of Purkinje neurons is notable. (Hematoxylin-eosin stain; ×139.) (Courtesy the Washington Animal Disease Diagnostic Laboratory, Pullman, WA.)

unremarkable. However, because mentation is normal in foals with cerebellar abiotrophy, EEG examination is not necessary to make a diagnosis and is primarily useful to exclude seizure disorders as a cause of the tremors observed.

Antemortem diagnosis of cerebellar abiotrophy is based on a typical history and the clinical signs of intention tremor, lack of menace, failure to blink to bright light, and ataxia in Arabian, part-Arabian, or Gotland pony foals. The differential diagnoses for cerebellar abiotrophy include cranial malformations; congenital spinal malformations, including atlantoaxial malformations and stenotic myelopathy; inflammation or infection of the cerebellum; and trauma. One can rule out these conditions based on the neurologic examination, CSF analysis, and radiography. The signs of characteristic ataxia and head tremor without weakness in the appropriate breed are nearly pathognomonic.

Postmortem examination provides a definitive diagnosis of this disorder. Generally, no gross abnormalities are notable; however, careful examination of the cerebellum might reveal an increased lobular pattern with prominent folia. In the Gotland pony, the weight ratio of the cerebellum to the cerebrum is reduced significantly in foals with cerebellar abiotrophy.[226] Normal foals had a 13% ratio, and affected foals had a 10% ratio. In the degenerative cerebellar condition in the Paso Fino and Thoroughbred foals, a decrease in the cerebellar-to-whole brain weight ratio was evident.[231] This ratio in normal foals was 8% and in affected foals was 6%.

Histologic abnormalities are consistent in cases of cerebellar abiotrophy. The most prominent finding is the widespread loss of Purkinje neurons[202,223-229] (Fig. 11.34). Degenerative changes, such as shrunken and angular neurons with hyperchromasia and dispersion of Nissl's substance, are apparent. One may observe occasional "baskets" or clear spaces where the Purkinje neuron is lost. Thinning of the molecular layer occurs with gliosis. The granular layer is also thin with a loss of cellularity. Similar histologic findings were found in Thoroughbred and Paso Fino foals with vacuolation and proliferation of Bergmann's glia in the Purkinje cell layer.[231]

The pathogenesis of cerebellar abiotrophy is unknown. Viral, toxic, and genetic causes have been investigated.[202,223,225,228] To date no evidence has been found to support an infectious cause. No virus has been isolated from the CSF or brain of affected foals, and no viral inclusions have been observed on histologic examination. No toxin has been associated consistently with cerebellar abiotrophy of Arabian foals. Experimental breedings of Arabian horses indicate an autosomal recessive mode of inheritance, but an exact genetic basis for disease has not been determined. A pedigree analysis of Gotland ponies similarly suggests an autosomal recessive mode of inheritance[226]; however, a high degree of inbreeding was noted, making definitive conclusions difficult. Attempts to breed affected individuals in this study were unsuccessful.

No treatment exists for cerebellar abiotrophy. As noted previously, signs may be progressive until the foal reaches maturity. Signs may stabilize or improve slightly with time.

Gomen Disease

Gomen disease is a degenerative cerebellar condition recognized in the northwest part of New Caledonia.[237] Gomen disease is a progressive cerebellar disease that causes mild to severe ataxia. Horses that are indigenous or are introduced to the region may be affected. The disease occurs only in horses that are allowed to roam free, and signs may take 1 to 2 years to develop once a horse is introduced into an endemic area. Horses that are confined generally are unaffected. Clinical signs consist of ataxia, which is most prominent in the pelvic limbs; toe dragging; and a wide-based stance. As the disease progresses, horses might have difficulty rising. The signs are primarily referable to involvement of the cerebellum; however, weakness likely is caused by brainstem or spinal cord involvement. Nystagmus is not observed. Ataxia is progressive over 3 to 4 years until the horse dies or is euthanized.

Mild cerebellar atrophy might be apparent on gross examination of the brain. Histologically, severe depletion of Purkinje neurons is evident throughout the cerebellum.[238] Purkinje neurons might contain lipofuscin pigment and vacuoles. Moderate to severe lipofuscin pigmentation of neuron cell bodies occurs throughout the brain and spinal cord. Although lipofuscin accumulation might be considered a normal variation of aging, the degree of pigment accumulation is far more severe than that in horses of similar age. The pathogenesis of this disease is unknown. Pedigree analysis has not revealed any genetic component for susceptibility to development of disease.[237] A condition of neuronal lipofuscinosis in dogs has some similarities to this disease.[238] The accumulation of lipofuscin pigment and association with free-ranging horses suggest a metabolic disorder, perhaps resulting from toxicity.[237,238]

Developmental Abnormalities

Dandy-Walker syndrome is characterized by a midline defect of the cerebellum and cystic dilation of the fourth ventricle, which separates the cerebral hemispheres.[239] Frequently, all or portions of the cerebellar vermis fail to form, and the corpus callosum may be absent. The condition is rare in horses and has been observed in Thoroughbred and Arabian foals.[240] Foals with this syndrome might be abnormal neurologically from birth, with difficulty rising, seizures, and absence of the suckle reflex.[240] The forehead might be domed excessively. Ataxia, nystagmus, aggression, and difficulty in training might persist as the foal ages.[240] Diagnosis generally is made at postmortem examination; however, one case was diagnosed antemortem using CT.[240]

Several individual cases of equine developmental cerebellar abnormalities have been described. Cerebellar hypoplasia has been described in a Thoroughbred foal that had difficulty rising and developed seizures shortly after birth.[241] Bilateral focal cerebellar cortical hypoplasia has been reported in a 6-year-old Thoroughbred gelding.[242] No gait abnormalities were detected in this horse, although it had fallen over repeatedly before euthanasia. The relationship between falling and the cerebellar abnormality is unclear. Possibly the abnormality in this adult horse resulted from a secondary problem, such as vascular injury, rather than a developmental defect.[233]

A single case of cerebellar dysplasia has been described in a 4-year-old Thoroughbred horse.[243] This horse had a 7-month history of circling and collapsing to the left side. In this case the horse had hyperplasia of the right side of the cerebellum with no associated central white matter. Histologically, the granule layer was thinning, with increased thickness of the molecular layer and cavitation of the white matter.

Additional reported developmental disorders include cerebellar hypoplasia with internal hydrocephalus and cerebellar aplasia with hydranencephaly in two fetuses from Haflinger mares with hydrops allantois.[244] Mild cerebellar degenerative changes consisting of Purkinje neuron granularity have been noted in a Standardbred filly with a chromosomal abnormality.[245] This abnormality was accompanied by mild spongiotic degeneration of the cerebrum. Abnormal neurologic signs in this filly included difficulty standing at birth, mental dullness, and a head tilt. Growth retardation, small inactive ovaries, and a consistently wrinkled muzzle accompanied these signs.

Infectious Conditions

Unlike in many other large animals, no infectious agents have the cerebellum as their primary target; however, a number of agents might affect the cerebellum. Any agent that targets the CNS, especially those that have a multifocal distribution, also may involve the cerebellum. Occasionally, disseminated *Streptococcus equi* subsp. *equi* infection (bastard strangles) may result in a cerebellar abscess.[246] Neurologic abnormalities in one reported case included proprioceptive deficits in the right thoracic limb, nystagmus, and a head tilt. Meningitis contributed to other CNS signs such as depression, blindness, and recumbency. Diagnosis of this condition can be based on a history of previous *S. equi* subsp. *equi* infection, evidence of severe suppurative inflammation in the CSF, and culture of *S. equi* subsp. *equi* from the CSF. Treatment consists of penicillin. The prognosis is guarded; however, successful surgical drainage of a cerebral *S. equi* subsp. *equi* abscess has been reported.[247]

Focal involvement of the cerebellum has been associated with aberrant parasite migration in the horse.[224,248] In a 6-year-old pony, infection with *Halicephalobus gingivalis* resulted in severe ataxia.[248] Histologic study showed lesions scattered throughout the cerebellum, brainstem, thalamus, and pituitary gland, and nematodes were observed throughout the lesions. A second case involving a 1-year-old Thoroughbred colt had a sudden onset of severe ataxia.[224] Multifocal malacia with numerous eosinophils was observed throughout the cerebellar white matter. No nematode was detected, although parasitic involvement was suspected based on the eosinophilic inflammation.

Miscellaneous Conditions

A familial neurologic condition in newborn Thoroughbred foals has been reported.[249] This syndrome affected three of five foals of a Thoroughbred mare. The foals were normal at birth and developed signs of severe incoordination, a wide-based stance, and recumbency at 2 to 5 days of age. The condition appeared more severe when the foals became excited or struggled; consequently, they were treated symptomatically with diazepam. The signs would improve with this treatment and return as the sedation diminished. These foals improved with stall rest over 7 to 10 days. The cause of the clinical signs in these foals is unknown; however, the authors suggested possible viral or toxic causes.

Cerebellar ataxia in two Thoroughbred fillies has been associated with hematoma in the fourth ventricle.[250] These two horses demonstrated fever, dysmetria, spasticity, and weakness. Clinical signs most likely resulted from compression of the adjacent cerebellum. CSF analyses in these cases revealed xanthochromia, elevated RBC and white blood cell counts, and elevated total protein concentrations. The cause of the hematomas was not identified; damage to regional small vessels and a vascular anomaly were suspected.

Chronic methylmercurial poisoning in horses can cause a number of clinical abnormalities, including cerebellar ataxia.[251] Severe poisoning can result in incoordination, dysmetria, and gross head nodding in the experimental setting. Associated clinical signs include lethargy, anorexia, exudative dermatitis, and laminitis. Lesions in the cerebellum consisted of focal atrophy and cellular depletion in the granular layer with little to no involvement of Purkinje cells. Additional abnormalities included neuronal necrosis and gliosis in the cerebrum, lymphocytic perivascular cuffing, and swollen axons in the spinal cord. Preferential accumulation of inorganic mercury in the brain and resulting cell injury most likely led to the neurologic signs observed. Diagnosis of methylmercurial poisoning can be based on clinical signs and measurement of mercury in the liver and kidney.

SHIVERS

Monica Aleman

Shivers is a chronic progressive equine movement disorder of unknown etiology. Affected horses display difficulty walking backward, assume hyperflexed pelvic limb posture, and have tremors during backward movement.[252,253] The condition might progress to involve thoracic limb movement. The condition has been reported in draft breeds, Thoroughbreds, Warmbloods, and others with less frequency such as Connemara, Welsh, Quarter Horses, Standardbreds, Saddlebreds, Tennessee Walking horses, Missouri Fox Trotters, Paint, Morgan, and mixed breeds.[252] Gait patterns of shivering were characterized in one study as (1) hyperextension when backing and lifting the limb, (2) hyperflexion and abduction during backward walking, (3) shivering hyperflexion with abduction during backward walking, and (4) shivering-forward hyperflexion including intermittent hyperflexion and abduction with forward walking.[253] Horses with shivering-hyperflexion, shivering-forward hyperflexion, and stringhalt had a prolonged swing phase duration compared with control horses and others with other types of shivers. Initially forward movements are normal.[253] Other signs might include muscle atrophy, reduced muscle strength, exercise intolerance, facial twitching, elevated tail head, and problems picking up feet for farrier (common complaint).[252] Shivering signs usually begin at less than 5 years of age and progress in the majority of cases (74% reported in one study).[252] Horses with shivers are significantly

taller (mean 173 ± 6.2 cm) with a higher male-to-female reatio (3:1) than a control population.[252]

There are no histologic lesions in the thoracic and pelvic limb skeletal muscle. In a study, eosinophilic segmented axonal spheroid and calretinin-positive axonal spheroids were found to be common lesions in the CNS, particularly in the nucleus cuneatus lateralis.[254] Calretinin-negative, calbindin-positive, and glutamic acid decarboxylase-positive spheroids were found increased 80-fold in Purkinje cell axons within the deep cerebellar nuclei in affected horses.[254] Unusual lamellar structures resembling marked myelin decompaction were observed between myelin sheaths of presumed Purkinje cell axons in the deep cerebellar nuclei of shivers but not control horses.[254] The authors concluded that shivers is characterized by end-terminal neuroaxonal degeneration in the deep cerebellar nuclei, which results in context-specific hypermetria and myoclonus.[254]

CERVICAL VERTEBRAL COMPRESSIVE MYELOPATHY

Yvette S. Nout-Lomas

Cervical vertebral compressive myelopathy (CVCM) is a common cause of ataxia and weakness in horses. It is the most common cause of ataxia in horses in Europe and Australia and is an important differential diagnosis in regions affected by inflammatory diseases such as EPM and WNV. Differentiating CVCM from other conditions that cause ataxia is important for prognostic and therapeutic considerations. The disease is also referred to as cervical vertebral malformation, cervical vertebral stenotic myelopathy, cervical stenotic myelopathy, cervical spondylotic myelopathy, equine sensory ataxia, equine incoordination, spinal ataxia, and wobbler syndrome. Compression of the spinal cord occurs secondary to stenosis, or narrowing, of the vertebral canal anywhere from C1 to T1. Stenosis of the cervical vertebral canal is a result of bone and joint malformations, the pathogenesis of which remains unclear but is likely multifactorial.

CVCM is most frequently seen in young, well-fed, rapidly growing horses. Analysis of over 800 horses with CVCM confirmed what previous studies have shown: males (stallions and geldings) and Thoroughbreds, Tennessee Walking Horses, and Warmbloods have a significantly higher likelihood of having CVCM compared with mares and other breeds. CVCM has been shown to affect 1.3% to 2% of Thoroughbred horses.[255,256] Furthermore, horses that ranged from less than 6 months to less than 7 years of age had significantly higher odds of having CVCM compared with horses 10 years of age or older.[257] Developmental bone disease in younger horses can lead to deformation and malarticulation or malalignment of vertebral bodies (Fig. 11.35A). In older horses, spinal cord compression more commonly occurs secondary to vertebral malformation because of osteoarthritis of caudal cervical articular process joints, although there may be some overlap in this distinction. Male horses and horses of Warmblood or Tennessee Walking Horse breeds may be predisposed to development of CVCM at an older age.[258] Spinal cord compression has traditionally been separated into two categories: (1) dynamic vertebral compressions, whereby the spinal cord compression is intermittent and occurs when the cervical vertebrae are flexed (more cranial vertebral) or extended (more caudal vertebrae), and (2) static compressions, in which spinal cord compression is continuous regardless of cervical position.[259] Both syndromes are likely to occur in individual cases or can occur simultaneously. The clinical significance of distinguishing between the two types of CVCM is unknown, but it is important for imaging studies because significant flexion or extension may be necessary to demonstrate dynamic compressions. Proposed etiologic factors that result in developmental bone disease include high planes of nutrition,

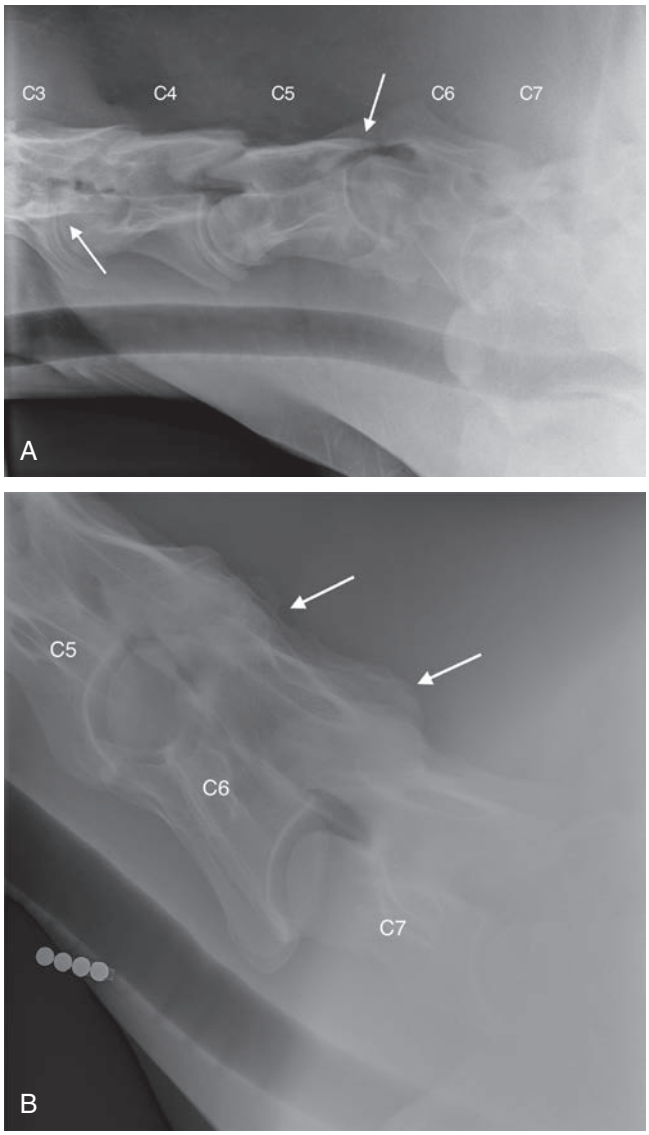


FIG. **11.35** A shows a lateral cervical radiograph taken from an 8-month-old Quarter Horse colt with grade 4 out of 5 ataxia. Cervical vertebrae 3–7 are indicated with evidence of malformation at C3–4 and C5–6 (arrows). Collapsing of the dorsal aspect of the disk space is seen at C3–4 with surrounding bony proliferation. Caudal extension of the dorsal aspect of the vertebral arch of C5 (arrow) and obvious malalignment and stenosis of the vertebral canal at C5–6 are shown. B shows a lateral cervical radiograph taken from a 17-year-old Thoroughbred gelding with a chronic bilateral front limb lameness and a 1-month history of progressive ataxia. Shown are the C5–C7 vertebrae with extensive bony proliferation at the articular processes (arrows) of both C5–C6 and C6–C7.

accelerated growth rates, altered ratios of copper and zinc, and inherited genetic determinants. None of these has so far been shown to be a direct cause of CVCM.

Clinical Signs

Most horses are diagnosed with CVCM when they are between 12 and 23 months of age.[257] Cervical vertebral compressive myelopathy is characterized by symmetric ataxia, upper motor neuron paresis, and dysmetria, which are usually worse in the pelvic limbs than thoracic limbs.[40] Physical examination sometimes reveals palpable cranial cervical vertebral flexion-fixation, and affected horses tend to be large for their age and breed. Male animals appear to be more frequently affected. Often a history of increased clumsiness occurs, and mild to moderately affected horses show symmetric ataxia with circumduction of the pelvic limbs, especially when the animal is made to walk in small circles. Proprioceptive deficits, toe dragging (flexor paresis), and varying degrees of upper motor neuron extensor paresis (assessed by pulling on the horse's tail at a walk) are also present. Thoracic limbs often have a hypometric, stiff (spastic) component to the strides, especially while walking the horse down a slope and with the head elevated. Thoracic limb deficits are usually less severe than pelvic limb deficits because of the more superficial location of pelvic limb spinocerebellar proprioceptive tracts in the spinal cord. Horses with chronic disease tend to show less paresis than those with recent-onset clinical signs.[4] Neck pain or stiffness may be present in some older horses. An accident or injury to the neck is sometimes in the history, and clinical signs may have an abrupt onset or indeed the horse may be tetraplegic, especially if external trauma plays a major role. The ataxia, however, was likely present before the traumatic incident.

Clinical signs of concurrent developmental orthopedic disease of the appendicular skeleton, such as physeal enlargement of the long bones, joint effusion after osteochondrosis, and flexural limb deformities, are often present in young horses with CVCM.[40] It is worth remembering that sometimes horses affected with CVCM may have another concurrent neurologic disease such as equine protozoal encephalomyelitis.

Pathogenesis

Compression of the spinal cord is a result of stenosis, or narrowing, of the vertebral canal anywhere from C1 to T1. Stenosis of the cervical vertebral canal is a result of bone and joint malformations, the pathogenesis of which remains unclear. Currently it is thought that in some horses there is an underlying developmental disorder of bone and cartilage morphogenesis and maturation that leads to cervical vertebral malformations, whereas in other horses malformations are thought to be the result of abnormal biomechanical stresses and forces on the cervical column.[94] Neurologic signs result from progressive spinal cord compression secondary to structural changes to vertebral and surrounding tissues. Pathologic changes of the cervical vertebrae leading to spinal cord compression consist of malformation including angular deformity of the vertebrae, malalignment between adjacent vertebrae, stenosis of the vertebral canal, flare or epiphysitis of the caudal vertebral epiphysis of the vertebral body, extension of the dorsal aspect of the vertebral arch, abnormal ossification of the articular processes, and degenerative joint disease of the articular processes (Fig. 11.35B).[40,260] Recently, detailed MRI and histopathologic analyses have also identified bone cysts within the articular processes of horses with CVCM.[94] The abnormalities found in the necks of horses with CVCM are generalized and not limited to the site(s) of spinal cord compression. Histopathologic examination of the spinal cord reveals wallerian-like degeneration, loss of axons and fibrosis at the sites of spinal cord compression, and axon loss in the ascending and descending white matter tracts within adjacent segments of the spinal cord cranial and caudal to the site of spinal cord compression.[40]

A high dietary energy intake is almost certainly necessary for expression of the disease, and trauma to the neck probably plays a role as young, large, fast-growing animals (often male animals) are most commonly affected. CVCM resulting from developmental bone disease almost certainly has a genetic basis; however, a breeding trial using horses with CVCM that had had corrective surgery resulted in offspring that did not have CVCM but did have a higher frequency of developmental orthopedic diseases than expected.[261]

CVCM in older horses is frequently associated with arthritis of the articular processes of the caudal (C5–T1) cervical vertebrae, and articular process osteophytes are the most commonly seen radiographic lesions.[258] Biomechanical forces are likely to play a part in the onset of the disease. Compression of the spinal cord is due to vertebral malformation at C5, C6, and C7, resulting in cone-shaped caudal cervical vertebrae with a reduction in a cross-sectional area of the vertebral canal and resultant impingement on the spinal cord by proliferating articular and periarticular soft tissues. There may be concurrent formation of epidural and periarticular cysts, which can result in sudden onset of symmetric or asymmetric signs without a contemporaneous episode of external injury. Bleeding into one of these cysts has been observed and adds to the compression. Occasionally these cause acute-onset severe clinical signs that can abate within a few hours only to reappear later.

Diagnosis

Fortuitously, the narrowing of the cervical vertebral canal and associated spinal cord compressive lesions in horses occur most commonly in a dorsoventral orientation, allowing the diagnosis to be made using (lateral) cervical myelography (Fig. 11.36). Good-quality, standing plain lateral radiographs of C1 to T1 vertebrae provide information on anatomy of the vertebral bodies and width of the vertebral canal but do not show the spinal cord. It should be remembered that the lack of dorsoventral radiographic projections effectively makes traditional lateral imaging an incomplete diagnostic series. The assessment of qualitative features of vertebral malformation, including physitis of the caudal vertebral body, caudal extension of the dorsal arch of the vertebral body, intervertebral malalignment, and arthropathy of the articular processes, is useful; however, alone these findings offer poor positive and negative predictive values.

The most important factor in the diagnosis of CVCM in adult horses and foals is the identification of a decrease in the diameter of the vertebral canal, be it within a vertebra or, more commonly, between two vertebrae. Corrected intravertebral and particularly intervertebral sagittal ratios (SRs) from C2 to C7 calculated from lateral standing cervical radiographs can be used to accurately diagnose CVCM in horses.[262,263] The intravertebral and intervertebral sagittal diameter of the vertebral canal is measured at the narrowest

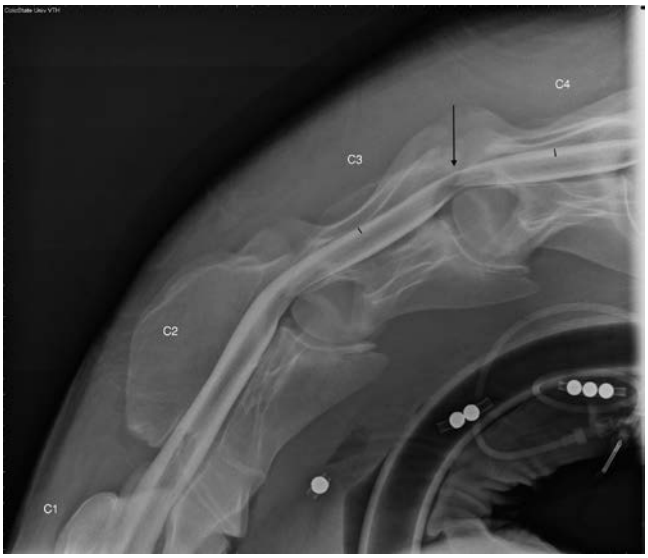


FIG. **11.36** A lateral cervical myelogram showing compression of the cervical cord at C3–C4 (arrow). At C3–C4 there is complete attenuation of the ventral contrast column with significant narrowing (>50%) of the dorsal contrast column.

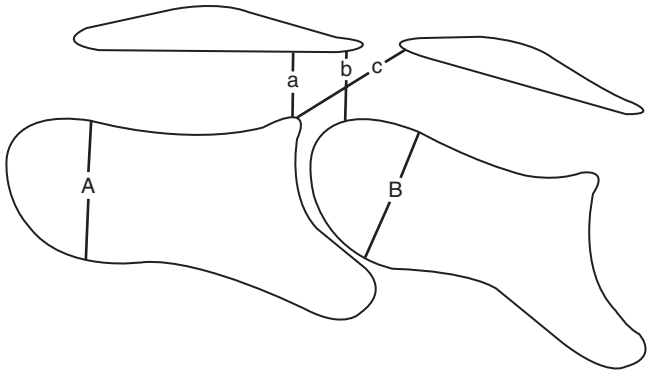


FIG. **11.37** Two cervical vertebra showing sites for measuring the intravertebral and intervertebral sagittal diameter and the width of the vertebral body *(A) (B).* For a particular vertebra, the minimum intravertebral diameter could be anywhere along the vertebral canal *(a),* and the minimum intervertebral sagittal diameter is the smallest of the two measurements *(b)* or *(c)* compared with the diameter of the more caudal vertebral diameter *(B).*

points and corrected for radiographic magnification and size of horse by being expressed as a ratio of the maximal height of the cranial vertebral physis (Fig. 11.37). The minimum intravertebral diameter is measured at the point along the vertebral canal where the diameter is at a minimum, whereas the minimum intervertebral sagittal diameter ratio is made from the caudal aspect of the dorsal lamina of the vertebral arch of the more cranial vertebra to the dorsocranial aspect of the body of the more caudal vertebra, or from the caudal vertebral body of the more cranial vertebra to the cranial dorsal lamina of the vertebral arch of the more caudal vertebra, whichever was smaller. For vertebrae C2–C3 to C5–C6, the minimum diameter is invariably found to be the former measurement (see Fig. 11.37).

Reference figures for intravertebral and intervertebral SR in a limited number of horses are shown in Tables 11.9 and 11.10. A specific site can be considered very likely to be compressed if it is more than 2 standard deviations smaller than the reference values. As a general rule of thumb, horses with signs of cervical spinal cord disease that have SR values at any intervertebral or intravertebral site that are less than 50% of reference values have a greatly increased risk of having cervical vertebral canal stenosis. One must consider, however, that there is poor agreement among observers when performing these measurements, which is likely influenced by experience. This may limit the accuracy of this method and could result in discrepancies of diagnosis.[264]

In older horses with CVCM, spinal cord compression is most often the result of coning of affected caudal cervical vertebrae. The measurements can be difficult to perform on caudal cervical vertebrae, particularly if the radiographs in large horses are underexposed. In addition, these horses have enlarged and remodeled intervertebral joints of the caudal cervical vertebrae. Many horses without neurologic signs have degrees of cervical vertebral osteoarthritis and considerable arthritic enlargement of articular processes that can occur well lateral and dorsal to the vertebral canal with no spinal cord compression.[4] Cervical scintigraphic examinations similarly cannot determine whether active arthritis is actually causing spinal cord compression. Spinal magnetic motor evoked responses[265] may be used more in the future to objectively grade the degree of electric conductivity across a spinal lesion site.

Before deciding on surgical decompression or vertebral fusion, positive contrast myelographic evidence of spinal cord compression is mandatory. It should be remembered, however, that myelography under general anesthesia is not an innocuous procedure in the horse. Adverse reactions have been shown to occur in one third of horses undergoing myelography, with the procedure having a mortality rate of 2%, which is similar to any type of surgery under general anesthesia.[266] Neurologic complications including worsening of neurologic grade and seizures are the most common adverse reaction seen.[266] Myelography has traditionally been considered the gold standard antemortem diagnostic test; however, sagittal diameter ratio analysis may be more sensitive and specific than myelography, at least if the 50% or 2-mm reduction of the dorsal contrast medium myelographic rules are used.[267,268] Myelography can result in acceptable sensitivity and specificity for detecting sites of compression by using a 20% reduction of the total dural diameter on a neutral myelographic view for the midcervical sites, and a 20% reduction of the same measurement at C6–C7 with the neck in either neutral or flexed position.[267] In some horses with CVCM, particularly those with caudal cervical arthropathy, spinal cord compression may occasionally occur in a lateral or transverse plane with no dorsoventral compression. In these cases, more subjective assessments, such as observing a blanching of the contrast column or split dorsal margins, may be the only myelographic abnormalities.

More recently a technique has been described for cervical vertebral canal endoscopy during which a flexible videoendoscope with an external diameter of 4.9 mm is introduced into the subarachnoid space dorsal to the spinal cord.[269] This technique allows for direct visualization of the structures in the vertebral canal and may allow for recognition of more subtle abnormalities in the epidural space. In the future this

TABLE **11.9 Summary Data for Sagittal Diameter Ratio by Vertebral Site in Eighteen Horses without Cervical Vertebral Malformation**

	Intravertebral						Intervertebral				
Site	C2	C3	C4	C5	C6	C7	C2–C3	C3–C4	C4–C5	C5–C6	C6–C7
Mean	7.2	6.1	5.9	6.1	6.0	6.2	9.1	7.1	7.4	8.0	7.1
Standard deviation	0.79	0.56	0.54	0.54	0.62	0.52	1.17	0.93	1.11	0.95	0.88

TABLE **11.10 Summary Data for Sagittal Diameter Ratio by Vertebral Site in Eight Horses with Cervical Vertebral Malformation**

	Intravertebral						Intervertebral				
Site	C2	C3	C4	C5	C6	C7	C2–C3	C3–C4	C4–C5	C5–C6	C6–C7
Mean	6.3	4.8	4.8	4.9	4.8	4.8	7.0	5.5	5.7	5.8	5.9
Standard deviation	1.19	0.33	0.37	0.26	0.44	0.17	1.93	1.28	0.59	1.14	0.68

technique may prove to be reliable for localization of vertebral canal stenosis and diagnosis of CVCM.[270]

Treatment

Stall rest, glucocorticoids, DMSO, and other antiinflammatory drugs may provide transient improvement in clinical signs in horses with CVCM, especially if acute exacerbation of disease secondary to trauma is seen. However, continued compression of the spinal cord is inevitable, and clinical signs will persist. For most horses for which surgical treatment is not an option or with multiple intervertebral site involvement and severe, chronic clinical signs, the prognosis is poor to grave and humane destruction is advised. A recent review of 103 horses with presumptive CVCM showed that 33 were euthanized after diagnosis, and of the remaining horses 30% had at least one racing start. Horses were more likely to race if they had a neurologic grade less than 1 in the thoracic limbs and less than 2 in the pelvic limbs.[11] In a controlled field study, in which growing foals with CVCM were fed a diet restricted in protein and energy (65%–75% of National Research Council recommendations), clinical signs and radiographic lesions resolved in some foals and significantly improved in others.[271] A positive effect of dietary modification and changing the rate of growth is likely to be seen only in young horses less than 1 to 2 years of age.[11]

Young patients with mild clinical signs of short duration and with only one site of cervical spinal cord compression have a good prognosis for return to athletic function with surgery. No controlled trials have been performed; however, it is estimated that more than 60% of such horses return to athletic function.[272-274] Ventral intervertebral fusion using a stainless steel fenestrated basket or threaded cylinder can allow expansion of the vertebral canal, atrophy of articular enlargements, and resolution of some or all the clinical signs in selected cases. This procedure has been most successful in horses with acute clinical signs resulting from spinal cord compression at only one intervertebral site between C2 and C7 and in horses with absolute stenosis and enlarged articular processes of the caudal cervical vertebrae. Horses with persistent neck pain associated with cervical osteoarthritis but no evidence of fractures to the bodies of the vertebrae are also well suited to cervical intervertebral fusion surgery.

EQUINE NEUROAXONAL DYSTROPHY/ EQUINE DEGENERATIVE MYELOENCEPHALOPATHY

Yvette S. Nout-Lomas

Neuroaxonal dystrophies (NADs) are a group of neurodegenerative diseases described in human beings and animals that are characterized by dystrophic alterations of neurons and axons and development of spheroid bodies. In the horse, equine degenerative myeloencephalopathy (EDM) is considered a more severe form of equine neuroaxonal dystrophy (ENAD) where CNS lesions are thought to be on a continuum, with EDM lesions being more widely distributed than those seen in ENAD.[3,275,276] Clinically, ENAD and EDM are indistinguishable, and both have the same basic underlying neuropathologic lesions.[277-279] ENAD/EDM is a noncompressive, diffuse, symmetric, degenerative neurologic disease characterized by symmetric ataxia, weakness, and dysmetria in young horses of many breeds and both genders. Mayhew and colleagues first described the disease in 1976,[280] and it has been subsequently diagnosed in many other breeds of horses. In addition, identical syndromes have been observed in Mongolian wild horses *(Equus przewalskii)*[281] and Grant's zebras.[282] Earlier work suggested that the pathogenesis of the disease involved a familial vitamin E deficiency,[283,284] and a series of recent studies supports the theory that ENAD/EDM is inherited as a complex trait and affects genetically predisposed horses that undergo a period of α-tocopherol deficiency.[279,285] ENAD/EDM was one of the most prevalent causes of spinal cord disease in horses in the United States during an approximate 15-year span after it was first recognized. Although no recent epidemiologic surveys exist, the prevalence of EDM appears to have declined since about 1990.[3] In Europe the disease has been reported only sporadically. However, it should be noted that clinical signs of ENAD/EDM are similar to those seen in CVCM, and the diagnosis can only be made after careful histologic evaluation of the CNS.

Clinical Signs

The onset of clinical signs may be abrupt but is usually insidious, and the age at which signs are noticed varies from less than 1 month to several years, with most horses manifesting

signs within the first year of life. One older study of 128 horses with EDM showed the age of onset ranged between 1 month to 20 years, with 16% of the horses showing signs at older than 28 months of age.[286] A more recent evaluation of a group of 88 horses with neurologic deficits suspected to be secondary to ENAD/EDM showed that 42% of the horses were older than 3 years at the time of examination; however, no specific information on age of onset of clinical signs was provided.[279] There is no sex predilection.

Signs are referable to upper motor neuron and general proprioceptive deficits and include symmetric ataxia, weakness, and dysmetria, most notably hypometria (spasticity), of all four limbs, often worse in the pelvic limbs.[3,287] Signs may begin in the pelvic limbs and progress to the thoracic limbs. Postural placing reactions may show conscious proprioceptive deficits. The gait is characterized by dysmetria and stabbing of the ground with the limbs. Horses may walk with a two-beat lateral gait, referred to as *pacing,* as also sometimes seen in horses with any spinal cord disease.[3] Often, hindlimb interference and dragging or scuffing of the toes are present. When walking backward, the horse may resist or rock back on the pelvic limbs and dog sit. When circling, affected horses often pivot on the inside hindlimb and circumduct the outside limb. Affected horses may have trouble stopping, and it is not unusual for them to fall while running in the pasture or being worked. Cranial nerve involvement, muscle atrophy, or changes in skin sensation or tail tone are absent in ENAD/EDM[280]; however, a recent evaluation of 88 horses with suspected ENAD/EDM found 60% of those horses to have an abnormal mentation ranging from quiet to obtunded, and 38% had a decreased or inconsistent to absent menace without loss of vision.[279] In addition, the presence of a pigment retinopathy in 4 out of 10 related Warmblood horses that had clinical signs of ENAD/EDM was recently demonstrated.[288] Because previous work from this same group had not found evidence of ocular disease in a group of Quarter Horses with ENAD/EDM it is suspected that the development of ocular lesions is associated with genetic differences and/or severity of α-tocopherol deficiency. It should be noted that none of the horses in which ocular lesions were identified had clinical signs of visual deficits; however, given these findings it is highly recommended to perform a careful ophthalmologic examination on cases in which ENAD/EDM is suspected. Lower motor neuron signs such as hyporeflexia over the neck and trunk with diminished to absent cervical, cervicofacial, cutaneous trunci, and laryngeal adductor reflexes may be found, especially in severe and long-standing cases.[283] The disease is considered not or very slowly progressive, and most horses stabilize at around 3 years of age. Horses do not recover from this neurodegenerative condition.

ENAD/EDM should also be included in the differential diagnosis of young horses presenting with different neurologic deficits, because it is possible for ENAD/EDM to occur in conjunction with other developmental brain diseases. For example, a 2009 report described a foal with confirmed ENAD that had clinical signs of cerebellar disease, most noticeably a head tilt and head tremor and pendular nystagmus, in addition to signs consistent with ENAD.[289] A detailed postmortem examination showed a prominent external granular layer of the cerebellum, in addition to the histopathologic features of ENAD. The authors suggested that the cerebellar lesion might have been a type of migration disorder, although this could not be proven to have occurred in conjunction with ENAD. Migration disorders are another complex group of diseases resulting from failure of embryologic neuronal cells to migrate properly to their final location.

Pathology

Gross necropsy findings in EDM are unremarkable. Classic histologic lesions are evident in the brainstem and spinal cord.[40,279,287,290] In ENAD, lesions are restricted to certain nuclei in the brainstem and spinal cord gray matter, whereas in EDM, lesions also occur in white matter tracts of the spinal cord.[3,279,290] Specific brainstem nuclei in which lesions have been found are the lateral and medial cuneate nuclei, gracile nucleus, nucleus of the solitary tract, trigeminal nucleus, vestibular nuclei, olivary nuclei, and reticular formation. Lesions have also been identified in the medial lemniscus of the medulla oblongata and cerebellar vermis. Spinal cord gray matter lesions have been found in the lateral cervical and thoracic nuclei, and lumbosacral and cervical intermediate gray columns.[279,287] Ascending spinal cord white matter tracts involved in horses with EDM are the spinocuneocerebellar tract (unconscious proprioception; thoracic limbs), the spinocerebellar tract (unconscious proprioception; trunk and pelvic limbs), and the dorsal column-medial lemniscal tract (conscious proprioception).[287,290] Lesions in the ventromedial funiculi of the spinal cord containing descending tracts have also been described.[287,291] With chronicity, the dorsal and ventral spinocerebellar tracts and the medial part of the ventral funiculi of the thoracic segments are more severely affected.[40,287] Astrocytosis, astrogliosis, vacuolization, myelin loss, spheroid formation (axonal swelling), and lipofuscin-like pigment accumulation are present in these areas.[287,290] Recently, histopathologic changes characteristic of EMND were identified in horses with clinical signs of ENAD/EDM.[292] EMND is also attributable to α-tocopherol deficiency but is characterized by clinical signs of lower motor neuron disease and seen in older horses. Although clinical signs of EMND and ENAD/EDM are very different, this report shows that there is overlap possible in the histopathologic lesions identified in horses with these diseases.

Pathophysiology

For years it has been proposed that there is a familial tendency to develop EDM and that there was an association between disease development and vitamin E deficiency. Breeds in which a familial tendency to develop EDM has been suggested include the Arabian,[287] Thoroughbred,[283] and wild horses,[281] and breeds in which this has been demonstrated include Morgans,[293] Appaloosas,[278] Standardbreds and Paso Finos,[283] Lusitanos,[276] American Quarter Horses,[285] and Warmbloods.[288] Pedigree analyses support a genetic cause for increased susceptibility to α-tocopherol deficiency with subsequent development of NAD/EDM; however, the mode of inheritance of NAD/EDM has been difficult to ascertain. From previous studies the mode of inheritance was most likely considered to be an autosomal dominant one with variable expression or a polygenic one, based on a breeding trial in Morgan horses and pedigree analysis in Lusitano horses.[276,286] Most recent work indeed suggests the disease is being inherited as a complex trait,[285] whereby effects of more than one gene along with environmental influences determine the

phenotype. Currently, it is hypothesized that the genetic variant of ENAD/EDM is a susceptibility locus, and the amount of α-tocopherol received by a foal during the first year of life may play a role in determining the final phenotype.[285]

In humans a condition referred to as ataxia with vitamin E deficiency occurs that is caused by various mutations in the α-tocopherol transfer protein gene (TTPA). Clinical signs and neuropathologic findings in people are similar to those seen in horses with ENAD/EDM. In a group of 88 American Quarter Horses pedigree analysis demonstrated inheritance as a polygenic trait with high heritability; however, this study excluded TTPA as a candidate gene for ENAD in this group of horses.[285]

Degenerative myelopathy in other species, which bears clinical and histopathologic similarities to EDM, has been linked to vitamin E and copper deficiencies, hereditary factors, and toxic insults.[294] An older study involving 56 affected and 179 control horses identified three risk factors associated with the development of EDM: (1) use of insecticides, (2) exposure to wood preservatives, and (3) spending frequent time on a dirt lot.[295] This same study showed that spending time on green pastures was found to be a protective factor, and a foal was 25 times more likely to develop EDM if its dam had any other foals diagnosed with EDM. More recently an epidemiologic study involving 3 index case horses with ENAD and 145 randomly selected horses from a single farm showed that most affected and unaffected horses had low serum vitamin E concentrations, hepatic vitamin E concentrations were low, and the pastures on the farm were deficient in vitamin E and selenium.[279] Further, serum concentrations of other trace minerals were within normal limits, and environmental samples were negative for insecticides and ionophores. Heavy metal concentrations in samples were within normal limits. Studies over the years support the hypothesis that exposure of genetically predisposed young foals to environmental oxidants and lack of antioxidants, including vitamin E, are important factors in the pathophysiology of ENAD/EDM.

Oxidative stress is caused by the imbalance between production of prooxidants and the antioxidant defenses. ROS (e.g., superoxide anion, hydrogen peroxide, hydroxyl radical) are formed during the reduction of oxygen to water in normal cellular metabolism (Fig. 11.38A). Aerobic cells have antioxidant defense mechanisms that protect them from oxidative stress (Fig. 11.38B). The brain's high consumption of oxygen, high metabolic activity, and high concentration of polyunsaturated fatty acids, which can easily be oxidized to ROS, make the CNS extremely vulnerable to oxidative attack by ROS. Another source of ROS is through the metabolism of excitatory amino acids and neurotransmitters such as glutamate and aspartate. When present in excess, excitatory amino acids can trigger a series of events including an increase in intracellular calcium, which can lead to the production of free radicals and subsequent neuronal damage and death. Other sources of free radicals that arise from brain metabolism include cytochrome P-450 electron transport, monoamine oxidase activity, and endogenous guanidine compounds. Lipid peroxidation of cellular membranes and the direct oxidation of amino acids leading to inactivation of enzymes, receptors, and structural proteins are the main consequences of oxidative injury (Fig. 11.39A). Increased evidence exists for a role of redox signaling by oxygen radicals that targets mitochondrial cytochrome *c* release, DNA repair enzymes, and transcriptional factor nuclear factor–κβ. Neuronal damage occurs once these physiologic systems are disrupted.[296]

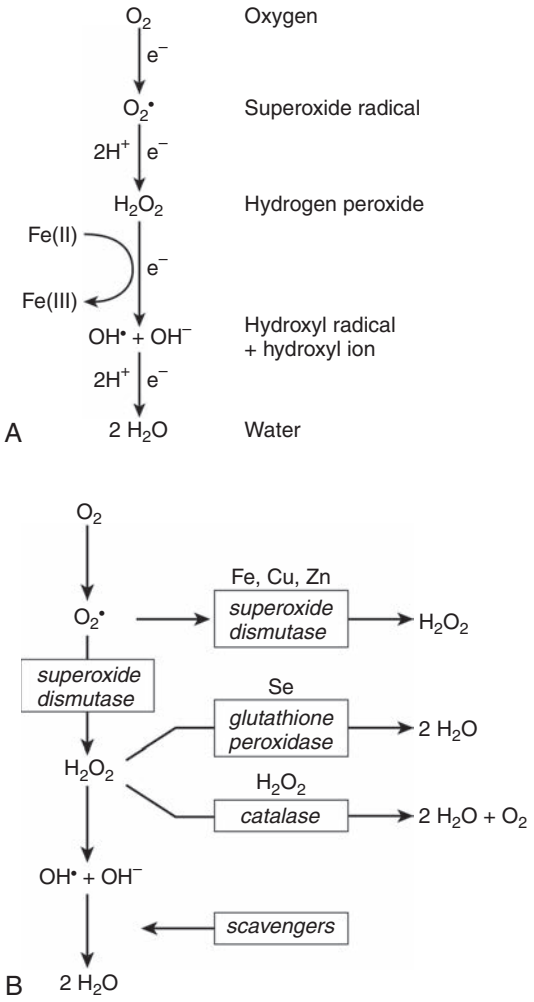


FIG. **11.38** A, Physiologic metabolism of molecular oxygen to water. B, The mechanisms of action of four antioxidant systems. Superoxide dismutase may act as a prooxidant by increasing the formation of hydrogen peroxide and as an antioxidant by decreasing the superoxide radical concentration. Cofactors for superoxide dismutase are iron, zinc, and copper. The cofactor for glutathione peroxidase is selenium.

The main endogenous antioxidants are superoxide dismutase, catalase, glutathione peroxidase (that contains selenium), α-tocopherol (vitamin E), and ascorbic acid (vitamin C) (see Fig. 11.38B and Fig. 11.39B). Another protective mechanism is the existence of iron-binding proteins that keep iron in a less reactive form and prevent iron from catalyzing free radical reactions. Vitamin E reacts directly with OH^- and prevents oxidant injury to polyunsaturated fatty acids and thiol-rich proteins in cellular membranes (see Fig. 11.39B). Insufficient vitamin E may result in oxidative damage and lipid peroxidation of cell membranes and may therefore cause the accumulation of lipopigment. Accumulation of lipofuscin-like pigment is a common feature of experimentally induced vitamin E deficiency in rats and monkeys[297] and in human and equine motor neuron disease.[298] Lipofuscin accumulation in the CNS of ENAD/EDM-affected horses has been reported,[283,290,299] and the amount exceeds the amount found in age-matched controls.[299] Further evidence for oxidative injury within the spinal cord of horses with EDM has been provided by positive immunoreactivity for peroxynitrite (3-NT) and unsaturated

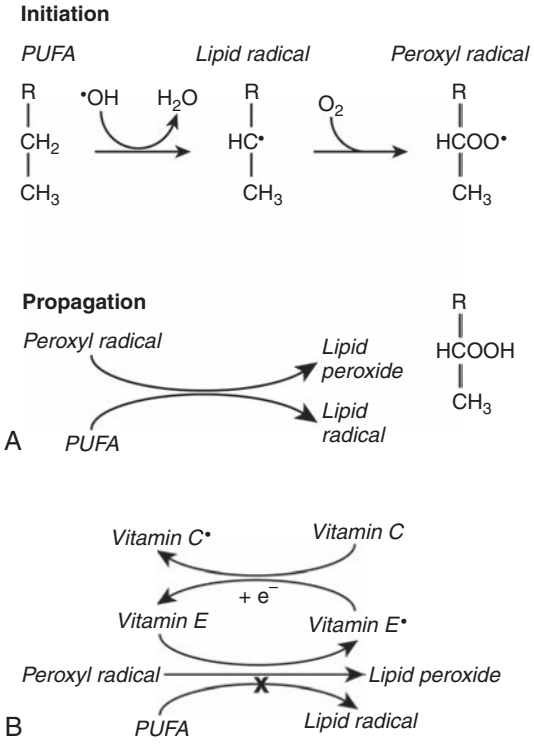


FIG. **11.39** A, Mechanism of peroxidation of polyunsaturated fatty acids in cell membranes. B, Antioxidant mechanisms of α-tocopherol (vitamin E) and ascorbic acid (vitamin C). Polyunsaturated fatty acids are spared from oxidation because vitamin E is oxidized to a free radical instead. This prevents the propagation of lipid peroxidation in cell membranes and is referred to as the *chain-breaking action of vitamin E*. Vitamin C is a reducing agent that donates electrons to free radicals.

aldehyde (4-NHE).[291] These substrates markedly increase in neurons in conditions involving oxidative stress and mediate neuronal apoptosis.

The association between vitamin E deficiency and ENAD/EDM is based on epidemiologic studies, the similarities between ENAD/EDM and vitamin E deficiency in human beings and other animals, the reduced incidence of ENAD/EDM seen after prophylactic treatment with vitamin E, and the response to treatment with vitamin E in affected horses. However, vitamin E deficiency has not been a consistent finding in horses with ENAD/EDM.[300] Two studies have shown a high incidence of ENAD/EDM on farms in which low serum vitamin E concentrations were found, and in both studies affected and unaffected horses were vitamin E deficient.[279,283] Furthermore, vitamin E supplementation decreased the incidence and severity of disease seen in both studies. Another study documented significantly lower vitamin E concentrations and clinical signs compatible with EDM in eight of nine foals sired by an EDM-affected stallion. Age-matched control foals raised in the same environment had normal serum vitamin E concentrations and no signs of EDM. Oral vitamin E absorption tests were performed on both groups, and no significant differences were found between the groups.[284] Thus an inability to absorb vitamin E from the gastrointestinal tract does not appear to be a factor in the low serum vitamin E concentrations in EDM-affected horses.

In horses with EDM evidence has been found for severe axonal transport impairment. Abnormal expression of synaptic proteins and abnormal accumulation of neurofilaments in dystrophic axons has been described in two Arabian horses with EDM.[301] Presynaptic proteins such as synaptophysin, synaptosomal-associated protein 25 kDa (SNAP-25), syntaxin-1, and α-synuclein participate in trafficking, docking, and fusion of the synaptic vesicle to the plasma membrane, facilitating synaptic transmission and exocytosis of neurotransmitter. Findings in this study are similar to what has been reported in dogs with NAD and suggest a loss of synapse and accumulation of synaptic proteins in dystrophic neurons.

Diagnosis

Definitive diagnosis of ENAD/EDM can be made only with histopathologic examination of the spinal cord and brainstem. Antemortem diagnosis is based on clinical signs and ruling out other neurologic diseases (especially CVM and EPM). In ENAD/EDM, CSF analysis and cervical spinal radiographs are usually within normal limits, although increased CSF creatine kinase (CK) levels have been found in horses affected with EDM.[40]

Measuring serum/plasma α-tocopherol concentrations may be unreliable when the animal is examined after the critical deficient period. Moreover, it should be noted that a single serum α-tocopherol sample may not adequately reflect the true vitamin E status of the horse, because up to 12% variation in concentrations can occur normally.[302] However, a low serum/plasma α-tocopherol concentration (less than 1.5 mg/mL) in a horse with clinical signs is supportive of the diagnosis. One can also measure the α-tocopherol concentration in the CSF, which is significantly correlated to the serum[303] and brain tissue concentration.[304] Reference ranges for serum/plasma and CSF α-tocopherol concentrations are available.[305]

Treatment and Prevention

Oral supplementation with vitamin E to genetically susceptible foals in the first year of life may reduce the incidence and severity of disease[279,283,295,299]; however, one of these studies showed that new cases did still occur despite vitamin E supplementation.[279] Typically animals are supplemented at 1000–2000 IU/450 kg/day of vitamin E. There are mixed reports on efficacy of supplementation with vitamin E in affected horses, where some reports suggest improvement of clinical signs after treatment with vitamin E at 6000 IU/day,[306] and others report no effect.[279] Additionally, one study reports improvement of clinical signs after gaining access to fresh grass.[307] The NRC requirements for dietary vitamin E concentrations are 500 to 1000 IU/kg dry weight for a 500-kg horse. Additional supplementation for healthy adult horses appears not necessary.[305] Heat-treated pellets, stored oats, and sun-baked forages have marginal vitamin E concentrations, and horses fed a diet of these or horses kept on dry lots should have frequent access to fresh green forage or vitamin E supplementation to meet their reported needs. Horses with clinical signs of ENAD/EDM may benefit from large doses of vitamin E (6000–10,000 IU/day) for an extended period of time; however, more recent work suggests that this does not lead to resolution of clinical signs.[279] The current recommendation is that a natural RRR-α-tocopherol (nonacetate) form of vitamin E is used to supplement deficient horses with ENAD/EDM because this form is the most biologically available, is the most readily absorbed, and has the most potent antioxidant activity.[305] Vitamin E toxicity associated with supplementation in horses has not been reported, and the risk is considered minimal in horses;

however, adverse effects have been reported with doses greater than 10 times NRC requirements.[305]

Prognosis

Although clinical signs of ENAD/EDM typically appear to stabilize by 2 to 3 years of age, affected horses are neurologically abnormal and often unfit for any performance activity. Generally, horses with ENAD/EDM do not progress to a state of recumbency. Severely affected horses usually have an earlier age of onset and rapid disease progression, whereas mildly affected horses usually have a later age of onset and the disease has a less rapid course.

EQUINE PROTOZOAL MYELOENCEPHALITIS

Yvette S. Nout-Lomas

Equine protozoal myeloencephalitis is a commonly diagnosed condition of the horse, which J. Rooney originally described in 1964 and termed *segmental myelitis.* Later descriptions used the terminology *focal myelitis encephalitis,* recognizing that lesions within the brain occurred.[308] Eventually, an organism that resembled toxoplasma was seen in histologic sections, and in 1976, Dubey first suggested that EPM was caused by a member of the genus *Sarcocystis.*[309] A *Sarcocystis* organism was eventually cultured from the spinal cord of an affected horse and named *Sarcocystis neurona* because it often develops within neurons.[310] Since that time, *S. neurona* or *S. neurona*–like organisms have been cultured from several ataxic horses, as well as numerous other animals including the zebra, domestic cat, Canadian lynx, sea otter, straw-necked ibis, mink, raccoon, and skunk. Another protozoan parasite *(Neospora hughesi)* has also been shown to be a cause of EPM in the horse; however, the majority of cases are because of infection with *S. neurona.*[311-313] The *S. neurona* genome has now been sequenced and annotated, potentially leading to discovery of better diagnostic methods and therapies.[314] Recently a panel of experts has provided a comprehensive review of this disease[315] and up-to-date information on the pathophysiology, diagnosis, and treatment of this clinically important disease in the form of a consensus statement.[313]

Epidemiology

EPM is one of the most commonly diagnosed neurologic diseases of the horse in the Western Hemisphere. Given the complexity of clinical diagnosis, and the difficulty of finding conclusive lesions in the CNS, the true incidence of EPM is difficult to state conclusively. Two older studies reported that 25% of equine neurologic cases were diagnosed with EPM,[40,316] and a national study conducted by the United States Department of Agriculture (USDA/APHIS NAHMS Equine '98 study) in 1998 found an average incidence of 14 + 6 cases per 10,000 horses per year. The overall prevalence was estimated at 0.5% to 1% of the horse population. The lowest incidence was in farm and ranch horses (1 + 1 case/10,000 horses), with 6 + 5 cases/10,000 horses for pleasure horses. A much higher incidence of 51 + 39 and 38 + 16 cases/10,000 horses was seen for competition and racing horses, respectively. An intermediate incidence of 17 + 12 cases was reported for breeding animals. This is similar to the findings of other studies showing racing and showing animals to have a higher risk than breeding and pleasure horses.[317]

Numerous seroprevalence studies have been performed, casting light on the distribution of the infection and life cycle of the organism. Horses in the United States have a variable but generally high seropositive incidence to *S. neurona,* ranging from 15% to 89%, depending on geographic location.[312,313] Seroprevalence increases with increasing age, and an association with temperature whereby the lowest seroprevalence was found in regions with a higher number of days of freezing temperature or during the colder months.[312,315,317,318] Seroprevalence against *N. hughesi* is low and has been reported to vary from less than 3% to 10%.[312,313] A large seroprevalence study recently demonstrated that some horses are seropositive for both *S. neurona* and *N. hughesi.*[312]

All horses are susceptible to the development of EPM, but epidemiologic surveys have suggested that the average age of affected horses is approximately 4 years.[319,320] More than 60% of cases were less than 4 years old, and less than 20% were 8 years or older.[320] Another study found that young horses (1–5 years) and older horses (greater than 13 years) had a higher risk of developing EPM.[317] The age range of reported cases is from 2 months[321] to 24 years.[322] Data from 364 horses with histologically confirmed EPM taken from across the United States and Canada revealed that Thoroughbreds, Standardbreds, and Quarter Horses were most commonly affected, although many other breeds were represented; no gender predilection exists.[320]

Most cases appear to be individual cases, and "outbreaks" of EPM appear to be very rare, although one author has described an epizootic of EPM on a single farm.[323] A report from Ohio, however, did find that previous diagnosis of the disease on the farm increased a horse's risk of subsequently developing EPM (greater than 2.5 times higher).[317] This finding suggests clustering of cases may occur when all the risk factors for EPM are present. Analysis from data collected during the USDA/APHIS NAHMS Equine '98 study[324] and another epidemiologic study[317] demonstrated the following risk factors for EPM:

1. Environmental and management factors that affect exposure to opossums. A higher risk for disease occurs in the fall of the year.
2. Type of housing for horses (decreased risk with pasture housing), stocking density (increased risk with increased numbers of horses), the choice of bedding material (wood products lower odds than straw or corn stalks), or safe storage of concentrate feeds (lower odds at operations that stored feeds in rodent-proof containers).
3. Horses that were used primarily for racing had a greater risk compared with horses used primarily for pleasure. Thoroughbreds, Standardbreds, and Warmblood horses had markedly greater odds of disease compared with Quarter Horses. Young horses had a higher risk of developing EPM than older ones, and recent transportation increased odds of disease.
4. Larger number of resident horses and finding evidence of wildlife on premises increased the risk of EPM. Premises near a marsh/creek/river decreased the risk for EPM.
5. Horses were more than twice as likely to have EPM if they resided in Kentucky, Michigan, Missouri, New Jersey, New York, Ohio, Pennsylvania, and Tennessee.

EPM is a disease of the Western Hemisphere, with cases reported from many states within the United States, as well as Canada, Mexico, Panama, Argentina, and Brazil. Most

horses with a diagnosis of EPM outside this region appear to have spent some time in the endemic area. EPM has been reported in England among horses imported from the eastern United States and in an 8-month-old Arabian horse in South Africa that had been imported from the United States approximately 5 months before the onset of signs.[325] Another report concerned a horse from California that developed clinical signs of EPM after 10 months in Hong Kong.[326] These cases demonstrate the probability of persistent, subclinical, latent infections. However, a few reports exist of neurologic horses with consistent clinical signs, positive immunoblot test results, and no history of travel in the American continent.[327,328] The nature of the infection in these horses is unclear and may be because of cross-reacting antigens.

Immune suppression because of stress or advanced age might predispose a horse to development of EPM. Stressful events such as heavy exercise, transport, injury, surgery, or parturition have all been found to increase the risk of EPM.[317] Racehorses and show horses had a higher risk of developing EPM compared with breeding and pleasure horses. Not surprisingly, horses with EPM that were treated with an anticoccidial drug were 10 times more likely to improve than untreated horses.

Life Cycle

Sarcocystis spp. belongs to the phylum Apicomplexa, which includes several genera of coccidia that use an obligatory predator-prey or scavenger-carrion life cycle.[329] *Sarcocystis* spp. produce sporulated oocysts by sexual reproduction (gametogony) in the gut wall of the appropriate predator or definitive host. Infective sporocysts are introduced into the food and water supply of the prey animal or intermediate host by fecal contamination from the predator. In the *S. neurona* life cycle, the definitive host is the opossum, whereas the intermediate hosts are skunks, raccoons, and armadillos, among others. Once ingested by the intermediate host, sporocysts excyst, releasing four sporozoites that penetrate the gut and enter arterial endothelial cells in various organs. Meronts develop rapidly and eventually rupture the host cell, releasing merozoites into the bloodstream, which usually is followed by a second round of merogony in capillary endothelial cells throughout the body. Second-generation merozoites are released into the bloodstream and usually enter skeletal muscle cells, where they develop into specialized meronts known as *sarcocysts.* Mature sarcocysts contain bradyzoites, which are able to complete the life cycle only when ingested by the appropriate predator or scavenger.

S. neurona may infect a large number of intermediate hosts aberrantly, unlike most *Sarcocystis* spp. Several species of animals and birds have been reported to exhibit signs similar to those in horses with EPM. This feature made the experimental elucidation of the *S. neurona* life cycle quite challenging. At the present time, domestic cats *(Felis domesticus),* nine-banded armadillos *(Dasypus novemcinctus),* striped skunks *(Mephitis mephitis),* raccoons *(Procyon lotor),* and sea otters *(Enhydra lutris nereis)* are considered to be viable intermediate hosts. Feeding studies, as well as epidemiologic and seroprevalence data, have determined this. Initially the domestic cat was thought to be only a laboratory intermediate host; however, several epidemiologic studies have now incriminated domestic cats as natural intermediate hosts.[330] The life cycle of *S. neurona* has been completed in a laboratory setting.[331] The horse has traditionally been considered an aberrant, dead-end host, in that sarcocysts have never been reported. One report, however, describes mature sarcocysts that were well characterized as *S. neurona* and found on the tongue of a horse suffering from EPM.[332] This suggests that horses can be intermediate hosts but requires further confirmation.

Horses are infected with *S. neurona* by ingesting food or water that has been contaminated with feces from an infected opossum. Some evidence suggests that the organism may be transmitted by methods other than direct contact with opossum feces. It has been suggested that birds may mechanically disseminate sporocysts and that secondary transmission via "pass-through" of infective sporocysts in the feces of budgerigars, canaries, mice, and chickens fed opossum feces has occurred. At least some organisms appeared to retain viability and infectivity after transit through the digestive tract.[333] This is most likely to be a laboratory phenomenon; its importance in nature is unknown but likely to be small. Importantly, *S. neurona* is not transmitted horizontally among horses, nor can it be transmitted to horses from nonequine intermediate hosts. Antibodies against *S. neurona* in foals before suckling have been reported,[334,335] but current evidence indicates that transplacental or lactogenic transmission of *S. neurona* is very uncommon or absent.[313,315]

The life cycle of *Neospora hughesi* in horses is understood poorly. The definitive host of *N. caninum* has been demonstrated to be the dog,[336] but whether the dog is the definitive host of *N. hughesi* is not known. Tachyzoites, as well as tissue cysts, have been found in other horse tissues in two of the horses reported to have EPM caused by *Neospora* spp.[337] Vertical transmission of *N. caninum* is very efficient in cattle, and several recent studies indicate that *N. hughesi* can be transmitted transplacentally in horses.[335,338]

Pathogenesis

Despite the often-high rate of exposure to the organism, only a small percentage of horses develop clinical illness. This suggests that immune clearance of the parasite is very effective but that unknown factors must exist in certain cases to allow clinical disease to be expressed. Parasite dose is likely to be a factor, and in fact this has been experimentally demonstrated.[339] Other factors that have been considered to have a role in the induction of EPM include physiologic stress associated with shipping, training, showing, and pregnancy that may make animals more susceptible to EPM.[317] Indeed, one reliable model of inducing EPM incorporates long-range shipping as a stressor, performed immediately before infection.[340] Other attempts to induce EPM using the oral infection route that do not incorporate stressors such as shipping have led to inconsistent and only mild illness.[341] The assumption is that these stressors lead to some degree of immune suppression, which is a commonly implicated factor in protozoan parasite infections. However, treatment of horses with immunosuppressive doses of steroids associated with oral infection with *S. neurona* did not result in significantly worse histopathologic changes in the CNS, although clinical signs were slightly more severe than in non–steroid-treated horses.[341] Additional evidence for the role of physiologic stress is found in the observation that stressed horses develop more severe clinical signs than naturally infected (nonstressed) horses.[342] It thus appears likely

that stress has a role in the development of EPM; however, the interaction is complex and not fully understood at this time. Further, genetic variation has been observed among the strains of *S. neurona* that have been analyzed,[343,344] and although there is evidence that specific parasite genotypes may be particularly virulent in marine mammals,[345] such an association has not been found in isolates from horses clinically affected by EPM. Recent studies have demonstrated differences among isolates in the expression of surface receptors, which may have substantial implications for immunodiagnosis.[346]

After infective sporocysts of *S. neurona* are passed from the definitive host and subsequently ingested by a horse, infection proceeds. Clinical disease results from the inflammation and neuronal necrosis associated with infection of the CNS with live organisms. The mechanisms by which *S. neurona* enters the CNS are unclear but are thought to involve either infection of endothelial cells or leukocytes.[347-350] One hypothesis is that *S. neurona* organisms that have been phagocytosed by leukocytes in the periphery are transported across the blood-brain barrier in the process of normal immune surveillance. Once within the CNS, the organisms proliferate. This "Trojan horse" hypothesis of CNS infection is attractive and has support from experimental work in which clinical disease can be induced by administering horses autologous lymphocytes, which have been infected with *S. neurona*.[348] There does not appear to be "targeting" of the CNS by the organism; rather the organism is not cleared from the CNS tissue while it is cleared elsewhere. Once organisms enter the CNS, it is likely that the immunosuppressive environment of the CNS plays a role by diminishing clearance of the organism in this immune-privileged tissue. The parasite itself may also induce some immunosuppression.

Resistance to *Sarcocystis neurona* is presumed to be the result of the combined effects of humoral and cellular immunity. After infection, a relatively rapid production of antibodies occurs. In horses challenged with live *S. neurona* organisms orally, all horses seroconverted within 32 days,[341] whereas in another study, horses challenged with a larger number of organisms seroconverted by day 13 after infection (if stressed by transport) and by day 30 if unstressed.[340] It has been demonstrated with various apicomplexan parasites that antibody is at least partially protective.

Although the effects of circulating antibody are likely to be very important, cell-mediated immunity is necessary for the elimination of intracellular forms of most organisms. Mouse studies have confirmed the importance of CD8+ T cells in protection against *S. neurona* encephalopathy in that species; it is presumed to be similar in horses. Endothelitis and meningoencephalitis developed in CD8 knockout mice after challenge with *S. neurona,* highlighting the importance of this cell subset in protection against *S. neurona,*[351] at least in the mouse. The CD8+ T cell is one important source of interferon-gamma (IFN-γ), which is critical for protection against *S. neurona*–induced neurologic disease in mice.[315,352] Infection of IFN-γ knockout mice leads to fulminant neurologic disease. These findings support the critical importance of IFN-γ in protection against *S. neurona.* Recent research demonstrated that *S. neurona*–infected horses have altered immune cell subset expression and immune cell function that changes during disease progression. *S. neurona* infection alters the ability of antigen-presenting cells to stimulate CD4+ and CD8+ proliferation, and the data suggest that the process between antigen-presenting cells (monocyte/dendritic cells) and/or T-cell antigen recognition may be impaired in *S. neurona*–infected horses.[350]

Pathology

Confirmation of disease is through demonstration of protozoa in CNS lesions. However, the diagnosis is frequently made presumptively when organisms are not detected if the characteristic inflammatory lesions are found.[313] Gross lesions consist of multifocal areas of hemorrhage in the acute setting and foci of malacia and areas of discoloration ranging from pale to dark tan in the subacute to chronic phase.[315] Lesions are most commonly seen in the spinal cord. The brainstem is more often involved than other parts of the brain, and, in rare cases, lesions are found in both spinal cord and brain. Microscopically, lesions are characterized by focal to diffuse areas of nonsuppurative inflammation and necrosis with perivascular infiltration of mononuclear cells, including lymphocytes, macrophages, and plasma cells. Giant cells, eosinophils, and gitter cells also are present in inflammatory infiltrates. Gray or white matter (or both) is affected. Organisms have been found in neurons, leukocytes, and vascular endothelium, although they tend to develop most often in neurons.

Clinical Signs

The clinical signs associated with *S. neurona* infection vary from acute to chronic with insidious onset or focal or multifocal signs of neurologic disease involving the brain, brainstem, or spinal cord. Clinical signs are variable, which reflects the random distribution of the lesions that may occur within the CNS. Signs of gray matter involvement include focal muscle atrophy and severe muscle weakness, whereas damage to white matter frequently results in ataxia and weakness in limbs caudal to the lesion.

Usually the physical examination is within normal limits, and the horse appears bright and alert, although one may observe focal muscle atrophy. Neurologic examination typically reveals asymmetric ataxia, weakness, and spasticity involving all four limbs. The most common signs of brain/brainstem involvement are obtundation, head tilt, facial nerve paralysis, and difficulty swallowing. Abnormal airway function, unusual lameness, and seizures are also sometimes seen, and visual deficits and behavioral abnormalities have also been reported. A typical history is a slowly progressive ataxia, which may initially have been identified as a musculoskeletal disorder, since early signs may include stumbling and frequent interference between limbs. Commonly horses exhibit a gradual progression in severity and range of clinical signs. Sometimes clinical signs stabilize and then relapse days or weeks later, and sometimes a gradual onset of clinical signs suddenly worsens, resulting in recumbency. Occasional acute and rapidly progressive disease is seen; empirically this presentation seems more commonly associated with brainstem disease, although this has not been formally evaluated.

The presence of ataxia, asymmetry, and atrophy (the "three *A*'s of EPM") suggests multifocal or diffuse disease, which is characteristic of, although not pathognomonic for, EPM. Less commonly observed are diseases of the cerebrum, cerebellum, or brainstem resulting in a variety of cranial nerve deficits including dysphagia, head tilt, tongue or master paralysis, or masseter atrophy. The clinician needs to recognize that all of the clinical signs listed previously may be caused by conditions other than EPM, all of which should be considered in the differential diagnosis.

Diagnosis

An important point to remember is that EPM is an easy diagnosis to make but difficult to substantiate and confirm in a live patient. Experts recommend the following steps to be taken before a diagnosis of EPM is rendered.[313]

1. A thorough neurologic examination must reveal clinical signs arising from disease of the nervous system that are consistent with EPM as described previously.
2. Other conditions that could give rise to the clinical signs observed should be ruled out to the degree possible. This may require radiographs of the cervical spine or head, nuclear scintigraphy, CSF evaluation, or testing for EHV, for example. These tests will be determined based on a careful physical and neurologic examination, as well as a consideration of the horse's history and progression, risk factors, and related information.
3. Immunodiagnostic testing of serum and CSF should be conducted to confirm intrathecal antibody production against *S. neurona* or *N. hughesi.*

At the present time there are several immunodiagnostic tests in use for EPM diagnosis. These tests are all based on detection of antiprotozoal antibodies in serum, CSF, or both. EPM only occurs in a small portion of horses infected with *S. neurona* so serologic testing has minimal diagnostic value unless test results are negative (low positive predictive value, high negative predictive value). A negative serum test usually indicates that the horse has not been infected; however, a recently infected horse may display clinical signs before seroconversion, and retesting in 10 to 14 days is recommended. Detection of antibodies in the CSF alone is not a definitive indicator of EPM because there is passive transfer of antibody across a healthy blood-brain barrier and blood contamination of CSF samples can cause false-positive results.[353,354] Intrathecal antibody production, indicative of active parasite infection in the CNS, can be determined by tests of proportionality that assess whether the amount of pathogen-specific antibody in the CSF is greater than should be present from normal passive transfer across the blood-brain barrier. The Goldman-Witmer coefficient (C-value) and the antigen-specific antibody index (AI) are such tests.[313,354]

The immunodiagnostics that have been developed over the years include a Western blot, a direct agglutination test, an indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assays (ELISAs) based on single or multiple *S. neurona* surface antigens (SnSAGs). The first test developed to aid in the diagnosis of EPM was immunoblot analysis (Western blot) of serum and CSF to provide antemortem information regarding exposure to *S. neurona.*[355] The test uses cultured merozoites to detect antibodies directed against proteins considered unique to *S. neurona.* Antibodies produced to proteins shared with *S. fayeri* or other organisms found in North America can be differentiated with this test. False-positive results are obtained through hemorrhage occurring in the brain or through blood contamination of CSF samples. In an effort to differentiate false-positive CSF antibody tests, a number of techniques have been described, including determination of the albumin quotient (AQ) and the IgG index; however, the sensitivity of these tests appeared to be too low, and their use is no longer recommended as a diagnostic aid for EPM.[356] False-negative results have been rare but may occur. The possible causes of false-negative responses are important to consider so that one does not misdiagnose affected horses. Some horses simply may fail to respond to the *S. neurona*–specific proteins identified by the immunoblot. Horses that initially tested positive have become negative after several weeks of treatment and apparently have recovered. A chronically affected horse may test negative and still be infected, or the horse still may exhibit neurologic signs. Persistent neurologic damage (i.e., scarring) may be present in some cases, resulting in permanent neurologic damage in the absence of active infection. One should retest acute cases that initially test negative in 2 to 3 weeks. However, the incubation period appears to be sufficiently long to allow production of detectable amounts of IgG before the onset of clinical signs in most cases.

A whole organism IFAT was developed and is currently available from the University of California Diagnostic Laboratory.[357] Serum titers of greater than 1:100 and CSF titers of greater than 1:5 are considered positive and diagnostic of active infection. This test is considered to be robust in the presence of blood contamination of the CSF sample. However, the superiority of the IFAT as a diagnostic for EPM is not clearly established and it has been demonstrated that the test was unable to differentiate between *S. neurona* and *S. fayeri* (a nonpathogenic *Sarcocystis*) infection.[358]

Most recently the SnSAG ELISAs, quantitative (end-point titer) tests based on *S. neurona* surface antigens, have been evaluated as a diagnostic tool.[354,359,360] These surface antigen molecules have proven to be good serologic targets in the assays because of their high level of expression in the parasite and their immunogenicity in infected horses. The SnSAG2 ELISA and the SnSAG4/3 ELISA accurately detect antibodies against *S. neurona* in equine serum and CSF samples,[361,362] and although none of the tests described here is considered a gold standard,[313] most recent studies suggest the SAG2, 4/3 ELISA serum:CSF titer ratio, should be utilized for antemortem testing for EPM.[359,360] A study that used 59 sets of samples from 57 horses compared the SnSAG ELISAs to the IFAT and showed that using serum alone was least accurate, and that the overall accuracy was highest for the SnSAG2, 4/3 ELISA with a titer ratio set at 0.97. The sensitivity was 0.88 and the specificity was 1. The IFAT CSF and titer ratio results also showed high accuracy at 0.88 but lower sensitivity at 0.65.[359] Another study confirmed the value of detecting intrathecal antibody production, and the authors showed excellent diagnostic sensitivity and specificity obtained from the SnSAG2, 4/3 serum:CSF titer ratio with sensitivity and specificity of 93.2 and 81.1%, respectively, using a ratio cutoff of ≤100. If a more rigorous cutoff of ≤50 was used sensitivity and specificity were 86.4% and 95.9%, respectively.[313] Two studies have also shown that modest blood contamination of the CSF, up to 10,000 RBCs/L, will have minimal effect on SnSAG2 test results.[353,354]

There is an NhSAG1 ELISA and an IFAT available for immunodiagnostic testing for *Neospora hughesi.* Neither one of these tests has been fully validated for EPM diagnosis because of inadequate number of samples from EPM cases caused by this parasite.[313]

As previously noted, most horses with EPM do not show constitutional signs of illness, such as fever, depression, or anorexia. Changes in the CBC are not recognized, and changes in serum biochemistry analysis are not noted, unless the severity of the neurologic signs is such that the horse falls or is recumbent, or it is dysphagic and cannot drink or eat. In these

horses, secondary changes in the serum biochemistry panel may be seen.

A key part of the diagnosis of EPM is ruling out other conditions that may be present; evaluation of CSF is necessary for this process. Conditions such as intrathecal hemorrhage, neoplasia, meningitis, neuroborreliosis, and verminous encephalitis can be ruled out when evaluating EPM suspects using CSF analysis. CSF analysis should be considered a key part of the full diagnostic evaluation of horses with CNS disease. Most horses with EPM have normal CSF cytology. Early work identified the occasional increase in RBC count; however, in more recent times this finding has been considered to be spurious and a result of unrecognized blood contamination from the collection. The severe and fulminant case may have a mildly increased total protein or white blood cell count, but these situations are rare.

Differential Diagnosis

Given the variety of clinical abnormalities that may be expressed in horses with EPM, the differential diagnosis includes virtually all diseases of the equine CNS. However, the results of a careful history, physical examination, and neuroanatomic localization help to limit the number of rule outs and guide further diagnostic efforts. The most common and likely rule out is probably cervical vertebral compressive myelopathy (CVCM). In contrast to EPM, CVCM usually results in symmetric gait deficits, which are worse in the pelvic limbs and are characterized by spasticity and hypermetria, with good retention of strength and no muscle wasting.

Infectious diseases such as WNV encephalitis, equine encephalitis (eastern equine encephalitis [EEE] and western equine encephalitis [WEE]), and equine herpesvirus–1 (EHV-1) may all cause neurologic disease that could resemble EPM. Horses affected with these conditions are typically systemically ill, demonstrating fevers and alterations in the leukogram. The neurologic deficits of EHV-1 are fairly characteristic in that dysuria is a common component that is not often seen in horses with EPM. CSF cytology is abnormal in most such cases (e.g., WNV, EEE, EHV-1), in contrast to EPM, and a variety of specific diagnostic tests exist for each of these conditions, such as IgM capture ELISA for WNV and PCR testing for EHV-1.

Polyneuritis equi and ENAD/EDM may also be confused with EPM and can have signs of multifocal disease, ataxia, and muscle atrophy. Less common conditions, such as verminous encephalitis, bacterial meningitis, or CNS abscessation, can be seen, but alterations in the leukogram and the CSF are usually present, distinguishing these cases from EPM.

Treatment

The cornerstone of treatment for horses with EPM is antiprotozoal medication. At the present time the Food and Drug Administration (FDA) has approved a number of compounds for the treatment of EPM. The first compounds used for the treatment of EPM were the sulfonamide drugs combined with pyrimethamine to achieve a synergistic effect. Clinical efficacy studies have been performed using sulfadiazine (20 mg/kg) and pyrimethamine (1 mg/kg) orally once per day, marketed and FDA approved as Re-Balance (PRN Pharmacal). Using this dose in well-characterized cases of EPM, the overall success rate (e.g., improvement by one clinical grade) is 60% to 70%.[313,363] The duration of treatment varied between 90 to 270 days, and complications of this drug regimen were reported as anemia (22%), leukopenia (19%), and neutropenia (5%).[363] These signs are usually self-limiting and resolve with cessation of treatment. Folic acid supplementation has been advised by some authors to limit the degree of anemia; however, no support for this practice exists, and research has demonstrated increased toxicity when folic acid supplementation is provided. Hence its use is discouraged. Use of sulfadiazine in breeding animals is controversial, although one study has shown no effect on pregnancy rates or early embryonic death.

The first FDA-approved drug for the treatment of EPM was ponazuril (Marquis, Bayer Animal Health) to be administered as an oral paste. Ponazuril is a member of the benzeneacetonitrile compounds, which have demonstrated broad-spectrum anticoccidial activity in many avian and mammalian species. These drugs are related to the herbicide atrazine and are thought to target the parasite's apicoplast organelle. Ponazuril is well absorbed orally and within 3 days achieves a therapeutic steady-state concentration in the CSF of horses treated with 5-mg/kg body weight.[364] A field efficacy study of 101 horses with well-characterized EPM and treated with ponazuril demonstrated successful treatment in 60% of treated animals, success defined as improvement by at least one neurologic grade. A 90-day relapse rate of 8% after the termination of treatment was found.[365] Animals typically responded within 10 days and often continued to improve even after treatment stopped at 28 days.

Diclazuril is chemically similar to ponazuril and is also a member of the benzeneacetonitrile compounds. Diclazuril is FDA approved as Protazil, antiprotozoal oral pellets to be administered as a top dressing. In one study, treatment of EPM-affected horses with diclazuril resulted in improvement in 58% of the treated animals, when given for 28 days.[363] Because ponazuril and diclazuril are highly selective against apicomplexan parasites, little to no toxicity is to be expected at therapeutic doses.

Regardless of the drug used, the duration of treatment necessary and when to stop treatment are considerations in all EPM cases. Duration of treatment depends on response to antiprotozoal administration, and although the FDA-approved products are labeled for a treatment course of 28 days, the majority of horses with EPM are treated for a longer period of time, generally 6 to 8 weeks or longer if clinical improvement is still apparent.[313] Attempting to treat until the horse is negative on immunodiagnostic testing is currently not recommended.[313] Other treatments that have been investigated or used in the past have been reviewed elsewhere.[315,366]

Administration of nonsteroidal antiinflammatory drugs, corticosteroids, and dimethyl sulfoxide is justified in certain conditions—for example, to prevent worsening of neurologic signs during the early antiprotozoal treatment or in horses that are in danger of falling down or exhibit signs of brain disease. Similarly, vitamin E is sometimes used; however, some information suggests that this may not be efficacious if the horse is not vitamin E deficient.[305] Furthermore, some people include immunomodulators in the treatment of this disease based on the thought that horses with EPM may be immune compromised; however, there are no studies to evaluate efficacy of this practice.[313]

Relapses are a concern and are probably dependent on a number of factors, including the exact drug and dose used and the duration of treatment. Extended dosing may reduce the occurrence of relapse.[363] The relapse rate for horses treated for 28 days with ponazuril was 8%, when examined 90 days after cessation of therapy.[365] Relapse rates for the other drugs are not reported. Relapse implies that the horse responded well to the initial drug treatment; hence horses that relapse can be treated with the same drug that was initially used but for a longer period.

No studies have been conducted to determine the effectiveness of the currently approved antiprotozoal medications against *N. hughesi.* Ponazuril has demonstrated in vitro effectiveness against *N. caninum.*[367,368] Ponazuril (5 mg/kg body weight, once per day for 28 days) was used in three horses with *N. hughesi* EPM. All three horses showed clinical improvement, and one showed complete resolution.[369]

The prognosis for horses diagnosed with EPM appears to be similar regardless of the treatment used, because most reports suggest an approximate improvement rate of 60% to 75% with the standard therapy.

Prevention

The widespread distribution of the parasite and the variety of intermediate hosts make control of EPM complicated. A vaccine was available, but it has been removed from the market because of failure to demonstrate efficacy. Decreasing stress and preventing contamination of feed and water with opossum feces is essential to prevent EPM in the horse. Opossums can produce millions of sporocysts that can be excreted in feces for months. Sporocysts are resistant to environmental influences, and most commonly used disinfectants do not kill *S. neurona* sporocysts.[315] Practical approaches including not feeding off the ground, providing separate sources of fresh water for horses and preventing wildlife access to horse pastures, paddocks, and stalls may also help reduce the incidence of protozoal infections in horses.[313]

Monitoring high-risk age groups such as young and old horses closely for evidence of neurologic disease may help detect EPM early. That EPM may be the cause of the clinical signs when horses are presented for treatment of neurologic disease in the warmer months should raise the index of suspicion. Because many major horse competitions take place in the fall of the year, monitoring of horses before transport and competition may be helpful. Close monitoring of broodmares close to foaling and horses that develop a major illness or injury is important, because it may help early diagnosis of EPM cases.

Intermittent use of coccidiostatic and coccidiocidal drugs is another approach used to prevent EPM. Two studies that evaluated prophylactic use of ponazuril showed that daily or intermittent treatment minimized but did not eliminate infection in horses experimentally infected with *S. neurona.*[370,371] Recently it was shown that the daily administration of a low-dose diclazuril pellet top dressing to healthy foals from a farm with a high exposure rate to *S. neurona* significantly reduced the monthly seroprevalence to *S. neurona* compared with untreated foals.[372,373] This preventive strategy has the potential to be used in high-risk horses in an attempt to reduce the incidence of EPM, although future longitudinal studies will be required before establishing a standard protocol.[313]

EQUINE HERPESVIRUS–1 MYELOENCEPHALOPATHY

EHV-1 is an economically important pathogen of horses and exerts its major effect by inducing abortion storms or sporadic abortions in pregnant mares, early neonatal death in foals, and respiratory disease in young horses.[374-381] Myeloencephalopathy is an uncommon manifestation of EHV-1 infection but can cause devastating losses during outbreaks on individual farms, racetracks, veterinary hospitals, or boarding stables.[382-384] Although EHV-4 rarely causes clinical manifestations of disease in organs other than the respiratory tract, isolated cases of myeloencephalopathy and sporadic abortions have been reported in EHV-4 infections.[374-376,385,386] Clinical signs of neurologic disease reflect a diffuse multifocal myeloencephalopathy after vasculitis, hemorrhage, thrombosis, and ischemic neuronal injury. Sudden onset and early stabilization of signs including ataxia, paresis, and urinary incontinence; involvement of multiple horses on the premises; and a recent history of fever, abortion, or viral respiratory disease in the affected horse or herdmates are typical features, although considerable variation exists between outbreaks concerning epidemiologic and clinical findings.[387] Prevention is difficult because many horses are latently infected with EHV-1, allowing the virus to circulate silently in horse populations, and current vaccines do not confer protection against neurologic manifestations of infection.[381,388] The distribution of lesions that can result after infection with neurotropic EHV-1 results in the need to include many conditions on the differential diagnosis list, including EPM, cervical vertebral instability, cervical stenotic myelopathy, vertebral or CNS trauma, polyneuritis equi, fibrocartilaginous emboli, aberrant parasite migration, degenerative myelopathy, togaviral encephalitis, rabies, botulism, toxins, and other disorders.

Virologic Findings

Of the five distinct herpesviruses that are known to infect horses, three are typical α-herpesviruses with a double-stranded DNA genome and are designated EHV-1 (equine abortion virus, formerly known as *EHV-1, subtype 1*), EHV-4 (equine rhinopneumonitis virus, formerly known as *EHV-1, subtype 2*), and EHV-3 (equine coital exanthema virus), and two are γ-herpesviruses, designated EHV-2 (formerly called *equine cytomegalovirus*) and EHV-5 (which has recently been associated with interstitial pulmonary disease).[375,376,389-391] In addition, three asinine α-herpesviruses (AHV1, AHV2, and AHV3) have been isolated from donkeys. Of these, AHV3 has been shown by many criteria to be related closely to EHV-1. Indeed, EHV-1 and AHV3 are related more closely to each other than either is to EHV-4.[375,391-393] Phylogenetic analysis and epidemiologic evidence suggest that EHV-1 recently has been derived from AHV3 and that donkeys may remain an alternate host for EHV-1, serving as a reservoir to infect horses.[389,391]

EHV-1 and EHV-4 are distinguishable from EHV-2, EHV-3, and EHV-5 by biologic properties and virus neutralization tests (and distinguishable from each other by restriction endonuclease fingerprinting of DNA, DNA sequences, and several immunologic tests based on monoclonal antibodies to each virus).[374-376,389,390,394] EHV-1 and EHV-4 produce eosinophilic intranuclear inclusion bodies in infected cells in vivo and in vitro. Several strains have been identified within EHV-4 and

EHV-1, although the epidemiologic, immunologic, and pathogenic significance of this finding is not known. The 1-p and 1-b subtypes of EHV-1 likely are capable of inducing neurologic disease. Apart from differences in endotheliotropism, genetic and antigenic fingerprinting and experiments in baby mice have not yielded clear markers distinguishing EHV-1 strains that induce neurologic disease or abortion (or both).[389,395-399] However, a recent analysis of EHV-1 isolates from neurologic and nonneurologic disease outbreaks revealed a point mutation within the DNA polymerase gene that was strongly associated with neuropathogenic disease.[400,401] A hamster model has been described showing some potential for discrimination between abortigenic and neuropathogenic EHV-1 strains.[402]

Epidemiology and Immunity

EHV-1 and EHV-4 are enzootic in most horse populations, and the majority of horses show serologic evidence of exposure to these viruses. Most horses become infected via the respiratory tract with EHV-1 or EHV-4 (or both) during the first year of life. After an incubation period of 2 to 10 days, clinical signs of respiratory disease of variable severity develop and resolve within 1 to 2 weeks in uncomplicated cases.[374-376] Resolution of clinical signs is coincident with development of virus-specific neutralizing antibody directed primarily against surface viral glycoproteins. The development of cell-mediated responses is probably critical for recovery.[403] Resistance to reinfection with homologous virus is demonstrable after recovery but generally persists for only 3 to 4 months. Subsequent infections typically induce milder clinical signs or subclinical infection, although virus shedding from the nasopharynx occurs.[374,375] The immune response frequently is not successful in clearing herpesviral infection, and the majority of clinically recovered horses remain latently (asymptomatically) infected with EHV-1 or EHV-4 (or both) for life.[374,375,404,405] EHV-1 has been shown to evade the host immune system in part by downregulating major histocompatibility complex class I expression at the cell surface. This process may be a prerequisite to the establishment of latency.[406]

Recrudescence of latent infection is important in the epidemiology of EHV-1 and EHV-4 and explains why these diseases can occur in closed populations without the introduction of new horses.[374,375,405,407] Signs of EHV-1 infection may occur in the horse in which stress-associated recrudescence of infection has occurred, or the horse may remain asymptomatic but shed infectious virus in nasal secretions to infect other horses. Natural infection with EHV-1 occurs by inhalation or ingestion of aerosolized infective virus or by direct contact with virus shed in the products of abortion or in the nasal and ocular discharges and saliva of horses with overt clinical disease, subclinically infected horses, or shedding carrier horses.[374,375,394] Infectious EHV-1 virus was detected in the feces of experimentally infected foals that developed diarrhea, suggesting that fecal spread is a possibility.[408] Virus may be shed by clinically affected and inapparently infected horses for 3 weeks or more, and EHV-1 may remain infective in the environment for up to 14 days and on horse hair for 35 to 42 days.[375,409,410]

The first definitive association between EHV-1 and myeloencephalopathy was made in 1966 in Norway with the isolation of the virus from the brain and spinal cord of a horse that showed signs of severe neurologic dysfunction.[411] The myeloencephalopathic form of EHV-1 infection now is considered to have a worldwide distribution, having been recognized in Denmark, The Netherlands, Germany, Sweden, Austria, Britain, Ireland, Australia, India, the United States, and Canada.[382,383,387,398,412,413] In view of the ubiquitous occurrence of EHV-1 infection in horse populations, outbreaks of EHV-1 myeloencephalopathy are rare. In many instances, cases of neurologic EHV-1 infection occur in association with outbreaks of abortion or respiratory disease, although some outbreaks occur in the absence of other manifestations of EHV-1 infection and without the introduction of new horses into the group.[394,407,414,415]

The myeloencephalopathic form of EHV-1 infection may occur as sporadic individual cases or, more often, as outbreaks involving multiple individuals over a period of several weeks on one or more premises within a limited geographic region. Secondary or tertiary waves of clinical disease may occur as previously unexposed horses become infected from a common source over a short period.[383,394,416,417] There has been an increased reporting of occurrences of EHV-1 myeloencephalopathy[418] in congregations of horses around the United States. Most of these outbreaks have been associated with a mutant strain of herpesvirus, which appears to replicate rapidly, leading to a very high level of viremia and an apparent increased incidence of neurologic manifestations of this disease.[400,401,419] Morbidity rates ranging from less than 1% to almost 90% of exposed individuals and mortality rates ranging from 0.5% to 40% of in-contact horses have been reported. Neurologic EHV-1 infection can occur at any time of year, but the highest incidence is in the late winter, spring, and early summer, perhaps reflecting the seasonal occurrence of abortigenic EHV-1 infections during the same months.[219]

The neurologic form of EHV-1 infection has been observed in pregnant mares, barren mares, geldings, stallions, and foals, although foals frequently do not show neurologic manifestations of infection during outbreaks that involve severe neurologic disease in adult horses.[382,416] The disease also appears to less commonly affect pony breeds. Pregnant mares and mares nursing foals appear be at increased risk for developing neurologic manifestations of EHV-1 infection, and the stage of gestation may be important in determining the outcome of infection in pregnant mares.[377,382,416,420,421] Mares infected during the first 2 trimesters of gestation appear to be more likely to develop neurologic signs without abortion, whereas mares infected during the last trimester are more likely to abort without showing neurologic signs.[377,394,420,422,423]

All breeds of horses are susceptible to the neurologic form of EHV-1 infection, and other Equidae also may be affected. EHV-1 was the suspected cause of myeloencephalopathy that developed in a zebra 1 week after an in-contact onager *(Equus hemionus onager)* aborted an EHV-1 infected fetus.[424] The authors are unaware of reports of neurologic EHV-1 affecting donkeys and mules, although donkeys and mules have shown seroconversion indicating infection with EHV-1 while in contact with affected horses during outbreaks.[425-427] Indeed, donkeys and mules returning from a show were thought to be responsible for dissemination of EHV-1 and propagation of multiple outbreaks of neurologic EHV-1 infection in Southern California in 1984 (and in several subsequent years), suggesting that a donkey-adapted variant of EHV-1 with an increased neuropathogenicity for horses may have been involved.[427]

A modified live EHV-1 vaccine of monkey cell line origin was associated with neurologic disease in 486 of 60,000 recipients, prompting its withdrawal from the U.S. market

in 1977.[406] No reports of EHV-1 myeloencephalopathy have been associated with use of the modified live vaccine currently approved for use in horses in the United States.

Pathogenicity and Pathogenesis

Natural infection with EHV-1 occurs by inhalation or ingestion, after which the virus attaches to and rapidly replicates in cells of the nasopharyngeal epithelium and associated lymphoreticular tissues, causing necrosis, exudation, and infiltration of phagocytic cells. Bronchial and pulmonary tissues also become infected, particularly in foals, thus predisposing them to secondary bacterial pneumonia.[374,375,394,403,428] Migration of virus-infected phagocytes into the circulation results in viremia associated with mononuclear cells (primarily T lymphocytes) of the buffy coat.[374,375,394,429] The immunologically privileged intracellular location of the virus appears to protect it from inactivation by circulating antibody and permits dissemination to other tissues, including the CNS, even in the presence of high levels of antibody.[430] EHV-1 is capable of spreading directly from one infected cell to contiguous cells without an extracellular phase.[394] Vascular endothelium is the initial site of infection in the CNS and appears to be the predilection site for replication of EHV-1 after transfer of the virus from circulating leukocytes.[377,431,432] Viremia, which may be of prolonged duration, can occur during primary and all successive infections with EHV-1, even when no clinical signs are apparent; thus all EHV-1 infections pose a threat of inducing neurologic disease or abortion.[374,382]

The acute onset of clinical signs of EHV-1 myeloencephalopathy appears to result from vasculitis and thrombosis of arterioles in the brain and especially the spinal cord. This causes functional impairment of blood flow and metabolic exchange and, in severe cases, hypoxic degeneration and necrosis (malacia) with hemorrhage into adjacent neural tissues of the white and, to a lesser extent, gray matter. This proposed pathogenesis, based primarily on interpretation of the prominent vasculitis seen histopathologically in infected horses and the lack of definitive evidence of viral multiplication in neural tissues, contrasts greatly with the well-established pathogenesis of encephalitis caused by herpesviruses in other species.[389] The propensity of certain EHV-1 isolates to induce myeloencephalopathy does not appear to reflect specific neurotropism but rather a significant endotheliotropism. The finding of chorioretinopathy and neural lesions in experimentally infected specific pathogen-free ponies, however, suggests that at least some strains of EHV-1 may exhibit neurotropism.[433] Furthermore, strong evidence indicates that in addition to circulating T lymphocytes, epithelial cells of the respiratory tract, and lymphoid tissues draining the respiratory tract, trigeminal ganglia are important sites for establishing and maintaining the lifelong state of latency that occurs in most, if not all, horses during primary infection with EHV-1 and EHV-4.[388,433-435] The ubiquitous EHV-2 has been proposed to play an important role in promoting reactivation of EHV-1 and EHV-4 from these sites in latently infected horses.[404]

No satisfactory explanation exists as to why some outbreaks of EHV-1 infection are associated with a high incidence of neurologic disease, whereas others are not, or why different horses show different clinical manifestations of EHV-1 infection during outbreaks.[382,383,417,436] A genetic strain of EHV-1 with an adenine-to-guanine mutation at the open reading frame 30, causing a mutation in the DNA polymerase, is associated with replicative aggressiveness and a greater potential for causing neurologic disease.[419] The nature and extent of lesions resulting from EHV-1 infection appear to be influenced by the age, gender, reproductive status (including stage of pregnancy), and immune status of the horse; the magnitude of challenge; strain variations; and perhaps the route of infection.[382,436,437] In one carefully monitored outbreak of EHV-1 infection on a stud farm in England, less than 17% of infected horses developed neurologic manifestations of infection, even though almost 60% of the horses on the farm were confirmed to have been infected.[382] Endothelial cell infection and perivascular cuffing within the CNS appeared to be at least as pronounced in foals that died during this outbreak without showing neurologic signs as in profoundly paretic mares with severe CNS lesions; however, parenchymal neural lesions were minimal in the foals.[382,436] A notable finding during this outbreak was that mares and stallions that developed neurologic signs had considerable antibody responses, whereas the majority of foals did not, despite experiencing a prolonged period of viremia.[382]

The majority of EHV-1 infections that cause neurologic signs probably represent reinfection rather than a new infection.[412,438,439] Infection occurs in horses with significant preexisting serum EHV-1 antibody titers. Affected horses frequently have high titers at the onset of neurologic signs, and those horses that develop the most severe clinical signs are frequently the ones that show the most rapid increase in antibody titer after infection.[77,382,421] In addition, the characteristic vascular lesions in neural tissues of affected horses are typical of type III (Arthus) hypersensitivity reactions, and circulating immune complexes have been demonstrated at the onset of neurologic signs, suggesting that they may result from an immune-complex vasculitis. An immune-mediated mechanism is supported further by the difficulty experienced in isolating the virus from neural tissues of affected horses.[413,414,436] In addition, assessment of risk factors during outbreaks of neurologic EHV-1 infection in Southern California in 1984 revealed that horses vaccinated with killed or modified live EHV-1 vaccine within the previous year were significantly (9–14 times) more likely to develop neurologic manifestations of infection than were nonvaccinated horses.[427]

The finding of circulating antibodies to the myelin protein P2 in the serum of horses that died from EHV-1 myeloencephalopathy (but not in horses that recovered) has led to the suggestion that an alternate immune-mediated mechanism may play a role in the pathogenesis of neurologic EHV-1.[440] The presence of this antibody, however, may represent a response to leakage of the protein after damage induced by another mechanism.

Despite the foregoing observations, evidence for an immune-mediated pathogenesis for EHV-1 myeloencephalopathy is by no means conclusive. In experimental EHV-1 infections in which the onset of neurologic signs 8 to 9 days after infection correlated with a peak in the level of circulating immune complexes, vasculitis was not present in vessels in which endothelial cells did not support viral replication or in organs such as the kidney that one would expect to trap circulating immune complexes.[431] The finding of greatly depressed platelet counts several days before the onset of clinical signs, presumably the result of consumption in thrombi after endothelial damage, suggests that the neuropathologic changes are initiated before circulating immune complexes peak and

that the action of immune complexes may be secondary and localized. Failure to isolate the virus from the CNS may be attributable to high levels of circulating antibody and to the endotheliotropism of the virus.[431]

Clinical Signs

In natural and experimental infections, neurologic signs appear 6 to 10 days after infection by the intranasal route. The onset of neurologic signs may be preceded or accompanied by signs of upper respiratory disease, fever, inappetence, or hindlimb edema within the previous 2 weeks, although in many instances no antecedent signs are notable unless one routinely monitors rectal temperature. However, frequently one finds a herd or stable history of current or recent cases of respiratory tract infection, fever, inappetence, distal limb edema, abortion, neonatal death, foal diarrhea, or neurologic disease, and for one to encounter different signs of EHV-1 infection in different groups of horses on a particular farm is not unusual.[382]

Affected horses are occasionally febrile at the onset of neurologic disease, although most are normothermic and some are hypothermic. Neurologic signs are generally of acute or peracute onset, after which they tend to stabilize rapidly and generally do not progress after the first 1 or 2 days.[219,412,416,420,441] Clinical signs vary depending on the location and severity of lesions, but in most horses, signs reflect predominant involvement of the spinal white matter.[219] Ataxia and paresis of the limbs are the most common signs, with hypotonia of the tail and anus, tail elevation, and urinary incontinence being common but not invariable findings. Clinical signs are usually bilaterally symmetric or only mildly asymmetric, although hemiparesis or sudden onset of unilateral hind- or forelimb lameness progressing to unilateral or more generalized ataxia, paresis, and recumbency have been reported.[219,442-445] Lesions in peripheral nerves and spinal cord were observed in some of these cases.[442] The hindlimbs generally are affected more severely and earlier in the disease course than the forelimbs. In mildly affected horses, transient ataxia and stiffness of the pelvic limbs or dribbling of urine after overflow from a distended atonic bladder may be the only signs noted.[219,420] One may note conscious proprioceptive deficits in these cases as reluctance to move, clumsiness, toe dragging, knuckling, stumbling, pivoting, and circumduction in one or more limbs on circling, with spasticity evident in some cases.[44,52,85] These signs are often subtle and may go unnoticed. More severely affected horses show profound limb weakness and swaying of the hindquarters, and a small proportion show complete paralysis of affected limbs, manifested as paraplegia and sitting like a dog, complete recumbency, or tetraplegia.[219,382,394,446]

Distention of the urinary bladder is common and may cause signs of colic or dribbling of urine, which frequently results in scalding of the perineum, legs, and other areas.[426] Cystitis is a frequent complication, particularly when repeated catheterization is necessary to relieve bladder distention.[441] Affected stallions and geldings may develop penile flaccidity and paraphimosis or repeated erections, whereas mares may develop vulvar flaccidity. In addition, stallions may experience reduced libido and swelling of the testes.[382,436] Scrotal edema may accompany hindlimb edema at the onset of neurologic signs in some cases.[382,416,420,423,436] Sensory deficits are uncommon, but perineal hypalgia or analgesia has been noted, and analgesia of the caudal half of the body was observed in one affected horse.[219,412,420,446] Consistent with predominant involvement of the white matter of the spinal cord, flexor reflexes are normal and perineal reflexes are preserved. In recumbent horses, spinal tendon reflexes can be tested and may be increased. Atrophy is rarely seen, even in the later stages of the disease.[219] Affected horses usually remain alert and have good appetites, even when recumbent, although some show modest depression and inappetence.[416,447] Severe depression, when it occurs, is more often caused by secondary complications than by brain involvement.[447] Unequivocal signs of brain disease are rare, although infarction of the brainstem may cause depressed sensorium, altered behavior, and cranial nerve damage leading to vestibular signs and to lingual, mandibular, and pharyngeal paresis, which may manifest as dysphagia.[422,445,447-449] Strabismus, nystagmus, circling, and head tilt have been observed on occasion.[219,387,422,446]

Affected horses show variable progression of clinical signs. Those horses that are affected mildly frequently stabilize rapidly over a period of hours to a few days as edema and hemorrhage resolve; generally they recover completely over a period of days to several weeks.[382,383,412,416,417] If recumbency occurs, it generally does so during the first 24 hours, with some horses showing such complete motor paralysis that they are unable to lift their heads.[442] Severely affected horses may show progression of signs during the first few days and may die in coma or convulsion or be euthanized because of secondary complications.[382]

Laboratory Findings

CSF analysis typically, although by no means always, reveals xanthochromia, an increased protein concentration (100–500 mg/dL), and increased AQ (ratio of CSF to serum albumin concentration), reflecting vasculitis and protein leakage into CSF. The white blood cell count in CSF is usually normal (0–5 cells/μL) but occasionally is increased. Abnormalities in CSF are not present at the onset of clinical signs in some horses, and changes resolve rather quickly; thus the CSF may be normal within 2 weeks of onset of clinical signs.[219,377,441]

The presence of antibodies to EHV-1 in the CSF of affected horses strongly suggests a diagnosis of EHV-1 myeloencephalopathy, although such antibodies are absent in many cases.[219,377,387,422,450] One should take into account the albumin concentration, IgG concentration, and EHV-1 antibody titer in serum and CSF when interpreting positive antibody titers in CSF.[451] Because the AQ usually is elevated in affected horses and the IgG index is normal, the presence of EHV-1 antibodies in CSF reflects leakage of protein across a damaged blood-brain or blood-CSF barrier after vasculitis rather than intrathecal antibody production.[440,450,451] Antibodies therefore are more likely to be present in the CSF of affected horses with concomitantly high serum titers.[219,377,440,450,451] Blood contamination during collection of CSF and other diseases that cause an increase in the permeability of the blood-brain barrier or bleeding into the subarachnoid space may elevate CSF antibody titers falsely if serum titers are also high. Isolation of EHV-1 from the CSF of affected horses would confirm a diagnosis but is rarely successful.[219,426]

Virus isolation and identification of EHV-1 from nasopharyngeal swabs or buffy coat samples strongly support a diagnosis of EHV-1 myeloencephalopathy in a horse with compatible clinical signs and should be attempted by submission of nasopharyngeal swabs in viral transport medium

and an uncoagulated blood sample (citrated or heparinized). Diagnosis may be achieved more rapidly using real-time PCR for identification of EHV DNA from nasopharyngeal swabs or buffy coat samples. The likelihood of isolating EHV-1 during outbreaks of neurologic disease increases by monitoring in-contact horses and collecting nasal swab and buffy coat samples during the prodromal febrile phase before neurologic signs develop.[416] Even so, interpretation of positive results can be confusing because EHV-1 and EHV-4 have been isolated from the respiratory tract of normal horses.[409] Application of new diagnostic methods such as polymerase chain reaction (PCR), in situ hybridization, antigen-capture ELISA, and dot immunobinding to nasal swabs or scrapings, buffy coat samples, or pathologic specimens have improved the speed and specificity greatly with which one can diagnose EHV-1 infection.[452-460] Many conventional PCR protocols targeting specific genes of EHV-1 have been published in recent years for molecular detection of EHV-1 in nasopharyngeal swabs or buffy coat samples.[405,455-459] Although considerable progress has been made in developing PCR protocols for clinical use, quality control of nucleic acid amplification techniques remains an ongoing challenge because of lack of protocol standardization among laboratories.[461] Furthermore, the majority of PCR assays targeting genomic EHV-1 DNA are unable to differentiate between a lytic and a latent infection. Novel technologies such as real-time PCR that allow quantitation of viral DNA and detection at the level of gene expression likely will feature prominently as molecular diagnostic approaches to EHV-1 infection are refined further in the future.

Serologic testing that demonstrates a fourfold or greater increase in serum antibody titer using serum-neutralizing or complement fixation tests on acute and convalescent samples collected 7 to 21 days apart provides presumptive evidence of infection. Many horses with EHV-1 myeloencephalopathy, however, do not show a fourfold rise in serum-neutralizing titer, and some actually show a decline.[426] This may be explained by the finding that when antibody titers rise, they do so rapidly within 6 to 10 days of infection and already may have peaked by the time neurologic signs appear. Although serologic testing has limitations in confirming a diagnosis of EHV-1 myeloencephalopathy in an individual horse, testing of paired serum samples from in-contact horses is recommended because a significant proportion of affected and unaffected in-contact horses seroconvert, providing indirect evidence that EHV-1 is the causative agent. Interpretation of the results of serologic tests is complicated by the fact that the serum-neutralizing, complement-fixation, and ELISA tests in use at most diagnostic laboratories do not distinguish between antibodies to EHV-1 and EHV-4 because of cross-reaction between these viruses. A specific ELISA test based on the C-terminal portion of glycoprotein G of both viruses has been developed and should prove valuable in the investigation and management of disease outbreaks in the future.[462-464]

Diagnosis

The multifocal distribution of lesions results in variability of clinical presentation, which necessitates inclusion of a number of conditions in the differential diagnosis. These conditions include EPM, cervical stenotic myelopathy, and cervical vertebral instability (wobbler syndrome), cervical vertebral fracture or other CNS trauma, neuritis of the cauda equina, fibrocartilaginous infarction, aberrant parasite migration, degenerative myelopathy, other viral encephalitides (flaviviruses and alphaviruses), rabies, botulism, CNS abscess, and a variety of plant and chemical intoxications.[219,387] Sudden onset and early stabilization of neurologic signs including ataxia, paresis, and urinary incontinence; involvement of multiple horses on the premises; and a recent history of fever (most consistently reported clinical sign before onset of ataxia), abortion, or viral respiratory disease in the affected horse or herdmates are sufficient to make a tentative diagnosis of EHV-1 myeloencephalopathy.[387] Antemortem diagnosis is supported by ruling out other conditions; demonstrating xanthochromia and elevated protein concentration in CSF; identifying EHV-1 in or isolating EHV-1 from the respiratory tract, buffy coat, or CSF; and demonstrating a fourfold increase in antibodies using serum-neutralizing, complement fixation, or ELISA tests performed on acute and convalescent serum samples collected from affected or in-contact horses 7 to 21 days apart.[383,417,465] Antemortem confirmation of a diagnosis of EHV-1 myeloencephalopathy is frequently not possible, however, particularly when an individual horse is affected, because the foregoing tests do not yield consistent results in all cases. Hematologic abnormalities with EHM are inconsistent but may include mild anemia and lymphopenia, followed by increased plasma concentration of fibrinogen.

Treatment and Prognosis

Because EHV-1 is a contagious and potentially devastating infection, horses suspected of being affected should be isolated promptly and strictly until EHV-1 is ruled out by confirmation of an alternate diagnosis.[447] No specific treatment is available; thus management of horses with EHV-1 myeloencephalopathy aims toward supportive nursing and nutritional care and reduction of CNS inflammation.[387] One should encourage horses that are not recumbent to remain standing and should protect them from self-inflicted trauma by the provision of good footing, such as a grass paddock, by placement of food and water in an accessible location at a convenient height above ground level, and by other measures, including the use of padded hoods and elimination of obstacles. Patients that become recumbent should be maintained in a sternal position on a thick cushion of dry absorbent bedding and should be rolled frequently (at least every 2–4 hours) to reduce the risk of myonecrosis and decubital ulcers. Whenever possible, one should lift and support the horse in the standing position using an appropriately fitting sling.[383] Slings are most beneficial for moderately affected horses that are too weak to rise but are able to maintain a standing position with minimal assistance.

Affected horses usually maintain a good appetite, even when recumbent, although hand feeding may be necessary to encourage some horses to eat. Maintenance of hydration is important, and provision of a laxative diet or the administration of laxatives such as bran mashes, mineral oil, or psyllium may be necessary to reduce intestinal impaction. One usually can meet the caloric and water needs of anorectic patients by feeding gruels of alfalfa-based or similar pelleted feeds in water or balanced electrolyte solution via nasogastric tube. If oral intake is insufficient to meet the daily water needs of 60 to 80 mL/kg of body mass per day, one can maintain hydration by intravenous administration of balanced electrolyte solutions.[219] Partial or total parenteral nutrition can also be used to meet the caloric needs of anorectic, recumbent horses.

If affected horses are unable to stand and posture to urinate or if bladder function is impaired significantly, manual evacuation of the bladder by application of pressure per the rectum may be necessary. If these measures are unsuccessful, judicious urinary catheterization is indicated and should be performed aseptically with the collection tubing attached to a sterile closed bag to minimize the risk of inducing urinary tract infection.[387,446,447] Cystitis is, however, a frequent complication, particularly in recumbent horses, and can lead to bladder wall necrosis, bladder rupture, and systemic sepsis. Urine scalding can become a major problem, particularly in mares that dribble urine. Prevention involves regular washing of the perineum, tail, and hind legs with water, application of water-repellent ointments, and braiding or wrapping the tail to simplify cleaning.[387] Administration of enemas or manual emptying of the rectum also may be necessary to promote defecation and improve patient comfort.[387]

Because vasculitis, hemorrhage, and edema are prominent early lesions and may have an immune basis, treatment with corticosteroids early in the disease course is recommended by some clinicians, although no objective data are available to document the efficacy of these or other antiinflammatory drugs.[219,416,447] A short course of treatment with prednisolone acetate (1–2 mg/kg/day) or dexamethasone (0.05–0.25 mg/kg parenterally twice daily) for 2 to 3 days with decreasing doses over another 1 to 3 days may be beneficial.[219,387,446] Flunixin meglumine (1.1 mg/kg body mass every 12 hours) is indicated to treat CNS vasculitis. DMSO at a dose of 0.5 to 1.0 g/kg administered intravenously as a 10% to 20% solution in normal saline or 5% dextrose once daily for up to 3 days commonly is used to treat horses with suspected CNS trauma or inflammatory disease, such as EHV-1.[384,387] Although the efficacy of DMSO for treating herpesvirus myeloencephalopathy has not been evaluated, its reported ability to inhibit platelet aggregation and scavenge free radicals supports its continued use. Because of the high risk of development of cystitis and other secondary bacterial infections, administering broad-spectrum antibiotics such as potentiated sulfonamides (trimethoprim-sulfamethoxazole 30 mg/kg body mass orally every 12 hours) or ceftiofur (2.2 mg/kg body mass intramuscularly or intravenously every 12 hours) is advisable, particularly when corticosteroid treatment is used.[19,383,387] One should base the choice of antibiotics for treating established secondary bacterial infections of the urinary tract, respiratory tract, or other areas on the results of culture and susceptibility testing.

Acyclovir, a synthetic purine nucleoside analog with inhibitory activity against several human herpesviruses, has been shown to exert an inhibitory effect on EHV-1 in vitro.[466] Apparent efficacy of acyclovir was suggested by a successful treatment outcome in occasional outbreak situations.[417,467] Although these anecdotal reports seemed promising, data describing the pharmacokinetics, bioavailability, and safety of acyclovir in horses do not support claims of efficacy, especially after oral administration. Bioavailability of acyclovir after oral administration to horses is extremely low; in contrast, bioavailability of valacyclovir, a prodrug for acyclovir, is much higher.[468,469] Clearly, additional studies are needed to define the appropriate dose for valacyclovir in horses and to document its efficacy for the treatment of EHV-1 myeloencephalopathy. Another nucleoside analog, penciclovir, has been shown to have excellent activity against EHV-1 in tissue culture and in a mouse model of EHV-1 infection.[470]

Affected horses that remain standing have a good prognosis for recovery, and improvement generally is apparent within a few days, although a period of several weeks to more than a year may be required before horses with severe deficits show complete recovery. In these instances, control of urination frequently returns before gait abnormalities resolve completely.[447] Some horses may be left with permanent residual neurologic deficits, including urinary incontinence and ataxia, that may necessitate euthanasia many months beyond onset of neurologic signs.[219,383,425,426] Horses that become recumbent have a greatly increased likelihood of developing complications such as myonecrosis, urinary tract infection, decubital ulcers, respiratory tract infection, gastrointestinal obstruction and ulceration, injuries, and complications of dehydration and malnutrition. Their prognosis for recovery is therefore poor, particularly if they remain recumbent for more than 24 hours and they are unable to stand after being lifted with a sling.[382,383] One should not elect euthanasia prematurely in valuable horses, however, because reports document horses standing again and recovering completely to race successfully after being recumbent for several days to 3 weeks.[416,423,442] Most mildly affected mares return to breeding soundness in the same season, whereas fertility is likely to be compromised in more severely affected mares that experience urinary retention.[382] Recurrence or exacerbation of neurologic signs in horses that have recovered completely has not been documented, even though the majority likely remain latently infected.[219,394,407,447]

Pathologic Findings

When horses with suspected EHV-1 myeloencephalopathy die or are euthanized, one should submit the whole carcass or at least the head, spine, spleen, thyroid, and lung for postmortem examination because lesions frequently are not confined to the CNS of horses with EHV-1 myeloencephalopathy.[471] Gross pathologic lesions in the CNS frequently are not found, but small (2–6 mm) focal areas of hemorrhage distributed randomly throughout the meninges and parenchyma of the brain and spinal cord may be observed. More diffuse dural hemorrhage is notable in some cases and may extend to spinal nerve roots and the cauda equina.[377,421,414] Small plum-colored areas of degeneration and hemorrhage are sometimes grossly visible in fresh tissue at various levels of the spinal cord (white matter), and malacic foci may be visible macroscopically in the gray and white matter in sliced fixed sections of brain.[377,414,421,423,428]

The gross and histologic lesions in the CNS reflect vasculitis, congestion, and secondary ischemic degeneration of nervous tissue. Although vasculitis is a consistent finding, degeneration of nervous tissue is evident chiefly in those horses with clinical signs of severe neurologic disease.[377,421,436] The vasculitis is often severe and has a widespread, random, multifocal distribution, with the most severe lesions usually in the brainstem and spinal cord. In the brain the meningeal and penetrating or radiating vessels in gray matter are the major sites of vascular involvement. Thus foci of axonal swelling and malacia develop in the gray and white matter, particularly adjacent to the meningeal surface and in the deep cortex adjacent to the white matter.[377,421,414] In the spinal cord a similar orientation to meningeal vessels results in degeneration of white matter within ovoid, linear, or wedge-shaped foci, affecting predominantly the lateral and the ventral white columns.[377] In some instances, sheaths

of nerve roots and nerves and capsules of ganglia also are involved.[219,377,421,436] Trigeminal ganglionitis might be present but usually is not manifest clinically.[442]

Ocular lesions, including uveal vasculitis with perivascular mononuclear cell cuffing in the ciliary body and optic nerve or extensive retinal degeneration, have been observed in foals showing signs of bilateral hypopyon and iritis or severe visual impairment and chorioretinopathy without anterior segment involvement during field and experimental EHV-1 infections.[377,382,414,433,436] In some foals, EHV-1 appears to induce ocular and neural damage in the absence of gross signs of neurologic or visual impairment.[433] During paralytic infections, secondary viral replication occurs in blood vessels of the testis and epididymis in addition to CNS and may be responsible for reported signs of scrotal edema and loss of libido in affected stallions.[382,472]

EHV-1 infrequently is isolated from the CNS of affected horses that show typical lesions. Thus one should attempt to isolate the virus from other sites to support the diagnosis. Those sites most likely to yield virus or to contain viral antigen include the turbinates and nasal passages, as well as lymph nodes draining the upper respiratory tract, lung, thyroid, spleen, and endometrium, in addition to the brain.[219,436,473] Immunofluorescent antibody testing of brain and spinal cord sections is considered to be more sensitive than virus isolation, but false-negative results have been observed.[219,413,436] An indirect immunoperoxidase method using orthodox light microscopy was highly sensitive for identifying individual antigen-containing cells in the CNS and other areas of the body, even at sites containing few or no lesions or inclusion bodies and in which virus could not be detected by immunofluorescent antibody testing or virus isolation.[436] Similarly, the PCR technique is more sensitive than virus isolation for detecting viral antigen in nasopharyngeal swabs collected from horses with respiratory tract disease caused by EHV-1 or EHV-4.[455] Both of these techniques—as well as antigen-capture ELISA tests, dot immunobinding, and in situ DNA hybridization, which are sensitive and readily differentiate between EHV-1 and EHV-4—show great promise for routine application to samples collected antemortem or at necropsy from affected horses.[432,436,453,456,460]

Control and Prevention

Control measures during outbreaks of EHV-1 infection aim at reducing spread by infectious aerosols, direct contact, and fomites, as well as at reducing stress-induced recrudescence of latent EHV-1 infection.[374,375,394] If neurologic signs or other clinical signs suggestive of EHV-1 infection occur, one should isolate affected animals promptly and completely in a well-ventilated airspace separate from the remainder of the herd. In-contact horses should be isolated in their current location in small groups for at least 1 month; pregnant mares should be isolated preferably until they foal.[374-376,382,394,416,449] On breeding farms, one should suspend covering.[382] Aborted fetuses and fetal membranes are rich sources of infectious virus; therefore they should be collected and placed in leak-proof containers (e.g., heavy-gauge plastic bags) for submission for diagnostic evaluation or disposal by burning.[374,375] Similarly, bedding and dirt contaminated with fetal fluids should be disposed of or burned, and stalls or other areas occupied by infected horses should be cleaned thoroughly, disinfected with an iodophor or a phenolic product, and left empty for several weeks.[394] Equipment used to handle, groom, feed, water, muck out, or transport affected horses also should be cleaned and disinfected or disposed of properly. Thereafter separate equipment and personnel should be used for affected and unaffected horses, or at least caretakers should handle affected horses last and wear disposable gloves, surgical masks, and protective clothing that can be changed or disinfected between groups.[394] Although control measures are frequently successful in stopping further spread of infection during outbreaks, one should note that transmission of infection before control measures are implemented may result in a secondary wave of disease 1 to 2 weeks later.[382,394,416]

Traffic of horses and human beings on the premises should be minimized, and movement of horses onto and off of the infected premises should be suspended until at least 3 weeks after resolution of acute signs in the last clinical case or until tests show that virus transmission is no longer occurring.[374-376,387,394] Collection of nasal swabs and uncoagulated blood (buffy coat) samples from clinically affected and exposed horses within each group and demonstration of stable or declining antibody titers in serum samples proved to be helpful in determining patterns of exposure and spread and in establishing when virus transmission had ceased in one reported outbreak. Protracted viremia lasting several weeks or months occurs in some horses and extends the period during which movement of horses should be restricted.[382]

If horses must enter the farm, they should be current on EHV-1 vaccination and should be isolated away from the resident population. Although giving booster vaccinations to exposed pregnant mares during outbreaks of abortigenic EHV-1 infection is common practice,[374,375,394] vaccination of exposed horses during outbreaks of EHV-1 myeloencephalopathy has not been investigated and cannot be recommended at this time because of the possibility of an immune-mediated pathogenesis. Administering booster doses of inactivated EHV-1 vaccine to all unexposed horses that have not been vaccinated within the previous month is common practice, however.

Preventive measures should include routine management practices aimed at reducing the chances of introducing and disseminating infection.[376,394,444,474] New arrivals should be isolated for at least 3 weeks before joining the herd, distinct herd groups should be maintained based on the age and use of horses, and care should be taken to minimize or eliminate commingling of resident horses with visiting or transient horses. In particular, pregnant broodmares should be maintained in groups separate from the remaining farm population. In addition, minimizing stress associated with overcrowding and handling procedures is prudent in an attempt to reduce recrudescence of latent EHV-1 infection.[394,407,444,474]

No method is known that reliably prevents the neurologic form of EHV-1 infection. None of the EHV-1 or EHV-4 vaccines currently available carries a claim that they prevent EHV-1 myeloencephalopathy, and the disease has been observed in horses vaccinated regularly at 3- to 5-month intervals with inactivated or modified live vaccines.[219,387,417,426,448] Repetitive administration of currently available EHV-1 and EHV-4 vaccines appears to induce some immunity to respiratory disease and reduce the incidence of abortion but does not block infection and induction of viremia or eliminate the possibility of clinical disease and establishment of the carrier state.[374,375,394,448,474-479] Although the protection induced by inactivated EHV-1 vaccines is incomplete and of short duration, the vaccine reduces virus excretion in horses that do

become infected.[476,479] To maintain appropriate vaccination procedures in an attempt to reduce the incidence of other manifestations of EHV-1 infection and reduce the magnitude of challenge experienced by in-contact horses is logical. This indirectly may help prevent EHV-1 myeloencephalopathy.[219] An attenuated live virus vaccine based on the temperature-sensitive and host range mutant clone 147 of EHV-1 has been evaluated recently. The novelty of this vaccine lies in the fact that a low dose administered intranasally replicated in conventional target species and conferred exceptional efficacy against respiratory disease, virus shedding, viremia, and abortion caused by a severe EHV-1 challenge.[480-482] Intranasal vaccine also claims to protect against less common manifestations of EHV-1 infection such as paresis and jaundice.[381]

POLYNEURITIS EQUI

Polyneuritis equi is an uncommon neurologic disease of all equine species that is characterized by tail and anal sphincter paralysis, often accompanied by cranial and peripheral nerve damage.[4,483-493] Previous reports referred to the disease as *neuritis of the cauda equina* because of the susceptibility of this region, but frequent involvement of the cranial and peripheral nerves led to the term *polyneuritis equi*.[375] Although the disease has been recognized more readily in Europe, where Dexler first reported it in 1897, cases have now been reported in Great Britain, Canada, and the United States.[485,486,488,494] The disease does not appear to have a breed, gender, or age predilection, but the youngest horse affected was 17 months of age.[484,487,488,494,495]

The cause of this disease is unknown. Primary immune reaction and viral inflammatory disease have been suggested, although possibly one may be a consequence of the other.[484] Several infectious agents have been suggested, such as EHV-1, equine adenovirus, and streptococcal and *Campylobacter* spp. bacteria.[491,493,496] The pathologic lesions resemble those of Guillain-Barré syndrome in human beings, and the disease is similar to experimental allergic neuritis in rats.[491] Evidence suggests that the immune system is involved because horses with polyneuritis equi have circulating antibodies to P2 myelin protein, which is present in rats with experimental allergic neuritis.[491,497] Inflammatory lesions contain both T and B lymphocytes, suggesting the possibility of an immune-mediated reaction to myelin.[495,498]

Clinical Signs

Although the disease manifests itself in two forms, signs are generally slow and progressive: (1) the acute or early signs include hyperesthesia of the perineal or head regions (or both), and (2) in the chronic form, horses show paralysis of the tail, anus, rectum, and bladder. Paralysis often is accompanied by fecal and urinary retention, urinary scalding of the pelvic limbs, and penile paralysis in male horses.[4,483-485,488,490,493]

The pelvic limb signs are often symmetric, whereas the signs involving the head and cranial nerves are often asymmetric.[488] Muscle atrophy in the gluteal region is sometimes present along with mild degrees of ataxia.[484,485,487,488,490] Muscle atrophy associated with cranial nerve involvement may occur in the head region. Damage to peripheral motor nerves might result in gait deficits and abnormal use of thoracic or pelvic limbs.[483,493]

Although cranial nerve involvement is reported primarily to affect cranial nerves V, VII, and VIII, any of cranial nerves II, III, IV, VI, IX, X, and XII also may be involved.[4,484,486,487] As a result of damage to the cranial nerves, horses might have trouble with mastication and swallowing.[493] A head tilt, ear droop, lip droop, and ptosis are common signs.[4,493] One report describes a horse with brachial neuritis along with involvement of cranial nerves V, VII, and XII. The horse in that report also exhibited mild ataxia and weakness in all limbs. The horse performed the hopping test poorly on the right thoracic limb, and the horse resented palpation in the right caudal cervical and prescapular region.[484]

Colic caused by fecal retention may be the primary sign when one initially examines horses with polyneuritis equi. Fecal retention leads to an impaction caused by the flaccid anal sphincter, often accompanied by an atonic, distended bladder.[483] If the clinician sees these signs in the acute or hyperesthetic form, they usually progress to hypalgesia or anesthesia. An area of hyperesthesia might surround the area of anesthesia.[4,483,484]

Diagnosis

The definitive diagnostic test is a postmortem examination. Blood work might be normal or reveal a mature neutrophilia with hyperfibrinogenemia, mild to moderate anemia, and an increased total protein—all indications of a chronic inflammatory process.[485,487] Results of CSF are variable and nonspecific, ranging from mild mixed mononuclear to primarily neutrophilic pleocytosis, with normal to increased protein concentrations (70–300 mg/dL). There could be a mixed pleocytosis with predominance of lymphocytes and marked lymphoid reactivity supportive of humoral response to inflammation.[495] Cytologic examination of CSF might be normal in the acute stage of disease.[4,484,485,487,488,492]

Radiography might be helpful to rule out trauma to the tailhead or cranial nerve involvement, such as a fractured petrous temporal or other bones of the skull.[4,484]

Some horses with clinical signs exhibit circulating serum antibodies to P2 myelin.[491,497] However, the presence of this antibody is only weakly supportive of the diagnosis because the same antibody has been detected in horses with EHV-1 and equine adenovirus infections.[440,483,491,496]

Classically, the primary pathologic lesions involve the extradural nerve roots but also might involve the intradural nerve roots.[484,485,487,493] The lesions are granulomatous with various degrees of inflammation and infiltration of lymphocytes, eosinophils, macrophages, giant cells, and plasma cells. This inflammation leads to myelin degeneration, subsequent axonal degeneration, and thickening of the epineurium, endoneurium, and perineurium with proliferation, which causes obliteration of the neural architecture by the fibrous tissue.[483,484,490] The most severe lesions are in the cauda equina, but swelling, edema, and hemorrhage of cranial nerves may occur. The fibrous tissue formation might lead to adhesions between the meninges and the periosteum of the vertebral bodies. Reports describe involvement of the autonomic nervous system, but no changes in clinical signs have been reported (postmortem only).[484,486]

The polyneuritis lesions are similar to those observed with Guillain-Barré syndrome in human beings, experimental allergic neuritis in rats, and coonhound paralysis in dogs.[484,487,492,493] This similarity might indicate a combination of inflammatory and immune-mediated mechanisms in the pathogenesis of polyneuritis equi.

Differential Diagnosis

The most important differential diagnosis is trauma to the sacrococcygeal area of the spinal canal, which can be differentiated by radiography of the area looking for fractures or displacements.[4,484,488] EPM is the second most common disease in the differential diagnosis of polyneuritis equi. The usual signs of EPM include asymmetric multifocal signs of brain and spinal cord injury causing cranial nerve deficits with alterations in mentation, whereas the cranial nerve deficits of polyneuritis equi are peripheral, with no change in mentation or behavior.[313,488]

EHV-1 myeloencephalitis should be another rule out of polyneuritis equi. However, signs of fever in the acute stage of EHV-1 infection does not develop in horses with polyneuritis equi. This condition frequently affects more than one horse on a farm. Herpesvirus has a rapid onset with ataxia as a predominant or common sign, which is usually not the case in horses with polyneuritis equi. Urinary incontinence can commonly develop in horses with EHV-1 infection along with ataxia and pelvic limb neurologic deficits. The ataxia and weakness are usually symmetric and might result in recumbency. Occasionally, affected horses sit like a dog because of profound pelvic limb weakness. Cranial nerve involvement is not common but might occur in between 10% and 15% of affected horses.[375,421,471,499]

One should consider verminous myeloencephalitis as a differential diagnosis for polyneuritis equi. The signs vary and depend on the migratory pathway of the parasite. Diffuse or multifocal brain and spinal cord lesions have been reported. The onset is usually sudden, with rapid deterioration and death. The incidence of this disease is low, perhaps because of more intense parasite control.[248,500,501]

One should consider EMND in the differential diagnosis. Horses with motor neuron disease have symmetric muscle wasting or atrophy and weight loss with significant weakness, sweating, and muscle fasciculations. However, these horses are usually not ataxic, and their unique clinical feature is that they walk better than they stand. This disease is a denervation atrophy of both muscle fiber types (1 and 2) with a predominance of type 1 muscle fibers. The diagnosis is through a spinal accessory nerve biopsy or sacrodorsalis caudalis muscle biopsy.[502] However, any muscle containing both fiber types is useful for diagnosis.

Treatment

The primary therapy is palliative. No treatment for the disease is known. Removing feces from the rectum and evacuating the bladder are usually necessary. If cystitis caused by bladder distention occurs, systemic antibiotics might be indicated. Some attempts have been made at treating the inflammation with corticosteroids, but the effects have been short lived. The prognosis is poor. Some animals might be maintained for a few months.

ACQUIRED EQUINE POLYNEUROPATHY IN SCANDINAVIA

Monica Aleman

Acquired equine polyneuropathy (AEP), also known as Scandinavian knuckling syndrome, is characterized by acute onset of bilateral pelvic limb digital extensor dysfunction and knuckling.[503,504] Ataxia is not a feature of this polyneuropathy. The disease has been described in horses of various breeds during the months from December to April in Sweden, Norway, and Finland. The estimated prevalence of disease on farms in Norway and Sweden is 27% with a case fatality rate of 29%. Duration of clinical signs in survivors varies from 1 to 17 months (median 4.4 months). Survivors can return to full work within 19 months (median 6.6 months). Younger horses have a greater chance of survival than older horses. However, the disease was less prevalent in horses over 12 years old according to an epidemiologic study.[503] The exact etiology of disease is unknown but suspected to be forage-related toxicity. Histologic lesions consist of inflammatory demyelinating polyneuropathy and intracisternal Schwann cell inclusions.[505]

NEUROMUSCULAR DISORDERS

The neuromuscular system is an important part of the nervous system. This system has central and peripheral components. The central components include motor neurons located in the central nervous system (brain [nuclei of cranial nerves III–VII, IX–XII] and spinal cord). Its peripheral part includes all structures located outside the brain and spinal cord. The peripheral nervous system can be classified into two major functional systems: afferent or sensory and efferent or motor systems. The neuromuscular system is composed of motor units. A motor unit consists of a single lower motor neuron, its axon and supportive cells (Schwann cells), neuromuscular junction, and all the muscle fibers innervated by the motor neuron. The neuromuscular junction can be further divided in presynaptic (axonal terminal), synaptic (synaptic space), and postsynaptic (muscle membrane) regions.

A single lower motor neuron innervates muscle fibers contained within one muscle and not of different muscle groups. One muscle fiber is innervated by one motor neuron. Large muscles (postural and locomotion muscles) on which fine movements are not required generally have a few hundred to thousands of muscle fibers per motor unit. Whereas muscles required for more dexterous movement such as those for ocular movement have fewer muscle fibers per motor unit. A single discharge of a motor neuron will result in the contraction of all muscle fibers innervated by its axon. All motor neurons innervating skeletal muscle are exclusively excitatory. However, interneurons located within the spinal cord can have either excitatory or inhibitory effects on the motor neurons.

Neuromuscular disorders are those affecting any component of the neuromuscular system and their supporting cells. Signs of dysfunction can be variable depending on the specific area affected. Lower motor neuron dysfunction results in muscle weakness, paresis to paralysis, decreased muscle tone, decreased to absent reflexes, and neurogenic muscle atrophy. Definition of a few terms will be provided for clarification. Neuronopathy is the abnormality of the neuron cell body, whereas neuropathy is the abnormality of the nerve. A nerve might be motor or sensory or both. Neuropathies can be characterized by degeneration of its axons (axonopathy), demyelination (e.g., Schwannopathy), or both. Junctionopathies are disorders involving any of the presynaptic, synaptic,

and postsynaptic regions of the motor end plate. Examples of neuromuscular disorders are shown in Table 11.11.[30]

Diagnostic Approach

Complete signalment and history are the essential first step in the investigation of any disorder. A history of a few or several horses affected in the same premises should raise suspicion of nutritional, toxic, or infectious causes. A thorough physical and neurologic examination must be performed for the evaluation of patients with suspected neuromuscular dysfunction. Neuroanatomic localization is essential, and disorders that may present with clinical signs similar to those of neuromuscular dysfunction must be ruled out, particularly in the sick neonatal foal on which weakness, inability to rise, apparent decreased muscle tone, among others, might be a common presentation for various disorders. Full blood work (CBC, chemistry panel, blood gases and pH), and urinalysis should be part of a minimum database collection. Neuromuscular disorders on which muscle enzymes may be elevated include ionophores and OP toxicity and those associated with tick infestation. Electrolyte analysis must also include ionized calcium (Ca^{++}) and magnesium (Mg^{++}) because these are physiologically active ions essential for neuromuscular homeostasis and function. Cerebrospinal fluid (CSF) cytology is usually normal in neuromuscular disorders. Toxicologic screening of the diet, water, plants, soil, blood, stomach contents, feces, and body fluids including CSF might add useful information. Consider imaging modalities such as radiographs, ultrasound, scintigraphy, computed tomography, and magnetic resonance if indicated by a problem-oriented diagnostic approach. A full body necropsy and thorough evaluation of the nervous system by a trained neuropathologist are essential. Neuroelectrodiagnostics are essential in the evaluation and diagnosis of neuromuscular dysfunction. Examples of electrodiagnostics include electromyography (EMG), nerve conduction studies (NC), single fiber EMG (SF-EMG), and repetitive nerve stimulation (RNS—more specific for neuromuscular disorders).

TABLE **11.11** **Neuromuscular Disorders Reported or Suspected in Horses**[30]

CENTRAL
Interneuron (Inhibitory: Renshaw cells)
Tetanus (*C. tetani* toxins)
Motor Neuron Cell Body
Equine motor neuron disease
PERIPHERAL
Neuropathy: Mononeuropathy/Multiple Mononeuropathy/ Polyneuropathy
Anesthesia associated (compression, ischemia, hypoxia)
Drugs (cisplatin, colchicine, metronidazole, vincristine)
Endocrinopathies/metabolic
Guttural pouch infection associated
Iatrogenic (drugs, alcohol blocks, neurolytics, postsurgical)
Immune-mediated/inflammatory
Polyneuritis equi
Infection of adjacent tissues
Neoplasia (primary: peripheral nerve sheath tumor, secondary: lymphoma)
Neuroma (postsurgical)
Neuropathic pain
Recurrent laryngeal neuropathy
Toxic
Arsenic
Lead
Ionophores (monensin, salynomycin, narasin)
Mercury
Organophosphates (delayed motor polyneuropathy)
Polyneuropathy of Scandinavian horses (presumed toxic)
"Stringhalt" (distal axonopathy)
Idiopathic, toxic, traumatic
Presynaptic
Reduced Acetylcholine (ACh) Release
Botulism (*C. botulinum* toxins A, B, C, D)
Drugs (e.g., aminoglycosides, tetracyclines, antiarrhythmics, anticholinesterases)
Hypermagnesemia
Hypocalcemia
Tick paralysis (females of *Ixodes holocyclus* [Australia], *Dermacentor* sp. [USA])
Increased ACh Release
Envenomations (e.g., black widow spider [*Latrodectus matans,* alpha-latrotoxin])
Hypomagnesemia
Hyperkalemic periodic paralysis
Myasthenia gravis–like
Myotonic disorders
Tick myotonia *(Otobius megnini)*, myotonia congenital/dystrophica
Snake envenomation (rare: coral snake, rattlesnake)
Myopathy
Non-Inflammatory
Multiple subcategories
Drugs (long-term steroids)
Inflammatory
Immune-mediated
Infectious
Paraneoplastic

VIRAL ENCEPHALITIS

Monica Aleman

A wide variety of viruses can affect the CNS of horses, resulting in encephalitis, meningoencephalitis, encephalomyelitis, or myeloencephalitis. Table 11.12 depicts reported viral diseases that affect the CNS in equids. Viruses can spread to the CNS from distal sites through adjacent tissues, nerves or via blood. To spread from blood, viruses must cross the blood-brain barrier, which can occur via infected leukocytes or by active or passive transport through the vascular wall. Viruses can infect neurons and glial cells (astrocytes, microglia, oligodendrocytes). Lytic infections to neurons are caused by togaviruses, flaviviruses, herpesviruses, and others leading to encephalitis or encephalomyelitis. Lytic infections are characterized by neuronal necrosis, neuronophagia, perivascular inflammation. In contrast to noncytocidal infection of neurons as in the case of rabies virus on which little to no inflammation is observed. EHV-1 can produce infection of the respiratory system; reproductive tract; fetus or embryo causing abortion or fetal or early neonatal

death; and the CNS. The type of nucleic acid and genomic structure are used to classify viruses into RNA or DNA viruses (Table 11.13). This section describes disease resulting from infections with members of the Togaviridae family of viruses (eastern, western, and Venezuelan encephalitis) and CNS infections caused by viruses from miscellaneous viral families.[506]

TABLE **11.12 Viruses That Affect the Central Nervous System in Equids**

BORNAVIRIDAE
Borna disease virus[a]

BUNYAVIRIDAE
Shuni virus
California serogroup viruses[a]
Bunyamwera virus[a]

FLAVIVIRIDAE

Mosquito-borne[a]
Japanese encephalitis virus
St. Louis encephalitis virus
Murray Valley encephalitis virus
West Nile virus
Kunjin virus

Tick-borne[a]
Louping ill virus
Powassan virus
Tick-borne encephalitis virus

HERPESVIRIDAE
Equine herpesvirus 1 (D_{752} and N_{752})

PARAMYXOVIRIDAE
Nipah virus[a]

REOVIRIDAE
Equine encephalosis virus
Peruvian horse sickness virus
Elsey virus
Yunnan orbivirus

RETROVIRIDAE
Equine infectious anemia virus

RHABDOVIRIDAE
Rabies virus[a]
Bat lyssaviruses[a]

TOGAVIRIDAE
Equine alphaviruses[a]
- Eastern equine encephalomyelitis (EEE) virus
- Western equine encephalomyelitis (WEE) virus
- Venezuelan equine encephalomyelitis (VEE) virus
- Ross River virus
- Getah virus

[a]May also affect humans.

Togaviral Encephalitis

Togaviruses are small lipid- and protein-enveloped RNA viruses. Viruses in the family Togaviridae include the genera *Alphavirus* and *Rubivirus. Rubivirus* exclusively affects humans (rubella virus). The alphavirus equine encephalitides are mosquito-borne infections that cause neurologic disease and death in humans and horses (Table 11.14). Togaviral encephalitis can cause severe encephalitis and has been commonly referred to as sleeping sickness due to the degree of obtundation that affected horses develop. The incubation period is variable and depends on virus type but usually ranges from 5 to 14 days.

EEE, WEE, and Venezuelan (VEE) equine encephalitis viruses are the most frequently isolated alphaviruses from epidemics of encephalitis in horses and human beings in the Western Hemisphere (see Table 11.14). The first recorded epidemic of EEE in North America likely occurred in Massachusetts in 1831.[507] The viruses have been isolated from infected mosquitoes, horses, humans, birds, and rodents and are transmitted by hematophagous arthropods.[508]

Epizootiology

The alphaviruses EEE and WEE are specific and discrete togaviral species; North and South American antigenic variants of EEE exist.[509] WEE is a recombinant between an EEE-like virus and a Sindbis-like virus.[510] Two antigenic subtypes of WEE virus exist: (1) WEE and (2) Highlands J viruses. The Highlands J virus causes most infections that occur east of the Mississippi River. Although these alphaviruses have different antigenic properties, there is extensive overlap in geographic distribution.[506,511] VEE virus has six distinct subtypes designated by Roman numerals I through VI. Subtypes IAB, IC, and IE have been responsible for large outbreaks of encephalitis in horses in the Western Hemisphere in the past 20 years. Subtypes ID and IF have been reported in Central America and Brazil, respectively; type II (Everglades) virus in Florida; and types II, IV, V, and VI viruses.[512-515] Encephalitis has had a high morbidity and mortality in the United States.[516-519] Horses immunized with strains of virus isolated from infected horses from the East or West were protected when vaccinated with attenuated virus.[519-522] Table 11.15 summarizes the most common types of alphaviruses associated with encephalitic disease in horses and their geographic distribution.

Distribution

Disease associated with EEE, WEE, and VEE is restricted to the Western Hemisphere and ranges from temperate to desert climates. Many seropositive horses do not have recognizable clinical disease.[522-549] The presence of EEE virus has

TABLE **11.13 Properties of Viral Families**

Family	Genome	Configuration	Size (kb)	Envelope	Morphology	Virion size (nM)
Herpesviridae	dsDNA	1 linear	125–240	+	Isometric	150
Reoviridae	dsRNA	10–12 segments	19–32	–	Isometric	60–80
Rhabdoviridae	NssRNA	1 – segment	11–15	+	Bullet-shaped	100–430 × 45–100
Bornaviridae	NssRNA	1 – segment	9	+	Spherical	80–100
Bunyaviridae	NssRNA	3 – or +/– segments	11–19	+	Spherical	80–120
Togaviridae	ssRNA	1 + segment	10–12	+	Spherical	70
Flaviviridae	ssRNA	1 – circular	3–4	+	Spherical	40–60

TABLE **11.14 A Summary of the Major Togaviral Equine Encephalitides**[a]

Virus	Major Disease Vector	Zoonotic Potential	Amplification from Horses	Disease Spread	Viremia	Equine Mortality
EEE	*Aedes* spp.	Unlikely	Unlikely	Vector	Low	75%–100%
WEE	*Culex tarsalis*	Unlikely	Unlikely	Vector ± secretions	Low	20%–50%
VEE	*Culex melanconium, Aedes* spp. *Phosphora* spp.	Occurs	Occurs	Vector ± secretions	High	40%–80%

EEE, Eastern equine encephalitis virus; *WEE,* western equine encephalitis virus; *VEE,* Venezuelan equine encephalitis virus.
[a]The statements made are generalizations, and some degree of variation occurs.

been reported from Canada to Central and South America. In the United States disease has been reported primarily in the southeastern states, some western states, and east of the Mississippi River. Other parts of the world include the Philippines and some areas in Europe.[550,551] Historically large outbreaks of WEE have been described in horses in California and other western states, and uncommonly in eastern states.[552] Venezuelan equine encephalitis is a very important human and veterinary pathogen in the Western Hemisphere that can cause large outbreaks of disease in human beings and horses over large geographic areas. Disease due to VEE virus has been reported in Mexico, Venezuela, Colombia, Trinidad, French Guiana, Peru, and Brazil.

Epidemic

For outbreaks to occur several factors play a role such as presence of reservoir animals, intermediate hosts, insect vectors, and susceptible horse and human populations.[450,553] Prediction of outbreaks has been attempted but without success,[507,524,546] which indicates that other unknown factors may exist. In the environment, Togaviridae persist by asymptomatically infecting wild animals (sylvatic hosts) such as birds, small mammals, and reptiles.[554] The viruses may survive during the winter or nonvector season in sylvatic populations.[524,555]

Vectors

Specificity of the viruses for particular vectors occurs. The vectors for EEE include *Culiseta melanura, Aedes* spp., and *C. melanura.*[522,539,556,557] *Aedes* spp. appear to be more important in epizootics and epidemics. *Culex tarsalis* is the primary vector that maintains WEE virus. *Dermacentor andersoni* ticks,[558,559] *Triatoma sanguisuga* (assassin bug),[560,561] and the cliff swallow bug *(Oeciacus vicarius)*[558,562] may also be involved as vectors or overwintering reservoirs for WEE. Several species of mosquitoes from at least 11 genera have been determined to be naturally infected with epidemic strains of VEE virus, including *Culex melanconium,* and *Mansonia, Aedes,* and *Psorophora* spp.[526,527,534,535] Ticks may also be capable of virus transmission. Although less likely, EEE and VEE may spread by nasal secretions.[526,527,561,563] Vectors transmit viral particles between sylvatic hosts when taking a blood meal. Mosquitoes remain infected for life.[557,564] Seasonal incidence depends on peak of seasonal vectors. The highest number of cases occurs between June and November in temperate climates. If vector season is longer such as in warmer climates, disease outbreaks last longer.[565]

Outbreaks of disease because of EEE have been described in emus, ostriches, and swine, with isolated cases in cattle, sheep, and nondomestic ungulates.[566,567] Pigs may also be affected by VEE.[567] The signs of disease in these species are similar but are milder than disease in horses for the respective viruses. Burros and mules may contract all three diseases, and the disease is as severe as that identified in horses.[568] Experimental models of infection have included mice, hamsters, guinea pigs, and rhesus monkeys.[507]

TABLE **11.15 Alphavirus Types and Geographic Distributions**

Eastern Equine Encephalomyelitis Virus
- Multiple lineages
  - Lineage 1, subtype I in North America
  - Lineage 2, subtypes II to IV in South America
- Horse is a dead-end host

Western Equine Encephalomyelitis Virus
- North American and South American lineages with multiple variants
- Primarily found in western United States
- Highlands J variant in eastern North America
- Horse is a dead-end host

Venezuelan Equine Encephalomyelitis Virus
- Genetically diverse with six primary subtypes (I–VI)
- Epidemic or "epizootic" subtypes (IAB, IC, IE) in Central America and South America
- Endemic subtype II (Everglades) in Florida has low pathogenicity
- Horse is amplifying host

Pathogenesis

Alphaviruses can replicate to very high titers and cause severe cytopathic changes in many cells of vertebrates.[506] In mammalians and birds, infection causes complete shutdown of host-cell protein and nucleic acid synthesis. In mosquitoes, cell division can continue, with cells becoming persistently infected and continuously shedding virus.[506] After viruses are inoculated, they multiply in muscle, enter the lymphatic circulation, and localize in lymph nodes. Viruses replicate in macrophages and neutrophils and subsequently are shed in small numbers. Many of the viral particles are cleared at this time. If clearance mechanisms are successful, no further clinical signs develop. Neutralizing antibodies still will be produced. Several mechanisms of viral immunologic avoidance exist and include erythrocyte and leukocyte absorption. If viral elimination is not complete, the remaining viruses infect endothelial cells and concentrate in highly vascular organs such as the liver and spleen. Viral replication in these tissues subsequently is associated with circulating virus. The second viremic period often is associated with early clinical signs of disease. Infection of the CNS occurs within 3 to 5 days.[514,527,569,570]

Clinical Signs

Alphavirus clinical infection in humans is associated with insect vector contact and usually involves old or young persons.[507] Morbidity and mortality rates are virus specific.[571] Clinical signs in human beings include acute fulminant encephalitis, headache, altered consciousness, and seizures. The mortality rate in human patients varies depending on specific virus.[571] Human beings and horses are terminal hosts for WEE. A transient viremia occurs in horses in the acute stages of disease.

EEE virus is probably the most virulent of the alphaviruses with a case fatality in humans of 50% to 70%.[508,571] In humans, the virus causes fever, muscle pain, headache, disorientation, stupor, vomiting, respiratory signs, leukocytosis, hematuria, seizures, and coma.[508,571] MRI has shown lesions in the basal ganglia and thalamus.[508] Horses could be a natural amplifier of EEE, and infection in equines precedes human cases by 2 to 5 weeks.[572,573] Thus horses are sentinels for human beings in a given area. In animals, acute clinical signs of EEE and WEE include mild fever to severe pyrexia, anorexia, and stiffness. Viremia occurs during this period. The incubation period (1–3 weeks) is often shorter with EEE than WEE. Early signs of the disease include fever and mild obtundation. With EEE, progression is more common. Once nervous signs develop, the viremia is past, and animals are unlikely to be able to amplify the disease. Cerebrothalamic signs such as compulsive walking, altered behavior, hyperesthesia, excitability, and obtundation occur. Cranial nerve dysfunction can also occur, with signs such as vestibular, facial, pharyngeal, laryngeal, and tongue dysfunction. Death often is preceded by recumbency for 1 to 7 days. Comatose animals rarely survive. If animals survive, slow gradual improvement is observed over weeks to months.[574-577] Clinical signs are more profound in unvaccinated animals.[573,576]

VEE virus circulates in enzootic cycles between rodent host and mosquito vector.[578] Clinical signs with VEE vary depending on virus subtype from mild to severe to fatal. In general, severe encephalitis in humans infected with VEE virus is less common than with EEE and WEE virus infection. Neurologic disease in humans can be detected in up to 14% of cases, mainly children, and fatality rate is about 1%.[508,571] VEE viral infections in human epidemics and equine epizootics have been associated with subtypes ID and IE, which are less virulent for horses. In contrast, subtypes IAB and IC are highly pathogenic to horses with case fatality of 20% to 80%. Epidemic strains are associated with severe pyrexia and leukopenia.[514,570] Neurologic signs occur at approximately 4 days after infection and include severe obtundation and recumbency. Diarrhea can occur before neurologic deficits. Other associated signs include abortion, oral ulceration, pulmonary hemorrhage, and epistaxis.[526,527] Horses with VEE have sufficient circulating viral concentrations that act as amplifiers of disease.[534,579] Ocular and nasal secretions from infected horses contain high concentrations of VEE virus.[526,527] Infection via entry through the respiratory tract may occur by direct contact with infected animals. Equine and human survivors of VEE infection and clinical disease may develop chronic relapsing viremias and serve as chronic disease amplifiers.[566] Clinical signs in human beings include fever, headache, myalgia, and pharyngitis.

Accidental cases in humans have occurred in laboratory conditions.[508] Viral load in infected tissue could be high, therefore, precautions must be taken when performing necropsies or handling tissue from diseased animals. Alphavirus infections are reportable diseases to state health officials.[507,579] EEE virus has poor ability to replicate in lymphoid tissues, whereas VEE does so efficiently. Therefore EEE virus can avoid IFN-α and IFN-β induction in vivo, which may allow EEE to evade the host's innate immune responses and thereby enhance neurovirulence. Inhibition of genome translation restricts EEE infectivity for myeloid but not mesenchymal cells. This factor likely contributes to the observed differences in disease cause and presentation.[580]

Diagnosis

The diagnosis of equine encephalitides can be made by viral isolation or detection through virus-specific antibody response, viral nucleic acid, and presence of viral antigen in tissue samples. Clinically, a presumptive diagnosis is based on clinical findings of acute onset of altered behavior, obtundation, preceded by fever; season of the year, presence of vectors and hosts. IgM antibody-capture enzyme-linked immunosorbent assay can detect recently infected horses and humans.[508] These antibodies are not produced in response to vaccine.[581] The antibodies disappear by 21 days after infection.[582] The assay for confirming acute VEE infection should be used when one cannot collect convalescent serum samples. Detection of IgG antibodies is not predictive of recent infection and may be confused with vaccine-induced antibodies.[506] Viral antibodies are commonly present within 24 hours after the initial viremia, and their presence often precedes clinical encephalitis.[551] The concentration of antibodies increases rapidly and then decreases over 6 months.[582] An initial sample often is taken when encephalitic signs are present, which may be after titers have peaked. Therefore a second sample possibly may have a decreased titer compared with the initial sample. Hemagglutination-inhibition and neutralization assay can also detect within a few days after onset of infection.[578] Fourfold antibody rise in IgG antibody 10 days to 2 weeks apart supports infection. Complement fixation has also been used for the diagnosis of alphaviruses.[583] The virus can also be isolated from vertebrates and mosquitos. Immunohistochemistry of brain tissue can be done for the detection of virus. More recently, RT-PCR has been used for the diagnosis.[584] Viral cultures are unlikely to be fruitful, except in the case of acute VEE. One may isolate the virus from CSF of horses with acute infections.[527] The usefulness of CSF viral titers in light of a negative viral isolation is questionable. Fluorescent antibody, ELISA, and viral isolation are useful in identifying virus in brain tissue.[585,586]

Colostral antibodies may interfere with diagnosis in foals. The antibody titers to VEE, WEE, and EEE viruses in the sera of 2- to 8-day-old foals are similar to those of dams. The serum half-life of maternal antibodies in foals is approximately 20 days.[587]

Clinical Pathology

The CSF changes associated with togaviral infections are similar to those of other viral encephalitides and include most commonly lymphocytic pleocytosis (50–400 mononuclear cells/μL) and protein concentration (100–200 mg/dL).

Necropsy Findings

With any of the alphaviral equine encephalitides, sufficient viral particles for infection may be present in CNS and other tissues, and one should take precautions when performing a necropsy examination on suspect cases. The brain and spinal cord often have a normal gross appearance. In some cases

TABLE **11.16** **Vaccination Protocol for EEE/WEE Recommended by the American Association of Equine Practitioners Guidelines 2015**

Horse Group	Unvaccinated or Unknown (EEE/WEE)	Seasonal Exposure	Year-Round Exposure
Adults	2 doses 4–6 weeks apart	Annual before vector season	Every 6 months
Pregnant mares	2 doses 4–6 weeks apart	4–6 weeks before foaling or before vector season	
Foals (vaccinated mare)	3 doses at 4–6 months of age with a 4–6-week interval between 1st and 2nd vaccination; if high risk start at 2–3 months of age	Third dose at 10–12 months of age or before vector season	
Foals (unvaccinated mare)	3 doses with a 4-week interval	4th dose at 10–12 months of age or before vector season	

vascular congestion and discoloration of the CNS is evident. Histologic findings include nonseptic mononuclear cell and neutrophilic inflammation of the entire brain.[526,588-590] Severe lesions are evident in the cerebral cortex, thalamus, and hypothalamus. Specific lesions include significant perivascular cuffing with mononuclear and neutrophil cell infiltration, gliosis, neuronal degeneration, and mononuclear cell meningeal inflammation. With VEE, liquefactive necrosis and hemorrhage of the cerebral cortex, atrophy of the pancreatic acinar cells, and hyperplasia of the pancreatic duct cells commonly occur.[589] Immunohistochemistry can be diagnostic on necropsy samples.[591,592]

Differential Diagnosis

The differential diagnosis for EEE, WEE, and VEE should include other acute conditions associated with diffuse or multifocal neurologic deficits such as other viral diseases including other togaviral encephalitides, trauma, hepatoencephalopathy, rabies, WNV, and leukoencephalomalacia. Others such as bacterial meningoencephalitis, EPM, and verminous encephalitis should also be considered.

Treatment

There is no specific treatment for alphavirus infection. However, appropriate management includes supportive therapy, treatment of other concurrent disease, and prevention of complications. Supportive therapy includes meeting needs of hydration, nutrition, urination, defecation, and bedding. NSAIDs (phenylbutazone, 2–4 mg/kg every 12 hours; flunixin meglumine, 1 mg/kg every 12–24 hours) control pyrexia, inflammation, and discomfort. DMSO given at 1 g/kg intravenously in a 20% solution may be useful in controlling inflammation. The use of corticosteroids is controversial because beneficial effects are short term, and the risk of developing secondary bacterial infections increases. One might control seizures as described in this chapter previously. One should monitor hydration and administer balanced isotonic fluid solutions orally or intravenously as needed. Other supportive care should include dietary supplementation and administration of laxatives to minimize the risk of gastrointestinal impaction. If anorexia persists for more than 48 hours, enteral or parenteral supplementation should be used. For the short term, pelleted feeds may be put into suspension for oral administration. Protection from self-induced trauma may require protective limb wraps and head protection. If the horse is recumbent, one should attempt to provide support in a sling, and all animals should be bedded heavily.

Prognosis

Complete recoveries from the neurologic deficits associated with these viruses are reported, but they are rare.[591] Animals that have recovered from EEE often have residual neurologic deficits that commonly include ataxia, obtundation, and abnormal behavior. Neurologic sequelae are similar but less common in horses that recover from WEE. For horses that develop neurologic disease, the mortality rate for EEE is between 75% and 100%; for WEE, 20% and 50%; and for VEE, 40% and 80%.[526,551] If horses recover from any of the diseases, they seem to be protected variably for up to 2 years after infection. One would wisely assume that infection affords no protection.

Prevention

Prevention of alphavirus encephalitis should aim at reducing the concentration of insect vectors and implementing vaccination programs.[543-545,568,593-596] EEE, WEE, VEE are included as core vaccines in endemic areas as defined by the American Veterinary Medical Association. Current commercial vaccines are formalin inactivated viruses (Table 11.16). Significant increases in antibody titer occur at 3 days after vaccination.[526,527,595-598] One should vaccinate susceptible horse populations with monovalent, divalent, or trivalent vaccines containing EEE, WEE, or VEE. Administration of trivalent vaccines increases specific antibody production to all viruses. Some cross-protection exists between EEE and WEE and between EEE and VEE, but none exists between WEE and VEE.[587,599,600] If one is to give VEE vaccine, simultaneous administration of all three vaccines is recommended.[601-603] The response to VEE vaccination alone is poorer in horses previously vaccinated against WEE and EEE.[597,598,602,603] VEE vaccination does not seem to interfere with responses to EEE or WEE vaccination.[604] One should complete annual vaccinations in late spring or several months before the beginning of the encephalitis season. Adequate titers appear to last for 6 to 8 months. In areas in which the mosquito problem is prolonged or continuous, biannual or triannual vaccination is suggested. Vaccination of susceptible horses in the face of an outbreak is recommended. If vaccinated horses develop disease, the affected individuals are often young or old. Vaccination of mares 1 month before foaling enhances colostral antibody concentrations. Antibody concentrations in foals born to immunized mares appear by 3 hours after colostrum is fed and persist for 6 to 7 months.[592] Vaccination may begin at any age, but if they are vaccinated early, one should revaccinate foals at 6 months

and 1 year to ensure adequate protection. Foals respond to vaccination with VEE in utero.[605] A prospective study to determine the serologic response of previously vaccinated horses to revaccination against EEE and WEE identified that horses responded variably to each antigen, and some horses had low or undetectable antibodies 6 months after vaccination. Some horses did not develop increasing titers to EEE or WEE despite recent vaccination. Geometric mean titers peaked 2 weeks after revaccination and were significantly increased from before revaccination.[606,607]

Novel vaccines have been developed experimentally, such as modified live, chimeric gene-deleted live mutants, DNA, and adenovirus-vectored alphavirus vaccines.[581] The results have appeared promising. The development in new vaccines has been the result of concerns involved in the production of inactivated vaccines. These concerns mainly involve the manipulation of large quantities of viral particles under biosafety level 3 containment laboratories, which poses a possible risk of infection for operators producing vaccines. Further, these vaccines have not been able to induce long-lasting immunity.

Owners should use insecticides and repellents when possible and practical, should eliminate standing water, and in endemic areas or during an outbreak should implement environmental insecticide application and should screen stalls. Horses with VEE can be persistently viremic and should be quarantined for 3 weeks after complete recovery. Cases of VEE must be reported to regulatory authorities in the United States. Public health officials may institute other measures of disease control.

Flavivirus Encephalitis

Monica Aleman

The family *Flaviviridae* comprises three genera: *Flavivirus, Pestivirus,* and *Hepacivirus.*[608-620] Although these viruses have similar genomic organization, they are genetically and biologically different. At least half of the members of *Flaviviridae* are zoonotic. The genus *Flavivirus* has at least 70 viruses. Of importance in equine species are Japanese encephalitis, St. Louis encephalitis, West Nile, Powassan, and louping ill viruses. Arthropod-borne (e.g., *Culex* spp.) diseases include Japanese encephalitis, St. Louis encephalitis, and West Nile virus infection. Powassan and louping ill viruses are transmitted as tick-borne (*Ixodes* spp.) diseases.[608-614] The mosquito- and tick-borne flaviviruses are maintained in nature in arthropod-vertebrate-arthropod cycles, whereas the nonarthropod-borne viruses are transmitted directly among bats or rodents.[608]

Serocomplex Japanese encephalitis viruses include Japanese encephalitis, St. Louis encephalitis, Murray Valley encephalitis, Kunjin virus, and West Nile virus. These group of viruses are vector borne, with transmission occurring to avian and mammalian hosts from blood meal–seeking mosquitoes.[621] Virus is either maintained or cycled between vectors, and biologic amplification occurs within the vector species. Vertical transmission within vectors must occur for maintenance of virus within a geographic area.[621] The distribution of Japanese encephalitis occurs in most countries of southeast Asia and has extended to India, Pacific islands, and northern Australia. Japanese encephalitis virus causes between 30,000 and 50,000 human encephalitis cases annually worldwide, with endemic areas including China, the southeast region of the Russian Federation, South and Southeast Asia, and Australia. Exact numbers of horses with clinical Japanese encephalitis are difficult to ascertain; however, reports exist of Japanese encephalitis isolation from horses in Taiwan, China, Pakistan, and Australia in the literature since the 1980s. Outbreaks in horses have also been reported in India, Nepal, the Philippines, Sri Lanka, and northern Thailand. Seroconversion of young horses over their first year of exposure in Hong Kong is as high as 63% in some locales. The disease has a high mortality rate of 10% to 40%, with 40% to 70% of survivors having permanent neurologic deficits.[621] Japanese encephalitis should be suspected in horses with compatible clinical signs that reside in an area of virus activity. Diagnostic confirmatory tests include serologic assays such as neutralizing, complement fixation, hemagglutination inhibition, and ELISA tests.[621] All single sera testing, including IgM assays, must be interpreted with caution in horses from areas with other endemic flaviviruses. In fatal Japanese encephalitis cases, viral isolation, PCR assays, and immunohistochemistry for detection of virus in CNS tissues are confirmatory.

St. Louis encephalitis virus is associated most commonly with encephalitis in human beings and may rarely be involved in equine disease. Experimental inoculation in horses produces viremia but no clinical signs. Neutralizing antibody is often present. *Culex pipiens* and *C. tarsalis* are the major vectors. Wild birds seem to be the primary reservoir.[608,613]

Experimental infection with Murray Valley virus results in transient pyrexia, myalgia, and ataxia. Horses are unlikely to be efficient amplifiers of this virus.[610-612] In Australia, the virus is more commonly a disease of human beings. An epidemic in human beings was associated with significant titers in horses. Some horses with clinical signs, significant titers, and histologic evidence of viral encephalitis were identified.[611,612]

Powassan virus has been associated with nonsuppurative, focal necrotizing meningoencephalitis in horses.[609] Antibodies for Powassan virus commonly are identified in Ontario and the eastern United States. *Ixodes cookei, I. marxi,* and *Dermacentor andersoni* appear to be important vectors, with snowshoe hares and striped skunks as major reservoirs. Zoonoses occur after bites by infected ticks. Approximately 13% of horses sampled across Ontario in 1983 were serologically positive for the virus.[609] Experimental infection with Powassan virus strain M794 in horses was associated with neurologic deficits within 8 days. A nonsuppurative encephalomyelitis, neuronal necrosis, and focal parenchymal necrosis occur. Signs include tremors of the head and neck, ptyalism, myalgia, ataxia, and recumbency. No clinical signs were identified in inoculated rabbits, but widespread encephalitis characterized by lymphoid perivascular cuffing, lymphocytic meningitis, and lymphocytic choroiditis occurred.[609]

The other two genera have viruses of veterinary and human importance, with the genus *Pestivirus* containing the ubiquitous bovine diarrhea virus (BVD) and *Hepacivirus* containing the human pathogen hepatitis C virus (HCV). There was no known *Pestivirus* affecting the nervous system in horses until recently when a *Hepacivirus* was implicated as causing central nervous system disease in horses as previously mentioned in this chapter.[622]

West Nile Virus

Monica Aleman

West Nile virus (WNV) is an important cause of neurologic disease in humans and horses worldwide.[506,620,623] The virus is neurotropic, and access to the brain is proposed to

be hematogenous and transneural through the disruption of the blood-brain barrier and retrograde axonal transport. Historically, WNV was first recognized in humans in Uganda in 1937 and the Middle East as a febrile illness. Since then, the infection has spread globally to other parts of Africa, the Middle East, Asia, Australia, Eastern Europe, and North, Central, and South America.[506,623] The first reports of encephalitis in humans from North America occurred in August 1999 in Queens, New York.[506] These patients developed fever, altered mental status, flaccid paralysis causing respiratory failure, and weakness. All patients were over 50 years old, spent time outdoors, and lived close to mosquito-breeding sites. These patients were positive for IgM on ELISA testing on CSF. There are two major genetic lineages (1 and 2) of West Nile virus. Kunjin virus is a variant of lineage 1 WNV. WNV lineage in North America is 1. Before 1999 veterinarians, trainers, and horse owners on the American continent had little familiarity with flaviviruses. Since that time, several thousands of equine cases have been reported to have disease, with an estimated 30% to 40% case fatality rate in the United States. Horses represent 96.9% of all reported nonhuman mammalian cases of WNV.

Etiology. All flaviviridae are positive-sense single-stranded RNA viruses measuring approximately 50 nm.[506] The virions are spherical and enveloped with the C protein, making up a nucleocapsid of about 25 nm. Electron microscopy reveals an icosahedral symmetry of the envelope and capsid of these viruses. An approximately 11-kb genome contains a single open reading frame that is translated in its entirety and cleaved into 10 viral proteins by both cell and viral proteases.[506] Three structural and seven nonstructural proteins exist; the structural proteins include the capsid (C), premembrane (prM) and membrane (M), and envelop (E) proteins. The nonstructural (NS) proteins, numbered 1 through 5, are cleaved after translation and are required for viral replication and assembly.

The final M protein and the E protein are important for virulence.[506,620,623] The M protein is formed from a precursor protein (prM protein) that is modified as immature virions are secreted through the Golgi network of the cell. The E protein is only secreted in its native conformation through association with the prM protein. The E protein is the immunodominant viral protein and is important in receptor ligand binding and fusion to host cells.

Epidemiology. WNV is generally transmitted via bite from infected mosquitoes. There are several species of mosquito capable of acquiring the virus after feeding in infected vertebrate amplifying hosts (most common birds), but the most important in the United States include *Culex* genus *(C. pipiens, C. restuans, C. salinarius, C. tarsalis)*.[506,620,624-626] WNV penetrates the mosquito gut and replicates in multiple tissues including nervous system and salivary glands, infecting the mosquito for life. The virus is transmitted from females transovarially to offspring, continuing its persistence in the environment. Mosquitoes transmit WNV to over 300 species of birds and at least 30 vertebrates.[627] Birds include crows, magpies, jays, house sparrows, finches, and grackles. Vertebrates include humans, horses, mules, donkeys, camelids, pigs, dogs, cats, birds, and harbor seal, among others.[506,620] Seropositive free-ranging mammals include the big brown bat, little brown bat, eastern chipmunk, eastern gray squirrel, eastern striped skunk, white-tailed deer, and the brown bear.[506,620,627] Neurologic disease has been confirmed as WNV in gray squirrels and fox squirrels. Alligators can have an extremely high titer of viremia and may be an important reservoir for WNV in the Southeast. Reports exist of both farmed and free-ranging alligators with neurologic signs from which WNV has been isolated. In farm-raised alligators, cloacal shedding of virus has been demonstrated with oral infection likely.

The virus may also be transmitted through contaminated blood transfusion or organ transplantation if donors are viremic. Vertical transmission through placenta and milk has been demonstrated in people.[628-630]

The outbreaks are seasonal and reflect mosquito activity. In the northeastern United States, more than half of the WNV-positive mosquito pools are *C. pipiens.*[506] In the West, populations of the highly efficient *C. tarsalis* constitute the majority of positive pools, with *C. pipiens* the next most commonly found *Culex* spp.[506,624,626] In the Southeast, *C. quinquefasciatus* and *C. nigripalpus* have the highest WNV infection rates. In the Southwest, epidemics are most commonly associated with positive mosquito pools of *C. quinquefasciatus, C. tarsalis,* and *C. pipiens.*

A reservoir host (mostly birds) is one in which a pathogen is amplified in vivo so that it can be transmitted to a vector species.[631] Horses and human beings are considered dead-end (nonreservoir) hosts for WNV.[631] To date, more than 300 species of birds have been reported WNV-positive in the United States. High levels of viral amplification occur in many bird species.

Because transmission is via vectors, transmission could be seasonal in temperate regions and year-round in subtropical regions. Intense virus activity in the United States begins in July, with a peak incidence in September and October.[506,620] A drop in ambient temperature with soft frost usually results in a rapid decrease in reporting activity.

Older people appear more susceptible to neuroinvasive disease from WNV. Similarly, older horses appeared to be more susceptible.[632] Although men are more frequently affected with neuroinvasive disease, there appears to be no breed or gender predilection in horses. In one study of horses with WNV encephalomyelitis, female horses were 2.9 times more likely to die than male horses with neurologic signs.[633]

Clinical Signs. Neurologic disease has been reported in horses naturally and experimentally infected.[634-639] Most horses seroconvert without clinical disease after infection with WNV. However, approximately 8% to 10% of infected naïve horses develop clinical disease with neurologic signs.[623] Neurologic signs usually develop 5 to 22 days post infection. The most common clinical signs are weakness (94–100%), ataxia (72%), altered mentation (67%), fever early in disease (65%), fasciculations (61%), cranial nerve deficits (44%), and recumbency (30%). Other common signs include anorexia (47%) and bruxism (20%).[634,636,637]

Alterations in behavior such as hyperexcitability, apprehension, aggression, and compliance occur mainly due to thalamic involvement. Interspersed during periods of hyperexcitability, some horses appear to have abnormalities of sudden sleeplike activity resembling narcolepsy. This can occur to the point of cataplexy, and horses may partially or completely collapse for a short period of time. Because WNV affects predominantly the brainstem, signs such as abnormal state of consciousness, mainly obtundation due to injury to the reticular formation, is common. In some horses a change

of mentation is persistent, and a state of nonresponsiveness resembling coma results. Both gray and white matter can be infected. In the brain, gray matter of the midbrain and hindbrain is affected, resulting in cranial nerve deficits (mainly VII, IX, and XII) along with altered mentation.[4,639] Injury to these cranial nerves results for short periods of time in weakness of the tongue, muzzle deviation, and head tilt. Dysphagia has been reported with choke as a sequela. A cauda equina syndrome consisting of stranguria and rectal impaction is infrequently reported. Involvement of lower motor neurons in the spinal cord results in weakness, gait abnormalities, and proprioceptive deficits.

WNV can affect sensory and motor tracts within the spinal cord, and the signs could be diffuse or multifocal symmetric or asymmetric. However, motor and sensory deficits could also be caused by involvement of relay tracts and centers within the brain (thalamus and basal nuclei).[635-637,639-642] These two clinical signs are likely a reflection of brain and spinal cord disease through direct infection of the spinal cord, interruption of motor tracts in the hindbrain, and loss of fine motor control through infection of the large nuclei of the thalamus and the basal nuclei. General proprioceptive ataxia, paresis, weakness, and proprioceptive deficits result from lesions in the spinal cord. Many horses might have difficulty standing primarily because of profound weakness.

Involuntary skin and muscle fasciculations, tremors, and hyperesthesia, extremely common in this disease, likely result from loss of fine motor control, which is regulated mainly by the basal nuclei.[643,644] Movement disorders are detected with flavivirus infection in a long-term Parkinson-like syndrome in rats and experimental infection in monkeys.[644] Fine and coarse fasciculations of the muscles of the face and neck are very common. Fasciculations can be quite severe and involve all four limbs and trunk, affecting normal activities such as walking, eating, and interactions with handlers and other horses. The fasciculations are most notable at the muzzle and eyelids. Eyelid activity during this period is enhanced with light, and at times it appears that horses are quite photophobic.

Horses develop clinical signs when infected with the neurally invasive lineage type I WNV, whereas infection with the African lineage type II viruses is universally subclinical in nature. A mild to moderate increase in rectal temperature (38.6°C–39.4°C), anorexia, and depression are the most common initial systemic signs.[634] Abdominal pain is not an uncommon initial presenting complaint.[631,633,634,636] Gait abnormalities, including overt lameness or dragging of a limb before development of an obvious neurologic syndrome, have also been reported.

Overall, the combination, severity, and duration of clinical signs can be highly variable. After initial signs abate, about 30% of horses experience a recrudescence in clinical signs within the first 7 to 10 days of apparent recovery. Irrespective of recrudescence, about 30% of affected horses progress to complete paralysis of one or more limbs overall. Most of these horses are euthanized for humane reasons or die spontaneously.

Many horses will improve within 3 to 7 days of displaying clinical signs. If the horse demonstrates significant improvement, full recovery within 1 to 6 months can be expected in 90% of patients. Residual weakness and ataxia appear to be common, with long-term loss of the use of one or more limbs infrequently described. Mild to moderate persistent fatigue on exercise has been observed.

Diagnosis. CBC and serum biochemistry profiles are usually within reference values. Occasionally, creatine kinase might be elevated due to trauma and/or recumbency. A frequent finding is hyponatremia, which has also been described in human beings with encephalitis, potentially caused by inappropriate release of antidiuretic hormone.[645,646] CSF analysis could be normal or reflect lymphocytic pleocytosis with elevated protein. In contrast, the most numerous cells in the CSF of horses with EEE are neutrophils, especially during the initial stages of disease.[647]

Differential diagnosis should include alphavirus encephalitis, rabies, EPM, EHV-1, botulism, and verminous meningoencephalomyelitis (e.g., *Halicephalobus gingivalis, Setaria* spp., *Strongylus vulgaris*). Noninfectious causes to consider include hypocalcemia, tremorgenic toxicities, hepatoencephalopathy, and leukoencephalomalacia.

Confirmation of WNV infection with encephalitis in horses begins with assessment of whether or not a horse meets the case definition based on clinical signs, as well as whether or not the horse resides in an area in which WNV has been confirmed in the current calendar year in mosquitoes, birds, human beings, or horses.[641] Serologic testing developed by the National Veterinary Services Laboratory (NVSL) is based on detection of the IgM antibody response that uniformly occurs in acutely infected horses. The preferred test is an IgM capture ELISA (MAC-ELISA).[648] Horses develop a very intense IgM response on exposure to WNV that lasts approximately 6 weeks. This immunologic reaction is much more reliable than in human infection in which a more persistent IgM response is common. Most diagnostic laboratories use the WNV IgM capture ELISA (MAC) for actual confirmation of disease (increases in IgM rarely occur after vaccination). The sensitivity and specificity of this test are 81% and 100%, respectively.

In nonvaccinated horses, a fourfold increase in neutralizing antibody titers is confirmatory of a diagnosis of WNV infection. The most common neutralizing antibody test formats are the classic plaque reduction neutralizing antibody response (PRNT) and a more recently developed microwell format.[648,649] Vaccination induces formation of neutralizing antibody that likely confounds interpretation of the PRNT.

Other methods for confirmation of a diagnosis of WNV include postmortem detection of WNV by PCR, culture, and immunohistochemistry in tissues of the CNS. Nested PCR targeting the E protein has demonstrated sensitivity for relatively low viral load in equine tissues.[650,651] Real-time PCR methodology has been used to detect WNV in equine tissues.[651] The E-protein target appears less sensitive; however, the NS5 target has detected WNV nucleic acids in CNS tissues, heart, and intestine of clinically affected horses.

Pathologic Findings. Flaviviruses cause polioencephalomyelitis (inflammation of the gray matter) with lesions that increase in number from the diencephalon through the hindbrain and frequently increase in severity caudally through the spinal cord. The basal nuclei, thalamus, pons, and medulla have the highest numbers of lesions with two to several cell layers of mononuclear perivascular cuffing. Neuronal damage includes chromatolytic neurons and neuronophagia. In long-standing disease, areas of neuronal dropout may exist. By contrast, lesions are limited in the cortex and cerebellum. In the spinal cord, perivascular cuffing, gliosis, and damaged neurons may be seen.

Therapy. The goals of therapy are supportive and preventive. Treatment is mainly supportive care as there are no

known antiviral medications.[634,636,652-654] Survival of horses with WNV is higher compared with other viral encephalitis. Horses might start recovering between 3 to 5 days post onset of clinical signs. Most cases that have been euthanized have been the result of progressive disease resulting in recumbency. Therapy of recumbent horses is generally more aggressive and might include dexamethasone sodium (0.05–0.1 mg/kg IV every 24 hours) and mannitol (0.25–2.0 g/kg IV every 24 hours). Controversy remains as to whether corticosteroids enhance peripheral and CNS viral load.[655-658] Detomidine hydrochloride (0.02–0.04 mg/kg IV or IM) is effective for prolonged tranquilization. Low doses of acepromazine (0.02 mg/kg IV or 0.05 mg/kg IM) provide excellent relief from anxiety in both recumbent and standing horses. In addition, treatment of concurrent disease is recommended. IFN-α has been used in humans and veterinary medicine. However, studies on its efficacy are limited.[653,654,659-661] Plasma containing WNV-specific immunoglobulins for intravenous administration has been used, but plasma treatment did not change outcome and severity of WNV disease.

Prevention. Experimental models of disease have shown that 70% to 100% of nonvaccinated healthy horses become viremic upon viral challenge through mosquitoes, needle, or intrathecal injection. Epidemiologic and experimental evidence exists regarding the effectiveness of vaccination.[648,662] Several commercially available vaccines are licensed by the USDA as an aid in prevention of viremia, encephalitis, and mortality and reduction of viremia, encephalitis, and mortality. Available vaccines are inactivated whole WNV or a nonreplicating live canary pox recombinant vector vaccine. An inactivated flavivirus chimera vaccine is no longer commercially available. Vaccination before the mosquito season is critical. Manufacturer's labeling instructions must be followed for induction of immunity with initial immunization. All available vaccines are labeled for administration every 12 months after the initial series; however, only the modified live chimera vaccine has published 12-month efficacy data.[663] More frequent vaccination in areas with year-round mosquito seasons is recommended for most vaccines because it is not expected that the initial vaccine series will provide long-term protection, especially with killed virus vaccines with which antibody levels rapidly decrease after 4 to 6 months. Where these viruses are endemic, vaccination schedules should be maintained even when a decrease in the incidence of overt disease exists.

Public Health Considerations. WNV is a zoonotic disease. Bird reservoirs maintain the virus in an endemic life cycle, allowing for transmission by mosquitoes to human beings. Therefore control of the mosquito population is important. Little risk exists of disease by direct contact with an infected horse, except during postmortem examination with inappropriate handling of infected tissues. Blood-borne transmission can occur between viremic hosts. In addition, occupational infection has occurred through necropsy of avian hosts.

Bornavirus

Borna disease was first described in the town of Borna in Saxony, Germany, in 1766 as "sad horse disease" due to the obtunded appearance of diseased horses. However, the virus was not identified until 1926. The virus is a member of the *Bornavirus* genus of the Bornaviridae family, a group of enveloped viruses with a nonsegmented, negative-sense, single-stranded RNA genome. It is the cause of a naturally occurring infectious, usually fatal (80%–100% mortality rate), progressive meningopolioencephalitis that affects horses and sheep most commonly.[664] Less often the virus affects other equids, cattle, goats, camelids, fox, dogs, cats, rabbits, rodents, birds, primates, and humans. The disease is recognized to date in Germany, Switzerland, Liechtenstein, and Austria. Antibody-positive horses are present in the Middle East, Asia, Australia, and the United States. Reservoirs include rodents, rabbits, and deer. The route of transmission includes nasal secretions and saliva, and vectors are believed to be rodents. Virus is shed in body secretions, gaining entrance to a new host through exposed nerve endings in the nasal and pharyngeal mucosa.[665,666]

Borna disease is caused by a virus-induced immunopathologic reaction.[665-667] The virus is a noncytolytic, neurotropic, single-stranded RNA virus. The glycoproteins GP43 and GP84 are important in pathogenesis of disease. GP84 serves for attachment to cells and GP43 for the internalization to cells via endocytosis. The virus gets access to nasal mucosa and migrates to olfactory bulbs via axonal transport mechanisms. The virus has tropism for the limbic system, particularly the hippocampus. Once in the central nervous system, the virus migrates centrifugal to the peripheral nervous system. Main histologic lesions occur in Ammon's horn, olfactory bulb, caudate nuclei, thalamus, and less commonly the cerebellar nuclei. The virus causes progressive severe immune-mediated nonsuppurative meningoencephalitis. Natural infection in horses results in peracute, acute, or subacute meningoencephalitis leading to death in 1 to 4 weeks of affected animals. In endemic areas (Germany), the prevalence of disease is estimated to be 12%.[664,665] There can be subclinical carriers. Specific neurologic signs are variable but may include slow-motion eating, chewing motions of the mouth, head pressing, somnolence and stupor, hyperexcitability, fearfulness, aggressiveness, hypokinesia, abnormal posture, hyporeflexia, head tilt, neurogenic torticollis, and inability to swallow. Diagnosis is made through serology such as Western blot, ELISA, IFAT; virus isolation; IMHC; and RT-PCR.

Miscellaneous Viral Encephalitides

Other mosquito-borne encephalitides reported in California have been caused by a group of closely related viruses belonging to the *Orthobunyavirus* genus of the Bunyaviridae family.[506,668] Snowshoe hare and Jamestown Canyon viruses have been isolated in Canada and California.[669-672] Snowshoe hare virus is the most widely occurring arbovirus in Canada and is maintained in an amplification cycle involving small mammals and mosquitoes primarily of the *Aedes* genus.[669,671] Seroconversion without clinical disease is widespread. One report exists of acute encephalitis with complete recovery in a horse that seroconverted to the snowshoe hare serotype of California encephalitis viruses.[669] Jamestown Canyon virus has been isolated from vesicular lesions in a horse.[670,672]

Main drain virus, a member of the *Orthobunyavirus* genus of the Bunyaviridae family that is not part of the California encephalitis serogroup, was isolated from the brain of a horse with encephalitis in Sacramento County, California.[673] Signs included incoordination, ataxia, stiffness of the neck, head pressing, dysphagia, fever, and tachycardia. The major vector is *Culicoides variipennis,* which transmits the virus from infected rabbits and rodents.[673] The Cache Valley virus, a member of the *Bunyamweravirus* genus of the Bunyaviridae

family, has been isolated from a clinically normal horse; a high seroprevalence exists for this virus among horses in some geographic areas.[674]

Nipah virus, a member of the *Henipavirus* genus of the Paramyxoviridae family, causes encephalitis in human beings and pigs in Southeast Asia. It is transmitted from bats to pigs and then spreads horizontally to other pigs and human beings. One anecdotal report exists of dilated meningeal vessels in a horse from which Nipah virus was isolated.[675]

Equine encephalosis virus, a member of the *Orbivirus* genus of the Reoviridae family, is an insect-borne virus transmitted by a variety of *Culicoides* spp.[676] Horses, donkeys, and zebra in Southern Africa are frequently seropositive. The clinical importance of equine encephalosis virus as a cause of neurologic disease in equids appears to be limited despite the fact that it was originally isolated from a horse with clinical neurologic disease. Clinical signs that have been attributed to equine encephalosis virus include fever, depression, edema of the lips, acute neurologic signs, enteritis, and abortion.

Other viruses, identified in areas around the world, that have been implicated in equine encephalitis or that are associated with encephalitis in other species and for which significant titers have been identified in horses include the louping ill,[677,678] Maguari,[679,680] Aura,[680] Una,[680] Highlands J,[681] Semliki forest,[682] and Getah[619] viruses.

Three recently identified viruses (anellovirus, hepacivirus, and parvovirus) were found through PCR in CSF from horses with neurologic signs predominantly involving brainstem and spinal cord (personal observation, Aleman).[622] Cytologic analysis of CSF showed lymphocytic pleocytosis in these horses.

RABIES

Monica Aleman

The family *Rhabdoviridae* includes six genera of viruses that infect a variety of species such as mammals, birds, fish, insects, and plants. The genera consist of *Lyssavirus, Vesiculovirus, Ephemerovirus, Novirhabdovirus, Cytorhabdovirus,* and *Nucleorhabdovirus.* There are other unclassified rhabdoviruses. Rhabdoviruses are enveloped with single-stranded RNA.[506,683] The family *Rhabdoviridae* includes viruses of importance to animal and human disease such as rabies, vesicular stomatitis, and bovine ephemeral fever viruses. Rabies virus belongs to the genus *Lyssavirus.*[506] Other related viruses such as Mokola, Lagos bat, European bat lyssaviruses 1 and 2, and Australian bat lyssavirus also belong to the genus *Lyssavirus* and cause rabies-like disease in animals and humans. The rabies virus is a large, cylindric, bullet-shaped neurotropic rhabdovirus.[683] Rhabdoviruses are relatively stable in the environment. However, rabies virus is inactivated by detergent-based disinfectants, iodine-containing solutions, radiation, strong acids, alkalis, lipid solvents, and anionic solvents.[506,683]

Rabies was first recognized as a fatal disease in Egypt before 2300 BC and in ancient Greece. The virus has a worldwide distribution except for Australia, New Zealand, Japan, Antarctica, and certain islands. The virus was recently eradicated from portions of Europe (e.g., Switzerland, France) and Scandinavia.[506] Although rabies is uncommonly diagnosed in equids due to its zoonotic potential, it should be considered as a differential diagnosis in horses with acute progressive neurologic signs. Rabies is estimated to cause 35,000 to 70,000 human deaths worldwide annually.[506] Rabies surveillance in 2009 showed that 49 states and Puerto Rico reported 6690 rabid animals and 4 human rabies.[684]

The most common route of transmission of the virus is via saliva-contaminated wounds or bites from a wild carnivorous or insectivorous bat carrying the virus. In Africa and Asia most of the human cases result from bites from rabid dogs, whereas in North America they result more commonly from wildlife.[506] The most common reservoir hosts in the United States are skunks, raccoons, and the red fox.[683] However, domestic dogs, cats, and other horses may transmit rabies to horses by bite wounds. An important reservoir of rabies in the Caribbean islands is the gray mongoose. Furthermore, rabies virus can be transmitted by droplet inhalation, orally, or transplacentally. Droplet transmission has been reported to have occurred in foxes, coyotes, opossums, and raccoons in a bat cave in Texas. In that report the virus was isolated from the air in the cave.[685] Aerosolization of the virus also caused an outbreak of rabies in a laboratory. Transmission via aerosol has also been reported in a few humans working in bat caves.[506] Transplacental transmission of the virus has occurred in naturally infected cattle and experimentally infected mice and bats.[683]

Virus entry into host cells occurs by receptor-mediated endocytosis via coated pits, pH-dependent fusion of the viral envelope with the endosomal membrane releasing the viral nucleocapsid into the cytoplasm where replication occurs.[506] Rabies virus infects and replicates in myocytes at the inoculation site and might remain undetectable for weeks or months before moving centrally. The virus infects peripheral nerves by traversing neuromuscular and neurotendinous spindles. Progression along the nerve is thought to occur in the tissue spaces of the nerve fasciculus.[685] After progressing centripetally up the peripheral nerve by axoplasmic flow, the virus replicates in spinal and dorsal root ganglia of the corresponding peripheral nerve. Once the virus reaches the CNS, spread occurs rapidly through multiplication in neurons of the brain, spinal cord, sympathetic trunk, and glial cells. Spread of rabies virus also can occur through passive transport within CSF or blood.[683] Finally, the virus reaches tissues outside the CNS via centrifugal movement of the virus along nerve axons.[683]

The incubation period is usually between 14 and 90 days, but it could be longer, up to a year. The incubation period can be affected by the virus strain, host species, inoculum size, and proximity of the inoculation site to the CNS.[683] Retention of virus in myocytes at the inoculation site may be a mechanism for variation in the incubation period. A shorter incubation period also might be explained by the virus entering peripheral nerves soon after exposure and rapidly migrating centripetally to the CNS without replication in non-neural tissue.[683]

Clinical Signs

No signs are pathognomonic for rabies infection in horses. There is usually a prodromal phase on which signs are often overlooked or not appearing as neurologic in origin. This also occurs in horses. Clinical signs on presentation vary and range from lameness to sudden death.[686-689] Hyperesthesia, ataxia, behavior change, anorexia, paralysis or paresis, and

colic have been reported as initial clinical signs.[686-691] One rarely finds a bite wound, and the horse might or might not be febrile. The site of inoculation and its proximity to the CNS influence what clinical signs one observes.[683,687,688] The neurologic signs exhibited in rabies-infected horses can be classified into three forms, depending on the neuroanatomic location in the CNS infected by the virus. First, in the cerebral or furious form, one might see aggressive behavior, photophobia, hydrophobia, hyperesthesia, self-mutilation, straining, muscular tremors, convulsions, and blindness.[4] Second, in the brainstem or dumb form, one commonly sees obtundation, anorexia, head tilt, circling, excess salivation, facial and pharyngeal paralysis (giving the name of "hydrophobia").[4,690] Finally, in the paralytic or spinal form, one sees progressing ascending paralysis, ataxia, shifting lameness with hyperesthesia, self-mutilation of an extremity, flaccid tail and anus, and urinary incontinence.[4,691,692] Most affected animals with the paralytic form become recumbent in 3 to 5 days. The neurologic signs may vary as the virus spreads to other portions of the CNS. Thus horses may have clinical signs of two or all forms of rabies. Regardless of form presented, progression occurs to severe altered behavior and mentation and paralysis leading to recumbence. Antiinflammatory therapy can delay virus progression,[4] but death usually occurs within 5 to 10 days after onset of clinical signs.[506,689]

Diagnosis

Antemortem diagnosis of rabies is difficult, but one should consider the disease in horses showing rapidly progressing or diffuse neurologic signs. Other diseases that one should consider include other viral encephalitis such as togaviral encephalitis, hepatoencephalopathy, protozoal encephalomyelitis, nigropallidal encephalomalacia, botulism, lead poisoning, polyneuritis, meningitis, space-occupying mass, trauma to the brain or spinal cord, and esophageal obstruction.[685] Clinical laboratory data of body fluids are nonspecific. CSF might be within normal reference range or might show a moderate increase in total protein concentration (60–200 mg/dL) and lymphocytic pleocytosis (5–200 mg/dL).[685] Fluorescent antibody testing of tactile hair follicles of facial skin taken on biopsy or from corneal epithelium might help diagnose rabies antemortem. The fluorescent antibody technique detects the rabies virus antigen in these tissues. However, a negative test does not exclude rabies as a differential diagnosis.[689] One can achieve definitive postmortem diagnosis by submitting half the brain in 10% formaldehyde for histologic examination and the other half frozen to a public health diagnostic laboratory for direct immunofluorescent antibody tests, mouse inoculation, and monoclonal antibody techniques. The whole brain might be shipped unfrozen on ice for further rabies evaluation and testing. One should examine the rest of the carcass only with careful precautions against transmission of the virus, if present, by wearing gloves, caps, and masks until a negative rabies diagnosis is made.

Common histopathologic changes are a mild, nonsuppurative encephalomyelitis; perivascular cuffing by mononuclear cells; gliosis; glial nodules; and neuronal degeneration. These lesions occur most commonly in the hippocampus, brainstem, cerebellum, and gray matter of the spinal cord. Large intracytoplasmic eosinophilic inclusions within neurons and ganglion cells, known as *Negri bodies,* are pathognomonic for rabies.[683] However, in 15% to 30% of confirmed rabies cases, Negri bodies are not present in histopathologic sections, especially if the animal died or was euthanized early in the disease process.[683] The most commonly used and fastest diagnostic test for rabies is the direct fluorescent antibody test. This technique may identify 98% of infected brain specimens.

A recent histologic study characterized the lesions caused by rabies virus in cattle and horses.[693] Inflammatory lesions in horses were moderate at the thoracic, lumbar, and sacral levels, and marked at the lumbar enlargement level. Gitter cells were present in large numbers in the lumbar enlargement region. Immunohistochemistry staining intensity ranged from moderate to strong in the spinal cord. Only two horses exhibited lesions in the brain, which were located mainly in the obex and cerebellum. The study concluded that the odds of detecting lesions in horses with rabies are 3.5 times higher in spinal cord compared with brain alone.[693]

The mouse inoculation test is the most accurate method for diagnosing rabies virus infection but requires 5 to 6 days to complete. The mouse inoculation test involves the injection of suspect brain or salivary gland tissue homogenates intracerebrally in mice and observation of clinical and neurologic signs or death.[683] The monoclonal antibody test has been used most recently for rabies diagnosis in horses. The test can differentiate specific street, fixed, or vaccinal strains of rabies virus by their glycoprotein or nucleocapsid antigens.[683] This is important in postexposure vaccination of human beings and animals when using the specific strain of virus.

Rapid and accurate laboratory diagnosis of rabies is essential for timely administration of postexposure prophylaxis in humans and control of the disease in animals. RT-qPCR assays have been developed and proved to be a fast and useful diagnostic method. One study evaluated 211 biologic samples from humans including saliva, skin, and brain biopsies. RT-qPCR had a sensitivity of 91.5% for skin biopsies and 54% for saliva. This methodology has been implemented in a few countries as an antemortem diagnostic aid.[694]

This method was also evaluated for the diagnosis of human rabies on 211 biologic samples (positive $n = 76$ and negative $n = 135$) including saliva, skin, and brain biopsies. It detected all 41 human cases of rabies tested and confirmed the sensitivity and the interest of skin biopsy (91.5%) and saliva (54%) samples for intra vitam diagnosis of human rabies. Finally, this method was successfully implemented in two rabies reference laboratories in enzootic countries (Cambodia and Morocco). This combined RT-qPCR method constitutes a relevant, useful, validated tool for the diagnosis of rabies in both humans and animals and represents a promising tool for lyssavirus surveillance.

Recently a pan-lyssavirus (multiplex) Taqman RT-PCR assay named LN34 for the detection of highly variable rabies virus and other lyssaviruses was developed. LN34 assay uses a combination of degenerate primers and probes along with probe modifications to achieve identification of the *Lyssavirus* genus. The LN34 assay was successfully used for both antemortem and postmortem diagnosis of over 200 clinical samples.[695]

Treatment and Prevention

As for most viral encephalitis, there is no specific treatment for rabies. Aggressive supportive therapy might prolong the disease course. However, this increases the risk of exposure

to those involved with the horse's care. Rabies should always be considered as a differential diagnosis for acute progressive neurologic disease, and all measures to minimize exposure to personnel must be taken. These measures include isolation of the animal and minimal handling of the animal. Horses that are known to have been exposed to rabies should have all wounds cleaned and lavaged with iodine or quaternary ammonium disinfectant (and rabies antiserum, if available, infiltrated around the bite wound). No postexposure protocol exists for unvaccinated or vaccinated horses. However, exposed horses should be quarantined for 6 months and observed for the occurrence of neurologic signs. Unvaccinated horses should not receive postexposure prophylaxis until after the 6 months of quarantine.

Currently, there are three inactivated (tissue culture–derived products with an adjuvant) vaccines licensed for rabies to be administered intramuscularly. The American Association of Equine Practitioners recommends that all horses in the United States be vaccinated against rabies. Foals from vaccinated mares should be vaccinated no earlier than 6 months of age. The second dose of vaccine should be given 4 to 6 weeks after the first. A third dose should be given at 10 to 12 months of age. The first dose of vaccine should be given to foals from unvaccinated mares at 3 to 4 months of age. A second dose should be given at 10 to 12 months of age. Adult horses should receive annual revaccination. Pregnant mares might be vaccinated annually before breeding or 4 to 6 weeks before foaling. Testing for rabies antibodies using the rapid fluorescence focus inhibition test (RFFIT) is available through the Kansas State Veterinary Diagnostic Laboratory.

Recently a study investigating the serologic response to vaccination in 48 horses with undocumented vaccination history was conducted.[696] These horses were vaccinated and blood collected before, 3 to 7 weeks, and at 6-month intervals for 2 to 3 years post vaccination. Serum rabies virus–neutralizing antibody (RVNA) values were measured. A protective RVNA value (≥0.5 U/mL) as defined by the World Health Organization for humans was maintained for 2 to 3 years in horses inferred to have been previously vaccinated on the basis of prevaccination RVNA values. The authors found significant differences between horses inferred to have been previously vaccinated and horses inferred to be naïve before the study. Based on these results, the conclusion was that a rabies vaccination interval greater than 1 year may be appropriate for previously vaccinated horses but not for horses vaccinated only once.[696]

EQUINE MOTOR NEURON DISEASE

Yvette S. Nout-Lomas

Equine motor neuron disease (EMND) is an acquired neurodegenerative disease of adult horses that has been reported in North and South America, Japan, and Europe.[697-700] Lower motor neuron disease was first recognized in 1988 based on histologic changes in skeletal muscle,[701] and EMND was first described in 1990.[697] In the 1990s, EMND was recognized worldwide in an apparently increasing frequency that was estimated to have peaked in 1997.[3] A study conducted between 1997 and 2007 suggested incidence was still decreasing,[702] and currently the disease is seen only sporadically. The decrease may be the result of preventive management measures taken for horses at risk of developing the disease.

The disease affects primarily the motor neurons in the spinal cord ventral horn cells and brainstem and leads to characteristic clinical signs, including generalized neuromuscular weakness and neurogenic muscle atrophy.[697,699,703] EMND closely resembles progressive spinal muscular atrophy, a variant of *amyotrophic lateral sclerosis (ALS)* or Lou Gehrig's disease.[697,704] However, EMND only affects lower motor neurons, which is not the case in ALS. A chronic lack of antioxidants is implicated in the pathogenesis of EMND, but experimental and clinical studies also suggest that there is an individual predisposition to this neurodegenerative disease.[705]

Clinical Signs

EMND occurs in adult horses 2 years and older with a mean age of onset of clinical signs of 9 years.[704] The risk for EMND increases with age, peaking at around 15 years.[706] No gender predilection is apparent, and although initial studies showed the Quarter Horse to be at increased risk for disease, this likely was a reflection of management factors.[706]

Clinical signs reflect motor denervation of skeletal muscles. Muscle wasting despite a normal or ravenous appetite, trembling or muscle fasciculations, and excessive recumbency are the predominant signs of EMND, and each of these was shown to be present in greater than 90% of 77 cases.[705] Muscle wasting is most noticeable in the quadriceps, triceps, and gluteal areas. Onset of muscle wasting usually occurs before the other clinical signs do, but sometimes signs of muscle weakness are seen in normally muscled horses. Generally onset is acute, and, in addition to the aforementioned signs, gait abnormalities such as short-stridedness and weight shifting in the pelvic limbs are frequently seen. Ataxia is not a clinical sign of EMND. A horse with EMND is said to move better than it stands. Other abnormalities that are seen in horses with EMND include a base-narrow stance and abnormal sweating. Also, in more than 50% of cases, horses may display a lower than normal head carriage with muscle wasting of the cervical musculature (hangdog appearance) and an elevated tail head carriage (Fig. 11.40).[697,699] Elevated tail head carriage is likely secondary to denervation atrophy and fibrotic contracture of the sacrococcygeus dorsalis medialis muscle. Occasionally horses are presented for poor performance or development of noncharacteristic gait



FIG. **11.40** Characteristic appearance of a horse with equine motor neuron disease (EMND), including muscle wasting, sweating, base-narrow stance, low head carriage (hangdog appearance), and tailhead elevation.

abnormalities (stringhalt-like movement). Despite the frequent occurrence of cranial nerve nuclei pathology clinical signs of cranial nerve dysfunction are very uncommon; in 2013 a case of acute-onset EMND was discussed that had marked reduction of tongue tone.[707] In nearly 40% of EMND cases ocular funduscopic examination reveals a yellow-brown-black pigment that has a reticulated appearance and is located in a horizontal band above the optic disc at the tapetal-nontapetel junction.[705,708] Loss of vision has not been reported.

Pathologic Findings

Gross necropsy findings in EMND include diffuse muscle atrophy and pallor (especially of the intermediate vastus and medial head of the triceps muscles). Despite the obvious weight loss seen in horses with EMND, fat deposits are usually within normal limits.[697,703] The CNS and peripheral nerves are grossly normal.

Histologically, one may detect noninflammatory neuronal degeneration and neuronal loss at all levels of the spinal cord, but such loss is most obvious in the cervical and lumbar intumescence. In addition, degenerative axonal changes can be found in the ventral roots and peripheral nerves. Degenerative lesions and neuronal loss are found in the ventral horn cells (lower motor neurons) of the spinal cord gray matter; the nucleus ambiguous, and all brainstem cranial nerve somatic motor nuclei, except III, IV, and VI. Most affected neurons are swollen, markedly chromatolytic, and diffusely argyrophilic, and severely affected neurons are shrunken or vacuolated. In chronic cases there are glial scars consisting of astrocytes and lipofuscin-laden microglia. In the retinal epithelium, liver, and intestine additional deposits of ceroid-lipofuscin can be found.

Skeletal muscle changes include nonspecific myopathic changes such as excessive fiber size variation, internal nuclei, and cytoarchitectural alterations. Scattered fiber degeneration and necrosis are consistent findings in EMND. Atrophy of type I and type II muscle fibers occurs in severely affected muscles in EMND and is pathognomonic of denervation atrophy.[697,703] EMND predominantly affects type I fibers, in contrast to denervating diseases in other species, and has not been reported in ALS. Motor neurons supplying the type I fibers have a higher oxidative activity and thus may be more susceptible to oxidative injury. Similar to ALS, in which at least 30% of motor axons must be destroyed before clinical evidence of atrophy occurs, in horses with muscle wasting caused by EMND, a mean motor neuron loss of 31% was recorded.[709] There have been some reports of horses with EMND having small intestinal lesions including evidence of lymphocytic or eosinophilic-lymphocytic infiltration, edema, and catarrhal enteritis.[699,710]

Pathogenesis

The clinical signs of neuromuscular weakness result from the generalized denervation muscle atrophy found in horses with EMND. Oxidative stress is believed to be the underlying mechanism for development of EMND, either through a deficiency in antioxidant elements, such as vitamin E, and/or an excess of prooxidant elements such as copper and iron.[706,711-714] However, besides oxidative stress it appears there must be other factors involved in the pathogenesis of this disease.

A chronic vitamin E deficiency is thought to be the most important contributor to the state of oxidative stress in horses with EMND, although recently it has been shown that EMND occurred in a horse with high tissue iron concentrations,[715] and in five horses that were on pasture at the onset of the disease.[716] The latter studies suggest that, besides inadequate intake of vitamin E, abnormal bioavailability or excessive utilization of vitamin E may play a role in the pathogenesis of EMND. Evidence of chronic oxidative stress in horses with EMND includes the pronounced type I muscle fiber atrophy and the lipopigment deposition in the capillaries of the spinal cord and the epithelium of the retina. In addition, there is overwhelming epidemiologic and experimental evidence that a diet low in vitamin E is a strong risk factor for EMND.[705,713,717]

Although EMND is the only naturally occurring model for ALS and oxidative injury is implicated in the cause of both diseases, some important differences exist between the two.[703] In contrast to EMND, in which the disease process is limited to the lower motor neurons, ALS affects upper and lower motor neurons. Only mild degeneration of the pyramidal tracts is present in horses with EMND, but the pyramidal tracts in horses are less extensive and poorly developed compared with human beings. Why both these diseases affect motor neurons so selectively is unclear. Motor neurons are particularly susceptible to oxidative injury by having high energy requirements associated with the maintenance of long axons. The high concentration of polyunsaturated fatty acids in neuronal cell membranes make these cells particularly susceptible to lipid peroxidation.[718] About 10% of human ALS cases are considered familial, of which about 20% are associated with mutations in the Cu/Zn superoxide dismutase gene. Mutations of this gene were not found in horses with EMND.[711] Pathologic protein aggregates that are found in neural tissues of humans with ALS or other neurodegenerative diseases may reflect a common pathway for a variety of destructive processes, including oxidative injury. In 2012, one of those proteins, transactive response DNA binding protein of 43 kDa, was shown to be present in neural tissues of EMND-affected horses but not in those of control horses.[719] In addition to gene mutations, other causal factors for ALS include excess iron and copper intake and exposure to lead, solvents, and chemicals. For some of these there is evidence these may play a role in the pathogenesis of EMND as well. For example, a case report and an experimental feeding trial have shown that excessive copper and iron may increase the risk of development of EMND.[715,717] Moreover, elevated copper concentrations were found in the spinal cords of EMND-affected horses compared with controls.[720] Further, similarities in the clinicopathologic features of EMND and lead toxicosis suggest a trace element toxicosis is possible.[721] Another recent hypothesis involved a neurotoxin called β-*N*-methylamino-L-alanine, which can be found in the brains of human patients with ALS or other neurodegenerative conditions such as Alzheimer disease and Parkinson dementia complex. This toxin is produced by cyanobacteria that can be present in the intestinal tract of humans and horses. Altered intestinal conditions may lead to overgrowth of cyanobacteria and resultant toxin production.[722]

A temporal vitamin E deficiency is also implicated in the pathophysiology of NAD/EDM. NAD/EDM develops in genetically susceptible animals if vitamin E deficiency occurs during the first year of life,[279,723] whereas EMND occurs in adult horses after an extended period of vitamin E deficiency.[713] Clinical signs and histopathologic findings are very different in these two diseases, and they are therefore considered distinct entities. However, recently evidence was provided for the concurrent presence of NAD/EDM and EMND

in three young horses.[292] Although the authors did not find evidence of mutations in the superoxide dismutase gene, they did suggest that there could be overlap in genetic susceptibility to developing both NAD/EDM and EMND in certain families when a nutritional vitamin E deficiency is present.

Diagnosis

Clinical signs and a history of other EMND cases in the stable and absence of pasture or green hay may lead to a tentative diagnosis of EMND. One must be aware of the fact that a recent European study showed EMND to occur in horses that did have access to pasture.[716] Ophthalmoscopic examination may reveal fundic lesions in approximately 30% of cases. Serum enzyme activities of AST and CK are generally mildly to moderately increased. The plasma vitamin E concentration is consistently low (less than 1 μg/mL); however, a 2006 case report described a horse with EMND that had normal serum vitamin E concentrations and was hypothesized to have developed the disease secondary to high tissue iron concentrations.[715] CSF analysis has been performed in horses with EMND and demonstrated elevated IgG concentrations in approximately 50% of cases. Intrathecal production of IgG also occurs in ALS; however, in both diseases this is considered a secondary effect of the disease process rather than a cause. The AQ was normal in most horses examined, indicative of normal blood-brain barrier function.[699] EMG of cervical, facial, triceps, rear limb, and tailhead muscles may be useful in acute cases. However, the electromyographic changes, which include positive sharp waves and fibrillation potentials, may be difficult to evaluate because they are expected in any peripheral nerve disease, myopathy, or myositis.[697,724] In approximately 50% of horses with EMND, the plasma glucose curve is decreased after oral glucose tolerance testing, whereas results of xylose absorption tests are normal or only slightly abnormal.[699,725] Research suggests that increased glucose metabolism, rather than reduced intestinal absorption, is the cause of abnormal glucose tolerance in horses with EMND.[726] The clinical significance of this finding is unknown.

An antemortem diagnosis of EMND can be reached through examination of muscle and/or nerve biopsies. A biopsy of the sacrocaudalis dorsalis muscle is easy to obtain in a standing horse, and histopathologic examination of this muscle reveals changes consistent with denervation muscle atrophy and scattered muscle necrosis.[703] This test has a sensitivity of approximately 90%, and it has recently been hypothesized that horses with a syndrome of vitamin E–responsive muscle atrophy and weakness may account for the less than 10% of false negatives. This is because vitamin E–related pathology is only detectable using mitochondrial stainings in fresh frozen sections, and not in formalin-fixed tissue, whereas evidence of denervation can be seen in formalin-fixed tissue. Horses with this syndrome have clinical signs of EMND but do not have evidence of lower motor neuron involvement in biopsies.[727] Advanced quantitative analytical techniques have shown that muscle biopsy samples from EMND-affected horses have a lower percentage of type I fibers and higher percentages of type II fibers, reduced oxidative capacity, increased glycolytic capacity, diminished intramuscular glycogen, lower capillary-to-fiber ratio, a higher ratio of myofibers expressing sarcoendoplasmic Ca^{2+}-ATPase 1a to the 2a isoform, and a lower percentage of fibers expressing phospholamban. The extent of the changes observed in this study appeared to be related to the duration of the disease.[728]

Examination of a biopsy of the ventral branch of the spinal accessory nerve may be more sensitive in chronic cases. Nerve biopsies must be placed on a tongue depressor and in 10% formalin or another fixative suitable for electron microscopy. An experienced neuropathologist should examine nerve biopsies carefully; wallerian degeneration, Schwann cell proliferation, and sometimes only evidence of smaller Bungner's bands (columns of proliferated Schwann cells) can be detected.[703,729] This nerve biopsy technique is described postmortem and in horses under general anesthesia, but it is also possible in standing sedated horses.[730]

A definitive diagnosis is based on postmortem examination of spinal cord, brainstem, nucleus ambiguus, and skeletal muscle. The most important differential diagnoses that one should consider are laminitis, rhabdomyolysis, and colic. Other diseases that may cause similar signs are botulism, EPM, polysaccharide storage myopathy, iliac thrombosis, equine grass sickness, and lead toxicosis.[502,721]

Treatment, Prognosis, and Prevention

The only recommended treatment is based on the idea that this disorder is caused by oxidative injury and on the fact that horses with EMND consistently have low plasma vitamin E concentrations. Supplementation with vitamin E (5000–7000 IU/horse/day) results in an increase of plasma vitamin E concentrations to 2.0 μg/mL or greater after 4 to 6 weeks.[502] Treatment with vitamin E has been associated with improvement of clinical signs in about 40% of cases within 6 weeks of treatment, of which many horses can make a near full recovery in 3 months[502]; however, no published studies examining the effect of treatment exist at this time. Response to treatment is thought to depend on the number of neurons that are damaged versus those that are dead; in the case of neuronal death, full recovery is unlikely because this is irreversible.

The prognosis is poor for return to performance and guarded for life. Although no published investigations exist regarding the survival rate and follow-up of horses with EMND, horses with EMND generally have been shown to follow one of three possible clinical courses.[502] Approximately 20% of horses continue to deteriorate, and the severe weakness and excessive recumbency necessitate humane euthanasia. In approximately 40% of horses, clinical signs appear to stabilize; however, these horses do not regain muscle mass and may develop severe gait abnormalities. Continued clinical abnormalities frequently lead to euthanasia within 1 year of onset of clinical signs. The third group of horses (approximately 40%) show dramatic improvement after treatment with vitamin E, and many may regain a normal muscle mass. These horses may remain stabilized, that is, appear normal, for 1 to 6 years or more; however, many relapse, resulting in euthanasia. This relapse appears associated with return to exercise, training, and competition. The group of horses that recover and appear normal may be affected by a more recently discovered syndrome of vitamin E–responsive muscle atrophy and weakness that may be a separate disease entity or a predecessor to development of EMND.[727] Horses affected by this syndrome have clinical signs of EMND but they lack evidence of neurogenic atrophy in the sacrocaudalis dorsalis medialis muscle. It is suggested that such undiagnosed cases may be the result of a specific myogenic presentation of vitamin E deficiency that can only be identified in mitochondrial stains of frozen sacrocaudalis dorsalis medialis muscle.[727]

In healthy young and middle-aged horses receiving adequate dietary vitamin E intake, there is no research-based evidence for the need for additional vitamin E supplementation above 500 IU/day.[305] The goal of vitamin E supplementation in horses at risk of developing EMND (horses at risk for oxidative stress) is to increase α-tocopherol concentrations in the CNS. A dose of 10,000 IU RRR-α-tocopherol/500-kg horse/day was shown to do this[303]; however, it is important to consider that this dose equals the NRC upper limit safety recommendation.[305] Periodic monitoring of blood antioxidant status is recommended in horses that are at risk for developing EMND.[713,731]

TETANUS

Yvette S. Nout-Lomas

Tetanus is an infectious disease of all domestic animals and human beings. Tetanus has been recognized since ancient times, including mention in the writings of Hippocrates. Tetanus (from the Greek *tetanos,* to contract) was first identified as a neurologic disease more than 20 centuries ago by Greek physicians. A causative infectious agent was first hypothesized in the 1860s. In 1884 Nicolaire produced tetanus with injections of garden soil into mice and transfer of tetanus by injection of infected material from a human wound into a rabbit was demonstrated. Reinforcing the transmissible nature of an infectious agent, smears of wounds from infected patients showed a bacillus-like organism under microscopy. Pure culture of the toxigenic organism was obtained by Kitasato in 1889, and it was subsequently shown that animals immunized with modified tetanus toxin generated neutralizing serum antibodies.[732] Passive immunization of horses against tetanus using horse serum antitoxin was practiced by Nocard as early as 1892. In 1986 the amino acid sequence of tetanus toxin was revealed,[733] with the mechanism of action elucidated in 1992.[734]

Although equine tetanus is an increasingly rare disease, tetanus occurs worldwide and is considered enzootic in many warmer countries in the developing world where vaccination programs for both man and equids are not established. Tetanus is a distressing and often-fatal disease caused by 3 protein exotoxins liberated by the bacterium *Clostridium tetani* of the family Bacillaceae. Equids are particularly susceptible to the tetanus exotoxin.

Biology of *Clostridium Tetani*

C. tetani is a large gram-positive bacillus and is an obligate anaerobic spore former. It is part of the normal flora of the intestinal tract of humans and animals and can be readily isolated from the intestinal tract and feces of a wide range of animals, with fecal surveys in North America, Brazil, and Canada revealing between 30% and 42% positive samples.[732] *C. tetani* is found ubiquitously and in adverse environmental conditions, *C. tetani* produces round terminal spores able to survive in the environment for a prolonged period (years) in the absence of direct sunlight. Spores are highly resistant to environmental changes, acid, alkali, boiling water, and many standard disinfection techniques. The vegetative form is susceptible to heat and numerous disinfectants. *C. tetani* spores are terminal and oval, giving the bacillus a characteristic drumstick or tennis racket appearance.[732] The organisms may occur singly or in filamentous chains. Organisms are highly motile by means of flagellae uniformly distributed over the entire bacterial surface, allowing them to swarm over a culture plate. Optimal growth occurs at 37°C. Colonies have irregular margins and are flat, translucent, and gray with a matte surface on blood agar. Colonies are 4 to 6 mm in diameter with a narrow zone of clear (β-type) hemolysis.

Toxins

Under anaerobic conditions, *C. tetani* spores germinate and produce three exotoxins: tetanolysin, tetanospasmin, and nonspasmogenic toxin. Tetanospasmin is the most powerful and most important clinically.

Tetanospasmin

Tetanospasmin is produced in the bacterial cell as a single polypeptide chain of 1315 amino acids[733] with a molecular mass of approximately 150 kd.[735] The amino acid sequence of tetanus toxin shares marked similarities to the amino acid sequences of botulinum toxins A, B, and E, suggesting that, although neurotoxins from *C. tetani* and *C. botulinum* have different clinical effects, they are derived from a common ancestral gene.[733] After release from the cell, the molecule is cleaved into two polypeptide fragments by proteases produced by the organism—a heavy chain of about 100 kd and a light chain of about 50 kd that remain joined by a disulfide bond.[736] Toxin spreads from the infected site by diffusing into the adjacent tissues and is subsequently transported by the lymphatic system, allowing entrance to the bloodstream.[732] Toxin also travels up (centripetal transport) peripheral nerve endings, sensory and autonomic fibers, into the central nervous system through retrograde axonal transport.[732] Tetanospasmin localizes in the ventral horn of the gray matter of the spinal cord and binds to inhibitory interneurons, called Renshaw cells. There are three steps that result in action of this toxin: (1) binding to the neuronal cell membrane; (2) internalization by endocytosis; and (3) the intracellular blockade of neurotransmitter release.[737] Binding of the toxin to receptors on the nerve endings is the initial step in the paralytic process. Membrane receptors for these toxins consist of membrane gangliosides GT and GD1b and a receptor protein. This binding is almost irreversible and thus accounts for the prolonged nature of the signs. Once toxin is bound, it is internalized by endocytosis. The light chain, where it displays its zinc-endopeptidase activity specific for protein components of the neuroexocytosis apparatus, is translocated into the cytosol where it can exert its effect.[738,739] This process of internalization makes toxin unavailable to be bound and neutralized by circulating antibodies. Finally, tetanospasmin blocks the postsynaptic inhibitory signal of the spinal cord motor neurons by preventing release of the inhibitory neurotransmitters glycine and GABA. This disinhibition leads to continued stimulation of motor and reflex arcs, which results in the characteristic muscular spasms and contractions, hyperesthesia, and eventually convulsions, respiratory arrest, and death seen in patients with tetanus.

The specific target of tetanus toxin is synaptobrevin, also known as vesicle-associated membrane protein (VAMP).[740] This is one of three proteins that comprise the soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE), an integral membrane protein of the synaptic vesicles of nerve cell terminals crucial for normal vesicle physiology.[737,741] SNAREs are essential for a variety of vesicle docking and

fusion events,[739] and mutant studies have shown that any alteration in synaptobrevin leads to disturbed vesicle function.[741] Two copies of a nine-residue motif situated in tandem, each with three negatively charged residues, appear to be responsible for toxin specificity.[741] Cleavage of synaptobrevin by proteolytic action of the zinc-dependent endopeptidase of tetanospasmin light chain is the mechanism of toxicity, incapacitating cellular exocytotic machinery and blocking neurotransmitter release.[742] Both binding and internalization are mediated only by the heavy chain of the toxin, whereas the intracellular blockade of neurotransmitter release involves the light chain alone.[737]

Tetanospasmin is poorly absorbed across mucous membranes, is destroyed by gastric juices, and is unable to cross the placenta as result of its high molecular weight.

Tetanolysin and Nonspasmogenic Toxin

The effect of tetanospasmin is amplified by tetanolysin and nonspasmogenic toxin. Tetanolysin facilitates the spread of infection by increasing the amount of local tissue necrosis. The mechanism of action is to cause permeability changes in liposomes and biologic membranes resulting in cell lysis.[743] Tetanolysin is an oxygen-sensitive hemolysin similar to streptolysin and can affect a variety of cells including erythrocytes, neutrophils, macrophages, fibroblasts, and platelets. Tetanolysin has an affinity for cholesterol and related sterols that inhibit its lytic and lethal actions. The amount of tetanolysin produced by the organism in vivo is unknown.[732] The role of nonspasmogenic toxin in the pathogenesis of tetanus is not fully known but may involve blocking transmission in peripheral neuromuscular junctions.

Risk Factors for Disease

A 2007 report on tetanus in equids in a group of clinics in Morocco showed that tetanus cases represented 0.07% of the total patient load and 2% of all hospitalized patients.[744]

Animal species vary in their susceptibility to tetanus toxin. The horse is the most susceptible animal, and the cow is among the least. Neither age nor sex influences susceptibility. Incidence of disease depends on susceptibility and opportunity for exposure to the organism. Entry of organisms into the animal is usually by inoculation into a contaminated deep-penetrating wound. From two retrospective studies it appears that tetanus in equids is mostly associated with wounds to the distal limbs and hooves, which were found in 32 out of a combined 74 cases.[744,745] Other sites of wounds that were found in these studies were the head, proximal limbs, tail, hobble wounds, fistulous withers, rectal prolapse, and castration wounds. Other potential sites of entry include lacerations, surgical sites, the umbilicus of the neonatal foal, and the postfoaling reproductive tract. What may complicate or delay the diagnosis of tetanus is that the wound that served as the initial site of introduction of *C. tetani* may involve unbroken skin or be healed by the time clinical signs are observed. In fact, one study showed this had occurred in 2 out of 18 cases,[745] and another series showed that 18 out of 56 equids with tetanus had no visible wound at presentation.[744] Devitalized tissue is essential for development of tetanus. Once traumatized or devitalized tissue has become contaminated with spores, the spores can remain dormant until necrosis of the tissue provides the strict anaerobic environment necessary for germination to the vegetative, toxin-producing form.

Clinical Signs and Course of Disease

Tetanus is manifest as hypertonia of the striated muscles, with clonic paroxysmal muscular spasms superimposed. Muscular activity may be increased to the point that rectal temperature becomes markedly elevated. Clinical signs may be generalized or localized. Localized tetanus involves muscular rigidity and spasms in the vicinity of the infected wound. With time, this usually progresses to a more generalized tetanus affecting the entire body; however, the initial manifestation of tetanus is most often generalized rather than localized.[732] With generalized tetanus, a characteristic "sawhorse" stance, with an extended, rigid tail, may be present, and the gait is stiff if the horse remains ambulatory (Fig. 11.41). Difficulty in standing or lying down is due to extensor muscle rigidity that is exacerbated by external stimuli. As a result of hyperesthesia, painful reflex muscle spasms progressing to generalized tonic contractions with opisthotonus may be the result of even mild stimulation. Progression of the disease makes voluntary movement impossible because of marked extensor rigidity of all four limbs, often leading to recumbency.

Tetanospasmin affects both the sympathetic and parasympathetic nervous systems. Sympathetic hyperactivity

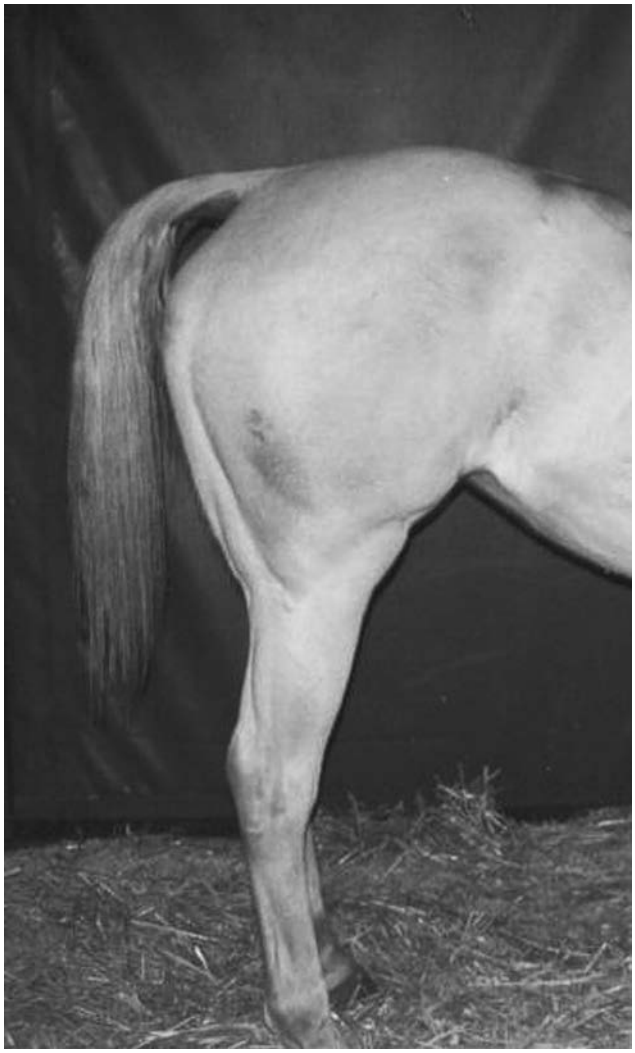


FIG. **11.41** The extended, rigid tail seen in this horse is characteristic of generalized tetanus.

associated with adrenergic stimulation may lead to tachycardia, cardiac arrhythmias, and peripheral vasoconstriction. Parasympathetic hyperactivity increases vagal tone that may result in bradyarrhythmias, atrioventricular block, and sinus arrest. Furthermore, tetanospasmin can act upon cranial motor nuclei, resulting in spasms of cranial muscles. Trismus is due to contraction of the masticatory muscles, with sustained facial muscle contracture and lip retraction resulting in risus sardonicus (a sardonic grin). Dorsomedial retraction of the ears and excessive wrinkling of the skin of the forehead can be seen. Prolapse of the nictitating membrane and enophthalmos is due to retraction of the globe by the hypertonic extraocular muscles (Fig. 11.42). Miosis, dysphagia, ptyalism, and laryngeal spasm may also occur.

A series of 20 cases showed that hyperesthesia (17 of 20, 85%) and prolapse of the third eyelid (17 of 20, 85%) were the most consistently observed clinical signs. A stiff gait, fever, tachycardia, tachypnea, erect ears and tail, elevated head, trismus, extensor spasms, sweating, and convulsions were seen in 50% (10 of 20) of the horses.[745] In an attempt to correlate clinical signs to survival a disease severity scoring system has been proposed using a clinical score from 1 indicating mild disease to 4 indicating terminal disease (Table 11.17).[744]

Complications from tetanus include decubital ulcers after periods of recumbency, regurgitation because of dysphagia, dysuria because of a hypertonic urethral sphincter, and constipation with gaseous distention because of a hypertonic anal sphincter and lack of exercise. Death may result from respiratory failure secondary to the spasm of respiratory muscles or central respiratory arrest from medullary intoxication. Aspiration pneumonia secondary to dysphagia or increased airway secretions may also be fatal. No characteristic necropsy lesions can be ascribed to the tetanus toxins themselves.



FIG. **11.42** This horse exhibits enophthalmus, prolapsed nictitans, and rigidity of facial muscles, typical of generalized tetanus.

The prognosis for survival from this disease is reported to depend on several factors, including the immune and vaccination status of the host, the dose of clostridial organism inoculated, and the availability and duration of aggressive treatment and supportive care.[745] From the three reports available on survival of equine tetanus, the oldest describes a case fatality rate of 75%, with previous vaccination strongly associated with survival.[745] Two other reports describe a mortality rate of 68%[746] and 59%.[744] Severity of clinical signs is clearly associated with survival across these three studies.[744-746] In particular, the presence of dyspnea, dysphagia, and recumbency were significantly more common in nonsurvivors than survivors and can be considered indicators of a poor prognosis.[746] When using the clinical scoring system (see Table 11.17), animals categorized as grade 1 or 2 had a survival rate of over 60%, whereas this was less than 10% for animals categorized in grade 3.[744] One report showed that younger horses were significantly less likely to survive than older horses[746]; however, this was not the case in the other study that looked at this.[744] A study involving 56 cases showed that factors such as sex, age, and chronicity at presentation had little relationship with prognosis.[744] Heart rate at presentation had little value as a prognostic indicator, except where elevated above 80 beats/min. Furthermore, survival was not different among donkeys, horses, and mules.

Diagnosis

There is no definitive antemortem test for tetanus. There are also no postmortem findings pathognomonic for the disease. Finding microscopic evidence of the bacteria or its toxin at the site of a wound is an unreliable diagnostic indicator. A biologic assay for antemortem diagnosis is possible in some cases by injecting infected material into the tail base of mice and observing for onset of clinical signs. A presumptive diagnosis of tetanus is based on history, clinical signs, and response to treatment. Confirmation is difficult. On physical examination, a recent or healed wound may be found; however, absence of a detectable wound at the time of clinical onset is not

TABLE **11.17** **Clinical Scoring System for Equids with Tetanus, According to Kay and Knottenbelt**

Clinical Score	Disease Severity	Clinical Signs
1	Mild	Nictitans membrane flashing, ears pulled caudally, slightly stiff gait but still walking and eating without difficulty
2	Moderate	Nictitans membrane flashing, ears pulled caudally, limbs stiff and walking with difficulty, trismus and generalized muscle spasm, animal still capable of eating and drinking voluntarily albeit slowly
3	Severe	Capable of standing but incapable of walking, severe trismus, difficulty with prehension, mastication and swallowing of food, and drinking voluntarily
4	Terminal	Animal recumbent, incapable of standing, incapable of eating; animals presenting in this situation are considered candidates for immediate euthanasia

From Kay G, Knottenbelt DC. Tetanus in equids: a report of 56 cases. *Equine Vet Educ*. 2007;19:107-112.

uncommon. Devitalization of deep tissue undetectable externally may be the site of toxin production.

Gram staining of samples from the wound is of limited diagnostic value. Sporulated and vegetative forms of *C. tetani* appear similar to other anaerobic bacteria. Furthermore, *C. tetani* may be present as a wound contaminant, with some strains of *C. tetani* nontoxigenic because they lack the 75-kilobase plasmid containing the toxin gene.[733,741] Bacteriologic confirmation of tetanus is not often attempted but would be obtained by isolating *C. tetani* from the infected wound. However, isolation of *C. tetani* can be difficult and often fails because of low concentration of organisms present in the wound and strict anaerobic conditions required for culture.

Hematology, serum chemistry, and CSF analysis are usually unremarkable. If a wound or aspiration pneumonia is present, neutrophilic leukocytosis with a left shift may be observed. Muscle enzymes may be elevated (CK and AST) because of muscle trauma from sustained contracture and prolonged recumbency.

Treatment

The binding of tetanospasmin to the Renshaw cells via ganglioside receptors is almost irreversible. Recovery is slow and does not occur until new interneuronal synapses develop to replace those that were inactivated by toxin. Therapeutic management of clinical cases of tetanus is centered around the following five goals.

Elimination of the Source of the Toxin

Local and parenteral antibiotic therapy is initiated to prevent further production of tetanospasmin by eradication of the vegetative form of *C. tetani* at the site of infection. Penicillin is the drug of choice for eliminating the vegetative form and is recommended to be administered at high dosages. Other antimicrobials that may be effective include the tetracyclines, macrolides (to be avoided in adults), and metronidazole. Metronidazole is indicated for deep and contaminated wounds because it is able to penetrate necrotic tissues without losing efficacy. Any visible wounds should be systematically cleaned, meticulously debrided, and lavaged thoroughly. If possible, aerobic conditions should be established at the infection site.

Neutralizing Unbound Toxin

Neutralizing any toxin that is not already bound to the ganglioside receptors is of primary importance. Tetanus antitoxin (TAT) is produced by hyperimmunization of horses with tetanus toxoid. Administration of 1500 U of antitoxin to unvaccinated horses provides immediate passive protection lasting approximately 3 weeks.[747] Higher doses result in longer duration of protection. In one study monitoring antitoxin levels as a measure of protection, combined active-passive immunization (tetanus toxoid concurrently with TAT) was effective at providing rapid and prolonged protection for previously unvaccinated horses.[748] Administration in separate, remotely located sites on the horse was recommended. Subclinical and clinical hepatic disease after TAT administration has been reported.[749,750] Known as idiopathic acute hepatic disease, this condition has also been called Theiler's disease, serum hepatitis, serum sickness, and postvaccinal hepatitis. Signs vary from hepatic enzyme elevation to hepatic encephalopathy in severe cases. Occurrence of this disease is rare and sporadic, but prognosis is poor in most affected horses.

Once TAT is administered to the affected horse, the passively acquired antibodies neutralize unbound toxin both circulating in the blood and present in the wound. Injection of TAT, both around and proximal to the wound site, has been suggested to be beneficial in neutralizing unbound toxin. It is important to realize that once the toxin is bound and then internalized into the neurons it can no longer be neutralized by antitoxin. Therefore the disease will likely continue to progress after the administration of TAT. Although the use of TAT for neutralization of unbound toxin has been reported to be an effective adjunct to therapy, dosages and routes of administration vary and there are few data for evidence-based therapy. Currently, recommended doses range from a single administration of 5000 to 2.5×10^6 IU/animal followed by lower doses over 5 days.[744] The use of TAT administered into the subarachnoid space (intrathecally) has also been advocated. One of the first reports to use this method showed a recovery rate of 77.5% in horses treated with 30,000 to 50,000 IU TAT intrathecally.[751] Such success has not been repeated in more recent reports.[744,745,752] The advantages of the intrathecal route seem to be questionable, and it appears that early diagnosis, nursing care, high doses of parenteral penicillin, and establishing aerobic conditions at the infected site may be the most important aspects of treatment.[744]

Relief of Pain and Control of Neuromuscular Derangements

Generalized muscle contractions can be painful and result in hyperthermia that must be managed. Typically a combination of sedatives and muscle relaxants is used to achieve the goals of pain relief and control of muscle spasms. Phenothiazine drugs are commonly used for sedation and relieve of muscle spasms. The relaxing effects appear to allow some affected animals to eat and drink again, which are considered good prognostic indicators. Phenothiazine drugs work at the level of the brainstem, depressing descending excitatory input on the lower motor neurons within the spinal cord. Although these medications have been reported to lower the seizure threshold, their effectiveness in the management of clinical cases strongly supports the use of these drugs. Phenothiazines potentiate barbiturates, which are also useful in the management of tetanus cases because of their ability to depress the motor areas of the brain and abolish spontaneous spinal cord activity. Muscle relaxants such as the benzodiazepines indirectly antagonize the effects of tetanospasmin. Diazepam has glycine mimetic effects and potentiates release of GABA, an inhibitory neurotransmitter. The combination of benzodiazepines and α_2-agonists such as xylazine may also be very effective in controlling muscle spasms. Methocarbamol or other central-acting muscle relaxants, such as guaifenesin or baclofen, would also be considered rational and useful therapeutic agents. Magnesium sulfate may also have beneficial effects in the treatment of tetanus.

Supportive Care

Supportive care is of utmost importance in the successful treatment of tetanus. Patients should be placed in a dark, quiet environment, with minimal stimulation and handling. Ear plugs can be placed to reduce auditory stimulation. If recumbent, supportive care for down horses needs to be provided.[9] Maintaining adequate hydration and nutritional status is of critical importance for care in these horses. In addition, providing deep soft bedding and regular turning (every 4 hours) to minimize decubital ulcers and pulmonary congestion are essential. Urinary catheterization, enemas, and manual rectal evacuation of feces may be required

because of hypertonic urethral and anal sphincters and lack of an effective abdominal press. If dysphagic, an esophagostomy or gastrostomy tube may be required for feeding. Tracheostomy may be required should laryngeal spasm and respiratory obstruction occur.

Generation of Active Immunity to Tetanus Toxins

Unfortunately, the concentration of tetanus toxin required to cause overt neurologic disease is insufficient to generate a protective immunologic response. Therefore at the initiation of treatment for tetanus, all horses should be immunized with tetanus toxoid to initiate a protective antibody response. An antibody response may take 2 to 4 weeks to develop.

Prognosis

Mortality rates of 54% to 75% have been reported for horses with tetanus.[744-746] The prognosis for survival depends on several factors such as the immune and vaccination status of the horse, the dose of clostridial organisms, and the availability and duration of treatment and supportive care. A short incubation period, short duration of onset, and rapid progression of signs is often associated with a poor prognosis. When attempting to treat clinical cases, it must be realized that the required supportive care is prolonged, labor intensive, and costly. One study reported an association between survival and previous prophylactic vaccination with tetanus toxoid; in that study none of the nonvaccinated horses survived.[745] One study showed that there was no association between sex, age, and duration of disease at presentation. However, what did affect prognosis was severity of clinical signs: animals with severe clinical signs (grade 3) had the lowest survival rate (less than 10%).[744]

Prevention

Where possible, management of contaminated or necrotic wounds should include thorough debridement, large volume flushing, aeration, and comprehensive cleansing. After this, rational antibiotic therapy should be instituted. Together these procedures, in conjunction with the following, should greatly minimize the occurrence of tetanus.

Low concentrations of tetanospasmin are capable of inducing clinical signs. As a result, neither exposure to tetanospasmin nor recovery from clinical disease induces the development of immunity. Prophylactic antibody formation and generation of immunity is stimulated by vaccination with tetanus toxoid, derived from inactivation of tetanospasmin with formaldehyde. Tetanus is a preventable disease, and vaccination with tetanus toxoid markedly reduces the occurrence of clinical disease. However, no vaccination is absolute,[745] with protection being dependent on proper administration and subject to breakdown in the face of overwhelming challenge. The adjuvanted tetanus toxoid vaccine is highly immunogenic, but efficacy of the vaccine relies heavily on the vaccination program. Current recommendations for adult horses are an initial vaccination followed by a booster 4 to 6 weeks later with annual revaccination. Significant differences have been found in antibody response (IgG titers) between commercially available multivalent vaccines,[753] and it was suggested that these differences were the result of variations in antigenic mass. Recently a prospective seroconversion study demonstrated that protective titers against tetanus were present up to 3 years after a series of vaccinations were administered (initial booster 4–6 weeks after primary vaccination and a second booster 15–17 months after the second vaccination).[754] The authors suggested our vaccination guidelines may need to be revisited and annual revaccination may not be necessary. In clinical cases of tetanus, experts recommend that tetanus toxoid be administered to horses with susceptible wounds if longer than 6 months since vaccination.

In foals, tetanus-specific IgG antibodies inhibiting the foal's response to tetanus toxoid are passively transferred via colostrum. High titers of IgGa, IgGb, and IgG(T) subisotypes were detected in postsuckling serum samples collected from foals born to mares that had received booster doses of multicomponent vaccines during the past 2 months of gestation. In addition, antibody response to vaccination of younger foals has been shown to be poor, necessitating multiple doses of toxoid. Current recommendations are to vaccinate foals born to vaccinated mares at 4 to 6 months of age with boosters 4 to 6 weeks later and a third vaccine at 10 to 12 months of age. Vaccination for foals born to unvaccinated mares should begin at a younger age: the current recommendations are to start at 1 to 4 months of age with a booster 4 weeks after the first dose and a third vaccine 4 weeks after the second dose.

BOTULISM

Yvette S. Nout-Lomas, (Stephen M. Reed)

Botulism is a severe neuroparalytic disease caused by botulinum neurotoxins (BoNTs) that are produced by BoNT-producing clostridia. The neurospecificity and toxic potency of BoNTs make them the most powerful known toxins, and they are potential bioterrorism weapons.[755,756] Horses are more susceptible to botulism than other species such as cattle, dogs, and humans, which are relatively resistant.[757] In horses, botulism has been most commonly associated with BoNT serotype B (BoNT/B) and BoNT/C, followed by BoNT/A and less commonly by BoNT/D.[755,758-760] The disease course is related to total toxin exposure and most commonly results in death unless the horse is treated promptly with specific antitoxin. A fast and accurate diagnosis is crucial because of the necessity of rapid treatment and the large potential of botulism outbreaks linked to a common feed source. Unfortunately, laboratory diagnosis has proven difficult in horses, and botulism is generally considered to be a clinical diagnosis.[758,759]

Biology of Bont-Producing Clostridia and their Toxins

There are six phylogenetically distinct groups of BoNT-producing clostridia (*Clostridium botulinum* groups I–III, *Clostridium argentinense* group IV, and some strains of *Clostridium baratii* and *Clostridium butyricum*) that produce seven serotypically distinct BoNTs (serotypes A–G).[755,756] The number of subtypes has grown in recent years, owing to the increased use of whole-genome sequencing and mass spectrometry, as well as the availability of high-affinity monoclonal antibodies. This has revealed the striking variety of distinct BoNT subtypes that are produced by clostridial species.[756] Notably, *C. botulinum* strains from groups I and II produce the widest range of neurotoxins; group I proteolytic *C. botulinum* produces BoNT/A, BoNT/B, and BoNT/F; and group

II nonproteolytic *C. botulinum* produces BoNT/B, BoNT/E, and BoNT/F. Group III *C. botulinum* produces BoNT/C, BoNT/D, and their mosaic BoNT/CD and BoNT/DC. Group IV *C. argentinense* produces BoNT/G, *C. butyricum* produces BoNT/E, and *C. baratti* produces BoNT/F. Each toxin serotype is categorized into various subtypes on the basis of their amino acid sequences, and, although most strains of *C. botulinum* express a single toxin serotype, some isolates produce more than one serotype; for example, some proteolytic *C. botulinum* group I isolates produce a mixture of Ab, Af, Ba, and Bf subtypes.[756] Investigators have shown that there are now more than 40 different BoNTs.[756]

Clostridia are sporulating and anaerobic gram-positive, rod-shaped bacteria that are widely distributed in the environment and in anaerobic regions of the intestines of several animals, where they are typically found as spores. Spores are resistant to physical and chemical stresses and can persist for long periods of time until favorable conditions enable germination. Under appropriate environmental conditions (such as humidity, nutrients, and the absence of oxygen), spores germinate into vegetative cells; conversely, exposure to oxygen, as well as water and nutrient deprivation, trigger sporulation.[756] Botulism outbreaks typically occur in environments that contain *C. botulinum* spores, which can germinate in decomposing organic material under anaerobic conditions. Environmental conditions that favor botulism outbreaks include warm temperatures, shallow alkaline waters that contain abundant invertebrate populations, and decomposing vertebrate carcasses.[755,756]

Human botulism is much rarer than animal botulism and is mostly caused by BoNT/A, BoNT/B, BoNT/E, and rarely by BoNT/F. Botulism mostly affects wild and domesticated animals, and outbreaks of animal botulism can spread rapidly, leading to the intoxication of hundreds of thousands of animals in just a few days. The primary contamination route for both humans and animals is the ingestion of preformed toxins in foods or feeds. Raw material, such as grass, hay, rotting vegetation, and slaughterhouse waste, as well as decay of vertebrate carcasses, invertebrates, and sewage may support BoNT-producing clostridial growth and toxin production. Animals may directly ingest decaying organic matter containing toxin, or they may ingest toxins through the consumption of zooplankton or invertebrates, such as larvae, that have consumed toxic material. Fly larvae and other invertebrates are unaffected by the toxin, but feeding on toxigenic carcasses may concentrate toxin. Ingestion of a single toxigenic maggot could be lethal. This is described as the carcass-maggot cycle of botulism.[755] Typically carrion-associated (birds, small animals) botulism is associated with contaminated water, feed, or the environment involving BoNT/C and BoNT/D. Non–carrion-associated botulism is caused by BoNT/A and BoNT/B.

In North America, over 85% of equine botulism cases are caused by BoNT/B.[757] BoNT/A and BoNT/C have been associated with fewer cases, although studies suggest that BoNT/A botulism may be more common in horses than previously assumed.[761] BoNT/D botulism has been suspected in horses and cattle in the United States[757] but has only been confirmed in equine botulism in Senegal.[762] Equine botulism in Europe appears to be mostly associated with BoNT/B and BoNT/C.[763] In North America, BoNT/B botulism is endemic in the mid-Atlantic states and Kentucky, and BoNT/A botulism is more common in the western states. This geographic distribution is consistent with outcomes from soil surveys, which have demonstrated a predominance of BoNT/B-producing clostridial spores in the northeast and central states, from Kentucky to Pennsylvania, whereas BoNT/A-producing clostridial spores were found in samples from western states.[764] BoNT/A and B botulism are associated with forage, whereas BoNT/C botulism is associated with decomposing carcasses. Ravens have been shown to serve as transport vectors by feeding off a decomposing animal carcass and then transporting the toxin to the feed buckets or feed troughs of horses up to 1.5 miles distant from the decomposing carcass.[765]

Mechanisms of Intoxication

Three routes of BoNT intoxication have been described in the horse: (1) food-borne botulism, (2) toxicoinfectious botulism, and (3) wound botulism. Food-borne botulism is caused by ingestion of the preformed toxin present in feed materials, most commonly roughage (hay, haylage, silage) but occasionally grain by adult horses. Typically, BoNT/B-producing clostridia directly proliferate and produce toxins in decaying vegetable matter. In contrast, BoNT/C-producing clostridial spores that can be present in the intestinal tract of animals or birds can undergo germination and toxin production in a decomposing carcass, which provides an excellent anaerobic environment. Toxins produced in the carcass over time leach out and contaminate the hay or other feed material.[757]

Toxicoinfectious botulism, or "shaker foal syndrome," is usually associated with BoNT/B and occurs in foals between 2 weeks and 8 months of age.[757] The syndrome is similar to what is seen in human infants whereby spores are ingested, germinate, and produce toxin once in the gastrointestinal tract. Under normal circumstances, the intestinal flora of adult animals and humans inhibits the intestinal proliferation of BoNT-producing clostridia, thereby limiting the occurrence of toxicoinfectious botulism to neonates.[766] Endemic foal botulism is thus considered to occur from ingestion of spores present in contaminated soil. Toxin can be detected in the feces of approximately 30% to 40% of foals with botulism but only during the acute clinical phase.[757,767]

Wound botulism occurs in horses when BoNT/B-producing clostridia infect a wound, germinate, proliferate, and release toxin under anaerobic conditions. In adult horses, wound botulism has been associated with an injection abscess and castration, and in foals, it has been diagnosed in conjunction with infected umbilical remnants and an infected limb wound.[757]

Mechanism of BoNT-Induced Neuroparalysis

BoNT-producing clostridia secrete toxins during their vegetative growth as progenitor, inactive, single-chain polypeptide toxins of approximately 150 kDa. Toxins are produced as heterodimers with proteins that effectively decrease the exposure of BoNT to external damaging agents. These proteins may also facilitate crossing epithelial cells.[755,756] BoNTs cross the intestinal epithelial barrier, disperse in extracellular fluid, and enter the lymphatic system and the blood circulation where they can remain for many days. They are unable to cross the blood-brain barrier. BoNTs are very specific and only bind to peripheral nerve terminals, particularly those of skeletal and autonomic cholinergic nerves.[768]

BoNT mature toxins consist of a light chain and a heavy chain. Internalization of the BoNT into nerve terminals occurs through binding of the carboxy-terminal end of the HC domain of the light chain to a polysialoganglioside (PSG) receptor that is present on the presynaptic membrane, followed

by binding to a protein receptor (either synaptotagmin or SV2) that is located either inside the exocytosed synaptic vesicle or on the presynaptic membrane. The light chain encodes the toxic moiety, which is a metalloprotease domain that specifically cleaves the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins that are necessary for neurotransmitter exocytosis. Some BoNTs cleave VAMP, some cleave SNAP25, and some cleave both SNAP25 and syntaxin. BoNTs thus act presynaptically at the peripheral cholinergic neuromuscular junction and result in the inhibition of neurotransmitter (acetylcholine) release and consequent neuroparalysis.[756]

The action of BoNTs is reversible likely because BoNTs do not cause neuronal death. The duration of BoNT-induced neuroparalysis is quite variable, depending in part on the lifetime of the metalloprotease within the nerve terminal cytosol, which is different for the different BoNTs (BoNT/A > BoNT/C > BoNT/B > BoNT/D). Also, the activity of the affected muscle and toxin dose determine duration of BoNT action. Duration of action determines the severity of disease, and in humans and horses there are data to suggest that BoNT/A causes more severe disease than BoNT/B.[756,761] Once the toxin is bound, improvement in clinical signs is achieved after sprouting of the presynaptic axon terminal and subsequent formation of a new synapse.[769]

Clinical Signs

BoNT intoxication results in diffuse, symmetric, flaccid paralysis and loss of muscle strength. Although there are some suggestions that there may be subtle differences in clinical signs depending on the BoNT involved, this remains unconfirmed in the horse, and the clinical signs of botulism in horses are similar, regardless of the type or source of BoNT.[758,761] The speed of progression of disease is variable, resulting in peracute, acute, and chronic forms of botulism. Some cases may present as sudden deaths. Depending on the amount of ingested toxin, initial signs may be subtle or overt. Generally, the clinical signs appear from 24 hours up to 17 days after exposure.[755] The incubation period may be associated with inoculum size, which suggests that the shorter the incubation, the more severe the disease. The disease usually affects motor nerves with high efferent traffic, resulting in weakness, dysphagia, and poor muscle tone. Usually generalized weakness, dysphagia, or both are the first clinical signs detected by observant horse owners. Early in some cases the owners will note prolongation of the time the horse takes to eat its grain, or they will note abdominal discomfort and colic. Some horses may have decreased salivation and occasionally urinary retention resulting from involvement of the cholinergic autonomic nervous system. Over time affected horses demonstrate muscle fasciculations, often first in the triceps region, that eventually progress to the entire body and result in recumbency. At first the horse appears to gain strength after lying down for a short time and may return to standing spontaneously or with coaxing. Eventually the horse is unable to rise. Death often results from respiratory failure. In foals, clinical signs are similar, although frequently foals are presented for excessive recumbency ad muscle fasciculations. Foals are typically bright and alert when they are lying down, but when they stand will develop fine muscle fasciculations that progress to severe muscle trembling, hence the term "shaker foal syndrome," before the foal stumbles and falls down. Foals are too weak to lie down in a normal fashion, yet they fall down. Similar to adult horses, foals regain strength after lying down until they are too weak and unable to rise anymore.

Clinical evaluation should include careful examination of cranial nerve and spinal cord function. Special attention should be paid to the palpebral and pupillary light responses, tongue tone, ability to prehend, and evidence of dysphagia, tail tone, gait and posture, and assessment of vital parameters and respiratory pattern. Vital signs are typically normal in the early stages, but heart rate and respiratory rate increase once the horse is recumbent, depending on the amount of struggling that occurs. Affected horses appear to maintain coordination and therefore appear weak but not ataxic. A low head and neck carriage and inability to lift the head can be seen and initially were thought to be more prevalent in BoNT/C botulism[770] but recently have been shown to occur in cases with BoNT/A botulism as well.[771,772] Occasionally these horses develop severe edema of the head and airways and then inspiratory stridor.

Mydriasis and ptosis are observed early in the disease process, as well as a sluggish pupillary light response. Reduced tongue tone and slow tongue retraction are characteristic early signs of botulism that typically occur before the onset of obvious muscle weakness. However, there are some cases of botulism that do not demonstrate clear dysphagia. Tail tone is frequently decreased in horses with botulism, but this can be difficult to assess given the wide variation in normal tail tone among horses. Profound tail tone reduction is frequently reported in advanced botulism.

The time to ingest a small quantity (usually a cup) of grain can be useful as a predictor of whether the horse has botulism. A botulism grain test has been described in which the suspect horse is offered 8 ounces of sweet feed in a large flat feeding tub on the floor, and the horse's ability to consume the feed is timed.[757] Most normal horses will consume an 8-ounce cup of sweet feed in less than 2 minutes and many horses faster than that. As the ability to retract the tongue diminishes, the time for the horse to eat the grain becomes longer. Also, grain mixed with saliva often falls out of the mouth through the horse's lips while eating. This is highly characteristic of botulism and is one of the earliest clinical signs.

Differential Diagnosis

The differential diagnosis for horses affected with botulism can be any cause of weakness and dysphagia in horses and should include EPM; EMND; EHV-1 myeloencephalopathy; WNV; rabies; several toxins such as lead, ionophore drugs, yew, and yellow star thistle; low blood calcium; and hyperkalemic periodic paralysis.[757] One also should consider less common clinical diseases such as tick paralysis, vascular accident, or even unusual examples of exercise intolerance.

Diagnosis

The diagnosis of botulism is difficult and frequently is based on compatible clinical signs of acute onset of flaccid paralysis, weak or poor eyelid tone, poor tail tone, slow or difficult eating, and dysphagia, as well as a compatible history of possible exposure to toxin. Clinical pathology results are mostly within normal limits. Some of the abnormalities seen may be reflective of dehydration, there may be elevated muscle enzymes detected in recumbent horses, and in the case of wound botulism there may be results consistent with infection. CSF evaluation is normal. Electrodiagnostic testing, specifically performing repetitive nerve stimulation, can demonstrate facilitation characterized by incremental

responses in both amplitude and area under the curve at a high stimulus rate.[37]

Laboratory support for a diagnosis of botulism requires at least one of the following: (1) detection of BoNT in serum, gastrointestinal contents, or from a wound; (2) detection of BoNT and/or BoNT-producing clostridial spores in the feed and/or gastrointestinal contents in addition to compatible clinical signs; or (3) detection of an antibody response in a convalescent patient.[757] BoNT is stable in frozen tissues or plasma and can be stored at −20°C for several weeks. The current gold standard test for botulism is the mouse bioassay.[755,759] This test involves the inoculation of mice with a sample from the suspect horse and subsequent observation of the mice for development of clinical signs of botulism. Because the horse is extremely susceptible to BoNT the circulating concentration in an affected horse is often below the threshold for detection by the mouse bioassay.[757] The mouse bioassay was recently reviewed and shown to have low sensitivity in adult horses (32%) and foals (53%) but high specificity (97% in adults and 100% in foals).[759] These results indicate that a positive result is highly suggestive of botulism but that negative results do not exclude the diagnosis. A higher sensitivity can be obtained by culture enrichment of the sample, which takes advantage of the presence of BoNT-producing clostridial spores that are not present in unaffected humans or animals.[758,759] The low sensitivity of the mouse bioassay is consistent with previous reports in horses that show that preformed BoNT is only found in approximately 20% of cases using this test.[757,760]

Quantitative real-time PCR assays for BoNT genes have been developed and validated and shown to have higher sensitivity with similar specificity as the mouse bioassay.[773,774] Other advantages of the qPCR test are that results are available much quicker (4 days vs. 2–3 weeks for culture-enriched mouse bioassay) and reduction of the live-animal use. The mouse bioassay would still be required to confirm gene expression by detection of BoNT in qPCR-positive samples.[773,774] Although ELISA tests have been developed these have not been shown to be superior to the mouse bioassay and have not been validated in the horse.[757] It is also possible to determine antibody titers in unvaccinated horses that have survived botulism. Despite the relatively low probability of confirming botulism through laboratory tests, testing remains an important component in managing botulism cases, first to identify the BoNT type, which may have important implications for treatment and prevention strategies and will provide information on epidemiologic characteristics, and second, if new botulism syndromes or toxin types were discovered, their full description would require as much testing data as we can provide.

Treatment and Prevention

Treatment of horses with botulism is expensive, time consuming, and often unrewarding. The first therapeutic objective is prompt administration of specific or multivalent antitoxin to bind circulating BoNT. A single dose of antitoxin is considered to be sufficient (30,000 IU for a foal and 70,000 IU for an adult horse) because that should provide passive protection for more than 60 days.[757,758] Antitoxin is not effective after BoNT has translocated into the cells; the earlier in the course of the disease the antitoxin is administered, the better. The horse should be confined to a stall, and physical activity should be kept to a minimum, if necessary with the use of sedatives. Antimicrobial therapy may be used to treat botulism or specific secondary complications. However, antimicrobials can potentiate neuromuscular blockage (aminoglycosides, tetracyclines, and procaine penicillin) and should be avoided. Mineral oil or other cathartics may be indicated to reduce impaction colic. Additional therapy includes appropriate nursing and supportive care. Of particular importance are maintaining the horse's hydration status and providing nutrient support through tube feeding in dysphagic animals. In severely affected foals or adults with respiratory failure, mechanical ventilation is indicated.

Prognosis for horses with untreated botulism is poor. In outbreak situations survival is typically reported at only 10% to 30%.[760,761,765,770] A recent review of 92 cases of botulism in adult horses showed that overall survival was 48%. Survival was significantly better for horses that arrived standing (67%) and even higher (95%) for horses that remained able to stand throughout hospitalization.[760] Treatment with antitoxin increased the odds of survival significantly, with treated patients being four times more likely to survive. Horses with abnormal respiratory effort or inability to stand had decreased odds of survival. Horses that lose the ability to stand have a poor chance of survival. Unfortunately there are horses that despite receiving antitoxin deteriorate over up to 5 days, likely due to the fact that the antitoxin binds only unbound BoNT and not the toxins already bound to the motor end plates. Horses in which treatment is initiated after they are recumbent only have a 13% chance of survival. Early diagnosis and treatment are critical for horses with botulism. Development of complications such as decubital ulcers, corneal ulcerations, muscle disease, pneumonia, and gastrointestinal disease during hospitalization for botulism is common but does not affect overall survival.[760] In horses that do survive, recovery is complete, but it can take months for the horse to regain full strength.

In foals, survival can be high with appropriate treatment. A review of 30 foals that were less than 6 months of age showed a survival of treated cases of greater than 96%. All foals, except one mildly affected one, received botulism antitoxin. Approximately 50% of the cases required oxygen therapy, and 30% required mechanical ventilation. Mean duration of hospitalization was 14 days.[767] It is possible that foals affected with BoNT/A may require longer term care and have slower recoveries than foals affected with other BoNTs.[772] Mechanical ventilation is an important aspect of treatment of botulism in human infants and, although this is not commonly performed in adult horses with botulism, it is a strategy that can be successfully used to reduce death from respiratory failure in foals. A study of nine foals with toxicoinfectious botulism employed mechanical ventilation early on in the course of hospitalization in foals that were progressively acidemic and had increased $Paco_2$ tensions. Arterial blood gas abnormalities were ameliorated with mechanical ventilation, and survival in treated foals was 87.5%.[775]

Type B botulism is preventable by vaccination using type B toxoid, which was initially developed to prevent disease in newborn foals. The current USDA-approved available product is a killed (toxoid) vaccine directed against *C. botulinum* type B. The AAEP recommends a vaccination schedule that includes an initial three-dose series at 1-month intervals followed by an annual booster. For brood mares they recommend administering the annual booster 4 to 6 weeks before foaling. Interestingly, a recent large study evaluating 92 cases of botulism showed that two horses with botulism were reportedly fully vaccinated. Both

of these horses had mild clinical signs and both survived. These were the first two horses reported in the literature to develop botulism despite an adequate vaccination history, of which one was confirmed positive for BoNT/B spores on mouse bioassay, representing a true vaccine failure.[760]

Additional preventive measures involve (1) providing safe and high-quality feed; (2) properly storing animal feed; (3) inspecting water sources for dying of dead small animals and birds; (4) avoiding spreading poultry litter that contains birds or dead animals on pastures; and (5) avoiding use of poultry litter as bedding material. Whenever more than one horse on a premise demonstrates clinical signs compatible with botulism, quick analysis of the entire ration and the feeding methodology to determine whether contamination has occurred is recommended. This aggressive approach may prevent additional horses or other animals on the farm from becoming infected. Beginning the investigation with the feed is always best, because this is the most common source of toxin. Conditions may be optimal for proliferation of botulism spores and toxin production in improperly prepared hay or hay stored in plastic bags.

EQUINE GRASS SICKNESS (EQUINE DYSAUTONOMIA)

Yvette S. Nout-Lomas

Equine grass sickness (EGS) is an acquired degenerative polyneuropathy that predominantly affects the neurons of the autonomic and enteric nervous system. As the name implies there is a strong association between EGS and grazing. The disease was recognized first in Scotland in 1909.[776] Since then it has been reported in other parts of England, continental Europe, the Falkland Islands, and Australia. Although there had been an earlier credible, but unpublished, report of EGS from the United States (Missouri),[777] the disease was not confirmed until 2010, when it was diagnosed based on clinical and pathologic findings in a mule in Kansas.[778] A clinically and histopathologically similar disease, mal seco, has been described in South America, predominantly in Chile. Britain has remained the country most affected by EGS, and a nationwide surveillance scheme has been instituted.[779]

The disease predominantly affects the prevertebral and paravertebral ganglia of the autonomic nervous system and enteric neurons (myenteric and submucosal plexuses) and is therefore characterized as a dysautonomia; however, given the fact that neuronal degeneration has also been identified in the brain and spinal cord of horses with EGS, the disease is likely better termed a *multisystem neuropathy.*[780] Clinical signs vary, and the severity is likely related to the extent of degenerative neuronal damage. The disease occurs sporadically and is often fatal. Although EGS has been studied extensively, the cause of the disease remains unknown.

Epidemiology

EGS has been reported in other equids such as the Przewalski's Horse, zebra, donkey, mule, and pony, but it occurs primarily in young, mature horses that have access to pasture. Horses with EGS are generally young adults between 2 and 7 years of age.[780] No gender predilection is apparent, but a study in Scotland found that there may be an increased susceptibility to EGS in native Scottish breeds.[781]

There is a strong association between EGS and grazing. Numerous epidemiologic studies have been performed over the years, and besides grazing, other consistently reported risk factors for development of the disease are age, recent movement to new pasture or premises, and time of year, with most cases occurring in spring and early summer.[780,782,783] Other factors that appear to increase the risk for disease, or recurrence thereof, include previous occurrence of cases on the premises, recent prior movement to a new premises (within the previous 2 weeks), increased stock density, and cooler drier weather and irregular ground frosts. Also, loam and sandy soils are associated with an increased risk of EGS, and abundance of *Ranunculus* species and higher herbage iron, lead, arsenic, and chromium concentrations, as well as increased soil nitrogen and high titatium/low zinc and high titanium/low chromium concentrations have been found in EGS pastures.[780,784] Although a previous occurrence of EGS on the premises is associated with an increased risk of diagnosing EGS on that premises again, previous contact and/or cograzing with a previous case of EGS appears to decrease the risk of EGS in horses. Also, chalk soil reduced recurrence of EGS. Interestingly, an increased risk of EGS is seen in horses with low serum antibody titers to BoNT/C and to surface antigens of BoNT/C-producing clostridia and *C. novyi* Type A,[785] and horses with higher titers to BoNT/C and BoNT/C-producing clostridia had reduced risk of developing EGS.[786]

Clinical Signs

Acute, subacute, and chronic forms of EGS are recognized, of which clinical signs are correlated with extent of neuronal damage and overlap from one form to the other.[780,787,788] Clinical signs are reflective of dysfunction of the autonomic nervous system, including the enteric nervous system, together with somatic dysfunction. Most horses are depressed, anorexic, dysphagic, and tachycardic. The dysphagia is likely caused by cranial nerve dysfunction and possibly esophageal dysfunction and is recognized by drooling of saliva, feed material in the nares, impacted feed material in the buccal pouches, and difficulty drinking. The drooling of saliva seen in EGS may also be caused by excessive salivation, which has been reported in some forms of human dysautonomia. Contributors to the tachycardia include increased adrenaline and noradrenaline and removal of vagal inhibitory input. Ptosis, sweating, and muscle fasciculations can also be present in all forms of EGS.

Acute Form

Clinical signs are related to acute onset of gastrointestinal ileus. The course of disease is generally less than 48 hours. Signs of abdominal pain are mild to moderate and are caused by gastric and small intestinal distention. Typically these cases produce large quantities of nasogastric reflux. Affected horses are hypovolemic, and the reduced circulating volume may cause death from cardiac failure. Considering the degree of gastrointestinal distention, the severity of abdominal pain is usually less than would be expected. Examination per rectum reveals small intestinal distention and often a mild secondary impaction of the large colon.

Subacute Form

The course of disease is 3 to 7 days, and clinical signs are similar but less severe than in acute EGS. Horses with subacute EGS do not develop gastric or small intestinal distention, and

nasogastric reflux is usually absent. Horses with subacute EGS often have large colon or cecal impactions.

Chronic Form

The course of the disease is weeks to months. Cachexia, with the development of a "tucked up" appearance, is the most prominent clinical abnormality in horses with chronic EGS. In addition, horses will develop evidence of progressive myasthenia, demonstrated by a base-narrow stance, leaning back against walls and weight shifting of the limbs. Unlike in botulism and equine lower motor neuron disease where horses spend more time recumbent, this is not the case in horses with EGS. Furthermore, in EGS muscle fasciculations are also present when animals are lying down, which is typically not the case in botulism or equine lower motor neuron disease. Development of rhinitis sicca is characteristic of chronic EGS and is thought to be caused by loss of innervation of the nasal mucosa. The entire gastrointestinal tract is empty in these horses, and they usually have dry mucus-covered feces in the rectum; the latter is a finding commonly seen in all three forms of EGS. Other clinical signs in animals with chronic EGS are gait abnormalities (short strided), snoring, pica, and penile prolapse. In a later stage, one may observe coat abnormalities such as areas of piloerection, growth of a long coat, and pallor of the coat.

Pathologic Findings

Gross necropsy findings are usually reflective of gastrointestinal dysfunction that was present antemortem.[789] Gross necropsy findings in acute EGS are consistent with gastrointestinal ileus. A large fluid-filled stomach and distended small intestine are present. Secondary impacted ingesta is often present in the large colon and cecum. Splenomegaly and erosions of the esophageal mucosa are other findings in acute EGS. Gross necropsy findings in subacute EGS are less severe and include colonic impaction. One may find inspissated mucus in the lumen of the small colon and rectum and evidence of intraluminal hemorrhage such as inspissated blood or black feces. In horses with subacute and chronic EGS, one may find rhinitis sicca.

Neuronal lesions are most severe in the autonomic ganglia (cranial cervical, stellate, celiacomesenteric) and enteric nerves. Lesions are also found in brainstem nuclei and the somatic efferent lower motor neurons of the spinal cord.[787-789] Specific histopathologic findings characteristic of neuronal degeneration include chromatolysis, loss of Nissl's substance, eccentricity or pyknosis of the nuclei, neuronal swelling and vacuolization, accumulation of intracytoplasmic eosinophilic spheroids, and axonal dystrophy. Although the locations of neuropathologic lesions found in EGS are very similar to those found in dysautonomias of other species, including finding lesions in brainstem nuclei and spinal cord, this latter fact gives rise to the question of whether EGS is a true dysautonomia or a multifocal neurologic disease.[780] This similarity in anatomic distribution of neuropathologic changes in EGS supports the hypothesis of a common factor sensitizing the neurons to specific insults. The insult in EGS appears to be specific and repeatable, consistent with findings in primary dysautonomias in other species.

The extent of lesions of the enteric nervous system largely determines the severity of disease, with most severe lesions seen in acute EGS. Specific lesions include neuronal loss in both the submucosal and myenteric plexuses throughout the gastrointestinal tract and reduction of interstitial cells of Cajal in the myenteric plexus regions of the gastrointestinal tract. Neuropathologic lesions within the autonomic nervous system have been reported at the ciliary ganglion, cranial cervical ganglion, caudal cervical ganglion, stellate ganglion, thoracic and abdominal sympathetic trunk, celiac/cranial mesenteric ganglion, caudal mesenteric ganglion, and parasympathetic terminal cardiac ganglion. Furthermore, CNS lesions have been identified in brainstem nuclei, spinal cord lower motor neurons, and spinal cord intermediolateral horn neurons.[780] The association between central neuronal pathologic findings and severity of clinical disease is unclear. In one study, increased neuronal pathologic findings were observed in milder clinical cases (chronic EGS), which contrasts to another study that demonstrated increased neuronal pathologic findings in acute cases of EGS.[790]

Pathogenesis

Clinical signs are related predominantly to dysfunction of the autonomic nervous system. The most severe lesions are found in the myenteric and submucosal plexuses of the ileum, and less severe changes occur in the celiacomesenteric ganglion. The altered autonomic activity results in cessation or decrease of intestinal peristalsis and subsequently leads to the development of ileus and colonic impaction. The dysphagia in most cases of EGS is caused by cranial nerve or brainstem involvement. Based on epidemiologic and pathologic findings in horses with EGS, an unidentified neurotoxin is implicated as the causative agent of the disease. Epidemiology supports the role of an ingested soil-borne agent capable, under certain conditions, of producing or liberating a putative neurotoxin. Some of the pathologic findings support retrograde axonal transport of the toxin from the gastrointestinal tract to the ganglia, whereas there are other studies that suggest that the widespread nature of the neuropathology in EGS is also consistent with more extensive distribution of the toxin, for example, via hematogenous dissemination.[780]

Multiple experimental studies over the past decades have failed to identify the causative agent or agents of EGS. Agents studied include alsike clover, *C. perfringens* enterotoxicity, insect vectors, and fungi. Toxicoinfection with BoNT/C-producing clostridia was proposed as an etiology for EGS in the 1920s,[776] and it has dominated recent research efforts as well.[780,786,791-795] The hypothesis is that a dietary trigger induces intestinal bacterial overgrowth and in vivo production of BoNT/C and/or BoNT/D. Despite the overwhelming evidence to support an association between BoNT-producing clostridia and EGS, there are a number of significant differences between the clinical and pathologic findings of botulism and EGS. Vaccine trials are under way in Britain and perhaps the results of those will provide answers about the involvement of BoNTs in the pathogenesis of EGS.[795,796] Given the differences between EGS and botulism, investigators are currently focusing investigative efforts on potential roles of other etiologic agents, for example, *Fusarium* spp. that are known to produce mycotoxins.[792,795]

Diagnosis

No noninvasive definitive test exists to obtain an antemortem diagnosis of EGS. A presumptive diagnosis is made based on the history, epidemiologic information, nature and progression of clinical signs, and elimination of other possible

diagnoses. Serum biochemical and hematologic examinations do not reveal specific changes in horses with EGS. Acute phase proteins and fibrinogen concentrations are significantly higher in horses with EGS compared to those with intestinal tract obstructions.[797] Other ancillary diagnostic tests that may be helpful include evaluation of esophageal motility by endoscopy or contrast imaging, electrodiagnostic testing, and the use of 0.5% phenylephrine applied to the cornea to confirm the presence of smooth muscle paralysis underlying the ptosis (ptosis should temporarily disappear).[798-800] A definite antemortem diagnosis can only be made following histopathologic examination of enteric ganglia.[787,801]

Because antemortem diagnosis requires collection of biopsies via exploratory laparotomy, often taken at the ileum, investigators have been evaluating alternative biopsy sites. Histopathologic examination of rectal biopsies was investigated as a less invasive and cost-prohibitive diagnostic tool; however, the sensitivity of this approach was disappointing.[802] Recently histopathologic examination of gustatory papillae via a lingual biopsy technique was evaluated in postmortem specimens of horses with EGS and was shown to have a sensitivity of 100% and a specificity of 98.2%.[803] This approach has the potential to offer a valuable and cost-effective means of antemortem disease confirmation but requires further prospective validation.

The most important differential diagnoses for acute EGS are a small intestinal strangulating lesion and duodenitis-proximal jejunitis. Clinical signs and neuronal lesions in chronic EMND and chronic EGS may be similar, and these similarities have led to the speculation that these two diseases are related. Further examination, however, reveals important differences such as the fact that EMND occurs in older horses that have not had access to pasture, and EGS occurs in young horses with access to pasture. EGS sometimes occurs along with EMND.[804]

Prognosis, Treatment, and Prevention

The case fatality rates for acute and subacute EGS are 100%. No effective cure exists. Horses with acute EGS may respond initially to gastric decompression, analgesic administration, and intravenous fluid therapy, and this supportive treatment can be provided until a more definitive diagnosis is achieved, at which point euthanasia should be recommended. The clinical signs that are generally associated with a grave prognosis include persistent gastric reflux or the presence of firm colonic impactions, both reflective of severe intestinal dysmotility resulting from extensive neuronal degeneration within the ENS and generally accepted as supporting a diagnosis of acute and subacute EGS, respectively.

Horses with chronic EGS often are euthanized because of weakness, inability to stand, and emaciation; however, when given appropriate care at a referral hospital, approximately 40% to 50% of horses may survive long term.[787,805] Nonsurvival is associated with severe rhinitis sicca and high subjective clinical scores based on the severity of dysphagia, anorexia, colic, and reduction in intestinal sounds.[806] More recently it was shown that rapidity and magnitude of body-weight loss were predictive of outcome, with nonsurvivors losing more weight than survivors.[807] The greatest obstacle for treatment of horses with chronic EGS is the profound anorexia that is often present. Providing palatable high-quality food is the cornerstone for treatment of chronic EGS. In addition, use of analgesics, antimicrobials, and providing light exercise may be indicated for selected cases. Unfortunately there has not been a clear benefit of using ancillary treatments such as appetite stimulants and prokinetics in the management of horses with chronic EGS.[780]

LYME DISEASE IN HORSES

Stephen M. Reed

Lyme disease is the most common vector-borne infectious disease in human beings in the United States. Initially recognized in the mid-1970s in Lyme, Connecticut, as the cause of unexplained rheumatoid arthritis in children, the causative agent of Lyme disease was discovered to be a spirochete, *Borrelia burgdorferi (sensu lato).* In addition to human beings, Lyme disease affects domestic animals such as dogs, cats, cattle, and horses.[808-814]

Human beings and animals acquire Lyme disease by transmission of *B. burgdorferi* through the bite of infected hard ticks (*Ixodes* spp.). In the Eastern and Midwestern United States the vector is the blacklegged tick or deer tick, *Ixodes scapularis* (formerly *I. dammini*), whereas in the Western United States the vector is the western blacklegged tick, *I. pacificus.* In Europe the sheep tick, *I. ricinus,* is the vector of Lyme borreliosis.

Not all ticks are infected with the spirochete, and infection varies by tick species and geographic region. These ticks have a 2-year, three-stage life cycle and feed once during each stage.[815,816] The larvae hatch in the spring and summer and are usually noninfective because transovarial transmission is rare.[817] The tick may become infected at any stage of the life cycle by feeding on small mammalian hosts, typically the white-footed mouse *(Peromyscus leucopus),* which is a natural host for the spirochete. The nymphal stage emerges the next spring and is most likely to transmit the disease because it is small, difficult to see, and engorges faster; engorgement is necessary to transmit *B. burgdorferi.* The white-tailed deer *(Odocoileus virginianus)* is the host for adult ticks. The life cycle of *I. ricinus* in Europe involves birds and mammals because immature stages feed on birds and reptiles. Nymphal *I. scapularis* are the main vectors of *B. burgdorferi* in human beings. These ticks are more active from May to July. Once the tick has engorged, *B. burgdorferi* is transmitted to the host via lymphatics or blood. Although larval and nymph stages are responsible for *B. burgdorferi* transmission to other mammals, the stage responsible for transmitting Lyme disease in horses is unknown.

Epidemiology

In endemic areas of the northeastern and midwestern United States, approximately 20% of nymphal stages and 30% to 40% of adult stages of *Ixodes scapularis* are infected with *Borrelia burgdorferi.* In contrast, *I. pacificus* often feeds on lizards that are poor reservoirs for *B. burgdorferi,* and only 1% to 3% of these ticks, including the nymphal stages, are infected with the spirochete.[817] This difference in the number of infected ticks might explain the difference in the prevalence of Lyme borreliosis between the eastern and western United States. Equine seroprevalence is high in the northeastern United States, but limited information is available for other parts of the country.[809,813] In Europe, clinical cases and equine serorologic surveys have documented Lyme borreliosis as a cause of equine disease in Poland, Germany, Scandinavia, and England.[812,818,819]

The apparent increasing incidence of disease in human beings and animals might result from an increased deer population, increased number of ixodid ticks, expansion of human and horse populations into previously rural woodland areas, or increased recognition of the disease manifestations.

The disease has a seasonal prevalence and is most common in spring, summer, and fall, with a peak incidence in June and July. In some climates such as California, ticks might be active throughout the year. The organism is maintained in a complex life cycle of small wild mammals and immature stages of the blacklegged tick. Larval and nymphal stages of the tick acquire the infection when they feed on infected mice. The white-tailed deer *(Odocoileus virginianus)* is not a reservoir for Lyme disease but rather the host for the adult stages of the tick. This is relevant to tick and Lyme disease control programs, because regional reductions in the number of *I. scapularis* has only been possible when the deer population has been decreased.[815]

Clinical Signs

Clinical signs associated with *Borrelia burgdorferi* in horses, as in human beings, are often nonspecific and involve multiple body systems. In human beings, clinical signs frequently include annular rash, erythema migrans, myalgia, aseptic meningitis, arthritis, and seventh cranial nerve palsy.[817] The disease usually begins as a skin rash and often progresses to involve joints and the nervous and cardiac systems but may involve other body systems as well. In human beings the skin rash may be slowly progressive and sometimes may take up to 1 month before becoming apparent.

In horses, reported clinical signs of Lyme borreliosis include chronic weight loss, sporadic lameness, laminitis, low-grade fever, swollen joints, muscle tenderness, anterior uveitis, encephalitis, and abortion.[810,811,820] Some horses might demonstrate clinical illness and obtundation and might go off feed in a short time. The bacteria might enter the CNS within a short time after exposure. Chronic arthritis might develop as a result of autoimmune mechanisms, although this is not fully understood. *Ixodid* spp. ticks are often coinfected with *Borrelia burgdorferi* and *Anaplasma phagocytophila;* however, dual infections remain to be documented in the horse. An important consideration is that limb swelling and response to tetracycline treatment, indicating Lyme borreliosis could be the result of *A. phagocytophila* infection. Infection with *Borrelia burgdorferi* is common but rarely results in neuroborreliosis. Neurologic signs could be variable. These signs might include behavioral changes, hyperesthesia, hyperreactivity, gait abnormalities, cranial nerve deficits, neck stiffness, muscle atrophy and muscle tremors or fasciculations.[821]

Diagnosis

The diagnosis of Lyme disease is often difficult. History of tick exposure or living in a Lyme disease–endemic area is helpful; when combined with the identification of clinical signs and the elimination of other diseases, this information allows the clinician to make a presumptive diagnosis. Examination of blood work for other diseases is important. In human beings an early increase in serum IgM often occurs. Response to therapy might help to support a presumptive diagnosis of Lyme disease, but it is not definitive. Diagnosis of Lyme disease in horses is difficult,[822] and in many cases one bases a presumptive diagnosis on history, clinical signs, and response to antibiotic therapy in an animal with probable cause to be infected (i.e., possible exposure). In human beings the skin lesions are obvious. Blood tests are of limited value; however, ELISA, kinetic ELISA, Western blot testing, and PCR on blood samples and synovial fluid from suspect animals have been evaluated.[809,812-814,822,823] One may test samples of joint fluid, CSF, and tissues from affected patients for the presence of organisms. Several pathologic conditions should be considered in the differential diagnosis of equine borreliosis, including *Anaplasma phagocytophila* infection, chronic diseases, vasculitis, and immune-mediated arthritis if limb or periarticular swelling is present, as well as causes of neurologic disease.

Neuroborreliosis is rarely diagnosed. CSF reveals neutrophilic or lymphocytic pleocytosis and increased total protein. Other tests include culture of the organism, immunohistochemistry, PCR of tissue samples and CSF, and others previously described. More recently a fluorescent bead–based multiplex assay (Multiplex) in serum and CSF has been developed for the diagnosis of borreliosis and neuroborreliosis.[824] Multiplex assay tests serum at a dilution of 1:400 whereas CSF is generally run undiluted. In serologic assays, a positive result indicates past or present infection (exposure) and not necessarily cause of clinical disease, and vaccination can also cause a positive result. Horses living in endemic areas are often seropositive. Development of an antibody ratio test to compare antibody concentration in blood to the concentration in CSF is being evaluated and may prove to be useful in the future (Amy Johnson, personal communication).

Pathogenesis

Borrelia burgdorferi organisms are capable of nonspecifically activating monocytes, macrophages, and synovial lining cells, as well as natural killer cells, B cells, and complement, leading to production of host proinflammatory mediators. These proinflammatory mediators seem to localize in joints, leading to chronic arthritis and lameness. *B. burgdorferi* has developed strategies to interact with the mammalian host, including adhesion to host cells and components of the extracellular matrix such as fibronectin, β_3 integrins, and glycosaminoglycans. The current thinking in the development of arthritis is that outer-surface proteins (Osps) of *B. burgdorferi* trigger an autoimmune reaction because antibodies to OspA and OspB have been detected in 50% to 80% of human patients with arthritis.[825,826] Natural killer cells also play a central role in joint inflammation by producing excessive amounts of TNF-α and IL-8.[825]

Treatment and Prevention

Recommended treatment includes oxytetracycline (6.6 mg/kg IV every 24 hours) or doxycycline (10 mg/kg PO every 12 hours).[827] Treatment can be started with tetracycline for 1 week followed with doxycycline for 3 to 4 weeks. Ceftiofur (2.2–4.4 mg/kg IV every 12 hours) has also been evaluated.[827] In animals infected with *B. burgdorferi,* a rapid clinical response (2–4 days) is expected with tetracyclines. In some horses the clinical signs might show an initial worsening as a response to toxins released after death of the organisms. Currently, no licensed vaccine is available to prevent Lyme disease in horses, although vaccines are available for dogs and human beings. A recombinant OspA vaccine was evaluated in horses with promising results.[828]

Aids to prevention include daily grooming with removal of ticks, along with the use of tick repellents that contain permethrin. One should apply tick repellents to the head, neck, legs, abdomen, and under the tail. Keeping pastures mowed and removing brush and woodpiles makes the environment

less hospitable for rodents, which in turn decreases the tick population. Regional programs to control Lyme disease are based on reducing the deer population.

TRIGEMINAL-MEDIATED HEADSHAKING

Monica Aleman

Idiopathic headshaking has been recognized for over 100 years.[829] The disorder is characterized by spontaneously occurring uncontrolled violent head shakes. One study showed that the maxillary branch of the trigeminal nerve, has a low threshold for firing, therefore even innocuous stimuli could trigger sensory activation and firing of this nerve, causing facial pain.[46] Therefore the authors of the study proposed calling this condition *trigeminal-mediated headshaking* to reflect more accurately the source of pain despite an uncertain etiology.[830] It is important to rule out other causes of headshaking (Table 11.18). Headshaking is a widely recognized disorder characterized by persistent or intermittent, spontaneous, and frequently repetitive vertical (most common), horizontal, or rotary movements of the head and neck.[831] The affected horse shakes, jerks, or flicks its head uncontrollably in the absence of obvious physical stimuli or apparently innocuous stimuli (i.e., light, wind, exercise), and the condition can be severe enough to render the horse unusable and dangerous.[832-836] Headshaking often is accompanied by snorting and sneezing, and many horses rub their noses on stationary objects or on the ground while moving. In addition to shaking the head up and down, flipping the upper lip, or shaking the head side to side, horses also might act as if a bee has flown up the nostril or might strike at the face with the thoracic limbs.[832,836,837] Headshaking horses also might exhibit avoidance behavior, with low head carriage, corner seeking, and nostril clamping.[831,838] To characterize the severity of clinical signs, a grading system of 1 to 5 has been described, with grade 1 being a rideable horse with intermittent and mild signs (facial muscle twitching) and grade 5 being an unrideable, uncontrollable, dangerous horse with bizarre behavior patterns.[831] Clinical signs are usually worse during exercise; however, headshaking can affect horses at rest.[831-835,837] Severely affected horses might inflict self-trauma and compromise welfare.[830] Mean age of onset is typically 7 to 9 years, and Thoroughbreds and geldings appear to be overrepresented in some studies.[832,833,836] Headshaking can be seasonal or nonseasonal. Early literature suggested an increased incidence of headshaking during the warmer months of the year,[834] and a later study indicated that the peak periods of onset were spring and early summer.[833] Studies have found that 64%[833] and 59%[832] of headshaking horses are affected seasonally, with the majority developing signs in the spring or early summer.

Pathogenesis

It is important to rule out other causes of headshaking before considering trigeminal-mediated headshaking (see Table 11.18).[839] Reported and suggested causes include behavioral resentment to rider-induced head and neck flexion, exercise-induced hypoxia, ear mites, cranial nerve dysfunction, otitis media or otitis interna, cervical injury, ocular disease, guttural pouch mycosis, dental periapical osteitis, vasomotor rhinitis, allergic rhinitis, *Trombicula autumnalis* (harvest mite) larval infestation, maxillary osteoma, and EPM.[833,835,839-844] A study of 100 headshaking horses revealed a potential specific cause in only 11 horses, and elimination of the abnormality resulted in resolution of the headshaking in only 2 of them.[833] Idiopathic headshaking has been used to describe the majority of cases in which no specific underlying cause is found, and necropsy results in these horses reveal no lesions.[845] Further, a study evaluated the possibility of herpesvirus infection as a potential cause of this disorder but found no association.[845] However, immune-mediated reaction or inflammation of the trigeminal ganglia can cause headshaking.

The clinical signs exhibited by most headshaking horses now generally are thought to result from sharp, electric-like, burning pain involving the trigeminal nerve.[831,832] Trigeminal nerve involvement is supported by the observations that some horses

TABLE **11.18** **Potential Causes of Headshaking in Horses**

Disorders of the Oral Cavity
Dental disorders
Pharyngeal lesions
Tongue or gingival lesions
Buccal ulceration
Disorders of the Eyes
Abnormalities affecting vision
Cysts, masses, foreign bodies, cataracts
Nasolacrimal duct disorders
Disorders of the Ear
Otitis
Ear ticks *(Otobius megnini)*
Mites
Mass lesion–neoplasia, abscess, granuloma
Foreign body
Upper Airway Disorders
Nasal passages–e.g., masses
Rhinitis (allergic, vasomotor)
Sinuses–masses, exudate, sinusitis
Guttural pouch disease
Laryngeal disorders
Disorders of the Skull
Fractures
Neoplastic lesions
Temporomandibular joint disorders
Temporohyoid osteoarthropathy
Other disorders of the hyoid apparatus
Neurologic Abnormalities
Trigeminal neuralgia
Equine protozoal myeloencephalitis
Photic headshaking
Cervical Pain
Osteoarthritis
Neuropathy
Myositis
Behavior
Stereotypical behavior
Classical conditioning response
Avoidance behavior
Objections to Rider or Tack
Bridle, bit, browband fit and comfort
Rider actions or interference
Excessive head and neck flexion

improve after blocking the infraorbital nerve (part of the maxillary branch)[846,847] and that most horses improve after blocking the caudal nasal nerve, a branch of the maxillary nerve commonly referred to as the posterior ethmoidal nerve (branch of ophthalmic nerve). Horses that headshake in response to light stimulation have been described as *photic headshakers*.[838] Most of these horses are affected seasonally (spring and summer), improve at night or when brought indoors, and improve when blindfolded or when gray lenses are placed over their eyes. Photic headshaking is hypothesized to be caused by optic-trigeminal summation (stimulation of the optic nerve that results in referred sensation to parts of the nose innervated by the trigeminal nerve), a mechanism similar to that proposed for photic sneezing in human beings.[832,838] Many features of headshaking in the horse are similar to trigeminal neuralgia in human beings—a severe chronic pain syndrome characterized by dramatic, brief stabbing or electric shock–like pain paroxysms felt in one or more divisions of the trigeminal distribution, spontaneously or on gentle tactile stimulation of a trigger point on the face or oral cavity. The disease is associated with microvascular compression and pathologic changes in the trigeminal root and trigeminal ganglion.[848] The trigeminal nerve was postulated to be hypersensitive and to fire in response to a variety of trigger factors, including wind, airway turbulence, increased blood flow, pollen, dust, warmth, cold, insects, allergies, or other irritations. These trigger factors appear to act intranasally in many horses but could act in any trigeminal sensory region.[831] As previously mentioned, there is evidence that the trigeminal nerve has a low threshold for firing.[849]

Diagnosis and Treatment

One should perform a complete physical examination, including neurologic, dental, ophthalmic, and otoscopic examinations, to rule out potential underlying causes (see Table 11.18). Other diagnostics could include endoscopy of the nasal passages, pharynx, and guttural pouches; radiography of the skull and cervical spine; CBC; and serum biochemistry profile. For photic headshaking horses, clinical signs should improve after blindfolding or after placement of a mask shielding the eyes from the sun.

Treatment of trigeminal-mediated headshaking could be challenging as shown in a recent owner survey (Table 11.19).[830] Because the etiology of headshaking is unknown, current treatments are neither specific nor curative; therefore the majority of horses with headshaking are managed, rather than cured. Cyproheptadine, an antihistamine and serotonin antagonist with anticholinergic effects, has been used (0.3 mg/kg PO twice daily) to treat headshaking horses, resulting in improvement in 5 of 7 horses in one study[838] and 43 of 61 horses in another.[832] Horses that respond do so within 1 week, and some might respond within 24 hours. Cyproheptadine also works as a calcium channel blocker in addition to blocking serotonin-mediated pain as a proposed mechanism of action.[838] Cyproheptadine has also been used with carbamazepine (4–8 mg/kg PO q 6–8 h), resulting in 80% to 100% improvement in seven of nine cases, with horses responding within 3 to 4 days.[831] Carbamazepine is a sodium channel–blocking antiepileptic drug and is the drug of choice for treating trigeminal neuralgia in human beings.[850] Carbamazepine alone was reported to be effective in headshaking horses, but results were unpredictable.[831] The elimination half-life of carbamazepine in the horse is less than 2 hours, making sustaining therapeutic concentrations difficult; the drug is therefore of more benefit diagnostically than therapeutically in headshaking horses (D. C. Knottenbelt, personal communication). Other medications and therapies, including NSAIDs, corticosteroids, antihistamines, acupuncture, chiropractic manipulation, homeopathy, and feed supplements, are generally ineffective in most horses.[832,851] Pulse therapy with corticosteroids has not been effective to manage trigeminal-mediated headshaking.[852]

Because the disorder affects more geldings than females and intact males, a study investigated the role of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by using a gonadotropin-releasing hormone vaccine.[853] This vaccine significantly decreased FSH and LH levels but did not affect headshaking in horses.

Diagnostic blockade of trigeminal nerve branches can be useful for identifying trigger points in headshaking horses. Blockade of the infraorbital nerve might improve some horses and might identify candidates for infraorbital neurectomy.[846,847] However, results of infraorbital blockade and infraorbital neurectomy do not correlate, and infraorbital neurectomy is not a recommended procedure because of neuroma formation, self-trauma, risk of infection, low success rate, and recurrence of pain that might require a second surgery.[846] A high percentage of horses improve after blockade of branches of the maxillary nerve, and sclerosis of this nerve results in temporary improvement in some horses.[831] More recently, a study investigated the neuromodulation of the trigeminal nerve by using percutaneous electrical nerve stimulation under sedation. Horses tolerated well the procedure, which proved to be safe and

TABLE **11.19 Drugs Used for the Treatment of Trigeminal-Mediated Headshaking**

Drug	Dosage (mg/kg)	Route	Frequency
Carbamazepine	2–8	PO	q 6–12 hours
Clonidine	0.0025	PO	q 12 hours
Cyproheptadine	0.3	PO	q 12 hours
Fluphenazine	0.1	IM	q 1–4 months
Gabapentin	5	PO	q 8–12 hours
Hydroxyzine	0.6–0.8	PO	q 12 hours
Magnesium	20–40	PO	q 24 hours
Melatonin	0.03–0.04	PO	q 24 hours at 5 pm
Phenobarbital	3	PO	q 12 hours
Sodium chromoglycolate	1 drop	Ophthalmic	q 6 hours

minimally invasive and provided pain relief in the short to medium term (months).[854]

Therapies aimed at decreasing the response to trigger factors can be effective and include tinted contact lenses; face masks or hoods that block sunlight, wind, insects, and dust, with mesh or dark eye cups; and nose nets.[831,832,838,851,855,856] An owner survey found that nose nets that cover the nostrils with mesh and include a drawstring or elastic band that applies pressure to the upper lip resulted in some improvement in 70% of head-shaking horses and that 70% or more improvement occurred in about 30% of the horses.[855]

VERMINOUS ENCEPHALOMYELITIS

Aberrant migration of helminth and fly larvae through the CNS of horses and donkeys is a rare but important cause of severe neurologic disease. Parasites that have been reported to affect the brain and spinal cord of equids include rhabditid nematodes *(Halicephalobus gingivalis),* strongyloid nematodes *(Strongylus vulgaris, S. equinus,* and *Angiostrongylus cantonensis),* protostrongyloid nematodes *(Parelaphostrongylus tenuis),* spiruroid nematodes *(Draschia megastoma),* filarid nematodes (*Setaria digitata* and other *Setaria* spp.), and warble fly larvae (*Hypoderma* spp.).

Antemortem diagnosis is often impossible; however, a high index of suspicion might be warranted for certain clinical signs (acute onset or rapidly progressive asymmetric, focal, or multifocal brain or spinal cord signs) and changes in CSF analysis, in which case the treatment regimen should include specific antiparasitic drugs (e.g., fenbendazole) in addition to the more routinely used symptomatic and antiinflammatory treatments (e.g., NSAIDs, DMSO, corticosteroids, antiprotozoal drugs).

Causes

Halicephalobus gingivalis

Halicephalobus gingivalis was previously known as *Micronema deletrix,* then *Halicephalobus deletrix.*[857] The parasite was referred to as *Micronema deletrix* until the 1970s and 1980s, with use of the new nomenclature beginning in 1990.[857] The life cycle of *Halicephalobus* spp. nematodes is unknown. This small roundworm generally is considered a saprophytic organism that occasionally acts as a facultative parasite in horses and human beings. The likely route of entry is through nasal and oral mucosa, followed by possible hematogenous spread to organs with high vascularization such as the brain, spinal cord, and kidneys.[858] *H. gingivalis* recently has been shown to be transmitted from a mare to her foal because of mammary infestation.[859] CSF is abnormal, showing mixed pleocytosis with elevated protein concentration. In one study, *H. gingivalis* was observed on cytologic examination of CSF.[860]

Organs affected by migration of *Halicephalobus* spp. include the brain, spinal cord, nasal and oral cavities, pituitary gland, and kidneys and less commonly the lymph nodes, heart, lungs, stomach, liver, and bones.[248,861] Organisms affecting the CNS have been reported to have a predilection for the basilar, pituitary region of the brainstem.[862] Characteristic histopathologic lesions in the CNS include malacia, granulomatous, and lymphohistiocytic inflammatory infiltration, meningitis, and vasculitis in addition to identification of the nematodes. Other clinical signs include oral and nasal granulomata, renal involvement (*H. gingivalis* is visible in the urine), granulomatous osteomyelitis,[863] and granulomatous chorioretinitis.[864]

Most of the cases of *Halicephalobus* encephalomyelitis in adult horses reported in the literature during the past 40 years describe simultaneous renal granulomatous lesions encapsulating the nematodes.[248,859,861-874] Granulomata within or adjacent to the renal parenchyma were observed on postmortem examination in 13 of 16 horses with neurologic disease; of the other 3 horses, in one case lesions were confined to the sacral spinal cord and cauda equine, and in another case only the skull and brain were examined at postmortem examination. In contrast, all three cases reported to date in foals showed no renal involvement but did show pulmonary granulomata.[859,862]

Strongylus vulgaris, Strongylus equinus, and Angiostrongylus cantonensis

Aberrant strongyle larval migration is a much less common cause of neurologic signs because of routine broad-spectrum antiparasitic treatment with ivermectin and moxidectin. The pathogenic mechanisms described for strongyle encephalomyelitis include aberrantly migrating fourth- and fifth-stage larvae in the intima of the aorta or left ventricle, which causes endothelial damage, stimulates the clotting cascade, and results in formation of a thrombus that often contains the parasitic larva.[875] Lesions are generally asymmetric because of random migration in the brain, although migration along the spinal cord has been reported in a donkey.[500,875]

Adult parasites of *Angiostrongylus cantonensis* are found in the right ventricle and pulmonary artery of rats. The pulmonary circulation carries eggs released by adult worms to the alveoli, where the first larval stage develops and migrates up the trachea, is swallowed, and passes in feces. Snails and slugs are intermediate hosts, and ingestion of the snail results in ingestion of the infective larvae that migrate through the CNS, and finally the larvae reach the heart via the circulation. Aberrant CNS migration of *A. cantonensis* larvae has been reported in two foals with tetraparesis in Australia,[876] and aberrant migration is a recognized cause of eosinophilic meningoencephalitis in human beings and dogs.[877]

Parelaphostrongylus tenuis

The meningeal worm *Parelaphostrongylus tenuis* causes neurologic disease in cervids, ovids, bovids, and camelids. There have been multiple reports of similar disease in horses ranging in age from 6 months to 9 years.[5,878,879] Affected horses have a history of sudden onset of scoliosis with no history or evidence of trauma.

Draschia megastoma

Adult *Draschia megastoma* worms are found in the stomach of equids where they cause mucosal granulomatous masses and mild chronic gastritis. The life cycle of *Draschia* is indirect because the organism uses flies as the intermediate host. Eggs and larvae are released into the gastric lumen, and the first larval stage passes in the feces and is ingested by fly larvae of the genus *Musca* in which *Draschia* spp. organisms develop to infective larvae. Finally, horses become infected when the third larval stage is deposited on the host by adult flies. Infective larvae that are ingested and reach the stomach develop into adults and complete the life cycle. Larvae deposited in damaged skin result in local inflammation and development of extensive granulation tissue, which is the typical lesion of cutaneous habronemiasis.

Stray *D. megastoma* larvae may be found anywhere throughout the body, and a case has been reported of *D. megastoma* migration in the brainstem of a horse in the southern United States, resulting in asymmetric brainstem disease.[880]

Setaria Species

Infestation with the filarial nematode *Setaria* is common in the abdominal cavity of ungulates where the organism does not cause significant clinical effects. *S. labiatopapillosa* is found in cattle, and *S. equina* is found in horses. Microfilariae gain access to peripheral circulation and then are transmitted to other potential hosts via mosquitoes. In a postmortem examination study of 305 horses in Japan, *S. equina* was recovered from 66 of those horses (approximately 22%).[881]

The cattle parasite *S. digitata* occurs only in Asia, and infestation in unnatural hosts (horses, sheep, goats, camels, and human beings) has been associated with cerebrospinal nematodiasis. This condition can be enzootic in India, Burma, and Sri Lanka, is called *Kumri* (weak back), and has a seasonal occurrence, usually during the fly season (late summer and fall).[882]

Two cases of aberrant *S. digitata* CNS migration have been reported in Japanese racehorses, and one case of *Setaria* spp. larval migration in the brainstem and cervical spinal cord has been reported in a 12-year-old Quarter Horse mare in the midwestern United States.[881,883]

Hypoderma Species

Cattle are normal hosts of *Hypoderma* spp., but horses are accidental hosts of warble fly larvae. In cattle, *Hypoderma* larvae penetrate the skin after hatching from eggs attached to hair in the rump and lower legs. These larvae migrate through connective tissues to reach the esophagus, in the case of *H. lineatum,* or to the vertebral canal around epidural fat, in the case of *H. bovis.* Finally, the larvae migrate back to the skin over the back where they create a breathing hole. Cutaneous myiasis is uncommon in horses, and warble fly larvae occasionally might migrate aberrantly into the brain. Tissue damage causing necrosis and hemorrhage is extensive because of the big size of the instars.[884-886] Instars can enter through large natural foramina such as the foramen magnum, optic foramen, and occasionally intervertebral foramina.

Clinical Signs

Severity and duration of clinical signs vary from mild, transient, and insidious to severe and fatal. In some cases, clinical signs progress over 2 to 4 months, with periods of improvement or stabilization.[861,865,880] Variability of clinical signs depends on the number of parasites (thromboembolic shower of *S. vulgaris*), on the size of the migrating organism (*Hypoderma* spp. larvae are large and cause severe necrosis and hemorrhage as they migrate in the CNS parenchyma), and neuroanatomic localization of the lesions. Horses suffering from larval migrations in the brain (*S. vulgaris, Halicephalobus deletrix, Draschia megastoma,* and *Hypoderma* spp.) might display head tilt, circling, recumbency, blindness, hyperesthesia, stiff neck, ataxia, head pressing, recumbency, seizures, and coma. In those cases in which larvae cause lesions in the spinal cord (*S. vulgaris, H. gingivalis, Setaria* spp., *Angiostrongylus cantonensis*), clinical signs might include focal or multifocal asymmetric ataxia, weakness, dog-sitting posture caused by paraparesis, increased patellar reflexes, atonic bladder, poor tail tone, and poor rectal tone with impaction of feces. A case of cauda equine neuritis was reported to be caused by *H. gingivalis* migration in the sacral spinal cord and cauda equina nerves.[871]

Clinical signs of *Parelaphostrongylus tenuis* infection are quite characteristic and include acute onset of scoliosis (observed in 90% of reported cases), with progressive gait deficits. Scoliosis is most common in the cervical area.[5,878,879] The affected area of the vertebral column is usually C shaped, with flaccid muscles and decreased cutaneous sensation on the convex side of the curve. Tetraparesis and ataxia is mild to moderate and predominantly ipsilateral to the convex side of the curve. If bilateral gait deficits are observed, they are worse on the convex side. The proposed pathogenesis for clinical signs involves unilateral weakness of the paraspinal epaxial muscles on the convex side of the curve.[879] A linear lesion that extends for several segments, most likely in the dorsal gray column, is most likely to cause the observed signs.

Differential Diagnosis

One should consider verminous encephalomyelitis in all cases of acute or progressive asymmetric disease of the spinal cord, cerebrum, cerebellum, or brainstem. If brain involvement is evident without other localizing signs, the differential diagnosis list might include equine togaviral encephalomyelitis, rabies, equine protozoal myelitis (EPM), trauma, cerebral abscess or basilar epidural empyema, bacterial meningitis, hepatic encephalopathy, neoplasia, and leukoencephalomalacia. If the neurologic signs are limited to spinal cord involvement, other diseases to include in the differential diagnosis list might be cervical stenotic myelopathy, EPM, EHV-1 myeloencephalopathy, trauma, WNV meningoencephalitis, EDM, trauma, spinal osteomyelitis or discospondylitis, vertebral fracture, and neoplasia. If the only signs present are related to cauda equina syndrome, other conditions to consider are polyneuritis equi, sacral or coccygeal fracture, EPM, EHV-1 myeloencephalopathy, sorghum or Sudan grass toxicity, epidural abscess from tail blocking, and neoplasia.

Initial diagnostics should include CBC, serum chemistry profile, urinalysis, and CSF collection for cytologic evaluation. Radiographs of the cervical or lumbosacral spine and myelography might be required to rule out more common causes of spinal cord disease (cervical stenotic myelopathy, trauma, fractures). Although only routinely available at referral hospitals, advanced imaging techniques (e.g., CT, MRI) might be useful in diagnosing parasitic encephalomyelitis or other conditions with similar clinical signs.

CSF analysis in cases of parasitic encephalomyelitis can be normal; however, CSF changes are common and include xanthochromia, increased protein, and neutrophilic and mononuclear pleocytosis, but eosinophils rarely occur (Table 11.20). Only in a case of *Angiostrongylus cantonensis* were eosinophils the predominant cell type on CSF cytologic examination. In addition, *Halicephalobus gingivalis* might be observed in CSF subjected to cytocentrifugation.[860,862] Similarly, *H. gingivalis* larvae might be visible on microscopic examination of urine sediment or semen in cases of renal and testicular involvement.[861] Analysis of CSF obtained from horses with *Parelaphostrongylus tenuis* neurologic disease has been within normal limits.[879] PCR is a highly sensitive diagnostic modality that can aid in the definitive diagnosis of disease as reported in one case of a horse with verminous encephalitis due to *P. tenuis.*[878]

TABLE **11.20** Cerebrospinal Fluid Analysis in Verminous Encephalomyelitis

Organism	White Blood Cells in CSF	Protein in CSF	Cells in CSF	Larvae Present in Urine	Reference
Halicephalobus	2030 cells/μL	89 mg/dL	Mostly PMNs	—	865
Halicephalobus	25 cells/μL	69 mg/dL	15% N, 56% L, 22% M, 5% E, 2% B	—	868
Halicephalobus	81 cells/μL	114 mg/dL	9% N, 41% L, 50% M	—	868
Halicephalobus	60 cells/μL	710 mg/dL	—	—	869
Halicephalobus	495 cells/μL	112 mg/dL	34% N, 37% L, 29% M	No	24
Halicephalobus	—	—	—	Yes	860
Halicephalobus	—	—	—	No	873
Halicephalobus	179 cells/μL	71 mg/dL	Predominantly N; few L, M, and E	No	861
Halicephalobus	35 cells/μL	100 mg/dL	25% N, 58% M, 17% E, 2% L	No	248
Halicephalobus	16 cells/μL	76 mg/dL	31% N, 22% L, 47% M	—	858
Setaria	Increased	Increased	—	—	874
Strongylus	Two of eight ponies had increased white blood cell counts: 42 cells/μL and 1080 cells/μL	One of eight ponies had high protein at 175 mg/dL	—	—	874
Strongylus	9988 cells/μL	550 mg/dL	72% N, 14% L, 12% M, 2% E	—	500
Draschia	Normal	Normal	—	—	876
Angiostrongylus	1560 cells/μL	—	1% N, 8% L, 14% M, 77% E	No	877

B, Basophils; *CSF,* cerebrospinal fluid; *E,* eosinophils; *L,* small lymphocytes; *M,* large mononuclear cells; *N,* neutrophils; *PMN,* polymorphonuclear neutrophil leukocytes.

Antemortem diagnosis might be possible in those cases in which renal or bony involvement is detected and nematodes are identified in biopsies of the affected tissues.[863] As described previously, renal involvement with granulomatous lesions encapsulating *Halicephalobus gingivalis* worms is observed in most cases of *H. gingivalis* encephalomyelitis. Therefore transabdominal renal ultrasound examination and ultrasound-guided biopsy (in those cases with renal lesions present), as well as microscopic examination of urine sediment, might prove useful in cases of acute-onset progressive asymmetric neurologic disease, especially if one suspects cerebrospinal nematodiasis caused by *H. gingivalis.*

Additionally, a PCR-based diagnostic test has been developed for confirmation of *Setaria* encephalomyelitis in goats, sheep, and horses.[887] This test is based on specific amplification of *Setaria* spp. filarial DNA from a blood sample from the host.

Pathologic Findings

The gross and histopathologic lesions depend on the parasite involved. Thorough postmortem examination with sectioning and histopathologic examination of all areas of the CNS relevant to the antemortem clinical signs are important to identify migrating parasites. *Strongylus vulgaris* and *Hypoderma* spp. larvae are easy to see, but other nematodes are only visible on microscopic examination. Some reports describe how one may recover and examine whole, fixed nematodes for distinctive morphologic features by centrifugation of formalin solution in which the brain had been fixed.[866,873] Gross examination of other tissues for evidence of *S. vulgaris* thrombi or presence of *Halicephalobus gingivalis* granulomatous lesions might help establish a postmortem diagnosis.

On histopathologic examination, one generally sees extensive tissue necrosis with mixed inflammatory response. *H. gingivalis* migration in the CNS typically results in histiolymphocytic infiltrates, malacia, glial proliferation, and perivascular lymphocytic cuffing. Migrations of warble fly and *S. vulgaris* larvae result in severe hemorrhage, large malacic tracts, and edema caused by their relatively larger size.

Treatment

Treatment of verminous encephalomyelitis in horses is often unrewarding. None of the cases reported in the literature has responded favorably to antiinflammatory drugs and anthelmintics. In one horse with a *Halicephalobus gingivalis* granuloma limited to the prepuce, therapy with ivermectin and diethylcarbamazine was successful.[888]

Therapeutic recommendations depend on the severity, progression, and localization of neurologic signs, as well as on consideration of possible contraindications (e.g., if one suspects a bacterial or viral cause, one should avoid using corticosteroids). In cases of acute neurologic disease use of the following antiinflammatory drugs might be warranted: NSAIDs such as phenylbutazone (2.2 mg/kg twice a day), flunixin meglumine (1 mg/kg twice a day), or ketoprofen (2 mg/kg twice a day); intravenously administered DMSO (1 g/kg as a 10% solution once daily for 3–5 days); and corticosteroids (dexamethasone at 0.1–0.25 mg/kg once daily or prednisolone

at 0.2–4.4 mg/kg once daily). If cerebral and/or brainstem signs are observed, one might administer mannitol (0.25–2 g/kg as a 20% solution once daily), or hypertonic saline might be warranted in an attempt to minimize cerebral edema. In countries in which EPM is known to occur, antiprotozoal treatment is recommended.

Specific antiparasitics suggested for treating verminous encephalomyelitis include benzimidazole compounds (oxfendazole, thiabendazole, fenbendazole, and mebendazole), diethylcarbamazine and ivermectin for the treatment of nematodes, and organophosphates with caution (trichlorfon and dichlorvos) for the treatment of warble fly larvae. Although ivermectin is effective against most equine parasites, it might not be the best choice because of its delayed method of killing, which might take as long as 10 to 14 days. Some authors have suggested administration of fenbendazole at 50 mg/kg by mouth once daily for 3 days[4,501]; however, specific data on the efficacy of anthelmintics in treating nematode or warble fly larvae migration through the CNS are not available.

MISCELLANEOUS NEUROLOGIC DISORDERS

Debra C. Sellon

In addition to the numerous neurologic problems discussed in detail earlier in this chapter, horses can develop a wide variety of acute or chronic neurologic disorders with clinical signs related to brain, spinal cord, or nerve dysfunction. Clinical signs can occur because of primary disease or dysfunction of the CNS or secondary to a variety of metabolic disorders or disorders of other body systems. This section will mention the most important of the miscellaneous CNS disorders and refer the reader to relevant chapters for more detailed information.

Neurologic Neoplasia

Many types of neoplastic lesions have been identified in the brain and spinal cord of horses. A comprehensive review of the clinical and pathologic aspects of equine CNS neoplasia, however, is lacking in the peer-reviewed veterinary literature. Available case reports and summaries of pathologic studies suggest that CNS neoplasia is quite uncommon in horses. In one Australian survey of 450 horses, with neurologic disease, the prevalence of neurologic disease secondary to neoplasia was less than 2%.[141] This percentage is likely to be much lower in horses in the United States because of the much higher incidence of encephalitic disorders and EPM.

Tumors of the nervous system can be classified on the basis of their cellular origin as tumors of nerve cells, neuroepithelium, glia, nerves and nerve sheaths, mesodermal structures, and endocrine organs. Neoplasms of nerve cell origin appear to be extremely uncommon in horses, with only a few reports of ganglioneuromas, complex tumors arising in peripheral ganglia and composed of well-differentiated neurons, nerve processes, Schwann cells, and glial cells.[889,890] These tumors might cause intestinal obstruction and signs of colic in affected horses.

Tumors of neuroepithelial origin in horses include ependymoma, choroid plexus papilloma, neuroepithelial tumor of the optic nerve, malignant medulloepithelioma, ocular medulloepithelioma, and pineoblastoma.[891-906] Glial cell tumors include glioblastoma multiforme, optic disc astrocytoma, retinoblastoma, and microglioma.[900,906-908] Neoplasms of nerves and nerve sheaths appear to be more common than CNS neoplasia and include neurofibromas and neurofibrosarcomas.[909-914] The most common sites for neurofibromas are in the cutaneous tissues, of the pectoral region, abdomen, neck, and face, but gastrointestinal tract lesions have also been described.[909] There are also reports of intracranial and mediastinal schwannomas in horses.[915,916]

The most common mesodermal neoplasm in horses appears to be meningioma.[892,905,917] Neoplastic reticulosis, lipoma in the mesencephalic aqueduct, angioma in the cervical spinal cord, primary meningeal lymphoma, and melanoblastoma of the cerebellar meninges have also been described.[918-920]

Older texts may refer to pituitary pars intermedia dysfunction (PPID) of older horses as a neoplastic lesion. This disorder is most likely a manifestation of oxidative damage to dopamine-producing neurons in the CNS with subsequent dysregulation of hormone production within the pars intermedia of the pituitary gland. PPID is discussed in detail in the Endocrinology chapter (Chapter 16).

Cholesterol granulomas (cholesteatomas, cholesterinic granulomas) are likewise not neoplastic lesions.[921-929] These lesions are found in the choroid plexus of up to 20% of older horses.[930] Although more common in the choroid plexus of the fourth ventricle, lesions in the lateral ventricles may be more likely to result in clinical signs. Lesions might represent a chronic granulomatous reaction to deposition of cholesterol crystals associated with chronic choroid plexus leakage. Grossly the granulomas are circumscribed, firm, granular, and yellow-brown with a glistening cut surface. They are most often recognized as incidental findings at necropsy of horses without noticeable clinical signs. If they are large enough, however, they might cause CNS signs as a result of direct pressure on cerebral tissues or obstructive hydrocephalus. In affected horses, reported clinical signs have included altered behavior, obtundation, somnolence, seizures, ataxia, weakness, and coma. CSF from affected horses might have an elevated protein and appear xanthochromic.[921-923]

Secondary neoplastic conditions affecting the CNS might penetrate the cranial vault or vertebrae, extend through osseous foramina, or metastasize via the vascular system. Clinical signs vary depending on the type, site, and extent of the lesion. Lymphoma is probably the most common secondary neoplasm of the CNS in horses.[920,931-936] Lesions might cause forebrain signs if present in the cranial vault or ataxia and paresis if causing compression of the spinal cord.

Melanoma of the spinal cord, meninges, and brain may occur with metastasis of primary cutaneous lesions.[937-939] Alternatively, clinical signs may represent spread from affected sublumbar lymph nodes.[920,940] Melanoma of the CNS is most common in gray horses.

Hemangiosarcoma and undifferentiated sarcoma have been reported to affect the CNS of horses.[920,941-943] There are numerous descriptions of adenocarcinomas and carcinomas (including squamous cell carcinoma) affecting the CNS of horses by direct spread from a primary site in the head or by metastasis from a distant site.[920,944-950] Tumors of the bone or bone marrow might involve the skull or vertebrae, resulting in compression of the brain or spinal cord, respectively.[920,951-953]

Malformation tumors, including epidermoids, dermoids, teratomas, and teratoids, might also affect the CNS.[920] They are often incidental findings at necropsy but might occasionally be clinically significant.

Supportive evidence for the presence of CNS neoplasia can be obtained with advanced imaging (i.e., CT, MRI) of the brain or spinal cord in select equine patients. Definitive diagnosis of primary CNS neoplasia in horses requires cytologic or histopathologic identification of neoplastic cells or tissues. This diagnosis necessitates biopsy, cytologic evaluation of an aspirate from a suspect lesion, or identification of neoplastic cells in CSF. Analysis of CSF is rarely diagnostic of neoplasia in horses because of the rarity of the presence of neoplastic cells in the fluid. Given the practical difficulties inherent in obtaining biopsy samples from the brain or spinal cord, definitive diagnosis of primary CNS neoplasia in horses rarely occurs antemortem. Definitive diagnosis is more likely in horses with secondary CNS neoplasia in which a diagnosis is made by biopsy or cytologic evaluation of primary lesions identified external to the CNS.

Toxic Disorders

A wide variety of toxic substances can affect the CNS of horses. Most of these are discussed in detail in the Toxicology chapter (Chapter 21). Three toxic neurologic conditions merit mention in this chapter because of their common occurrence in some geographic areas and their distinctive clinical appearance as demonstrated in the referenced video segments on the DVD accompanying this text.

Leukoencephalomalacia

Equine leukoencephalomalacia is a seasonal disorder of horses, ponies, donkeys, and mules, occurring most commonly in late fall through early spring. Most outbreaks are associated with a dry growing period followed by a wet period. The disorder is caused by ingestion of the mycotoxin fumonisin B1, a metabolite of *Fusarium moniliforme*.[954-976] Two clinical syndromes are associated with fumonisin B1 intoxication. The more common is a neurologic syndrome characterized initially by incoordination, aimless walking, intermittent anorexia, lethargy, obtundation, blindness, and head pressing. These signs might be followed by hyperexcitablity, belligerence, extreme agitation, profuse sweating, and delirium. Recumbency and clonic-tetanic seizures might occur before death. Less commonly, horses develop a hepatotoxic syndrome with swelling of the lips and nose, somnolence, severe icterus and petechiation of mucous membranes, abdominal breathing, and cyanosis. Gross lesions include liquefactive necrosis and degeneration of the cerebral hemispheres; changes might also occur in the brainstem, cerebellum, and spinal cord.

Nigropallidal Encephalomalacia

Yellow star thistle and Russian knapweed grow over much of the western United States in nonirrigated pastures during dry seasons of summer and fall. Ingestion of these plants can result in unilateral or more commonly bilateral softening and necrosis in areas of the globus pallidus and substantia nigra.[977-986] Lesions are often sharply defined and may be cavitary. Although the plants are generally considered unpalatable by most horses, some horses may develop a craving for the plants and selectively seek them out. The exact toxic principle in these plants has not been determined. Affected horses demonstrate variable degrees of impairment of eating and drinking with lack of coordination of movements of prehension, mastication, and deglutition. Most horses appear to be able to swallow if food or water is placed in the posterior pharynx. Severely affected horses might attempt to drink by immersing their muzzle deeply into the water in an apparent attempt to force water into the posterior pharynx. Hypertonicity of facial muscles is common, with the horse often holding the mouth partially open with the lips retracted. The tongue may protrude from the mouth, and many horses display constant chewing movements.

Fluphenazine Toxicity

Fluphenazine administration to horses may result in characteristic clinical signs of toxicity.[987-991] Fluphenazine is a highly potent phenothiazine neuroleptic that is widely used in human medicine for a variety of psychological disturbances. In horses, fluphenazine decanoate has been used to provide a persistent sedative effect. It binds avidly to dopamine D_2 receptors in the brain. The very slow dissociation of fluphenazine from these receptors is associated with a greater risk of adverse extrapyramidal signs than is observed with newer atypical antipsychotic medications.[987] Its administration to some horses can result in severe extrapyramidal effects and parkinsonism with clinical signs including agitation, profuse sweating, hypermetria, aimless circling, intense pawing and striking with the thoracic limbs, and rhythmic swinging of the head and neck alternating with periods of severe stupor. A study using liquid chromatography showed that fluphenazine decanoate administered intramuscularly can be detected in serum up to 14 days after administration, with the highest concentration detected in serum at 1.4 ng/mL.[992] The lower limit of detection was determined to be 0.05 ng/mL.[992] A long-acting depot formulation for intramuscular administration is available and appears to be most commonly used. One published report suggests a dosage of 0.05 to 0.08 mg/kg IM every 2 weeks with a warning to beware of idiosyncratic reactions.[993] Horses have exhibited signs of extrapyramidal effects of fluphenazine decanoate after receiving doses as low as 40 mg IM.[987] Given that recommended doses for adult humans range from 25 to 50 mg, it is reasonable to assume that at least some horses are much more susceptible than humans to the adverse effects of fluphenazine decanoate. The severity of clinical signs observed in some affected horses is sufficient to pose considerable safety risks for veterinarians and handlers treating these horses. This author is aware of at least one horse that was euthanized because of the extreme danger of clinical signs after administration of fluphenazine; other authors have indicated similar adverse outcomes.[987] Treatment consists of discontinuation of drug administration and enhancing cholinergic function by administering anticholinergic medications. Diphenhydramine chloride results in significant improvement in some, but not all, affected horses.[987,989] Benztropine mesylate (0.018 mg/kg IV every 8 hours) has also been helpful for treatment of some affected horses.[987] Other horses apparently benefited from sodium pentobarbital (2 mg/kg IV followed by a constant rate infusion at 2.5 mg/kg/h) in an attempt to control maniacal behavior without inducing recumbency or anesthesia.[987]

Metabolic Disorders

A wide variety of metabolic disorders can cause clinical signs indicative of central nervous system dysfunction. These signs include hepatic dysfunction, hypoglycemia, hypoxemia and ischemia, and severe abnormalities in plasma electrolyte concentrations.

Hepatoencephalopathy

An excellent review of the etiology, pathogenesis, clinical signs, diagnosis, and treatment of liver disease is included

elsewhere in this text. A normally functioning liver is necessary to maintain normal brain neuron and astrocyte function. With acute liver disease hepatoencephalopathy is most often a result of astrocyte swelling, acute cytotoxic cerebral edema, and intracranial hypertension. In chronic hepatic disease astrocytes are swollen but also show evidence of Alzheimer type II changes. Horses with hepatoencephalopathy show signs of cerebral cortex dysfunction. The earliest recognizable clinical signs in horses are often obtundation, lethargy, mild ataxia, and various forms of inappropriate behavior. These signs can progress to head pressing, circling, somnolence, and diminished responsiveness to external stimuli. Eventually affected horses become recumbent and comatose. Seizures might occasionally be observed, but they are not common (not clinically manifested). The type and severity of clinical signs do not correlate with type or reversibility of the underlying hepatic disease.

Hypoglycemia, Hypoxemia, and Ischemia

Severe hypoglycemia is not common in adult horses but is common in neonates, often as a complication of sepsis, prematurity, hypoxic ischemic encephalopathy, or hypothermia. When hypoglycemia does occur it might result in weakness, obtundation, ataxia, and eventually loss of consciousness. Seizure activity is not common in adult horses but might occasionally be observed in affected foals. Iatrogenic hypoglycemia can occur in horses treated with insulin or with abrupt cessation of intravenous glucose therapy. Clinical signs are rapidly reversed with intravenous administration of glucose-containing solutions. Guidelines for intravenous glucose therapy are included in Chapter 20.

Because of the brain's high and continuous demand for oxygen, the CNS is extremely susceptible to hypoxic or ischemic damage. This is most apparent in the neonate with hypoxic ischemic encephalopathy, as discussed in detail in Chapter 20. As nervous tissue switches to anaerobic glycolysis to meet its energy needs, brain glucose is depleted, and localized lactic acidosis occurs. Neurons swell, and cytotoxic edema exacerbates ischemia. The end result is irreversible cell damage and neuronal death. Clinical signs depend on what areas of the brain are affected. Localized hypoxia may occur with thromboembolic disorders or intracarotid injections. Deprivation of oxygen to the entire CNS results in more dramatic clinical signs of impaired cerebral function ultimately leading to seizures, coma, and death. This type of generalized severe tissue hypoxia may result from decreased inspired oxygen, ventilation/perfusion abnormalities, right-to-left shunting of blood, impairment of gas diffusion within the lungs, hypoventilation, severe anemia, impaired oxygen utilization by tissues, or profound hypotension secondary to sepsis, hemorrhage, anaphylaxis, or cardiac failure. These disorders are discussed in detail in the appropriate chapters elsewhere in this text.

Electrolyte Abnormalities

Severe hyponatremia or hypernatremia can result in CNS dysfunction. Hyponatremia most often results from water intoxication because of excessive IV or oral administration or consumption of hypotonic solutions. A common clinical scenario resulting in hyponatremia is oral consumption of large quantities of water by a patient with a disorder that results in significant concurrent electrolyte loss (e.g., diarrhea, excessive sweating, polyuric renal failure). Clinical signs are most common in horses with a serum sodium concentration of less than 110 mEq/L. Profound hypernatremia might result in clinical signs of spasticity, myoclonus, and obtundation. Severe hyponatremia was identified in 4% of foals (69 of 1718) presented to an intensive care unit.[994] Of those affected foals, 16% displayed neurologic signs attributed to hyponatremic encephalopathy.[994] Signs included seizures, obtundation, ataxia, and blindness. Severe hyponatremia was associated with renal disease, enterocolitis, and uroperitoneum. Survival rate to discharge was 72.5% (50 of 69 foals).[994] Long-term survival in a 12-month follow-up was 76%.[994] A different study in 4 foals with hyponatremic encephalopathy reported resolution of neurologic signs in 3 of 4 foals.[995] Signs were attributed to increased intracranial pressure as the result of severe hyponatremia. Azotemia due to acute renal failure in these foals was slow to resolve with intensive therapy. Therapy of foals affected with profound hyponatremia or hypernatremia is discussed in Chapter 20.

REFERENCES

1. de Lahunta A, Glass E, Kent M. *Veterinary Neuroanatomy and Clinical Neurology*. 4th ed. St. Louis: Saunders; 2014.
2. Divers TJ, Johnson AL. Clinical neurology. *Vet Clin North Am Equine Pract*. 2011;27:ix–x.
3. Furr M, Reed S. *Equine Neurology*. 2nd ed. Philadelphia: Wiley-Blackwell; 2015.
4. Mayhew J. *Large Animal Neurology*. 2nd ed. Philadelphia: Wiley-Blackwell; 2008.
5. Van Biervliet J, de Lahunta A, Ennulat D, et al. Acquired cervical scoliosis in six horses associated with dorsal grey column chronic myelitis. *Equine Vet J*. 2004;36:86–92.
6. Irby NL. Neuro-ophthalmology in Horses. *Vet Clin North Am Equine Pract*. 2011;27:455.
7. Mayhew IG. Neuro-ophthalmology: a review. *Equine Vet J Suppl*. 2010:80–88.
8. Rooney JR. Two cervical reflexes in the horse. *J Am Vet Med Assoc*. 1973;162:117–118.
9. Nout YS, Reed SM. Management and treatment of the recumbent horse. *Equine Vet Educ*. 2005;7:416–432.
10. Olsen E, Dunkel B, Barker WH, et al. Rater agreement on gait assessment during neurologic examination of horses. *J Vet Intern Med*. 2014;28:630–638.
11. Hoffman CJ, Clark CK. Prognosis for racing with conservative management of cervical vertebral malformation in thoroughbreds: 103 cases (2002-2010). *J Vet Intern Med*. 2013;27:317–323.
12. Edsbagge M, Tisell M, Jacobsson L, et al. Spinal CSF absorption in healthy individuals. *Am J Physiol Regul Integr Comp Physiol*. 2004;287:R1450–R1455.
13. MacKay RJ. Developments in ultrasound-guided thecal puncture in horses. *Vet Rec*. 2014;174:43–44.
14. Aleman M, Borchers A, Kass PH, et al. Ultrasound-assisted collection of cerebrospinal fluid from the lumbosacral space in equids. *J Am Vet Med Assoc*. 2007;230:378–384.
15. Depecker M, Bizon-Mercier C, Courouce-Malblanc A. Ultrasound-guided atlanto-occipital puncture for cerebrospinal fluid analysis on the standing horse. *Vet Rec*. 2014;174:45.
16. Pease A, Behan A, Bohart G. Ultrasound-guided cervical centesis to obtain cerebrospinal fluid in the standing horse. *Vet Radiol Ultrasound*. 2012;53:92–95.
17. Andrews FM, Geiser DR, Sommardahl CS, et al. Albumin quotient, IgG concentration, and IgG index determinations in cerebrospinal fluid of neonatal foals. *Am J Vet Res*. 1994;55:741–745.
18. Beech J. Cytology of equine cerebrospinal fluid. *Vet Pathol*. 1983;20:553–562.
19. Furr M, Chickering WR, Robertson J. High resolution protein electrophoresis of equine cerebrospinal fluid. *Am J Vet Res*. 1997;58:939–941.

20. Furr MO, Bender H. Cerebrospinal fluid variables in clinically normal foals from birth to 42 days of age. *Am J Vet Res*. 1994;55:781–784.
21. Hayes TE. Examination of cerebrospinal fluid in the horse. *Vet Clin North Am Equine Pract*. 1987;3:283–291.
22. Mayhew IG, Whitlock RH, Tasker JB. Equine cerebrospinal fluid: reference values of normal horses. *Am J Vet Res*. 1977;38:1271–1274.
23. Mozaffari AA, Samadieh H. Analysis of serum and cerebrospinal fluid in clinically normal adult miniature donkeys. *N Z Vet J*. 2013;61:297–299.
24. Darien BJ, Belknap J, Nietfeld J. Cerebrospinal fluid changes in two horses with central nervous system nematodiasis (Micronema deletrix). *J Vet Intern Med*. 1988;2:201–205.
25. Pusterla N, Wilson WD, Conrad PA, et al. Comparative analysis of cytokine gene expression in cerebrospinal fluid of horses without neurologic signs or with selected neurologic disorders. *Am J Vet Res*. 2006;67:1433–1437.
26. Andrews FM, Maddux JM, Faulk D. Total protein, albumin quotient, IgA and IgG index determinations for horse cerebrospinal fluid. *Prog Vet Neurol*. 1990;1:197–204.
27. Furr MO, Tyler RD. Cerebrospinal fluid creatine kinase activity in horses with central nervous system disease: 69 cases (1984-1989). *J Am Vet Med Assoc*. 1990;197:245–248.
28. Green EM, Green S. Cerebrospinal fluid lactic acid concentration: reference values and diagnostic implications of abnormal concentrations in adult horses. *Ann Forum Am Coll Vet Int Med*. 1990.
29. Vickroy TW, Chang SK, Chou CC. Caffeine-induced hyperactivity in the horse: comparisons of drug and metabolite concentrations in blood and cerebrospinal fluid. *J Vet Pharmacol Ther*. 2008;31:156–166.
30. Aleman M. Miscellaneous neurologic or neuromuscular disorders in horses. *Vet Clin North Am Equine Pract*. 2011;27:481–506.
31. Aminoff MJ. Clinical electromyography. In: Aminoff MJ, ed. *Electrodiagnostics in Clinical Neurology*. 4th ed. Philadelphia: Churchill Livingston; 1999:223–252.
32. Daube JR, Rubin DI. Needle electromyography. *Muscle Nerve*. 2009;39:244–270.
33. Kimura J. *Electrodiagnostics in Diseases of Nerve and Muscle: Principles and Practice*. 3rd ed. New York: Springer; 2001.
34. Wijnberg ID, Franssen H. The potential and limitations of quantitative electromyography in equine medicine. *Vet J*. 2016;209:23–31.
35. Jose-Cunilleras E, Wijnberg ID. Quantitative motor unit action potential analysis of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles in adult Royal Dutch sport horses. *Equine Vet J*. 2016;48:234–239.
36. Aleman M, Watson JL, Williams DC, et al. Myopathy in horses with pituitary pars intermedia dysfunction (Cushing's disease). *Neuromuscul Disord*. 2006;16:737–744.
37. Aleman M, Williams DC, Jorge NE, et al. Repetitive stimulation of the common peroneal nerve as a diagnostic aid for botulism in foals. *J Vet Intern Med*. 2011;25:365–372.
38. Sims MH. Electrodiagnostic techniques in the evaluation of diseases affecting skeletal muscle. *Vet Clin North Am Small Anim Pract*. 1983;13:145–162.
39. Andrews FM, Spurgeon TL, Reed SM. Histochemical changes in skeletal muscles of four male horses with neuromuscular disease. *Am J Vet Res*. 1986;47:2078–2083.
40. Mayhew IG, deLahunta A, Whitlock RH, et al. Spinal cord disease in the horse. *Cornell Vet*. 1978;68(suppl 6):1–207.
41. Moore MP, Andrews F, Reed SM, et al. Electromyographic evaluation of horses wtih laryngeal hemiplegia. *J Equine Vet Sci*. 1988;8:424–427.
42. Reed SM, Hegreberg GA, Bayly WM, et al. Progressive myotonia in foals resembling human dystrophia myotonica. *Muscle Nerve*. 1988;11:291–296.
43. Wettstein A. The origin of fasciculations in motoneuron disease. *Ann Neurol*. 1979;5:295–300.
44. Kornegay JN, Gorgacz EJ, Dawe DL, et al. Polymyositis in dogs. *J Am Vet Med Assoc*. 1980;176:431–438.
45. Spier SJ, Carlson GP, Holliday TA, et al. Hyperkalemic periodic paralysis in horses. *J Am Vet Med Assoc*. 1990;197:1009–1017.
46. Aleman M, Williams DC, Brosnan RJ, et al. Sensory nerve conduction and somatosensory evoked potentials of the trigeminal nerve in horses with idiopathic headshaking. *J Vet Intern Med*. 2013;27:1571–1580.
47. Anor S, Espadaler JM, Monreal L, et al. Electrically elicited blink reflex in horses with trigeminal and facial nerve blocks. *Am J Vet Res*. 1999;60:1287–1291.
48. Blythe LL, Engel Jr HN, Rowe KE. Comparison of sensory nerve conduction velocities in horses versus ponies. *Am J Vet Res*. 1988;49:2138–2142.
49. Blythe LL, Kitchell RL, Holliday TA, et al. Sensory nerve conduction velocities in forelimb of ponies. *Am J Vet Res*. 1983;44:1419–1426.
50. Henry RW, Diesem CD. Proximal equine radial and median motor nerve conduction velocity. *Am J Vet Res*. 1981;42:1819–1822.
51. Henry RW, Diesem CD, Wiechers DO. Evaluation of equine radial and median nerve conduction velocities. *Am J Vet Res*. 1979;40:1406–1410.
52. Huntington PJ, Jeffcott LB, Friend SC, et al. Australian Stringhalt—epidemiological, clinical and neurological investigations. *Equine Vet J*. 1989;21:266–273.
53. Whalen LR, Wheeler DW, LeCouteur RA, et al. Sensory nerve conduction velocity of the caudal cutaneous sural and medial cutaneous antebrachial nerves of adult horses. *Am J Vet Res*. 1994;55:892–897.
54. Wheeler SJ. Influence of limb temperature on sensory nerve conduction velocity in horses. *Am J Vet Res*. 1989;50:1817–1819.
55. Wheeler SJ. Effect of age on sensory nerve conduction velocity in the horse. *Res Vet Sci*. 1990;48:141–144.
56. Zarucco L, Driessen B, Scandella M, et al. Sensory nerve conduction and nociception in the equine lower forelimb during perineural bupivacaine infusion along the palmar nerves. *Can J Vet Res*. 2010;74:305–313.
57. Collins N, Milne E, Hahn C, et al. Correlation of the Havemeyer endoscopic laryngeal grading system with histopathological changes in equine Cricoarytenoideus dorsalis muscles. *Ir Vet J*. 2009;62:334–338.
58. Ducharme NG, Horney FD, Partlow GD, et al. Attempts to restore abduction of the paralyzed equine arytenoid cartilage. I. Nerve-muscle pedicle transplants. *Can J Vet Res*. 1989;53:202–209.
59. Delauche AJ. Brain-stem evoked responses as a diagnostic tool for deafness; a neurophysiological test with potential? *Br Vet J*. 1996;152:13–15.
60. Aleman M, Holliday TA, Nieto JE, et al. Brainstem auditory evoked responses in an equine patient population: part I—adult horses. *J Vet Intern Med*. 2014;28:1310–1317.
61. Aleman M, Madigan JE, Williams DC, et al. Brainstem auditory evoked responses in an equine patient population. Part II: foals. *J Vet Intern Med*. 2014;28:1318–1324.
62. Aleman M, Puchalski SM, Williams DC, et al. Brainstem auditory-evoked responses in horses with temporohyoid osteoarthropathy. *J Vet Intern Med*. 2008;22:1196–1202.
63. Harland MM, Stewart AJ, Marshall AE, et al. Diagnosis of deafness in a horse by brainstem auditory evoked potential. *Can Vet J*. 2006;47:151–154.
64. Magdesian KG, Madigan JE, Williams DC. Deafness of suspected congenital origin in American Paint Horses. *J Vet Intern Med*. 1998;12:208.
65. Magdesian KG, Williams DC, Aleman M, et al. Evaluation of deafness in American Paint Horses by phenotype, brainstem auditory-evoked responses, and endothelin receptor B genotype. *J Am Vet Med Assoc*. 2009;235:1204–1211.
66. Marshall AE. Brain stem auditory-evoked response in the nonanesthetized horse and pony. *Am J Vet Res*. 1985;46:1445–1450.

67. Marshall AE, Byars TD, Whitlock RH, et al. Brainstem auditory evoked response in the diagnosis of inner ear injury in the horse. *J Am Vet Med Assoc*. 1981;178:282–286.
68. Mayhew IG, Washbourne JR. A method of assessing auditory and brainstem function in horses. *Br Vet J*. 1990;146:509–518.
69. Mayhew IG, Washbourne JR. Short latency auditory evoked potentials recorded from non-anaesthetized thoroughbred horses. *Br Vet J*. 1992;148:315–327.
70. Mayhew IGJ. The clinical utility of brainstem auditory evoked response testing in horses. *Equine Vet Educ*. 2003;15:31–33.
71. Rolf SL, Reed SM, Melnick W, et al. Auditory brain stem response testing in anesthetized horses. *Am J Vet Res*. 1987;48:910–914.
72. Steiss JE, Brendemuehl JP, Wright JC. Nerve conduction velocities and brain stem auditory evoked responses in normal neonatal foals, compared to foals exposed to endophyte-infected fescue in utero. *Prog Vet Neurol*. 1991;2:252–260.
73. Wilson WJ, Mills PC, Dzulkarnain AA. Use of BAER to identify loss of auditory function in older horses. *Aust Vet J*. 2011;89:73–76.
74. Aleman M, Davis E, Williams DC, et al. Electrophysiologic Study of a Method of Euthanasia Using Intrathecal Lidocaine Hydrochloride Administered during Intravenous Anesthesia in Horses. *J Vet Intern Med*. 2015;29:1676–1682.
75. Aleman M, Gray LC, Williams DC, et al. Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985-2005). *J Vet Intern Med*. 2006;20:1443–1449.
76. Aleman M, Williams DC, Guedes A, et al. Cerebral and brainstem electrophysiologic activity during euthanasia with pentobarbital sodium in horses. *J Vet Intern Med*. 2015;29:663–672.
77. Grint NJ, Johnson CB, Clutton RE, et al. Spontaneous electroencephalographic changes in a castration model as an indicator of nociception: a comparison between donkeys and ponies. *Equine Vet J*. 2015;47:36–42.
78. Haga HA, Dolvik NI. Electroencephalographic and cardiovascular variables as nociceptive indicators in isoflurane-anaesthetized horses. *Vet Anaesth Analg*. 2005;32:128–135.
79. Johnson CB, Bloomfield M, Taylor PM. Effects of midazolam and sarmazenil on the equine electroencephalogram during anaesthesia with halothane in oxygen. *J Vet Pharmacol Ther*. 2003;26:105–112.
80. Lacombe VA, Podell M, Furr M, et al. Diagnostic validity of electroencephalography in equine intracranial disorders. *J Vet Intern Med*. 2001;15:385–393.
81. Murrell JC, White KL, Johnson CB, et al. Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Vet Anaesth Analg*. 2005;32:212–221.
82. Mysinger PW, Redding RW, Vaughan JT, et al. Electroencephalographic patterns of clinically normal, sedated, and tranquilized newborn foals and adult horses. *Am J Vet Res*. 1985;46:36–41.
83. Otto KA, Voight S, Piepenbrock S, et al. Differences in quantitated electroencephalographic variables during surgical stimulation of horses anesthetized with isoflurane. *Vet Surg*. 1996;25:249–255.
84. Sogaro-Robinson C, Lacombe VA, Reed SM, et al. Factors predictive of abnormal results for computed tomography of the head in horses affected by neurologic disorders: 57 cases (2001-2007). *J Am Vet Med Assoc*. 2009;235:176–183.
85. Wijnberg ID, van der Ree M, van Someren P. The applicability of ambulatory electroencephalography (AEEG) in healthy horses and horses with abnormal behaviour or clinical signs of epilepsy. *Vet Q*. 2013;33:121–131.
86. Williams DC, Aleman M, Holliday TA, et al. Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *J Vet Intern Med*. 2008;22:630–638.
87. Williams DC, Aleman M, Tharp B, et al. Qualitative and quantitative characteristics of the electroencephalogram in normal horses after sedation. *J Vet Intern Med*. 2012;26:645–653.
88. Williams DC, Aleman MR, Brosnan RJ, et al. Electroencephalogram of healthy horses during inhaled anesthesia. *J Vet Intern Med*. 2016;30:304–308.
89. Audigie F, Tapprest J, George C, et al. Magnetic resonance imaging of a brain abscess in a 10-month-old filly. *Vet Radiol Ultrasound*. 2004;45:210–215.
90. Beltran E, Grundon R, Stewart J, et al. Imaging Diagnosis—Unilateral Trigeminal Neuritis Mimicking Peripheral Nerve Sheath Tumor in a Horse. *Vet Radiol Ultrasound*. 2016;57:E1–E4.
91. Ferrell EA, Gavin PR, Tucker RL, et al. Magnetic resonance for evaluation of neurologic disease in 12 horses. *Vet Radiol Ultrasound*. 2002;43:510–516.
92. Goncalves R, Malalana F, McConnell JF, et al. Anatomical Study of Cranial Nerve Emergence and Skull Foramina in the Horse Using Magnetic Resonance Imaging and Computed Tomography. *Vet Radiol Ultrasound*. 2015;56:391–397.
93. Janes JG, Garrett KS, McQuerry KJ, et al. Comparison of magnetic resonance imaging with standing cervical radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. *Equine Vet J*. 2014;46:681–686.
94. Janes JG, Garrett KS, McQuerry KJ, et al. Cervical vertebral lesions in equine stenotic myelopathy. *Vet Pathol*. 2015;52:919–927.
95. Javsicas LH, Watson E, MacKay RJ. What is your neurologic diagnosis? Equine protozoal myeloencephalitis. *J Am Vet Med Assoc*. 2008;232:201–204.
96. Kraft CN, Scharfstadt A, Yong M, et al. Correlation of back pain and magnetic resonance imaging of the lumbar spine in elite horse vaulters. *Sportverletz Sportschaden*. 2007;21:142–147.
97. Manso-Diaz G, Dyson SJ, Dennis R, et al. Magnetic resonance imaging characteristics of equine head disorders: 84 cases (2000-2013). *Vet Radiol Ultrasound*. 2015;56:176–187.
98. Maulet BEB, Bestbier M, Jose-Cunilleras E, et al. Magnetic resonance imaging of a cholesterol granuloma and hydrocephalus in a horse. *Equine Vet Educ*. 2008;20:74–79.
99. Mitchell CW, Nykamp SG, Foster R, et al. The use of magnetic resonance imaging in evaluating horses with spinal ataxia. *Vet Radiol Ultrasound*. 2012;53:613–620.
100. Sleutjens J, Cooley AJ, Sampson SN, et al. The equine cervical spine: comparing MRI and contrast-enhanced CT images with anatomic slices in the sagittal, dorsal, and transverse plane. *Vet Q*. 2014;34:74–84.
101. Spoormakers TJ, Ensink JM, Goehring LS, et al. Brain abscesses as a metastatic manifestation of strangles: symptomatology and the use of magnetic resonance imaging as a diagnostic aid. *Equine Vet J*. 2003;35:146–151.
102. Tucker RL, Farrell E. Computed tomography and magnetic resonance imaging of the equine head. *Vet Clin North Am Equine Pract*. 2001;17:131–144. vii.
103. Berendt M, Gram L. Epilepsy and seizure classification in 63 dogs: a reappraisal of veterinary epilepsy terminology. *J Vet Intern Med*. 1999;13:14–20.
104. Trinka E, Cock H, Hesdorffer D, et al. A definition and classification of status epilepticus—Report of the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia*. 2015;56:1515–1523.
105. Berendt M, Farquhar RG, Mandigers PJ, et al. International veterinary epilepsy task force consensus report on epilepsy definition, classification and terminology in companion animals. *BMC Vet Res*. 2015;11:182.
106. Lacombe VA, Mayes M, Mosseri S, et al. Epilepsy in horses: aetiological classification and predictive factors. *Equine Vet J*. 2012;44:646–651.
107. Lacombe VA, Mayes M, Mosseri S, et al. Distribution and predictive factors of seizure types in 104 cases. *Equine Vet J*. 2014;46:441–445.
108. Aleman M, Pickles KJ, Conley AJ, et al. Abnormal plasma neuroactive progestagen derivatives in ill, neonatal foals presented to the neonatal intensive care unit. *Equine Vet J*. 2013;45:661–665.
109. Furr M. Perinatal asphyxia in foals. *Comp Contin Educ Pract Vet*. 1996;18:1342–1351.

110. Toth B, Aleman M, Nogradi N, et al. Meningitis and meningoencephalomyelitis in horses: 28 cases (1985-2010). *J Am Vet Med Assoc*. 2012;240:580–587.
111. Viu J, Monreal L, Jose-Cunilleras E, et al. Clinical findings in 10 foals with bacterial meningoencephalitis. *Equine Vet J Suppl*. 2012:100–104.
112. Adler EM, Selzer ME. Cellular pathophysiology and pharmacology of epilepsy. In: Asbury AK, McKhann GM, McDonald WI, eds. *Disease of the Nervous System*. Philadelphia: W.B. Saunders; 1986.
113. Delgado-Escueta AV, Ward Jr AA, Woodbury DM, et al. New wave of research in the epilepsies. *Adv Neurol*. 1986;44:3–55.
114. Podell M. Antiepileptic drug therapy. *Clin Tech Small Anim Pract*. 1998;13:185–192.
115. Prince DA. Neurophysiology of epilepsy. *Annu Rev Neurosci*. 1978;1:395–415.
116. Trommer BL, Pasternak JF. NMDA receptor antagonists inhibit kindling epileptogenesis and seizure expression in developing rats. *Brain Res Dev Brain Res*. 1990;53:248–252.
117. Williams DC, Brosnan RJ, Fletcher DJ, et al. Qualitative and Quantitative Characteristics of the Electroencephalogram in Normal Horses during Administration of Inhaled Anesthesia. *J Vet Intern Med*. 2016;30:289–303.
118. Duran SH, Ravis WR, Pedersoli WM, et al. Pharmacokinetics of phenobarbital in the horse. *Am J Vet Res*. 1987;48:807–810.
119. Knox DA, Ravis WR, Pedersoli WM, et al. Pharmacokinetics of phenobarbital in horses after single and repeated oral administration of the drug. *Am J Vet Res*. 1992;53:706–710.
120. Norman WM, Court MH, Greenblatt DJ. Age-related changes in the pharmacokinetic disposition of diazepam in foals. *Am J Vet Res*. 1997;58:878–880.
121. Spehar AM, Hill MR, Mayhew IG, et al. Preliminary study on the pharmacokinetics of phenobarbital in the neonatal foal. *Equine Vet J*. 1984;16:368–371.
122. Madigan JE. Drug formulary. In: Madigan JE, ed. *Manual of Equine Neonatal Medicine*. 4th ed. Woodland, CA: Live Oak Publishing; 2013:387–388, 395–396.
123. Borris DJ, Bertram EH, Kapur J. Ketamine controls prolonged status epilepticus. *Epilepsy Res*. 2000;42:117–122.
124. Mayhew IG. Rules of thumb in managing epileptic horses. *North Am Vet Conf*. 2003.
125. Ravis WR, Duran SH, Pedersoli WM, et al. A pharmacokinetic study of phenobarbital in mature horses after oral dosing. *J Vet Pharmacol Ther*. 1987;10:283–289.
126. Fielding CL, Magdesian KG, Elliott DA, et al. Pharmacokinetics and clinical utility of sodium bromide (NaBr) as an estimator of extracellular fluid volume in horses. *J Vet Intern Med*. 2003;17:213–217.
127. Raidal SL, Edwards S. Pharmacokinetics of potassium bromide in adult horses. *Aust Vet J*. 2005;83:425–430.
128. Dirikolu L, Dafalla A, Ely KJ, et al. Pharmacokinetics of gabapentin in horses. *J Vet Pharmacol Ther*. 2008;31:175–177.
129. Cesar FB, Stewart AJ, Boothe D, et al. *Disposition of levetiracetam in healthy adult horses. Deaprtment of Clinical Sciences*. Auburn, AL: Auburn University; 2014.
130. Aleman M. Sleep and sleep disorders in horses. *Proc Amer Assoc Equine Pract*. 2008;54:180–185.
131. Bertone JJ. Excessive drowsiness secondary to recumbent sleep deprivation in two horses. *Vet Clin North Am Equine Pract*. 2006;22:157–162.
132. Dauvilliers Y, Arnulf I, Mignot E. Narcolepsy with cataplexy. *Lancet*. 2007;369:499–511.
133. Tonokura M, Fujita K, Nishino S. Review of pathophysiology and clinical management of narcolepsy in dogs. *Vet Rec*. 2007;161:375–380.
134. McFarlane D, Maidment NT, Lam H. Cerebrospinal fluid concentration of hypocretin-1 in horses with equine pituitary pars intermedia disease and its relationship to oxidative stress. *J Vet Intern Med*. 2007.
135. Mignot EJ, Dement WC. Narcolepsy in animals and man. *Equine Vet J*. 1993;25:476–477.
136. Sheather AL. Fainting in foals. *J Comp Pathol Ther*. 1924;37:106–113.
137. Lunn DP, Cuddon PA, Shaftoe S, et al. Familial occurrence of narcolepsy in miniature horses. *Equine Vet J*. 1993;25:483–487.
138. Peck KE, Hines MT, Mealey KL, et al. Pharmacokinetics of imipramine in narcoleptic horses. *Am J Vet Res*. 2001;62:783–786.
139. Bathen-Nothen A, Heider C, Fernandez AJ, et al. Hypocretin measurement in an Icelandic foal with narcolepsy. *J Vet Intern Med*. 2009;23:1299–1302.
140. Ludvikova E, Nishino S, Sakai N, et al. Familial narcolepsy in the Lipizzaner horse: a report of three fillies born to the same sire. *Vet Q*. 2012;32:99–102.
141. Tyler CM, Davis RE, Begg AP, et al. A survey of neurological diseases in horses. *Aust Vet J*. 1993;70:445–449.
142. Feige K, Furst A, Kaser-Hotz B, et al. Traumatic injury to the central nervous system in horses: occurrence, diagnosis and outcome. *Equine Vet Educ*. 2000;12:220–224.
143. Lyle CH, Uzal FA, McGorum BC, et al. Sudden death in racing Thoroughbred horses: an international multicentre study of post mortem findings. *Equine Vet J*. 2011;43:324–331.
144. Feary DJ, Magdesian KG, Aleman MA, et al. Traumatic brain injury in horses: 34 cases (1994-2004). *J Am Vet Med Assoc*. 2007;231:259–266.
145. MacKay RJ. Brain injury after head trauma: pathophysiology, diagnosis, and treatment. *Vet Clin North Am Equine Pract*. 2004;20:199–216.
146. Little CB, Hilbert BJ, McGill CA. A retrospective study of head fractures in 21 horses. *Aust Vet J*. 1985;62:89–91.
147. Reed SM. Management of head trauma in horses. *Comp Contin Educ Pract Vet*. 1993;15:270–273.
148. Reed SM. Medical and surgical emergencies of the nervous system of horses: diagnosis, treatment, and sequelae. *Vet Clin North Am Equine Pract*. 1994;10:703–715.
149. Stick JA, Wilson T, Kunze D. Basilar skull fractures in three horses. *J Am Vet Med Assoc*. 1980;176:228–231.
150. Ramirez 3rd O, Jorgensen JS, Thrall DE. Imaging basilar skull fractures in the horse: a review. *Vet Radiol Ultrasound*. 1998;39:391–395.
151. Martin L, Kaswan R, Chapman W. Four cases of traumatic optic nerve blindness in the horse. *Equine Vet J*. 1986;18:133–137.
152. Dunkel B, Corley KT, Johnson AL, et al. Pneumocephalus in five horses. *Equine Vet J*. 2013;45:367–371.
153. Ragle CA, de Mira MC, Pearson LK, et al. Pneumocephalus secondary to removal of an osteoma from the paranasal sinuses of a horse. *J Am Vet Med Assoc*. 2009;235:184–188.
154. Finnie JW. Pathology of traumatic brain injury. *Vet Res Commun*. 2014;38:297–305.
155. Andrews PJ, Citerio G. Intracranial pressure. Part one: historical overview and basic concepts. *Intensive Care Med*. 2004;30:1730–1733.
156. Verweij BH, Muizelaar JP, Vinas FC, et al. Impaired cerebral mitochondrial function after traumatic brain injury in humans. *J Neurosurg*. 2000;93:815–820.
157. Fishman RA. Brain edema. *N Engl J Med*. 1975;293:706–711.
158. Moore RM, Trims CM. Effect of xylazine on cerebrospinal fluid pressure in conscious horses. *Am J Vet Res*. 1992;53:1558–1561.
159. Lacombe VA, Sogaro-Robinson C, Reed SM. Diagnostic utility of computed tomography imaging in equine intracranial conditions. *Equine Vet J*. 2010;42:393–399.
160. Nollet H, Vanschandevijl K, Van Ham L, et al. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. *Vet Rec*. 2005;157:656–658.
161. Kortz GD, Madigan JE, Goetzman BW, et al. Intracranial pressure and cerebral perfusion pressure in clinically normal equine neonates. *Am J Vet Res*. 1995;56:1351–1355.

162. Brosnan RJ, LeCouteur RA, Steffey EP, et al. Direct measurement of intracranial pressure in adult horses. *Am J Vet Res*. 2002;63:1252–1256.
163. McConnell EJ, Rioja E, Bester L, et al. Use of near-infrared spectroscopy to identify trends in regional cerebral oxygen saturation in horses. *Equine Vet J*. 2013;45:470–475.
164. Finfer S, Bellomo R, Boyce N, et al. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *N Engl J Med*. 2004;350:2247–2256.
165. Investigators SS, Australian, New Zealand Intensive Care Society Clinical Trials G, et al. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. *N Engl J Med*. 2007;357:874–884.
166. Gantner D, Moore EM, Cooper DJ. Intravenous fluids in traumatic brain injury: what's the solution? *Curr Opin Crit Care*. 2014;20:385–389.
167. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med*. 2001;345:1359–1367.
168. Dietrich WD. The importance of brain temperature in cerebral injury. *J Neurotrauma*. 1992;9(suppl 2):S475–485.
169. Busto R, Dietrich WD, Globus MY, et al. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab*. 1987;7:729–738.
170. Dietrich WD, Bramlett HM. Therapeutic hypothermia and targeted temperature management in traumatic brain injury: clinical challenges for successful translation. *Brain Res*. 2016;1640:94–103.
171. Roberts I, Yates D, Sandercock P, et al. Effect of intravenous corticosteroids on death within 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *Lancet*. 2004;364:1321–1328.
172. Burgess S, Abu-Laban RB, Slavik RS, et al. A Systematic Review of Randomized Controlled Trials Comparing Hypertonic Sodium Solutions and Mannitol for Traumatic Brain Injury: Implications for Emergency Department Management. *Ann Pharmacother*. 2016;50:291–300.
173. Young B, Ott L, Twyman D, et al. The effect of nutritional support on outcome from severe head injury. *J Neurosurg*. 1987;67:668–676.
174. Kaufmann MA, Buchmann B, Scheidegger D, et al. Severe head injury: should expected outcome influence resuscitation and first-day decisions? *Resuscitation*. 1992;23:199–206.
175. Sarrafian TL, Case JT, Kinde H, et al. Fatal musculoskeletal injuries of Quarter Horse racehorses: 314 cases (1990-2007). *J Am Vet Med Assoc*. 2012;241:935–942.
176. Collar EM, Zavodovskaya R, Spriet M, et al. Caudal lumbar vertebral fractures in California Quarter Horse and Thoroughbred racehorses. *Equine Vet J*. 2015;47:573–579.
177. Gygax D, Furst A, Picek S, et al. Internal fixation of a fractured axis in an adult horse. *Vet Surg*. 2011;40:636–640.
178. Rashmir-Raven AM, DeBowes RM, Hudson L, et al. Vertebral fracture and paraplegia in a foal. *Prog Vet Neurol*. 1991;2:197–202.
179. Tutko JM, Sellon DC, Burns GA, et al. Cranial coccygeal vertebral fractures in horses: 12 cases. *Equine Vet Educ*. 2002;14:197–200.
180. Jeffcott LB. Disorders of the thoracolumbar spine of the horse—a survey of 443 cases. *Equine Vet J*. 1980;12:197–210.
181. Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg*. 1991;75:15–26.
182. Kwon BK, Tetzlaff W, Grauer JN, et al. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J*. 2004;4:451–464.
183. de la Torre JC. Spinal cord injury. Review of basic and applied research. *Spine (Phila Pa 1976)*. 1981;6:315–335.
184. Beattie MS. Inflammation and apoptosis: linked therapeutic targets in spinal cord injury. *Trends Mol Med*. 2004;10:580–583.
185. Beattie MS, Farooqui AA, Bresnahan JC. Review of current evidence for apoptosis after spinal cord injury. *J Neurotrauma*. 2000;17:915–925.
186. Smith PM, Jeffery ND. Spinal shock—comparative aspects and clinical relevance. *J Vet Intern Med*. 2005;19:788–793.
187. Ramer LM, Ramer MS, Bradbury EJ. Restoring function after spinal cord injury: towards clinical translation of experimental strategies. *Lancet Neurol*. 2014;13:1241–1256.
188. Lloyd D. Cervical spine injuries. *Equine Vet Educ*. 2009;21:533–535.
189. Mayhew IG. Cervical vertebral fractures. *Equine Vet Educ*. 2009;21:536–539.
190. Zurita M, Vaquero J, Oya S, et al. Effects of dexamethasone on apoptosis-related cell death after spinal cord injury. *J Neurosurg*. 2002;96:83–89.
191. Clayton HM. Core Training and Rehabilitation in Horses. *Vet Clin North Am Equine Pract*. 2016;32:49–71.
192. Davidson EJ. Controlled Exercise in Equine Rehabilitation. *Vet Clin North Am Equine Pract*. 2016;32:159–165.
193. King MR, Davidson EJ. Innovations in Equine Physical Therapy and Rehabilitation. *Vet Clin North Am Equine Pract*. 2016;32:xiii–xiv.
194. McGowan CM, Cottriall S. Introduction to Equine Physical Therapy and Rehabilitation. *Vet Clin North Am Equine Pract*. 2016;32:1–12.
195. Van Meeteren NL, Eggers R, Lankhorst AJ, et al. Locomotor recovery after spinal cord contusion injury in rats is improved by spontaneous exercise. *J Neurotrauma*. 2003;20:1029–1037.
196. King AS. *Physiological and Clinical Anatomy of the Domestic Animals: Central Nervous System*. Oxford: Blackwell Publishing; 2008.
197. Chrisman C. Disorders of the vestibular system. *Comp Contin Educ Pract Vet*. 1979;1:744–757.
198. Geiser DR, Henton J, Held J. Tympanic bulla, petrous temporal bone, and hyoid apparatus disease in horses. *Comp Contin Educ Pract Vet*. 1988;10:740–756.
199. Power HT, Watrous BJ, de Lahunta A. Facial and vestibulocochlear nerve disease in six horses. *J Am Vet Med Assoc*. 1983;183:1076–1080.
200. Blythe LL, Watrous BJ, Schmitz JA, et al. Vestibular syndrome associated with temporohyoid joint fusion and temporal bone fracture in three horses. *J Am Vet Med Assoc*. 1984;185:775–781.
201. Watrous BJ. Head tilt in horses. *Vet Clin North Am Equine Pract*. 1987;3:353–370.
202. Palmer AC. Pathogenesis and pathology of the cerebello-vestibular syndrome. *J Small Anim Pract*. 1970;11:167–176.
203. Firth EC. Vestibular disease, and its relationship to facial paralysis in the horse: a clinical study of 7 cases. *Aust Vet J*. 1977;53:560–565.
204. Raphel CF. Brain abscess in three horses. *J Am Vet Med Assoc*. 1982;180:874–877.
205. Aleman M, Spriet M, Williams DC, et al. Neurologic Deficits Including Auditory Loss and Recovery of Function in Horses with Temporohyoid Osteoarthropathy. *J Vet Intern Med*. 2016;30:282–288.
206. Divers TJ, Ducharme NG, de Lahunta A, et al. Temporohyoid osteoarthropathy. *Equine Pract*. 2006;5:17–23.
207. Walker AM, Sellon DC, Cornelisse CJ, et al. Temporohyoid osteoarthropathy in 33 horses (1993-2000). *J Vet Intern Med*. 2002;16:697–703.
208. Montgomery T. Otitis media in a thoroughbred. *Vet Med Small Anim Clin*. 1981;76:722–724.
209. Hilton H, Puchalski SM, Aleman M. The computed tomographic appearance of equine temporohyoid osteoarthropathy. *Vet Radiol Ultrasound*. 2009;50:151–156.
210. Ford J, Lokai MD. Complications of Streptococcus equi infection. *Equine Pract*. 1980;4:41–44.
211. Mittel L. Seizures in the horse. *Vet Clin North Am Equine Pract*. 1987;3:323–332.

212. Ueltschi G. Bone and joint imaging with 99mTC labelled phsophates as a new diagnostic aid in veterinary orthopedics. *Vet Radiol*. 1977;18:80–84.
213. Lamb C, Koblik P. Scintigraphic evaluation of skeletal disease and its application to the horse. *Vet Radiol Ultrasound*. 1988;29:16–27.
214. Devous Sr MD, Twardock AR. Techniques and applications of nuclear medicine in the diagnosis of equine lameness. *J Am Vet Med Assoc*. 1984;184:318–325.
215. Blythe LL, Watrous BJ, Shires GMH, et al. Prophylactic partial stylohyoidostectomy for horses wtih osteoarthropathy of the temporohyoid joint. *J Equine Vet Sci*. 1994;14:32–37.
216. Pease AP, van Biervliet J, Dykes NL, et al. Complication of partial stylohyoidectomy for treatment of temporohyoid osteoarthropathy and an alternative surgical technique in three cases. *Equine Vet J*. 2004;36:546–550.
217. Oliver ST, Hardy J. Ceratohyoidectomy for treatment of equine temporohyoid osteoarthopathy (15 cases). *Can Vet J*. 2015;56:382–386.
218. Cook WR. Skeletal radiology of the equine head. *Vet Radiol*. 1970;11:35–55.
219. Kohn CW, Fenner WR. Equine herpes myeloencephalopathy. *Vet Clin North Am Equine Pract*. 1987;3:405–419.
220. Teuscher E, Vrins A, Lemaire T. A vestibular syndrome associated with Cryptococcus neoformans in a horse. *Zentralbl Veterinarmed A*. 1984;31:132–139.
221. Mair TS, Pearson GR. Melanotic hamartoma of the hind brain in a riding horse. *J Comp Pathol*. 1990;102:239–243.
222. Holliday TA. Clinical signs of acute and chronic experimental lesions of the cerebellum. *Vet Sci Comm*. 1979;3:259–278.
223. Dungworth DL, Fowler ME. Cerebellar hypoplasia and degeneration in a foal. *Cornell Vet*. 1966;56:17–24.
224. Fraser H. Two dissimilar types of cerebellar disorder in the horse. *Vet Rec*. 1966;78:608–612.
225. Sponseller ML. Equine cerebellar hypoplasia and degeneration. *Proc Amer Assoc Equine Pract*. 1967;13:123–126.
226. Bjorck G, Everz KE, Hansen HJ, et al. Congenital cerebellar ataxia in the Gotland pony breed. *Transboundary Emerg Disord*. 1973;20:341–354.
227. Baird JD, Mackenzie CD. Cerebellar hypoplasia and degeneration in part-Arab horses. *Aust Vet J*. 1974;50:25–28.
228. Beatty MT, Leipold HW, Cash W, et al. Cerebellar disease in Arabian horses. *Proc Amer Assoc Equine Pract*. 1985;31:241–255.
229. DeBowes RM, Leipold HW, Turner-Beatty M. Cerebellar abiotrophy. *Vet Clin North Am Equine Pract*. 1987;3:345–352.
230. de Lahunta A. Abiotrophy in domestic animals: a review. *Can J Vet Res*. 1990;54:65–76.
231. Mayhew IG. Neurological and neuropathological observations on the equine neonate. *Equine Vet J Suppl*. 1988:28–33.
232. Brault LS, Cooper CA, Famula TR, et al. Mapping of equine cerebellar abiotrophy to ECA2 and identification of a potential causative mutation affecting expression of MUTYH. *Genomics*. 2011;97:121–129.
233. Blanco A, Moyano R, Vivo J, et al. Purkinje cell apoptosis in arabian horses with cerebellar abiotrophy. *J Vet Med A Physiol Pathol Clin Med*. 2006;53:286–287.
234. Adams R, Mayhew IG. Neurological examination of newborn foals. *Equine Vet J*. 1984;16:306–312.
235. Sherwin AL, Norris JW, Bulcke JA. Spinal fluid creatine kinase in neurologic disease. *Neurology*. 1969;19:993–999.
236. Culebras-Fernandez A, Richards NG. Glutamic oxaloacetic transaminase, lactic dehydrogenase, and creatine phosphokinase content in cerebrospinal fluid. *Ceve Clin Q*. 1971;38:113.
237. LeGonidec G, Kuberski T, Daynes P, et al. A neurologic disease of horses in New Caledonia. *Aust Vet J*. 1981;57:194–195.
238. Hartley WJ, Kuberski T, LeGonidec G, et al. The pathology of Gomen disease: a cerebellar disorder of horses in New Caledonia. *Vet Pathol*. 1982;19:399–405.
239. Jubb KVF, Kennedy P, Palmer N. *Pathology of Domestic Animals*. 4th ed. San Diego: Academic Press; 1993.
240. Cudd TA, Mayhew IG, Cottrill CM. Agenesis of the corpus callosum with cerebellar vermian hypoplasia in a foal resembling the Dandy-Walker syndrome: pre-mortem diagnosis by clinical evaluation and CT scanning. *Equine Vet J*. 1989;21:378–381.
241. Oliver RE. Cerebellar hypoplasia in a thoroughbred foal. *N Z Vet J*. 1975;23:15.
242. Wheat JD, Kennedy PC. Cerebellar hypoplasia and its sequela in a horse. *J Am Vet Med Assoc*. 1957;131:291–293.
243. Poss M, Young S. Dysplastic disease of the cerebellum of an adult horse. *Acta Neuropathol*. 1987;75:209–211.
244. Waelchli RO, Ehrensperger F. Two related cases of cerebellar abnormality in equine fetuses associated with hydrops of fetal membranes. *Vet Rec*. 1988;123:513–514.
245. Makela O, Gustavsson I, Hollmen T.A64,X,i(Xq) karyotype in a standardbred filly. *Equine Vet J*. 1994;26:251–254.
246. Bell RJ, Smart ME. An unusual complication of strangles in a pony. *Can Vet J*. 1992;33:400–401.
247. Allen JR, Barbee DD, Boulton CR, et al. Brain abscess in a horse: diagnosis by computed tomography and successful surgical treatment. *Equine Vet J*. 1987;19:552–555.
248. Blunden AS, Khalil LF, Webbon PM. Halicephalobus deletrix infection in a horse. *Equine Vet J*. 1987;19:255–260.
249. Mayhew IG, Schneiders DH. An unusual familial neurological syndrome in newborn thoroughbred foals. *Vet Rec*. 1993;133:447–448.
250. Miller LM, Reed SM, Gallina AM, et al. Ataxia and weakness associated with fourth ventricle vascular anomalies in two horses. *J Am Vet Med Assoc*. 1985;186:601–603.
251. Seawright AA, Costigan P. Chronic methylmercurialism in a horse. *Vet Hum Toxicol*. 1978;20:6–9.
252. Draper AC, Bender JB, Firshman AM, et al. Epidemiology of shivering (shivers) in horses. *Equine Vet J*. 2015;47:182–187.
253. Draper AC, Trumble TN, Firshman AM, et al. Posture and movement characteristics of forward and backward walking in horses with shivering and acquired bilateral stringhalt. *Equine Vet J*. 2015;47:175–181.
254. Valberg SJ, Lewis SS, Shivers JL, et al. The equine movement disorder "shivers" is associated with selective cerebellar Purkinje cell axonal degeneration. *Vet Pathol*. 2015;52:1087–1098.
255. Rooney JR. Disorders of the nervous system. In: Rooney JR, ed. *Biomechanics of Lameness in Horses*. Baltimore: Williams and Wilkins; 1969:219–233.
256. Oswald J, Love S, Parkin TD, et al. Prevalence of cervical vertebral stenotic myelopathy in a population of thoroughbred horses. *Vet Rec*. 2010;166:82–83.
257. Levine JM, Ngheim PP, Levine GJ, et al. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). *J Am Vet Med Assoc*. 2008;233:1453–1458.
258. Levine JM, Adam E, MacKay RJ, et al. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: a retrospective study (1992-2004). *J Vet Intern Med*. 2007;21:812–819.
259. Nout YS, Reed SM. Cervical vertebral stenotic myelopathy. *Equine Vet Educ*. 2003;15:212–223.
260. Reed SM, Rush BR. Developmental vertebral anomalies. In: Auer JA, Stick JA, eds. *Equine Surgery*. 2nd ed. Philadelphia: W.B. Saunders; 1999:423–428.
261. Wagner PC, Grant BD, Watrous BJ, et al. A study of the heritability of cervical vertebral malformation in horses. *Proc Amer Assoc Equine Pract*. 1985;31:43–50.
262. Moore BR, Reed SM, Biller DS, et al. Assessment of vertebral canal diameter and bony malformations of the cervical part of the spine in horses with cervical stenotic myelopathy. *Am J Vet Res*. 1994;55:5–13.
263. Hahn CN, Handel I, Green SL, et al. Assessment of the utility of using intra- and intervertebral minimum sagittal diameter ratios in the diagnosis of cervical vertebral malformation in horses. *Vet Radiol Ultrasound*. 2008;49:1–6.

264. Hughes KJ, Laidlaw EH, Reed SM, et al. Repeatability and intra- and inter-observer agreement of cervical vertebral sagittal diameter ratios in horses with neurological disease. *J Vet Intern Med*. 2014;28:1860–1870.
265. Nollet H, Deprez P, Van Ham L, et al. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. *Equine Vet J*. 2002;34:156–163.
266. Mullen KR, Furness MC, Johnson AL, et al. Adverse reactions in horses that underwent general anesthesia and cervical myelography. *J Vet Intern Med*. 2015;29:954–960.
267. Van Biervliet J. An evidence-based approach to clinical questions in the practice of equine neurology. *Vet Clin North Am Equine Pract*. 2007;23:317–328.
268. van Biervliet J, Scrivani PV, Divers TJ, et al. Evaluation of decision criteria for detection of spinal cord compression based on cervical myelography in horses: 38 cases (1981-2001). *Equine Vet J*. 2004;36:14–20.
269. Prange T, Derksen FJ, Stick JA, et al. Cervical vertebral canal endoscopy in the horse: intra- and post operative observations. *Equine Vet J*. 2011;43:404–411.
270. Prange T, Carr EA, Stick JA, et al. Cervical vertebral canal endoscopy in a horse with cervical vertebral stenotic myelopathy. *Equine Vet J*. 2012;44:116–119.
271. Donawick WJ, Mayhew IG, Galligan DT, et al. Results of a low-protein, low-energy diet and confinement on young horses with wobbles. *Proc Amer Assoc Equine Pract*. 1993;39:125–127.
272. Grant BD, Barbee DD, Wagner PC, et al. Long term results of surgery for equine cervical vertebral malformation. *Proc Amer Assoc Equine Pract*. 1985;31:91–96.
273. Moore BR, Reed SM, Robertson JT. Surgical treatment of cervical stenotic myelopathy in horses: 73 cases (1983-1992). *J Am Vet Med Assoc*. 1993;203:108–112.
274. Walmsley JP. Surgical treatment of cervical spinal cord compression in horses: a European experience. *Equine Vet Educ*. 2005;17:39–43.
275. Miller MM, Collatos C. Equine degenerative myeloencephalopathy. *Vet Clin North Am Equine Pract*. 1997;13:43–52.
276. Finno CJ, Higgins RJ, Aleman M, et al. Equine degenerative myeloencephalopathy in Lusitano horses. *J Vet Intern Med*. 2011;25:1439–1446.
277. Beech J. Neuroaxonal dystrophy of the accessory cuneate nucleus in horses. *Vet Pathol*. 1984;21:384–393.
278. Blythe LL, Hultgren BD, Craig AM, et al. Clinical, viral, and genetic evaluation of equine degenerative myeloencephalopathy in a family of Appaloosas. *J Am Vet Med Assoc*. 1991;198:1005–1013.
279. Aleman M, Finno CJ, Higgins RJ, et al. Evaluation of epidemiological, clinical, and pathological features of neuroaxonal dystrophy in Quarter Horses. *J Am Vet Med Assoc*. 2011;239:823–833.
280. Mayhew IG, De Lahunta A, Whitlock RH. Equine degenerative myeloencephalopathy. *Proc Amer Assoc Equine Pract*. 1976;22:103–105.
281. Liu SK, Dolensek EP, Adams CR, et al. Myelopathy and vitamin E deficiency in six Mongolian wild horses. *J Am Vet Med Assoc*. 1983;183:1266–1268.
282. Montali RJ, Bush M, Sauer RM, et al. Spinal ataxia in zebras. Comparison with the wobbler syndrome of horses. *Vet Pathol*. 1974;11:68–78.
283. Mayhew IG, Brown CM, Stowe HD, et al. Equine degenerative myeloencephalopathy: a vitamin E deficiency that may be familial. *J Vet Intern Med*. 1987;1:45–50.
284. Blythe LL, Craig AM, Lassen ED, et al. Serially determined plasma alpha-tocopherol concentrations and results of the oral vitamin E absorption test in clinically normal horses and in horses with degenerative myeloencephalopathy. *Am J Vet Res*. 1991;52:908–911.
285. Finno CJ, Famula T, Aleman M, et al. Pedigree analysis and exclusion of alpha-tocopherol transfer protein (TTPA) as a candidate gene for neuroaxonal dystrophy in the American Quarter Horse. *J Vet Intern Med*. 2013;27:177–185.
286. Beech J. Equine degenerative myeloencephalopathy. *Vet Clin North Am Equine Pract*. 1987;3:379–383.
287. Mayhew IG, deLahunta A, Whitlock RH, et al. Equine degenerative myeloencephalopathy. *J Am Vet Med Assoc*. 1977;170:195–201.
288. Finno CJ, Kaese HJ, Miller AD, et al. Pigment retinopathy in warmblood horses with equine degenerative myeloencephalopathy and equine motor neuron disease. *Vet Ophthalmol*. 2016.
289. Brosnahan MM, Holbrook TC, Ritchey JW. Neuroaxonal dystrophy associated with cerebellar dysfunction in a 5-month-old Pony of the Americas colt. *J Vet Intern Med*. 2009;23:1303–1306.
290. Finno CJ, Valberg SJ, Shivers J, et al. Evidence of the Primary Afferent Tracts Undergoing Neurodegeneration in Horses With Equine Degenerative Myeloencephalopathy Based on Calretinin Immunohistochemical Localization. *Vet Pathol*. 2016;53:77–86.
291. Wong DM, Ghosh A, Fales-Williams AJ, et al. Evidence of oxidative injury of the spinal cord in 2 horses with equine degenerative myeloencephalopathy. *Vet Pathol*. 2012;49:1049–1053.
292. Finno CJ, Miller AD, Siso S, et al. Concurrent equine degenerative myeloencephalopathy and equine motor neuron disease in three young horses. *J Vet Intern Med*. 2016;30:1344–1350.
293. Beech J, Haskins M. Genetic studies of neuraxonal dystrophy in the Morgan. *Am J Vet Res*. 1987;48:109–113.
294. Toenniessen JAG, Morin DE. Degenerative myelopathy: a comparative review. *Comp Contin Educ Pract Vet*. 1995;17:271–283.
295. Dill SG, Correa MT, Erb HN, et al. Factors associated with the development of equine degenerative myeloencephalopathy. *Am J Vet Res*. 1990;51:1300–1305.
296. Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. *J Cereb Blood Flow Metab*. 2001;21:2–14.
297. Blythe LL, Craig AM. Equine degenerative myeloencephalopathy 1: clinical signs and pathogenesis. *Comp Contin Educ Pract Vet*. 1992;14:1215–1221.
298. Cummings JF, de Lahunta A, Mohammed HO, et al. Histopathology of equine motor neuron disease (EMND). *First Int Workshop*. 1997:23–24.
299. Gandini G, Fatzer R, Mariscoli M, et al. Equine degenerative myeloencephalopathy in five Quarter Horses: clinical and neuropathological findings. *Equine Vet J*. 2004;36:83–85.
300. Dill SG, Kallfelz FA, deLahunta A, et al. Serum vitamin E and blood glutathione peroxidase values of horses with degenerative myeloencephalopathy. *Am J Vet Res*. 1989;50:166–168.
301. Siso S, Ferrer I, Pumarola M. Abnormal synaptic protein expression in two Arabian horses with equine degenerative myeloencephalopathy. *Vet J*. 2003;166:238–243.
302. Craig AM, Blythe LL, Lassen ED, et al. Variations of serum vitamin E, cholesterol, and total serum lipid concentrations in horses during a 72-hour period. *Am J Vet Res*. 1989;50:1527–1531.
303. Pusterla N, Puschner B, Steidl S, et al. α-Tocopherol concentrations in equine serum and cerebrospinal fluid after vitamin E supplementation. *Vet Rec*. 2010;166:366–368.
304. Vatassery GT, Brin MF, Fahn S, et al. Effect of high doses of dietary vitamin E on the concentrations of vitamin E in several brain regions, plasma, liver, and adipose tissue of rats. *J Neurochem*. 1988;51:621–623.
305. Finno CJ, Valberg SJ. A comparative review of vitamin E and associated equine disorders. *J Vet Intern Med*. 2012;26:1251–1266.
306. Blythe LL. Equine degeneratie myeloencephalopathy—genetics and treatment. *Int Equine Neurol Conf*. 1997.
307. Mayhew IG. Equine degenerative myeloencephalopathy (EDM): clinical findings and suspected aetiology. *Int Equine Neurol Conf*. 1997.
308. Rooney JR, Prickett ME, Delaney FM, et al. Focal myelitis-encephalitis in horses. *Cornell Vet*. 1970;60:494–501.
309. Dubey JP. A review of Sarcocystis of domestic animals and of other coccidia of cats and dogs. *J Am Vet Med Assoc*. 1976;169:1061–1078.
310. Dubey JP, Davis SW, Speer CA, et al. Sarcocystis neurona n. sp. (Protozoa: Apicomplexa), the etiologic agent of equine protozoal myeloencephalitis. *J Parasitol*. 1991;77:212–218.

311. Marsh AE, Barr BC, Madigan J, et al. Neosporosis as a cause of equine protozoal myeloencephalitis. *J Am Vet Med Assoc*. 1996;209:1907–1913.
312. Pusterla N, Tamez-Trevino E, White A, et al. Comparison of prevalence factors in horses with and without seropositivity to Neospora hughesi and/or Sarcocystis neurona. *Vet J*. 2014;200:332–334.
313. Reed SM, Furr M, Howe DK, et al. Equine protozoal myeloencephalitis: an updated consensus statement with a focus on parasite biology, diagnosis, treatment, and prevention. *J Vet Intern Med*. 2016;30:491–502.
314. Blazejewski T, Nursimulu N, Pszenny V, et al. Systems-based analysis of the Sarcocystis neurona genome identifies pathways that contribute to a heteroxenous life cycle. *MBio*. 2015:6.
315. Dubey JP, Howe DK, Furr M, et al. An update on Sarcocystis neurona infections in animals and equine protozoal myeloencephalitis (EPM). *Vet Parasitol*. 2015;209:1–42.
316. Reed SM, Granstrom D, Rivas LJ, et al. Results of cerebrospinal fluid analysis in 119 horses testing positive to the Western blot test on both serum and CSF to equine protozoal encephalomyelitis. *Proc Amer Assoc Equine Pract*. 1994;40:199.
317. Saville WJ, Reed SM, Morley PS, et al. Analysis of risk factors for the development of equine protozoal myeloencephalitis in horses. *J Am Vet Med Assoc*. 2000;217:1174–1180.
318. Tillotson K, McCue PM, Granstrom DE, et al. Seroprevalence of antibodies to Sarcocystis neurona in horses residing in northern Colorado. *J Equine Vet Sci*. 1999;19:122–126.
319. Boy MG, Galligan DT, Divers TJ. Protozoal encephalomyelitis in horses: 82 cases (1972-1986). *J Am Vet Med Assoc*. 1990;196:632–634.
320. Fayer R, Mayhew IG, Baird JD, et al. Epidemiology of equine protozoal myeloencephalitis in North America based on histologically confirmed cases. A report. *J Vet Intern Med*. 1990;4:54–57.
321. Gray LC, Magdesian KG, Sturges BK, et al. Suspected protozoal myeloencephalitis in a two-month-old colt. *Vet Rec*. 2001;149:269–273.
322. MacKay RL, Davis SW, Dubey JP. Equine protozoal myeloencephalitis. *Comp Contin Educ Pract Vet*. 1992;14:1359–1366.
323. Fenger CK, Granstrom DE, Langemeier JL, et al. Epizootic of equine protozoal myeloencephalitis on a farm. *J Am Vet Med Assoc*. 1997;210:923–927.
324. Morley PS, Traub-Dargatz JL, Benedict KM, et al. Risk factors for owner-reported occurrence of equine protozoal myeloencephalitis in the US equine population. *J Vet Intern Med*. 2008;22:616–629.
325. Ronen N. Putative equine protozoal myeloencephalitis in an imported Arabian filly. *J S Afr Vet Assoc*. 1992;63:78–79.
326. Lam KKH, Watkins KL, Chan CW. First report of equine protozoal myeloencephalitis in Hong Kong. *Equine Vet Educ*. 1999;11:54–56.
327. Goehring LS, Sloet van Oldruitenborgh-Oosterbaan MM. Equine protozoal myeloencephalitis in the Netherlands? An overview. *Tijdschr Diergeneeskd*. 2001;126:346–351.
328. Pitel PH, Pronost S, Gargala G, et al. Detection of Sarcocystis neurona antibodies in French horses with neurological signs. *Int J Parasitol*. 2002;32:481–485.
329. Fayer R, Dubey JP. Comparative epidemiology of coccidia: clues to the etiology of equine protozoal myeloencephalitis. *Int J Parasitol*. 1987;17:615–620.
330. Stanek JF, Stich RW, Dubey JP, et al. Epidemiology of Sarcocystis neurona infections in domestic cats (Felis domesticus) and its association with equine protozoal myeloencephalitis (EPM) case farms and feral cats from a mobile spay and neuter clinic. *Vet Parasitol*. 2003;117:239–249.
331. Dubey JP, Saville WJ, Lindsay DS, et al. Completion of the life cycle of Sarcocystis neurona. *J Parasitol*. 2000;86:1276–1280.
332. Mullaney T, Murphy AJ, Kiupel M, et al. Evidence to support horses as natural intermediate hosts for Sarcocystis neurona. *Vet Parasitol*. 2005;133:27–36.
333. Box ED. Recovery of Sarcocystis sporocysts from feces after oral administration. In: *Proceedings of the Helminthological Society of Washington*. 1983;50:348–350.
334. Pivoto FL, de Macedo Jr AG, da Silva MV, et al. Serological status of mares in parturition and the levels of antibodies (IgG) against protozoan family Sarcocystidae from their pre colostral foals. *Vet Parasitol*. 2014;199:107–111.
335. Pusterla N, Mackie S, Packham A, et al. Serological investigation of transplacental infection with Neospora hughesi and Sarcocystis neurona in broodmares. *Vet J*. 2014;202:649–650.
336. McAllister MM, Dubey JP, Lindsay DS, et al. Dogs are definitive hosts of Neospora caninum. *Int J Parasitol*. 1998;28:1473–1478.
337. Dubey JP. Recent advances in Neospora and neosporosis. *Vet Parasitol*. 1999;84:349–367.
338. Antonello AM, Pivoto FL, Camillo G, et al. The importance of vertical transmission of Neospora sp. in naturally infected horses. *Vet Parasitol*. 2012;187:367–370.
339. Sofaly CD, Reed SM, Gordon JC, et al. Experimental induction of equine protozoan myeloencephalitis (EPM) in the horse: effect of Sarcocystis neurona sporocyst inoculation dose on the development of clinical neurologic disease. *J Parasitol*. 2002;88:1164–1170.
340. Saville WJ, Stich RW, Reed SM, et al. Utilization of stress in the development of an equine model for equine protozoal myeloencephalitis. *Vet Parasitol*. 2001;95:211–222.
341. Cutler TJ, MacKay RJ, Ginn PE, et al. Immunoconversion against Sarcocystis neurona in normal and dexamethasone-treated horses challenged with S. neurona sporocysts. *Vet Parasitol*. 2001;95:197–210.
342. Njoku CJ, Saville WJ, Reed SM, et al. Reduced levels of nitric oxide metabolites in cerebrospinal fluid are associated with equine protozoal myeloencephalitis. *Clin Diagn Lab Immunol*. 2002;9:605–610.
343. Elsheikha HM, Schott 2nd HC, Mansfield LS. Genetic variation among isolates of Sarcocystis neurona, the agent of protozoal myeloencephalitis, as revealed by amplified fragment length polymorphism markers. *Infect Immun*. 2006;74:3448–3454.
344. Howe DK, Gaji RY, Marsh AE, et al. Strains of Sarcocystis neurona exhibit differences in their surface antigens, including the absence of the major surface antigen SnSAG1. *Int J Parasitol*. 2008;38:623–631.
345. Wendte JM, Miller MA, Lambourn DM, et al. Self-mating in the definitive host potentiates clonal outbreaks of the apicomplexan parasites Sarcocystis neurona and Toxoplasma gondii. *PLoS Genet*. 2010;6:e1001261.
346. Hyun C, Gupta GD, Marsh AE. Sequence comparison of Sarcocystis neurona surface antigen from multiple isolates. *Vet Parasitol*. 2003;112:11–20.
347. Speer CA, Dubey JP, Mattson DE. Comparative development and merozoite production of two isolates of Sarcocystis neurona and Sarcocystis falcatula in cultured cells. *J Parasitol*. 2000;86:25–32.
348. Ellison SP, Greiner EC, Brown KK, et al. Experimental infection of horses wtih culture-derived Sarcocystis neurona merozoites as a model for equine protozoal myeloencephalitis. *Intern J Appl Res Vet Med*. 2004;2:79–89.
349. Lindsay DS, Mitchell SM, Yang J, et al. Penetration of equine leukocytes by merozoites of Sarcocystis neurona. *Vet Parasitol*. 2006;138:371–376.
350. Lewis SR, Ellison SP, Dascanio JJ, et al. Effects of experimental Sarcocystis neurona-induced infection on immunity in an equine model. *J Vet Med*. 2014;2014:239495.
351. Witonsky SG, Gogal Jr RM, Duncan Jr RB, et al. Prevention of meningo/encephalomyelitis due to Sarcocystis neurona infection in mice is mediated by CD8 cells. *Int J Parasitol*. 2005;35:113–123.
352. Dubey JP, Lindsay DS. Isolation in immunodeficient mice of Sarcocystis neurona from opossum (Didelphis virginiana) faeces, and its differentiation from Sarcocystis falcatula. *Int J Parasitol*. 1998;28:1823–1828.

353. Finno CJ, Packham AE, David Wilson W, et al. Effects of blood contamination of cerebrospinal fluid on results of indirect fluorescent antibody tests for detection of antibodies against Sarcocystis neurona and Neospora hughesi. *J Vet Diagn Invest*. 2007;19:286–289.
354. Furr M, Howe D, Reed S, et al. Antibody coefficients for the diagnosis of equine protozoal myeloencephalitis. *J Vet Intern Med*. 2011;25:138–142.
355. Granstrom DE. Diagnosis of equine protozoal myeloencephalitis: western blot analysis. *Ann Forum Am Coll Vet Int Med*. 1993.
356. Furr M. Antigen-specific antibodies in cerebrospinal fluid after intramuscular injection of ovalbumin in horses. *J Vet Intern Med*. 2002;16:588–592.
357. Duarte PC, Daft BM, Conrad PA, et al. Evaluation and comparison of an indirect fluorescent antibody test for detection of antibodies to Sarcocystis neurona, using serum and cerebrospinal fluid of naturally and experimentally infected, and vaccinated horses. *J Parasitol*. 2004;90:379–386.
358. Saville WJ, Dubey JP, Oglesbee MJ, et al. Experimental infection of ponies with Sarcocystis fayeri and differentiation from Sarcocystis neurona infections in horses. *J Parasitol*. 2004;90:1487–1491.
359. Johnson AL, Morrow JK, Sweeney RW. Indirect fluorescent antibody test and surface antigen ELISAs for antemortem diagnosis of equine protozoal myeloencephalitis. *J Vet Intern Med*. 2013;27:596–599.
360. Reed SM, Howe DK, Morrow JK, et al. Accurate antemortem diagnosis of equine protozoal myeloencephalitis (EPM) based on detecting intrathecal antibodies against Sarcocystis neurona using the SnSAG2 and SnSAG4/3 ELISAs. *J Vet Intern Med*. 2013;27:1193–1200.
361. Hoane JS, Morrow JK, Saville WJ, et al. Enzyme-linked immunosorbent assays for detection of equine antibodies specific to Sarcocystis neurona surface antigens. *Clin Diagn Lab Immunol*. 2005;12:1050–1056.
362. Yeargan MR, Howe DK. Improved detection of equine antibodies against Sarcocystis neurona using polyvalent ELISAs based on the parasite SnSAG surface antigens. *Vet Parasitol*. 2011;176:16–22.
363. MacKay RJ. Equine protozoal myeloencephalitis: treatment, prognosis, and prevention. *Clin Tech Equine Pract*. 2006;5:9–16.
364. Furr M, Kennedy T. Cerebrospinal fluid and serum concentrations of ponazuril in horses. *Vet Ther*. 2001;2:232–237.
365. Furr M, Kennedy T, MacKay R, et al. Efficacy of ponazuril 15% oral paste as a treatment for equine protozoal myeloencephalitis. *Vet Ther*. 2001;2:215–222.
366. Dirikolu L, Foreman JH, Tobin T. Current therapeutic approaches to equine protozoal myeloencephalitis. *J Am Vet Med Assoc*. 2013;242:482–491.
367. Darius AK, Mehlhorn H, Heydorn AO. Effects of toltrazuril and ponazuril on Hammondia heydorni (syn. Neospora caninum) infections in mice. *Parasitol Res*. 2004;92:520–522.
368. Mitchell SM, Zajac AM, Davis WL, et al. The effects of ponazuril on development of apicomplexans in vitro. *J Eukaryot Microbiol*. 2005;52:231–235.
369. Finno CJ, Aleman M, Pusterla N. Equine protozoal myeloencephalitis associated with neosporosis in 3 horses. *J Vet Intern Med*. 2007;21:1405–1408.
370. Furr M, McKenzie H, Saville WJ, et al. Prophylactic administration of ponazuril reduces clinical signs and delays seroconversion in horses challenged with Sarcocystis neurona. *J Parasitol*. 2006;92:637–643.
371. Mackay RJ, Tanhauser ST, Gillis KD, et al. Effect of intermittent oral administration of ponazuril on experimental Sarcocystis neurona infection of horses. *Am J Vet Res*. 2008;69:396–402.
372. Pusterla N, Packham A, Mackie S, et al. Daily feeding of diclazuril top dress pellets in foals reduces seroconversion to Sarcocystis neurona. *Vet J*. 2015;206:236–238.
373. Hunyadi L, Papich MG, Pusterla N. Pharmacokinetics of a low dose and FDA-labeled dose of diclazuril administered orally as a pelleted topdressing in adult horses. *J Vet Pharmacol Ther*. 2015;38:243–248.
374. Ostlund EN, Powell D, Bryans JT. Equine herpesvirus 1: a review. In: *Proceedings of the thirty-sixth annual convention of the American Association of Equine Practitioners*. Lexington, KY: AAEP; 1991:387–395.
375. Ostlund EN. The equine herpesviruses. *Vet Clin North Am Equine Pract*. 1993;9:283–294.
376. Powell DG. *Viral respiratory disease*. Philadelphia: WB Saunders; 1992.
377. Jackson TA, Osburn BI, Cordy DR, et al. Equine herpesvirus 1 infection of horses: studies on the experimentally induced neurologic disease. *Am J Vet Res*. 1977;38:709–719.
378. Peet RL, Coackley W, Smith VW, et al. Equine abortion associated with herpesvirus. *Aust Vet J*. 1978;54:151.
379. Van Maanen C. Equine herpesvirus 1 and 4 infections: an update. *Vet Q*. 2002;24:58–78.
380. Reed SM, Toribio RE. Equine herpesvirus 1 and 4. *Vet Clin North Am Equine Pract*. 2004;20:631–642.
381. Patel JR, Heldens J. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4)—epidemiology, disease and immunoprophylaxis: a brief review. *Vet J*. 2005;170:6–7.
382. McCartan CG, Russell MM, Wood JL, et al. Clinical, serological and virological characteristics of an outbreak of paresis and neonatal foal disease due to equine herpesvirus-1 on a stud farm. *Vet Rec*. 1995;136:7–12.
383. van Maanen C, Sloet van Oldruitenborgh-Oosterbaan MM, Damen EA, et al. Neurological disease associated with EHV-1-infection in a riding school: clinical and virological characteristics. *Equine Vet J*. 2001;33:191–196.
384. Henninger RW, Reed SM, Saville WJ, et al. Outbreak of neurologic disease caused by equine herpesvirus-1 at a university equestrian center. *J Vet Intern Med*. 2007;21:157–165.
385. Meyer H, Thein P, Hubert P. Characterization of two equine herpesvirus (EHV) isolates associated with neurological disorders in horses. *Zentralbl Veterinarmed B*. 1987;34:545–548.
386. Thein P, Darai G, Janssen W, et al. Recent information about the etiopathogenesis of paretic-paralytic forms of herpesvirus infection in horses. *Tierarztl Praxis*. 1993;21:445–450.
387. Wilson JH. Neurological syndrome of rhinopneumonitis. In: *Proceedings of the ninth annual Veterinary Medicine Forum of the American College of Veterinary Internal Medicine*. San Diego; 1991:419–421.
388. Slater JD, Borchers K, Thackray AM, et al. The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. *J Gen Virol*. 1994;75:2007–2016.
389. Crabb BS, Studdert MJ. Equine herpesviruses 4 (equine rhino pneumonitis virus) and 1 (equine abortion virus). *Adv Virus Res*. 1995;45:153–190.
390. Agius CT, Nagesha HS, Studdert MJ. Equine herpesvirus 5: comparisons with EHV2 (equine cytomegalovirus), cloning, and mapping of a new equine herpesvirus with a novel genome structure. *Virology*. 1992;191:176–186.
391. Browning GF, Ficorilli N, Studdert MJ. Asinine herpesvirus genomes: comparison with those of the equine herpesviruses. *Arch Virol*. 1988;101:183–190.
392. Crabb BS, Studdert MJ. Comparative studies of the proteins of equine herpesviruses 4 and 1 and asinine herpesvirus 3: antibody response of the natural hosts. *J Gen Virol*. 1990;71:2033–2041.
393. Crabb BS, Allen GP, Studdert MJ. Characterization of the major glycoproteins of equine herpesviruses 4 and 1 and asinine herpesvirus 3 using monoclonal antibodies. *J Gen Virol*. 1991;72:2075–2082.
394. Allen GP, Bryans JT. Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infections. *Prog Vet Microbiol Immunol*. 1986;2:78–144.

395. Patel JR, Edington N. The pathogenicity in mice of respiratory, abortion and paresis isolates of equine herpesvirus-1. *Vet Microbiol*. 1983;8:301–305.
396. Palfi V, Christensen LS. Analyses of restriction fragment patterns (RFPs) and pathogenicity in baby mice of equine herpesvirus 1 and 4 (EHV-1 and EHV-4) strains circulating in Danish horses. *Vet Microbiol*. 1995;47:199–204.
397. van Woensel PA, Goovaerts D, Markx D, et al. A mouse model for testing the pathogenicity of equine herpes virus-1 strains. *J Virol Methods*. 1995;54:39–49.
398. Chowdhury SI, Kubin G, Ludwig H. Equine herpesvirus type 1 (EHV-1) induced abortions and paralysis in a Lipizzaner stud: a contribution to the classification of equine herpesviruses. *Arch Virol*. 1986;90:273–288.
399. Nowotny N, Burtscher H, Burki F. Neuropathogenicity for suckling mice of equine herpesvirus 1 from the Lipizzan outbreak 1983 and of selected other EHV 1 strains. *Zentralbl Veterinarmed B*. 1987;34:441–448.
400. Nugent J, Birch-Machin I, Smith KC, et al. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. *J Virol*. 2006;80:4047–4060.
401. Goodman LB, Loregian A, Perkins GA, et al. A point mutation in a herpesvirus polymerase determines neuropathogenicity. *PLoS Pathog*. 2007;3:e160.
402. Wernery U, Wade JF, Mumford JA, et al. *Equine infectious diseases VIII*. Dubai: R & W Publications; 1999.
403. Kydd JH, Smith KC, Hannant D, et al. Distribution of equid herpesvirus-1 (EHV-1) in respiratory tract associated lyphoid tissue: implications for cellular immunity. *Equine Vet J*. 1994;26:470–473.
404. Edington N, Welch HM, Griffiths L. The prevalence of latent equid herpesviruses in the tissues of 40 Abattoir horses. *Equine Vet J*. 1994;26:140–142.
405. Welch HM, Bridges CG, Lyon AM, et al. Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co-cultivation from lymphoid tissues. *J Gen Virol*. 1992;73:261–268.
406. Rappocciolo G, Birch J, Ellis SA. Down-regulation of MHC class I expression by equine herpesvirus-1. *J Gen Virol*. 2003;84:293–300.
407. Edington N, Bridges CG, Huckle A. Experimental reactivation of equid herpesvirus 1 (EHV 1) following the administration of corticosteroids. *Equine Vet J*. 1985;17:369–372.
408. Patel JR, Edington N, Mumford JA. Variation in cellular tropism between isolates of equine herpesvirus-1 in foals. *Arch Virol*. 1982;74:41–51.
409. Anonymous. EHV-1: a recurrent problem. *Vet Rec*. 1989;124:443–444.
410. Campbell TM, Studdert MJ. Equine herpesvirus type 1 (EHV 1). *Vet Bull*. 1983;53:135–146.
411. Saxegaard F. Isolation and identification of equine rhinopneumonitis virus (equine abortion virus) from cases of abortion and paralysis. *Nord Vet Med*. 1966;18:504–516.
412. Bitsch V, Dam A. Nervous disturbances in horses in relation to infection with equine rhinopneumonitis virus. *Acta Vet Scand*. 1971;12:134–136.
413. Thorsen J, Little PB. Isolation of equine herpesvirus type 1 from a horse with an acute paralytic disease. *Can J Comp Med*. 1975;39:358–359.
414. Platt H, Singh H, Whitwell KE. Pathological observations on an outbreak of paralysis in broodmares. *Equine Vet J*. 1980;12:118–126.
415. Bryans JT, Allen GP. Equine viral rhinopneumonitis. *Rev Sci Tech*. 1986;5:837.
416. Greenwood RE, Simson AR. Clinical report of a paralytic syndrome affecting stallions, mares and foals on a thoroughbred stud farm. *Equine Vet J*. 1980;12:113–117.
417. Friday PA, Scarratt WK, Elvinger F, et al. Ataxia and paresis with equine herpesvirus type 1 infection in a herd of riding school horses. *J Vet Intern Med*. 2000;14:197–201.
418. USDA-APHIS: Equine herpes virus myeloencephalopathy: a potentially emerging disease (website) https://www.aphis.usda.gov/animal_health/emergingissues/downloads/ehv1final.pdf.
419. Allen GP, Breathnach CC. Quantification by real-time PCR of the magnitude and duration of leucocyte-associated viraemia in horses infected with neuropathogenic vs. non-neuropathogenic strains of EHV-1. *Equine Vet J*. 2006;38:252–257.
420. Crowhurst FA, Dickinson G, Burrows R. An outbreak of paresis in mares and geldings associated with equid herpesvirus 1. *Vet Rec*. 1981;109:527–528.
421. Jackson T, Kendrick JW. Paralysis of horses associated with equine herpesvirus 1 infection. *J Am Vet Med Assoc*. 1971;158:1351–1357.
422. Thein P. Infection of the central nervous system of horses with equine herpesvirus serotype 1. *J S Afr Vet Assoc*. 1981;52:239–241.
423. Charlton KM, Mitchell D, Girard A, et al. Meningoencephalomyelitis in horses associated with equine herpesvirus 1 infection. *Vet Pathol*. 1976;13:59–68.
424. Montali RJ, Allen GP, Bryans JT, et al. Equine herpesvirus type 1 abortion in an onager and suspected herpesvirus myelitis in a zebra. *J Am Vet Med Assoc*. 1985;187:1248–1249.
425. Pursell AR, Sangster LT, Byars TD, et al. Neurologic disease induced by equine herpesvirus 1. *J Am Vet Med Assoc*. 1979;175:473–474.
426. Franklin TE, Daft BM, Silverman VJ, et al. Serological titers and clinical observations in equines suspected of being infected with EHV-1. *Calif Vet J*. 1985;39:22–24.
427. Hughes PE, Ryan CP, Carlson GP, et al. An epizootic of equine herpes virus-1 myeloencephalitis. *Unpublished observations*. 1987.
428. Kydd JH, Smith KC, Hannant D, et al. Distribution of equid herpesvirus-1 (EHV-1) in the respiratory tract of ponies: implications for vaccination strategies. *Equine Vet J*. 1994;26:466–469.
429. Scott JC, Dutta SK, Myrup AC. In vivo harboring of equine herpesvirus-1 in leukocyte populations and subpopulations and their quantitation from experimentally infected ponies. *Am J Vet Res*. 1983;44:1344–1348.
430. Bryans JT. On immunity to disease caused by equine herpesvirus 1. *J Am Vet Med Assoc*. 1969;155:294–300.
431. Edington N, Bridges CG, Patel JR. Endothelial cell infection and thrombosis in paralysis caused by equid herpesvirus-1: equine stroke. *Arch Virol*. 1986;90:111–124.
432. Edington N, Smyth B, Griffiths L. The role of endothelial cell infection in the endometrium, placenta and foetus of equid herpesvirus 1 (EHV-1) abortions. *J Comp Pathol*. 1991;104:379–387.
433. Slater JD, Gibson JS, Barnett KC, et al. Chorioretinopathy associated with neuropathology following infection with equine herpesvirus-1. *Vet Rec*. 1992;131:237–239.
434. Slater JD, Borchers K, Field HJ. Equine herpesvirus-1: a neurotropic alphaherpesvirus. *Vet Rec*. 1994;135:239–240 (letter).
435. Chesters PM, Allsop R, Purewal A, et al. Detection of latency-associated transcripts of equid herpesvirus 1 in equine leukocytes but not in trigeminal ganglia. *J Virol*. 1997;71:3437–3443.
436. Whitwell KE, Blunden AS. Pathological findings in horses dying during an outbreak of the paralytic form of equid herpesvirus type 1 (EHV-1) infection. *Equine Vet J*. 1992;24:13–19.
437. Studdert MJ, Fitzpatrick DR, Horner GW, et al. Molecular epidemiology and pathogenesis of some equine herpesvirus type 1 (equine abortion virus) and type 4 (equine rhinopneumonitis virus) isolates. *Aust Vet J*. 1984;61:345–348.
438. Dinter Z, Klingeborn B. Serological study of an outbreak of paresis due to equid herpesvirus 1 (EHV-1). *Vet Rec*. 1976;99:10–12.
439. Klingeborn B, Dinter Z. Measurement of neutralizing antibody to equid herpesvirus 1 by single radial hemolysis. *J Clin Microbiol*. 1978;7:495–496.

440. Klingeborn B, Dinter Z, Hughes RA. Antibody to neuritogenic myelin protein P2 in equine paresis due to equine herpesvirus 1. *Zentralbl Veterinarmed B*. 1983;30:137–140.
441. Braund KG, Brewer BD, Mayhew IG. *Equine herpesvirus type 1 infection*. Philadelphia: WB Saunders; 1987.
442. Little PB, Thorsen J. Disseminated necrotizing myeloencephalitis: a herpes-associated neurological disease of horses. *Vet Pathol*. 1976;13:161–171.
443. Little PB, Thorsen J, Moran K. Virus involvement in equine paresis. *Vet Rec*. 1974;95:575.
444. Mayhew IG. *Equine herpesvirus 1 (rhinopneumonitis) myeloencephalitis*. Philadelphia: Lea & Febiger; 1989.
445. deLahunta A. *Equine herpesvirus 1: myeloencephalopathy and vasculitis*. Philadelphia: WB Saunders; 1983.
446. George LW. *Equine herpesvirus 1 myeloencephalitis (rhinopneumonitis myelitis)*. St Louis: CV Mosby; 1990.
447. MacKay RJ, Mayhew IG. *Equine herpesvirus myeloencephalitis*. 4th ed. Goleta, CA: American Veterinary Publications; 1991.
448. Thomson GW, McCready R, Sanford E, et al. Case report: an outbreak of herpesvirus myeloencephalitis in vaccinated horses. *Can Vet J*. 1979;20:22–25.
449. Roberts RS. A paralytic syndrome in horses. *Vet Rec*. 1965;77: 404–405.
450. Keane DP, Little PB, Wilkie BN, et al. Agents of equine viral encephalomyelitis: correlation of serum and cerebrospinal fluid antibodies. *Can J Vet Res*. 1988;52:229–235.
451. Andrews FM, Granstrom D, Provenza M. Differentiation of neurologic diseases in the horse by the use of albumin quotient and IgG index determinations. In: *Proceedings of the forty-first annual conference of the American Association of Equine Practitioners*. Lexington, KY; 1995:215–217.
452. Whitwell KE, Gower SM, Smith KC. An immunoperoxidase method applied to the diagnosis of equine herpesvirus abortion, using conventional and rapid microwave techniques. *Equine Vet J*. 1992;24:10–12.
453. Schmidt P, Meyer H, Hubert P, et al. In-situ hybridization for demonstration of equine herpesvirus type 1 DNA in paraffin wax-embedded tissues and its use in horses with disseminated necrotizing myeloencephalitis. *J Comp Pathol*. 1994;110:215–225.
454. Sinclair R, Mumford JA. Rapid detection of equine herpesvirus type-1 antigens in nasal swab specimens using an antigen capture enzyme-linked immunosorbent assay. *J Virol Methods*. 1992;39:299–310.
455. Sharma PC, Cullinane AA, Onions DE, et al. Diagnosis of equid herpesviruses-1 and -4 by polymerase chain reaction. *Equine Vet J*. 1992;24:20–25.
456. Lawrence GL, Gilkerson J, Love DN, et al. Rapid, single-step differentiation of equid herpesviruses 1 and 4 from clinical material using the polymerase chain reaction and virus-specific primers. *J Virol Methods*. 1994;47:59–72.
457. Ballagi-Pordany A, Klingeborn B, Flensburg J, et al. Equine herpesvirus type 1: detection of viral DNA sequences in aborted fetuses with the polymerase chain reaction. *Vet Microbiol*. 1990;22:373–381.
458. Kirisawa R, Endo A, Iwai H, et al. Detection and identification of equine herpesvirus-1 and -4 by polymerase chain reaction. *Vet Microbiol*. 1993;36:57–67.
459. Wagner WN, Bogdan J, Haines D, et al. Detection of equine herpesvirus and differentiation of equine herpesvirus type 1 from type 4 by the polymerase chain reaction. *Can J Microbiol*. 1992;38:1193–1196.
460. Richa GYP, Charan S. A dot immunobinding assay in comparison with the gel diffusion test for the detection of equine herpesvirus-1 antigen from field samples. *Rev Sci Tech*. 1993;12:923–930.
461. Valentine-Thon E. Quality control in nucleic acid testing: where do we stand? *J Clin Virol*. 2002;25(suppl 3):S13–S21.
462. Drummer HE, Reynolds A, Studdert MJ, et al. Application of an equine herpesvirus 1 (EHV1) type-specific ELISA to the management of an outbreak of EHV1 abortion. *Vet Rec*. 1995;136:579–581.
463. Crabb BS, MacPherson CM, Reubel GH, et al. A type-specific serological test to distinguish antibodies to equine herpesviruses 4 and 1. *Arch Virol*. 1995;140:245–258.
464. Crabb BS, Studdert MJ. Epitopes of glycoprotein G of equine herpesviruses 4 and 1 located near the C termini elicit type-specific antibody responses in the natural host. *J Virol*. 1993;69:6332–6338.
465. Blythe LL, Mattson DE, Lassen ED, et al. Antibodies against equine herpesvirus 1 in the cerebrospinal fluid in the horse. *Can Vet J*. 1985;26:218–220.
466. Smith KO, Galloway KS, Hodges SL, et al. Sensitivity of equine herpesviruses 1 and 3 in vitro to a new nucleoside analogue, 9-[[2-hydroxy-1-(hydroxymethyl) ethoxy] methyl] guanine. *Am J Vet Res*. 1983;44:1032–1035.
467. Murray MJ, del Piero F, Jeffrey SC, et al. Neonatal equine herpesvirus type 1 infection on a thoroughbred breeding farm. *J Vet Intern Med*. 1998;12:36–41.
468. Garre B, Shebany K, Gryspeerdt A, et al. Pharmacokinetics of acyclovir after intravenous infusion of acyclovir and after oral administration of acyclovir and its prodrug valacyclovir in healthy adult horses. *Antimicrob Agents Chemother*. 2007;51:4308–4314.
469. Maxwell LK, Bentz BG, Bourne DW, et al. Pharmacokinetics of valacyclovir in the adult horse. *J Vet Pharmacol Ther*. 2008;31:312–320.
470. de la Fuente R, Awan AR, Field HJ. The acyclic nucleoside analogue penciclovir is a potent inhibitor of equine herpesvirus type 1 (EHV-1) in tissue culture and in a murine model. *Antiviral Res*. 1992;18:77–89.
471. Estell KE, Dawson DR, Magdesian KG, et al. Quantitative molecular viral loads in 7 horses with naturally occurring equine herpesvirus-1 infection. *Equine Vet J*. 2015;47:689–693.
472. Smith KC, Tearle JP, Boyle MS, et al. Replication of equid herpesvirus-1 in the vaginal tunics of colts following local inoculation. *Res Vet Sci*. 1993;54:249–251.
473. Mumford JA, Edington N. EHV1 and equine paresis. *Vet Rec*. 1980;106:277 (letter).
474. Mumford JA. Equid herpesvirus 1 (EHV 1) latency: more questions than answers. *Equine Vet J*. 1985;17:340–342 (editorial).
475. Eaglesome MD, Henry JN, McKnight JD. Equine herpesvirus 1 infection in mares vaccinated with a live-virus rhinopneumonitis vaccine attenuated in cell culture. *Can Vet J*. 1979;20:145–147.
476. Burrows R, Goodridge D, Denyer MS. Trials of an inactivated equid herpesvirus 1 vaccine: challenge with a subtype 1 virus. *Vet Rec*. 1984;114:369–374.
477. Burki F, Rossmanith W, Nowotny N, et al. Viraemia and abortions are not prevented by two commercial equine herpesvirus-1 vaccines after experimental challenge of horses. *Vet Q*. 1990;12:80–86.
478. Burki F, Nowotny N, Oulehla J, et al. Attempts to immunoprotect adult horses, specifically pregnant mares, with commercial vaccines against clinical disease induced by equine herpesvirus-1. *Zentralbl Veterinarmed B*. 1991;38:432–440.
479. Heldens JG, Hannant D, Cullinane AA, et al. Clinical and virological evaluation of the efficacy of an inactivated EHV1 and EHV4 whole virus vaccine (Duvaxyn EHV1,4): vaccination/challenge experiments in foals and pregnant mares. *Vaccine*. 2001;19:4307–4317.
480. Patel JR, Bateman H, Williams J, et al. Derivation and characterization of a live equid herpes virus-1 (EHV-1) vaccine to protect against abortion and respiratory disease due to EHV-1. *Vet Microbiol*. 2003;91:23–39.
481. Patel JR, Foldi J, Bateman H, et al. Equid herpesvirus (EHV-1) live vaccine strain C147: efficacy against respiratory diseases following EHV types 1 and 4 challenges. *Vet Microbiol*. 2003;92:1–17.
482. Patel JR, Didlick S, Bateman H. Efficacy of a live equine herpesvirus-1 (EHV-1) strain C147 vaccine in foals with maternally-derived antibody: protection against EHV-1 infection. *Equine Vet J*. 2004;36:447–451.

483. Reed SM. Neuritis of the cauda equina: polyneuritis equi in the horse. In: *JD Stewart Memorial Refresher Course for Veterinarians*. Sydney, Australia; 1992.
484. Vatistas NJ, Mayhew IG, Whitwell KE, et al. Polyneuritis equi: a clinical review incorporating a case report of a horse displaying unconventional signs. *Prog Vet Neurol*. 1991;2:67–72.
485. Rousseaux CG, Futcher KG, Clark EG, et al. Cauda equina neuritis: a chronic idiopathic polyneuritis in two horses. *Can Vet J*. 1984;25:214–218.
486. Wright JA, Fordyce P, Edington N. Neuritis of the cauda equina in the horse. *J Comp Pathol*. 1987;97:667–675.
487. White PL, Genetzsky RM, Pohlenz JFI, et al. Neuritis of the cauda equina in a horse. *Comp Contin Educ Pract Vet*. 1984;6:S217–S224.
488. Scarratt WK, Jortner BS. Neuritis of the cauda equina in a yearling filly. *Comp Contin Educ Pract Vet*. 1985;7:S197–S202.
489. Milne FJ, Carbonell PL. Neuritis of the cauda equina of horses: a case report. *Equine Vet J*. 1970;2:179–182.
490. Greenwood AG, Barker J, McLeish I. Neuritis of the cauda equina in a horse. *Equine Vet J*. 1973;5:111–115.
491. Fordyce PS, Edington N, Bridges GC, et al. Use of an ELISA in the differential diagnosis of cauda equina neuritis and other equine neuropathies. *Equine Vet J*. 1987;19:55–59.
492. Cummings JF, de Lahunta A, Timoney JF. Neuritis of the cauda equina, a chronic idiopathic polyradiculoneuritis in the horse. *Acta Neuropathol*. 1979;46:17–24.
493. Beech J. Neuritis of the cauda equina. *Proc Amer Assoc Equine Pract*. 1976;21:75–76.
494. Yvorchuk-St Jean K. Neuritis of the cauda equina. *Vet Clin North Am Equine Pract*. 1987;3:421–427.
495. Aleman M, Katzman SA, Vaughan B, et al. Antemortem diagnosis of polyneuritis equi. *J Vet Intern Med*. 2009;23:665–668.
496. Edington N, Wright JA, Patel JR, et al. Equine adenovirus 1 isolated from cauda equina neuritis. *Res Vet Sci*. 1984;37:252–254.
497. Kadlubowski M, Ingram PL. Circulating antibodies to the neuritogenic myelin protein, P2, in neuritis of the cauda equina of the horse. *Nature*. 1981;293:299–300.
498. van Galen G, Cassart D, Sandersen C, et al. The composition of the inflammatory infiltrate in three cases of polyneuritis equi. *Equine Vet J*. 2008;40:185–188.
499. Ostlund EN, Powell D, Bryans JT. Equine herpesvirus 1: a review. *Proc Am Assoc Equine Pract*. 1990;36:387–395.
500. Mayhew IG, Brewer BD, Reinhard MK, et al. Verminous (Strongylus vulgaris) myelitis in a donkey. *Cornell Vet*. 1984;74:30–37.
501. Lester G. Parasitic encephalomyelitis in horses. *Comp Contin Educ Pract Vet*. 1992;14:1624–1630.
502. Divers T, De Lahunta A, Hintz HF, et al. Equine motor neuron disease. *Equine Vet Educ*. 2001;13:63–67.
503. Grondahl G, Hanche-Olsen S, Brojer J, et al. Acquired equine polyneuropathy in Norway and Sweden: a clinical and epidemiological study. *Equine Vet J Suppl*. 2012:36–44.
504. Wolff C, Egenvall A, Hanche-Olsen S, et al. Spatial and temporal distribution of incidence of acquired equine polyneuropathy in Norway and Sweden, 1995-2012. *BMC Vet Res*. 2014;10:265.
505. Hahn CN, Matiasek K, Syrja P, et al. Polyneuropathy of Finnish horses characterised by inflammatory demyelination and intracisternal Schwann cell inclusions. *Equine Vet J*. 2008;40:231–236.
506. MacLachlan NJ, Dubovi EJ. *Fenner's Veterinary Virology*. New York: Elsevier; 2011.
507. Grady GF, Maxfield HK, Hildreth SW, et al. Eastern equine encephalitis in Massachusetts, 1957-1976. A prospective study centered upon analyses of mosquitoes. *Am J Epidemiol*. 1978;107:170–178.
508. Zacks MA, Paessler S. Encephalitic alphaviruses. *Vet Microbiol*. 2010;140:281–286.
509. Casals J. Antigenic Variants of Eastern Equine Encephalitis Virus. *J Exp Med*. 1964;119:547–565.
510. Hahn CS, Lustig S, Strauss EG, et al. Western equine encephalitis virus is a recombinant virus. *Proc Natl Acad Sci U S A*. 1988;85:5997–6001.
511. Trent DW, Grant JA. A comparison of New World alphaviruses in the western equine encephalomyelitis complex by immunochemical and oligonucleotide fingerprint techniques. *J Gen Virol*. 1980;47:261–282.
512. Calisher CH, Kinney RM, de Souza Lopes O, et al. Identification of a new Venezuelan equine encephalitis virus from Brazil. *Am J Trop Med Hyg*. 1982;31:1260–1272.
513. Martin DH, Dietz WH, Alvaerez Jr O, et al. Epidemiological significance of Venezuelan equine encephalomyelitis virus in vitro markers. *Am J Trop Med Hyg*. 1982;31:561–568.
514. Walton TE, Alvarez Jr O, Buckwalter RM, et al. Experimental infection of horses with enzootic and epizootic strains of Venezuelan equine encephalomyelitis virus. *J Infect Dis*. 1973;128:271–282.
515. Dietz Jr WH, Alvarez Jr O, Martin DH, et al. Enzootic and epizootic Venezuelan equine encephalomyelitis virus in horses infected by peripheral and intrathecal routes. *J Infect Dis*. 1978;137:227–237.
516. Udall DH. A report on the outbreak of "cerebro-spinal meningitis" (encephalitis) in horses in Kansas and Nebraska. *Cornell Vet*. 1913;3:17–43.
517. Meyer KF, Haring CM, Howitt B. Newer knowledge of neurotropic virus infections of horses. *J Amer Med Assoc*. 1931;79:376–389.
518. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg*. 2008;79:974–979.
519. TenBroeck C, Merrill MH. A serological difference between eastern and western equine encephalomyelitis virus. *Proc Soc Exp Biol Med*. 1933;85:89–95.
520. Records E, Vawter LR. Equine encephalomyelitis cross-immunity in horses between western and eastern strains of virus. *J Am Vet Med Assoc*. 1934;85:89–95.
521. Records E, Vawter LR. Equine encephalomyelitis crosss-immunity in horses between western and eastern strains of virus: supplemental report. *J Am Vet Med Assoc*. 1935;86:764–772.
522. Hoff GL, Bigler WJ, Buff EE, et al. Occurrence and distribution of western equine encephalomyelitis in Florida. *J Am Vet Med Assoc*. 1978;172:351–352.
523. Goldfield M, Sussman O, Black HC, et al. Arbovirus infection of animals in New Jersey. *J Am Vet Med Assoc*. 1968;153:1780–1787.
524. Shahan MS, Giltner LT. Equine encephalomyelitis studies. I. Cross-immunity tests between eastern and Western types of virus. *J Am Vet Med Assoc*. 1935;86:7664–7672.
525. Shahan MS, Giltner LT. A review of the epizootiology of equine encephalomyelitis in the United States. *J Am Vet Med Assoc*. 1945;107:279–288.
526. Kissling RE, Chamberlain RW. Venezuelan equine encephalitis. *Adv Vet Sci*. 1967;11:65–84.
527. Kissling RE, Chamberlain RW, Nelson DB, et al. Venezuelan equine encephalomyelitis in horses. *Am J Hyg*. 1956;63:274–287.
528. Gilyard RT. A clinical study of Venezuelan virus equine encephalomyelitis in Trinidad, BWI. *J Am Vet Med Assoc*. 1945;106:267–277.
529. Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: their geographic distribution and epidemiologic significance. *Am J Epidemiol*. 1969;89:286–307.
530. Scherer WF, Anderson K, Pancake BA, et al. Search for epizootic-like Venezuelan encephalitis virus at enzootic habitats in Guatemala during 1969-1971. *Am J Epidemiol*. 1976;103:576–588.
531. Scherer WF, Madalengoitia J, Flores W, et al. Ecologic studies of Venezuelan encephalitis virus in Peru during 1970-1971. *Am J Epidemiol*. 1975;101:347–355.
532. Sudia WD, Fernandez L, Newhouse VF, et al. Arbovirus vector ecology studies in Mexico during the 1972 Venezuelan equine encephalitis outbreak. *Am J Epidemiol*. 1975;101:51–58.

533. Sudia WD, McLean RG, Newhouse VF, et al. Epidemic Venezuelan equine encephalitis in North America in 1971: vertebrate field studies. *Am J Epidemiol*. 1975;101:36–50.
534. Sudia WD, Newhouse VF. Epidemic Venezuelan equine encephalitis in North America: a summary of virus-vector-host relationships. *Am J Epidemiol*. 1975;101:1–13.
535. Sudia WD, Newhouse VF, Beadle ID, et al. Epidemic Venezuelan equine encephalitis in North America in 1971: vector studies. *Am J Epidemiol*. 1975;101:17–35.
536. Monath TP. *The Arboviruses: epidemiology and ecology*. Boca Raton, FL: CRC; 1989.
537. Dietz Jr WH, Galindo P, Johnson KM. Eastern equine encephalomyelitis in Panama: the epidemiology of the 1973 epizootic. *Am J Trop Med Hyg*. 1980;29:133–140.
538. Srihongse S, Grayson MA, Morris CD, et al. Eastern equine encephalomyelitis in upstate New York: studies of a 1976 epizootic by a modified serologic technique, hemagglutination reduction, for rapid detection of virus infections. *Am J Trop Med Hyg*. 1978;27:1240–1245.
539. Bigler WJ, Lassing EB, Buff EE, et al. Endemic eastern equine encephalomyelitis in Florida: a twenty-year analysis, 1955-1974. *Am J Trop Med Hyg*. 1976;25:884–890.
540. Bast TF, Whitney E, Benach JL. Considerations on the ecology of several arboviruses in eastern Long Island. *Am J Trop Med Hyg*. 1973;22:109–115.
541. Bryant ES, Anderson CR, Van der Heide L. An epizootic of eastern equine encephalomyelitis in Connecticut. *Avian Dis*. 1973;17:861–867.
542. Morgante O, Vance HN, Shemanchuk JA, et al. Epizootic of western encephalomyelitis virus infection in equines in Alberta in 1965. *Can J Comp Med*. 1968;32:403–408.
543. Ellis RA. Emergency measures and mosquito control operations during the 1975 western encephalomyelitis outbreak in Manitoba. *Can J Public Health*. 1976;67(suppl 1):59–60.
544. Donogh NR. Public information on western encephalomyelitis and emergency mosquito control in Manitoba, 1975. *Can J Public Health*. 1976;67(suppl 1):61–62.
545. Lillie LE, Wong FC, Drysdale RA. Equine epizootic of western encephalomyelitis in Manitoba-1975. *Can J Public Health*. 1976;67(suppl 1):21–27.
546. Potter ME, Currier RW, Pearson JE, et al. Western equine encephalomyelitis in horses in the Northern Red River Valley, 1975. *J Am Vet Med Assoc*. 1977;170:1396–1399.
547. Morier L, Cantelar N, Soler M. Infection of a poikilothermic cell line (XL-2) with eastern equine encephalitis and western equine encephalitis viruses. *J Med Virol*. 1987;21:277–281.
548. Carneiro V, Cunha R. Equine encephalomyelitis in Brazil. *Arch Inst Biol Andina*. 1943;14:147–194.
549. Meyer KF, Wood F, Haring CM. Susceptibility of non-immune hyperimmunized horses and goats to eastern, western and Argentine virus of equine encephalomyelitis. *Proc Soc Exp Biol Med*. 1934;32:56–58.
550. Livesay HR. Isolation of eastern equine encephalitis virus from naturally infected monkey (Macacus philippensis). *J Infect Dis*. 1934;84:306–309.
551. Gibbs EP. Equine viral encephalitis. *Equine Vet J*. 1976;8:66–71.
552. Holden P. Recovery of western equine encephalomyelitis virus from naturally infected English sparrows of New Jersey, 1953. *Proc Soc Exp Biol Med*. 1955;88:490–492.
553. Sellers RF. Weather, host and vector—their interplay in the spread of insect-borne animal virus diseases. *J Hyg (Lond)*. 1980;85:65–102.
554. Smart DL, Trainer DO. Serologic evidence of Venezuelan equine encephalitis insome wild and domestic populations of Southern Texas. *J Wildl Dis*. 1975;11:195–200.
555. McLean RG, Frier G, Parham GL, et al. Investigations of the vertebrate hosts of eastern equine encephalitis during an epizootic in Michigan, 1980. *Am J Trop Med Hyg*. 1985;34:1190–1202.
556. Chamberlain RW, Kissling RE, Stamm DD, et al. Transmission of eastern equine encephalitis to horses by Aedes sollicitans mosquitoes. *Am J Trop Med Hyg*. 1956;5:802–808.
557. Crans WJ, McNelly J, Schulze TL, et al. Isolation of eastern equine encephalitis virus from Aedes sollicitans during an epizootic in southern New Jersey. *J Am Mosq Control Assoc*. 1986;2:68–72.
558. Hayes CG, Wallis RC. Ecology of Western equine encephalomyelitis in the eastern United States. *Adv Virus Res*. 1977;21:37–83.
559. Syverton JT, Berry GP. The tick as a vector for the virus disease equine encephalomyelitis. *J Bacteriol*. 1937;33:60.
560. Kitselman CH, Grundman AW. Equine encephalomyelitis virus isolated from naturally infected Triatoma sanguisuga. *Kans Agric Exp Station Tech Bull*. 1940;50:15.
561. Hardy JL. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945-1985. *Am J Trop Med Hyg*. 1987;37:18S–32S.
562. Hayes RO, Francy DB, Lazuick JS. Role of the cliff swallow bug (Oeciacus vicarius) in the natural cycle of a Western equine encephalitis-related alphavirus. *J Entomol*. 1977;14:257–262.
563. Vawter LR, Records E. Respiratory Infection in Equine Encephalomyelitis. *Science*. 1933;78:41–42.
564. Chamberlain RW. Vector relationships of the arthropod-borne encephalitides in North America. *Ann N Y Acad Sci*. 1958;70:312–319.
565. Ross WA, Kaneene JB. Evaluation of outbreaks of disease attributable to eastern equine encephalitis virus in horses. *J Am Vet Med Assoc*. 1996;208:1988–1997.
566. Pursell AR, Mitchell FE, Seibold HR. Naturally occurring and experimentally induced eastern encephalomyelitis in calves. *J Am Vet Med Assoc*. 1976;169:1101–1103.
567. Pursell AR, Peckham JC, Cole Jr JR, et al. Naturally occurring and artificially induced eastern encephalomyelitis in pigs. *J Am Vet Med Assoc*. 1972;161:1143–1147.
568. Byrne RJ. The control of eastern and Western arbovirus encephalomyelitis of horses. *Third Conference on Equine Infectious Diseases*. 1972:115–123.
569. Binn LN, Sponseller ML, Wooding WL, et al. Efficacy of an attenuated western encephalitis vaccine in equine animals. *Am J Vet Res*. 1966;27:1599–1604.
570. Henderson BE, Chappell WA, Johnston Jr JG, et al. Experimental infection of horses with three strains of Venezuelan equine encephalomyelitis virus. I. Clinical and virological studies. *Am J Epidemiol*. 1971;93:194–205.
571. Whitley RJ. Viral encephalitis. *N Engl J Med*. 1990;323:242–250.
572. Gahlinger PM, Reeves WC, Milby MM. Air conditioning and television as protective factors in arboviral encephalitis risk. *Am J Trop Med Hyg*. 1986;35:601–610.
573. McLintock J. The arbovirus problem in Canada. *Can J Public Health*. 1976;67(suppl 1):8–12.
574. Wilson JH, Rubin HL, Lane TJ, et al. A survey of eastern equine encephalomyelitis in florida horses: prevalence, eceonomic impact, and management practices, 1982-1983. *Prev Vet Med*. 1986;4:261–271.
575. Doby PB, Schnurrenberger PR, Martin RJ, et al. Western encephalitis in Illinois horses and ponies. *J Am Vet Med Assoc*. 1966;148:422–427.
576. Sponseller ML, Binn LN, Wooding WL, et al. Field strains of western encephalitis virus in ponies: virologic, clinical, and pathologic observations. *Am J Vet Res*. 1966;27:1591–1598.
577. Cox HR, Philip CB, Marsh H, et al. Observations incident to an outbreak of equine encephalomyelitis in the Bitterroot Valley of Western Montana. *J Am Vet Med Assoc*. 1938;94:225–232.
578. Taylor KG, Paessler S. Pathogenesis of Venezuelan equine encephalitis. *Vet Microbiol*. 2013;167:145–150.
579. Parker RL, Dean PB, Zehmer RB. Public health aspects of Venezuelan equine encephalitis. *J Am Vet Med Assoc*. 1973;162: 777–778.

580. Gardner CL, Burke CW, Tesfay MZ, et al. Eastern and Venezuelan equine encephalitis viruses differ in their ability to infect dendritic cells and macrophages: impact of altered cell tropism on pathogenesis. *J Virol*. 2008;82:10634–10646.
581. Carossino M, Thiry E, de la Grandiere A, et al. Novel vaccination approaches against equine alphavirus encephalitides. *Vaccine*. 2014;32:311–319.
582. Calisher CH, Mahmud MI, el-Kafrawi AO, et al. Rapid and specific serodiagnosis of western equine encephalitis virus infection in horses. *Am J Vet Res*. 1986;47:1296–1299.
583. Calisher CH, Emerson JK, Muth DJ, et al. Serodiagnosis of western equine encephalitis virus infections: relationships of antibody titer and test to observed onset of clinical illness. *J Am Vet Med Assoc*. 1983;183:438–440.
584. Vina-Rodriguez A, Eiden M, Keller M, et al. A Quantitative Real-Time RT-PCR Assay for the Detection of Venezuelan equine encephalitis virus Utilizing a Universal Alphavirus Control RNA. *Biomed Res Int*. 2016;2016:8543204.
585. Scott TW, Olson JG, All 3rd BP, et al. Detection of eastern equine encephalomyelitis virus antigen in equine brain tissue by enzyme-linked immunosorbent assay. *Am J Vet Res*. 1988;49:1716–1718.
586. Monath TP, McLean RG, Cropp CB, et al. Diagnosis of eastern equine encephalomyelitis by immunofluorescent staining of brain tissue. *Am J Vet Res*. 1981;42:1418–1421.
587. Ferguson JA, Reeves WC, Hardy JL. Studies on immunity to alphaviruses in foals. *Am J Vet Res*. 1979;40:5–10.
588. Roberts ED, Sanmartin C, Payan J, et al. Neuropathologic changes in 15 horses with naturally occurring Venezuelan equine encephalomyelitis. *Am J Vet Res*. 1970;31:1223–1229.
589. Monlux WS, Luedke AJ. Brain and spinal cord lesions in horses inoculated with Venezuelan equine encephalomyelitis virus (epidemic American and Trinidad strains). *Am J Vet Res*. 1973;34:465–473.
590. Hurst EW. The Histology of Equine Encephalomyelitis. *J Exp Med*. 1934;59:529–542.
591. Patterson JS, Maes RK, Mullaney TP, et al. Immunohistochemical diagnosis of eastern equine encephalomyelitis. *J Vet Diagn Invest*. 1996;8:156–160.
592. Devine EH, Byrne RJ. A laboratory-confirmed case of viral encephalitis (equine type) in a horse in which the animal completely recovered from the disease. *Cornell Vet*. 1960;50:494–497.
593. Eldridge BF. Strategies for surveillance, prevention, and control of arbovirus diseases in western North America. *Am J Trop Med Hyg*. 1987;37:77S–86S.
594. Spertzel RO, Kahn DE. Safety and efficacy of an attenuated Venezuelan equine encephalomyelitis vaccine for use in Equidae. *J Am Vet Med Assoc*. 1971;159:731–738.
595. Gochenour Jr WS, Berge TO, Gleiser CA, et al. Immunization of burros with living Venezuelan equine encephalomyelitis virus. *Am J Hyg*. 1962;75:351–362.
596. Berge T, Banks IS, Tigertt WD. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. *Am J Hyg*. 1961;73:209–218.
597. Ferguson JA, Reeves WC, Milby MM, et al. Study of homologous and heterologous antibody response in California horses vaccinated with attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). *Am J Vet Res*. 1978;39:371–376.
598. Baker Jr EF, Sasso DR, Maness K, et al. Venezuelan equine encephalomyelitis vaccine (strain TC-83): a field study. *Am J Vet Res*. 1978;39:1627–1631.
599. Walton TE, Jochim MM, Barber TL, et al. Cross-protective immunity between equine encephalomyelitis viruses in equids. *Am J Vet Res*. 1989;50:1442–1446.
600. Jochim MM, Barber TL. Immune response of horses after simultaneous or sequential vaccination against eastern, western, and Venezuelan equine encephalomyelitis. *J Am Vet Med Assoc*. 1974;165:621–625.
601. Barber TL, Walton TE, Lewis KJ. Efficacy of trivalent inactivated encephalomyelitis virus vaccine in horses. *Am J Vet Res*. 1978;39:621–625.
602. Vanderwagen LC, Pearson JL, Franti CE, et al. A field study of persistence of antibodies in California horses vaccinated against western, eastern, and Venezuelan equine encephalomyelitis. *Am J Vet Res*. 1975;36:1567–1571.
603. Calisher CH, Sasso DR, Sather GE. Possible evidence for interference with Venezuelan equine encephalitis virus vaccination of equines by pre-existing antibody to Eastern or Western Equine encephalitis virus, or both. *Appl Microbiol*. 1973;26:485–488.
604. Ferguson JA, Reeves WC, Hardy JL. Antibody studies in ponies vaccinated with Venezuelan equine encephalomyelitis (strain TC-83) and other alphavirus vaccines. *Am J Vet Res*. 1977;38:425–430.
605. Morgan DO, Bryans JT, Mock RE. Immunoglobulins produced by the antigenized equine fetus. *J Reprod Fertil Suppl*. 1975:735–738.
606. Waldridge BM, Wenzel JG, Ellis AC, et al. Serologic responses to eastern and western equine encephalomyelitis vaccination in previously vaccinated horses. *Vet Ther*. 2003;4:242–248.
607. Paweska JT, Venter GJ. Vector competence of Culicoides species and the seroprevalence of homologous neutralizing antibody in horses for six serotypes of equine encephalosis virus (EEV) in South Africa. *Med Vet Entomol*. 2004;18:398–407.
608. Kokernot RH, Hayes J, Will RL, et al. Arbovirus studies in the Ohio-Mississippi Basin, 1964-1967. II. St. Louis encephalitis virus. *Am J Trop Med Hyg*. 1969;18:750–761.
609. Little PB, Thorsen J, Moore W, et al. Powassan viral encephalitis: a review and experimental studies in the horse and rabbit. *Vet Pathol*. 1985;22:500–507.
610. Campbell J, Hore DE. Isolation of Murray Valley encephalitis virus from sentinel chickens. *Aust Vet J*. 1975;51:1–3.
611. Kay BH, Pollitt CC, Fanning ID, et al. The experimental infection of horses with Murray Valley encephalitis and Ross River viruses. *Aust Vet J*. 1987;64:52–55.
612. Kay BH, Young PL, Hall RA, et al. Experimental infection with Murray Valley encephalitis virus. Pigs, cattle, sheep, dogs, rabbits, macropods and chickens. *Aust J Exp Biol Med Sci*. 1985;63(Pt 1):109–126.
613. Bailey CL, Eldridge BF, Hayes DE, et al. Isolation of St. Louis encephalitis virus from overwintering Culex pipiens mosquitoes. *Science*. 1978;199:1346–1349.
614. Giltner LT, Shahan MS. Transmission of infectious equine encephalomyelitis in mammals and birds. *Science*. 1933;78:63–64.
615. Parkin WE. The occurrence and effects of the local strains of the California encephalitis group of viruses in domestic mammals of Florida. *Am J Trop Med Hyg*. 1973;22:788–795.
616. Artsob H, Wright R, Shipp L, et al. California encephalitis virus activity in mosquitoes and horses in southern Ontario, 1975. *Can J Microbiol*. 1978;24:1544–1547.
617. Campbell GL, Reeves WC, Hardy JL, et al. Distribution of neutralizing antibodies to California and Bunyamwera serogroup viruses in horses and rodents in California. *Am J Trop Med Hyg*. 1990;42:282–290.
618. Lynch JA, Binnington BD, Artsob H. California serogroup virus infection in a horse with encephalitis. *J Am Vet Med Assoc*. 1985;186:389.
619. Matsumura T, Goto H, Shimizu K, et al. Prevalence and distribution of antibodies to Getah and Japanese encephalitis viruses in horses raised in Hokkaido. *Nihon Juigaku Zasshi*. 1982;44:967–970.
620. Oyer RJ, David Beckham J, Tyler KL. West Nile and St. Louis encephalitis viruses. *Handb Clin Neurol*. 2014;123:433–447.
621. Mansfield KL, Hernandez-Triana LM, Banyard AC, et al. Japanese encephalitis virus infection, diagnosis and control in domestic animals. *Vet Microbiol*. 2017;201:85–92.

622. Li L, Giannitti F, Low J, et al. Exploring the virome of diseased horses. *J Gen Virol*. 2015;96:2721–2733.
623. Angenvoort J, Brault AC, Bowen RA, et al. West Nile viral infection of equids. *Vet Microbiol*. 2013;167:168–180.
624. Girard YA, Klingler KA, Higgs S. West Nile virus dissemination and tissue tropisms in orally infected Culex pipiens quinquefasciatus. *Vector Borne Zoonotic Dis*. 2004;4:109–122.
625. Girard YA, Popov V, Wen J, et al. Ultrastructural study of West Nile virus pathogenesis in Culex pipiens quinquefasciatus (Diptera: Culicidae). *J Med Entomol*. 2005;42:429–444.
626. Vanlandingham DL, Schneider BS, Klingler K, et al. Real-time reverse transcriptase-polymerase chain reaction quantification of West Nile virus transmitted by Culex pipiens quinquefasciatus. *Am J Trop Med Hyg*. 2004;71:120–123.
627. van der Meulen KM, Pensaert MB, Nauwynck HJ. West Nile virus in the vertebrate world. *Arch Virol*. 2005;150:637–657.
628. Overstreet M. Patient education series. West Nile virus. *Nursing*. 2005;35:64.
629. Centers for Disease C, Prevention. Possible West Nile virus transmission to an infant through breast-feeding—Michigan, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:877–878.
630. Centers for Disease C, Prevention. Intrauterine West Nile virus infection—New York, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:1135–1136.
631. Pantheir R, Hannoun C, Oudar J, et al. Isolation of West Nile virus in a Camarge horse with encephalomyelitis. *C R Acad Sci Hebd Seances Acad Sci D*. 1966;262:1308–1310.
632. Hayes EB, Gubler DJ. West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annu Rev Med*. 2006;57:181–194.
633. Schuler LA, Khaitsa ML, Dyer NW, et al. Evaluation of an outbreak of West Nile virus infection in horses: 569 cases (2002). *J Am Vet Med Assoc*. 2004;225:1084–1089.
634. Porter MB, Long MT, Getman LM, et al. West Nile virus encephalomyelitis in horses: 46 cases (2001). *J Am Vet Med Assoc*. 2003;222:1241–1247.
635. Cantile C, Del Piero F, Di Guardo G, et al. Pathologic and immunohistochemical findings in naturally occuring West Nile virus infection in horses. *Vet Pathol*. 2001;38:414–421.
636. Snook CS, Hyman SS, Del Piero F, et al. West Nile virus encephalomyelitis in eight horses. *J Am Vet Med Assoc*. 2001;218:1576–1579.
637. Cantile C, Di Guardo G, Eleni C, et al. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet J*. 2000;32:31–35.
638. Joubert L, Oudar J, Hannoun C, et al. Experimental reproduction of meningo-encephalomyelitis of horses with West Nile arbovirus. 3. Relations between virology, serology, and anatomo-clinical evolution. Epidemiological and prophylactic consequences. *Bull Acad Vet Fr*. 1971;44:159–167.
639. Oudar J, Joubert L, Lapras M, et al. Experimental reproduction of meningo-encephalomyelitis of horses with West Nile arbovirus. II. Anatomo-clinical study. *Bull Acad Vet Fr*. 1971;44:147–158.
640. Guillon JC, Oudar J, Joubert L, et al. Histological lesions of the nervous system in West Nile virus infection in horses. *Ann Inst Pasteur (Paris)*. 1968;114:539–550.
641. Ostlund EN, Crom RL, Pedersen DD, et al. Equine West Nile encephalitis, United States. *Emerg Infect Dis*. 2001;7:665–669.
642. Joubert L, Oudar J, Hannoun C, et al. Epidemiology of the West Nile virus: study of a focus in Camargue. IV. Meningo-encephalomyelitis of the horse. *Ann Inst Pasteur (Paris)*. 1970;118:239–247.
643. Ogata A, Tashiro K, Nukuzuma S, et al. A rat model of Parkinson's disease induced by Japanese encephalitis virus. *J Neurovirol*. 1997;3:141–147.
644. Asher DM. Movement disorders in rhesus monkeys after infection with tick-borne encephalitis virus. *Adv Neurol*. 1975;10:277–289.
645. Cernescu C, Ruta SM, Tardei G, et al. A high number of severe neurologic clinical forms during an epidemic of West Nile virus infection. *Rom J Virol*. 1997;48:13–25.
646. Jeha LE, Sila CA, Lederman RJ, et al. West Nile virus infection: a new acute paralytic illness. *Neurology*. 2003;61:55–59.
647. Wamsley HL, Alleman AR, Porter MB, et al. Findings in cerebrospinal fluids of horses infected with West Nile virus: 30 cases (2001). *J Am Vet Med Assoc*. 2002;221:1303–1305.
648. Ostlund EN, Andresen JE, Andresen M. West Nile encephalitis. *Vet Clin North Am Equine Pract*. 2000;16:427–441.
649. Davidson AH, Traub-Dargatz JL, Rodeheaver RM, et al. Immunologic responses to West Nile virus in vaccinated and clinically affected horses. *J Am Vet Med Assoc*. 2005;226:240–245.
650. Johnson DJ, Ostlund EN, Pedersen DD, et al. Detection of North American West Nile virus in animal tissue by a reverse transcription-nested polymerase chain reaction assay. *Emerg Infect Dis*. 2001;7:739–741.
651. Tewari D, Kim H, Feria W, et al. Detection of West Nile virus using formalin fixed paraffin embedded tissues in crows and horses: quantification of viral transcripts by real-time RT-PCR. *J Clin Virol*. 2004;30:320–325.
652. Scholle F, Mason PW. West Nile virus replication interferes with both poly(I: C)-induced interferon gene transcription and response to interferon treatment. *Virology*. 2005;342:77–87.
653. Engle MJ, Diamond MS. Antibody prophylaxis and therapy against West Nile virus infection in wild-type and immunodeficient mice. *J Virol*. 2003;77:12941–12949.
654. Anderson JF, Rahal JJ. Efficacy of interferon alpha-2b and ribavirin against West Nile virus in vitro. *Emerg Infect Dis*. 2002;8:107–108.
655. Leis AA, Stokic DS. Neuromuscular Manifestations of Human West Nile Virus Infection. *Curr Treat Options Neurol*. 2005;7:15–22.
656. Roos KL. West Nile encephalitis and myelitis. *Curr Opin Neurol*. 2004;17:343–346.
657. Jackson AC. Therapy of West Nile virus infection. *Can J Neurol Sci*. 2004;31:131–134.
658. Ben-Nathan D, Lachmi B, Lustig S, et al. Protection by dehydroepiandrosterone in mice infected with viral encephalitis. *Arch Virol*. 1991;120:263–271.
659. Drebot MA, Artsob H. West Nile virus. Update for family physicians. *Can Fam Physician*. 2005;51:1094–1099.
660. Romero JR, Newland JG. Viral meningitis and encephalitis: traditional and emerging viral agents. *Semin Pediatr Infect Dis*. 2003;14:72–82.
661. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med*. 2002;137:173–179.
662. Seino KK, Long MT, Gibbs EP, et al. Comparative efficacies of three commercially available vaccines against West Nile Virus (WNV) in a short-duration challenge trial involving an equine WNV encephalitis model. *Clin Vaccine Immunol*. 2007;14:1465–1471.
663. Long MT, Gibbs EP, Mellencamp MW, et al. Efficacy, duration, and onset of immunogenicity of a West Nile virus vaccine, live Flavivirus chimera, in horses with a clinical disease challenge model. *Equine Vet J*. 2007;39:491–497.
664. Sauder C, Staeheli P. Rat model of borna disease virus transmission: epidemiological implications. *J Virol*. 2003;77:12886–12890.
665. Staeheli P, Sauder C, Hausmann J, et al. Epidemiology of Borna disease virus. *J Gen Virol*. 2000;81:2123–2135.
666. Narayan O, Herzog S, Frese K, et al. Pathogenesis of Borna disease in rats: immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. *J Infect Dis*. 1983;148:305–315.
667. Stitz L, Bilzer T, Planz O. The immunopathogenesis of Borna disease virus infection. *Front Biosci*. 2002;7:d541–555.
668. Blitvich BJ, Saiyasombat R, Travassos da Rosa A, et al. Orthobunyaviruses, a common cause of infection of livestock in the Yucatan peninsula of Mexico. *Am J Trop Med Hyg*. 2012;87:1132–1139.

669. Heath SE, Artsob H, Bell RJ, et al. Equine encephalitis caused by snowshoe hare (California serogroup) virus. *Can Vet J*. 1989;30:669–671.
670. Nelson DM, Gardner IA, Chiles RF, et al. Prevalence of antibodies against Saint Louis encephalitis and Jamestown Canyon viruses in California horses. *Comp Immunol Microbiol Infect Dis*. 2004;27:209–215.
671. McFarlane BL, Embree JE, Embil JA, et al. Antibodies to snowshoe hare virus of the California group in the snowshoe hare (Lepus americanus) and domestic animal populations of Prince Edward Island. *Can J Microbiol*. 1981;27:1224–1227.
672. Sahu SP, Landgraf J, Wineland N, et al. Isolation of Jamestown Canyon virus (California virus group) from vesicular lesions of a horse. *J Vet Diagn Invest*. 2000;12:80–83.
673. Emmons RW, Woodie JD, Laub RL, et al. Main Drain virus as a cause of equine encephalomyelitis. *J Am Vet Med Assoc*. 1983;183:555–558.
674. McLean RG, Calisher CH, Parham GL. Isolation of Cache Valley virus and detection of antibody for selected arboviruses in Michigan horses in 1980. *Am J Vet Res*. 1987;48:1039–1041.
675. Hooper PT, Williamson MM. Hendra and Nipah virus infections. *Vet Clin North Am Equine Pract*. 2000;16:597–603. xi.
676. Howell PG, Groenewald D, Visage CW, et al. The classification of seven serotypes of equine encephalosis virus and the prevalence of homologous antibody in horses in South Africa. *Onderstepoort J Vet Res*. 2002;69:79–93.
677. Timoney PJ. Susceptibility of the horse to experimental inoculation with louping ill virus. *J Comp Pathol*. 1980;90:73–86.
678. Timoney PJ, Donnelly WJ, Clements LO, et al. Encephalitis caused by louping ill virus in a group of horses in Ireland. *Equine Vet J*. 1976;8:113–117.
679. Calisher CH, Monath TP, Sabattini MS, et al. A newly recognized vesiculovirus, Calchaqui virus, and subtypes of Melao and Maguari viruses from Argentina, with serologic evidence for infections of humans and horses. *Am J Trop Med Hyg*. 1987;36:114–119.
680. Sabattini MS, Monath TP, Mitchell CJ, et al. Arbovirus investigations in Argentina, 1977-1980. I. Historical aspects and description of study sites. *Am J Trop Med Hyg*. 1985;34:937–944.
681. Karabatsos N, Lewis AL, Calisher CH, et al. Identification of Highlands J virus from a Florida horse. *Am J Trop Med Hyg*. 1988;39:603–606.
682. Robin Y, Bourdin P, Le Gonidec G, et al. Semliki forest virus and equine encephalomyelitis in Senegal (author's transl). *Ann Microbiol (Paris)*. 1974;125A:235–241.
683. Martin ML, Sedmake PA. Rabies. I. Epidemiology, pathogenesis, and diagnosis. *Comp Contin Educ Pract Vet*. 1983;5:521–529.
684. Blanton JD, Palmer D, Rupprecht CE. Rabies surveillance in the United States during 2009. *J Am Vet Med Assoc*. 2010;237:646–657.
685. Baer GM. *The Natural History of Rabies*. New York: Academic Press; 1975.
686. West GP. Equine rabies. *Equine Vet J*. 1985;17:280–282.
687. Striegel P, Genetzky RM. Signs of rabies in horses: a clinical review. *Mod Vet Pract*. 1983;64:983–985.
688. Joyce JR, Russell LH. Clinical signs of rabies in horses. *Comp Contin Educ Pract Vet*. 1981;3:S56–S61.
689. Smith JM, Cox JH. Central nervous system disease in adult horses. III. Differential diagnosis and comparison of common disorders. *Comp Contin Educ Pract Vet*. 1987;9:1042–1054.
690. Sommardahl CS, Henton JE, Peterson MG. Rabies in a horse. *Vet Clin North Am Equine Pract*. 1990;12:11–14.
691. Siger L, Green SL, Merritt AM. Equine rabies with a prolonged course. *Vet Clin North Am Equine Pract*. 1989;11:6–8.
692. Meyer EE, Morris PG, Elcock LH, et al. Hindlimb hyperesthesia associated with rabies in two horses. *J Am Vet Med Assoc*. 1986;188:629–632.
693. Bassuino DM, Konradt G, Cruz RA, et al. Characterization of spinal cord lesions in cattle and horses with rabies: the importance of correct sampling. *J Vet Diagn Invest*. 2016;28:455–460.
694. Dacheux L, Larrous F, Lavenir R, et al. Dual Combined Real-Time Reverse Transcription Polymerase Chain Reaction Assay for the Diagnosis of Lyssavirus Infection. *PLoS Negl Trop Dis*. 2016;10:e0004812.
695. Wadhwa A, Wilkins K, Gao J, et al. A Pan-Lyssavirus Taqman Real-Time RT-PCR Assay for the Detection of Highly Variable Rabies virus and Other Lyssaviruses. *PLoS Negl Trop Dis*. 2017;11:e0005258.
696. Harvey AM, Watson JL, Brault SA, et al. Duration of serum antibody response to rabies vaccination in horses. *J Am Vet Med Assoc*. 2016;249:411–418.
697. Cummings JF, de Lahunta A, George C, et al. Equine motor neuron disease; a preliminary report. *Cornell Vet*. 1990;80:357–379.
698. Sustronck B, Deprez P, Roy Mv, et al. Equine motor neuron disease: the first confirmed cases in Europe. *Vlaams Diergeneeskundig Tijdschrift*. 1993;62:40–44.
699. Divers TJ, Mohammed HO, Cummings JF, et al. Equine motor neuron disease: findings in 28 horses and proposal of a pathophysiological mechanism for the disease. *Equine Vet J*. 1994;26:409–415.
700. Kuwamura M, Iwaki M, Yamate J, et al. The first case of equine motor neuron disease in Japan. *J Vet Med Sci*. 1994;56:195–197.
701. van den Hoven P, Meijer AE, Breukink HJ, et al. Enzyme histochemistry on muscle biopsies as an aid in the diagnosis of diseases of the equine neuromuscular system: a study of six cases. *Equine Vet J*. 1988;20:46–53.
702. DeVilbiss BA, Mohammed HO, Divers TJ. Perception of equine practitioners regarding the occurrence of selected equine neurologic disease in the northeast over a 10-year period. *J Equine Vet Sci*. 2009;29:237–246.
703. Valentine BA, de Lahunta A, George C, et al. Acquired equine motor neuron disease. *Vet Pathol*. 1994;31:130–138.
704. Mohammed HO, Cummings JF, Divers TJ, et al. Risk factors associated with equine motor neuron disease: a possible model for human MND. *Neurology*. 1993;43:966–971.
705. Divers T, Mohammed HO, Hintz HF, et al. Equine motor neuron disease: a review of clinical and experimental studies. *Clni Tech Equine Pract*. 2006;5:24–29.
706. de la Rua-Domenech R, Mohammed HO, Cummings JF, et al. Intrinsic, management, and nutritional factors associated with equine motor neuron disease. *J Am Vet Med Assoc*. 1997;211:1261–1267.
707. Robin M, Malbon A, Ricci E, et al. Reduced tongue tone associated with degeneration of the hypoglossal nerve nucleus in a horse with equine motor neuron disease. *Equine Vet Educ*. 2013;28:434–438.
708. Riis RC, Jackson C, Rebhun W, et al. Ocular manifestations of equine motor neuron disease. *Equine Vet J*. 1999;31:99–110.
709. Weber Polack E, King JM, Cummings JF, et al. Quantitative assessment of motor neuron loss in equine motor neuron disease (EMND). *Equine Vet J*. 1998;30:256–259.
710. Diez de Castro E, Zafra R, Acevedo LM, et al. Eosinophilic Enteritis in Horses with Motor Neuron Disease. *J Vet Intern Med*. 2016;30:873–879.
711. de la Rua-Domenech R, Wiedmann M, Mohammed HO, et al. Equine motor neuron disease is not linked to Cu/Zn superoxide dismutase mutations: sequence analysis of the equine Cu/Zn superoxide dismutase cDNA. *Gene*. 1996;178:83–88.
712. De la Rua-Domenech R, Mohammed HO, Cummings JF, et al. Association between plasma vitamin E concentration and the risk of equine motor neuron disease. *Vet J*. 1997;154:203–213.
713. Mohammed HO, Divers TJ, Summers BA, et al. Vitamin E deficiency and risk of equine motor neuron disease. *Acta Vet Scand*. 2007;49:17.
714. Mohammed HO, Divers TJ, Kwak J, et al. Association of oxidative stress with motor neuron disease in horses. *Am J Vet Res*. 2012;73:1957–1962.

715. Syrja P, Cizinauskas S, Sankari SM, et al. Equine motor neuron disease (EMND) in a horse wtihout vitamin E deficiency: a sequela of iron excess? *Equine Vet Educ*. 2006;18:122–126.
716. McGorum BC, Mayhew IG, Amory H, et al. Horses on pasture may be affected by equine motor neuron disease. *Equine Vet J*. 2006;38:47–51.
717. Divers TJ, Cummings JE, de Lahunta A, et al. Evaluation of the risk of motor neuron disease in horses fed a diet low in vitamin E and high in copper and iron. *Am J Vet Res*. 2006;67:120–126.
718. Hahn CN, Mayhew IG. Equine neurodegenerative diseases—stressed neurons and other radical ideas. *Vet J*. 1997;154:173–174.
719. El-Assaad I, Bari J, Yasuda K, et al. Differential expression of TAR DNA-binding protein (TDP-43) in the central nervous system of horses afflicted with equine motor neuron disease (EMND): a preliminary study of a a potential pathologic marker. *Vet Res Commun*. 2012;36:221–226.
720. Polack EW, King JM, Cummings JF, et al. Concentrations of trace minerals in the spinal cord of horses with equine motor neuron disease. *Am J Vet Res*. 2000;61:609–611.
721. Sojka JE, Hope W, Pearson D. Lead toxicosis in 2 horses: similarity to equine degenerative lower motor neuron disease. *J Vet Intern Med*. 1996;10:420–423.
722. Brenner SR. Blue-green algae or cyanobacteria in the intestinal micro-flora may produce neurotoxins such as Beta-N-Methylamino-L-Alanine (BMAA) which may be related to development of amyotrophic lateral sclerosis, Alzheimer's disease and Parkinson-Dementia-Complex in humans and Equine Motor Neuron Disease in horses. *Med Hypotheses*. 2013;80:103.
723. Finno CJ, Estell KE, Katzman S, et al. Blood and Cerebrospinal Fluid alpha-Tocopherol and Selenium Concentrations in Neonatal Foals with Neuroaxonal Dystrophy. *J Vet Intern Med*. 2015;29:1667–1675.
724. Kyles KW, McGorum BC, Fintl C, et al. Electromyography under caudal epidural anaesthesia as an aid to the diagnosis of equine motor neuron disease. *Vet Rec*. 2001;148:536–538.
725. Benders NA, Dyer J, Wijnberg ID, et al. Evaluation of glucose tolerance and intestinal luminal membrane glucose transporter function in horses with equine motor neuron disease. *Am J Vet Res*. 2005;66:93–99.
726. van der Kolk JH, Rijnen KE, Rey F, et al. Evaluation of glucose metabolism in three horses with lower motor neuron degeneration. *Am J Vet Res*. 2005;66:271–276.
727. Bedford HE, Valberg SJ, Firshman AM, et al. Histopathologic findings in the sacrocaudalis dorsalis medialis muscle of horses with vitamin E-responsive muscle atrophy and weakness. *J Am Vet Med Assoc*. 2013;242:1127–1137.
728. Palencia P, Quiroz-Rothe E, Rivero JL. New insights into the skeletal muscle phenotype of equine motor neuron disease: a quantitative approach. *Acta Neuropathol*. 2005;109:272–284.
729. Jackson CA, De Lahunta A, Cummings JF, et al. Spinal accessory nerve biopsy as an ante mortem diagnostic test for equine motor neuron disease. *Equine Vet J*. 1996;28:215–219.
730. Husulak ML, Lohmann KL, Gabadage K, et al. Equine motor neuron disease in 2 horses from Saskatchewan. *Can Vet J*. 2016;57:771–776.
731. Delguste C, de Moffarts B, Kirschvink N, et al. Change in blood antioxidant status of horses moved from a stable following diagnosis of equine motor neuron disease. *Can Vet J*. 2007;48:1165–1167.
732. Hatheway CL. Toxigenic clostridia. *Clin Microbiol Rev*. 1990;3:66–98.
733. Eisel U, Jarausch W, Goretzki K, et al. Tetanus toxin: primary structure, expression in E. coli, and homology with botulinum toxins. *EMBO J*. 1986;5:2495–2502.
734. Schiavo G, Benfenati F, Poulain B, et al. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature*. 1992;359:832–835.
735. Bizzini B. Tetanus toxin. *Microbiol Rev*. 1979;43:224–240.
736. Helting TB, Parschat S, Engelhardt H. Structure of tetanus toxin. Demonstration and separation of a specific enzyme converting intracellular tetanus toxin to the extracellular form. *J Biol Chem*. 1979;254:10728–10733.
737. Poulain B. Molecular mechanism of action of tetanus toxin and botulinum neurotoxins. *Pathol Biol (Paris)*. 1994;42:173–182.
738. Montecucco C, Papini E, Schiavo G. Bacterial protein toxins penetrate cells via a four-step mechanism. *FEBS Lett*. 1994;346:92–98.
739. Montecucco C, Schiavo G. Structure and function of tetanus and botulinum neurotoxins. *Q Rev Biophys*. 1995;28:423–472.
740. Grumelli C, Verderio C, Pozzi D, et al. Internalization and mechanism of action of clostridial toxins in neurons. *Neurotoxicology*. 2005;26:761–767.
741. Pellizzari R, Rossetto O, Lozzi L, et al. Structural determinants of the specificity for synaptic vesicle-associated membrane protein/synaptobrevin of tetanus and botulinum type B and G neurotoxins. *J Biol Chem*. 1996;271:20353–20358.
742. Humeau Y, Doussau F, Grant NJ, et al. How botulinum and tetanus neurotoxins block neurotransmitter release. *Biochimie*. 2000;82:427–446.
743. Blumenthal R, Habig WH. Mechanism of tetanolysin-induced membrane damage: studies with black lipid membranes. *J Bacteriol*. 1984;157:321–323.
744. Kay G, Knottenbelt DC. Tetanus in equids: a report of 56 cases. *Equine Vet Educ*. 2007;19:107–112.
745. Green SL, Little CB, Baird JD, et al. Tetanus in the horse: a review of 20 cases (1970 to 1990). *J Vet Intern Med*. 1994;8:128–132.
746. van Galen G, Delguste C, Sandersen C, et al. Tetanus in the equine species: a retrospective study of 31 cases. *Tijdschr Diergeneeskd*. 2008;133:512–517.
747. Jansen BC, Knoetze PC. The immune response of horses to tetanus toxoid. *Onderstepoort J Vet Res*. 1979;46:211–216.
748. Liefman CE. Combined active-passive immunisation of horses against tetanus. *Aust Vet J*. 1980;56:119–122.
749. Messer NTt, Johnson PJ. Serum hepatitis in two brood mares. *J Am Vet Med Assoc*. 1994;204:1790–1792.
750. Guglick MA, MacAllister CG, Ely RW, et al. Hepatic disease associated with administration of tetanus antitoxin in eight horses. *J Am Vet Med Assoc*. 1995;206:1737–1740.
751. Muylle E, Oyaert W, Ooms L, et al. Treatment of tetanus in the horse by injections of tetanus antitoxin into the subarachnoid space. *J Am Vet Med Assoc*. 1975;167:47–48.
752. Steinman A, Haik R, Elad D, et al. Intrathecal administration of tetanus antitoxin to three cases of tetanus in horses. *Equine Vet Educ*. 2000;12:237–240.
753. Holmes MA, Townsend HG, Kohler AK, et al. Immune responses to commercial equine vaccines against equine herpesvirus-1, equine influenza virus, eastern equine encephalomyelitis, and tetanus. *Vet Immunol Immunopathol*. 2006;111:67–80.
754. Kendall A, Anagrius K, Ganheim A, et al. Duration of tetanus immunoglobulin G titres following basic immunisation of horses. *Equine Vet J*. 2016;48:710–713.
755. Anniballi F, Fiore A, Lofstrom C, et al. Management of animal botulism outbreaks: from clinical suspicion to practical countermeasures to prevent or minimize outbreaks. *Biosecur Bioterror*. 2013;11(suppl 1):S191–S199.
756. Rossetto O, Pirazzini M, Montecucco C. Botulinum neurotoxins: genetic, structural and mechanistic insights. *Nat Rev Microbiol*. 2014;12:535–549.
757. Whitlock RH, Buckley C. Botulism. *Vet Clin North Am Equine Pract*. 1997;13:107–128.
758. Whitlock RH, McAdams S. Equine botulism. *Clin Tech Equine Pract*. 2006;5:37–42.
759. Johnson AL, McAdams-Gallagher SC, Aceto H. Accuracy of a Mouse Bioassay for the Diagnosis of Botulism in Horses. *J Vet Intern Med*. 2016;30:1293–1299.
760. Johnson AL, McAdams-Gallagher SC, Aceto H. Outcome of adult horses with botulism treated at a veterinary hospital: 92 cases (1989-2013). *J Vet Intern Med*. 2015;29:311–319.

761. Johnson AL, McAdams SC, Whitlock RH. Type A botulism in horses in the United States: a review of the past ten years (1998-2008). *J Vet Diagn Invest*. 2010;22:165–173.
762. Doutre MP. First observation of type D animal botulism in Senegal. *Rev Elev Med Vet Pays Trop*. 1969;22:25–27.
763. Goehring LS, Sloet van Oldruitenborgh-Oosterbaan MM. Botulism in a horse: a concrete possibility in the Netherlands? *Tijdschr Diergeneeskd*. 2002;127:82.
764. Meyer KF, Dubovsky BJ. The distribution of the spores of B. botulinus in the United States. IV. *J Infect Dis*. 1922;31:559–594.
765. Schoenbaum MA, Hall SM, Glock RD, et al. An outbreak of type C botulism in 12 horses and a mule. *J Am Vet Med Assoc*. 2000;217:365–368. 340.
766. Arnon SS, Midura TF, Damus K, et al. Intestinal infection and toxin production by Clostridium botulinum as one cause of sudden infant death syndrome. *Lancet*. 1978;1:1273–1277.
767. Wilkins PA, Palmer JE. Botulism in foals less than 6 months of age: 30 cases (1989-2002). *J Vet Intern Med*. 2003;17:702–707.
768. Dolly JO, Black J, Williams RS, et al. Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization. *Nature*. 1984;307:457–460.
769. Comella JX, Molgo J, Faille L. Sprouting of mammalian motor nerve terminals induced by in vivo injection of botulinum type-D toxin and the functional recovery of paralysed neuromuscular junctions. *Neurosci Lett*. 1993;153:61–64.
770. Kinde H, Bettey RL, Ardans A, et al. Clostridium botulinum type-C intoxication associated with consumption of processed alfalfa hay cubes in horses. *J Am Vet Med Assoc*. 1991;199:742–746.
771. Ostrowski SR, Kubiski SV, Palmero J, et al. An outbreak of equine botulism type A associated with feeding grass clippings. *J Vet Diagn Invest*. 2012;24:601–603.
772. Prutton JS, Magdesian KG, Plummer A, et al. Survival of a Foal with Type A Botulism. *J Vet Intern Med*. 2016;30:675–678.
773. Johnson AL, McAdams-Gallagher SC, Sweeney RW. Quantitative real-time PCR for detection of neurotoxin genes of Clostridium botulinum types A, B and C in equine samples. *Vet J*. 2014;199:157–161.
774. Johnson AL, Sweeney RW, McAdams SC, et al. Quantitative real-time PCR for detection of the neurotoxin gene of Clostridium botulinum type B in equine and bovine samples. *Vet J*. 2012;194:118–120.
775. Wilkins PA, Palmer JE. Mechanical ventilation in foals with botulism: 9 cases (1989-2002). *J Vet Intern Med*. 2003;17:708–712.
776. Tocher JF, Tocher JWB, W, Buxton JB. "Grass sickness" investigation report. *Vet Rec*. 1923;3:37–45 (75-89).
777. Wylie CE, Proudman CJ. Equine grass sickness: epidemiology, diagnosis, and global distribution. *Vet Clin North Am Equine Pract*. 2009;25:381–399.
778. Wright A, Beard L, Bawa B, et al. Dysautonomia in a six-year-old mule in the United States. *Equine Vet J*. 2010;42:170–173.
779. Wylie CE, Proudman CJ, McGorum BC, et al. A nationwide surveillance scheme for equine grass sickness in Great Britain: results for the period 2000-2009. *Equine Vet J*. 2011;43:571–579.
780. Pirie RS, Jago RC, Hudson NP. Equine grass sickness. *Equine Vet J*. 2014;46:545–553.
781. Wylie CE, Shaw DJ, Fordyce FM, et al. Equine grass sickness in Scotland: a case-control study of signalment- and meteorology-related risk factors. *Equine Vet J*. 2014;46:64–71.
782. McCarthy HE, Proudman CJ, French NP. Epidemiology of equine grass sickness: a literature review (1909-1999). *Vet Rec*. 2001;149:293–300.
783. Newton JR, Hedderson EJ, Adams VJ, et al. An epidemiological study of risk factors associated with the recurrence of equine grass sickness (dysautonomia) on previously affected premises. *Equine Vet J*. 2004;36:105–112.
784. Wylie CE, Shaw DJ, Fordyce FM, et al. Equine grass sickness in Scotland: A case-control study of environmental geochemical risk factors. *Equine Vet J*. 2016;48:779–785.
785. McCarthy HE, French NP, Edwards GB, et al. Equine grass sickness is associated with low antibody levels to Clostridium botulinum: a matched case-control study. *Equine Vet J*. 2004;36:123–129.
786. Hunter LC, Poxton IR. Systemic antibodies to Clostridium botulinum type C: do they protect horses from grass sickness (dysautonomia)? *Equine Vet J*. 2001;33:547–553.
787. Milne EM, Mayhew IG. Equine grass sickness: clinical findings and pathology. *Int Equine Neurol Conf.* Ithaca, NY. 1997.
788. Hahn CN, Mayhew IG, de Lahunta A. Central neuropathology of equine grass sickness. *Acta Neuropathol*. 2001;102:153–159.
789. Cottrell DF, McGorum BC, Pearson GT. The neurology and enterology of equine grass sickness: a review of basic mechanisms. *Neurogastroenterol Motil*. 1999;11:79–92.
790. Gilmour JS. Observations on neuronal changes in grass sickness of horses. *Res Vet Sci*. 1973;15:197–200.
791. Hunter LC, Miller JK, Poxton IR. The association of Clostridium botulinum type C with equine grass sickness: a toxicoinfection? *Equine Vet J*. 1999;31:492–499.
792. McGorum BC, Jago RC, Cillan-Garcia E, et al. Neurodegeneration in equine grass sickness is not attributable to niacin deficiency. *Equine Vet J*. 2016.
793. McGorum BC, Wilson R, Pirie RS, et al. Systemic concentrations of antioxidants and biomarkers of macromolecular oxidative damage in horses with grass sickness. *Equine Vet J*. 2003;35:121–126.
794. McGorum BC, Scholes S, Milne EM, et al. Equine grass sickness, but not botulism, causes autonomic and enteric neurodegeneration and increases soluble N-ethylmaleimide-sensitive factor attachment receptor protein expression within neuronal perikarya. *Equine Vet J*. 2016;48:786–791.
795. Pirie RS, McGorum BC. Equine grass sickness: Benefits of a multifaceted research approach. *Equine Vet J*. 2016;48:770–772.
796. Ireland JL, McGorum BC, Proudman CJ, et al. Designing a field trial of an equine grass sickness vaccine: a questionnaire-based feasibility study. *Vet J*. 2016;213:64–71.
797. Copas VE, Durham AE, Stratford CH, et al. In equine grass sickness, serum amyloid A and fibrinogen are elevated, and can aid differential diagnosis from non-inflammatory causes of colic. *Vet Rec*. 2013;172:395.
798. Greet TR, Whitwell KE. Barium swallow as an aid to the diagnosis of grass sickness. *Equine Vet J*. 1986;18:294–297.
799. Hahn CN, Mayhew IG. Phenylephrine eyedrops as a diagnostic test in equine grass sickness. *Vet Rec*. 2000;147:603–606.
800. Wijnberg ID, Franssen H, Jansen GH, et al. The role of quantitative electromyography (EMG) in horses suspected of acute and chronic grass sickness. *Equine Vet J*. 2006;38:230–237.
801. Scholes SF, Vaillant C, Peacock P, et al. Diagnosis of grass sickness by ileal biopsy. *Vet Rec*. 1993;133:7–10.
802. Mair TS, Kelley AM, Pearson GR. Comparison of ileal and rectal biopsies in the diagnosis of equine grass sickness. *Vet Rec*. 2011;168:266.
803. McGorum BC, Pirie RS, Shaw D, et al. Neuronal chromatolysis in the subgemmal plexus of gustatory papillae in horses with grass sickness. *Equine Vet J*. 2016;48:773–778.
804. Divers TJ. Comparing equine motor neuron disease (EMND) with equine grass sickness (EGS). *Equine Vet J*. 1999;31:90–91.
805. Doxey DL, Milne EM, Harter A. Recovery of horses from dysautonomia (grass sickness). *Vet Rec*. 1995;137:585–588.
806. Milne EM, Woodman MP, Doxey DL. Use of clinical measurements to predict the outcome in chronic cases of grass sickness (equine dysautonomia). *Vet Rec*. 1994;134:438–440.
807. Jago RC, Handel I, Hahn CN, et al. Bodyweight change aids prediction of survival in chronic equine grass sickness. *Equine Vet J*. 2016;48:792–797.
808. Chang YF, Novosol V, McDonough SP, et al. Experimental infection of ponies with Borrelia burgdorferi by exposure to Ixodid ticks. *Vet Pathol*. 2000;37:68–76.
809. Cohen ND, Heck FC, Heim B, et al. Seroprevalence of antibodies to Borrelia burgdorferi in a population of horses in central Texas. *J Am Vet Med Assoc*. 1992;201:1030–1034.

810. Butler CM, Houwers DJ, Jongejan F, et al. Borrelia burgdorferi infections with special reference to horses. A review. *Vet Q*. 2005;27:146–156.
811. Parker JL, White KK. Lyme borreliosis in cattle and horses: a review of the literature. *Cornell Vet*. 1992;82:253–274.
812. Stefancikova A, Adaszek L, Pet'ko B, et al. Serological evidence of Borrelia burgdorferi sensu lato in horses and cattle from Poland and diagnostic problems of Lyme borreliosis. *Ann Agric Environ Med*. 2008;15:37–43.
813. Bernard WV, Cohen D, Bosler E, et al. Serologic survey for Borrelia burgdorferi antibody in horses referred to a mid-Atlantic veterinary teaching hospital. *J Am Vet Med Assoc*. 1990;196:1255–1258.
814. Cohen D, Bosler EM, Bernard W, et al. Epidemiologic studies of Lyme disease in horses and their public health significance. *Ann N Y Acad Sci*. 1988;539:244–257.
815. Piesman J. Strategies for reducing the risk of Lyme borreliosis in North America. *Int J Med Microbiol*. 2006;296(suppl 40):17–22.
816. Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol*. 1991;36:587–609.
817. Shapiro ED, Gerber MA. Lyme disease. *Clin Infect Dis*. 2000;31:533–542.
818. Gall Y, Pfister K. Survey on the subject of equine Lyme borreliosis. *Int J Med Microbiol*. 2006;296(suppl 40):274–279.
819. Carter SD, May C, Barnes A, et al. Borrelia burgdorferi infection in UK horses. *Equine Vet J*. 1994;26:187–190.
820. Burgess EC, Mattison M. Encephalitis associated with Borrelia burgdorferi infection in a horse. *J Am Vet Med Assoc*. 1987;191:1457–1458.
821. Johnstone LK, Engiles JB, Aceto H, et al. Retrospective evaluation of horses diagnosed with neuroborreliosis on postmortem examination: 16 cases (2004-2015). *J Vet Intern Med*. 2016;30:1305–1312.
822. Magnarelli LA, Ijdo JW, Van Andel AE, et al. Serologic confirmation of Ehrlichia equi and Borrelia burgdorferi infections in horses from the northeastern United States. *J Am Vet Med Assoc*. 2000;217:1045–1050.
823. Johnson AL, Divers TJ, Chang YF. Validation of an in-clinic enzyme-linked immunosorbent assay kit for diagnosis of Borrelia burgdorferi infection in horses. *J Vet Diagn Invest*. 2008;20:321–324.
824. Wagner B, Goodman LB, Rollins A, et al. Antibodies to OspC, OspF and C6 antigens as indicators for infection with Borrelia burgdorferi in horses. *Equine Vet J*. 2013;45:533–537.
825. Nardelli DT, Callister SM, Schell RF. Lyme arthritis: current concepts and a change in paradigm. *Clin Vaccine Immunol*. 2008;15:21–34.
826. Puius YA, Kalish RA. Lyme arthritis: pathogenesis, clinical presentation, and management. *Infect Dis Clin North Am*. 2008;22:289–300. vi-vii.
827. Chang YF, Ku YW, Chang CF, et al. Antibiotic treatment of experimentally Borrelia burgdorferi-infected ponies. *Vet Microbiol*. 2005;107:285–294.
828. Chang Y, Novosol V, McDonough SP, et al. Vaccination against lyme disease with recombinant Borrelia burgdorferi outer-surface protein A (rOspA) in horses. *Vaccine*. 1999;18:540–548.
829. Williams WL. Involuntary twitching of the head relieved by trifacial neurectomy. *J Comp Med Vet Arch*. 1897;18:426–428.
830. Pickles K, Madigan J, Aleman M. Idiopathic headshaking: is it still idiopathic? *Vet J*. 2014;201:21–30.
831. Newton SA, Knottenbelt DC, Eldridge PR. Headshaking in horses: possible aetiopathogenesis suggested by the results of diagnostic tests and several treatment regimes used in 20 cases. *Equine Vet J*. 2000;32:208–216.
832. Madigan JE, Bell SA. Owner survey of headshaking in horses. *J Am Vet Med Assoc*. 2001;219:334–337.
833. Lane JG, Mair TS. Observations on headshaking in the horse. *Equine Vet J*. 1987;19:331–336.
834. Cook WR. Headshaking in horses. Part I. *Vet Clin North Am Equine Pract*. 1979;1:9–17.
835. Cook WR. Headshaking in horses: an afterword. *Comp Contin Educ Pract Vet*. 1992;14:1369–1371.
836. Mills DS, Cook S, Taylor K, et al. Analysis of the variations in clinical signs shown by 254 cases of equine headshaking. *Vet Rec*. 2002;150:236–240.
837. Madigan JE, Bell SA. Characterization of headshaking syndrome: 31 cases. *Equine Vet J Suppl*. 1998.
838. Madigan JE, Kortz G, Murphy C, et al. Photic headshaking in the horse: 7 cases. *Equine Vet J*. 1995;27:306–311.
839. Cook WR. Headshaking in horses. IV. Special diagnostic procedures. *Vet Clin North Am Equine Pract*. 1980;2:7–15.
840. Moore LA, Johnson PJ, Messer NT, et al. Management of headshaking in three horses by treatment for protozoal myeloencephalitis. *Vet Rec*. 1997;141:264–267.
841. Mair TS. Headshaking associated with Trombicula autumnalis larval infestation in two horses. *Equine Vet J*. 1994;26:244–245.
842. McGorum BC, Dixon PM. Vasomotor rhinitis with headshaking in a pony. *Equine Vet J*. 1990;22:220–222.
843. Kold SE, Ostblom LC, Philipsen HP. Headshaking caused by a maxillary osteoma in a horse. *Equine Vet J*. 1982;14:167–169.
844. Blythe LL, Watrous BJ, Pearson EG, et al. Otitis media/interna in the horse: a cause of head shaking and skull fractures. *Proc Amer Assoc Equine Pract*. 1990;36:517–528.
845. Aleman M, Pickles KJ, Simonek G, et al. Latent equine herpesvirus-1 in trigeminal ganglia and equine idiopathic headshaking. *J Vet Intern Med*. 2012;26:192–194.
846. Mair TS. Assessment of bilateral infra-orbital nerve blockade and bilateral infra-orbital neurectomy in the investigation and treatment of idiopathic headshaking. *Equine Vet J*. 1999;31:262–264.
847. Wilkins PA. Cyproheptadine: medical treatment for photic headshakers. *Comp Contin Educ Pract Vet*. 1997;19:98–99.
848. Devor M, Amir R, Rappaport ZH. Pathophysiology of trigeminal neuralgia: the ignition hypothesis. *Clin J Pain*. 2002;18:4–13.
849. Aleman M, Rhodes D, Williams DC, et al. Sensory evoked potentials of the trigeminal nerve for the diagnosis of idiopathic headshaking in a horse. *J Vet Intern Med*. 2014;28:250–253.
850. Sindrup SH, Jensen TS. Pharmacotherapy of trigeminal neuralgia. *Clin J Pain*. 2002;18:22–27.
851. Mills DS, Cook S, Jones B. Reported response to treatment among 245 cases of equine headshaking. *Vet Rec*. 2002;150:311–313.
852. Tomlinson JE, Neff P, Boston RC, et al. Treatment of idiopathic headshaking in horses with pulsed high-dose dexamethasone. *J Vet Intern Med*. 2013;27:1551–1554.
853. Pickles KJ, Berger J, Davies R, et al. Use of a gonadotrophin-releasing hormone vaccine in headshaking horses. *Vet Rec*. 2011;168:19.
854. Roberts VL, Patel NK, Tremaine WH. Neuromodulation using percutaneous electrical nerve stimulation for the management of trigeminal-mediated headshaking: a safe procedure resulting in medium-term remission in five of seven horses. *Equine Vet J*. 2016;48:201–204.
855. Mills DS, Taylor K. Field study of the efficacy of three types of nose net for the treatment of headshaking in horses. *Vet Rec*. 2003;152:41–44.
856. Pickles KJ, Aleman M, Adams VJ, et al. Owner-reported response to treatment of 130 headshaking horses. *Proc Amer Assoc Equine Pract*. 2014;60:176–183.
857. Anderson RC, Linder KE, Peregrine AS. Halicephalobus gingivalis (Stefanski, 1954) from a fatal infection in a horse in Ontario, Canada with comments on the validity of H. deletrix and a review of the genus. *Parasite*. 1998;5:255–261.
858. Henneke C, Jespersen A, Jacobsen S, et al. The distribution pattern of Halicephalobus gingivalis in a horse is suggestive of a haematogenous spread of the nematode. *Acta Vet Scand*. 2014;56:56.

859. Wilkins PA, Wacholder S, Nolan TJ, et al. Evidence for transmission of Halicephalobus deletrix (H gingivalis) from dam to foal. *J Vet Intern Med*. 2001;15:412–417.
860. Adedeji AO, Borjesson DL, Kozikowski-Nicholas TA, et al. What is your diagnosis? Cerebrospinal fluid from a horse. *Vet Clin Pathol*. 2015;44:171–172.
861. Kinde H, Mathews M, Ash L, et al. Halicephalobus gingivalis (H. deletrix) infection in two horses in southern California. *J Vet Diagn Invest*. 2000;12:162–165.
862. Spalding MG, Greiner EC, Green SL. Halicephalobus (Micronema) deletrix infection in two half-sibling foals. *J Am Vet Med Assoc*. 1990;196:1127–1129.
863. Ruggles AJ, Beech J, Gillette DM, et al. Disseminated Halicephalobus deletrix infection in a horse. *J Am Vet Med Assoc*. 1993;203:550–552.
864. Rames DS, Miller DK, Barthel R, et al. Ocular Halicephalobus (syn. Micronema) deletrix in a horse. *Vet Pathol*. 1995;32:540–542.
865. Alstad AD, Berg IE, Samuel C. Disseminated Micronema deletrix infection in the horse. *J Am Vet Med Assoc*. 1979;174:264–266.
866. Angus KW, Roberts L, Archibald DR, et al. Halicephalobus deletrix infection in a horse in Scotland. *Vet Rec*. 1992;131:495.
867. Brojer JT, Parsons DA, Linder KE, et al. Halicephalobus gingivalis encephalomyelitis in a horse. *Can Vet J*. 2000;41:559–561.
868. Buergelt CD. Halicephalobus (Micronema) deletrix infection in the horse. *Equine Pract*. 1991;13:7–12.
869. Cho DY, Hubbard RM, McCoy DJ, et al. Micronema granuloma in the gingiva of a horse. *J Am Vet Med Assoc*. 1985;187:505–507.
870. Ferris DH, Levine ND, Beamer PD. Micronema deletrix in equine brain. *Am J Vet Res*. 1972;33:33–38.
871. Johnson JS, Hibler CP, Tillotson KM, et al. Radiculomeningomyelitis due to Halicephalobus gingivalis in a horse. *Vet Pathol*. 2001;38:559–561.
872. Jordan WH, Gaafar SM, Carlton WW. Micronema deletrix in the brain of a horse. *Vet Med Small Anim Clin*. 1975;70:707–709.
873. Powers RD, Benz GW. Micronema deletrix in the central nervous system of a horse. *J Am Vet Med Assoc*. 1977;170:175–177.
874. Rubin HL, Woodard JC. Equine infection with Micronema deletrix. *J Am Vet Med Assoc*. 1974;165:256–258.
875. Little PB, Lwin US, Fretz P. Verminous encephalitis of horses: experimental induction with *Strongylus vulgaris* larvae. *Am J Vet Res*. 1974;35:1501–1510.
876. Wright JD, Kelly WR, Waddell AH, et al. Equine neural angiostrongylosis. *Aust Vet J*. 1991;68:58–60.
877. Mason KV. Canine neural angiostrongylosis: the clinical and therapeutic features of 55 natural cases. *Aust Vet J*. 1987;64:201–203.
878. Tanabe M, Gerhold RW, Beckstead RB, et al. Molecular confirmation of *Parelaphostrongylus tenuis* infection in a horse with verminous encephalitis. *Vet Pathol*. 2010;47:759.
879. Johnson AL, de Lahunta A, Divers TJ. Acquired scoliosis in equids: case series and proposed pathogenesis. *Proc Amer Assoc Equine Pract*. 2008;54:192–197.
880. Mayhew IG, Lichtenfels JR, Greiner EC, et al. Migration of a spiruroid nematode through the brain of a horse. *J Am Vet Med Assoc*. 1982;180:1306–1311.
881. Yoshihara T, Oikawa M, Wada R, et al. A survey of filarial parasites in the peritoneal cavity of horses in Japan. *Bull Equine Res Inst Japan*. 1988;25:25–28.
882. Innes JRM, Pillai CP. Kumri—so-called lumbar paralysis—of horses in Ceylon (India and Burma) and its identification with cerebrospinal nematodiasis. *Br Vet J*. 1955;3:233–235.
883. Frauenfelder HC, Kazacos KR, Lichtenfels JR. Cerebrospinal nematodiasis caused by a filariid in a horse. *J Am Vet Med Assoc*. 1980;177:359–362.
884. Hadlow WJ, Ward JK, Krinsky WL. Intracranial myiasis by Hypoderma bovis (Linnaeus) in a horse. *Cornell Vet*. 1977;67:272–281.
885. Baker DW, Monlux WS. Hypoderma myiasis in the horse: summary of a series of cases studied during spring and summer, 1939. *J Parasitol*. 1939;25(suppl):16.
886. Olander HJ. The migration of Hypoderma lineatum in the brain of a horse. A case report and review. *Pathol Vet*. 1967;4:477–483.
887. Wijesundera WS, Chandrasekharan NV, Karunanayake EH. A sensitive polymerase chain reaction based assay for the detection of Setaria digitata: the causative organism of cerebrospinal nematodiasis in goats, sheep and horses. *Vet Parasitol*. 1999;81:225–233.
888. Dunn DG, Gardiner CH, Dralle KR, et al. Nodular granulomatous posthitis caused by Halicephalobus (syn. Micronema) sp. in a horse. *Vet Pathol*. 1993;30:207–208.
889. Allen D, Swayne D, Belknap JK. Ganglioneuroma as a cause of small intestinal obstruction in the horse: a case report. *Cornell Vet*. 1989;79:133–141.
890. Porter BF, Storts RW, Payne HR, et al. Colonic ganglioneuromatosis in a horse. *Vet Pathol*. 2007;44:207–210.
891. Carrigan MJ, Higgins RJ, Carlson GP, et al. Equine papillary ependymoma. *Vet Pathol*. 1996;33:77–80.
892. Hayes HM, Priester Jr WA, Pendergrass TW. Occurrence of nervous-tissue tumors in cattle, horses, cats and dogs. *Int J Cancer*. 1975;15:39–47.
893. Heath SE, Peter AT, Janovitz EB, et al. Ependymoma of the neurohypophysis and hypernatremia in a horse. *J Am Vet Med Assoc*. 1995;207:738–741.
894. Szazados I. Ependymoma as the cause of severe brain symptoms in a horse. *Dtsch Tierarztl Wochenschr*. 1973;80:57.
895. Pirie RS, Mayhew IG, Clarke CJ, et al. Ultrasonographic confirmation of a space-occupying lesion in the brain of a horse: choroid plexus papilloma. *Equine Vet J*. 1998;30:445–448.
896. Bistner S, Campbell RJ, Shaw D, et al. Neuroepithelial tumor of the optic nerve in a horse. *Cornell Vet*. 1983;73:30–40.
897. Bistner SI. Medullo-epithelioma of the iris and ciliary body in a horse. *Cornell Vet*. 1974;64:588–595.
898. Eagle Jr RC, Font RL, Swerczek TW. Malignant medulloepithelioma of the optic nerve in a horse. *Vet Pathol*. 1978;15:488–494.
899. Dopke C, Grone A, von Borstel M, et al. Metastatic esthesioneuroblastoma in a horse. *J Comp Pathol*. 2005;132:218–222.
900. Knottenbelt DC, Hetzel U, Roberts V. Primary intraocular primitive neuroectodermal tumor (retinoblastoma) causing unilateral blindness in a gelding. *Vet Ophthalmol*. 2007;10:348–356.
901. Riis RC, Scherlie Jr PH, Rebhun WC. Intraocular medulloepithelioma in a horse. *Equine Vet J Suppl*. 1990:66–68.
902. Szymanski CM. Malignant teratoid medulloepithelioma in a horse. *J Am Vet Med Assoc*. 1987;190:301–302.
903. Ueda Y, Senba H, Nishimura T, et al. Ocular medulloepithelioma in a thoroughbred. *Equine Vet J*. 1993;25:558–561.
904. Yamate J, Izawa T, Ogata K, et al. Olfactory neuroblastoma in a horse. *J Vet Med Sci*. 2006;68:495–498.
905. Fankhauser. Tumours of the nervous system. *Bull World Health Organ*. 1974;50:53–69.
906. Holshuh HJ, Howard EB. Pineoblastoma, a primitive neuroectodermal tumor in the brain of a horse. *Vet Pathol*. 1982;19:567–569.
907. Gelatt KN, Leipold HW, Finocchio EJ, et al. Optic disc astrocytoma in a horse. *Can Vet J*. 1971;12:53–55.
908. Gericota B, Aleman M, Kozikowski TA, et al. A grade IV glioblastoma with an oligodendroglial component (GBM-O) in a horse. *J Comp Pathol*. 2010;142:332–335.
909. Pascoe PJ. Colic in a mare caused by a colonic neurofibroma. *Can Vet J*. 1982;23:24–27.
910. Schoniger S, Summers BA. Localized, plexiform, diffuse, and other variants of neurofibroma in 12 dogs, 2 horses, and a chicken. *Vet Pathol*. 2009;46:904–915.
911. Spritz RA, Itin PH, Gutmann DH. Piebaldism and neurofibromatosis type 1: horses of very different colors. *J Invest Dermatol*. 2004;122:xxxiv–xxxv.

912. Strubbe DT. Periocular neurofibrosarcoma in a horse. *Vet Ophthalmol*. 2001;4:237–241.
913. van den Top JG, de Heer N, Klein WR, et al. Penile and preputial tumours in the horse: a retrospective study of 114 affected horses. *Equine Vet J*. 2008;40:528–532.
914. Pascoe RR, Summers PM. Clinical survey of tumours and tumour-like lesions in horses in south east Queensland. *Equine Vet J*. 1981;13:235–239.
915. Andreasen CB, Hedstrom OR, Allison P. Mediastinal Schwannoma in a horse—cytologic, histologic, and immunochemical evaluation. *Vet Clin Pathol*. 1993;22:54–59.
916. Williamson LH, Farrell RL. Intracranial schwannoma in a horse. *Cornell Vet*. 1990;80:135–141.
917. Kreeger JM, Templer A, Tumquist SE, et al. Paranasal meningioma in a horse. *J Vet Diagn Invest*. 2002;14:322–325.
918. McEntee M, Summers BA, de Lahunta A, et al. Meningocerebral hemangiomatosis resembling Sturge-Weber disease in a horse. *Acta Neuropathol*. 1987;74:405–410.
919. Lester GD, MacKay RJ, Smith-Meyer B. Primary meningeal lymphoma in a horse. *J Am Vet Med Assoc*. 1992;201:1219–1221.
920. Mayhew IG, MacKay RJ. The nervous system. In: Mansmann RA, McAllister ES, eds. *Equine Medicine and Surgery*. 3rd ed. Goleta, CA: America Veterinary Publications; 1982:1159–1252.
921. Duff S. Cholesterinic granulomas in horses. *Vet Rec*. 1994;135:288.
922. Jackson CA, deLahunta A, Dykes NL, et al. Neurological manifestation of cholesterinic granulomas in three horses. *Vet Rec*. 1994;135:228–230.
923. Johnson PJ, Lin TL, Jennings DP. Diffuse cerebral encephalopathy associated with hydrocephalus and cholesterinic granulomas in a horse. *J Am Vet Med Assoc*. 1993;203:694–697.
924. Vanschandevijl K, Gielen I, Nollet H, et al. Computed tomography-guided brain biopsy for in vivo diagnosis of a cholesterinic granuloma in a horse. *J Am Vet Med Assoc*. 2008;233:950–954.
925. Vink-Nooteboom M, Junker K, van den Ingh TS, et al. Computed tomography of cholesterinic granulomas in the choroid plexus of horses. *Vet Radiol Ultrasound*. 1998;39:512–516.
926. Ivoghli B, Emady M, Rezakhani A. Motor paralysis associated with cholesteatoma in a mare. *Vet Med Small Anim Clin*. 1977;72:602–604.
927. Maxwell HA. so-called cholesteatoma in a horse. *Cornell Vet*. 1948;38:102.
928. Rooney JR. Cerebral cholesteatoma. *Mod Vet Pract*. 1979;60:726.
929. Sundberg JP, Burnstein T, Page EH, et al. Neoplasms of Equidae. *J Am Vet Med Assoc*. 1977;170:150–152.
930. Sullivan ND. The nervous system. In: Jubb KVF, Kennedy PC, Palmer N, eds. *Pathology of Domestic Animals*. 3rd ed. Orlando, FL: Academic Press; 1985:201–338.
931. Kannegieter NJ, Alley MR. Ataxia due to lymphosarcoma in a young horse. *Aust Vet J*. 1987;64:377–379.
932. Morrison LR, Freel K, Henderson I, et al. Lymphoproliferative disease with features of lymphoma in the central nervous system of a horse. *J Comp Pathol*. 2008;139:256–261.
933. Shamis LD, Everitt JI, Baker GJ. Lymphosarcoma as the cause of ataxia in a horse. *J Am Vet Med Assoc*. 1984;184:1517–1518.
934. Williams MA, Welles EG, Gailor RJ, et al. Lymphosarcoma associated with neurological signs and abnormal cerebrospinal fluid in two horses. *Prog Vet Neurol*. 1992;3:51–56.
935. Zeman DH, Snider 3rd TG, McClure JJ. Vertebral lymphosarcoma as the cause of hind limb paresis in a horse. *J Vet Diagn Invest*. 1989;1:187–188.
936. Hartmann E, Baumgartner W, Hungerland C. Spinal lymphosarcoma in a foal. *Tierarztl Prax*. 1988;16:175–178.
937. Schott HC, Major MD, Grant BD, et al. Melanoma as a cause of spinal cord compression in two horses. *J Am Vet Med Assoc*. 1990;196:1820–1822.
938. Traver DS, Moore JN, Thornburg LP, et al. Epidural melanoma causing posterior paresis in a horse. *J Am Vet Med Assoc*. 1977;170:1400–1403.
939. Covington AL, Magdesian KG, Madigan JE, et al. Recurrent esophageal obstruction and dysphagia due to a brainstem melanoma in a horse. *J Vet Intern Med*. 2004;18:245–247.
940. Kirker-Head CA, Loeffler D, Held JP. Pelvic limb lameness due to malignant melanoma in a horse. *J Am Vet Med Assoc*. 1985;186:1215–1217.
941. Ladd SM, Crisman MV, Duncan R, et al. Central nervous system hemangiosarcoma in a horse. *J Vet Intern Med*. 2005;19:914–916.
942. Berry S. Spinal cord compression secondary to hemangiosarcoma in a saddlebred stallion. *Can Vet J*. 1999;40:886–887.
943. Van Biervliet J, Alcaraz A, Jackson CA, et al. Extradural undifferentiated sarcoma causing spinal cord compression in 2 horses. *J Vet Intern Med*. 2004;18:248–251.
944. D'Angelo A, Bertuglia A, Capucchio MT, et al. Central vestibular syndrome due to a squamous cell carcinoma in a horse. *Vet Rec*. 2007;161:314–316.
945. Patterson LJ, May SA, Baker JR. Skeletal metastasis of a penile squamous cell carcinoma. *Vet Rec*. 1990;126:579–580.
946. Spoormakers TJ, J IJ, Sloet van Oldruitenborgh-Oosterbaan MM. Neurological signs in a horse due to metastases of an intestinal adenocarcinoma. *Vet Q*. 2001;23:49–50.
947. Wright JA, Giles CJ. Diffuse carcinomatosis involving the meninges of a horse. *Equine Vet J*. 1986;18:147–150.
948. Davis JL, Gilger BC, Spaulding K, et al. Nasal adenocarcinoma with diffuse metastases involving the orbit, cerebrum, and multiple cranial nerves in a horse. *J Am Vet Med Assoc*. 2002;221:1460–1463. 1420.
949. Martens J, Rosenbruch M. Hypophyseal adenocarcinoma in a horse. A case study. *Tierarztl Prax*. 1984;12:354–358.
950. Reynolds BL, Stedham MA, Lawrence 3rd JM, et al. Adenocarcinoma of the frontal sinus with extension to the brain in a horse. *J Am Vet Med Assoc*. 1979;174:734–736.
951. Livesey MA, Wilkie IW. Focal and multifocal osteosarcoma in two foals. *Equine Vet J*. 1986;18:407–410.
952. Harada K, Uozumi T, Kuwabara S, et al. Plasma cell tumor of the parieto-occipital bone; a case report. *No Shinkei Geka*. 1991;19:1067–1071.
953. Drew RA, Greatorex JC. Vertebral plasma cell myeloma causing posterior paralysis in a horse. *Equine Vet J*. 1974;6:131–134.
954. Brownie CF, Cullen J. Characterization of experimentally induced equine leukoencephalomalacia (ELEM) in ponies (Equus caballus): preliminary report. *Vet Hum Toxicol*. 1987;29:34–38.
955. Christley RM, Begg AP, Hutchins DR, et al. Leukoencephalomalacia in horses. *Aust Vet J*. 1993;70:225–226.
956. Kellerman TS, Marasas WF, Thiel PG, et al. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. *Onderstepoort J Vet Res*. 1990;57:269–275.
957. Lock TF. Leukoencephalomalacia in two quarter horses. *Mod Vet Pract*. 1974;55:464.
958. Marasas WF, Kellerman TS, Gelderblom WC, et al. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from Fusarium moniliforme. *Onderstepoort J Vet Res*. 1988;55:197–203.
959. Naranjo Cerrillo G, Soler Rodriguez F, Gomez Gordo L, et al. Clinical and pathological aspects of an outbreak of equine leukoencephalomalacia in Spain. *Zentralbl Veterinarmed A*. 1996;43:467–472.
960. Pienaar JG, Kellerman TS, Marasas WF. Field outbreaks of leukoencephalomalacia in horses consuming maize infected by Fusarium verticillioides (= F. moniliforme) in South Africa. *J S Afr Vet Assoc*. 1981;52:21–24.
961. Porter JK, Voss KA, Bacon CW, et al. Effects of Fusarium moniliforme and corn associated with equine leukoencephalomalacia on rat neurotransmitters and metabolites. *Proc Soc Exp Biol Med*. 1990;194:265–269.
962. Rosiles MR, Bautista J, Fuentes VO, et al. An outbreak of equine leukoencephalomalacia at Oaxaca, Mexico, associated with fumonisin B1. *Zentralbl Veterinarmed A*. 1998;45:299–302.

963. Ross PF, Ledet AE, Owens DL, et al. Experimental equine leukoencephalomalacia, toxic hepatosis, and encephalopathy caused by corn naturally contaminated with fumonisins. *J Vet Diagn Invest*. 1993;5:69–74.
964. Ross PF, Nelson PE, Owens DL, et al. Fumonisin B2 in cultured Fusarium proliferatum, M-6104, causes equine leukoencephalomalacia. *J Vet Diagn Invest*. 1994;6:263–265.
965. Ross PF, Rice LG, Reagor JC, et al. Fumonisin B1 concentrations in feeds from 45 confirmed equine leukoencephalomalacia cases. *J Vet Diagn Invest*. 1991;3:238–241.
966. Uhlinger C. Clinical and epidemiologic features of an epizootic of equine leukoencephalomalacia. *J Am Vet Med Assoc*. 1991;198:126–128.
967. Uhlinger C. Leukoencephalomalacia. *Vet Clin North Am Equine Pract*. 1997;13:13–20.
968. Voss KA, Norred WP, Plattner RD, et al. Hepatotoxicity and renal toxicity in rats of corn samples associated with field cases of equine leukoencephalomalacia. *Food Chem Toxicol*. 1989;27:89–96.
969. Wilkins PA, Vaala WE, Zivotofsky D, et al. A herd outbreak of equine leukoencephalomalacia. *Cornell Vet*. 1994;84:53–59.
970. Wilson BJ, Maronpot RR, Hildebrandt PK. Equine leukoencephalomalacia. *J Am Vet Med Assoc*. 1973;163:1293–1295.
971. Wilson TM, Nelson PE, Marasas WF, et al. A mycological evaluation and in vivo toxicity evaluation of feed from 41 farms with equine leukoencephalomalacia. *J Vet Diagn Invest*. 1990;2:352–354.
972. Wilson TM, Ross PF, Nelson PE. Fumonisin mycotoxins and equine leukoencephalomalacia. *J Am Vet Med Assoc*. 1991;198:1104–1105.
973. Wilson TM, Ross PF, Owens DL, et al. Experimental reproduction of ELEM. A study to determine the minimum toxic dose in ponies. *Mycopathologia*. 1992;117:115–120.
974. Wilson TM, Ross PF, Rice LG, et al. Fumonisin B1 levels associated with an epizootic of equine leukoencephalomalacia. *J Vet Diagn Invest*. 1990;2:213–216.
975. Wohlsein P, Hinrichs U, Brandt K, et al. Leukoencephalomalacia in two horses—moldy corn poisoning in Germany? *Tierarztl Prax*. 1995;23:582–587.
976. Marasas WF, Kellerman TS, Pienaar JG, et al. Leukoencephalomalacia: a mycotoxicosis of Equidae caused by Fusarium moniliforme Sheldon. *Onderstepoort J Vet Res*. 1976;43:113–122.
977. Cordy DR. Nigropallidal encephalomalacia in horses associated with ingestion of yellow star thistle. *J Neuropathol Exp Neurol*. 1954;13:330–342.
978. Farrell RK, Sande RD, Lincoln SD. Nigropallidal encephalomalacia in a horse. *J Am Vet Med Assoc*. 1971;158:1201–1204.
979. Fowler ME. Nigropallidal encephalomalacia in the horse. *J Am Vet Med Assoc*. 1965;147:607–616.
980. Gard GP, De Sarem WG, Ahrens PJ. Nigropallidal encephalomalacia in horses in New South Wales. *Aust Vet J*. 1973;49:107–108.
981. Larson KA, Young S. Nigropallidal encephalomalacia in horses in Colorado. *J Am Vet Med Assoc*. 1970;156:626–628.
982. Moret S, Populin T, Conte LS, et al. HPLC determination of free nitrogenous compounds of *Centaurea solstitialis* (Asteraceae), the cause of equine nigropallidal encephalomalacia. *Toxicon*. 2005;46:651–657.
983. Roy DN, Peyton DH, Spencer PS. Isolation and identification of two potent neurotoxins, aspartic acid and glutamic acid, from yellow star thistle. *(Centaurea solstitialis). Nat Toxins*. 1995;3:174–180.
984. Sanders SG, Tucker RL, Bagley RS, et al. Magnetic resonance imaging features of equine nigropallidal encephalomalacia. *Vet Radiol Ultrasound*. 2001;42:291–296.
985. Young S, Brown WW, Klinger B. Nigropallidal encephalomalacia in horses caused by ingestion of weeds of the genus Centaurea. *J Am Vet Med Assoc*. 1970;157:1602–1605.
986. Young S, Brown WW, Klinger B. Nigropallidal encephalomalacia in horses fed Russian knapweed—Centaurea repens L. *Am J Vet Res*. 1970;31:1393–1404.
987. Baird JD, Arroyo LG, Vengust M, et al. Adverse extrapyramidal effects in four horse given fluphenazine decanoate. *J Am Vet Med Assoc*. 2006;229:104–110.
988. Brashier M. Fluphenazine-induced extrapyramidal side effects in a horse. *Vet Clin North Am Equine Pract*. 2006;22: e37–e45.
989. Brewer BD, Hines MT, Stewart JT, et al. Fluphenazine induced Parkinson-like syndrome in a horse. *Equine Vet J*. 1990;22:136–137.
990. Kauffman VG, Soma L, Divers TJ, et al. Extrapyramidal side effects caused by fluphenazine decanoate in a horse. *J Am Vet Med Assoc*. 1989;195:1128–1130.
991. Rodriguez-Palacios A, Quesada R, Baird J, et al. Presumptive fluphenazine-induced hepatitis and urticaria in a horse. *J Vet Intern Med*. 2007;21:336–339.
992. Costello S, Heffron B, Taddei L, et al. Quantitation of fluphenazine in equine serum following fluphenazine decanoate administration. *J Anal Toxicol*. 2013;37:594–599.
993. Bertone JJ, Horspool LJI. Drugs and dosages for use in equines. In: Bertone JJ, Horspool LJI, eds. *Equine Clinical Pharmacology*. Edinburgh: W.B. Saunders; 2004:367–380.
994. Collins NM, Axon JE, Carrick JB, et al. Severe hyponatraemia in foals: clinical findings, primary diagnosis and outcome. *Aust Vet J*. 2016;94:186–191.
995. Hardefeldt LY. Hyponatraemic encephalopathy in azotaemic neonatal foals: four cases. *Aust Vet J*. 2014;92:488–491.

CHAPTER 12

Disorders of the Gastrointestinal System

L. Chris Sanchez*

Physical Examination

Examination of patients with disease of the gastrointestinal tract must include evaluation of the metabolic and cardiovascular status of the patient because acute conditions of the proximal or distal intestinal tract can lead to endotoxemia and sepsis. Examination of the cardiovascular system (heart, peripheral pulse, and mucous membranes), lungs, and abdomen is essential to detect clinical signs of systemic inflammation from endotoxemia, coagulation disorders, dehydration, ileus, shock, and other abnormalities resulting from injury to the small or large intestine. Clinical signs of systemic inflammation from endotoxemia and sepsis are described later in this chapter.

The physical examination of the abdomen should include auscultation, transabdominal ballottement, and transrectal palpation. Abdominal distention often indicates distention of the large intestine; small intestinal distention also can cause visible abdominal distention if a large proportion of the small intestine is involved. Abdominal palpation can be performed in neonatal foals. After several weeks of age the abdominal wall is too rigid to allow effective palpation of intraabdominal structures.

Abdominal auscultation is particularly useful for assessing the motility of the large intestine. Progressive motility of the small intestine, conversely, is difficult to distinguish by auscultation from nonprogressive motility. The distinct character of the borborygmi produced during propulsive contractions of the cecum and ascending colon allows evaluation of the frequency and strength of retropulsion and propulsion. Propulsive contractions of the cecum and ventral colon occur every 3 to 4 minutes and give rise to prolonged rushing sounds heard over long segments of intestine. Retropulsive sounds presumably are similar to propulsive sounds, but they occur less frequently. Distinguishing between propulsion and retropulsion is not important clinically because both types of contractions signify normal motility. Interhaustral and intrahaustral mixing contractions produce nonspecific sounds of fluid and ingesta movement that are difficult to distinguish from other borborygmi, such as small intestinal contractions or spasmodic contractions.[1]

Auscultation over the right flank and proceeding along the caudal edge of the costal margin toward the xiphoid allows evaluation of the cecal borborygmi. Auscultation over a similar area on the left side allows evaluation of the pelvic flexure and ascending colon. Typical progressive borborygmi heard every 3 to 4 minutes on both sides of the abdomen indicate normal motility of the cecum and ascending colon. Less frequent progressive sounds may indicate a pathologic condition of the large intestine or may result from anorexia, nervousness (sympathetic tone), or pharmacologic inhibition of motility (i.e., α_2-adrenergic agonists such as xylazine).[2-5] Absolute absence of any auscultable borborygmi suggests abnormal motility and indicates ileus resulting from a serious pathologic condition but is not specific to any segment of the intestine.[3,6] If borborygmi are audible but progressive sounds are not detectable, determining whether a significant abnormality exists is difficult, and such findings should not be overinterpreted.[6] Borborygmi heard more frequently than normal may result from increased motility following feeding; from excessive stimulation from irritation, distention, or inflammation; or after administration of parasympathomimetic drugs such as neostigmine. Large intestinal motility increases in the early stages of intestinal distention regardless of the site.[7] Mild inflammation or irritation of the large intestinal mucosa also can stimulate motility.[3] Parasympathomimetic drugs stimulate contractions and auscultable borborygmi in the large intestine; an increase in parasympathetic tone may result in segmental contractions, which actually inhibit progressive motility.[2]

Percussion of the abdomen during auscultation can reveal gas in the large intestine. The characteristic *ping* produced by simultaneous digital percussion and auscultation over a gas-filled viscus often is associated with abnormal accumulation of gas under pressure. This technique is particularly useful in foals, ponies, and Miniature Horses because of the limitations of rectal palpation.

Transabdominal ballottement can be used to detect large, firm masses or an abnormal volume of peritoneal fluid (PF). The usefulness of this technique is usually limited to animals too small to palpate rectally. Soft tissue masses or fetuses can be detected by bumping the structures with a hand or fist. If excessive PF is present, a fluid wave can be generated by ballottement; however, this technique is not as useful in horses older than 4 weeks because the abdominal wall is rigid.

*The editors and authors acknowledge and appreciate the contributions of Samuel L. Jones, Katharina I. Lohmann, Michelle Henry Barton, Laura Javsicas, Anthony T. Blikslager, and Dana N. Zimmel as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

Transrectal palpation is the most specific physical examination technique for investigation of intestinal disease and is particularly valuable when evaluating obstructive diseases.[8] The primary objectives of transrectal palpation are to assess the size, consistency, and position of the segments of the large intestine; to determine the presence of any distention of the small intestine; and to detect intraabdominal masses. Evaluation of the wall thickness and texture and the mesenteric structures (blood and lymphatic vessels and lymph nodes) also may aid in diagnosis of large intestinal disease. The interpretation of transrectal palpation findings in light of clinical signs and laboratory results is an important diagnostic aid for developing appropriate treatment strategies for intestinal diseases manifested by abdominal pain. Enlargement of one or more segments of large intestine detected by transrectal palpation provides evidence of obstruction at or distal to the enlarged segment. By systematically evaluating each segment, the site of obstruction may be determined. Obstruction of the pelvic flexure, for instance, results in enlargement of the pelvic flexure and ventral colon, but the dorsal and descending colons are of normal size. Enlargement of a segment of the large intestine usually is accompanied by abnormal consistency of the contents. It is possible to distinguish among gas, fluid, and ingesta and to detect foreign bodies in palpable segments. Accumulation of gas and fluid suggests complete and acute obstruction, whereas accumulation of ingesta suggests chronic and incomplete obstruction. Accumulation of fluid usually indicates ileus. The practitioner must evaluate the consistency of the contents in light of the size of the segment; ingesta in the ventral colon of a dehydrated patient may be firm, but the size of the ventral colon will be normal. Conversely, if the ingesta is firm because of a distal obstruction, the ventral colon will be enlarged.

Displacement of a segment of the large intestine may create an obstruction detectable by enlargement of the segment and accumulation of gas and fluid, even if the site of obstruction is not palpable. Torsion of the ascending colon at the sternal and diaphragmatic flexures results in acute accumulation of gas and fluid proximal to the torsion, causing distention of the left dorsal and ventral colons. Depending on the degree of torsion, the position of the ventral and dorsal colons may not be significantly abnormal. Displacement of a segment of large intestine often results in incomplete obstruction, and the diagnosis is either confirmed on detection of the displaced segment in an abnormal position or suspected when the segment is not palpable in a normal position. A determination should be made as to whether the segment that appears to be displaced is in a normal position but of smaller than normal size because of a decreased volume of ingesta. The cecum, right dorsal and ventral colons, pelvic flexure, and descending colon are palpable in most horses. The nephrosplenic space should be palpated to detect the presence of intestine, usually pelvic flexure, entrapped within the ligament.

Small intestine is not normally palpable in the horse. Distention indicates ileus with gas or fluid retention, usually following a strangulating or nonstrangulating obstruction. Strangulating obstructions often are accompanied by severe pain, dehydration, PF changes, and a varying degree of gastric fluid accumulation. The small intestine in these cases is turgid and firm on palpation. The mesentery and wall thickness should be assessed in the same manner as for large intestinal disorders. Careful palpation of the inguinal rings in stallions with small intestinal distention is crucial for determining inguinal herniation.

Evaluation of the wall thickness and mesenteric vessels can reveal venous congestion (mural edema and enlarged blood and lymphatic vessels) or inflammation (mural edema with normal vessels). Disruption of arterial blood flow does not cause venous congestion, but the arterial pulse is not detectable. Mesenteric tears may not be palpable, but the entrapped ischemic intestinal segment may be thickened. Enlargement of mesenteric lymph nodes also may be noted. Abnormalities in the wall or vessels should be interpreted in light of the size, consistency, and position of the segment of intestine and the clinical signs. Several conditions involving small intestinal strangulating lesions do not necessarily cause abnormal rectal examination findings until the disease has been present for an extended time. These conditions include diaphragmatic hernias and epiploic foramen entrapments (EFEs). PF analysis can be normal in these cases as well because the fluid is trapped in the thorax or cranial abdomen.

Nonstrangulating causes of small intestinal distention can be divided further into intraluminal and extraluminal obstructions. Ileal impactions are the most common cause of intraluminal obstruction, and on rare occasions the impaction can be palpated in the upper right quadrant, near the ileocecal opening. Intraluminal masses caused by lymphoma, eosinophilic enteritis, foreign bodies, or ascarid impactions often lead to small intestinal distention and are usually indistinguishable from one another on the basis of palpation alone. Small intestine in these cases can be moderately to severely distended, depending on the degree of obstruction. Extraluminal obstructions include abdominal masses and large colon displacement. The rest of the abdomen should be carefully palpated to help rule out these causes. Some cases of small intestinal distention result from physiologic ileus rather than mechanical obstruction. The bowel is usually mildly to moderately distended and almost always is accompanied by significant gastric fluid.

The small colon is easily distinguishable by the presence of normal fecal balls and an antimesenteric band. In horses with impaction of the small colon, a long, hard, tubelike structure is present in the caudal abdomen, and the band is palpable along the length. Fluid stool is often present in the rectum in these horses, as is tenesmus, and the rectal mucosa is often edematous and occasionally roughened. Rectal tears can be detected and evaluated with careful rectal palpation. Also detectable are mural masses in palpable segments of intestine or mesentery; if a mass causes obstruction, then it is possible to detect the result of the obstruction in proximal segments of intestine even if the mass is unreachable. Palpation of the mesenteric vessels may reveal thickening and thrombosis, which can lead to ischemia or infarction.

Visual inspection of the mucosa of the rectum and descending colon can be performed with the aid of a speculum or flexible endoscope. A flexible endoscope is also useful for evaluation of rectal tears or perforations, mural masses, strictures, or mucosal inflammation and obtaining biopsy specimens of the mucosa or masses. The obvious limitations are the amount of fecal material, which can interfere with the examination, and the distance of the lesion of interest from the anus. These techniques offer little advantage over palpation in many cases, unless the patient is too small to palpate.

Examination of the oral cavity in horses with dysphagia or weight loss is an important extension of the physical examination. The horse should be adequately sedated and a full-mouth speculum used to allow palpation and visual examination of all parts of the oral cavity and detection of abnormal dentition, foreign bodies, fractures, abscesses, or mucosal ulceration.

The presence of fluid accumulation in the stomach indicates a functional or mechanical obstruction of gastric

outflow. Fluid accumulation in the stomach is assessed by siphoning of the gastric contents with a nasogastric tube and examining the fluid for amount, color, and odor. Normal fluid is green and may contain foamy saliva. The net volume obtained by gastric lavage is usually less than 4 L. Large volumes (≥8–10 L) of foul-smelling fluid may indicate proximal enteritis. Horses with strangulating obstructions or luminal obstructions often accumulate moderate amounts of gastric fluid, but the amount is generally less than in horses with proximal enteritis or postoperative ileus (POI). Distinction between these conditions should not be made based on the volume and character of gastric fluid alone. Hemorrhage in the gastric fluid usually indicates devitalized small intestine, stomach wall, or severe gastric ulceration. Endoscopy or contrast radiography may aid in the diagnosis of gastric outflow obstruction.

DIAGNOSTIC EVALUATION

Clinical Pathology

Hematologic alterations associated with diseases of the gastrointestinal tract are often nonspecific, reflecting systemic response to inflammation, endotoxemia, or sepsis. Neutrophilic leukocytosis and normochromic, normocytic anemia with or without hyperfibrinogenemia commonly are associated with chronic inflammatory conditions of the intestine. Anemia from chronic blood loss occurs infrequently in adult horses because of the large iron stores and high concentrations of iron in their diet; anemia usually follows chronic inflammation, as do alterations in the leukon and plasma fibrinogen concentrations. Plasma protein concentrations vary depending on gastrointestinal losses of albumin and globulin and elevation of globulin concentration from antigenic stimulation. Protein-losing enteropathy may manifest as a hypoalbuminemia or panhypoproteinemia.

Significant alterations of the hemogram do not accompany acute disease of the intestine unless severe dehydration, endotoxemia, or systemic inflammatory response syndrome (SIRS) is present. During the early stages of SIRS, elevations in circulating concentrations of inflammatory mediators, epinephrine, and cortisol produce characteristic changes in the hemogram. Leukopenia, with neutropenia and a left shift, toxic changes in the neutrophil cytoplasm, and lymphopenia commonly occur early in the disease, but neutrophilic leukocytosis is more common during the later stages of SIRS. Hemoconcentration and hyperfibrinogenemia are also common. Thrombocytopenia and other coagulopathies are also features of SIRS.

Electrolyte imbalances and increased blood lactate are common biochemical abnormalities in horses with acute gastrointestinal disease. Decreased serum calcium concentrations are common and nonspecific.[9] Mucosal inflammation can disrupt electrolyte fluxes; diarrhea or gastric reflux greatly exacerbates sodium, potassium, calcium, magnesium, and bicarbonate loss. Large colon ischemia causes increased lactate and potassium concentrations and metabolic acidosis in the colonic vasculature and inflammation in the colonic and systemic vasculature.[10] Reduced perfusion of peripheral tissues from hypotensive shock and intestinal ischemia can cause increased blood lactate; intestinal obstruction during ischemia may also result in absorption of lactate from the lumen. Increased blood lactate can result from a variety of causes, including hypovolemia, and blood lactate alone should not be used for diagnostic or prognostic purposes in horses with colic.[11] Increases in blood lactate over time are greater in nonsurvivors, relative to survivors, in adult equine emergencies (many of which had gastrointestinal disease).[12] Portable lactate analyzers have demonstrated variable intraanalyzer reliability in equine blood so that caution should be exercised in interpretation and comparison of results reported from various searches.[13,14] Metabolic acidosis may accompany lactic acidemia, but an inconsistent association exists between the two, especially when mixed acid-base imbalances are present.[15,16] Increases in hepatic enzymes, specifically γ-glutamyl transferase (GGT), may occur with large colon displacements, duodenal strictures, or proximal enteritis. Increased GTT is more suggestive of right, rather than left, dorsal displacement.[17]

Relative polycythemia from hemoconcentration or splenic contraction and changes in red blood cell deformability from hypoxia or hypocalcemia may increase blood viscosity. Blood viscosity increases in patients with acute obstructive disease. Hyperviscosity reduces perfusion of capillary beds, exacerbating ischemia and tissue hypoxia.[18]

Peritoneal Fluid

Abdominocentesis and analysis of PF are performed on many patients with gastrointestinal disease and are especially helpful in differentiating strangulating from nonstrangulating disorders of the small intestine. Important quantifications include white and red blood cell counts and protein, lactate, and glucose concentrations. Cytologic evaluation can reveal cellular abnormalities, especially in horses with intestinal neoplasia. Results of PF analysis may help establish a specific diagnosis and, more important, may reflect inflammatory, vascular, or ischemic injury to the intestine, requiring surgical intervention.

Alteration of PF reflects a sequence of events during acute intestinal vascular injury. The PF protein concentration increases first, followed by increases in red blood cell count and fibrinogen concentration. A transudative process resulting from vascular congestion and increased endothelial permeability allows small macromolecules (albumin) to escape into the PF, followed by larger macromolecules (globulin and fibrinogen), and finally diapedesis of cells (red then white blood cells). Severe ischemic intestinal inflammation or visceral peritonitis result in an exudative process, with large quantities of protein and white blood cells (WBCs), primarily neutrophils, to escape into the PF.[19,20] Eventually, bacteria begin to translocate across the intestinal wall and appear in the PF as the mucosal barrier breaks down. If perforation occurs, bacteria and particles of ingesta appear in the PF, and the neutrophils become degenerate (i.e., pyknotic), with karyorrhexis, karyolysis, and smudge cells.

Increased PF protein concentration is an indicator of early inflammation, whereas increased red blood cell counts in the presence of normal WBC counts suggest vascular damage without significant tissue ischemia.[20] Of note, the anticoagulant potassium ethylenediamine tetraacetic acid, but not lithium heparin, can cause an increase in total protein as measured by a refractometer, relative to the value obtained from the same sample without anticoagulant.[21] The gross color of the PF can be helpful in detecting injury and necrosis of the intestine. A serosanguineous appearance indicates vascular injury, whereas orange or brown-red indicates necrosis with the release of pigments such as hemosiderin.

Tissue hypoxia and ischemia cause rapid increases in PF lactate dehydrogenase, creatine kinase, and alkaline phosphatase (AP) activity and lactate concentration.[22,23] Phosphate

concentration increases when cellular disruption occurs.[24] PF enzyme activities, phosphate, and lactate concentration increase faster and higher than serum activities.[16,22-24] PF pH and glucose concentration tend to decrease during intestinal ischemia but not as dramatically as in septic peritonitis.[25] Lactate concentrations in PF are commonly evaluated and are better predictors of strangulating small intestinal obstruction than blood lactate,[22] although increases in both lactate (the term by which L-lactate is commonly referred) and D-lactate are likely more accurate for predicting strangulating lesions as opposed to ruling them out.[26] Serial sampling of blood and PF lactate may be useful in cases in which clinical and diagnostic findings at initial presentation were not conclusive for either strangulating or nonstrangulating lesions and/or a horse's clinical condition deteriorates.[27]

Cytologic examination of PF may reflect chronic inflammatory intestinal conditions or neoplastic diseases.[28] Although culturing PF is recommended to distinguish bacterial infections from noninfectious inflammation unless bacteria are visible on cytologic examination, culturing PF is often unrewarding. Decreases in PF glucose concentrations (<30 mg/dL) and pH (<7.3) are early indicators of septic peritonitis. Glucose concentration and pH in PF should approximately equal blood values in normal horses.

Practically, the gross appearance and the total solids of PF in conjunction with a comparison of PF to serum lactate are most useful when distinguishing between strangulating and nonstrangulating small intestinal disorders in a horse presenting for acute colic. Potential risks associated with performing an abdominocentesis should be considered, and this procedure is performed only if results are likely to alter the treatment plan. For example, if other examination findings indicate that an exploratory laparotomy is clearly indicated or not in the case of a horse with acute colic, abdominocentesis is likely not indicated.

Fecal Examination

Gross examination of the feces can provide information about digestion and transit time in the large intestine. Large fiber particles in the feces represent poor mastication or poor digestion in the large intestine. Small, mucus-covered, hard fecal balls indicate prolonged transit through the descending colon, whereas increased fluidity implies decreased transit time. Feces containing sand or gravel are not necessarily abnormal. However, a significant amount of sand implies that large quantities are present in the colon. Alternatively, absence of sand in feces does not confirm an absence of sand in the colon. Frank blood indicates substantial bleeding into the distal colon (right dorsal colon, small colon, or both) resulting from mucosal damage.

Laboratory analysis of the feces can be performed for horses with diarrhea. Fecal cytologic examination and tests for occult blood detect mucosal inflammation, erosion, or ulceration. Increased fecal leukocyte counts have been documented in horses with diarrhea and salmonellosis, but specificity of this test is low.[29]

Fecal occult blood tests detect blood in the feces, presumably from erosion or ulceration of the mucosa, but do not distinguish the source of the blood. Large volumes of blood (1–2 L) given by nasogastric tube were required to produce a positive test for occult blood in the feces, but the amount of blood originating from the large intestine required to produce a positive test is unknown. Despite initial reports to the contrary,[30] there does not appear to be any correlation between endoscopic evidence of glandular or nonglandular gastric ulceration and detection of fecal albumin or hemoglobin.[31]

Bacteriologic examination of the fecal flora has been used in horses with diarrhea. Quantitation of clostridial species may be beneficial in diagnosing clostridial infection of the large intestine, although tests to detect clostridial toxins in intestinal contents or feces are important for determining whether clostridia cultured from the feces are causing disease. The most common bacterial pathogens isolated from the feces of horses are *Salmonella* spp. and *Clostridium* spp. The number of *Salmonella* organisms isolated from the feces of horses with clinical salmonellosis is usually higher than from horses with asymptomatic infections. However, the volume of feces in many cases of acute diarrhea is high, and the concentration of *Salmonella* organisms may be lower than would be expected, accounting for many false-negative fecal cultures. The sensitivity of fecal cultures for detecting *Salmonella* infection may be as low as 20%. Culture of five consecutive daily fecal samples is recommended to increase the sensitivity of the test. Real-time polymerase chain reaction (PCR) assays used on fecal samples can detect DNA from *Salmonella* spp. and have performed well in recent validation studies; accuracy of point-of-care tests remains elusive.[32-35] Currently, PCR assays can be used for detection of viral (rotavirus and coronavirus), bacterial (*Cryptosporidium* spp., *Salmonella* spp., *Neorickettsia risticii, Lawsonia intracellularis*), or bacterial toxin (*Clostridium difficile* toxins A and B, *Clostridium perfringens* enterotoxin A) DNA in feces or blood (*N. risticii*).

Qualitative fecal examination can detect nematode and cestode ova, protozoan oocysts, parasitic larvae, and protozoan trophozoites. A direct smear of fecal material can rapidly detect parasite larvae and trophozoites and motility of ciliates and parasite larvae. Fecal flotation with zinc sulfate or sucrose solutions is often used to concentrate less dense ova and oocysts. Zinc sulfate produces less distortion of trophozoites and larvae than sucrose solutions. Fecal sedimentation is particularly appropriate for ciliates, *Giardia* organisms, and trichomonads. Quantitative techniques such as the Cornell–McMaster method allow estimation of the number of eggs per gram of feces and are most appropriate in monitoring parasite control programs.

RADIOGRAPHY

Survey radiography of the normal esophagus is usually unrewarding. It is possible to detect foreign bodies or soft tissue masses and, in cases of esophageal rupture, free air and ingesta in the tissues surrounding the esophagus and pneumomediastinum. Thoracic radiographs may be necessary to detect megaesophagus or cranial mediastinal masses causing extraluminal obstruction. Barium swallows or double-contrast esophagrams may be used after resolution of an esophageal obstruction to determine whether a stricture, diverticulum, or other underlying disorder is present, although endoscopy can provide similar information. Barium sulfate is the usual contrast medium and can be administered orally by way of a dose syringe or nasogastric tube (50–100 mL of a 40% barium sulfate suspension or barium paste). Oral administration is preferred for evaluation of swallowing and lesions in the proximal esophagus. Administration of contrast using a nasogastric tube (preferably cuffed) allows for delivery of larger volumes of barium (up to 500 mL) but should be performed

without sedation if possible. Administration of contrast material can be followed with air insufflation to create a double-contrast effect. If rupture of the esophagus is suspected or if the contrast material is likely to be aspirated, barium should be avoided, and iodinated organic compounds in an aqueous solution should be used as contrast material to decrease the potential for adverse effects. When interpreting esophageal radiographs, the veterinarian should take particular care if the horse is sedated. Acepromazine or detomidine administration causes esophageal dilation in normal horses, especially after passage of a nasogastric tube.[36]

Radiography of the adult equine abdomen is an effective technique in detecting radiodense material in the large intestine, such as enteroliths, sand, and metallic objects.[37-40] One survey demonstrated that radiography has 76.9% sensitivity and 94.4% specificity for diagnosing enterolithiasis.[40] Recently, an objective scoring system demonstrated greater efficacy and less interobserver variability than a subjective assessment of radiographic sand accumulation in horses with or without a clinical diagnosis of sand colic.[38] The large size and density of the adult abdomen preclude evaluation of soft tissue structures because the detail and contrast of the radiographs are usually poor, and ultrasonography is a much more useful imaging modality in the equine abdomen.

Administration of contrast (barium sulfate 30% at 5 mL/kg) through a nasogastric tube or retrograde (20 mL/kg) through a 24-Fr Foley catheter inserted into the rectum may be helpful for the diagnosis of gastric outflow obstruction or disorders of the rectum, small colon, or transverse colon, respectively, in foals.[41-44]

ULTRASONOGRAPHY

Transcutaneous ultrasonographic evaluation of the abdomen is quick and noninvasive and can add valuable information in cases of acute or chronic gastrointestinal disease. Ultrasound has become a virtually indispensable tool for horses with acute or chronic gastrointestinal conditions. This section provides a brief summary. Examination of the adult horse requires a 2.5- to 5.0-MHz transducer, depending on size of the horse; a curvilinear transducer is preferred. A functional examination is often performed with isopropyl alcohol saturation alone, although clipping, with or without coupling gel, can be used to enhance evaluation, especially in large or overweight patients.

A protocol for fast localized abdominal sonography (FLASH) has been described and demonstrates good predictive value of the requirement for surgical intervention in the acute abdomen, even with relatively inexperienced examiners.[45] This examination evaluates seven locations (ventral abdomen, gastric window, splenorenal window, left middle third of the abdomen, duodenal window, right middle third of the abdomen, and thoracic window) and can be performed in less than 15 minutes. A complete examination requires a methodical approach to the evaluation of the entire abdomen; details of the examination can be variable based on equipment and experience of the examiner. A complete review of abdominal ultrasound is beyond the scope of this chapter; a thorough, detailed examination is reviewed elsewhere.[46]

In general terms, a quick examination of the acute abdomen should include estimation of gastric size and assessment of small intestinal diameter, wall thickness, motility, and location. Specific abnormalities, such as ascarid impaction or intussusception, may also be visible. Evaluation of the large colon and cecum should include estimation of mural thickening or increased fluidity of contents, and whether or not the colon obscures viewing of the left kidney in the left paralumbar fossa. It is important to remember that other causes of colonic distention can have the same result; thus, it is always important to combine physical, rectal, and ultrasonographic examination findings for any given case. Sand impactions may appear as hyperechoic bands on the ventral abdominal wall,[47] but according to the author and others,[46] ultrasound does not allow for consistent diagnosis of sand accumulation. Evaluation of the abdomen always should include assessment of the peritoneal space for any evidence of an increased amount or echogenicity of PF. Ultrasonography also can be useful in determining the ideal location for abdominocentesis.

NUCLEAR SCINTIGRAPHY

Nuclear scintigraphy has several proposed uses for evaluation of the gastrointestinal tract, although most have been replaced with cross-sectional imaging (dental disease[48,49]), ultrasonography (right dorsal colitis[50]), or other modalities (gastric emptying[51]).

CROSS-SECTIONAL IMAGING

Computed tomography (CT) and, less commonly, magnetic resonance imaging are extremely useful for evaluating dental disease as well as tumors and masses of the head, larynx, pharynx, and proximal esophagus in adult horses[52-55] and some abdominal disorders in foals.[56] Availability, gantry size, and table weight limits provide the biggest limitations for widespread use.

ENDOSCOPY

Endoscopic examination of the gastrointestinal tract begins with evaluation of the pharynx for signs of collapse, dysfunction, or dysphagia. The oral cavity should only be examined endoscopically with the use of heavy sedation or anesthesia and a full-mouth speculum. Indications include examination of the teeth, palate, and tongue for completeness, ulceration, masses, or foreign bodies. The most common gastrointestinal indication is evaluation of the stomach, proximal esophagus, and duodenum, typically with a 3-m flexible endoscope.

The esophagus should be examined aborad to orad because of its collapsible nature. The esophageal mucosa is normally smooth and light pink. Erosion or ulceration can occur secondary to obstruction, reflux esophagitis, or an indwelling nasogastric tube, among other causes. Erosions may be punctate, linear, or circumferential, and their extent (depth, length, etc.) should be evaluated carefully. Distinguishing normal peristaltic contractions from areas of stricture requires observation of the area and its motility over time. Diverticula also may be noted as outpouchings of the mucosa, sometimes associated with a stricture distally. Megaesophagus, although rare, appears as generalized dilation. Reevaluation after resolution of an obstruction is especially important for detecting the presence of complications (ulceration and rupture) or initiating causes (strictures, diverticula, masses).

Gastroscopy is best performed after a minimum 12-hour fast. Complete examination of the stomach, including the antrum and pylorus, and preferably proximal duodenum, is critical to avoid missing lesions in the more aborad regions. The

squamous mucosa should resemble the esophageal mucosa. The glandular mucosa should be glistening red and may have a reticulated pattern. The veterinarian should carefully examine it for evidence of ulceration or masses. Transendoscopic biopsy material can be easily obtained from esophageal, pharyngeal, or gastric masses, and because the biopsy size will be small, several samples should be taken for histopathologic examination. A complete description of gastroscopy and evaluation of gastric and gastroduodenal ulceration are available in Gastroduodenal Ulcer Disease.

TESTS OF ABSORPTION AND DIGESTION

Oral Glucose Tolerance Test

D-Glucose or D-xylose absorption tests are useful in determining malabsorption of carbohydrates from the small intestine in horses. For either test, the horse should be fasted for 14 to 18 hours before testing. Prolonged fasting (≥24 hours) results in a delayed and slightly lower peak plasma glucose concentration.[57] A dosage of 1 g/kg of D-glucose is administered as a 20% solution via nasogastric tube. Xylazine sedation does not appear to significantly alter D-xylose results.[58] Blood glucose or xylose concentrations are then measured 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after administration. Additional samples can be taken up to 6 hours after dosing if the results are questionable. Sodium fluoride and heparin are the preferred anticoagulants for glucose and xylose, respectively.

A normal D-glucose absorption test, also known as an *oral glucose tolerance test (OGTT),* should peak between 90 and 120 minutes at a glucose concentration >85% above the resting glucose concentration.[59] *Complete malabsorption* is defined as a peak less than 15% above the resting concentration, and *partial malabsorption* is defined as a peak between 15% and 85% above the resting concentration. Gastric emptying, gastrointestinal transit time, length of fasting, glucose and insulin metabolism, age, diet, and endocrine function can influence glucose absorption curves.[59,60] Higher glucose peaks are recorded from healthy animals eating grass or hay than from those eating concentrates[60] and from horses fed a pasture (clover and kikuyu) versus stable (oat hay, complete feed, and alfalfa/oat chaff) diet.[61] Results from the OGTT can also be affected by the content of nonstructural carbohydrate and fat in the diet[62] and other disorders, such as polysaccharide storage myopathy.[63]

Oral Xylose Tolerance Test

The xylose absorption test is performed as per the OGTT, except 0.5 g D-xylose per kilogram body weight is administered as a 10% solution via nasogastric tube. The pretest period of fasting and timing of sample collection are identical. The laboratory to which samples will be sent should be contacted before collection to ensure that heparinized plasma is acceptable for their laboratory. Plasma D-xylose should peak between 20 and 25 mg/dL between 60 and 90 minutes following administration, with occasional peaks as late as 120 minutes.[64,65] D-Xylose absorption testing is not confounded by hormonal effects or mucosal metabolism as is glucose, but it is altered by diet, length of fasting (which could also be influenced by recent appetite or degree of cachexia), and age. Horses fed a high-energy (oat chaff, oats, and corn) diet had a lower mean peak plasma D-xylose concentration (14.1 mg/dL versus 24.9 mg/dL) than those consuming a low-energy (alfalfa chaff) diet.[61] Healthy mares fasted for up to 96 hours had flatter curves and a slower decrease in plasma xylose than when fasted for 12 to 36 hours.[64] Kinetic analysis indicates that prolonged food deprivation does not alter renal or nonrenal excretion of D-xylose; thus, the effect of fasting on the curve is likely related to either intestinal transit, small intestinal absorption, or both.[66,67] Ponies may have lower peak D-xylose concentrations than horses, although the range is wide, and diagnostic discriminatory cutoff points for peak plasma xylose concentrations have not been determined. Foals normally have a higher mean peak xylose concentration at 1 and 2 months of age, but mean peak falls to a level similar to that seen in adults by 3 months of age.[68] Gastric emptying rate, intestinal motility, intraluminal bacterial overgrowth, and renal clearance can affect curve shape.[69]

Lactose Tolerance Testing

Milk intolerance is well documented in children and can result from primary or secondary lactase (neutral β-galactosidase) deficiency. Secondary lactase deficiency can occur when a small intestinal disorder, typically rotavirus, damages the epithelial cells, resulting in decreased brush border disaccharidase activity. Such an association has been reported secondary to clostridial enteritis in the foal.[40] In horses, lactase activity peaks at birth and declines slowly, such that activity is approximately 50% at 2 years of age and rapidly decelerates after 3 years of age, and it is barely detectable by 4 years of age.[41] The oral lactose tolerance test is performed in foals after a 4-hour fast, with a standard dose of 1 g/kg lactose monohydrate given via nasogastric tube as a 20% solution. Blood sampling is as per the OGTT. Plasma glucose typically peaks 60 minutes following lactose administration, with a range from 30 to 90 minutes, with a mean increase of 77 mg/dL, with a 35-mg/dL increase covering 2 standard deviations.[42] Of note, older foals (12 weeks) had a more significant increase in plasma glucose relative to 1-week-old foals.

Evaluation of Gastric Emptying

Assessment of gastric emptying may be useful in cases of gastric and esophageal ulceration or suspected gastric outflow obstruction, although accurate assessment can be challenging.

Contrast radiography can be used in foals. In the normal foal, a significant amount of liquid barium should empty within 2 hours.[41] Nuclear scintigraphy can be used for measurement of liquid[51] or solid phase emptying[70] by use of orally administered ^{99m}Tc pentetate (10 mCi) or a ^{99m}Tc-labeled pelleted ration, respectively.

Alternatively, absorption testing of glucose or xylose (discussed previously) or acetaminophen can be used as an indirect determination of liquid gastric emptying. Acetaminophen absorption is performed by administering 20 mg/kg of acetaminophen orally, sampling blood, then calculating the time to reach maximum serum concentrations and the absorption constant.[71,72] In humans the proximal small intestine absorbs almost all of the acetaminophen.[73] The median time to reach peak plasma levels using acetaminophen absorption in horses is 47.7 minutes.[72]

The ^{13}C-octane acid breath test offers an easy, noninvasive method of determining gastric emptying of solids.[70,74] This test is performed by feeding a standard ^{13}C-labeled test meal and then collecting breath samples using a modified mask. The breath is then analyzed for the ratio of the novel isotope, $13{:}CO_2$, to the normally produced $12{:}CO_2$.

HISTOPATHOLOGIC EXAMINATION

It is often necessary to perform a histopathologic examination of tissues from the intestine to diagnose chronic inflammatory, infiltrative, or neoplastic conditions, and such an examination can be useful in evaluating the extent of injury after obstruction or ischemia. Rectal mucosal biopsies are easy to collect, with few complications. Full-thickness biopsy often provides more thorough analysis and can be directed based on serosal appearance via a flank, ventral midline, or laparoscopic approach. When taking gastric or duodenal biopsies endoscopically for the putative diagnosis of inflammatory bowel disorders, multiple samples are recommended. The likelihood of establishing an accurate diagnosis varies with tissue quality, number of samples, skill of the endoscopist, and submission technique.[75]

Laparoscopy

Laparoscopic evaluation of the equine abdomen requires specialized equipment and training. The laparoscopic procedure can be done with the horse standing or recumbent. Advantages of this technique over a flank or ventral midline celiotomy include smaller incisions, shorter healing time, and shorter procedure time. Disadvantages include equipment and personnel needs, limited therapeutic potential, and limited visual field, especially relative to midline celiotomy. Potential gastrointestinal applications of abdominal laparoscopy include the correction of rectal tears; percutaneous abscess drainage, assessment of adhesions, displacements, and integrity of the serosa of various bowel segments; biopsy of abdominal masses; and closure of the nephrosplenic space.[76-80]

Pathophysiology of Gastrointestinal Inflammation

The inflammatory response of the gastrointestinal tract is a mechanism ultimately aimed at eliminating pathogens, initiating tissue repair, and restoring the gastrointestinal barrier. Blood flow is altered, endothelial permeability increases, cells are rapidly recruited into the tissue, plasma protein cascades are activated, and myriad soluble products are released that coordinate the response, trigger innate and adaptive immunity, and mobilize reparative elements. Although the cellular and vascular response and the secreted mediators of inflammation are important for killing pathogens and limiting invasion of injured tissues by commensal organisms, they can be quite damaging to host cells and proteins if not tightly regulated. If the inciting stimulus is not eliminated quickly, then the inflammatory response itself will cause significant tissue injury. The mechanism regulating inflammation has been the focus of a great deal of research to identify therapeutic targets to modulate the damage to host tissues that occurs in many gastrointestinal diseases. Recent work has provided some of the molecular and cellular details of this complex physiology and has led to novel therapeutic strategies for treating inflammation.

INITIATION OF THE INFLAMMATORY RESPONSE

Epithelium

The gastrointestinal epithelium interfaces with a luminal environment that is inhabited by potentially hostile microbial organisms. The epithelium presents a physical barrier to invasion by the flora of the gastrointestinal tract, consisting of the apical cellular membrane, intercellular tight junctions (the permeability of which is highly regulated), and a secreted layer of mucus. When invading pathogens breach the mucosal barrier, potent soluble and neural signals are generated that initiate an inflammatory response.[81] The epithelium can be conceptualized as a sensory organ that detects pathogen invasion to trigger an appropriate host defense and reparative response.

Noninfectious mucosal injury or invasion of epithelial cells by pathogenic organisms such as *Salmonella* activates the synthesis of proinflammatory chemokines (chemoattractants) by epithelial cells that triggers a robust influx of neutrophils into the tissue within hours of the damage.[81] Of the chemoattractants produced by epithelium, interleukin-8 (IL-8) has a particularly important role in initiating inflammation by recruiting neutrophils from blood[82] and regulating neutrophil migration through tissue matrix adjacent to epithelium.[83,84] Complement fragments such as C5a and bacteria-derived formylated chemotactic peptides also act as potent "end target" chemoattractants that are fully capable of stimulating a robust inflammatory response in the intestine if the epithelial barrier permits invasion of bacteria or the diffusion of bacterial peptides across the mucosa.

Epithelial cells activated during infection produce cytokines such as tumor necrosis factor–α (TNF-α), arachidonic acid metabolites, and other proinflammatory mediators that activate recruited leukocytes.[85] Microbial products, particularly lipopolysaccharide (LPS) and other bacterial cell wall components and microbial nucleic acids, are potent activators of leukocytes recruited into the tissue.[86] Mast cells are key sentinel leukocytes that sense microbial invasion, releasing TNF-α, which appears to be a critical initiator and regulator of the cellular phase of inflammation.[87] Once the inflammatory response has been initiated, TNF-α; IL-1β; and other proinflammatory products of neutrophils, monocytes, mast cells, and epithelial cells amplify the inflammatory response.

The enteric nervous system (ENS) has a key role in sensing and regulating inflammatory responses in the intestine. For example, *C. difficile* toxin A activates a neural pathway that triggers mast cell degranulation and neutrophil influx into the tissue.[88,89] Blockade of this neural pathway is sufficient to abolish the profound inflammatory response induced by toxin A as well as many of the effects of toxin A on enterocyte secretion. Other pathogens and immune-mediated hypersensitivity reactions similarly stimulate inflammation by mechanisms that involve the ENS. Thus the epithelium interacts in a highly complex manner with the intestinal milieu, the ENS, and inflammatory cells to regulate the tissue response to injury and infection.

Macrophages

Resident macrophages located in the lamina propria, submucosa, and intestinal lymphoid organs are among the first cells beyond the epithelium to respond to infection or injury.

Macrophages are activated by microbial products by way of pattern recognition receptors and begin to produce proinflammatory molecules important for recruiting and activating neutrophils and monocytes. Pattern recognition receptors recognize microbial molecules such as LPS, lipoproteins, flagellin, peptidoglycan, and nucleic acids to signal the invasion by pathogens.[86] Of the pattern recognition receptors, the LPS receptor complex is perhaps the best defined. LPS activates macrophages by way of the CD14 Toll-like receptor 4 (TLR-4) complex to initiate transcription of the inflammatory cytokines TNF-α and IL-1β, which synergize with LPS to amplify the macrophage response.[86] LPS, particularly in concert with inflammatory cytokines, stimulates macrophages to produce copious amounts of nitric oxide, which is both microbicidal and vasoactive.[90] Nitric oxide and other nitrogen radicals react with reactive oxygen intermediates (ROIs) generated by the activated oxidase complex to produce some of the most toxic molecules of the host defense system: the peroxynitrites.[90] IL-8 is produced as well to recruit neutrophils. As the response progresses, other inflammatory mediators, particularly the arachidonic acid–derived lipids dependent on inflammation-induced cyclooxygenase (COX)-2 and 5-lipoxygenase activity, are produced that have potent vasoactive and proinflammatory effects through the activation of endothelial cells, neutrophils, and platelets.[91]

VASCULAR RESPONSE DURING INFLAMMATION

Four important changes occur in the intestinal vasculature during inflammation: (1) alteration of blood flow; (2) increased vascular permeability; (3) increased adhesiveness of endothelial cells, leukocytes, and platelets; and (4) exposure of the basement membrane and activation of the complement, contact, and coagulation cascades.

A wide range of mediators alter blood flow during inflammation in the intestinal tract, ranging from gases such as nitric oxide (a major vasodilator of the intestinal vasculature) to lipids (prostaglandins, leukotrienes, thromboxanes, and platelet-activating factor [PAF]), cytokines, bradykinin, histamine, and others. The major sources for these mediators include activated leukocytes, endothelial cells, epithelial cells, and fibroblasts. The primary determinant of blood flow early in inflammation is vascular caliber, which initially decreases in arterioles but then quickly changes to vasodilation coincident with the opening of new capillary beds, increasing net blood flow. The increase in blood flow is relatively short-lived, because the viscosity of the blood increases from fluid loss and tissue edema resulting from leaky capillaries. Leukocyte margination, platelet adhesion to endothelial cells and exposed matrix, and areas of coagulation protein accumulation further decrease local circulation.

Increased vascular permeability is initially caused by inflammatory mediator actions on the endothelial cells. Histamine, leukotrienes, PAF, prostaglandins, bradykinin, and other mediators stimulate endothelial cell contraction, and interendothelial gaps form.[92,93] This stage of increased vascular permeability is readily reversible. Concurrently, mediators such as the cytokines TNF-α and IL-1β induce a structural reorganization of the interendothelial junctions, resulting in frank discontinuities in the endothelial monolayer.[94] Cytokines also stimulate endothelial cells to express adhesion molecules that support adhesion of leukocytes and platelets,[95] leading to the next and perhaps most devastating event. Leukocytes (primarily neutrophils) and platelets adhere to exposed basement membranes and activated endothelial cells. Adherent neutrophils and platelets are then exposed to the mediators of inflammation present in the surrounding milieu, which activates the cells to release oxidants and proteases (particularly elastase) that injure the endothelium and have the potential to cause irreparable harm to the microvasculature.[96-98] Marginated neutrophils begin to transmigrate between endothelial cells (as described in later sections), which, if in sufficiently large numbers, disrupts the integrity of the interendothelial junctions, worsening the vascular leakage.[97]

These stages of enhanced vascular permeability can be conceptualized as a mechanism to allow plasma proteins to enter the tissues and to potentiate the critical influx of leukocytes into tissues. However, if they are not regulated precisely, alterations in both hydrostatic and oncotic forces and irreversible damage to the vascular bed may have devastating consequences. Moreover, inappropriate activation of plasma protein cascades and leukocytes by activated endothelium and exposed matrix proteins can contribute to SIRS (see the later section Gastrointestinal Ileus for more information) characterized by hypotension, generalized vascular leak syndrome, and multiorgan dysfunction, which may be fatal. Phosphodiesterase inhibitors reduce endothelial permeability in ischemia–reperfusion injury and other models of inflammation-induced vascular leakage[99,100] by increasing endothelial tight junction integrity; thus, it may be a viable therapeutic strategy to prevent or reduce the permeability alterations associated with inflammation.

CELLULAR EFFECTORS OF INFLAMMATION

Endothelial Cells

Endothelial cells respond to products of activated epithelial cells and macrophages in the intestinal tissue to recruit cells and humoral mediators of inflammation into the tissue. Activated endothelial cells display a range of molecules critical for neutrophil and platelet adhesion. The role of endothelial cells in mediating neutrophil recruitment is discussed in more detail later in this chapter. Intercellular permeability is increased to expose basement membrane proteins that trigger humoral defense systems (complement, coagulation, and contact system cascades) and to provide access for these macromolecules to the tissue. Endothelial cells are an important source of inflammatory mediators that amplify the response and vasoactive substances (particularly nitric oxide) altering blood flow.

Neutrophils

Recruitment

Infection or injury to the gastrointestinal mucosa causes an influx of leukocytes from the blood that lay the foundation of the inflammatory response. Neutrophils, the first to arrive during inflammation, have a dominant role in the acute response. Within minutes neutrophils are recruited into the tissue, in which they are activated to release products that not only are lethal to pathogens and proinflammatory but also may damage host cells and tissues.[101] Not surprisingly, a great

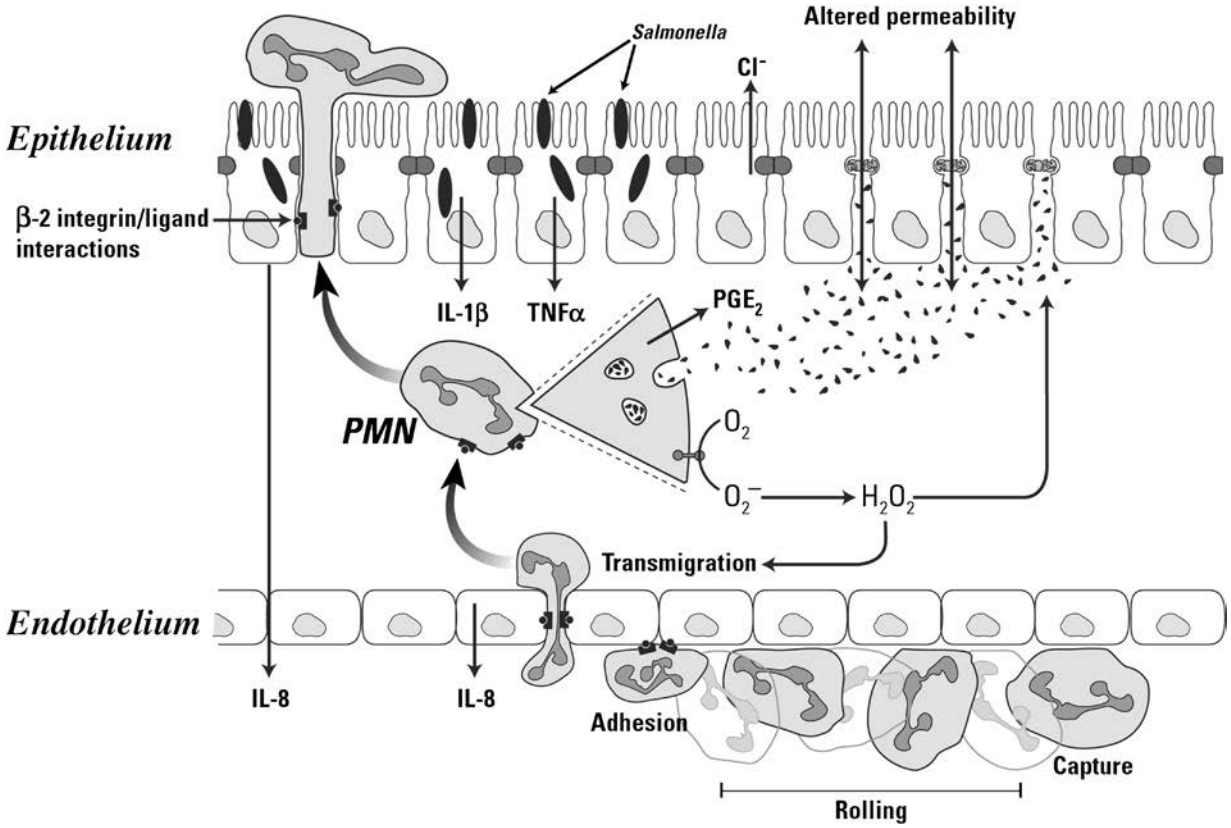


FIG. **12.1** Depiction of neutrophil responses during intestinal inflammation in response to *Salmonella* infection. Salmonellae infect epithelial cells, stimulating the production of chemokines (interleukin-8 [IL-8]), cytokines (IL-1β and tumor necrosis factor-α [TNF-α]), and other proinflammatory mediators. Endothelial cells stimulated by inflammatory mediators produce chemoattractants (such as IL-8) and display adhesion molecules that promote neutrophil emigration. The three steps of neutrophil (polymorphonuclear [PMN]) emigration capture/rolling (mediated by selectins), adhesion (mediated by β_2 integrins), and transendothelial migration (mediated by integrins and platelet/endothelial cellular adhesion molecule) occur on activated endothelium. Chemoattractant molecules such as IL-8 trigger neutrophil emigration. In inflamed tissues cytokines (IL-1β and TNF-α) and a variety of other proinflammatory mediators stimulate the neutrophil oxidase complex to produce reactive oxygen intermediates (ROIs; O_2^- and H_2O_2 and their derivatives). Activated neutrophils degranulate to release proteases and other hydrolases, cationic peptides (defensins), myeloperoxidase, and other products into the tissue. Activated neutrophils synthesize a variety of inflammatory mediators, including prostaglandins (PGE_2) that modulate the inflammatory response. The products of activated neutrophils (ROIs, proteases, and mediators) stimulate epithelial secretion and alter tight junction permeability, promoting diarrhea. Neutrophils eventually migrate across the infected epithelium by a mechanism that involves integrins, disrupting tight junction integrity and increasing permeability to bacterial products, thus exacerbating the inflammatory response.

deal of attention has been paid to the role of neutrophils in the pathophysiology of many inflammatory conditions.[102] Neutrophil depletion is protective in many models of gastrointestinal inflammatory disease. Of interest to clinicians, blockade of neutrophil migration into inflamed tissues prevents many of the pathophysiologic events associated with infectious enteritis, ischemia–reperfusion injury, and other gastrointestinal diseases.[98,103-106]

Neutrophil transendothelial migration is a multistep process that is temporally and spatially regulated and has a degree of cell type specificity (Fig. 12.1). The predominant sites of neutrophil transendothelial migration are in the postcapillary venules and, in some tissues, capillaries. Endothelial cells in these vessels respond to cytokines and other soluble signals by expressing molecules that promote neutrophil adhesion and transmigration, including selectins and counterreceptors for integrins. As neutrophils flow through these vessels, they are first tethered to activated endothelium. Tethering is mediated by selectin molecules expressed on neutrophils (L-selectin) and on activated endothelial cells (P- and E-selectins), which bind to PSGL-1, ESL-1, and other mucin counterreceptors.[107,108] The function of tethering is to increase the exposure of the neutrophil to activating chemokines presented on the surface of the endothelial cells.

Stimulation of neutrophils by IL-8 and other chemokines activate the second step of transendothelial migration. Chemokine binding to their receptors on the neutrophil generates signals that activate the binding of integrin adhesion receptors to their ligands, called *intracellular adhesion molecules* (ICAMs) or *vascular cell adhesion molecules*, expressed on endothelial

cells in inflamed mucosa. Integrin ligation to ICAMs arrests the tethered neutrophils, resulting in firm adhesion to the endothelium. Of the integrins expressed on neutrophils, the β_2-integrins have a particularly important role in transendothelial migration. Calves and people with the disorder leukocyte adhesion deficiency (LAD) illustrate the requirement for β_2-integrin–mediated adhesion in neutrophil function. LAD is a result of an autosomal recessive trait resulting in the lack of the β_2-integrin expression. The neutrophils from affected individuals cannot migrate into most tissues and do not function normally, resulting in poor tissue healing and profound susceptibility to infection, especially at epithelial barriers.[109,110] Other integrins also have a role in transendothelial migration. β_1-Integrins mediate transendothelial migration in some cells and seem to be particularly important for mediating emigration of monocytes into many tissues.[111]

Following this firm adhesion step, neutrophils migrate through the endothelium along a chemotactic gradient of IL-8 and other chemoattractants, such as C5a and leukotriene B_4 (LTB4).[82,97,112] Neutrophils migrate across the endothelial monolayer at intercellular junctions by way of a mechanism involving a series of integrin-ligand interactions mediated by both β_2- and β_1-integrins and other adhesion molecules,[108] which is generally capable of maintaining the integrity of the endothelial barrier.[113] However, a massive flux of neutrophils through the endothelium alters endothelial tight junctions and injures the basement membrane, resulting in increased endothelial permeability to molecules as large as plasma proteins and even endothelial cell detachment from the basement membrane.[96,97] Nonintegrin molecules such as platelet–endothelial cell adhesion molecules (PECAMs) also are involved in transendothelial migration of neutrophils.[108] Homotypic binding of PECAMs on adjacent endothelial cells form part of the intercellular junction. Neutrophils express an integrin of the β_3-family that can bind PECAMs, and through sequential binding of β_3-integrins to PECAMs, the neutrophil can "unzip" the intercellular junction and migrate through, closing it behind itself.

Activation

A key feature of neutrophils and other leukocytes is the requirement for integrin-mediated adhesion to extracellular matrix (ECM) proteins or other cells to achieve an optimal effector phenotype.[114] Critical components of the ECM in inflamed tissues include fibronectin, fibrinogen, and vitronectin, deposited in tissues as a result of plasma leakage and by synthesis of new proteins by stromal cells and resident macrophages in response to inflammatory mediator activation. The changing composition of the matrix proteins deposited in tissues during inflammation serves as a clue as to the nature of the tissue environment for recruited inflammatory cells as they become activated. Individual gene expression studies have demonstrated that adhesion to matrix proteins induces the expression of cytokines and chemokines and their receptors, arachidonic acid–derived lipid mediator synthases, metalloproteinases, growth factors, transcription factors, and other genes that influence the differentiation and activation of inflammatory cells.[115] ROI production, phagocytosis, degranulation, and other effector functions stimulated by inflammatory mediators and bacterial products are optimal only when neutrophils are adherent to the ECM.[114] Adhesion to distinct ECM proteins selectively activates signaling pathways and gene expression of neutrophils, monocytes, and other leukocytes with differing abilities to promote certain functions such that the composition of ECM in many ways controls the development of the ultimate effector phenotype. Thus integrin-mediated adhesion provides a mechanism in which neutrophils and other leukocytes can sense the complex tissue environment and respond appropriately.

Of the activators of neutrophils at sites of inflammation, complement (C3-opsonized particles), cytokines (TNF-α and IL-1β), PAF, immune complexes, and bacterial products are among the most potent stimuli. Other mediators produced during inflammation may modify neutrophil activity, particularly formylated bacterial peptides, chemokines, complement fragments (C5a), LTB_4, and prostaglandins. Activated neutrophils are highly phagocytic; produce large amounts of ROI; degranulate to release myeloperoxidase, cationic antimicrobial peptides (defensins), serine proteases (mainly elastase), and metalloproteinases; and secrete inflammatory mediators (TNF-α, IL-1β, prostaglandins, leukotrienes, and others) (see Fig. 12.1).

Mast Cells

Mast cells strategically reside in mucosal tissues, including the submucosa and lamina propria of the gastrointestinal tract, and constitute a crucial first line of defense at epithelial barriers. However, they are also important effector cells of the pathophysiology of inflammatory gastrointestinal diseases.[116] Experimental depletion of mast cells, genetic deficiency in the development of mast cells, or pharmacologic stabilization of mast cells to prevent degranulation all have a protective effect in a variety of models of gastrointestinal inflammatory disease, including dextran sulfate sodium–induced or trinitrobenzenesulfonic acid–induced colitis,[117,118] ischemia–reperfusion injury,[119,120] and immediate hypersensitivity responses.[121]

Mast cells are activated by a wide variety of microbial products and host-derived mediators.[122] Among the activators of mast cells, the so-called anaphylatoxins (complement fragments C3a, C5a, and C4a) are extremely potent stimuli causing release of mediators of inflammation. In addition, mast cells are the primary effector cells of IgE-mediated anaphylaxis (type I hypersensitivity reactions) by virtue of their high-affinity receptors for IgE. The cross-linking of a receptor-bound IgE on the mast cell surface by antigens (i.e., food antigens) causes rapid degranulation, which results in the explosive release of granule contents.[123] Neural pathways in the intestine also regulate mast cells, which respond to enteric pathogen invasion via neural reflexes that stimulate the release of inflammatory mediators.

Activated mast cells release preformed histamine, 5-hydroxytryptamine (5-HT), proteases, heparin, and cytokines from granules. Activation also stimulates de novo synthesis of a range of inflammatory mediators, including prostaglandins, PAF, and leukotrienes. Transcription of a number of peptide mediators, such as the cytokines TNF-α and IL-1β among many others, also increases stimulation of mast cells. Mast cell products have profound effects on the vasculature, increasing endothelial permeability and causing vasodilation.[124] Moreover, mast cell–derived mediators markedly enhance epithelial secretion by a mechanism that involves the activation of neural pathways and direct stimulation of epithelial cells.[123] In particular, the mast cell granule protease tryptase operating via the protease-activated receptor-2 is a key regulator of gastrointestinal physiologic responses during inflammation, including epithelial secretion and intercellular

junction integrity, motility, and pain responses.[125,126] Mast cell products significantly alter intestinal motility, generally increasing transit and expulsion of intestinal contents. Mast cell–derived leukotrienes and TNF-α also have crucial roles in host defense against bacterial pathogens, acting to recruit and activate neutrophils,[127,128] and are crucial players in the mechanism regulating dendritic cell function and adaptive immune responses.[129]

Mast cells have a role in host defense and inflammatory responses to bacterial pathogens, partly because of the release of proinflammatory mediators during bacterial infection, which is critical for recruiting and activating other innate host defense cells such as neutrophils.[87] Mast cells are also phagocytic, have microbicidal properties, and can act as antigen-presenting cells to the adaptive immune system.[87] The role for mast cells in host protective responses appears to be as a sensor of bacterial invasion. Unlike IgE-mediated responses, bacterial products seem to elicit a highly regulated and selective response from mast cells.

Humoral Mediators of Inflammation

Complement

The complement cascade is a fundamental part of the inflammatory response. Activation of the complement cascade, either by immune complexes (classical pathway) or by bacteria or bacterial products, polysaccharides, viruses, fungi, or host cells (alternative pathway), results in the deposition of complement proteins on the activating surface and the release of soluble proteolytic fragments of several complement components.[130] In particular, activation of either pathway results in the deposition of various fragments of the complement protein C3, which are potent activators of neutrophils and monocytes.[130] Opsonization of particles with C3 fragments constitutes a major mechanism of target recognition and phagocyte activation.[131] During the activation of the complement cascade culminating in deposition of C3, soluble fragments of C3 (C3a), C5 (C5a), and C4 (C4a) are liberated. These fragments, termed *anaphylatoxins,* have potent effects on tissues and cells during inflammation. Perhaps most notably, they are chemotactic for neutrophils (particularly C5a), activate neutrophil and mast cell degranulation, and stimulate reactive oxygen metabolite release from neutrophils.[130] The termination of the complement cascade results in the formation of a membrane attack complex in membranes at the site of complement activation. If this occurs on host cells such as endothelium, the cell may be irreversibly injured. Although the primary source of complement is plasma, epithelial cells of the gastrointestinal tract also produce C3, suggesting that local production and activation of the complement cascade during inflammation occurs in intestinal tissues.

It is clear that if the regulatory mechanisms of the complement cascade fail, then the inflammatory response may be inappropriate and tissue injury can occur. The role of complement in gastrointestinal inflammation has been most extensively studied in models of ischemia–reperfusion injury. Activation of the complement cascades has a major role in altered endothelial and epithelial permeability in these models. Several lines of evidence support the importance of complement in intestinal injury. Mice deficient in C3 or C4 are protected against ischemia–reperfusion injury.[132] Moreover, administration of monoclonal antibodies against C5 reduced local and remote injury and inflammation during intestinal reperfusion injury in a rat model.[133] Administration of a soluble form of complement receptor 1, a regulatory protein that halts the complement cascade by dissociating C3 and C5 on host cell membranes, reduced mucosal permeability, neutrophil influx, and LTB4 production during ischemia–reperfusion injury in rats and mice.[132,134] Although neutrophils and mast cells mediate many of the pathophysiologic effects of the complement cascade, the membrane attack complex may have a primary role in altered vascular permeability during ischemia–reperfusion injury.[135]

Contact System

The contact system of plasma is initiated by four components: Hageman factor (HF), prekallikrein, factor XI, and high-molecular-weight kininogen. HF is a large plasma glycoprotein that binds avidly to negatively charged surfaces.[136] Bacterial cell walls, vascular basement membranes, heparin, glycosaminoglycans, and other negatively charged surfaces in the intestine capture HF and the other three important initiators of the contact system in a large multimolecular complex. Of the surfaces that bind HF, the ECM is an extremely potent activator of the contact system. Once bound, HF is converted to HF-α, which cleaves prekallikrein to kallikrein and factor XI to factor XIa. The ultimate result is further cleavage of HF by kallikrein and triggering of the contact system cascade, activation of intrinsic coagulation by factor XIa, activation of the alternative pathway by HF, and proteolytic cleavage of high-molecular-weight kininogen by kallikrein, releasing biologically active kinins.

The products of the contact system, particularly bradykinin, have several important biologic properties that drive many of the vascular and leukocyte responses during inflammation.[136] Bradykinin induces endothelial cell contracture and intracellular tight junction alterations that increase vascular permeability to fluid and macromolecules. Bradykinin also affects vascular smooth muscle contracture, resulting in either vasoconstriction or vasodilation, depending on the location. Bradykinin also increases intestinal motility, enhances chloride secretion by the intestinal mucosa, and intensifies gastrointestinal pain. In neutrophils kinins stimulate the release of many inflammatory mediators, including cytokines, prostaglandins, leukotrienes, and ROIs.[137] Kallikrein cleaves C5 to release C5a, a potent chemotactic factor for neutrophils, and thus has a role in recruiting and activating inflammatory leukocytes.

The plasma kallikrein–bradykinin system is activated in a variety of acute and chronic inflammatory diseases of the gastrointestinal tract.[138,139] Blockade of the pathophysiologic effects of bradykinin has clinical applications. Oral or intravenous (IV) administration of the bradykinin receptor antagonist icatibant reduces the clinical signs, onset of diarrhea, and many of the histopathologic changes in experimental models of colitis in mice.[140] Inhibition of kallikrein by oral administration of P8720 attenuated the intestinal inflammation, clinical score, and systemic manifestations in a model of chronic granulomatous enterocolitis.[138] Thus the contact system is a potential therapeutic target for inflammatory diseases of the intestine.

TISSUE INJURY DURING INFLAMMATION

Changes in blood flow to the mucosa and other regions of the intestine that reduce perfusion of the tissues can potentiate the initial damage caused by infection or injury. For example,

reperfusion of ischemic tissues is associated with platelet and neutrophil clumping in the small vessels of the mucosa, which can impede blood flow.[141] Platelets are activated and adhere to exposed basement membrane and activated endothelial cells and provide a surface for leukocyte adhesion. The accumulation of platelets and leukocytes can significantly reduce vessel diameter and blood flow while potentiating local coagulation and thrombus formation.

Soluble mediators released by activated leukocytes and endothelial cells also affect blood flow. Histamine and the vasoactive lipids derived from arachidonic acid (leukotrienes, prostaglandins, thromboxane, prostacyclin, and PAF) have a prominent role in regulating local perfusion during inflammation and may have systemic effects on blood flow as well. Procoagulant mediators released by inflammatory cells in response to the inflammatory process (i.e., tissue factor produced by macrophages or endothelial cells), exposed basement membrane proteins, and bacterial components can trigger the contact system and the coagulation and complement cascades, the products of which affect blood flow. Nitric oxide, whether produced by endothelial cells or leukocytes (macrophages), is a potent regulator of blood flow and has a significant role in the control of perfusion during inflammation.[142] Many of the mediators that affect perfusion also affect endothelial permeability, altering osmotic and hydrostatic balance and tissue edema. In extreme cases local and systemic coagulopathies initiated by vascular injury and absorption of microbial products and inflammatory mediators induce a hypercoagulable state, leading to microthrombus formation, which can reduce blood flow or macrothrombus formation, causing tissue infarction.

The cellular mediators of inflammation have the potential to inflict severe injury to intestinal tissues. Neutrophils have an important role in the pathophysiology of many intestinal diseases, including ischemia–reperfusion injury,[98] infectious enterocolitis,[105,143] nonsteroidal antiinflammatory drug–induced mucosal ulceration,[106] and others. Depletion of neutrophils, blockade of their emigration into tissues, or inhibition of neutrophil activation reduces the severity of these and other inflammatory diseases.[144] Many antiinflammatory therapies are emerging that specifically target neutrophil adhesion, migration, and activation.

Migration of neutrophils through endothelium during emigration into inflamed tissues is remarkable in that the permeability of the endothelial monolayer is preserved under most circumstances. However, there is a limit above which neutrophil migration alters the permeability characteristics of the endothelium. The effect is in part physical in that the mere movement of large numbers of neutrophils through the endothelium is sufficient to mechanically disrupt the tight junctions and in part because of toxic products of neutrophils that damage endothelial cells and basement membranes.[141,145] Serine proteases (particularly elastase) and metalloproteinases released by degranulating neutrophils liquefy tissue matrix proteins and cleave cell-surface proteins that make up endothelial intercellular junctions to ease neutrophil migration to the site of infection.[101] These degradative enzymes are particularly damaging to basement membranes and the cellular barriers of the endothelium, thus contributing to vascular permeability (and local tissue edema) and thrombosis. The permeability may be affected to the extent that not only water but also macromolecules (e.g., albumin, matrix proteins, complement) leak into the interstitium. Blockade of neutrophil adhesion to endothelium with anti–β_2-integrin antibodies has a sparing effect on the microvasculature in experimental intestinal ischemia–reperfusion injury, reducing the alterations in vascular permeability and histopathologic evidence of microvascular damage.[141]

Similar to the endothelium of inflamed tissues, massive neutrophil transmigration occurs across the epithelium in response to infection or injury. Neutrophil transepithelial migration increases epithelial permeability by disrupting tight junctions.[145] Like the endothelium, neutrophils disrupt the epithelial barrier mechanically as they migrate through (see Fig. 12.1). Proteases, particularly elastase, degrade basement membrane components and tight junction proteins. Protease activated receptor-2 activated by neutrophil granule serine proteases alter epithelial and endothelial tight junction integrity. Proinflammatory products of activated neutrophils (TNF-α and IFN-γ) increase tight junction permeability by direct effects on enterocytes. Prostaglandins released by activated neutrophils stimulate epithelial secretion, thus contributing to diarrhea. Subepithelial accumulation of neutrophils can lead to deadhesion of the epithelial cells from the basement membrane and mild to severe ulceration. The physiologic result of the effects of neutrophils and their products on the epithelial barrier includes protein-losing enteropathy and absorption of bacterial cell wall constituents, which potentiates the local and systemic inflammatory responses.

Neutrophils in inflamed tissues stimulated by potent host-derived activators (such as IL-1β and TNF-α) and bacterial products (LPS) release copious amounts of ROIs (see Fig. 12.1). Although these oxygen and oxyhalide radicals are important for killing pathogens, they are also potentially toxic to epithelial and endothelial cells and matrix proteins. Reactive nitrogen intermediates, produced primarily by macrophages during inflammation, combine with ROIs to form peroxynitrites, which are particularly toxic.[90] In addition to injury to mucosal tissues, ROIs also have an as yet ill-defined role in recruiting and activating neutrophils, potentiating the inflammatory response.[146] In support of the role of ROIs in inflammatory diseases of the gastrointestinal tract, administration of inhibitors of ROI production or pharmacologic ROI scavengers can be protective in many models of reperfusion injury or enterocolitis. Many therapies are aimed at inhibiting neutrophil activation, and effector functions in tissues have been evaluated for use in intestinal diseases. Phosphodiesterase inhibitors, by causing cyclic adenosine monophosphate (cAMP) accumulation in neutrophils, are antiinflammatory by virtue of their ability to suppress neutrophil activation and ROI production. New phosphodiesterase inhibitors selective for the predominant neutrophil isoform of phosphodiesterase hold promise for use in many inflammatory diseases.

Subepithelial mast cells also have an important role in altering epithelial permeability in inflamed intestine. During the intestinal hypersensitivity response subepithelial mast cell release of mast cell protease tryptase by degranulation increases epithelial permeability via an effect on tight junctions.[147,148] This alteration in tight junction permeability results in enhanced transepithelial flux of macromolecules, including proteins and bacterial products. Cytokines released by mast cells and phagocytes also regulate tight junction permeability. IL-4, a product of mast cells and macrophages, has been demonstrated to increase epithelial permeability.[149] Moreover, TNF-α and IFN-γ, products of many inflammatory cells, synergistically increase tight junction permeability.[150]

PATHOPHYSIOLOGY OF DIARRHEA

Acute equine colitis causes rapid, severe debilitation and, often, death in horses. Diarrhea associated with acute equine colitis occurs sporadically and is characterized by intraluminal sequestration of fluid, moderate to severe colic (abdominal pain), and profuse watery diarrhea with resultant endotoxemia, leukopenia, and hypovolemia.[151,152] Causes of acute colitis and therapeutic options are discussed later in this section.

Although all mechanisms responsible for fluid losses are not known, inflammatory cells likely play an integral role; colitis is characterized by granulocyte infiltration of the large intestinal mucosa.[153-157] Equine cecal and colonic tissues collected during the acute stages of experimentally induced acute equine colitis (Potomac horse fever, lincomycin with and without *Clostridium* spp. inoculation, nonsteroidal antiinflammatory drug administration) reveal the presence of numerous neutrophils and eosinophils in the lamina propria and submucosa.[153,156,158,159] Granulocyte-derived ROIs are crucial to antimicrobial defenses in the gut and stimulate chloride and water secretion by interactions with enterocytes.[160,161] Normal equine intestinal tissue is unique compared with that in most other mammalian species for a preponderance of eosinophils located in the intestinal mucosa and submucosa.[162,163] Production of ROIs by stimulated phagocytic granulocytes following mucosal barrier disruption may be responsible for the massive fluid secretory response that occurs during the early stages of acute equine colitis.

Colitis refers to inflammation and mucosal injury of the colon and cecum (typhlocolitis) that may occur in response to a number of causes.[164] The cause of the colonic injury may be well defined such as in naturally occurring infectious or experimentally induced colitis. However, many cases of human and animal diarrhea have a speculative or unknown diagnosis or no diagnosis. Irrespective of the underlying or initiating cause of colonic injury, the colon apparently has a limited repertoire of responses to damage because most forms of colitis demonstrate similarities in histopathologic appearance and clinical presentation. Various degrees of mucosal erosion and ulceration, submucosal/mucosal edema, goblet cell depletion, and the presence of an inflammatory cellular infiltrate within the mucosa and submucosa are common to many types of human and animal colitis.[163,164] Characteristic clinical manifestations include intraluminal fluid sequestration; abdominal discomfort; hypovolemia; and most often profuse, watery diarrhea.

PATHOPHYSIOLOGY OF COLITIS

Large bowel diarrhea results from abnormal fluid and ion transport by cecal and colonic mucosa. Loss of fluid by the large intestine can result from malabsorptive or hypersecretory processes and is often a combination of the two.[165] Colonic secretory processes are a function of the crypt epithelium, whereas absorptive processes are limited to surface epithelial cells.[166,167] Under normal baseline conditions, an underlying secretion by crypt epithelium is masked by a greater rate of surface epithelial cell absorption. Abnormal forces influencing the rates of secretion and absorption can result in massive, uncontrolled secretion and malabsorption by large intestinal mucosal epithelial cells, leading to rapid dehydration and death.[165-167]

Two intracellular processes control colonic secretion: the cyclic nucleotide (cAMP and cyclic guanosine monophosphate [cGMP]) and the calcium systems.[168,169] Agents may activate adenyl cyclase (vasoactive intestinal peptide, prostaglandin E2 [PGE_2]) or guanylyl cyclase (bacterial enterotoxins) and induce increases in cAMP or cGMP, respectively. This reaction causes phosphorylation of specific protein kinases that induce the actual apical and basolateral membrane transport events. Increases in intracellular free calcium may arise from cyclic nucleotide–dependent release of stored calcium within the cell or from increased calcium entry across the cell membrane.[165-167] Calcium may act through calmodulin, which then can activate membrane-phosphorylating protein kinases.

At least four central systems control intestinal secretion: (1) the hormonal system, (2) the ENS, (3) bacterial enterotoxins, and (4) the immune system.[169,170] Hormonal control of colonic electrolyte transport is exerted primarily through the renin–angiotensin–aldosterone axis.[171] The ENS controls transport through three separate components: (1) extrinsic nerves of the parasympathetic and sympathetic pathways; (2) intrinsic ganglia and nerves, secreting a variety of neurotransmitters including peptides; and (3) neuroendocrine cells (intraepithelial lymphocytes) that reside in the epithelium and release messengers onto the epithelial cells in a paracrine manner.[165,169-171] Many bacterial enterotoxins can induce intestinal secretion by cAMP or cGMP signal transduction.[172] Bacterial enterotoxins can stimulate release of mediators (such as substance P) from primary afferent neurons, which then affect enteric neurons, often propagating neurogenic inflammation.[173]

Preformed inflammatory mediators such as histamine, serotonin, or adenosine and newly synthesized mediators such as prostaglandins, leukotrienes, PAF, various cytokines, the inducible form of nitric oxide, and reactive oxygen metabolites can initiate intestinal secretion by directly stimulating the enterocyte and by acting on enteric nerves indirectly to induce neurotransmitter-mediator intestinal secretion.[170] Prostaglandins of the E and F series can cause an increase in chloride secretion in intact tissue and isolated colonic cells.[174,175] Leukotrienes, PAF, and a number of cytokines have been shown to have no effect on T84 cell secretion but have a significant effect on electrolyte transport in intact tissue, suggesting that intermediate cell types may be involved in these secretory responses.[176-178]

The epithelial cell chloride secretory response occurs via prostaglandin-mediated and adenosine-mediated increases in cellular cAMP, whereas histamine acts by H_1 receptor induction of phosphatidylinositol turnover, production of inositol triphosphate, and mobilization of intracellular calcium stores.[170] Lipoxygenase products (leukotrienes) are capable of activating a colonic secretory response and do not appear to involve the cyclic nucleotides or calcium ions.[176] Phagocyte-derived reactive oxygen mediators (ROMs) can induce colonic electrolyte secretion in vitro, suggesting that oxidants may contribute directly to the diarrhea associated with colitis.[179] Reactive oxygen species initiate the secretory response by increasing cellular cAMP or stimulating mesenchymal release of PGE_2 or prostacyclin, which in turn stimulates the epithelial cell or enteric neuron, respectively.[179-182] Sodium nitroprusside, an exogenous source of nitric oxide, stimulated an increase in chloride secretion in rat colon that was mediated by COX products and enteric neurons.[183] Table 12.1 summarizes inflammatory mediator–induced epithelial cell chloride secretion.

TABLE **12.1 Inflammatory Mediators That Stimulate Epithelial Cell Chloride Secretion**

Mediator	Action
Prostaglandin E_2	Increases Cl secretion
	Decreases neutral NaCl absorption
Vasoactive intestinal peptide	Increases cAMP-mediated NaCl secretion
	Activates cholinergic nerves
Endotoxin	Increases Na absorption
	Increases cell membrane permeability
Serotonin	Increases fluid and electrolyte secretion
Interferon-γ	Decreases tight junctions and causes increase in cell membrane permeability
Interleukin-1 and interleukin-1β	Increase prostaglandins E_2 and $F_1\alpha$ and thromboxane B_2
Histamine (H_1)	Increases Cl secretion via Ca-mediated pathways
Bradykinin	Increases Cl secretion through prostaglandin-mediated pathways
Reactive oxygen mediators	Increase Cl secretion
Thromboxanes	Increase Cl secretion
	Decrease neutral NaCl absorption
Lipoxygenase products	Increase Cl secretion via prostaglandin-mediated pathways
Platelet-activating factor	Increases Isc (Cl secretion)
Adenosine	Increases Cl secretion

cAMP, Cyclic adenosine monophosphate.

ROLE OF INFLAMMATORY CELLS

Acute colitis rarely develops by a simple cause or effect phenomenon but is influenced by many extrinsic and intrinsic host and microorganism factors. Inflammatory mediators released from mast cells and monocytic or granulocytic phagocytes cause intestinal chloride and water secretion and inhibit neutral sodium and chloride absorption.[169,170,184] Inflammatory cells, particularly the phagocytic granulocytes, play an important role in mucosal pathophysiology in cases of colitis.[161,185] Large numbers of these cells are observed on histopathologic examination of tissues from human and animal cases of colitis. Products of cell activation stimulate direct and indirect secretory responses in intestinal cells and tissues.[169,170] Products of phagocyte secretion may amplify the inflammatory signal or have effects on other target cells in intestine such as enterocytes and smooth muscle cells.

ROLE OF PHAGOCYTE-DERIVED REACTIVE OXYGEN METABOLITES

The nicotinamide adenine dinucleotide phosphate (NADPH)-NADPH oxidase system of phagocytes (neutrophils, eosinophils, and monocytes/macrophages) is a potent inducer of superoxide radicals used as a host defense mechanism to kill invading microorganisms.[161] During inappropriate stimulation such as inflammation, trauma, or ischemia followed by reperfusion, increased levels of toxic oxygen species are produced, causing damage to host tissues. Engagement of any of several receptor and nonreceptor types including phagocytosis mediators, chemotactic agents, various cytokines, and microbial products can stimulate phagocytes.[161] Resident phagocytes or those recruited to colonic mucosa early in the disease process are considered to augment mechanisms causing fluid and electrolyte secretory processes, a so-called amplification process.[104,186]

Activation of the respiratory burst results in the production and release of large amounts of superoxide anion (O_2^-) and H_2O_2.[187] In addition to these ROMs, activated phagocytes secrete peroxidase enzyme (myeloperoxidase from neutrophils and eosinophil peroxidase from eosinophils) into the extracellular space. The peroxidases catalyze the oxidation of Cl^- by H_2O_2 to yield HOCl, the active ingredient in household bleach products. The peroxidase–H_2O_2–halide system is the most cytotoxic system of the phagocytes; HOCl is 100 to 1000 times more toxic than O_2^- or H_2O_2. HOCl is a nonspecific oxidizing and chlorinating agent that reacts rapidly with a variety of biologic compounds including DNA, sulfhydryls, nucleotides, amino acids, and other nitrogen-containing compounds. It reacts rapidly with primary amines to produce the cytotoxic *N*-chloramines. The mechanisms by which these substances damage cells and tissue remain speculative, but possibilities include direct sulfhydryl oxidation, hemoprotein inactivation, protein and amino acid degradation, and inactivation of metabolic cofactors of DNA.[188] Luminal perfusion of specific ROMs increased mucosal permeability, and serosal application caused increases in Cl^- secretion in vitro.[189] Tissue myeloperoxidase activity, an index of tissue granulocyte infiltration, is used experimentally to assess intestinal inflammation.[190] Myeloperoxidase activity is elevated in acute flare-ups of human inflammatory bowel disease and various animal models of acute colitis.[190-192] The acute inflammatory response in these conditions is characterized predominantly by neutrophils, although this assay measures total hemoprotein peroxidase, which includes monocyte and eosinophil peroxidase in addition to neutrophils.[193] Moreover, levels of peroxidase activity in equine circulating eosinophils are greater than in circulating neutrophils,[194] and this may apply to resident tissue eosinophils as well.

Arachidonic acid metabolites are thought to play a role in intestinal inflammation in diarrheal disease.[170] Elevated levels of these intermediate metabolites have been demonstrated in natural disease and experimental models of colitis and appear to parallel increases in ROMs in inflamed intestine.[195] The addition of H_2O_2 or HOCl to rat colonic tissue in Ussing chambers induces PGE_2 release and active Cl^- secretion.[182,196] Prostaglandins can stimulate increases in Cl^- secretion in intact intestinal tissue[181,196,197] and in isolated colonic T84 cells.[180,182] Interactions between ROMs and mesenchymal release of PGE_2/PGI_2 may be relevant to the mechanisms producing the diarrheic condition. Fibroblasts cocultured or juxtaposed to colonic T84 cells greatly increased the Cl^- secretory response to H_2O_2 in vitro through the release of PGE_2.[180] In addition, equine colonic mucosa has an increased sensitivity to endogenously released prostaglandin by exhibiting a significant secretory response under in vitro conditions.[198]

ROLE OF OTHER FACTORS

Endotoxin

Endotoxin, the LPS component of the outer cell wall of gram-negative bacteria, is present in large quantities in the large intestine of healthy horses.[194,198] The intact bowel forms an effective barrier to the transport of significant amounts of these highly antigenic toxins, but the diseased gut absorbs these macromolecules in large amounts, causing the subsequent adverse systemic effects that are often life-threatening.[199] A complete review of endotoxemia is presented later in this chapter.

Endotoxins trigger mucosal immune cells and subsequent release of inflammatory mediators in cases of colitis. In vitro studies on the effects of endotoxin on intestinal water and electrolyte transport in adult male rats showed a significant decrease in net colonic sodium absorption and increased colonic permeability.[200] In horses, endotoxin negatively affects gastrointestinal motility, including in the cecum and right ventral colon, and perfusion.[201]

Immunodeficiency

The importance of a normal immune system to the defense of the mucosal surface of the gastrointestinal tract is evident in the immunosuppressed state. Primary immunodeficiencies affecting the gastrointestinal tract are well documented. Common agammaglobulinemia is the most frequently reported gastrointestinal immunodeficiency and causes B-cell deficiency–associated giardiasis in other species.[202] In horses, severe combined immunodeficiency can result in diarrhea secondary to adenovirus, coronavirus, and/or *Cryptosporidium* infection.[203,204] Interestingly, selective immunoglobulin A (IgA) deficiency rarely results in intestinal disease because of a speculated increase in mucosal IgM response. However, combined IgA and IgM deficiencies with a higher incidence of intestinal disease occur. A selective deficiency of secretory IgA has been associated with intestinal candidiasis in other species. Certain mucosal pathogens may enhance their pathogenicity by producing IgA proteases.[202] Acquired immunodeficiency or immunosuppression in adults can result from infectious diseases (particularly viral), nutritional deficiencies, aging phenomena, and drugs (corticosteroids, azathioprine, and cyclophosphamide). Chronic salmonellosis was documented secondary to adult-onset B-cell deficiency in a Quarter Horse.[205]

Nutritional Deficiencies

Nutrition is a critical determinant of immunocompetence and risk of illness in many species.[206] Impaired systemic and mucosal immunity contributes to an increased frequency and severity of intestinal infections observed in patients with undernourishment. Abnormalities occur in cell-mediated immunity, complement system, phagocytic function, mucosal secretory antibody response, and antibody affinity. Morbidity caused by diarrheal disease is increased, particularly among individuals with stunted growth rate because of malnourishment.[207] The interaction between nutritional deficiency and intestinal health in the horse requires further investigation.

Intestinal Microbiota

Recently knowledge regarding the equine intestinal microbiota has expended dramatically. The intestinal microbiome is complex, and additional discussion is provided in Chapter 1 of this text. Briefly, fecal microbiota varies widely between healthy horses and those with colitis,[208] and changes can precede episodes of colic in postpartum mares.[209] The intestinal microbiota is affected by antimicrobial administration,[210] transport, fasting and anesthesia,[211] and dietary starch,[212] among other factors; many of these have been associated with the development of colitis.

Factors Affecting Motility

Disturbances in motility patterns occur during inflammatory diseases of the colon, but the role of motility alterations in the pathogenesis of diarrhea remains unclear. Invasive bacteria cause characteristic motor patterns in the colon consisting of rapid bursts of motor activity that appear to decrease transit time through the large intestine. The result is reduced clearance of bacteria from the large intestine, which may contribute to the virulence of the organism.[213] Absorption of endotoxin and the release of inflammatory mediators such as prostaglandins disrupts the motility patterns of the large intestine, resulting in less coordinated contractions, and may contribute to the alterations in motility seen with invasive bacteria. Although the effect of endotoxin and prostaglandins on transit time is not profound, the disruption of coordinated activity may play a role in causing diarrhea.[214] Thorough mixing and prolonged retention time of ingesta are important not only in microbial digestion of nutrients but also in absorption of microbial byproducts and fluid.[171] The ingesta is viscous and therefore must be mixed to bring luminal ingesta in contact with the mucosa for absorption. In addition, poor mixing increases the thickness of the unstirred layer, decreasing contact of ingesta with the mucosa and decreasing absorption.[171]

Progressive motility must be present, however, if a diarrheal state is to occur.[171] Ileus may be accompanied by increased fluid in the lumen of the large intestine, but without progressive motility the fluid is not passed. Acute colitis is often associated with a period of ileus characterized by scant stool, which may be the reason for signs of colic in the early stages of colitis. Diarrhea is apparent only when motility returns. Increased progressive motility has been suggested to contribute to diarrhea by decreasing transit time; this is thought to play a role in irritant catharsis and in the mechanism of action of some laxatives.[3]

Endotoxemia

Endotoxemia is literally defined as the presence of endotoxin in the bloodstream. Most often, however, the term is used to refer to the associated clinical manifestations caused by an excessive and unbalanced inflammatory reaction. Endotoxemia is not a disease in its own right; rather, it is a complication of many septic and nonseptic disease processes affecting horses and other animals. Its diagnosis and management must therefore be discussed in the context of underlying primary pathologic conditions.

In its pathophysiologic consequences the innate immune response to endotoxin (LPS) is similar to the response to other stimuli (e.g., bacterial infection, viral infection, severe trauma). Moreover, the clinical presentation and clinical pathologic abnormalities of patients suffering from severe systemic inflammation are consistent regardless of the etiologic cause, and outcome may be predicted more accurately by the severity of the inflammatory response than the nature of the

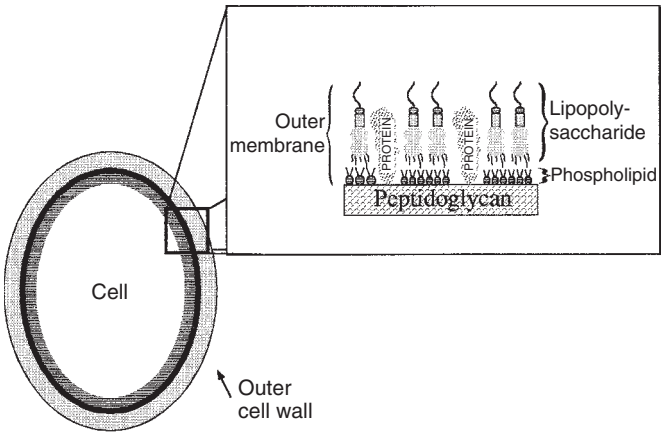


FIG. **12.2** Lipopolysaccharide.

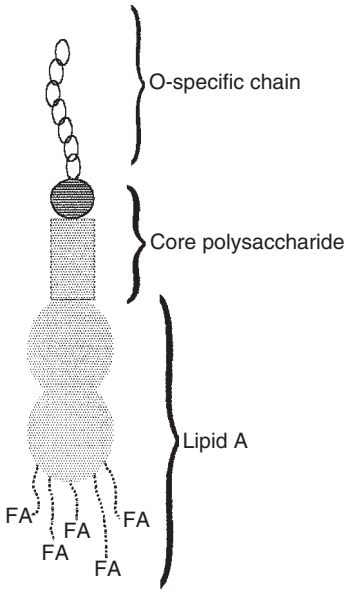


FIG. **12.3** Three domains of the lipopolysaccharide molecule. *FA*, fatty acid.

inciting insult.[215] In 1992, therefore, the term *systemic inflammatory response syndrome* (SIRS) was introduced in a consensus statement of chest physicians and critical care physicians to account for these similarities and provide a case definition for clinical and research purposes. Fairly soon thereafter, however, the 1992 SIRS definition was criticized as being too sensitive while providing little specificity,[216] and its usefulness for clinical practice was challenged because it failed to predict outcome and to discriminate patients at a high risk of morbidity and mortality.[217] The definition of *SIRS* may therefore be valuable from a conceptual standpoint, but it has little value in daily clinical practice.

Sepsis was defined as the systemic inflammatory response to infection, and *septic shock* as "sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction."[215] According to these definitions the diagnosis of sepsis requires documentation of infection by culture in addition to two or more of the following findings: hypothermia or hyperthermia; tachycardia; tachypnea or hypocapnia; and leukocytosis, leukopenia, or an increased proportion of immature leukocyte forms.

Organ failure is a common sequela of endotoxic or septic shock, and the term *multiple organ dysfunction syndrome* describes insufficiency of two or more organ systems, as evident by clinical or clinicopathologic changes. In horses the laminae of the feet should be included in the list of organs susceptible to failure.

German scientist Richard Pfeiffer (1858–1945), in working with *Vibrio cholerae,* first described endotoxin as a toxin "closely attached to, and probably integral of, the bacterial body."[218] He observed this toxin to be distinct from the actively secreted, heat-labile, and proteinaceous bacterial exotoxins. Endotoxin later was found to be a heat-stable LPS structure, and the terms *endotoxin* and *LPS* now are often used interchangeably.

LPS is a major structural cell wall component of all gram-negative bacteria, including noninfectious species (Fig. 12.2). With 3 to 4 × 10^6 molecules per cell, LPS makes up about 75% of the outer layer of the outer cell membrane and is a key functional molecule for the bacterial outer membrane, serving as a permeability barrier against external noxious agents. The LPS molecule consists of four domains that are essential for the virulence of gram-negative bacteria.[219] Three of the domains (inner core, outer core, and O-specific chain) represent the hydrophilic polysaccharide portion of the molecule, whereas the lipid A portion represents the hydrophobic lipid portion (Fig. 12.3). Combined, these domains confer the overall amphiphilic properties of the molecule that lead to the formation of micellar aggregates in aqueous solutions.

O-specific chains (also called O-antigen polysaccharides or O-chains) are characteristic of any given type of LPS and show enormous structural variability among bacterial serotypes.[220] O-chains are synthesized by the addition of preformed oligosaccharide blocks to a growing polymer chain and therefore have a repetitive structure. O-specific chains determine part of the immunospecificity of bacterial cells[221] and, on interaction with the host immune system, serve as antigens for the production of species-specific antibodies.[222] O-specific chains are further responsible for the smooth appearance of gram-negative bacterial colonies on culture plates,[219] and LPS molecules containing an O-chain are termed *smooth lipopolysaccharide.*

The inner (lipid A-proximal) and outer (O-chain-proximal) core oligosaccharide portion is more conserved among different strains of gram-negative bacteria than the O-specific chain.[220] The core of all LPS molecules contains the unusual sugar 3-deoxy-D-manno-oct-2-ulopyranosonic acid (KDO), which links the core region to the lipid A molecule. Synthesis of a minimal core is essential for the survival of bacteria,[223] and the smallest naturally occurring LPS structure consists of lipid A and KDO.[224] In contrast to the S-form colonies, colonies of gram-negative bacteria with LPS molecules that lack the O-specific chain but contain a core region show a rough appearance on culture plates. Rough LPS molecules are denoted further as Ra, Rb, and so on to indicate the length of the core region. In Re-LPS (also called *deep rough LPS*), the core region is reduced to a KDO residue. Re-mutants often are used to raise antibodies against the core region in an attempt to provide cross-protection against a variety of bacterial species. The lipid A portion, which serves to anchor the LPS molecule in the bacterial outer membrane, has been identified as the toxic principle of LPS,[225] and its structure is highly conserved among gram-negative bacteria. The common structure

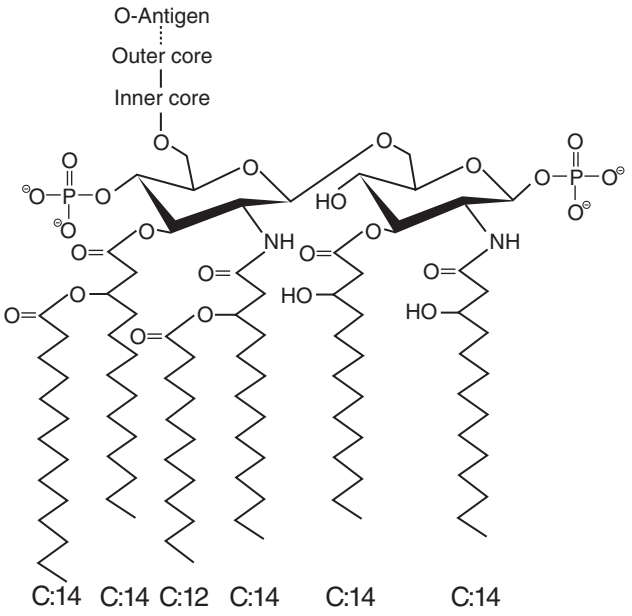


FIG. **12.4** Acylation pattern for *Escherichia coli* lipopolysaccharide.

shared by lipid A molecules is a 1,4′-bisphosphorylated β1,6-linked D-glucosamine disaccharide backbone (lipid A backbone), which is acylated by up to six fatty acids.[220] Fig. 12.4 shows the acylation pattern for *Escherichia coli* LPS. Variation in the lipid A structure between gram-negative bacteria affects the number, length, and position of fatty acids and the backbone structure and the substitution of phosphate by other polar groups.[222]

CAUSES OF ENDOTOXEMIA IN HORSES

According to its nature as a structural cell wall component, the presence of endotoxin implies the presence of gram-negative bacteria as a source. Depending on the nature of the underlying disease, these bacteria may circulate in the bloodstream in their intact form (i.e., bacteremia), may be confined to a localized infectious process, or may be part of the endogenous bacterial florae colonizing the gastrointestinal tract. In any of these scenarios endotoxin molecules are released as a byproduct of bacterial growth and in large numbers on bacterial cell death.[226] Common infectious conditions associated with endotoxemia in horses include neonatal gram-negative sepsis, bacterial pneumonia and pleuropneumonia, endometritis, peritonitis, and infectious colitis with bacteria such as *Salmonella* spp. that are not part of the normal intestinal florae. In one study, for example, endotoxin was detectable in plasma of 50% of foals evaluated for presumed sepsis.[227]

The term *translocation* describes entry of endogenous bacteria and bacterial products from the gastrointestinal tract into tissues and the systemic circulation.[228] The natural intestinal flora of horses consists mainly of gram-negative, anaerobic bacteria; thus, large amounts of endotoxin normally exist in the lumen of the equine intestinal tract.[229] Even in healthy horse small amounts of endotoxin probably cross the intact mucosal barrier and reach the portal circulation and the liver.[230] These molecules are cleared, however, by the mononuclear phagocytic system in the liver and lead only to a localized and restricted activation of the host immune system. For endotoxin translocation to become detrimental, excessive amounts have to cross the intestinal barrier and overwhelm the mononuclear phagocytic system, or the capacity of the liver to detoxify LPS must be compromised. The latter may be a concern in conditions such as hepatitis, cholangiohepatitis, or portosystemic shunting of blood.

Permeability of the intestinal mucosal barrier frequently increases in cases of acute gastrointestinal disease. Colic patients are prime candidates for developing endotoxemia, and plasma endotoxin was detectable in 10% to 40% of colic patients on admission.[231,232] The studies differed in their inclusion criteria, and the higher percentage of horses testing positive for plasma endotoxin was observed when only patients presenting for surgical intervention were evaluated.[232] Aside from gastrointestinal rupture, increased permeability to intact bacteria or free endotoxin molecules is thought to be associated most commonly with ischemic insults such as strangulating obstruction and bowel infarction; severe inflammation, as in proximal enteritis and colitis; bacterial overgrowth; and intraluminal acidosis, which occurs with grain overload.[233,234] One study, however, found no difference in plasma endotoxin detection among disease groups, emphasizing the fact that any disease of the abdominal cavity can induce endotoxemia in horses. In the same study endotoxin was approximately three times more likely to be detected in PF as opposed to plasma samples. Similarly, higher cytokine concentrations have been measured in PF than in plasma. The likely explanation for these findings is a local inflammatory response in the peritoneal cavity elicited by translocated bacteria or LPS molecules before their absorption into the systemic circulation.[231]

Although gastrointestinal disease is likely the most important cause of endotoxin translocation in horses, other conditions may also result in translocation of endotoxin and bacteria. In experimental studies using laboratory animals, entry of gut-associated bacteria into the lymphatic system was demonstrated after hypovolemic shock, burn injuries, trauma, malnutrition, and starvation.[235-237] Furthermore, endotoxin itself caused bacterial translocation into mesenteric lymph nodes after intraperitoneal administration to mice.[238] These findings have received much attention in the literature concerning human patients because they serve to explain cases of endotoxic shock in the absence of demonstrable bacterial infection. The veterinarian should keep in mind the possibility of translocation when evaluating cases of presumed SIRS in horses, in which bacterial infection or gastrointestinal disease cannot be demonstrated. Endotoxin translocation also may be associated with strenuous exercise, which results in reduced splanchnic blood flow, hypoxemia, and a higher body temperature. In fit racehorses a significantly increased mean plasma LPS concentration was found after racing, whereas anti-LPS IgG levels were decreased. Fit horses showed significantly higher anti-LPS IgG concentrations at rest than sedentary controls, suggesting leakage of small amounts of endotoxin from the intestinal lumen during training and racing.[239] A study in endurance horses demonstrated detectable LPS concentration in approximately 50% of horses, and correlation between plasma endotoxin and lactate concentration suggested that plasma endotoxin concentration may be reflective of the vigor of exercise.[240] Surprisingly, the latter study failed to show a correlation between plasma endotoxin concentration and levels of inflammatory mediators such as TNF-α and IL-6,[240] such that the clinical significance of endotoxin translocation during exercise requires further investigation. At least

experimentally, however, alterations in innate immune function during and after strenuous exercise in horses have been demonstrated.[241]

Mechanisms of Cellular Activation by Lipopolysaccharide

The initiating event in the pathophysiology of endotoxemia is the activation of LPS-responsive cells by endotoxin, resulting in altered cellular functions and increased expression of inflammatory mediators. Immune cells such as macrophages respond to minute amounts of LPS, which usually allows them to eliminate gram-negative bacteria and free LPS molecules efficiently. An important factor in the exquisite sensitivity to LPS is the presence of LPS-binding protein (LBP).[220] LBP is an approximately 60-kDa plasma glycoprotein[242] synthesized primarily by hepatocytes[243] and belongs to the family of lipid transfer/LBPs. LBP is an acute phase protein, and inflammatory agents and cytokines such as IL-1 increase LBP plasma concentration 10- to 100-fold within 24 to 48 hours of an inflammatory stimulus.[244,245] The main function of LBP is to transfer LPS to endotoxin-responsive cells, which include mononuclear phagocytes, neutrophils, lymphocytes, and endothelial cells. The importance of a highly sensitive response to LPS for protection against gram-negative bacterial infection is demonstrated in experiments using LBP knockout mice—that is, mice that lack the LBP gene and are therefore unable to synthesize LBP. Although these animals are resistant to the effects of purified LPS, they are unable to control infection with viable bacteria and rapidly succumb.[246] Despite its crucial importance for an effective host defense, LBP is not essential for LPS-receptor interaction per se, because high concentrations of LPS can activate cells in the absence of LBP.[247]

Aside from its role as a catalyst of cellular activation by LPS, LBP has opsonizing activity[248] and participates in the phagocytosis of LPS by macrophages and neutrophils.[249,250] Although phagocytosis of LPS is receptor dependent, it appears to be uncoupled from intracellular signaling events and occurs in the absence of cell activation.[251] LBP further catalyzes transfer of LPS to lipoproteins, such as high-density lipoprotein, which neutralizes LPS activity.[252] This detoxifying effect may become important when large amounts of LPS are present. A protective effect of LBP against LPS challenge and infection has been demonstrated in a murine model of septic shock,[253] and further studies investigating potential therapeutic uses of LBP are under way. More recently, LBP has also been investigated as a diagnostic and prognostic indicator in human patients, in which it may differentiate between SIRS and sepsis and predict outcome of septic patients.[245] One study investigating serum amyloid A (SAA) and LBP in horses with colic[254] found no correlation between serum concentration of LBP with outcome, type of disease process, or affected portion of the gastrointestinal tract.

The most important LPS receptors are cluster differentiation antigen 14 (CD14)[247] and TLR-4.[255] Both are classified as pattern-recognition receptors,[256] which means that they recognize LPS as a structural motif common to all gram-negative bacteria. CD14 is a 53-kDa protein that in its membrane-bound form (mCD14) is inserted into the cell membrane via a glycosyl–phosphatidyl–inositol anchor.[257] CD14 is expressed primarily on monocytes and tissue macrophages and to a lesser extent on neutrophils.[258] It also is found in a soluble form (sCD14)[259] that can bind to cell types lacking CD14, such as endothelial cells, and make them LPS responsive. In addition to this proinflammatory effect, high concentrations of sCD14 can sequester and neutralize LPS.[260] The amount of circulating sCD14 greatly increases during inflammation, which makes it a useful marker of acute and chronic inflammation.[258]

Although CD14 is known to be crucial for cellular activation, it cannot transmit signals to the inside of the cell because it lacks a transmembrane domain. The missing link between CD14 and the cytosolic environment is a Toll-like receptor in association with the molecule MD-2.[261] The name *Toll-like receptor* stems from the homology of the mammalian receptor with a receptor type in *Drosophila* (Toll) that is important for dorsoventral orientation and immune responses in the fly. A number of Toll-like receptors have been identified in mammalian species thus far, but TLR4 appears to be the receptor subtype most important for LPS signaling.[255] The importance of CD14 and TLR4 in the cellular response to LPS has been demonstrated in a number of experiments. Mice deficient in CD14 are incapable of mounting a normal inflammatory response to LPS,[260] whereas mutation or deletion of the gene encoding for TLR4 causes LPS hyporesponsiveness.[262-264]

After binding of LPS to cellular receptors, TLR4 undergoes oligomerization and recruits downstream adaptor proteins to activate intracellular signaling pathways.[265] Signaling pathways are characterized by sequential phosphorylation and activation of enzymatic activities and ultimately result in the alterations of cellular metabolism known as *cell activation*. A typical result of intracellular signaling is the activation of transcription factors—that is, proteins that bind to DNA and promote gene transcription. Translational mechanisms are activated in a similar manner.

TLR4-dependent cell signaling pathways can be differentiated according to the use of adaptor proteins that bind to the intracellular domain of TLR4.[265] The myeloid differentiation primary response gene 88 (MyD88)-dependent pathway results in activation of IKK (Iκβ kinase) and mitogen-activated protein kinase (MAPK) pathways and ultimately in the expression of proinflammatory cytokine genes. IKK activates the well-described transcription factor NF-κβ by phosphorylating an inhibitor protein complex (IκB) that sequesters and inactivates NF-κβ in the cytoplasm. On phosphorylation IκB is ubiquitinated and degraded, and NF-κβ is translocated to the nucleus, in which it unfolds its activity.[266] The MyD88-independent pathway, on the other hand, is activated by interaction of TLR4 with the adaptor protein Toll-IL-1 receptor domain-containing adaptor inducing interferon-β (TRIF). Although the MyD88-independent pathway also activates MAPK and NF-κβ (in addition to a transcription factor called *IRF3*), this pathway primarily results in activation of type I interferons, which are important for antiviral and antibacterial responses.[265] Despite the characterization of seemingly separate and "ordered" pathways, one should recognize that interaction and synergy between pathways are likely to occur. Similarly, inhibitory pathways are required for regulation of the cell response and can target multiple levels of TLR4 signaling.[265] The potential manipulation of signaling pathways for therapeutic use in septic patients is under investigation.

Inflammatory Mediators

Although endotoxin can exert some direct effects, cytokines are a primary mediator of LPS effects. Cytokines are glycoprotein molecules that regulate inflammatory and immune responses by acting as a signal between cells.[267] Cytokines of major interest

in the pathogenesis of endotoxemia include TNF-α, the interleukins, chemokines, and growth factors such as granulocyte-monocyte colony-stimulating factor. TNF-α is thought of as the most "proximal" cytokine released in response to LPS. Studies corroborate this by showing that administration of recombinant TNF-α mimics the effects of LPS[268] and that antibodies directed against TNF-α protect against the lethal effects of endotoxin.[269] Increased plasma activity of TNF is associated with increased mortality in equine patients with acute gastrointestinal disease and in septic neonates.[231] Despite being a structurally diverse group of proteins, cytokines share several characteristics that allow them to execute their complex functions in the inflammatory response.[267] Any individual cytokine generally is produced by several different cell types, can act on different cell types, and has multiple effects on any given cell. Furthermore, cytokine effects are redundant, meaning that different cytokines can share the same effect. In endotoxemia this is particularly true for the effects of IL-1 and TNF-α.[270] Many of the biologic activities of cytokines in vivo result from synergistic or antagonistic actions involving two or more cytokines.[271] Within itself the cytokine response is highly regulated: cytokines induce or suppress synthesis of other cytokines, including their own (feedback regulation); regulate expression of cytokine receptors; and regulate cytokine activities. Additional regulatory mechanisms include the release of specific cytokine inhibitors such as soluble IL-1 and TNF-α receptors; cytokine receptor antagonists such as the IL-1 receptor antagonist; and antiinflammatory cytokines, including IL-10, IL-4, IL-13, and transforming growth factor-β (TGF-β). Glucocorticoids, which are produced increasingly in response to endotoxin, also inhibit the production of cytokines.[272] During a "controlled" inflammatory response, therefore, cytokine secretion is a self-limited event, whereas excessive stimulation of cytokine production can lead to the perpetuation of the inflammatory response even after the initial stimulus has been removed. Aberrations from the controlled, self-regulated inflammatory response have been described as predominantly proinflammatory (SIRS), antiinflammatory or hypoinflammatory (compensatory antiinflammatory response syndrome [CARS]), or combined (mixed antagonist response syndrome) responses.[273] That these responses may not represent a continuum in response to infection was demonstrated in a recent study investigating cytokine profiles in an experimental model of sepsis.[274] Here, increased plasma concentration of antiinflammatory mediators such as IL-1 receptor antagonist and IL-10 in the early phase of sepsis predicted early mortality almost as accurately as the typical proinflammatory cytokines such as IL-6. Cytokine profiles failed to predict mortality in the later stages of sepsis, thereby suggesting that late outcome is not "preprogrammed" early on in the disease. A progression from proinflammatory (SIRS) to antiinflammatory (CARS), as previously proposed, could not be demonstrated.[274] The authors concluded that use of biomarkers may be helpful in determining the inflammatory status of clinical patients suffering from sepsis and that the success of treatments aimed at suppressing or stimulating immune responses may depend on the individual patient's inflammatory profile.

Interestingly, tolerance to endotoxin develops after repeated exposure to LPS.[275] Tolerance can be demonstrated in vitro and in vivo and encompasses decreased production of cytokines and a diminished clinical response.[275,276] Tolerance may be a protective mechanism and was shown to reduce mortality in some experimental models; however, impaired resistance to infectious processes was demonstrated by other investigators.[277] Mechanisms that likely are responsible for the development of endotoxin tolerance include receptor downregulation,[278] downregulation of intracellular signaling pathways,[277,279] and mediators such as glucocorticoids and IL-10.[277] The development of endotoxin tolerance in horses has been reported.[280,281] More recently, the term *cellular reprogramming* has been introduced to describe altered inflammatory cell functions in septic and SIRS patients. This term better accounts for the observation that cellular LPS-sensing ability appears to be maintained, whereas intracellular signaling and cytokine production are modified toward an antiinflammatory rather than a proinflammatory response.[277]

Aside from cytokines, a number of other molecules function as inflammatory mediators in the pathogenesis of endotoxemia, the synthesis and release of which are stimulated by endotoxin and by cytokines. These mediators include the arachidonic acid metabolites or prostanoids, PAF, oxygen-derived free radicals, nitric oxide, histamine, kinins, and complement components. Table 12.2 summarizes the origins, targets, and effects of the most important inflammatory mediators involved in the pathogenesis of endotoxemia. Fig. 12.5 shows the pathways of arachidonic acid metabolism by COX and lipoxygenase. COX products are the prostaglandins, prostacyclin (PGI_2) and thromboxanes, and the lipoxygenase produces the leukotrienes.

TABLE **12.2** **Important Mediators of the Systemic Inflammatory Response to Endotoxin**

Mediator	Origin	Effects
TNF	Macrophages	Induces synthesis of TNF, IL-1, IL-6, and GM-CSF
	Monocytes	Activates neutrophils
	Neutrophils	Activates fibrinolysis and coagulation
	CD4+ T cells	Activates contact and complement system
	Natural killer cells	Induces a catabolic state
		Induces insulin resistance
		Is a pyrogen (direct action and via IL-1 induction)
		Induces synthesis of TNF, IL-1, IL-6, PGI_2, PAF, and GM-CSF
IL-1	Activated macrophages	Activates pyrogen
	Endothelial cells	Induces malaise
	Fibroblasts	Activates neutrophils and chemotaxis

Continued

TABLE **12.2 Important Mediators of the Systemic Inflammatory Response to Endotoxin—cont'd**

Mediator	Origin	Effects
	Dendritic cells	Activates fibrinolysis and coagulation
	Lymphocytes	Activates contact and complement system
	Keratinocytes	Induces acute phase response
		Increases activity of lipoprotein lipase
		Mobilizes amino acids
		Induces muscle proteolysis
IL-6	Activated macrophages	Induces acute phase response
	Fibroblasts	Induces stress response
	Keratinocytes	Is a weak pyrogen
	T lymphocytes	
IL-8	Macrophages	Activates neutrophils and chemotaxis
	Endothelial cells	
Thromboxane A_2	Platelets	Induces vasoconstriction
		Activates platelet aggregation
PGE_2	Most nucleated cells	Induces vasodilation
		Activates platelet aggregation
		Induces fever
PGI_2	Vascular endothelial cells	Induces vasodilation
		Inhibits platelet aggregation
PAF	Macrophages	Activates platelet aggregation
	Platelets	Activates macrophages and neutrophils
	Neutrophils	Induces hypotension
	Mast cells	Increases vascular permeability
	Eosinophils	Aids recruitment of leukocytes
		Induces visceral smooth muscle contraction
		Is a negative inotrope and arrhythmogenic
		Induces ileus
$PGF_2\alpha$	Most nucleated cells	Induces vasoconstriction
		Activates luteolysis
Leukotriene B_4	Most nucleated cells	Is a chemoattractant
		Promotes neutrophil interaction with endothelial cells
Leukotrienes C_4, D_4, E_4	Most nucleated cells	Increase vascular permeability
		Induce bronchoconstriction
		Induce vasoconstriction
Kinins	Produced from serum precursors	Increase vascular permeability
		Induce smooth muscle contraction cause pain
Complement components (C3a, C5a)		Activate neutrophils and chemotaxis
		Induce smooth muscle constriction
		Induce mast cell degranulation
		Induce release of histamine and serotonin
		Increase vascular permeability
Oxygen-derived free radicals	Macrophages	Damage cell membranes
	Neutrophils	Inactivate enzymes
		Damage tissues
GM-CSF		Induces rebound neutrophilia

GM-CSF, Granulocyte-monocyte colony-stimulating factor; *IL,* interleukin; *PAF,* platelet-activating factor; *PG,* prostaglandin; *TNF,* tumor necrosis factor.

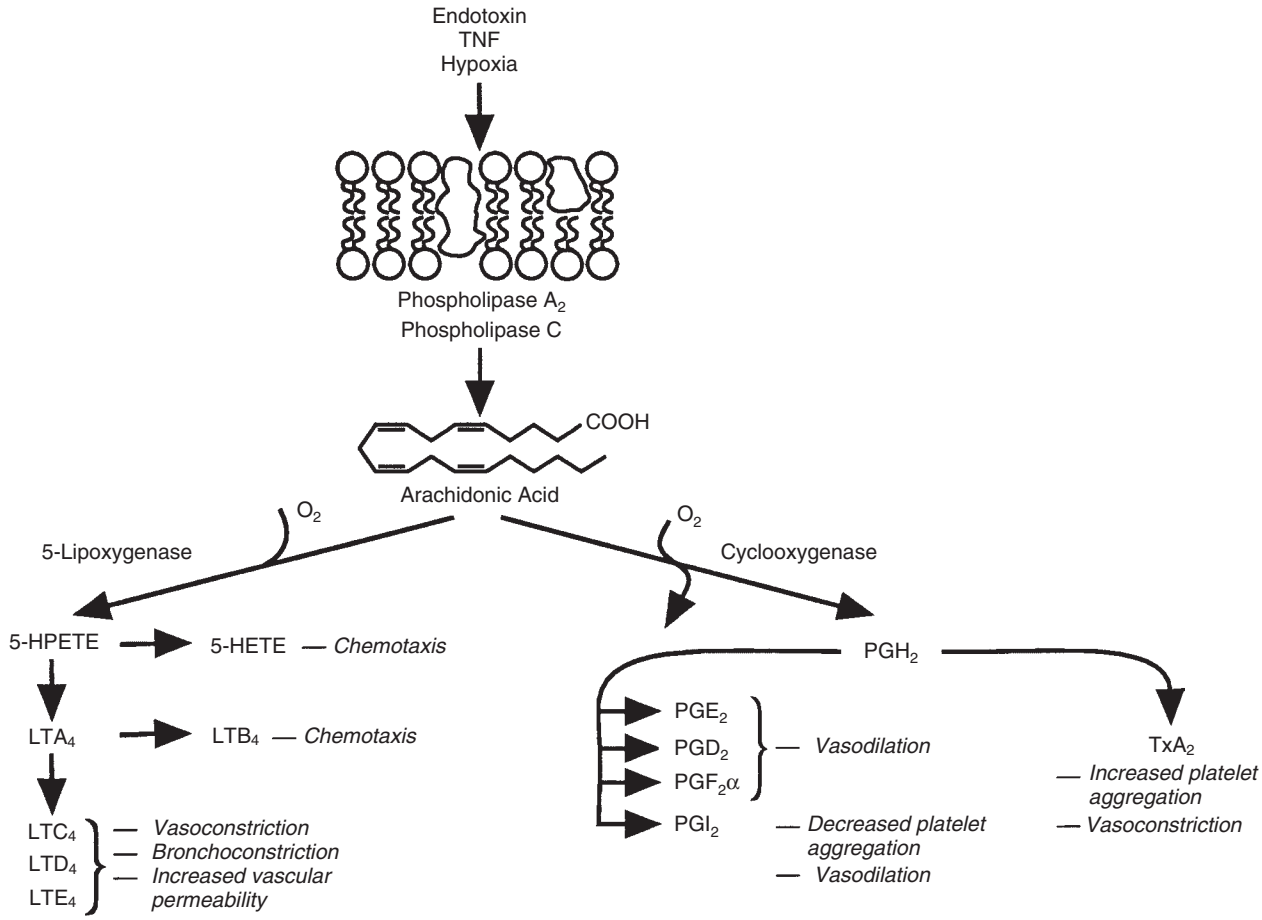


FIG. **12.5** Pathways of arachidonic acid metabolism by cyclooxygenase and lipoxygenase.

Pathogenesis

The innate immune response to LPS is an efficient defense mechanism that provides maintenance of homeostasis and therefore health in the face of an almost continuous exposure to microorganisms and their products.[282] Detrimental consequences of this immune response occur only if excessive and uncontrolled mediator output results in endothelial damage, neutrophil-mediated tissue damage, and uncontrolled activation of the coagulation and fibrinolytic cascades and the complement system. Ultimately, the combination of these events culminates in cardiovascular instability, impaired hemostasis, organ failure, shock, and death. The following discussion addresses the various pathophysiologic events in the development of endotoxemia and shock and the role of inflammatory mediators.

Endothelial Dysfunction and Damage

Normal endothelium plays an important role in regulating blood pressure and regional tissue perfusion and provides an anticoagulant surface. Endothelial dysfunction and damage result in a decreased responsiveness to vasoactive agents (vasoplegia), increased vascular permeability, and a tendency for clot formation in the microvasculature. If the basement membrane and underlying matrix are compromised, microvascular hemorrhage can occur. Endothelial cell damage is primarily neutrophil mediated. More specifically, damage is caused by oxygen-derived free radicals, which are produced within endothelial cells through reactions involving neutrophil-derived elastase and hydrogen peroxide molecules, endothelial cell enzymes such as xanthine oxidase, and endothelial cytosolic iron. The hypochloric anion radical ($HO^{\cdot}$) is thought to be responsible most directly for endothelial cell cytotoxicity. Nitric oxide, which is produced by constitutively expressed nitric oxide synthase in endothelial cells and scavenges superoxide radicals in a reaction to form peroxynitrite, may afford protection from oxygen radical–induced endothelial cell damage. Variations in the ability to produce nitric oxide may explain why vascular beds in different organs vary in their susceptibility to neutrophil-mediated damage.[283] Excessive production of nitric oxide by an inducible form of nitric oxide synthase (iNOS), however, contributes to tissue damage, and increased peroxynitrite concentrations may be responsible in part for PAF-induced increases in vascular permeability.[284] In addition to oxygen-derived free radicals, activated neutrophils release matrix metalloproteinases, which contribute to tissue damage.[272] Vascular endothelial cells are further susceptible to direct effects of various cytokines, most prominently TNF-α and IL-1. These cytokines are thought to cause damage via the induction of COX activity and production of prostanoids and through generation of free radicals. Endothelial cell damage in response to endotoxin infusion and the association with leukocyte attachment have been demonstrated in horses.[285]

Neutrophil Activation, Margination, and Transmigration

Neutrophil activation by LPS and cytokines results in stimulation of phagocytosis and the respiratory burst, release of lysosomal enzymes and inflammatory mediators, and expression of adhesion molecules. Perhaps the single most specific clinicopathologic indicator of endotoxemia is pronounced neutropenia,[286] which temporally correlates with peak plasma concentrations of TNF.[287] Neutropenia is caused primarily by margination of neutrophils in the vasculature, especially of the lungs,[288] whereas significant loss through active migration into peripheral tissues likely is limited to the presence of a localized source of infection. In fact, neutrophils exposed to endotoxin or inflammatory mediators exhibit reduced capacity to respond to chemotactic stimuli and extravasate.[288] Margination is made possible by adhesion molecules on endothelial cells and leukocytes that interact and allow sticking of leukocytes to the endothelial lining of blood vessels. The details of neutrophil margination and transmigration are reviewed in several excellent texts.[289,290] Recent evidence demonstrates a role for TLR-4 in neutrophil margination in the lung microvasculature during endotoxemia, and suggests that activation and TLR-4 expression of endothelial cells may be more important than that of circulating neutrophils.[288] Although neutrophil activation during infections provides the body with an effective defense system against microbial invaders, margination and reduced migratory ability of neutrophils during endotoxemia results in the accumulation of activated cells at the endothelial surface. These cells are then positioned to effect endothelial injury, increase vascular permeability, and in some cases cause parenchymal cell death and organ dysfunction.[291] In addition, inhibition of transmigration deprives the body of phagocytic cells at sites of infection, resulting in reduced ability to fight bacterial infection. This latter complication of endotoxemia may have significant clinical impact, because defective neutrophil recruitment has been demonstrated at low endotoxin doses that do not yet result in tissue damage.[291,292] In human patients endotoxemia and impaired neutrophil migration have been associated with infectious complications of surgical procedures.[291] Decreased phagocytic function and oxidative burst activity have also been suggested in sick and septic hospitalized foals,[293,294] in which a beneficial effect of plasma transfusion on neutrophil function could be demonstrated. The mechanisms of migration inhibition during endotoxemia are incompletely understood but may include occupation of neutrophil chemotactic receptors by cytokines and complement components, resulting in an inability of activated cells to respond to a chemotactic gradient.[291] Rebound neutrophilia, which is observed frequently following episodes of endotoxemia, is caused by neutrophil release from the bone marrow reserve pool and by stimulation of myeloid cell proliferation via granulocyte-macrophage colony-stimulating factor and is mediated primarily by TNF and IL-1.[270]

Coagulopathy and Disseminated Intravascular Coagulation

In health coagulation and fibrinolysis underlie stringent control mechanisms that allow appropriate clot formation and their resolution. Coagulopathies frequently are observed in horses with colic[233,295,296] and foals with sepsis[227] and are likely attributable to endotoxemia. In human medicine virtually all septic patients are considered to have some degree of coagulopathy, which may range from subclinical abnormalities of the clotting profile to fulminant disseminated intravascular coagulation (DIC).[297,298] DIC results from a widespread activation of the coagulation and fibrinolytic systems and failure of their control mechanisms. Ultimately, this leads to disseminated fibrin deposition in the microvasculature, consumption of platelets and clotting factors, and accumulation of fibrin degradation products (FDPs). Depending on the underlying disease process and the relative impairment of the coagulation and fibrinolytic systems, DIC can manifest as a diffuse thrombotic syndrome leading to ischemic organ failure, a fibrinolytic syndrome with uncontrolled hemorrhage, or a combination of both.[299] A procoagulant state characterized by clinicopathologic abnormalities of the clotting profile precedes DIC.

The intrinsic and extrinsic arms of the coagulation cascade are activated in endotoxemia. The intrinsic pathway is initiated by activation of coagulation factor XII (HF), prekallikrein, and high-molecular-weight kininogen, which compose the contact system.[300] Although direct activation of coagulation factor XII by endotoxin has been demonstrated,[301] the extrinsic pathway likely is more important for the development of coagulopathy in endotoxemia and sepsis.[300] Activation of the extrinsic pathway depends on the interaction of coagulation factor VII with tissue factor, which is the only coagulation factor not constitutively present in blood. Tissue factor is present in subendothelial tissues and is exposed on vascular injury but also is expressed on endothelial cells and mononuclear phagocytes in response to LPS.[302,303] Increased expression of monocyte tissue factor (also described as increased procoagulant activity) was significantly associated with coagulopathy and poor prognosis in horses with colic.[304] Furthermore, LPS-induced tissue factor expression by equine peritoneal macrophages may be associated with the development of intraabdominal adhesions.[280]

Regulatory mechanisms of the coagulation cascade include tissue factor pathway inhibitor, antithrombin III (AT III) and the protein C system.[300] Protein C acts as an anticoagulant by inactivating clotting factors V and VIII and promotes fibrinolysis by inactivating plasminogen activator inhibitor (PAI).[305] Protein C activation by thrombin-thrombomodulin complexes is important for the anticoagulative properties of normal endothelium,[300] and downregulation of endothelial thrombomodulin expression by TNF and IL-1 along with decreased expression of AT III and tissue factor pathway inhibitor by damaged endothelial cells contribute to the procoagulant state in endotoxemia and sepsis.[306-308] In addition, activation of vascular endothelial cells leads to a loss of prostacyclin and nitric oxide production and an increased release of thromboxane A_2 (TXA_2). As a result, platelets are stimulated to aggregate and release TXA_2 and PAF, further promoting clot formation.[234]

The crucial step in the fibrinolytic cascade is the conversion of plasminogen to plasmin, a fibrin-degrading enzyme.[300] Tissue-type (tPA) and urokinase-type (uPA) plasminogen activator are the major initiators of fibrinolysis, whereas PAI and α_2-antiplasmin are the main regulatory components.[309,310] TNF and IL-1 have been shown to induce the release of uPA and tPA and the synthesis of PAI.[300] Activation of fibrinolysis leads to consumption of α_2-antiplasmin and accumulation of FDPs, which if present in high concentrations can interfere with platelet aggregation, fibrin polymerization, and thrombin formation and can promote bleeding. Additionally, FDPs mediate an increase in vascular permeability. LPS infusion in rabbits[311] and humans[312] resulted in an early increase in plasma

tPA activity, followed by a later profound rise in PAI activity and fall in tPA activity. Increased plasma PAI concentrations also were found in horses with colic compared with controls.[313,314] Although fibrinolysis may compensate initially for accelerated coagulation, its subsequent inhibition contributes to clot formation.

Cross-activation between the inflammatory and coagulation cascades plays an important role in endotoxemia and sepsis, and the presence of coagulopathies has been associated with an increased risk of organ failure and poor outcome in septic human patients.[297,298] Activated protein C reduces mortality in septic human patients.[315] Activated protein C reduces inflammation by inhibiting leukocyte activation and cytokine production and was shown to lower plasma concentration of IL-6.[315] Although bleeding complications were increased in treated patients, incidence of severe bleeding was not increased significantly.

Complement Activation

Activation of the complement system in endotoxemia occurs via the alternative pathway through interaction with LPS. Increased concentrations of plasmin and kallikrein (caused by activation of the fibrinolytic and contact system) further promote this pathway by directly activating complement factors C3a and C5a. Aside from being key molecules in the complement cascade, C3a and C5a are anaphylatoxins and cause an increase in vascular permeability via mast cell degranulation. C5a further activates the lipoxygenase pathway in neutrophils and monocytes, acts as a chemotaxin for leukocytes and monocytes, and promotes neutrophil adhesion to endothelial cells.

Acute Phase Response

In response to acute inflammation, synthesis and secretion of a number of proteins called the *acute phase proteins* increase in hepatocytes, whereas synthesis of albumin decreases. The primary function of this acute phase response may be to suppress and contain inflammatory processes.[272] IL-6 and IL-1 are the most important cytokines that induce the acute phase response,[316] which typically begins within a few hours of the insult and subsides within 24 to 48 hours,[317] unless the initiating cause persists. In horses fibrinogen (the most commonly evaluated acute phase protein), haptoglobin, transferrin, ferritin, ceruloplasmin, coagulation factor VIII:C, SAA, C-reactive protein, α_1-acid glycoprotein, and phospholipase A_2 are considered part of the acute phase response.[318] SAA is a sensitive indicator of experimentally induced inflammation[319] and may be helpful in the diagnosis of inflammatory gastrointestinal disease,[254] neonatal weakness and diarrhea,[320] and various infectious diseases. A commercially available turbidimetric immunoassay for human SAA is reliable for measuring equine SAA.[321]

The effect of acute inflammation on the serum concentration of several coagulation factors must be considered when evaluating coagulation profiles. Serum fibrinogen concentration is determined primarily by the acute phase response, although fibrinogen is consumed increasingly on activation of the clotting cascade.

Hemodynamic Changes, Development of Shock, and Organ Failure

Shock is characterized by a loss of homeostasis attributable to the breakdown of hemodynamic control mechanisms, decreases in cardiac output and the effective circulating volume, and inadequate perfusion of vital organs. Shock caused by endotoxemia is classified as distributive shock[322] and is largely initiated by vascular dysfunction in the periphery. Peripheral vascular beds are of major importance for the regulation of local tissue perfusion and affect systemic blood pressure by regulating total peripheral resistance. Normally, vascular smooth muscle tone is regulated by endothelin-1 (vasoconstriction), nitric oxide, and prostacyclin (vasodilation) released from vascular endothelial cells.[323] Detrimental effects of nitric oxide are attributable to overproduction of nitric oxide by iNOS in macrophages and other cell types, rather than endothelial-derived nitric oxide. Peripheral vasomotor effects of endotoxin manifest as vasodilation and vasoplegia and are mediated by PGI_2, nitric oxide, and mediators such as bradykinin. Widespread vasodilation leads to vascular blood pooling, decentralization of blood flow, decreased venous return, and in effect decreased effective circulating volume and cardiac output.[322] Compensatory responses in the form of an initial hyperdynamic phase include tachycardia, increased cardiac output and central venous pressure, pulmonary hypertension, peripheral vasoconstriction, and increased peripheral vascular resistance.[322,324,325]

The early vasoconstrictive phase corresponds to an increased serum concentration of TXA_2,[234] but additional vasoconstrictors such as arginine vasopressin, angiotensin II, serotonin, endothelin, and norepinephrine likely are implicated in the pathogenesis of shock and organ failure.[272] With progression of disease the animal enters a stage of decompensated shock and progressive systemic hypotension, which corresponds to increased plasma concentrations of prostacyclin, PGE_2, and bradykinin.[234,272] Inadequate blood flow and oxygen delivery to tissues caused by hypotension are confounded by direct myocardial suppression via nitric oxide,[322] increased vascular permeability,[234] intravascular microthrombosis, and impaired tissue oxygen extraction[322] and results in progressive metabolic acidosis and inhibition of normal cellular metabolism.

CLINICAL SIGNS AND DIAGNOSIS

Quantification of endotoxin in plasma samples is possible. The Limulus amebocyte lysate assay is an activity assay based on the endotoxin-sensitive hemolymph coagulation cascade in the horseshoe crab *Limulus polyphemus.* In *Limulus,* this reaction is thought to be a defense mechanism against gram-negative infection.[326] Although frequently used as a research tool, the assay is not convenient enough to become a routine clinical test. The clinician therefore must appreciate the primary disease processes associated with a high risk of endotoxemia and rely on clinical signs and clinicopathologic data to achieve a diagnosis. In a survey of board-certified internal medicine specialists and surgeons, colitis/enteritis, small intestinal strangulation and obstruction, retained fetal membranes/metritis, grain overload, and pleuropneumonia were among the most frequently cited conditions associated with endotoxemia.[327] In some cases, endotoxemia may be the first indication of disease or may be the most overt of otherwise subtle clinical manifestations. With colitis or proximal enteritis, for example, one may detect signs of endotoxemia before the development of colic, diarrhea, or gastric reflux, which more specifically indicate the nature of the primary illness. The presence of neutropenia should always prompt the clinician to investigate causes of endotoxemia.

In vivo LPS challenge experiments in horses clearly show that many of the clinical signs associated with acute gastrointestinal disease and sepsis are attributable to endotoxemia. On administration of sublethal doses of LPS, the clinical response can be divided into the early hyperdynamic and the later hypodynamic or shock phases. Clinical signs during the first phase, which begins within 15 to 45 minutes after LPS administration, include anorexia, yawning, sweating, depression, evidence of abdominal discomfort, muscle fasciculations, and recumbency. Heart and respiratory rates increase, and decreased borborygmi suggest ileus. Hyperemia of the mucous membranes and an accelerated capillary refill time indicate the hyperdynamic state.[286] If large amounts of LPS are administered or if exposure is ongoing, depression worsens progressively, anorexia persists, and feces develop a diarrheic character. Signs of colic typically abate after the initial stage. Fever develops as a result of direct action of TNF on the thermoregulatory center and IL-1–induced local production of PGE_2 in or near the hypothalamus.[328,329] Because of compromised peripheral perfusion, mucous membrane color changes to brick red or purple, a dark "toxic" line appears, and capillary refill time is prolonged.[286] Inadequate peripheral perfusion and compromised organ function finally characterize the hypodynamic shock phase. Body temperature may become subnormal, and the skin, especially on extremities, is cool to the touch. The arterial pulse weakens, and venous fill is decreased. Vascular endothelial damage and increased capillary permeability result in a muddy mucous membrane color and diffuse scleral reddening. Similar changes are evident in horses suffering from endotoxemia associated with natural disease. In the previously mentioned survey,[327] tachycardia, fever, abnormal mucous membrane color, and increased capillary refill time were named by most specialists as indicating endotoxemia.

Hemostatic abnormalities can manifest in the form of thrombosis or increased bleeding tendency with mucosal petechiation or ecchymoses and prolonged bleeding from venipuncture sites.[299] Bleeding also may occur in the form of spontaneous epistaxis or prolonged hemorrhage after nasogastric intubation.[234] Additional clinical signs typically reflect the development of organ failure. Renal failure and laminitis[327] appear to be common complications of endotoxemia in horses, and endotoxemia was identified as the only clinical condition significantly associated with the development of acute laminitis in one retrospective case-control study of horses admitted to a referral center.[330] Other potential complications include liver failure,[299] respiratory failure, colic and ischemia-induced gastrointestinal ulceration,[234] cardiac failure, and abortion in pregnant mares.[331,332] Renal failure results from ischemic cortical necrosis and acute tubular necrosis caused by coagulopathy-induced afferent arteriolar obstruction. Clinical signs may include oliguria, anuria, or hematuria caused by renal infarction. Laminitis may lead to lameness, increased digital arterial pulsation, increased warmth of the hoof wall, and sensitivity to hoof tester pressure. The exact nature of the association between endotoxemia and laminitis is not understood, and, interestingly, experimental endotoxin infusion does not reliably induce laminitis. Studies have shown, however, that endotoxin administration decreases digital blood flow and laminar perfusion[333] coincident with increased plasma concentrations of 5-HT and thromboxane B_2 (TXB_2).[334] In addition, in vitro vascular reactivity of digital vessels is altered following sublethal endotoxin administration to horses.[335] In addition to the response to circulating mediators, LPS exposure also alters the production of vasoactive mediators by digital vascular endothelial cells.[336] Alterations in vascular reactivity are, therefore, likely responsible for development of laminitis in endotoxemia; however, other mechanisms may apply depending on the underlying clinical disease.

CLINICAL PATHOLOGIC TESTING

Leukopenia caused by neutropenia may be the most specific indicator of acute bacterial sepsis or endotoxemia.[286] In prolonged cases an increased proportion of immature neutrophil forms (bands) and toxic changes are observed. Toxic changes resulting from neutrophil activation include vacuolation, cytoplasmic granulation, basophilic cytoplasm, and Döhle bodies. Because neutropenia occurs early in the development of endotoxemia, it also may be a useful parameter for monitoring horses at risk.[286] On recovery neutropenia typically is followed by a pronounced rebound neutrophilia. Other alterations in the hemogram and serum biochemical profile mainly reflect the underlying disease process and the occurrence of organ failure.

An elevated hematocrit and total serum protein concentration are frequently interpreted as evidence of dehydration; however, splenic contraction caused by increased sympathetic stimulation, increased production of acute phase proteins, or protein losses also influence these parameters. Hyperproteinemia may be observed as a result of increases in the fibrinogen or globulin concentration, and determination of protein fractions, including protein electrophoresis, is indicated in hyperproteinemic patients. Hypoproteinemia and hypoalbuminemia can occur because of loss via the gastrointestinal or urinary tract or with pleural or peritoneal cavity effusion. Increased vascular permeability and edema formation contribute to hypoproteinemia.

Serum electrolyte abnormalities primarily depend on the nature and duration of underlying disease processes and need to be evaluated individually. In human patients, gram-negative sepsis frequently is associated with hypocalcemia, more specifically a decrease in serum ionized calcium concentration. Endotoxin is thought to be a causative factor, and proposed mechanisms include acquired parathyroid gland insufficiency, dietary vitamin D deficiency, impaired calcium mobilization, and renal 1-hydroxylase insufficiency, leading to decreased 1,25-hydroxylation of vitamin D. Hypocalcemia in septic human patients was associated with hypotension and poor outcome.[337] In horses with surgically managed gastrointestinal disease, decreased serum ionized calcium concentration was a common finding and was most severe in patients with strangulating or nonstrangulating infarctions. In some horses, ionized calcium concentration decreased further throughout surgery. Treatment with calcium gluconate resulted in normalization of serum ionized calcium concentrations in all cases.[338]

Septic neonatal patients are frequently hypoglycemic, which may be attributable to decreased oral intake, generally increased metabolism, glucose use by the infecting bacteria, inhibition of gluconeogenesis by endotoxin, and insulin-like activity produced by macrophages.[234] Interestingly, experimental endotoxin administration results in transient hyperglycemia in adult horses,[324] whereas profound hypoglycemia occurs in foals.[339] Because of the high incidence of coagulopathies in endotoxemic and septic patients, clinicians should consider monitoring coagulation parameters. The most

significant changes can be expected with severe inflammatory disease such as colitis,[295,296] devitalized intestine as with strangulating obstruction,[296,340] and with increased duration of disease. In 30 horses with acute gastrointestinal disease, coagulation profiles were considered normal in only two horses.[295] Although coagulation times may be shortened during the procoagulant state, commonly observed abnormalities with developing DIC include an increased concentration of FDPs and soluble fibrin monomer, prolonged prothrombin time indicative of factor VII consumption, prolonged activated partial thromboplastin time indicative of factor VIII:C and IX consumption, prolonged thrombin time, decreased AT III activity, thrombocytopenia, and decreased protein C and plasminogen activities. Fibrinogen concentration appears to reflect the acute phase response rather than coagulation abnormalities in horses and is frequently increased.[304] Some clinicians make a diagnosis of DIC if three or more coagulation parameters (specifically AT III, FDPs, platelet count, prothrombin time, and activated partial thromboplastin time) are abnormal,[340] whereas others require overt clinical signs of hemorrhage and concomitant thrombosis in addition to classic laboratory findings.[296] The prognostic value of coagulation parameters has been evaluated.[233,296,314] Overall, persistence or worsening of abnormalities in the face of treatment appears to be more indicative of poor outcome than alterations in any specific parameter. In one study decreased serum AT III concentration was the parameter most commonly associated with fatal outcome in mature horses with colic.[295]

Hypoxemia observed in response to endotoxin infusion is thought to be caused by an increase in ventilation-perfusion mismatch rather than pulmonary edema, as occurs in human patients with acute respiratory distress syndrome. Pulmonary edema may occur in patients with associated sepsis or complications such as DIC.[341]

MANAGEMENT

The ideal treatment for endotoxemia is prevention. Recognition and close monitoring of patients at risk are crucial because doing so allows institution of timely, possibly proactive treatment, which may reverse the effects of endotoxin before the inflammatory response has developed a dynamic of its own. Unfortunately, endotoxemia can develop rapidly, and horses are exquisitely sensitive to the effects of endotoxin; therefore, many equine patients are not presented for evaluation until they have reached more severe stages of endotoxemia or shock. Prognosis and patient outcome then frequently depend on the severity of complications associated with endotoxemia.[234]

Treatment of endotoxemia involves multiple aspects, and the following strategies have been proposed[342]:

- Inhibition of endotoxin release into the circulation
- Scavenging of LPS molecules to prevent direct effects and interaction with inflammatory cells
- Inhibition of cellular activation by LPS
- Inhibition of mediator synthesis
- Interference with the effects of inflammatory mediators
- General supportive care

In addition, treatment must also address the primary disease process as well as any complications.

When evaluating reports concerning the efficacy of any one treatment, the clinician should keep in mind differences in underlying disease processes and the complexity of the inflammatory cascade. A "one for all" treatment most likely will not be found; similarly, any one treatment can address only a few pathophysiologic aspects of endotoxemia at most.

Inhibition of Endotoxin Release into the Circulation

Inhibition of endotoxin release requires identification and removal of its source. Identification of responsible microorganisms and their antimicrobial sensitivity spectrum are crucial steps toward effective therapy; however, one should not necessarily delay treatment to obtain culture results. Specimen containers with antimicrobial removal devices may be useful in cases for which initiation of treatment precedes specimen collection. Once a diagnosis is reached, correction of the primary disease process is critical. Examples are removal of devitalized sections of bowel or infected umbilical remnants, drainage of infected pleural or PF, and gastric lavage followed by administration of intestinal adsorbents in cases of grain overload. Di-tri-octahedral smectite (DTO; Bio-Sponge, Platinum Performance Inc., Buellton, CA) was shown to remove endotoxin in an in vitro assay[343] and may be useful in preventing endotoxemia of intestinal origin. Septic processes must be addressed with appropriate antimicrobial therapy, and principles of antimicrobial therapy should be followed. Regarding endotoxemia specifically, antimicrobial therapy has been suggested to increase the amount of circulating endotoxin by inducing endotoxin release on cell death of gram-negative bacteria. An in vitro study comparing endotoxin release and inflammatory mediator activity among antimicrobials commonly used to treat *E. coli* bacteremia in foals evaluated amikacin, ampicillin, amikacin plus ampicillin, ceftiofur, and imipenem. Although these antimicrobials showed no difference in the ability to kill bacteria, amikacin and the amikacin–ampicillin combination resulted in the lowest, and ceftiofur in the greatest, release of endotoxin. Endotoxin release appeared to be dose dependent in that lesser amounts were released at higher antimicrobial concentrations.[344] On the basis of these results and clinical experience, combining antimicrobial therapy with endotoxin-binding agents such as polymyxin B may be beneficial, especially when using β-lactam antimicrobials.

Scavenging of Lipopolysaccharide Molecules

Endotoxin typically has a short plasma half-life and is removed rapidly by mononuclear phagocytes or neutralized by binding to serum proteins and lipoproteins. Many conditions responsible for the development of endotoxemia in horses, however, may be associated with an ongoing release of endotoxin. Examples include severe gastrointestinal inflammation as in proximal enteritis or colitis, grain overload, or uncontrolled sepsis. Therapy directed against endotoxin itself may be able to interrupt the continuous activation of the inflammatory cascade in these cases. Further benefits of antiendotoxin treatment may be derived if large amounts of endotoxin have been released before the inciting cause can be addressed.

Immunotherapy

An important consideration regarding the efficacy of immunotherapy is the region of the LPS molecule against which antibodies are raised. The O-chain of LPS acts as a potent antigen[222]; however, antibodies directed against the O-chain are serotype specific and cannot afford significant cross-protection against heterologous gram-negative bacterial strains. The core and lipid A region, both of which show a much

higher degree of homology between LPS derived from different bacterial strains, offer a more promising target for immunotherapy. Active immunization against endotoxins has been reported for horses. Vaccination with a bacterin-toxoid vaccine prepared from rough mutants of *Salmonella typhimurium* or *S. enteritidis* protected horses against homologous and heterologous endotoxin challenge[345,346] and carbohydrate overload.[346] Despite these encouraging results and the availability of a vaccine for use in horses (Endovac-Equi, Immvac Inc., Columbia, MO), active immunization against endotoxin does not appear to be a common practice. In comparison, passive immunization with anti-LPS antibodies is used widely. Rough bacterial mutants, most commonly J5 of *E. coli* O111:B4 and *S. minnesota* Re595, are used to immunize donor horses and subsequently prepare serum or plasma products. Proposed mechanisms of action after binding of the antibodies to LPS include steric blockade of lipid A interaction with cellular receptors and enhanced bacterial clearance by opsonization.[347-349] Studies concerning the efficacy of antibody administration in equine patients vary in their results. Beneficial effects have been described in experimental models of endotoxemia, acute gastrointestinal disease, and neonates with sepsis,[346,350-353] whereas in other studies antibodies failed to protect foals and horses against endotoxin effects.[354-356] Administration of an *S. typhimurium* antiserum to foals was associated with an increased respiratory rate and higher serum activities of IL-6 and TNF.[354]

Various equine serum and plasma products are commercially available. An antiserum raised against the LPS core of *S. typhimurium* (Endoserum, Immvac Inc., Columbia, MO) is available for administration to endotoxemic horses at a recommended dosage of 1.5 mL/kg body mass. Diluting the serum 10- to 20-fold in crystalloid IV solutions, administering it slowly over 1 to 2 hours, and monitoring the patient for adverse reactions is advisable. Although the product is marketed for use in foals with failure of passive transfer, adverse effects have been reported,[354] and one should use caution when administering it to neonates. Plasma from donors inoculated with J5 *(E. coli)* and *S. typhimurium* (Re-mutant) is available under a California license (Equiplas J, Plasvacc USA Inc., Templeton, CA). The manufacturer recommends administration of at least 1 to 2 L in cases of endotoxemia. Hyperimmune plasma, which has a guaranteed minimum IgG content but does not contain specific antiendotoxin antibodies (HiGamm Equi, Lake Immunogenics, Inc., Ontario, NY; Equiplas and Equiplas Plus, Plasvacc USA Inc.,), is marketed for treatment of failure of passive transfer, and many clinicians use it to treat endotoxemia and sepsis. In addition to antibodies and protein, plasma contains active constituents such as complement components, fibronectin, clotting factors, and AT III[351] and therefore may be particularly useful in patients with endotoxemia-induced coagulopathy. Volumes of 2 to 10 mL/kg body mass of hyperimmune plasma have been recommended for use in endotoxemic patients.[272,357]

Polymyxin B

Polymyxin B is a cationic polypeptide antibiotic that binds to the anionic lipid A portion of LPS and neutralizes its endotoxin capacity.[358] At dosages required for antimicrobial activity, polymyxin B carries the risk of respiratory paralysis and ototoxic, nephrotoxic, and neurotoxic side effects; however, a much lower dose is required for endotoxin-binding activity. The effects of polymyxin B in horses have been evaluated in different experimental models.[354,358,359] In an in vivo study in foals, treatment with polymyxin B at a dosage of 6000 U/kg body mass before infusion with *S. typhimurium* LPS resulted in significantly less severe elevations of body temperature, respiratory rate, and serum activities of TNF and IL-6 compared with untreated controls.[354] Similarly, polymyxin B treatment of adult horses given endotoxin ameliorated clinical signs and decreased plasma TNF activity.[360] In the latter study benefits of treatment were also evident at lower dosages of polymyxin B (1000 and 5000 U/kg body mass) and administration of polymyxin B 1 hour after the start of endotoxin infusion. Conversely, polymyxin B failed to ameliorate clinical signs of endotoxemia or prevent the development of coagulopathy, acidosis, lameness, and shock in experimental carbohydrate overload.[361] Adverse effects suggestive of neurotoxicity appeared after repeated administration of 5 mg/kg body mass (36,000 U/kg) and in a milder form after administration of 2.5 mg/kg body mass (18,000 U/kg). Nephrotoxicity was not observed. Currently, use of polymyxin B in equine patients is recommended at dosages of 1000 to 6000 U/kg body mass every 8 to 12 hours administered as a slow bolus.[362,363] Treatment should be initiated as early in the disease process as possible because the beneficial effects of LPS scavenging may be limited to the first 24 to 48 hours after the onset of endotoxemia, before endotoxin tolerance develops. Adverse effects in the form of neuromuscular blockade and apnea, which necessitate slow infusion of the drug in human patients, have not been observed in horses. If treating horses with hypovolemia, dehydration, or azotemia, the clinician should attempt to improve peripheral tissue perfusion, minimize the polymyxin B dose, and closely monitor patients for nephrotoxicity. Close monitoring is also important if medications such as aminoglycoside antibiotics, which share a similar spectrum of potential side effects, are administered concurrently. Azotemic neonates are more susceptible to the nephrotoxic effects of polymyxin B than adult horses.[360]

In an attempt to decrease the risk for adverse effects while preserving the LPS-neutralizing ability, a conjugate of polymyxin B with dextran has been developed.[364] In conjugated form, polymyxin B is prevented from extravasation into tissues, in which it exerts toxic effects by interaction with cell membranes. In addition, conjugation increases the residence time of polymyxin B in the circulation; therefore, it should prolong the antiendotoxic effect. The polymyxin B–dextran combination was evaluated at a total dose of 5 mg/kg body mass of polymyxin B in 6.6 g/kg body mass dextran, given 15 minutes before administration of endotoxin in horses.[365] Treatment blocked the development of tachycardia, tachypnea, fever, and neutropenia completely and prevented increases in serum concentrations of TNF, IL-6, TXB_2 (a TXA_2 metabolite), and the prostacyclin metabolite 6-keto-$PGF_1\alpha$. Although mild adverse effects in the form of tachypnea, sweating, and increased systolic blood pressure were observed, these were transient and could be prevented by pretreatment with ketoprofen. To the author's knowledge, the polymyxin B–dextran combination is not commercially available at this time.

Natural Endotoxin-Binding Substances

Natural endotoxin-binding proteins such as LBP, lipoproteins, and sCD14 have been evaluated experimentally. Results of these studies are somewhat contradictory, and detrimental effects occurred in some cases.[366] A protein receiving a great deal of attention regarding potential therapeutic efficacy is

the bactericidal permeability-increasing (BPI) protein. This protein is structurally similar to LBP but is expressed exclusively in myeloid precursors of polymorphonuclear (PMN) leukocytes.[367] BPI is stored in primary granules of mature neutrophils and during inflammation is expressed on their cell membranes and secreted into the extracellular environment.[368] BPI has an even higher affinity for LPS than LBP[369] and shows antibacterial activity specific for gram-negative bacteria.[219] Binding of BPI to the gram-negative bacterial membrane results in growth arrest and is an important factor in the antibacterial activity of intact neutrophils. Furthermore, BPI binding disrupts normal membrane organization and makes bacteria more susceptible to hydrophobic substances, including antimicrobials.[370] Experimentally, recombinant BPI protects against the toxic and lethal effects of isolated LPS and intact gram-negative bacteria, and clinical trials in human patients show promising results regarding its therapeutic use.[371] The biology and potential use of BPI in horses has not been evaluated.

Phospholipid emulsions have recently been evaluated for treatment of experimentally induced endotoxemia in horses. Phospholipid infusion improved clinical parameters, ameliorated neutropenia, and reduced inflammatory mediator production in response to an endotoxin challenge.[372,373] Because hemolysis was a complication of phospholipid infusion in some horses in these studies, optimization of dose and time of administration will be necessary before evaluating this treatment for potential clinical use.

Inhibition of Cellular Activation by Lipopolysaccharides

Treatments aimed at inhibiting LPS interaction with cells or turning off intracellular signaling pathways are under investigation. Nontoxic LPS or lipid A structures can act as endotoxin antagonists, if they competitively inhibit binding to LBP or cellular receptors or inhibit cellular activation by other mechanisms. Of the potential antagonists that have been evaluated experimentally, LPS and lipid A of the phototrophic bacterium *Rhodobacter sphaeroides* and the synthetic compounds E5531 and E5564 have been most promising.[373-379] Unfortunately, species differences exist regarding cellular response to these structures, and *R. sphaeroides* LPS as well as E5531 have been found to have agonist activity in equine cells.[380,381] In cell transfection experiments, TLR-4 was shown to be responsible for this phenotypic variation regarding *R. sphaeroides* LPS.[382] Given these results, any future potential LPS antagonists must be evaluated in equine systems.

Inhibition of Mediator Synthesis

Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are probably the most commonly used drugs to treat endotoxemia in horses. The rationale for their use is inhibition of COX, which inhibits prostanoid production (see Fig. 12.5). Additional beneficial effects may include scavenging of oxygen-derived free radicals and iron chelation; however, side effects may occur at dosages required to achieve these effects.[374] Prostanoids have been identified as important mediators in the inflammatory response in a number of studies, and inhibition of their synthesis is associated with beneficial effects. Generally speaking, two COX isoforms are recognized: constitutively expressed COX-1 and inducible COX-2. Upregulation of COX-2 expression results from various proinflammatory stimuli, including LPS, TNF-α, and IL-1.[383] Constitutively expressed COX products are likely important for maintenance of homeostasis, whereas increased production of prostanoids by COX-2 is thought to be responsible for detrimental effects during inflammation and shock.

In horses, the most commonly used NSAID to treat endotoxemia is flunixin meglumine. Beneficial effects of flunixin meglumine have been described in experimental models of endotoxemia[384-386] and in clinical cases. In equine colic patients treatment with flunixin meglumine before exploratory surgery resulted in reduced plasma concentrations of TXB_2 and PGE_2 and had a favorable effect on cardiovascular parameters.[387] Flunixin meglumine was shown further to maintain cardiac output and systemic arterial blood pressure, improve blood flow to vital organs, reduce pulmonary endothelial damage, and improve survival on endotoxin challenge.[285,388-390] Conversely, in vitro studies have suggested that flunixin meglumine impairs recovery of intestinal barrier function in intestinal segments subjected to ischemia–reperfusion injury and may increase mucosal permeability to LPS.[391,392] This effect may be reduced or eliminated by concurrent administration of continuous rate infusion of lidocaine.

NSAID use in horses carries the risk of adverse effects, the most significant of which are development of gastrointestinal ulceration and renal papillary necrosis (renal crest necrosis). Differences may exist among NSAIDs in their propensity to induce adverse effects,[393] but all NSAIDs must be used cautiously. Because of the potential concerns for masking of cardiovascular effects of endotoxin in horses with colic, a reduced dose of flunixin meglumine (0.25 mg/kg body mass thrice daily) has been suggested and is used widely in horses.[327] At this dosage flunixin meglumine inhibits eicosanoid synthesis efficiently in an in vivo model of endotoxemia.[394] Reduction of clinical signs, however, was dose dependent, and lower doses provide minimal, if any, analgesia (see Chapter 2 for further discussion of NSAID therapy in horses).

Some researchers have suggested that ketoprofen offers superior effects because of a proposed dual inhibitory effect on COX and lipoxygenase and may carry a decreased risk of adverse effects compared with flunixin meglumine and phenylbutazone. A comparison of cytokine and eicosanoid production by LPS-stimulated isolated monocytes in vitro, however, showed no significant difference between horses pretreated with flunixin meglumine (1.1 mg/kg body mass) or ketoprofen (2.2 mg/kg body mass), respectively.[395]

Given 15 minutes before LPS infusion, eltenac at a dosage of 0.5 mg/kg protected against changes in clinical, hemodynamic, and hematologic parameters and blunted the LPS-induced rise in plasma cytokine concentrations compared with controls in an experimental model of endotoxemia.[396] Some parameters, however, including heart rate, leukocyte count, lactate concentration, and plasma TNF activity, were not improved. Ibuprofen may have beneficial effects superior to those of the other NSAIDs because it may be possible to achieve tissue concentrations safely that allow iron chelation to occur. According to a study in healthy foals, dosages of ibuprofen up to 25 mg/kg every 8 hours can be given safely for up to 6 days.[397] To the author's knowledge, the COX-2 inhibitor firocoxib has not yet been evaluated critically for treatment of endotoxemia.

Corticosteroids

The use of corticosteroids for antiinflammatory therapy in sepsis and endotoxemia has been controversial in human and equine patients, and beneficial effects superior to the ones achieved by NSAIDs have not been demonstrated consistently. Corticosteroids inhibit the activity of phospholipase A_2 and the release of arachidonic acid from cell membrane phospholipids, as well as the production of TNF, IL-1, and IL-6 in response to an LPS stimulus. Experimentally, beneficial effects of dexamethasone in equine endotoxemia have been demonstrated.[398,399] To inhibit TNF production by equine peritoneal macrophages, however, the required concentration of dexamethasone was high and corresponded to an in vivo dosage (approximately 3 mg/kg body mass) greatly exceeding current recommendations.[398] Although single doses of corticosteroids are unlikely to carry a disproportionate risk of adverse effects, the clinician should consider the suggested association of laminitis with corticosteroid use in horses. In cases of sepsis immunosuppressive effects could also be detrimental.

In human patients with certain types of septic shock, dysfunction of the hypothalamic–pituitary–adrenal axis has been recognized and successfully treated with hydrocortisone replacement therapy.[400] Hypothalamic–pituitary–adrenal axis dysfunction has been suggested to occur in septic foals[401]; however, the use of low-dose corticosteroids for this indication remains to be investigated in horses.

Pentoxifylline

Pentoxifylline, a methylxanthine derivative and phosphodiesterase inhibitor, has been suggested for use in endotoxemia because of its effects on neutrophil function and its ability to inhibit the production of various cytokines, interferons, and thromboplastin. Decreased production of TNF, IL-6, TXB_2, and thromboplastin in response to endotoxin was shown in an equine ex vivo model.[402] In horses given endotoxin followed by treatment with pentoxifylline (7.5 mg/kg body mass followed by continuous infusion of 3 mg/kg/h for 3 hours), however, only minimal beneficial effects were observed.[403] Treatment significantly improved body temperature, respiratory rate, and whole blood recalcification time, but no effect was observed regarding heart rate, blood pressure, leukocyte count, plasma fibrinogen concentration, and serum cytokine concentrations. The conclusion was that benefits of treatment with pentoxifylline might be restricted to administration of high bolus doses or continuous infusion early in the pathophysiologic process. In an in vivo endotoxemia model in horses, the combination of pentoxifylline (8 mg/kg body mass) and flunixin meglumine (1.1 mg/kg body mass) had greater benefit than each treatment on its own.[404] Because of its rheologic properties (i.e., the ability to increase erythrocyte deformability and microvascular blood flow), pentoxifylline has been suggested for use in endotoxemic patients showing evidence of laminitis; however, no effect on blood flow to the hoof was demonstrated after administration to healthy horses.[405] An IV preparation of pentoxifylline is not commercially available.

Antioxidants

Dimethyl sulfoxide (DMSO) is used by some clinicians in an attempt to scavenge oxygen-derived radicals. However, DMSO failed to show beneficial effects in an experimental model of intestinal ischemia when administered on reperfusion of the ischemic intestine.[406] DMSO at a dosage of 1 g/kg body mass increased mucosal loss after ischemia and reperfusion of the large colon,[407] and a reduced dosage of 0.1 g/kg body mass has been proposed for horses with intestinal ischemia. DMSO failed to show significant benefit in an experimental model of endotoxemia in horses, although it ameliorated the effect on fever, and many clinicians do not advocate its use.[408] The xanthine oxidase inhibitor allopurinol has been suggested as a treatment to prevent oxygen radical–induced tissue damage. During periods of ischemia tissue xanthine dehydrogenase is converted to xanthine oxidase, which on reperfusion catalyzes the generation of superoxide radicals.[409,410] Evaluation in horses showed beneficial effects of allopurinol 5 mg/kg body mass administered 12 hours before endotoxin challenge.[411] In another study mucosal damage attributable to oxygen-derived free radicals was not attenuated by allopurinol in an experimental ischemia–reperfusion model.[407]

Lidocaine

Lidocaine given IV has been suggested as an antiinflammatory, analgesic, and prokinetic agent. In an experimental endotoxemia model in rabbits, lidocaine inhibited hemodynamic and cytokine responses to endotoxin profoundly if given immediately after LPS infusion.[412] Lidocaine further ameliorated the inhibitory effects of flunixin on recovery of mucosal barrier function following ischemic injury in equine small intestine.[413] Use of lidocaine therefore may have merit in endotoxemic patients. A common regimen for lidocaine use in horses is the administration of an initial bolus (1.3 mg/kg body mass) followed by continuous infusion at a rate of 0.05 mg/kg/min.

ω-3 Fatty Acids

High concentrations of ω-3 fatty acids can alter the phospholipid composition of cellular membranes toward a decreased ratio of ω-6 to ω-3, affecting membrane functions such as phagocytosis, receptor binding, and activities of membrane-bound enzymes.[286] Most important for the treatment of endotoxemia, ω-3 fatty acid incorporation into cell membranes decreases the availability of arachidonic acid (an ω-6 fatty acid) for eicosanoid synthesis[414] and provides alternative substrates. Metabolism of ω-3 fatty acids via the COX and lipoxygenase pathway leads to the production of 3-series prostaglandins and 5-series leukotrienes, which have less biologic activity than their 2-series and 4-series counterparts derived from arachidonic acid. Aside from these mechanisms, ω-3 fatty acids prevent LPS-induced upregulation of CD14 in monocytic cells; therefore, they may be able to block transmembrane signaling of LPS.[415] Cells from horses given linseed oil (high in ω-3 fatty acids) for 8 weeks before blood collection showed significantly decreased expression of procoagulant activity, TXB_2, and TNF in response to LPS stimulation.[416,417] In an in vivo experimental model of endotoxemia in horses, treatment resulted in prolonged activated partial thromboplastin time and whole blood recalcification time, suggesting an anticoagulant effect; however, a significant beneficial effect on clinical response and serum eicosanoid concentrations was not observed.[418] Because dietary addition of ω-3 fatty acids requires several weeks of treatment, IV infusion was evaluated and shown to alter the composition of cell membrane phospholipids rapidly.[419] Further evaluation of this treatment for use in horses is necessary before specific dosage recommendations can be made.

Interference with the Effects of Specific Inflammatory Mediators

Antibodies Directed Against Tumor Necrosis Factor

Monoclonal and polyclonal antibodies against equine TNF have been evaluated in horses.[420-422] Administration of a monoclonal antibody preparation before LPS infusion resulted in significantly reduced plasma TNF activity, improved clinical abnormality scores, lower heart rate, and higher leukocyte count compared with controls.[421] Plasma concentrations of lactate and 6-keto-PGF1α were reduced significantly, whereas TXA_2 production was not affected.[420] In another study[422] administration of a rabbit polyclonal antibody against recombinant human TNF did not improve clinical and hematologic parameters when given shortly (15 minutes) after LPS infusion, although inhibition of TNF activity was present in vitro.[422,423] Findings in horses are in agreement with studies in other species and suggest that beneficial effects of TNF inhibition may be limited to administration before LPS exposure. Widespread clinical use therefore is unlikely to become feasible. Clinical trials in septic human patients have not shown significant benefits of TNF antibody treatment.[424,425]

Platelet-Activating Factor Receptor Antagonists

The effects of selective PAF receptor antagonists have been evaluated. PAF is implicated in the development of systemic hypotension,[426] LPS-induced platelet aggregation,[427] ileus,[428] and increased vascular permeability[429] and may mediate recruitment of leukocytes to inflamed tissues.[430,431] A study in horses using the PAF receptor antagonist SRI 63-441 before LPS infusion showed significant decreases in heart rate and shorter elevation of lactate concentrations in response to the treatment. Although not statistically significant, additional beneficial effects included delayed onset of fever, a shortened period of neutropenia, and reduced maximal platelet aggregation.[432]

Supportive Care

Fluid Therapy and Cardiovascular Support

Fluid therapy is a mainstay of therapy of most endotoxemic patients suffering from the cardiovascular effects of systemic inflammation. Many endotoxemic equine patients require fluid therapy for treatment of the underlying disease process and correction of dehydration and electrolyte and acid-base abnormalities. Principles of fluid therapy are discussed in Chapter 4 of this text.

Patients with severe hypovolemia and shock present management challenges, especially because increased vascular permeability in endotoxemic patients requires careful consideration of fluid therapy plans. A rapid increase in total body fluid volume may be detrimental in patients with compromised cardiac and peripheral vasomotor function and may increase the severity of vascular pooling in peripheral organs. In these patients hypertonic solutions or colloids may be more appropriate means of stabilization than large volumes of crystalloid solutions. Hypertonic saline solution (7.5% sodium chloride) is the most commonly used hypertonic solution in horses and has beneficial effects in endotoxemic patients.[433] A dosage of 4 mL/kg is recommended, which should be given as a bolus infusion over 10 to 15 minutes, followed by administration of an isotonic solution to restore total body fluid volume. The clinician should use hypertonic saline with caution in patients with sodium or chloride derangements and should monitor serum electrolyte concentrations in the case of repeated administration. Failure of urination despite appropriate fluid resuscitation should result in critical evaluation of renal function. In one recent study small-volume resuscitation with hypertonic saline plus hydroxyethyl starch failed to alleviate hemodynamic responses in experimental endotoxin infusion in horses.[434]

Plasma is an ideal colloid and should be administered to maintain a serum total protein concentration above 4.2 g/dL.[357] To raise plasma protein concentration and colloid osmotic pressure significantly, however, horses often require large volumes of plasma (7–10 L or more in a 450-kg horse), and alternative colloids should be considered. High-molecular-weight polymers are thought to provide superior oncotic effects in cases of sepsis and endotoxemia, when vascular permeability is increased. Hetastarch, or hydroxyethyl starch (Hespan), is commercially available as a 6% solution in 0.9% sodium chloride. Hetastarch molecules have a very high molecular weight, and degradation must occur before renal excretion.[435] These properties result in a longer plasma half-life and prolonged oncotic effects compared with other colloids; persistence of the oncotic effect for 24 hours was observed in hypoproteinemic horses.[436] A dosage of 5 to 15 mL/kg given by slow IV infusion along with an equal or greater volume of crystalloid fluids has been recommended.[435,437] In human patients prolonged activated partial thromboplastin time, decreased factor VIII activity, and decreased serum fibrinogen concentration have been described in association with hetastarch use.[438] In the limited number of equine studies, bleeding times were not affected[439,440]; however, patients treated with hetastarch should be monitored for coagulopathy.

Metabolic acidosis in endotoxic shock is attributable to lactic acidemia and inadequate tissue perfusion.[441] Acid-base balance often improves considerably after fluid resuscitation alone; however, additional sodium bicarbonate may be required in cases in which serum bicarbonate concentration remains below 15 mEq/L.

Foals with sepsis are frequently hypoglycemic, and 5% dextrose solutions are useful as initial resuscitation fluids. The clinician should reduce the glucose concentration of IV solutions according to the blood glucose concentration to avoid prolonged hyperglycemia. Administration of hyperimmune plasma (20–40 mL/kg body mass) is highly recommended in foals with evidence of partial or complete failure of passive transfer.

One should consider positive inotropic and vasomotor agents in patients with persistently inadequate tissue perfusion. Lower dosages of dopamine (0.5–2 μg/kg/min) result in vasodilation of the renal, mesenteric, coronary, and intracerebral vasculature via dopaminergic effects, whereas higher dosages (up to 10 μg/kg/min) also exert stimulation of α_1-adrenergic receptors, resulting in increased myocardial contractility and heart rate.[442] Dobutamine is a direct α_1-adrenergic agonist and does not appear to have significant vasodilator properties. Dosages for dobutamine of 1 to 5 μg/kg/min as a continuous IV infusion have been recommended for use in horses. Norepinephrine was evaluated in hypotensive critically ill foals that were refractory to the effects of dopamine and dobutamine.[443] At dosages up to 1.5 μg/kg/min administered concurrently with dobutamine, six of seven foals showed an increase

in mean arterial pressure, and all foals had increased urine output. Because of the risk of cardiac side effects, close monitoring of heart rate and rhythm should accompany infusion of inotropes. Indirect blood pressure measurements using a tail cuff may be used to monitor the effects of treatment.

Management of Coagulopathy

More frequently than overt thrombosis or bleeding attributable to DIC, hemostatic abnormalities occur in the form of alterations in the coagulation profile. A procoagulant state with shortened bleeding times or prolonged bleeding times caused by consumption of clotting factors may be evident. One should address abnormalities in the coagulation profile as early as possible but especially if they persist more than 24 hours after initiation of therapy. Because of the complex interactions of coagulation and fibrinolysis during endotoxemia, it might be necessary to combine anticoagulant therapy with the administration of fresh frozen plasma to replace clotting and fibrinolytic factors. Heparin acts as an anticoagulant by activation of AT III and subsequent inhibition of thrombin, release of tissue factor pathway inhibitor from endothelial cells, and inhibition of platelet aggregation.[444] Because endogenous AT III levels frequently are decreased in patients with coagulopathy, the addition of heparin to fresh frozen plasma may be the most effective route of administration. An initial dosage of 100 IU/kg body mass followed by 40 to 80 IU/kg body mass thrice daily has been recommended.[357] Anemia caused by erythrocyte agglutination occurs in some patients during therapy with unfractionated heparin[445,446] but typically resolves within 96 hours if therapy is discontinued.[357] Because of the risk of microthrombosis associated with erythrocyte agglutination, use of low-molecular-weight heparin (50 IU/kg body mass subcutaneously [SC] every 24 hours) has been recommended[447] but may be cost prohibitive. Aspirin can be given orally (10–20 mg/kg body mass every 48 hours), which irreversibly inhibits platelet COX activity, to inhibit platelet aggregation and microthrombosis. Platelet hyperaggregability has been implicated in the pathogenesis of carbohydrate-induced laminitis,[448] and heparin and aspirin have been recommended to prevent the development of laminitis. In an in vitro study, however, aspirin did not inhibit endotoxin-induced platelet aggregation.[449]

Other Considerations

Luteolysis caused by increased concentrations of $PGF_2\alpha$ may lead to pregnancy loss in endotoxemic mares before day 55 of pregnancy (see Chapter 19).[450] Daily administration of altrenogest (Regu-Mate, Hoechst-Roussel Agri-Vet, Somerville, NJ) at a dosage of 44 mg orally consistently prevented fetal loss in mares if administered until day 70 of pregnancy.[331] Treatment with flunixin meglumine, by blockade of $PGF_2\alpha$ release,[332] also may contribute to the maintenance of pregnancy in endotoxemic mares. The pathogenesis of fetal loss and abortion caused by endotoxemia, surgery, or systemic disease later in gestation is not understood completely. Proposed mechanisms include direct effects on the fetus, placental function, or placental progesterone production.[451]

Decreased nitric oxide production by vascular endothelial cells in response to endotoxin has been suggested as a mechanism for vasoconstriction and decreased blood flow leading to laminitis)[452]; however, use of nitric oxide donors remains controversial. Maintenance of adequate peripheral perfusion and anticoagulant and antiinflammatory therapy may be helpful in preventing and treating laminitis caused by endotoxemia.

CRYOTHERAPY

Digital cryotherapy is a critical component of supportive care in the equine patient with suspected endotoxemia, including those suffering from many of the inflammatory diseases discussed in this chapter. Cryotherapy has been shown to reduce the severity of laminitis lesions in the oligofructose model,[453-455] including when initiated after the onset of lameness.[456] It has also been shown to decrease the incidence of laminitis in horses diagnosed with colitis.[457] When initiated, digital cryotherapy should include immersion of the hoof and pastern, at a minimum.[458]

Inflammatory Diseases of the Small Intestine

DUODENTITIS-PROXIMAL JEJUNITIS

Duodenitis-proximal jejunitis (DPJ) is an inflammatory condition affecting the upper small intestine and resulting in distention, abdominal pain, gastric reflux caused by excessive fluid and electrolyte secretion, and increased PF protein concentration without a significant elevated nucleated cell count. Other terms for this condition are *anterior enteritis* and *proximal enteritis.* Clinical signs of DPJ mimic those of a small intestinal obstruction. The clinical syndrome of DPJ was well described in the 1980s,[19,459-461] but the severity of clinical signs, especially duration of disease, is variable. Although not typical, DPJ can occur in conjunction with gastritis, ileitis, typhlitis, and colitis.

PATHOPHYSIOLOGY

Typical pathologic findings in horses with DPJ include involvement of the duodenum and usually the proximal jejunum.[19] The ileum and large colon usually are grossly normal. Gastric distention is common because of hypersecretion in the proximal small intestine combined with functional ileus. Small intestinal diameter often measures 5 to 7 cm, filled with malodorous, red to brown-red fluid. Duodenal and jejunal serosal surfaces may have varying degrees and distribution of bright red to dark red petechial and ecchymotic hemorrhages and yellow to white streaks. The enteric mucosal surfaces are usually hyperemic with varying degrees of petechiation and ulceration.

Microscopically, the most severe lesions are located in the duodenum and proximal jejunum but may extend proximally to the gastric mucosa and aborally to the large intestinal mucosa and submucosa.[19] Microscopic lesions include varying degrees of mucosal and submucosal hyperemia and edema, villous degeneration with necrosis and, more severely, sloughing of villous epithelium. The lamina propria, mucosa, and submucosa may have varying degrees of granulocyte infiltration (predominantly neutrophils), and the muscular layers and serosa may contain small hemorrhages. Proximal small intestinal serosal fibrinopurulent exudate is a common finding in the more severe cases; therefore the term *hemorrhagic fibrinonecrotic duodenitis-proximal jejunitis* has been suggested as a more descriptive name for this syndrome.[461]

Horses with DPJ can have hepatic changes including hepatocellular vacuolization, cholestasis, inflammatory infiltrate (either in association with centrilobular necrosis or periportal), and biliary hyperplasia.[462] Hepatic disease is thought to result from ascending infection via the common bile duct, local absorption of endotoxin via the portal circulation, systemic consequences of endotoxin absorption, metabolic imbalances, and hypoperfusion or hypovolemia.

In most cases, an underlying etiology cannot be determined. In some cases, *Salmonella* spp. or *Clostridium* spp. can be isolated from culture of gastric reflux. Salmonellosis has never been consistently identified in a majority of cases, and many horses with documented infections by these organisms do not develop DPJ. Alternatively, toxigenic strains of *C. difficile* were isolated from the reflux of five of five horses with DPJ and none of six control horses with other causes of nasogastric reflux.[463] To the author's knowledge, such an association has not been identified in other geographic locations. Another suspected infectious agent is *Fusarium* spp.[464] Alteration of the gastrointestinal microflora caused by recent dietary change has been suggested as a predisposing cause for DPJ; in one report, horses with DPJ were fed significantly more grain and were more likely to have grazed pasture, relative to horses with other forms of colic, but these associations were not deemed strong enough to allow clinical differentiation.[465]

Two intracellular processes control intestinal secretion: the cyclic nucleotide (cAMP and cGMP) and calcium systems.[169] Inflammatory mediators, microorganisms, and toxins can activate adenyl cyclase (vasoactive intestinal peptide and PGE_2) or guanylyl cyclase (bacterial enterotoxins) and induce increases in cAMP and cGMP, respectively. This reaction causes phosphorylation of specific protein kinases, which induce the actual mucosal membrane transport events. Increases in intracellular free calcium may arise from cyclic nucleotide–dependent release of stored calcium within the cell or from increased calcium entry across the cell membrane. Calcium may act through calmodulin, which then can activate membrane-phosphorylating protein kinases. The net effect is increased movement of sodium and chloride into the mucosal cell from the interstitium, with secretion of sodium and chloride into the intestinal lumen. Water follows the directional flux of sodium and chloride through highly permeable intercellular spaces. Several bacterial toxins and endogenous mediators can cause active secretion and contribute to a synergistic mucosal secretory response. Passive secretion of protein-rich fluid into the lumen occurs after damage to the mucosal epithelium, capillary endothelium, and submucosal inflammation in the proximal small intestine. The clinically relevant events that result from active and passive fluid secretion are proximal small intestinal distention and nasogastric reflux, dehydration, and circulatory shock.[466]

The concentration of protein in the PF from horses with DPJ is usually higher than in horses with small intestinal obstruction. A disproportionate increase in total protein concentration relative to nucleated cell count occurs, probably by leakage of blood or plasma into the peritoneal cavity without a significant stimulus for leukocyte chemotaxis. Suggested mechanisms for increased abdominal fluid protein concentration include serositis associated with inflamed intestine and small intestinal distention causing passive congestion and increased capillary hydrostatic pressure of visceral peritoneal vessels.[467] Small intestinal ileus is another hallmark sign of DPJ, and the pathophysiology is complicated, involving primary and secondary dysfunction of the central, autonomic, and ENSs and their purported roles in governing intestinal motility. Further detail regarding the effect of inflammation on small intestinal motility is provided in Inflammatory Disease of the Small Intestine later in this chapter.

CLINICAL AND CLINICOPATHOLOGIC SIGNS

Differentiating horses with DPJ from horses with small intestinal obstructive lesions can be challenging because there is no single distinguishing feature, and all information must be considered collectively. Horses with obstructive lesions of the small intestine usually show consistent signs of abdominal pain until the affected viscus is repaired surgically or ruptures. In contrast, signs of acute abdominal pain typically subside after gastric decompression and volume replacement in horses with DPJ. They are replaced by signs of lethargy and general malaise. On rectal examination the degree of small intestinal distention may be subjectively less with DPJ than with obstructive lesions, particularly following gastric decompression. Although the color and odor of gastric reflux can be similar, horses with DPJ tend to have a larger volume (≥4–20 L with each decompressive effort) of reflux than horses with obstructive lesions. Horses with DPJ often have a mild fever of 38.6°C to 39.1°C (101.5°F–102.5°F), whereas horses with obstructive lesions are typically normothermic or hypothermic.

Abnormalities in the leukogram are more common in horses with DPJ than with an acute obstructive lesion.[19,460] In addition, hyponatremia, hypochloremia, hypokalemia, prerenal azotemia, and elevated hepatic enzymes (GGT, alanine transaminase, and AP) are often evident.[462] Hyperlactemia from poor tissue perfusion and hypovolemia is common with both conditions.

PF analysis may be helpful in distinguishing DPJ from an obstructive lesion. The typical findings with DPJ include an increased PF protein concentration (often ≥3.5 g/dL) and a mild to moderate elevation of the peritoneal WBC count, although the count usually is less than 10,000 cells/μL; PF lactate concentration typically mirrors peripheral blood concentrations. The disproportionate increase in PF total protein compared with peritoneal nucleated cell count may be caused by leakage of blood or plasma without a marked leukotactic response.[19] The PF is usually yellow and turbid, but in severe cases, diapedesis can occur, resulting in a serosanguineous color. Strangulating lesions typically result in more severe changes in the PF. Serosanguineous PF with increased protein, lactate, and white and red blood cells is typical. Horses with intraluminal obstructions such as ileal impactions typically have grossly normal PF with a mildly elevated protein resulting from intestinal distention.[419,460,466]

Ultrasonographic findings in horses with DPJ typically include gastric and duodenal distention and segments of small intestine containing hypoechoic to anechoic fluid. The wall of the small intestine can be normal or thickened with time. Peristalsis can be decreased, normal, or increased. Differentiation from a small intestinal obstructive lesion remains difficult. More long-standing obstructions typically result in increased wall thickness, sedimentation of ingesta within the small intestinal lumen, and distended small intestine proximal to the lesion with collapsed intestine distally.



FIG. **12.6** Endoscopic image from a 15-year-old Quarter Horse gelding. The horse was diagnosed with duodenitis-proximal jejunitis (DPJ) and developed mild respiratory stridor and gagging and retching behavior after an indwelling nasogastric tube had been in place for 48 hours to allow for intermittent drainage of gastric reflux. The horse responded well to medical therapy for DPJ and pharyngitis-esophagitis.

In most cases, the diagnosis is suspected on the basis of clinical and clinicopathologic signs and response to therapy; a definitive diagnosis of DPJ requires gross examination of the duodenum and proximal jejunum at surgery or at necropsy

TREATMENT

Horses with DPJ appear to share a common characteristic clinical presentation. Treatment is supportive and should include volume replacement, analgesic and antiinflammatory therapy, gastric decompression, antiendotoxin therapy, antimicrobial therapy if indicated, nutritional support, and nursing care.

Gastric Decompression

Although the signs of abdominal pain usually resolve after gastric decompression, most horses remain lethargic. Horses with DPJ often require gastric decompression at 2-hour intervals, with 2 to 10 L of fluid recovered each time. Nasogastric tubes left in place for long periods cause varying degrees of pharyngitis (Fig. 12.6), laryngitis, and esophagitis, and maintenance of an indwelling nasogastric tube may further delay gastric emptying.[466,468,469] Thus one should use the smallest, softest nasogastric tube needed for gastric decompression and remove the tube as soon as possible.

Supportive Care

Much of the supportive care for horses with DPJ mirrors that described for other cases of endotoxemia and is discussed in detail in Endotoxemia earlier in this chapter. Specific fluid replacement should take ongoing losses through reflux into consideration. Horses should not receive food or water until they remain comfortable and reflux ceases. Occasional sips of water or rinsing of the mouth may decrease halitosis. The refeeding plan should be conservative, because horses refed quickly may relapse.

Antiinflammatory and Analgesic

NSAIDs are the most frequently used group of drugs for treatment of abdominal pain in horses (flunixin meglumine 0.25–1.1 mg/kg IV every 8–12 hours, with the higher dose given only at the least frequent interval). These agents also have beneficial antiinflammatory and antiendotoxic effects. The clinician must weigh the benefit of these drugs against their negative effects on gastrointestinal mucosa and renal function. Another popular analgesic option is butorphanol, an opioid agonist-antagonist, given at 0.02 to 0.1 mg/kg intramuscularly every 6 to 8 hours or as a constant rate infusion at 13 μg/kg/h.[470] This route appears to have minimal effects on gastrointestinal motility.

Antimicrobial

Because *Clostridium* spp. are suspected as a causative agent of DPJ, penicillin, metronidazole, or both may be administered to affected horses in geographic locations in which said organisms appear prevalent. Broad-spectrum antimicrobial therapy may be considered for horses with DPJ and significant leukopenia.

Nutritional Support

The veterinarian should consider the nutritional needs of horses with DPJ. Most horses have a total body protein loss because of cachexia and a protein-losing enteropathy. Total or partial parenteral nutrition may be indicated in horses that remain anorectic for more than 3 to 4 days. Parenterally administered solutions containing glucose, balanced amino acid solutions, lipid emulsions, balanced electrolyte and trace minerals, and vitamins have been administered to adult horses with small intestinal ileus or enterocolitis. Based on a small number of horses, this therapy has proved promising in terms of minimizing protein losses and decreasing the duration of illness. Postoperative parenteral nutrition ameliorates clinicopathologic evidence of starvation following small intestinal resection and anastomosis in adult horses.[471,472] Providing for part of the nutritional requirements of the horse (8000–12,000 kcal/day) is possible with glucose–amino acid solutions, which are of moderate cost. It is reasonable to suppose that providing nutritional support to an anorectic, severely ill horse will facilitate healing and even shorten the duration of illness. Thus the overall cost of providing parenteral nutritional supplementation to horses with DPJ may be offset by quicker recovery and diminished requirements for other, expensive treatments, but further data are necessary to accurately assess this point.

Prokinetics

Normal (healthy) intestine is necessary for optimal performance of most prokinetic agents in horses. Many motility-modifying agents likely are ineffective in cases of DPJ. Inflamed jejunal tissue has been shown to have downregulation and decreased production of motilin receptors, which may alter the prokinetic response to erythromycin.[473] However, some benefit may come of the judicious use of prokinetic agents in inflammatory conditions of the equine intestine, particularly if the agent provides additional effects. Lidocaine has shown particular promise for this use. Although the exact mechanism of action is unknown, the beneficial effects in horses with ileus are likely caused by antiinflammatory effects because a direct prokinetic effect has not been demonstrated.[474] In horses with nasogastric reflux attributable to POI or enteritis, a constant rate infusion of lidocaine was shown to reduce the hourly

volume of reflux and shorten the time to cessation of reflux compared with saline.[475] Prokinetic therapy with erythromycin lactobionate, metoclopramide, or bethanechol can also be considered.[476,477] Motility-modifying agents and the influence of inflammation on their effects are discussed in Gastrointestinal Ileus later in this chapter.

Surgical Therapy

Medical therapy is successful in most cases of DPJ. For horses with DPJ that continue to produce copious enterogastric reflux despite aggressive medical treatment, or when a mechanical obstruction cannot be satisfactorily ruled out, surgery may be considered. Refractory cases have improved with surgical intervention; however, some horses with refractory DPJ have been observed to recover after prolonged (up to 20 days) supportive care and gastric decompression. The latter is not recommended in the author's experience. For a horse exhibiting abdominal discomfort with small intestinal distention palpable per rectum and more than 2 L of gastric reflux, the veterinarian should recommend referral to an appropriate surgical facility. Typically, the main determinants for surgical intervention are degree and duration of abdominal pain, PF analysis, and results of (often repeated) rectal palpation and ultrasonography.

Short-term mortality rates have been reported to be 37% for horses in which manual evacuation of the small intestine into the cecum was performed surgically, compared with 40% for horses that received medical treatment.[478] A 95% recovery rate was reported in horses with DPJ that underwent laparotomy and manual evacuation of the small intestine into the cecum, combined with treatment for *C. perfringens* consisting of IV metronidazole and intramuscular procaine penicillin.[479] Drawbacks of surgery include the expense, risks of anesthesia, prolonged return to training, and risk of incisional complications. However, when an obstruction cannot be ruled out, these concerns are of minor consequence.[466]

PROGNOSIS

Survival rates for DPJ range between 25%[460] and 94%.[19,460] At present the survival of horses with DPJ that undergo surgery is much greater than previously described, and certainly greater than that of horses with small intestinal obstruction that do not have surgery.[478,479] Horses with DPJ that receive appropriate therapy have a reasonably good chance of making a full recovery, and recurrence is rare. Horses that continue to have frequent episodes of voluminous nasogastric reflux and systemic signs of endotoxemia and sepsis have a poorer prognosis for recovery. Frequent complications of DPJ include laminitis, thrombophlebitis, and weight loss. There appears to be geographic variation in the severity and prognosis for DPJ, with horses in the southeastern United States most severely affected.

EQUINE CORONAVIRUS

Equine coronavirus (ECoV) is classified as a betacoronavirus and is associated with fever, lethargy, anorexia, and enteric disease (colic and/or alteration in fecal consistency) in some horses.[480-482] A definitive association between ECoV and diarrhea in foals was not recognized before the year 2000[483,484] or in adult horses before 2011.[480,481] Diagnosis is confirmed via fecal PCR.[480,482] Mortality is typically low (7%),[480,482] with death caused by suspected[482] or confirmed[485] hyperammonemia and neurologic disease or severe endotoxemia and sepsis.[482,485] In some outbreaks, the fatality rate has been higher (27%), with fecal viral load higher in nonsurvivors.[486] Histologically, ECoV is associated with necrotizing enteritis, particularly in the jejunum and ileum, with hemorrhage in the ventral colon in nonsurviving horses in one report.[485]

INFLAMMATORY SMALL INTESTINAL DISEASES ASSOCIATED WITH MALABSORPTION AND MALDIGESTION

Malabsorption and maldigestion are commonly recognized clinical problems in humans and small animals and documented clinical entities in the horse. The term *malabsorption* implies impairment of digestive and absorptive processes arising from functional or structural disorders of the small intestine and related organs, the pancreas, liver, and biliary tract. The condition can affect absorption of carbohydrates, proteins, fats, vitamins, minerals, and to a lesser extent, water and electrolytes. In horses, the resulting pathophysiologic changes may influence large intestinal function adversely through alterations in the substrate presented for fermentation or through direct infiltration of the large colon.

Differentiation between carbohydrate, protein, or fat malabsorption is not possible in the horse because of the herbivorous diet and the contribution of large intestinal functions. The rarity of pancreatic problems, such as exocrine pancreatic insufficiency, in horses along with their herbivorous diet makes maldigestion less problematic, but maldigestion can contribute to chronic weight loss in horses with severe infiltrative small intestinal disease and exacerbate diarrhea in the suckling foal through reduced intestinal bile salt concentrations from hepatic or ileal dysfunction.

Malabsorption is not synonymous with diarrhea, although diarrhea may be a feature. Adult horses rarely exhibit diarrhea with small intestinal problems unless large intestinal involvement is concomitant. Chronic diarrhea is predominantly a large intestinal disorder that reflects an overload of water and electrolytes and thus may be considered a state of impaired absorption. Primary small intestinal disease is more likely to occur in neonates and young foals. For example, acquired small intestinal brush border lactase deficiency may result in increased lactose fermentation in the large intestine and induction of osmotic diarrhea.[487]

The clinical signs of chronic wasting and poor body condition, although nonspecific for a diagnosis of malabsorption antemortem, can typically be attributed to proliferative or inflammatory intestinal disorders, often collectively referred to as *chronic inflammatory bowel diseases* (CIBD).[488]

Clinical Assessment

The primary clinical sign associated with diseases of malabsorption and maldigestion is weight loss with or without diarrhea. Physical, ultrasonographic, and laboratory assessment is as described for other gastrointestinal diseases and detailed in Diagnostic Evaluation earlier in this chapter.

Rectal biopsy is easy to perform and may provide an indication of cellular infiltration that could be present at more proximal locations, but interpretation is often difficult. In one retrospective study, inflammatory bowel disease was

diagnosed from rectal biopsy specimens in approximately 50% of cases.[489] In that report, simple proctitis (neutrophils in the crypt or surface epithelium) was associated with inflammatory disorders, whereas only mild scattered neutrophil infiltration was seen in controls. Rectal biopsy aided diagnosis for 3 of 7 horses with lymphocytic-plasmacytic enterocolitis[490] and 1 of 2 horses with eosinophilic enterocolitis.[491] Tables 12.3 and 12.4 present the clinicopathologic and pathologic features of the diseases most commonly associated with malabsorption. In the same animal the extent and severity of pathologic changes differ in different regions of the small and large intestines, influencing the severity of clinical signs and abnormalities in tests of intestinal function. Early diagnosis remains a challenge, and even multiple intestinal biopsies taken at exploratory laparotomy may prove unhelpful.

Biopsies of skin, liver, lymph node, or lung may reveal evidence of multisystemic disease and can be easily obtained in the standing horse, with organ biopsies typically obtained with ultrasonographic guidance. Duodenal mucosal biopsies can be obtained endoscopically. Exploratory laparotomy facilitates rigorous inspection of the gastrointestinal tract and associated organs to obtain multiple biopsies from intestinal sites and lymph nodes. Cost and potential postoperative complications may limit surgical procedures for diagnosis. Laparoscopy may provide an alternative means to facilitate biopsy of certain tissues but is typically more helpful from a diagnostic rather than therapeutic standpoint. From this perspective, surgical exploration should be considered as an option early in the process rather than as a last resort.

Carbohydrate absorption tests, described in Diagnostic Evaluation earlier in this chapter, can provide a practical and inexpensive means to assess the absorptive capability of the small intestine. Pathologic changes in the mucosa and submucosa must be extensive and widely distributed to greatly affect the peak plasma concentration and shape of the curve. In one report of 42 mature horses with chronic weight loss, a normal OGTT (peak glucose concentration at 120 minutes >85% baseline) was not associated with abnormal small intestinal morphology in any of the 5 horses in which it was documented. When peak glucose concentration was between 15% and 85% of baseline at 120 minutes (considered partial malabsorption), approximately 72% had small intestinal infiltrative disease, and when peak concentration at 120 minutes was less than 15% above baseline (total malabsorption), all had severe small intestinal infiltrative disease.[59] Other reports have documented horses with flat OGTT curves that subsequently showed more normal OGTT responses and resolved clinical condition.[492] Carbohydrate absorption tests probably have poor diagnostic sensitivity to detect small intestinal involvement in horses with chronic diarrhea and predominantly large intestinal problems.[493] An abnormal absorption test and weight loss can occur in the horse as a transient event and without significant morphologic changes in the small intestine.

The intestinal sugar active transport system has a low affinity for D-xylose in the equine jejunum in vitro, hence D-xylose absorption likely occurs primarily by convection or diffusion.[494] An abnormal D-xylose absorption test likely indicates abnormal mucosal surface area or permeability and has been observed in horses with CIBD, parasitism, and idiopathic villous atrophy.[488,495] Abnormal absorption curves have been detected in the absence of small intestinal histologic changes,[496] and interpretation is clouded further by findings from small intestinal resection studies in healthy ponies. One study demonstrated a progressive decline in mean peak xylose concentration after 70% distal small intestinal resection in ponies despite their normal clinical appearance and absence of diarrhea.[497] In another study, a similar decline in peak xylose concentration following extensive (≥60%) small intestinal resection was accompanied by weight loss, diarrhea, and ill thrift.[498] Peak xylose concentrations were much lower in horses with granulomatous enteritis than those with eosinophilic granulomatosis (EG), whereas in EG the absorption curve shifted to the right, with the peak occurring at 240 minutes.[499] This is not surprising given the typical lesion distribution with these disorders. As with the OGTT, results of the xylose absorption test can improve following therapy.[500]

Alimentary Lymphosarcoma

Alimentary lymphosarcoma of the horse may represent a primary neoplasia of the gut-associated lymphoid tissue with significant cellular infiltration of the small intestine and associated lymph nodes with minimal large intestinal or systemic involvement. Case series and pathology reports indicate that young horses 2 to 4 years of age primarily are affected, although the age range can be broad.[501-503] No breed or sex predilection has been documented, and disease prevalence is unknown. Despite the progressive nature of lymphomata, onset of clinical signs can be rapid, and the animal may become acutely ill. As with all adult cases of CIBD, antemortem diagnosis is by a process of exclusion and usually is confirmed postmortem. Frequent abnormalities include anemia, thrombocytopenia, neutrophilia or neutropenia, and hypoalbuminemia with hyperglobulinemia, resulting in either a normal elevated serum protein. Lymphocytosis is rare. Intraabdominal masses such as enlarged mesenteric lymph nodes may be palpated per rectum. Abdominocentesis and rectal biopsy can provide a diagnosis but are not sensitive indicators of disease. Carbohydrate absorption tests usually reveal partial to total malabsorption indicative of the severely reduced surface area resulting from significant villous atrophy and the extensive mucosal or transmural infiltration. Confirmation of a diagnosis requires exploratory laparotomy to obtain multiple intestinal and lymph node biopsies if rectal biopsy and/or abdominocentesis are normal. Prognosis is poor, especially because most horses are presented in an advanced state of disease. Immunosuppressive drugs or chemotherapy may afford temporary improvement, but long-term outcome is unaffected.

Granulomatous Enteritis

Granulomatous enteritis was first described as a chronic wasting condition in 1974[504]; 9 of 10 horses were young Standardbreds. Most affected horses are 2 to 3 years of age. Case reports from many countries revealed a predominance of Standardbred over Thoroughbred horses by three to one.[499,505] Some of the Standardbreds were related, implicating a genetic predisposition, but this has not been proven. Prevalence of this disease is low. The condition is sporadic and has an insidious onset, and the course can be protracted. Significant diagnostic features include anemia, slight increases or decreases in WBC counts, hypoalbuminemia, normal serum protein or hypoproteinemia, occasional increases in serum AP activity, normal serum GGT activity, and enlarged mesenteric lymph nodes on rectal palpation. Partial or complete malabsorption is typically documented via carbohydrate absorption testing. One can attribute the low proportion of horses exhibiting diarrhea

TABLE **12.3** **Predominant Clinical and Clinicopathologic Features of Horses with Proliferative and Infl ammatory Bowel Diseases**

Condition	Breed	Age Range	Clinical Signs	Dermatitis/Coronitis	Hematology	Chemistry	Absorption Tests
Alimentary lymphosarcoma	None	2 yr to aged; Majority ≤4 yr	Weight loss, poor appetite, edema, depression, occadional fever, occasional diarrhea or colic	+/– Scurfy skin	Anemia, neutrophilia; lymphocytosis rare	Decreased albumin; TP normal to increased; increased globulin	Reduced absorption; partial to complete malabsorption
Granulomatous enteritis	Standardbred	1-6 yr; Majority ≤3 yr	Severe wasting, edema, variable appetite, depression, infrequent diarrhea, occasional slight fever	+/– Scurfy skin; severe lesions rare	Anemia; Leukocytes normal to slightly increased or decreased	Decreased albumin; TP normal to decreased; GGT normal, ALP normal to increased	Reduced absorption; partial to complete malabsorption
Multisystemic eosinophilic epitheliotropic disease	Standardbred, Thoroughbred	1 yr to aged; Majority ≤4 yr	Severe wasting, edema, appetite poor to ravenous, slight fever, diarrhea or soft feces common, rare colic, depression, oral ulcers	++++ Severe skin lesions and ulcerative coronitis prominent	Anemia rare to slight; neutrophilia and eosinophilia rare	Decreased albumin; TP normal to decreased; GGT and ALP normal to increased	Delayed absorption (peak shifted to right); reduced or normal peak concentration
Lymphocytic plasmacytic enterocolitis	None	3 yr to aged	Inappetence, depression, colic, edema		Normal	Decreased albumin and TP; increased fibrinogen	Inadequate absorption
Proliferative enteropathy	None	3-8 months; sporadic reports older	Depression, colic, diarrhea, edema, appetite often normal, concurrent infection	+/– Scurfy skin	Anemia, leukocytosis	Decreased albumin and TP; increased CK	Often normal

**GGT,* γ-Glutamyltransferase; *ALP,* alkaline phosphatase; *CK,* creatine kinase; *none,* no predominant breed.

TABLE **12.4 Pathologic Features of Proliferative and Inflammatory Bowel Diseases of Horses**

Condition		Small Intestine	Large Intestine	Other Organs/Systems
Alimentary lymphosarcoma	G	*Constant;* extensive thickening, thickened mucosa, fissures, serosal plaques, nodules, congestion	*Infrequent;* unremarkable to thickened segments	MLNs massively enlarged; occasional enlargement of other LNs
	H	Villous atrophy (partial to total); crypts disappear with hyperplasia; infiltrate of pleomorphic lymphoid cells, plasma cells; transmural	Nothing evident to diffuse mucosal infiltration	Extensive infiltration of MLNs; moderate in other LNs (liver, spleen, stomach rare)
Granulomatous enteritis	G	*Constant;* thickened wall and mucosa, fissures, widespread ulceration (tiny ulcers)	*Common,* generally discrete	MLNs enlarged, edematous; stomach commonly affected (generally discrete); liver/pancreas rare
	H	Villous atrophy (partial to total), crypt hyperplasia and abscesses, diffuse granulomatous inflammation; mononuclear cells (lymphoid), giant cells, epithelioid foci; lymphangiectasia	Similar infiltrate usually discrete; mucosa, submucosa	Similar infiltrate; stomach discrete; MLNs discrete to florid macrophage infiltration; diffuse cortical hyperplasia
Multisystemic eosinophilic epitheliotropic disease	G	*Common;* diffusely thickened, especially proximal duodenum and distal ileum; serosal nodules or granularity; ulceration	*Constant;* severe; segmental or multifocal granuloma; mucosal (predominantly) and transmural thickening; extensive ulcers	MLNs and other LNs enlarged; stomach and esophagus commonly affected; liver/pancreas commonly affected; may be hyperkeratotic Skin: exudative dermatitis, ulcerative coronitis
	H	Villous atrophy rare; lymphocytic and eosinophilic infiltration most severe in cranial duodenum, ileum, ileocecal junction; infiltrate more widespread than gross lesions	Segmental/multifocal lesions, severe infiltration, reactive fibrosis, tissue eosinophilia, walled-off granulomata, central necrotic core of eosinophilic material	Similar infiltration with fibrosis of MLNs, liver, pancreas Skin: acanthosis, hyperkeratosis, diffuse infiltrate of eosinophils, lymphocytes in dermis; focal eosinophilic accumulations
Lymphocytic-plasmacytic enterocolitis	G	*Constant;* mucosal/submucosal edema; prominent folds	*Common;* edema, congestion, areas of mucosal ulceration	MLNs enlarged
	H	Villous blunting to atrophy; moderate to severe infiltration of lymphocytes, plasma cells; edema, dilated lymphatics	Similar infiltrate, less remarkable	Minimal evidence
Proliferative enteropathy	G	*Constant;* significant mucosal thickening, corrugated appearance from proximal jejunum to distal ileum	*Uncommon;* submucosal edema	MLNs unremarkable
	H	Villous shortening, severe hyperplasia of crypt epithelium, small curved bacteria in apical cytoplasm, mononuclear infiltrate	No evidence	No evidence

G, Gross pathologic findings; *H,* histopathologic findings; *LNs,* lymph nodes; *MLNs,* mesenteric lymph nodes.

to the preferential distribution of inflammatory infiltration in the small intestine.[506] Rectal biopsy can be a useful aid to diagnosis.[489]

The cause of granulomatous enteritis is unknown. Several infectious agents have been implicated, including *Mycobacterium avium.*[507] The condition may represent a granulomatous hypersensitivity reaction. Immunomediated responses to dietary, parasitic, or bacterial antigens may be important initiating factors.[488] Six horses purported to have granulomatous enteritis were linked to environmental contamination with aluminum,[508] although problems existed regarding the case definition, data, and interpretation.[509]

Treatment of horses with granulomatous enteritis with a variety of drugs, particularly corticosteroids, has not affected the long-term outcome in the majority of cases.[510] Prolonged (5 months) corticosteroid administration produced clinical

remission and a favorable athletic outcome in a 6-year-old Standardbred gelding based on improvement in clinical signs and in D-xylose absorption.[500] Surgery may be indicated with localized disease. Two young horses underwent resection of the thickened terminal small intestine; one horse died 4 months after surgery, and the other remained clinically normal for at least 10 years.[505]

Multisystemic Eosinophilic Epitheliotropic Disease

Multisystemic eosinophilic epitheliotropic disease (MEED) encompasses disorders characterized by a predominant eosinophilic infiltrate in the gastrointestinal tract, associated lymph nodes, liver, pancreas, skin, and other structures accompanied by some degree of malabsorption and enteric protein loss. The disorders include chronic eosinophilic gastroenteritis,[511] EG,[499] chronic eosinophilic dermatitis,[512] and probably basophilic enterocolitis.[513]

Although prevalence is low, MEED appears to be more common than granulomatous enteritis. Most affected horses are 2 to 4 years of age, and Standardbreds and Thoroughbreds are reported to predominate. The condition is sporadic, has an insidious onset, and often a protracted course (duration of 1–10 months). Diarrhea is common. Severe skin lesions with exudative dermatitis and ulcerative coronitis are prominent and frequently are the principal presenting complaint. Despite extensive tissue eosinophilia, systemic eosinophilia is rare, and hematologic values are usually unremarkable. Notable features include hypoalbuminemia and elevations in serum GGT and AP activities. Most reports of carbohydrate absorption test findings indicate a reduced or normal peak concentration delayed for at least 180 minutes. Morphologic changes are less pronounced in the small intestine than in the large intestine,[514] and small intestinal lesions predominate segmentally in the proximal duodenum and distal ileum. Significant hyperkeratosis of the fundic region may contribute to gastric muscle contractile disruption. Diarrhea can be a consequence of the severe segmental or multifocal granulomatous lesions in the large intestine with mucosal and transmural thickening and extensive ulceration. Abundant fibrosis is a feature of all affected tissues.

The cause of MEED is unknown, and the disease may represent a chronic ongoing immediate hypersensitivity reaction against undefined antigens ingested or excreted into the lumen from parasitic, bacterial, or dietary sources. Infectious agents have not been identified.[511,512] Eosinophilia is a feature of parasitism in the equine intestinal tract, although nematodes rarely have been identified in any lesions of MEED.[511,515] Failure to detect larval structures in these lesions, however, may be attributable to chronicity of the disease and destruction of the parasites in tissue.[505] Biopsies of the rectal mucosa[489] or of the skin, liver, intestinal tract, and lymph nodes may assist in diagnosis. Unlike the other conditions associated with malabsorption/maldigestion in horses, MEED has definitive liver and pancreatic involvement; thus, maldigestion may contribute to the wasting disease.

Treatment has been attempted with a variety of drugs, including antibiotics, corticosteroids, and anthelmintics with larvicidal activity. Although some horses can improve briefly, the long-term prognosis is poor.

Eosinophilic Enterocolitis

Idiopathic eosinophilic enterocolitis affects segmental lesions in the small or large intestine, inducing signs of colic, often requiring surgical intervention.[505,516,517] This problem may not involve evidence of malabsorption and does not have multisystem involvement. Because the problem is often associated with signs of colic and not signs of malabsorption, eosinophilic enterocolitis differs from the other conditions discussed in this section and is often diagnosed at the time of surgery. It carries a much better prognosis than the other inflammatory bowel diseases.

Lymphocytic-Plasmacytic Enterocolitis

The morphologic findings in lymphocytic-plasmacytic enterocolitis reflect the predominant infiltrative cellular elements of this rarely encountered condition. No specific clinical or clinicopathologic features differentiate this condition antemortem from other inflammatory diseases of adult horses. In a retrospective study of 14 horses, carbohydrate absorption was abnormal or delayed in 9 of 12 horses, consistent with the predominance of small intestinal pathologic changes.[490] Rectal biopsies were abnormal in 3 of 7 horses, two of which were reported as having lymphocytic-plasmacytic proctitis. Prognosis is typically reported as poor, likely caused by the advanced nature of the condition at the beginning of treatment. In the author's experience, some horses with small intestinal mural thickening, lymphocytic-plasmacytic infiltrate evident on duodenal biopsy, and signs of abdominal pain and/or weight loss may improve with dietary modification and/or corticosteroid therapy.

Proliferative Enteropathy

Proliferative enteropathy (PE) typically affects weanling foals from 3 to 8 months of age and has been reported in North America, Europe, and Australia, causing disease in individuals or outbreaks of multiple affected animals on the same premise.[518-522] PE is uncommon in yearlings and adult horses.[518,523] The disease affects many other species, namely swine, and is caused by *L. intracellularis,* an obligate intracellular bacterium found in the cytoplasm of proliferative crypt epithelial cells of the jejunum and ileum.[518,524,525]

Like pigs, horses are affected as weanlings. The incubation period is 2 to 3 weeks in nonequine species and is presumed to be similar in horses. In some epidemiologic investigations, close proximity to swine operations was apparent, but in most instances such an association was not evident.[518,523] Comparisons of epidemiologic findings from the swine disease indicated that overcrowding, feed changes, antibiotic usage, and mixing and transportation were potential risk factors at two of the farms in one study, and recent weaning appears to be a common risk factor.[518] The window for exposure appears narrow,[526] and multiple cases on a given farm are common. Affected animals shedding the organism in the feces serve as a source of infection for herdmates. It is possible that nonequine species serve as reservoirs contributing to outbreaks on horse farms.

Profound hyperplasia of the mucosa associated with proliferation of crypt epithelium and crypt hyperplasia is induced locally in infected islands of tissue that eventually extend to the entire distal jejunum and ileum. *L. intracellularis* preferentially infects proliferating cells; thus, it is the tropism for the crypt epithelium. Infected cells proliferate far more rapidly than uninfected cells, suggesting that *L. intracellularis* directly

induces the proliferative response, but the molecular basis for enhanced proliferation is not known. *L. intracellularis* penetrates epithelial cells in a membrane-bound vesicle but eventually escapes the vacuole and is found free in the cytoplasm, concentrated at the apical pole of the cell. The gross pathologic lesions of equine PE are quite characteristic.[518] Lesions may be segmental and are most commonly found in the ileum and terminal jejunum in horses, but the duodenum may also be affected. Severe mucosal hypertrophy is often observed but may wane during the chronic stages of the disease. The mucosa may become corrugated with focal erosions or ulcers. Submucosal edema is often readily identified on cut sections of affected segments. Moderate to severe crypt hyperplasia with atrophy of intestinal villi is a consistent feature. Hyperplastic crypts are branched and may herniate into the submucosa. Necrosis, edema of the submucosal and lamina propria, hemorrhage, mononuclear inflammation, and muscular hypertrophy have been reported in affected intestinal segments but are not consistent. Special stains such as silver stain are required to detect intracellular organisms. The organisms are curved or comma-shaped rods found clustered in the apical cytoplasm of hyperplastic crypt epithelium. The proliferative response of the intestinal mucosa alters absorption of nutrients and fluid secretion by disrupting the architecture of the villi and by altering the maturation of epithelial cells into absorptive cells, accounting for the secretory diarrhea and often severe weight loss. The combined effects of the inflammatory response and malabsorption may account for the clinically observed protein-losing enteropathy.

Clinical signs include depression, rapid and significant weight loss, edema, diarrhea, and colic.[518] Poor body condition, a rough hair coat, and potbelly appearance are also reported. Not all clinical signs are present in all cases, and diarrhea has only been observed in approximately half of reported cases. Other problems often were concurrent, including respiratory tract infection, dermatitis, intestinal parasitism, and gastric ulceration. The most significant laboratory finding is profound hypoproteinemia, predominantly characterized by hypoalbuminemia; panhypoproteinemia can also occur.[518,521,527] Leukocytosis and hyperfibrinogenemia are also common, with occasional alterations in electrolytes (hyponatremia, hypokalemia, and hypochloremia) and elevated serum creatine kinase concentrations. Abdominal ultrasound commonly reveals increased small intestinal mural thickness.[521,527] Although small intestinal mural thickness >3 mm, in conjunction with clinical and clinicopathologic signs, is highly suggestive, PE should not be ruled out in the absence of this finding. Colloid oncotic pressure, if measured, is typically low.[527]

PE should be considered in a weanling foal with compatible clinical signs and severe hypoalbuminemia with exclusion of common enteric infections. Fecal PCR has very high specificity but variable sensitivity for confirmation of diagnosis.[528] Serum immunoperoxidase monolayer assay or indirect enzyme-linked immunosorbent assay (ELISA) are highly specific for exposure; submission of fecal PCR and serologic testing is recommended, although both tests are quite specific, because they can lack sensitivity, especially early in the course of disease (serology) or with prior antimicrobial therapy (fecal PCR).[518,527,529] Of note, fecal PCR can become negative in affected foals within 4 days of antimicrobial therapy.[530] PE is not typically associated with abnormal carbohydrate absorption test results.[518,530] In horses with diarrhea, other infectious causes should be ruled out. A definitive postmortem diagnosis can be confirmed by identifying characteristic mural thickening and intracellular bacteria within the apical cytoplasm of proliferating crypt epithelial cells using silver stains, PCR, and/or immunohistochemical testing.[518]

Antimicrobial therapy with erythromycin, alone or with rifampin, azithromycin, clarithromycin, oxytetracycline, doxycycline, metronidazole, or chloramphenicol has been reported.[518,519,527] Macrolides should not be used in adults or older foals because of an increased risk of colitis.[529] Recent reports favor the use of IV oxytetracycline, followed by oral doxycycline or minocycline, with apparent success.[519,527] Duration of therapy is typically 2 to 3 weeks. Affected foals often need supportive therapy including crystalloid fluid and electrolyte replacement and, potentially, colloid support. Nonsteroidal antiinflammatory therapy can be used as needed for significant pyrexia. Corticosteroid therapy is not indicated, because inflammation is not a significant pathologic finding. Response to therapy has been good, with reported survival rates between 82% and 93%.[518,519,527] Rapid improvement in clinical signs, even within 24 hours, precedes the rise in plasma protein concentration.

Foals with PE should be isolated from unaffected animals for at least 1 week after institution of antimicrobial therapy to avoid shedding of organisms into the environment. Intrarectal vaccination with an avirulent live vaccine marketed for use in pigs has demonstrated a protective effect in foals experimentally challenged with *L. intracellularis*.[531]

Miscellaneous Conditions

Abnormal D-xylose absorption has also been noted in association with AA amyloid-associated gastroenteropathy in an 18-year-old Morgan stallion[532] and a horse with a gastric mass and secondary small intestinal villous atrophy.[533]

Management, Therapy, and Outcome

The chronic wasting horse with suspected malabsorption and probable enteric protein loss generally has a guarded to poor prognosis. Diseases with a good prognosis include eosinophilic enteritis and PE. Prognosis may be improved through early and aggressive investigation to achieve a diagnosis. The owner must be cognizant from the start that the outcome may not be altered, even after protracted therapy; only a few case reports of successful responses with long-term follow-up have been documented.

Nutrition

Some level of digestive and absorptive capability remains in the diseased small intestine. Interval feeding of small quantities of easily digestible food may be beneficial. Diet may include feeds with a high fiber, but potentially low bulk. Some commercially available complete pelleted feeds offer high-fiber rations based on beet pulp and soybean hulls. Some affected horses may tolerate increased dietary fat. Consultation with an equine nutritionist may provide additional options for a given horse and, in the author's experience, can prove beneficial. The objective for an affected horse is to sustain, and preferably increase, dietary intake, value, and efficiency. The owner of an affected horse must be prepared to experiment with feeds slowly and deliberately, must be patient, and must keep records. Exposure to a feed component may contribute to the problem as an allergen eliciting a hypersensitivity reaction. Identifying the potential allergen through immunologic testing or by stepwise removal and outcome assessment over a longer period may be difficult.

Pharmacologic Therapy

Immunosuppressive agents have produced the most promising responses to ameliorate the effects of conditions associated with malabsorption, particularly CIBD. Short-duration, and in some cases more prolonged and sustained, improvements in body condition, weight gain, demeanor, energy, and activity levels have occurred following corticosteroid administration. Treatment should be initiated as early as possible in the course of disease. Initial parenteral (intramuscular or IV) loading doses of dexamethasone (sodium phosphate) should be followed with a series of depot injections, or orally administered prednisolone, on a tapered dose protocol over a period of months. Interval low-dose therapy may be necessary if clinical signs return after treatment ends. The lowest dose necessary to control the clinical signs with alternate-day therapy should be used. Clinical benefits far outweigh concerns over potential adverse effects. Chemotherapeutic agents such as vincristine, cytosine, cyclophosphamide, and hydroxyurea have been tried in a few cases of CIBD or lymphosarcoma with no apparent success, probably related to the advanced stage of the disease when treatment was initiated and the dose selected.

SURGERY

Resection of a segment of intestine that is edematous, hemorrhagic, or constricted is an option in localized forms of CIBD,[505,517] particularly if gross changes are not discernible in adjacent or distant parts of the intestinal tract—that is, malabsorption is not a feature. Long-term outcome has been favorable. Removal of a substantial proportion of the diseased small intestine may be indicated in a horse with malabsorption, considering that resection of 70% distal small intestine was performed in healthy animals without inducing adverse effects.[497] Because pathologic changes may exist in normal-appearing small or large intestine that is not resected or biopsied, the prognosis remains guarded. Two young horses with granulomatous enteritis had the thickened terminal small intestine resected with positive outcomes; one survived 4 months, the other has a follow-up extending more than 10 years.[505]

Inflammatory Diseases of the Large Intestine

Acute diarrhea caused by colitis in adult or young horses is a potentially life-threatening disorder with a variety of possible etiologies (Table 12.5) characterized by hypersecretion of fluid, motility disturbances, and an impaired mucosal barrier resulting from direct injury or inflammation. Many of the clinical and clinicopathologic features are similar regardless of the underlying cause. Severe dehydration with profound electrolyte abnormalities is common, as is systemic inflammation secondary to absorption of endotoxin or other bacterial products through compromised gastrointestinal mucosa. Severe cases may be complicated by serosal inflammation and mural ischemia and infarction as a direct extension of mucosal inflammation or secondary to coagulopathies. The diagnostic approach for horses with acute diarrhea is aimed at determining the underlying etiology but must be accompanied by clinical and laboratory assessment of hydration, electrolyte and acid-base balance, organ function, and evaluation of the degree of systemic inflammation and the integrity of the intestinal wall. The therapeutic approach for horses with colitis, regardless of cause, consists primarily of controlling local and systemic inflammation, maintaining fluid and electrolyte balance, and promoting mucosal repair. In addition, some horses with acute colitis require specific therapy aimed at the underlying etiology.

INFECTIOUS DISEASES

Salmonellosis

Pathogenesis

S. enterica is a species of gram-negative facultatively anaerobic bacteria that is a common gastrointestinal pathogen in horses. Many serovars of *S. enterica* have been reported to infect horses, but those classified in group B appear to be more commonly associated with disease than those in other groups. Group B includes *S. enterica* var. Typhimurium and *S. enterica* var. Agona, two of the species most frequently isolated from horses.[534-536] *S. enterica* var. Typhimurium is the most pathogenic serotype in horses and is associated with a higher case fatality rate than other serovars of *S. enterica*.[534] The number of horses that are inapparently infected with and actively shed *S. enterica* in their feces has been reported to be as high as 10% to 20%, but actual prevalence of *S. enterica* shedding in the general horse population is likely to be much lower, less than 2%.[537] Horses shedding *S. enterica* are a potential source of infection to susceptible horses,[534,538] as are environmental reservoirs.[539-541] For these reasons salmonellosis is one of the most common nosocomial diseases in horses. Nosocomial salmonellosis significantly affects morbidity and mortality in hospitalized horses.[542] The emergence of multidrug resistance in equine *S. enterica* isolates has been a cause of concern because of the importance of salmonellosis as a nosocomial disease and because a number of serovars of *S. enterica* are significant zoonotic pathogens.[540,543-546]

The virulence of the bacteria varies tremendously with serotype and even among strains of the same serotype. This is because of the important role of host susceptibility in the pathogenicity of particular organisms. The infective dose is generally on the order of millions of organisms inoculated orally, but various environmental and host factors can reduce the infective dose to a few thousand or even hundreds of organisms.[547-549] Environmental factors or stresses that increase susceptibility to *S. enterica* infection are not well defined, but it is known that high ambient temperature, for example, can greatly increase the prevalence of salmonellosis in horses.[539,548,549] Indeed, the peak incidence of salmonellosis in horses occurs in late summer and fall.[539,548,549] Other environmental and host factors that are associated with salmonellosis or shedding of *S. enterica* organisms in feces include transportation, antibiotic administration before or during hospitalization, gastrointestinal or abdominal surgery, general anesthesia, preexisting gastrointestinal disease (e.g., colic, diarrhea), the presence of leukopenia or laminitis during hospitalization, prolonged hospital stay, change in diet, and immunosuppression.[534,541,549-551] Interestingly, foals with gastrointestinal disease are more likely to shed *S. enterica* organisms than are adult horses with gastrointestinal disease.[548]

Host factors that restrict gastrointestinal colonization and invasion by pathogens include gastric pH, commensal

TABLE **12.5 Differentials and Diagnosis of Some Causes of Acute Diarrhea in Adult Horses**

Category	Differentials	Diagnosis
Infectious	Salmonellosis	Fecal culture (five consecutive) Fecal PCR
	Clostridium perfringens	Quantitative fecal culture Fecal toxin immunoassay or PCR
	C. difficile	Fecal culture Fecal toxin immunoassay or PCR
	Neorickettsia risticii	Fecal or blood PCR Serology
	Coronavirus	Fecal PCR
Parasitic	Strongylosis	Fecal egg counts Cranial mesenteric artery palpation Serum IgG(T)
	Cyathostominosis	Fecal egg count Rectal biopsy Cecal or colonic biopsy
Toxic	NSAID	History and clinical signs Right dorsal colon ultrasonography Laparoscopy or laparotomy
	Cantharidin	History of exposure Fecal or urine cantharidin concentrations
	Arsenic	History of exposure Fecal, blood, urine, or tissue arsenic concentrations
Miscellaneous	Carbohydrate overload	History of inappropriate ingestion of carbohydrate Blood lactate concentration
	Sand enteropathy	Auscultation of ventral colon Fecal sand content Abdominal radiography

Ig, Immunoglobulin; *NSAID,* nonsteroidal antiinflammatory drugs; *PCR,* polymerase chain reaction.

gastrointestinal flora, gastrointestinal motility, the mucosal barrier, and mucosal immunity.[534,552] Gastric acidity is an important defense mechanism for preventing live organisms from reaching the intestine.[552] Altering the gastric pH, with histamine H_2 receptor antagonists, for example, may increase susceptibility to infection. Gastrointestinal flora inhibit the proliferation and colonization of *S. enterica* by secreting bacteriocins, short-chain fatty acids (SCFAs), and other substances that are toxic to *S. enterica.*[552] Elements of the normal flora compete for nutrients and space, especially on the mucosa.[552] Being predominantly anaerobic, the normal flora maintain a low oxidation-reduction potential in the environment of the large intestine, which inhibits the growth of many bacterial pathogens.[553] The importance of normal host gastrointestinal ecology is illustrated by the fact that disturbances of the colonic flora with antibiotics, changes in feed, ileus, or other underlying gastrointestinal disease markedly increases the susceptibility of the host to infection by *S. enterica,* often resulting in serious disease.

The immune status of the host may be one of the most important factors determining not only the susceptibility to *S. enterica* infections but also the degree of invasion and subsequent outcome of the infection. Local immunity, such as mucosal antibody secretion and enterocyte-derived cationic peptides, prevents colonization of the mucosa.[552,554,555] Opsonizing antibodies and activation of the complement cascade are important in fighting systemic invasion by *S. enterica* by increasing the efficiency of phagocytosis and by direct bactericidal activity. Humoral immunity, however, is often ineffective in preventing disease and dissemination once invasion occurs and *S. enterica* is established in its intracellular niche. Following invasion, *S. enterica* is capable of surviving and multiplying within macrophages, rendering the humoral (noncellular) immune systems ineffective.[556,557] Specific cellular immunity may be the most effective defense mechanism in the host arsenal against dissemination and systemic infection by *S. enterica.*[557,558] Protective immunity in horses and calves may be induced by oral inoculation with small numbers of virulent organisms, but the duration of the immunity is not known.[559,560] Oral and parenteral vaccines using killed or attenuated organisms and bacterial products have been promising but are effective only against homologous organisms and are usually not cross-protective among different serogroups.[559-561]

In adult horses *S. enterica* primarily infects the cecum and proximal colon, causing enterocolitis, with limited likelihood of dissemination beyond the intestine. In foals, however, salmonellosis is often associated with septicemia. The ability of *S. enterica* to cause enterocolitis depends on the ability of the bacteria to invade the gastrointestinal mucosa.[552,556] Invasion of the gastrointestinal mucosa occurs preferentially through specialized enterocytes called *M cells* that overlie intestinal lymphoid tissues such as Peyer's patches in nonequine species. M cells are exploited by a variety of enteric pathogens

during infection of intestinal tissue.[562] Invasion of the epithelium occurs by self-induced uptake via the apical membrane of the M cell, often killing the cell in the process.[556] *S. enterica* then invades neighboring cells via the basolateral membrane, eventually spreading the destruction of the epithelium beyond the principal area of attack. Virulent *S. enterica* have a well-developed invasion mechanism that involves the generation of an apparatus called a type III secretory system that enables virulence gene products to be injected directly into enterocytes.[563] Virulence proteins injected by *S. enterica* into enterocytes engage the cellular machinery and induce the cell to engulf the bacteria by macropinocytosis. *S. enterica* virulence gene products also induce enterocyte chloride and fluid secretion and upregulate enterocyte transcription of inflammatory cytokines (TNF-α and IL-1β) and chemokines that trigger a mucosal inflammatory response.[81,556,563]

Once *S. enterica* has invaded the mucosa, organisms are quickly phagocytosed by macrophages and dendritic cells in the lamina propria and in lymphoid tissues. The ability of *S. enterica* to disseminate systemically and cause enteric fever is associated with the ability to survive and proliferate in macrophages. Indeed, phagocytes have an important role in dissemination to blood, lymph nodes, liver, and spleen.[564] The majority of *S. enterica* in the blood and tissues of animals infected with a strain of *S. enterica* that is competent to cause enteric fever are within phagocytic cells.[564] In adult horses with salmonellosis, dissemination appears to be limited to the intestine and mesenteric lymph nodes, and *S. enterica* is rarely cultured from blood. However, in foals and in some adults, *S. enterica* causes an enteric fever–like disease with dissemination to mesenteric lymph nodes, liver, spleen, and blood.

Specific virulence gene clusters called *pathogenicity islands* encoded on the chromosome or on plasmids confer the main virulence traits of *S. enterica:* invasion, enteropathogenesis, intracellular survival, and proliferation.[556] Some of the genes encoded within these islands or virulence factors are sensors that signal to the bacteria that it has entered an intracellular environment and to turn on other genes required for intracellular survival. Others, such as invasion genes, are transported from the bacteria and injected into macrophage cytosol by a type III secretory system apparatus to prevent phagosome-lysosome fusion and subvert other essential macrophage-killing mechanisms. Virulent *S. enterica* may also possess multiple genes that enable adhesion to target cells or confer resistance to reactive oxygen and nitrogen metabolites, which is, perhaps, the most lethal antimicrobial mechanisms of macrophages.[565]

Diarrhea associated with salmonellosis has multiple causes. An *S. enterica* cytotoxin inhibits protein synthesis in mucosal cells, causing morphologic damage and altered permeability.[566] Virulent *S. enterica* also produce an enterotoxin that is similar to the heat-labile (LT) toxin produced by *E. coli*.[567,568] This enterotoxin contributes to, but is not required, in the pathogenesis of diarrhea.[569,570] *S. enterica* enterotoxin increases secretion of chloride and water by colonic mucosal cells in many species, including horses, by increasing intracellular cAMP concentrations.[567,568,571]

The ability of virulent *S. enterica* to cause diarrhea appears to be most closely associated with the ability to invade enterocytes and to trigger an inflammatory reaction in the intestinal tissue.[103,556] Gene products injected into enterocyte cytosol by the type III secretory system of invading *S. enterica* stimulate chloride and fluid secretion.[563] *S. enterica* invasion of enterocytes is also a potent activator of inflammatory chemokine and cytokine production, resulting in the recruitment of leukocytes, particularly neutrophils, and activation of resident macrophages and mast cells. Products of these activated leukocytes, including prostaglandins, leukotrienes, reactive oxygen metabolites, and histamine, are potent stimulators of chloride secretion in the colon of many species.[213,552,572,573] The ENS integrates the diverse processes of pathogen recognition, triggering of the inflammatory response, and induction of enterocyte fluid secretion.[213]

Many of the inflammatory mediators studied stimulate colonic secretion by prostaglandin-dependent mechanisms, resulting in either increased intracellular cAMP or calcium concentrations, or both, in mucosal cells.[213] These mediators and the ENS may also stimulate secretion by prostaglandin-independent mechanisms, inhibit sodium and water absorption, cause motility disturbances, and potentiate tissue injury, all of which enhance the pathogenicity and dissemination of *S. enterica* and contribute to the pathogenesis of diarrhea.[213,573] Neutrophils recruited to the mucosa by signals generated by the infected enterocytes physically contribute to mucosal injury by producing a variety of products that are lethal to pathogens but are also toxic to host cells.[84,574] Neutrophils attracted to infected epithelial cells accumulate beneath the monolayer, lifting it off the basement membrane in sheets. Neutrophils also migrate across the epithelial monolayer in potentially massive numbers—enough to be detectable in feces as a marker of inflammatory diarrhea. Although the transepithelial migration of neutrophils has a benefit, positioning the host defense cell at the apical membrane to ward off attacks by invading bacteria, the mechanical disruption to the epithelial barrier may be significant enough to increase the permeability to macromolecules, bacterial products, and even bacteria.[574] Potentially massive losses of electrolytes, water, and protein can occur, depending on bacterial and host factors. Perhaps most devastatingly, mucosal injury and altered permeability allow systemic absorption of bacterial products and dissemination of bacteria, resulting in life-threatening sepsis.

Clinical Signs and Diagnosis

Four clinical syndromes of *S. enterica* infection have been documented clinically and reproduced experimentally in horses: (1) inapparent infections with latent or active carrier states; (2) depression, fever, anorexia, and neutropenia without diarrhea or colic; (3) fulminant or peracute enterocolitis with diarrhea; and (4) septicemia (enteric fever) with or without diarrhea.[575] Inapparent infections can be activated to clinical disease in compromised horses, such as horses with colic or horses being treated with antibiotics, causing mild to severe enterocolitis. Latent infections (nonshedding) can become active infections (shedding) under certain conditions, such as transportation stress and antibiotic treatment. Horses with depression, anorexia, fever, and neutropenia without diarrhea generally have a good prognosis and recover in several days without specific treatment.[575] The septicemic form is mostly restricted to neonatal foals and is uncommon in adult horses. The focus of this discussion is acute enterocolitis.

Acute enterocolitis is characterized by severe fibrinonecrotic typhlocolitis, with interstitial edema and variable degrees of intramural vascular thrombosis that may progress to infarction.[534] Severe ulceration of the large intestinal mucosa may occur, with serosal ecchymoses and congestion. The earliest signs of enterocolitis are usually fever and anorexia.[534,549] Signs of colic may be seen early in the course of the disease,

especially if ileus is present. Clinical signs of endotoxemia are common and range from fever, elevated heart and respiratory rates, poor peripheral perfusion, and ileus to fulminant and rapidly progressive signs of endotoxemic shock. Oral mucous membranes are often pale with perigingival hyperemia (a toxic rim) but may be brick red or cyanotic, with prolonged capillary refill time. Weakness, muscle fasciculations, cold extremities, and other signs suggestive of hypotensive shock; synchronous diaphragmatic flutter; abdominal pain; and marked metabolic and electrolyte abnormalities may be noted in severe cases of enterocolitis. Signs of mild dehydration may be observed before diarrhea is seen. Once diarrhea is evident, dehydration may rapidly become severe. Occasionally, horses die peracutely, without developing diarrhea.

Diarrhea may not occur for several days but usually is evident by 24 to 48 hours after the onset of fever.[534,549] The duration of diarrhea may be days to weeks. The character of the first diarrheal feces is usually watery, with particles of roughage, but may rapidly become fluid without solid material. Finding frank blood and fibrin in the feces is unusual. The volume of feces is often large, with frequent defecation. Straining or signs of colic may be observed when the patient is defecating, and rectal prolapse may occasionally occur. Persistent straining and rectal prolapse may be signs of colonic infarction. Abdominal borborygmi are often absent early in the course of the disease because of ileus but become evident later, usually when diarrhea begins. Fluid and gas sounds are commonly auscultated, but normal progressive motility is less frequently heard. Transrectal palpation may reveal edematous rectal and colonic mucosa and fluid-filled colon and cecum. Gastric reflux may be obtained, especially early in the course, when ileus is evident.

Hematologic abnormalities early in the course of the disease include moderate to severe neutropenia, lymphopenia, and leukopenia; a mild to moderate left shift; and toxic changes in the neutrophils.[534,549] Thrombocytopenia, moderate to severe hemoconcentration, and hyperfibrinogenemia are also common. Neutropenia is an early but nonspecific indicator of salmonellosis, often occurring concurrently with the onset of fever.[534] Later in the course of disease, neutrophilic leukocytosis may be seen, indicating recovery. A degenerating left shift, with metamyelocytes and myelocytes seen in the peripheral blood, is a poor prognostic sign.

Serum biochemical analysis may reveal azotemia, increases in serum sorbitol dehydrogenase and γ-glutamine aminotransferase activity, and increased blood lactic acid concentration. Azotemia is often prerenal, but acute hemodynamic renal failure may be seen in severely dehydrated, endotoxemic, or septic patients. Indeed, elevation of creatinine concentration is a poor prognostic indicator in horses with acute colitis.[576] Hemodynamic renal disease may be complicated by toxic injury caused by the administration of nephrotoxic drugs. Hyponatremia may also contribute to prerenal azotemia. Elevations in hepatocellular enzymes are usually mild and reflect damage to the hepatocytes from absorbed toxins, such as endotoxin, and from poor perfusion resulting from hypotensive shock, dehydration, or both. Lactic acidemia may be present, reflecting poor tissue perfusion. Plasma protein drops rapidly as protein is lost in the gastrointestinal tract, resulting in moderate to severe hypoalbuminemia and hypoglobulinemia. Peripheral or organ edema (vascular leak syndrome) may occur if hypoproteinemia is severe, coupled with systemic inflammation-induced increases in endothelial permeability.

Hypokalemia, hyponatremia, hypochloremia, and hypocalcemia are common electrolyte abnormalities in patients with enterocolitis. Metabolic acidosis may also be present, and DIC is common. Urinalysis may reveal isosthenuria, proteinuria, hematuria, cylindruria, or glucosuria if hemodynamic or toxic renal injury is present. The number of leukocytes in the feces is usually increased, and occult blood may be detected. PF is usually normal except when severe mural inflammation or colonic infarction occurs.

S. enterica in feces is routinely detected by analyzing five daily cultures of large samples (10–30 g) of feces using enrichment techniques.[534,577,578] The sensitivity of fecal culture can be as low as 30% to 50%, even if several fecal samples collected daily are cultured.[578] Concurrent culture of rectal biopsy specimens and feces increases the sensitivity of culture techniques to 60% to 75%.[578] Currently, the PCR test is the most sensitive and rapid way to detect *S. enterica* in feces. A single PCR test applied early in the course of disease is a more sensitive test for the presence of *S. enterica* than repeated fecal cultures,[579,580] with as high as 100% sensitivity and 98% specificity for detection of organisms in some reports.[581] Although detection of *S. enterica* organisms in feces does not prove a diagnosis of salmonellosis, the positive predictive value of either a positive PCR or culture result is high in horses with compatible clinical signs. Culture of peripheral blood may allow isolation of the organism if bacteremia or septicemia is present, but blood cultures are not a sensitive test for salmonellosis in adult horses. Foals are more likely than adults to become septicemic, and so blood culture is recommended in all foals with signs of sepsis. Increased numbers of fecal leukocytes suggest an invasive process in the colon but are not specific for salmonellosis.

Early in the course of the disease, dehydration, electrolyte and acid-base imbalances, endotoxemia, and sepsis may be life-threatening. Aggressive treatment during the acute stages to replace fluids lost in the diarrhea and to control sepsis and endotoxemia is often effective in controlling the primary disease. Weight loss and hypoproteinemia are often severe. Possible complications include multiorgan dysfunction, vascular leak syndrome with peripheral and organ edema, laminitis, acute renal failure, venous thrombosis and septic phlebitis, irreversible protein-losing enteropathy or chronic malabsorption, pulmonary aspergillosis, and gastrointestinal infarction. The reader is referred to the section Endotoxemia in this chapter for additional information regarding treatment of horses with severe endotoxemia and SIRS.

In many instances horses recover from acute salmonellosis with aggressive treatment, only to succumb to complications of the disease, which partially explains the high fatality rate of equine salmonellosis compared with that of human salmonellosis. Chronic mild to moderate diarrhea is occasionally seen in horses after a bout of severe salmonellosis, usually with protein-losing enteropathy. If the chronic diarrhea persists beyond 4 to 5 weeks after the onset of signs, the prognosis for recovery is poor.[549]

Potomac Horse Fever

Pathogenesis

Potomac horse fever is caused by the obligate intracellular rickettsial organism *N. risticii* (formerly called *Ehrlichia risticii*).[582-586] The disease is most common from late summer to early fall, with a peak incidence in July and August.[583,584] Potomac horse fever was first described in the northeastern

United States but has since been described now in most areas of the continental United States, with a particularly high prevalence in the Northeast and Midwest. The geographic distribution is characterized by a significantly higher percentage of cases found along waterways and rivers.[583,584] The disease occurs sporadically, both temporally and geographically, and can affect any age group of horses. The case fatality rate ranges from 5% to 30%.[583]

Transmission of *N. risticii* has been reproduced experimentally by oral, intramuscular, intradermal, SC, and IV routes.[583,587] Attempts to transmit the disease experimentally with ticks *(Dermacentor variabilis)* or biting flies *(Stomoxys calcitrans)* were unsuccessful.[588,589] *N. risticii* infects virgulate cercariae, larval stages of trematodes that use operculate freshwater snails of the family Pleuroceridae (*Juga* spp. in California and *Elimia* spp. in Ohio and Pennsylvania), as intermediate hosts in their life cycle.[590-593] Infected virgulate cercariae have been identified in aquatic snails collected in other parts of the world as well.[594] Although the trematode species infected with *N. risticii* remain to be definitively identified, at least two species have been identified as potential vectors,[595] and at least two potential definitive hosts were identified when *N. risticii* DNA was detected in the blood, liver, or spleen of 23 of 53 little and big brown bats harboring gravid trematodes in their intestinal tracts.[596]

Aquatic snails release large numbers of infected cercariae into water, in which they seek their next intermediate host, which is any of a variety of aquatic insects.[593,597] Successful transmission of *N. risticii* to horses was accomplished experimentally using trematode stages collected from *Juga yrekaensis* snails.[598] The number of PCR-positive snails in endemic regions corresponds to the seasonal incidence of Potomac horse fever and may be as high as 26%.[599] Preliminary studies suggest that *N. risticii* may in fact be naturally transmitted to horses through the ingestion of caddisflies and mayflies.[593,600]

The pathogenesis of *N. risticii* is not completely understood. The organism infects and survives in monocytes and monocyte-derived leukocytes and can be found in blood monocytes during natural infections, but the sequence of events resulting in enterocolitis remains open to speculation. The organism appears first to infect blood monocytes in experimentally infected horses, which may be the vehicle of organ infection.[585,601] It is unclear whether leukocytes of the monocytic lineage or epithelial cells are infected first in naturally infected horses. The target organ is the gastrointestinal mucosa, with the most severe lesions found in the large intestine.[156,601] Infection of human colonic cells in vitro does not cause major cytopathologic effects for several days.[602] Disruption of the microvilli in the region of the plasma membrane in which sodium chloride channels are located has been observed in human colonic cell cultures.[602] Infection in horses is associated with variable degrees of morphologic damage.[156,601] Mild morphologic damage and mononuclear cell infiltration of the lamina propria occur early during the infection, but fibrinous, necrotizing typhlocolitis with severe mucosal ulceration and inflammation of the lamina propria may occur later in the disease. Vasculitis and intravascular coagulation are consistent features in the large intestine, with perivascular edema.[156] *N. risticii* can be observed in mucosal cells and macrophages and mast cells of the lamina propria.[156,601] *N. risticii* can survive and multiply in macrophages by inhibiting the production of ROIs and avoiding lysosomal digestion by blocking phagosome–lysosome fusion.[603-605]

Some researchers have suggested that impaired sodium chloride absorption in the colon contributes to diarrhea in infected horses and may be related to destruction of the enterocyte membrane structure in the region of sodium chloride channels.[602,606] Direct injury to the mucosa by *N. risticii* and colonic inflammation are likely to be prominent features leading to diarrhea, especially later in the disease.[156] Loss of fluid, protein, and electrolyte is likely caused by mucosal injury and effects on enterocyte fluid secretion caused by the inflammatory response. Like other inflammatory conditions of the colon, systemic inflammation caused by absorption of bacteria and bacterial products is a potential complication of *N. risticii* infections if mucosal injury is severe, which contributes to the clinical signs seen during the disease.

Clinical Signs and Diagnosis

N. risticii infection is clinically similar to other forms of enterocolitis and is characterized by anorexia, depression, and fever.[156,583,607] Experimental infections produce a biphasic fever in which the second febrile phase occurs 6 to 7 days after the first.[607,608] Decreased gastrointestinal motility, manifested as reduced borborygmi, occurs during the early stages, before the onset of diarrhea. Diarrhea is seen in 75% of cases and occurs 2 days after the second fever episode during experimental infections.[607,608] The diarrhea can be moderate to severe and dehydrating. Ileus can develop at any stage of the disease and can cause signs of moderate to severe colic. Systemic signs of endotoxemia, shock, and peripheral edema may occur and are similar to those described for salmonellosis. Experimental and natural infection with *N. risticii* can cause abortion of infected fetuses in pregnant mares.[609,610] Laminitis is a complication in 20% to 30% of naturally occurring cases and is often severe.[584] Other complications include protein-losing enteropathy, thrombosis, and renal failure, as described for salmonellosis.

Hematologic abnormalities reflect endotoxemia, dehydration, and sepsis and are essentially identical to those described for salmonellosis. Neutropenia with a left shift is a consistent feature and occurs concurrently with or soon after the onset of diarrhea.[608] Thrombocytopenia is common and often severe.[608] Neutrophilic leukocytosis occurs later in the course of the disease. Hyperfibrinogenemia is usually more pronounced than that seen with salmonellosis. Serum electrolyte, acid-base, and biochemical abnormalities are also similar to those described for salmonellosis. Coagulopathies are commonly seen during *N. risticii* infection and reflect activation of coagulation pathways. DIC is not uncommon and may be the cause of the high frequency of laminitis associated with *N. risticii* infection.[611]

Diagnosis of *N. risticii* infection cannot be based solely on clinical signs because the disease is clinically similar to other forms of enterocolitis. In endemic areas, acute colitis is likely to be caused by *N. risticii;* thus, the clinical signs of acute inflammatory colitis may in fact have a high predictive value in these areas. Serologic evidence of infection, such as rising antibody titers to *N. risticii* detected by indirect immunofluorescence (IFA) or ELISA in paired serum samples, may be helpful in establishing a diagnosis.[584,612] Care should be taken when interpreting the IFA serologic test for *N. risticii* because the test appears to have a high false-positive rate.[613] Culture of the organism from blood is possible but difficult and is generally useful only in the research laboratory. Recently developed PCR tests for *N. risticii* DNA are rapid, highly sensitive (as

sensitive as culture), and there are specific tests for *N. risticii* infection that can be applied to blood or feces.[614-616]

Prevention

Prevention of the disease by reducing exposure to the etiologic organism is difficult because the mode of transmission is not known. A killed vaccine has been developed that is relatively effective in preventing clinical illness other than fever in 80% of experimentally challenged horses using the vaccine strain. Field studies, however, suggest the vaccine has limited benefit for preventing natural infection or decreasing its severity.[617,618] Vaccine failures have been attributed to strain differences in antigenicity or to poor antibody responses to the vaccine.[617,618]

Equine Intestinal Clostridiosis

Pathogenesis

Clostridiosis is an important cause of acute enterocolitis in foals and adult horses. *C. perfringens* and *C. difficile* are most commonly associated with intestinal clostridiosis in horses, but other clostridial species, including *C. septicum, C. cadaveris,* and *C. sordellii* have also been isolated from horses with enterocolitis.[619-624] In horses of all ages, clostridial enterocolitis appears to be a common antibiotic-associated and nosocomial cause of enterocolitis.[623,625,626] Hemorrhagic enterocolitis caused by *C. perfringens* in neonatal foals is a distinct clinical entity and will be discussed in more detail in Chapter 20. This discussion focuses on adult intestinal clostridiosis.

Clostridium organisms are obligate anaerobic to aerotolerant spore-forming gram-positive rods that are ubiquitous in the environment in the spore form.[624] They are elements of the normal flora of horses of all ages and are among the first bacteria acquired after birth. *Clostridium* organisms inhabiting the gastrointestinal tract are normally found in very low numbers and do not produce enterotoxins. Clostridiosis is associated with an increase in the number of a particular species of *Clostridia* in the gastrointestinal tract and, perhaps most important, exotoxin production. Although the conditions resulting in exotoxin production are not fully understood, several factors increase clostridial numbers in the gastrointestinal tract. Dietary factors affect the numbers of *Clostridium* species shed in horse feces.[619] Experimental induction of colic increases fecal shedding of *Clostridium* species in the absence of diarrhea.[627] Antibiotics, particularly those administered orally or recycled via the enterohepatic system, increase the recovery of *Clostridia* colony-forming units (CFUs) in equine feces and clinical clostridiosis.[620,622,628-630] Clostridiosis associated with *C. difficile* is likely to be the most important cause of antibiotic-induced enterocolitis in the horse.

Clostridium perfringens. *C. perfringens* includes many genetically distinct strains of variable virulence that produce one or more of a large group of exotoxins. The pattern of exotoxin production is used to classify *C. perfringens* into five types: A, B, C, D, and E. *C. perfringens* type A is the most common clostridial isolate from healthy and diarrheic horses of all ages. *C. perfringens* types A, B, C, and D have all been associated with hemorrhagic enteritis in foals younger than 10 days of age, with type C being the most common cause in North America.

The primary toxin produced by *C. perfringens* type A is α-toxin (phospholipase C), which interferes with glucose uptake and energy production and activates arachidonic acid metabolism and signaling pathways in enterocytes.[624] Oral administration of α-toxin does not cause tissue necrosis but causes increased secretion by small intestinal mucosal cells.[631,632] The β-toxin of types B and C is a cytotoxin that causes enterocyte necrosis, ulceration, and ultimately severe intestinal inflammation and hemorrhage.[624,632] A novel toxin designated β_2 may also have a role in *C. perfringens* enterocolitis.[633] The biologic activity of the β_2-toxin is similar to that of β-toxin, but β_2-toxin is not related to β-toxin in its genetic sequence. The β_2-toxin was prevalent in two groups of horses with acute enterocolitis but not in healthy horses.[634] It is predominantly associated with *C. perfringens* that would have otherwise been classified as type A but that may in fact represent a previously undescribed type.

Virulent strains of *C. perfringens* type A and, to a lesser extent, type C may produce enterotoxin. Enterotoxin is a cytotoxin that inserts into cell membranes to form pores, which alter permeability to water and macromolecules and ultimately lead to cellular necrosis.[635] Massive desquamation of the intestinal mucosa that is a result of enterotoxin cytotoxicity triggers an inflammatory response, intestinal edema, mural hemorrhage, and systemic inflammation.[158] Enterotoxin also alters tight junction integrity, resulting in increased paracellular permeability by a noncytotoxic mechanism.[636]

Clostridium difficile. *C. difficile* produces several toxins, only two of which, toxin A and toxin B, have been studied in detail. Toxin B is a potent cytotoxin in vitro, but its role in enterocolitis is less clear than that of toxin A. It does not induce fluid secretion, inflammation, or characteristic alterations in intestinal morphology. *C. difficile* toxin A is an enterotoxin that induces an inflammatory response with hypersecretory diarrhea.[637] Toxin A induces neutrophil influx into intestinal tissue, mast cell degranulation, and secretion of prostaglandins, histamine, cytokines, and 5-HT by these activated leukocytes.[142,637,638] The products of neutrophils and mast cells have a significant role in the vasodilatory and secretory responses in the intestine during *C. difficile* infection.

The ENS is central to the induction of intestinal inflammation and mucosal secretion by toxin A. A model for toxin A–induced secretory diarrhea has emerged in which toxin A stimulates substance P–containing afferent sensory nerve fibers, which in turn stimulate mast cell degranulation, recruitment and activation of PMNs, and vasodilation.[88,89,639] Toxin A–induced stimulation of enterocyte secretion can occur via secretomotor neuronal stimulation by substance P–containing sensory neurons or products of mast cells and PMNs. Mast cell degranulation, PMN influx, and enterocyte secretion are all abolished by neural blockade or depletion of substance P. How toxin A triggers the sensory component of the ENS remains unknown, but it is likely that toxin A–induced necrosis of enterocytes exposes afferent neurons to the noxious milieu of the intestinal contents.

Clinical Signs and Diagnosis

Equine intestinal clostridiosis is clinically similar to other forms of acute enterocolitis in horses.[619,624] Although the clinical course is usually acute, peracute colitis with rapid death may occur. Occasionally, a milder, more prolonged clinical course is seen. Fever, anorexia, and depression may be observed before the onset of gastrointestinal signs, but the absence of prodromal signs is more common. Signs of endotoxemia and shock may accompany acute signs of colic and severe, dehydrating diarrhea. Diarrhea may not be profuse but is usually dark and foul. Like the clinical signs, hematologic and

serum biochemical abnormalities are similar to those associated with other forms of enterocolitis and reflect fluid, protein, and electrolyte loss and systemic inflammation resulting from endotoxemia. Neutropenia, leukopenia, and hemoconcentration are common. Hypoproteinemia may be profound. Hyponatremia, hypokalemia, hypochloremia, hypocalcemia, and a mixed prerenal–renal azotemia are often noted, as well as metabolic acidosis and coagulopathies. Serum concentrations of hepatocellular enzymes, such as sorbitol dehydrogenase, may be elevated, and liver function may be reduced.

Preliminary diagnosis of equine intestinal clostridiosis caused by *C. perfringens* is based on the isolation of greater than 100 CFUs of *C. perfringens* type A per gram of feces from patients with diarrhea and signs suggestive of toxemia.[619,640] Similar criteria are used to screen human patients for *C. perfringens* type A infection. Normal horses shed less than 100 CFU/g of feces, and usually horses with intestinal clostridiosis shed greater than 10^6 CFU/g.[619,640] Identification of high numbers of *Clostridium* organisms in the feces does not prove infection as the cause of the observed clinical signs. Detection of *C. perfringens* toxins in feces or intestinal contents in horses with high numbers of fecal CFUs and clinical signs of enterocolitis is more conclusive evidence of an enterotoxigenic infection than that based on culture alone.[624] Immunoassays are available to detect *C. perfringens* enterotoxin,[624] but the reliability (specificity) of some immunoassays has come into question. PCR multiplex and gene probe assays are now available for detection of the major lethal toxins in bacterial isolates or fecal samples to determine the pattern of toxin production and are currently the preferred methods of detection.[641-643]

Like *C. perfringens,* diagnosis of *C. difficile* infection depends on the culture of the organism from feces and identification of toxins in the feces. Bacterial culture of *C. difficile* may be difficult; therefore, it is an insensitive diagnostic test in horses.[644,645] Enrichment techniques and culture of multiple fecal samples may be required.[645,646] Detection of toxin A or B (or both) in feces by cell cytotoxicity assay or immunoassay is the preferred test for diagnosis of *C. difficile* infection in humans.[624] These tests are more sensitive than bacterial culture for identifying *C. difficile* infection in adult horses.[644,645] Sensitive PCR methods may also be used to identify genes for toxins A and B in fecal samples from diarrheic horses.[624]

Strongylosis

Pathogenesis

Strongyle infections in horses are caused by two groups of nematodes: large and small strongyles (see later section Cyathostomiasis). Large strongyles that are pathogenic in horses include *Strongylus vulgaris, S. edentatus,* and *S. equinus.* Of these species *S. vulgaris* is by far the most important cause of disease in the large intestine and is the most pathogenic parasitic infection in horses.[647] *S. vulgaris* infection in horses is manifested as acute or chronic disease.[647] The age and resistance of the host, the infective dose, and the size and function of the affected arteries influence the type and degree of disease that occurs. Sudden ingestion of large numbers of infective larvae by a naive host causes acute strongylosis, whereas ingestion of fewer infective larvae over a long period of time by an older, more resistant host causes chronic strongylosis. Acute strongylosis is more likely to cause colic than diarrhea and may be rapidly fatal. Chronic strongylosis tends to cause debilitation and signs of colic but may also cause diarrhea.

Diarrhea associated with acute strongylosis occurs within several days of infection and is likely to be caused by migration of the larvae through the intestinal wall. Fourth-stage larvae migrate through the mucosa and submucosa into the arterioles of the intestine, causing mural edema, hemorrhage, and infiltration of inflammatory cells.[647,648] Increased secretion and decreased absorption of fluid and electrolytes, stimulated by inflammatory mediators such as prostaglandins and histamine, may play a role in the diarrhea induced by *S. vulgaris.* Interstitial edema and damage to the interstitial matrix and mucosa may occur as a result of inflammation and migration of the parasites, causing increased secretion of fluid and albumin loss. Abnormal gastrointestinal motility may also play a role in the development of diarrhea. Migration of larvae through the intestinal wall early in the course of infection affects myoelectrical activity and motility in the large intestine and may affect retention of ingesta and absorption of fluid.[649,650] The cause of death in acute strongylosis has not been addressed, but it may be related to massive migration through the vasculature, causing thrombosis with ischemia and infarction of the intestine.

Chronic strongylosis causes typical verminous arteritis and is more commonly associated with natural infections in horses than acute strongylosis.[647] Lesions of the large intestinal vasculature caused by migration of larvae through the intima are characterized by thrombus formation, narrowing of the arterial lumen, fibrosis, and thickening of the arterial wall.[647,648] Embolization may occur, causing acute segmental infarction of the large intestine, but more commonly, reduced blood flow without embolization causes ischemia and occasionally infarction.[648,651] Postmortem examination of horses with colonic infarction failed to reveal embolization as the cause in the majority of cases.[651] Reduced blood flow in the tissues of the intestine usually results from narrowing of the arterial lumen by the thrombus and formation of microthrombi at sites independent of the parasites. Release of vasoconstrictive inflammatory mediators, such as leukotrienes, from platelets, neutrophils, and eosinophils, as well as elaboration of parasitic antigens or toxins, may cause vasoconstriction and ischemia.[652] Horses with experimental strongylosis had a 50% reduction of blood flow in the colonic vasculature.[653]

Clearly, reduced blood flow is an important effect of chronic strongylosis, but the relationship between blood flow and diarrhea is unclear. Disrupted motility resulting from ischemia may lead to diarrhea by reducing the retention of ingesta and absorption of fluid. Acute infarction and mucosal ulceration cause severe, chronic diarrhea in naturally infected horses.[654] Release of inflammatory mediators, such as prostaglandins, histamine, and kinins, from inflammatory cells associated with thrombi and inflamed intestine may also affect secretion, absorption, and motility, leading to diarrhea.

Clinical Signs and Diagnosis

The clinical signs of acute strongylosis caused by *S. vulgaris* infection include depression, moderate to severe colic, and fever.[655] Diarrhea is less often a feature of acute strongylosis than colic.[647] Most cases of acute strongylosis occur in young naive horses introduced to an infested environment or inoculated experimentally with infective larvae. This form of strongylosis is not often recognized naturally. Chronic strongylosis is most commonly observed as a natural syndrome. Weight loss or poor weight gain; chronic, intermittent colic; fever; poor appetite; and diarrhea are frequently observed.[647,648] Diarrhea may be profuse and watery, or the feces may be soft

but of normal volume. Transrectal palpation may reveal thickening and fremitus in the cranial mesenteric artery. Young horses are most commonly affected, but older horses may also be too. Horses with acute infarction or large intestinal ulceration secondary to chronic strongylosis may have signs of severe abdominal pain, sepsis, and endotoxemia, and profuse, watery diarrhea is common.

Hematologic abnormalities associated with strongylosis include neutrophilic leukocytosis and eosinophilia.[655-657] Neutrophilia appears to be an early event during the course of the disease, and eosinophilia tends to appear later.[655,657] Hyperfibrinogenemia may also occur, especially later in the course of the disease. Serum α-globulin and β-globulin and IgG(T) concentrations are characteristically elevated.[656,658] Horses with chronic ulcerative colitis secondary to strongylosis may develop severe hypoalbuminemia.[654] PF analysis may reveal an elevated protein concentration and eosinophilia.[656,657] Tentative diagnosis is based on clinical signs, hematologic abnormalities, and PF analysis. Elevated serum α-globulin and β-globulin concentrations and IgG(T) concentration support the diagnosis.[658] Fecal analysis may reveal strongyle eggs, but fecal egg counts are often unreliable because nonpatent larvae cause the disease.

Prevention

Appropriate preventive measures are important in controlling this disease, including such management procedures as preventing overcrowding, reducing exposure of susceptible individuals, and instituting proper deworming schedules. Ivermectin is the preferred anthelmintic used to control strongylosis in horses. Monitoring fecal egg counts as a means of evaluating the efficacy of parasite control measures is recommended.

Cyathostomiasis

Pathogenesis

Infection with small strongyles (cyathostomiasis) is well recognized as a cause of diarrhea and large intestinal disease in horses of all ages.[659-664] Clinical disease is caused by intramural larval stages of more than 50 species of small strongyles (cyathostomes). The cyathostome life cycle requires migration by fourth-stage larvae through the mucosa of the large intestine and may include a period of hypobiosis, during which the larvae remain encysted within the mucosal layer of the large intestine.[659] After a period of hypobiosis, the larvae emerge in response to a largely unknown stimulus. Most cases occur when larval emergence takes place, classically in the late winter and spring in the northern temperate zones and in the late fall or winter months in the southeastern United States and subtropical regions.[659] Sudden emergence of encysted larvae causes mucosal injury, ulceration, and an inflammatory reaction, which are largely responsible for the clinical disease.[659,665] Migration of the larvae as they penetrate the mucosa affects motility patterns and can cause inflammation that may contribute to diarrhea.[659] Chronic, eosinophilic, granulomatous colitis and diarrhea with histopathologic evidence of hypobiotic cyathostome larvae in the large intestine have been reported in two horses during a period in which the emergence of larvae would not be expected to occur (early winter).[659]

Natural emergence of cyathostome larvae causes fibrinous inflammation of the large intestine, focal necrosis, mural hemorrhage, and ulceration of the large intestinal mucosa, which may even result in bleeding into the lumen.[648,665] Mild to moderate eosinophilic and mononuclear inflammation of the lamina propria is seen.[648,665] Moderate to severe interstitial edema is frequently observed.[648,665] Colonic inflammation and interstitial edema may contribute to diarrhea, in conjunction with the loss of the mucosal barrier, by causing increased active and passive secretion of fluid, electrolytes, and protein. Protein loss is often significant, resulting in profound hypoalbuminemia and interstitial edema of skin and other organs. Chronic granulomatous colitis has been reported to occur in response to encysted larvae and may cause diarrhea by increased secretion secondary to granulomatous inflammation or disruption of the interstitium by granulomatous infiltration. Administration of an anthelmintic to horses with a heavy load of encysted larvae may also cause rapid larval death and acute and often severe inflammation similar to natural emergence.

Clinical Signs and Diagnosis

Cyathostomiasis may be the most commonly identified cause of chronic diarrhea in the horse.[493,666,667] However, an acute syndrome has also been associated with cyathostomiasis.[664] Clinical signs of cyathostomiasis are characterized by moderate to severe weight loss or poor weight gain, ill thrift, ventral edema, intermittent fever, and intermittent mild colic.[659-664,667] Acute onset of diarrhea is typically profuse and progresses to chronic diarrhea that is often mild, the consistency of bovine feces, and may be intermittent.[659-664,667] Appetite is usually normal, but some affected horses have a ravenous appetite. Transrectal palpation usually does not reveal any abnormalities. Horses of any age may be affected, and clinical signs are more common during periods of emergence of larvae, corresponding to late winter and spring in northern temperate zones. The deworming history may appear to be adequate.

Neutrophilic leukocytosis is typically evident, but the WBC count may be normal.[659-664] Profound hypoalbuminemia is a characteristic feature of cyathostomiasis, manifested clinically by ventral edema. Plasma α-globulin and β-globulin concentrations may be elevated, which can result in a normal total plasma protein concentration in spite of hypoalbuminemia.[658-660] The serum IgG(T) concentration, however, has been reported to be normal, which may help distinguish cyathostomiasis from *S. vulgaris* infection.[659,661,662] PF analysis does not usually reveal any abnormalities, in contrast to horses with *S. vulgaris* infection. Fecal analysis may be unrewarding because the infection is often not patent when clinical signs are apparent. Measurement of plasma fructosamine may provide a measure of protein catabolism or protein loss in the absence of hypoalbuminemia.[667,668] Plasma fructosamine concentrations are significantly lower in horses with experimental cyathostomiasis than in normal controls,[667,668] suggesting that this test may be a useful diagnostic tool. However, the test has not yet been validated in naturally occurring cases, and neither the specificity nor the sensitivity is known. Rectal scrapings or rectal mucosal biopsies may reveal evidence of cyathostome larvae.[659,662] Definitive diagnosis usually requires microscopic examination of biopsy specimens of the cecum and ascending colon, collected by laparotomy. Examination of biopsy specimens collected from the small intestine is recommended to rule out other causes of weight loss and diarrhea. Appropriate diagnostic tests, such as culture of feces for pathogenic bacteria, should be included in the workup to further rule out other causes.

Prevention

Preventive measures are appropriate for other horses on the premises known to have a problem with cyathostomiasis. These include frequent deworming (every 6 weeks) during times of high infectivity (spring and summer in the north and fall, winter, and early spring in the south) to eliminate parasites before they become patent.[659] Because of high levels of resistance to benzimidazoles, avermectins (ivermectin or moxidectin) are often the drugs of choice for cyathostome control.[669-671] Resistance to ivermectin has been demonstrated, but the prevalence of ivermectin resistance appears to remain low.[669] Although daily pyrantel pamoate administration has also been reported to effectively reduce worm burdens and pasture infectivity in young and mature horses,[672] cyathostome resistance has been reported and is a concern for the use of this drug as a routine preventive anthelmintic.[670,673,674] Because of the rapid emergence of resistant strains to even ivermectin, targeted treatment, based on fecal egg counts and careful monitoring for the development of resistance to any anthelmintics used for cyathostome control, is warranted.[674]

TOXICOLOGIC DISEASES

Antibiotic-Associated Diarrhea

Pathogenesis

Antibiotic-associated diarrhea has been reported in many species, including horses.[675] Certain antibiotics, such as trimethoprim-sulfonamide combinations, erythromycin, penicillins, tetracyclines, clindamycin, and lincomycin, are associated with naturally occurring and experimental enterocolitis syndromes in horses.[620,675-678] In some cases, such as those seen with trimethoprim-sulfonamide combinations, the geographic incidence of antibiotic-associated diarrhea appears to differ markedly.

C. perfringens, C. difficile, and serovars of *S. enterica* are apparently the most common causes of antibiotic-associated diarrhea in horses. Outbreaks of *C. difficile* have been reported in hospitalized horses being treated with antibiotics.[622,626] In Sweden accidental erythromycin ingestion has been associated with *C. difficile* enterocolitis in mares in which their foals were being treated for *R. equi*.[629,677,679] Tetracycline administration has been associated with an increase in the numbers of gram-negative enteric bacteria and *C. perfringens* in the feces of horses as well as reactivation of salmonellosis and prolongation of fecal shedding of serovars of *S. enterica*.[619,680]

The most common mechanism by which antibiotics cause diarrhea is disruption of the gastrointestinal flora. The normal large intestinal flora, composed of mainly obligate anaerobes and streptococci, protects the host from pathogenic bacteria by colonization resistance.[553] Ecologic factors play an important role in colonization resistance. For example, surface bacteria in the large intestine interact with receptors on the mucosal cells, facilitating adherence to the mucosa.[545,681] In doing so, the normal organisms compete more successfully for this important niche. Competition for space and nutrients is an important means of preventing colonization and proliferation of pathogenic bacteria.[552,553,681] Anaerobic bacteria produce SCFAs and other metabolites that are toxic to facultative anaerobic bacteria, especially in the conditions of the large intestine.[552,553,681] Organisms of the normal flora produce bacteriocins that inhibit growth of potential pathogens.[552]

Antibiotics that deplete the population of obligate anaerobes and streptococci efficiently decrease colonization resistance.[552] Production of fatty acids is diminished, and competition for space and nutrients is reduced. As a result, gram-negative enteric bacteria, such as *S. enterica* var. Typhimurium, are able to proliferate. Pathogenic anaerobes normally found in low numbers can also proliferate. Antibiotic-resistant strains of bacteria, especially gram-negative enteric bacteria and possibly clostridia, may be selected by antibiotic administration, allowing proliferation of pathogenic bacteria resistant to many antibiotics.[682] Obligate anaerobic commensal organisms, perhaps the most critical group of microbes for maintaining colonization resistance, are usually susceptible to macrolides, tetracyclines, β-lactams, and lincosamides, which may explain the high incidence of diarrhea associated with the administration of these antibiotics.[624]

In addition to reduction of colonization resistance, depletion of the normal anaerobic microbial population in the intestine decreases carbohydrate fermentation and production of SCFAs, which contributes to the pathogenesis of antibiotic-associated diarrhea by decreasing absorption of sodium and water by the colonic mucosa.[171] Ampicillin decreases colonic fermentation of carbohydrates in humans.[683] Human patients with antibiotic-associated diarrhea have markedly impaired colonic fermentation and very low production of SCFAs.[684] Erythromycin, ampicillin, or metronidazole treatment is associated with decreased production of SCFAs in patients with and without diarrhea.[684] Absorption of sodium and water is stimulated by absorption of SCFAs in the equine colon, suggesting that reduction of colonic SCFA content by antibiotic-induced depletion of anaerobic flora has similar effects in horses as in humans.[171]

Broad-spectrum antibiotics exert a more profound effect on the gastrointestinal flora than narrow-spectrum antibiotics.[685] Antibiotics administered orally, especially those that are poorly absorbed, are more likely to cause diarrhea than parenterally administered antibiotics.[685] For instance, clindamycin is less likely to cause diarrhea in humans when administered IV than when administered orally.[685] Antibiotics with extensive enterohepatic circulation, such as tetracyclines and erythromycin, are excreted in high concentrations in the bile and are more commonly associated with diarrhea than antibiotics that do not undergo enterohepatic circulation.[685]

Antibiotics may cause diarrhea by other means than by disrupting the normal flora. Direct toxic effects may play a role in producing irritation, increasing secretion, and disrupting motility patterns. Tetracyclines are irritating to the gastrointestinal mucosa and may cause inflammation and increase secretion.[685] Erythromycin interacts with smooth muscle cells, stimulating gastrointestinal motility.[685,686] Normal peristalsis plays an important role in suppressing the population size of potentially pathogenic bacteria. Normally, bacteria that are prevented from adhering to the mucosa by colonization resistance are swept aborally by peristalsis and excreted in the feces. Disruption of normal motility patterns may prevent clearance of pathogenic bacteria, contributing to the colonization of mucosal surfaces.

Clinical Signs and Diagnosis

Diarrhea induced by antibiotics usually occurs within 7 days of initiation of antibiotic administration but may occur several days after cessation of antibiotic treatment. The clinical syndrome of antibiotic-associated diarrhea varies from mild

diarrhea to fulminant enterocolitis with severe diarrhea. Mild diarrhea is common, especially in foals receiving erythromycin, trimethoprim-sulfa combinations, or rifampin[677,687] and are usually not clinically significant. Acute, severe enterocolitis can occur in horses of all ages receiving antibiotics and can be life-threatening. Clinical signs are identical to those resulting from other causes of acute enterocolitis. Severe, dehydrating diarrhea; endotoxemia; sepsis; and shock may occur. Hemoconcentration, neutropenia, hypoproteinemia, and electrolyte and acid-base imbalances are common. Severe hyponatremia may occur in foals with antibiotic-associated diarrhea, especially if trimethoprim-sulfa and rifampin combinations are the cause.[687] More detailed descriptions of the clinical and laboratory findings were given earlier. Diagnosis is presumptive because definitive diagnosis of antibiotic-associated diarrhea is impossible. Fecal culture or PCR testing may reveal *S. enterica* or *Clostridium* spp. infection.

Nonsteroidal Antiinflammatory Drug Toxicity

Pathogenesis

Toxicity resulting from NSAID administration has been well documented in several species, including horses, and is discussed in Chapter 2.[688-694] In horses and humans NSAID toxicity is manifested by renal and gastrointestinal disease. Foals are considered to be more susceptible than adult horses to gastrointestinal disease secondary to NSAID administration, and ponies may be more susceptible than horses. NSAID toxicity varies primarily as a result of properties that influence distribution to sensitive tissues and relative selectivity for COX-1 or COX-2. All nonselective NSAIDs are capable of inducing gastrointestinal and renal damage at toxic concentrations. Aspirin has been suggested to be more toxic than other NSAIDs because it irreversibly inactivates COX by acetylation, whereas other NSAIDs reversibly inhibit COX.[688] Phenylbutazone is the drug most commonly reported to cause gastrointestinal toxicity in horses, perhaps because of its widespread usage by veterinarians and horse owners or perhaps because of bona fide differences in toxicity in horses compared with other nonselective NSAIDs. Acute phenylbutazone toxicity in horses resulting from overdose is characterized by mucosal ulceration throughout the gastrointestinal tract, oral ulceration, renal papillary necrosis, vasculopathy, thrombosis, and protein-losing enteropathy with hypoalbuminemia.[690-692] COX-2–selective NSAIDs appear to be much less toxic in the equine gastrointestinal tract.[392,695] The focus of this discussion is on the toxic effects of NSAIDs on the large intestine, but this necessarily includes elements of upper gastrointestinal and renal disease.

Horses with large intestinal disease resulting from NSAID toxicity generally are receiving inappropriately large doses or have underlying disorders that predispose the large intestine to the toxic effects of NSAIDs, even at appropriate dosages. The dosage regimen recommended for phenylbutazone (4.4 mg/kg every 12 hours for 1 day, then 2.2 mg/kg every 12 hours) is considered safe. Experimental studies in horses, however, have shown toxicity to occur when amounts exceeding the recommended dosage (6.6 mg/kg/day) are administered for several days.[688,689] Most reported cases of phenylbutazone toxicosis occurred in horses receiving higher than recommended dosages.[692,694,696] Regardless, administration of phenylbutazone at the recommended dosage has been reported to cause a significant decrease in plasma protein concentration and gastrointestinal disease.[691,697] Moreover, signs of NSAID toxicity have been reported in normovolemic horses treated with appropriate doses of phenylbutazone.[697,698] Dehydration, sepsis, endotoxemia, and other conditions that alter hemodynamic homeostasis exacerbate renal and gastrointestinal toxicity of NSAIDs.[688] Underlying inflammation of the intestinal tissues may increase the likelihood of gastrointestinal ulceration resulting from NSAIDs.

Gastrointestinal disease induced by NSAIDs is manifested by mucosal ulceration, inflammation, bleeding, and protein-losing enteropathy.[690,691,694,697] In addition to direct effects on the mucosal barrier, NSAID administration causes an acute relapse of preexisting colonic inflammatory disease and worsens colonic inflammation in humans with inflammatory bowel disease.[688,699,700]

It is not clear whether the previously mentioned NSAID effects occur in horses. The mechanism by which NSAIDs induce mucosal damage is probably multifactorial. Direct irritation may play a role in oral and gastric irritation and ulceration; however, parenteral administration of NSAIDs produces oral and gastric ulceration as well. Inhibition of prostaglandin synthesis by inhibition of both COX-1 and COX-2 appears to be the most important mechanism of mucosal injury. Prostaglandins, particularly PGE_2 and PGI_2, are critical for mucosal health and repair after injury.[701,702] PGE_2 increases mucosal blood flow; increases secretion of mucus, water, and bicarbonate; increases mucosal cell turnover rate and migration; stimulates adenyl cyclase activity; and exerts other protective effects in the gastric mucosa of several species.[688,701,702] Perhaps most important, PGE_2 and PGI_2 have roles in maintaining epithelial tight junction integrity, which is indispensable for mucosal barrier function and repair after mucosal injury.[701]

In spite of the overwhelming amount of information about the role of prostaglandins in maintaining the mucosal barrier in other species and clear clinical and experimental evidence that NSAIDs injure the equine colonic mucosa, the role of prostaglandins in mucosal protection in the equine colon is not yet well defined. Inhibition of COX-1 and COX-2 in equine colonic mucosa with flunixin meglumine results in reduced electrical resistance of the mucosa and increased permeability to macromolecules in vitro,[703] suggesting that flunixin treatment disrupts the epithelial tight junctions in the equine colon. This was correlated with a profound inhibition of PGE_2 and PGI_2 concentrations in the treated tissues. Administration of a PGE_2 analog prevents the gastrointestinal manifestations of phenylbutazone toxicosis in ponies.[691]

The recent development of NSAIDs that specifically inhibit COX-2 has markedly reduced the frequency and severity of gastrointestinal side effects in humans taking NSAIDs for chronic musculoskeletal conditions.[704] COX-2–specific NSAIDs such as firocoxib hold promise for use in horses to treat arthritis[705] and other conditions, with reduced incidence of toxicity. For example, the relatively COX-2–specific inhibitors meloxicam and firocoxib are less harmful to equine intestinal mucosa than flunixin meglumine in vitro.[392,695] Moreover, COX-2–selective inhibitors are significantly more permissive than flunixin for recovery of the mucosa in equine ischemic-injured intestinal tissues; recovery is no different from that for control tissues.[392,695]

NSAID-induced mucosal injury is associated with a marked inflammatory response to microbial products exposed to the lamina propria.[706] This inflammation exacerbates mucosal dysfunction and injury associated with NSAID toxicity. For

example, depletion of neutrophils or blockade of neutrophil influx into gastrointestinal tissues or inhibition of neutrophil activation and release of toxic products prevents many of the pathophysiologic effects of NSAID toxicity in the gastrointestinal tract.[106,707-709] The inflammatory response alone may result in moderate to severe gastrointestinal ulceration, mural vascular thrombosis and edema, fluid secretion, protein-losing enteropathy, and mucosal hemorrhage.

Clinical Signs and Diagnosis

NSAID colitis manifests as two clinical syndromes: right dorsal ulcerative colitis (RDUC) and generalized NSAID toxicity. As its name implies, RDUC is a disorder isolated to the right dorsal segment of the large intestine.[693,694,698] The most prominent clinical signs of RDUC are anorexia, lethargy, and colic. Anorexia, depression, diarrhea, fever, and signs of endotoxemia may also be features. If RDUC is chronic, weight loss, intermittent colic, lethargy, anorexia, and ventral edema are common clinical signs with soft and unformed feces. Ulceration of the right dorsal colonic mucosa results in protein-losing enteropathy and significant hypoproteinemia attributable mainly to hypoalbuminemia. Hypoproteinemia may be one of the earliest clinical manifestations of RDUC and can be sufficiently severe to cause peripheral (usually ventral) edema. In some horses, dehydration, electrolyte abnormalities, neutropenia or anemia, azotemia, and biochemical abnormalities may be noted if the ulceration and diarrhea are severe or if systemic inflammation is present.

Clinical signs of generalized NSAID toxicity vary from mild diarrhea with no systemic signs to severe dehydrating diarrhea with anorexia, fever, depression, peripheral edema, oral ulceration, and colic.[691,692,696]

Clinical signs of systemic inflammation caused by endotoxemia may occur, manifested as poor peripheral perfusion, tachycardia and tachypnea, weakness, trembling, and cyanotic or hyperemic oral mucous membranes. Hematuria or oliguria may be present with renal involvement. Complications associated with other forms of severe enterocolitis, such as laminitis, thrombophlebitis, and severe weight loss, may occur.

Although phenylbutazone has been associated specifically with bone marrow depression resulting in abnormalities in one or more blood cell lines,[710] hematologic abnormalities of generalized NSAID toxicity are usually nonspecific and include neutropenia with a left shift or leukocytosis and hemoconcentration. Serum biochemical analysis is characterized by profound hypoproteinemia, hyponatremia, and metabolic acidosis.[696,697] Hypocalcemia, hypokalemia, hypochloremia, and elevated hepatocellular enzyme activities may also be seen. Hypoproteinemia may occur without signs of diarrhea. Azotemia may be prerenal as a result of dehydration, but it is frequently caused by renal failure that results from a combination of hemodynamic effects of NSAIDs and direct toxic renal injury. Urinalysis frequently reveals hematuria, proteinuria, cylindruria, and isosthenuria. Fecal occult blood is frequently detected.

Diagnosis of either form of NSAID colitis is often presumptive, with a history of overdose of NSAIDs being strong evidence of NSAID toxicity. As discussed earlier, toxicity may occur with dosage regimens that are not considered inappropriate, particularly if the horse experiences a concurrent period of dehydration. Ultrasonographic examination of the right dorsal colon can be used to confirm a diagnosis of RDUC, but the sensitivity of this method appears to be low.[711] Ultrasonography (3.5- to 5-MHz transducer at the right twelfth through fifteenth intercostal spaces below the margin of the lung axial to the liver) may reveal a thickened right dorsal colon (>0.5 cm) and evidence of colonic edema in horses with RDUC.[711,712] Nuclear scintigraphy of horses after infusion with technetium-99–labeled WBCs can be used to document inflammation of the right dorsal colon.[50] Laparotomy or laparoscopic examination of the right dorsal colon may be required for definitive diagnosis of RDUC. Other causes of enterocolitis, such as salmonellosis, Potomac horse fever, clostridiosis, and antibiotic-associated diarrhea, must be ruled out.

Cantharidin Toxicity

Pathogenesis

Cantharidin is the toxic substance found in beetles of the genus *Epicauta,* commonly known as *blister beetles.*[713-715] Ingestion of the beetles causes release of the toxin and absorption through the gastrointestinal tract. Transcutaneous absorption may occur but appears to be rare in horses. Blister beetles feed on the flowers of alfalfa and may be incorporated into processed alfalfa hay if the hay is cut and processed simultaneously, as by crimping.[713-715] The beetles often swarm, and large numbers of beetles may be found in relatively small portions of hay. The lethal dose of cantharidin is less than 1 mg/kg, but the concentration of cantharidin varies among species of blister beetles and between sexes.[713,714] As many as 100 to as few as 6 beetles may be lethal. Usually, only one or a few horses fed contaminated hay will ingest beetles because they are concentrated in a small portion of the hay. However, outbreaks involving many horses on a farm have occurred. Most cases have occurred in Texas and Oklahoma, but horses in other states may be affected as well, especially if hay is imported from states in which blister beetles are common. Peak incidence is in late summer and fall.[716] The fatality rate may be 50% or greater,[713,717] but if the patient survives several days, recovery is probable.

Cantharidin is absorbed from the gastrointestinal tract and excreted by the kidneys. It is a potent irritant, causing acantholysis and vesicle formation when applied topically.[713,715,717] The chemical is thought to disrupt the oxidative metabolism in the mitochondria, causing mitochondrial swelling, plasma membrane damage, and changes in membrane permeability.[713] The mucosa of the gastrointestinal tract is most commonly affected in horses because they ingest the toxin. Cell swelling and necrosis occur, resulting in mucosal ulceration. Oral, esophageal, gastric, and small and large intestinal ulceration have been observed in natural and experimental cantharidiasis.[713,715,717] Severe fibrinous to pseudomembranous inflammation and submucosal edema of the intestine have also been reported. Diarrhea probably results from the severe ulceration and inflammation of the large intestine, causing increased secretion of water, electrolytes, and protein and decreased absorption of fluid. Large volumes of fluid and protein are lost in the gastrointestinal tract, causing hemoconcentration and profound hypoalbuminemia in some affected horses.[713,715,717]

Cystitis and myocarditis occur in natural and experimentally induced cases of cantharidin toxicity.[713,715,717] The toxin is excreted by the kidneys, and high concentrations of cantharidin in the urine induce cystitis. Occasionally, hemorrhagic cystitis may occur, resulting in hematuria or frank hemorrhage into the bladder.[713] The cause of myocarditis and myocardial necrosis is unknown but may be a direct effect of toxin on the myocardium. Increased plasma creatine kinase activity

is often observed and has been postulated to arise from the damaged myocardium.[713,714] Affected horses have a characteristically stiff gait, but histopathologic evidence of skeletal muscle injury that explains the elevated plasma creatine kinase activity has not been observed.[714] The kidneys are often pale, swollen, and moist, with occasional infarcts.[715]

Hypocalcemia and hypomagnesemia are biochemical features of cantharidin toxicity in horses that have not been explained.[713,714,717] Hypocalcemia may result from hypoalbuminemia, but the ionized calcium concentration is often decreased, indicating that hypoalbuminemia is not responsible for the hypocalcemia.[714]

Clinical Signs and Diagnosis

Cantharidin toxicity can cause a range of clinical signs, from mild depression and abdominal discomfort to fulminant signs of toxemia and rapid death, depending on the ingested dose of toxin.[713,714,717] Most commonly, clinical signs include depression, sweating, irritability, abdominal pain, elevated heart and respiratory rates, fever, polyuria, polydipsia, and profuse diarrhea.[713,714,717] Blood is rarely seen in the feces. Stranguria and pollakiuria are common.[713] Signs of hypocalcemia include synchronous diaphragmatic flutter and tremors. A stiff and stilted gait may be evident. Neurologic signs such as head pressing, swaying, and disorientation may be noted.[717] Signs of systemic inflammation resulting from endotoxemia may be seen in severe cases. Some horses develop severe depression and toxemia and may die within hours of ingesting cantharidin without developing diarrhea.[713,717]

Hematologic abnormalities include hemoconcentration and neutrophilic leukocytosis.[713,714] Occasionally, neutropenia and leukopenia may accompany endotoxemia. Serum biochemical analysis usually reveals increased creatine kinase activity, hypocalcemia, and hypoalbuminemia.[713,714] Biochemical abnormalities include hypocalcemia (both ionized and total calcium concentrations), hypomagnesemia, and azotemia.[713,714,717] Urine specific gravity is characteristically in the hyposthenuric range.[713,714] Microscopic hematuria and mild proteinuria may be evident. Fecal occult blood is often present, but hematochezia is unusual.

A tentative diagnosis can be made on the basis of clinical signs and identification of blister beetles in the hay. Determining the species of the insects may be necessary to estimate the amount of cantharidin ingested. All species of *Epicauta* contain cantharidin, but some have small amounts. Definitive diagnosis requires the measurement of the cantharidin concentration in gastric or intestinal contents and urine.[713,716]

Arsenic Toxicosis

Pathogenesis

Arsenic toxicosis is an unusual cause of diarrhea in horses, resulting from ingestion of arsenic-containing herbicides, insecticides, and other pest-control products contaminating water or roughage used as a food source.[718] The toxicity of arsenic depends on the valence of the element.[718,719] Arsenate may be reduced to arsenite in mammalian systems.[719] It is thought to be more toxic than arsenate and less rapidly excreted in urine.[719] Arsenate and arsenite uncouple oxidative phosphorylation, leading to the breakdown of energy metabolism in the cells of many tissues.[719] Widespread cellular injury and death occur rapidly during acute arsenic toxicosis. Multiorgan failure is usually the result. Cardiomyopathy and pulmonary disease are common causes of death in humans.[720] Damage to the large intestine is probably caused in part by direct cellular toxicity and corrosion by the compound. However, vasculitis is a hallmark of the disease in humans and horses and is thought to be the most important mechanism of large intestinal disease in humans.[718,721] Acute hemorrhagic colitis is a feature of arsenic toxicosis, with severe mural edema and mucosal ulceration.[718] Profuse hemorrhagic diarrhea and abdominal pain result. Chronic arsenic toxicity can occur but appears to be rare in horses.

Clinical Signs and Diagnosis

Depression, weakness, abdominal pain, hemorrhagic diarrhea, and shock are characteristic of acute arsenic toxicosis in horses.[718] Death may occur before diarrhea is evident. Initial clinical signs may be difficult to distinguish from other peracute forms of colitis and are related to endotoxic shock, metabolic disturbances, and dehydration. Later, cardiac arrhythmias, pulmonary edema, acute renal failure, and neurologic deficits (ataxia and stupor) may develop.[718] Anuria or polyuria may be observed. Hemolytic anemia caused by preferential binding of arsenic compounds to red blood cells is a feature of arsenic poisoning in humans.[720] Hematologic abnormalities resulting from injury to bone marrow cells and ongoing hemolysis may be seen after the peracute stage. Leukopenia and thrombocytopenia have been described in human patients.[720] Serum biochemical analysis may reveal azotemia, hepatocellular enzyme activities higher than generally attributed to endotoxemia, and increased creatine kinase activity.[718] Urine specific gravity may be in the isosthenuric range, with hematuria, cylindruria, and proteinuria evident by urinalysis.

Diagnosis may be possible by measuring blood and urine arsenic concentration, but these tests may not be diagnostic. Postmortem diagnosis is confirmed by measuring arsenic concentrations in liver and kidney samples.[718] History of exposure and clinical signs remain the primary means of diagnosis.

MISCELLANEOUS INFLAMMATORY DISORDERS OF THE LARGE INTESTINE

Intestinal Anaphylaxis

Pathogenesis

Severe intestinal anaphylaxis is a syndrome in horses characterized by peracute, rapidly fatal colitis.[722] The severe syndrome is clinically and pathologically similar to other known causes of peracute colitis. Some cases are less severe and manifest as mild to moderate diarrhea or colic (or both). The syndrome of intestinal anaphylaxis can be produced by either an IgE-mediated type I hypersensitivity or an IgE-independent anaphylactoid reaction.[723,724] Intestinal anaphylaxis is usually induced by local gastrointestinal exposure to a food, environmental, drug, or other allergen[723,725] but may also occur with systemic exposure to an allergen.[726-728] Massive mast cell degranulation, secretion of inflammatory mediators, and activation of enteric neural reflexes in the intestine cause profound alterations in blood flow, increased vascular permeability and interstitial edema, recruitment of neutrophils, altered motility, mucosal injury, absorption of microbial products, and mucosal hypersecretion.[729-733] Systemic signs may be caused by the anaphylactic reaction or may be associated with SIRS.

The peracute form is characterized by severe intramural edema and hemorrhagic inflammation of the large intestine, often producing submucosal thickening on the order of many centimeters.[162,722] Vascular thrombosis may be widespread, with mucosal and serosal petechiae and ecchymoses. Less severe forms of intestinal anaphylaxis may manifest as patchy areas of intestinal edema and congestion.[726] Diarrhea results from intestinal inflammation initiated by the type I hypersensitivity response. Many of the mediators of type I hypersensitivity, such as histamine and 5-HT, have well-documented stimulatory effects on mucosal secretory activity, vascular and epithelial permeability, and motility[729-731] in the intestine. Systemic inflammation resulting from endotoxemia may be overwhelming once the mucosal barrier breaks down. Infarction of intestinal segments and other organs may result from intravascular coagulation. Ileus, abdominal distention, and moderate to severe abdominal pain may result from motility disturbances and infarction of the large intestine.

Clinical Signs and Diagnosis

The clinical signs are similar to those described for other forms of peracute colitis. Severe diarrhea is possible, but death may occur before diarrhea is evident. Multiorgan failure resulting from DIC is not unusual. The rapid onset of weakness, staggering, and trembling commonly precedes death. The syndrome may cause death in 4 to 24 hours.

Diagnosis is based on clinical signs, postmortem findings, and exclusion of other causes. Most diagnostic tests are inconclusive. If an antigen is suspected to be the trigger of the anaphylaxis, a Prausnitz-Kustner passive cutaneous anaphylaxis sensitization test can confirm the presence of antigen-specific IgE in the patient's serum.[726]

Carbohydrate Overload

Pathogenesis

Overeating of soluble carbohydrates, especially so-called hot grains such as corn, overwhelms the digestive capability of the small intestine, resulting in a high percentage of soluble carbohydrates entering the large intestine. The amount of soluble carbohydrates that will produce diarrhea varies according to the previous dietary history of the individual. Horses fed diets higher in soluble carbohydrates are more resistant to the deleterious effects of carbohydrate overload. Gradual accommodation to a diet high in carbohydrates can be accomplished over several weeks. Horses fed an unusually large amount of grains or other form of soluble carbohydrates often develop diarrhea and may, depending on the amount ingested, develop severe colitis, systemic inflammation resulting from endotoxemia, metabolic acidosis, and laminitis.[229,734-736]

The pathogenesis of colitis from carbohydrate overload is primarily caused by the toxic effects on the microbial flora in the large intestine.[735] A sudden delivery of soluble carbohydrates to the large intestine causes rapid fermentation by gram-positive lactic acid–producing bacteria and a sudden increase in organic acid production.[229] The cecal pH level rapidly decreases, and the lactic acid concentration rapidly increases.[229] Rapid organic acid production overwhelms the buffering capacity of the large intestine, not only by directly depleting the buffers found in the contents but also by reducing the efficiency of buffer secretion. Bicarbonate secretion is linked to absorption of volatile fatty acids, which are produced in low amounts by fermentation of soluble carbohydrates. The contents of the large intestine become profoundly acidic, resulting in unfavorable conditions for the microbial flora. Lactic acid–producing bacteria flourish, whereas the gram-negative bacteria, especially the Enterobacteriaceae, are killed in large numbers by the acids. Large quantities of endotoxin are released from the dying bacteria.[229]

The osmotic load from lactic acid produced in the large intestine is an important factor in the development of diarrhea because organic acids such as lactic acid are poorly absorbed. Mild cases of carbohydrate overload may result purely from osmotic diarrhea. In more severe cases the acidic contents of the large intestine are toxic to the mucosa, causing necrosis of the mucosal tissues similar to that seen in ruminal acidosis. Mucosal ulceration allows absorption of large quantities of endotoxin and lactic acid produced by the massive die-off of acid-intolerant microbes and fermentation of soluble carbohydrates, normally poorly absorbed by intact mucosa.[736] Systemic inflammation resulting from endotoxemia may be overwhelming; clinical and laboratory findings are consistent with that described for endotoxemia. Laminitis is a frequent complication; carbohydrate overload is used to induce laminitis as an experimental model because of the consistency of laminitis produced.[229,735,736]

Clinical Signs and Diagnosis

Clinical signs of colitis from carbohydrate overload can vary according to the amount of carbohydrate ingested and accommodation of the flora to a high-carbohydrate diet. Mild cases may result in a transient osmotic diarrhea, with no systemic effects. Severe cases are characterized by signs similar to those described for other forms of colitis, including abdominal pain, moderate to severe diarrhea, and dehydration. Signs of endotoxemia and sepsis are frequently present in severe cases. Nasogastric intubation may yield significant acidic gastric reflux. Particles of grain may be noted in the gastric reflux and the feces if grain overload is the source of the carbohydrate overload. Laminitis is a common complication.

Sand Enteropathy

Sand enteropathy is often associated with acute intestinal obstruction due to abnormally large amounts of sand in the large intestine.[737] However, chronic sand-induced diarrhea is a distinct syndrome that can occur at any age as a result of the abnormal accumulation of sand in the large intestine.[738,739] Chronic diarrhea and signs of colic may be seen without obstruction. Diagnosis is usually based on the presence of abnormal amounts of sand in the feces, although some horses with sand enteropathy may not be passing sand in the feces at any given moment. Occasionally, radiography may be required to detect sand in the colon.[738]

PRINCIPLES OF THERAPY FOR ACUTE DIARRHEA

The principles of therapy for acute diarrhea resulting from colitis are similar regardless of the cause and include replacement of fluid and electrolyte losses, control of colonic inflammation, reduction of fluid secretion, promotion of mucosal repair, control of endotoxemia and sepsis, and reestablishment of normal flora. The reader is referred elsewhere in this chapter for a discussion of general treatment of endotoxemia, for which many of the principles of therapy overlap.

Fluid Replacement and Circulatory Support

Replacement of fluid and electrolyte losses is of primary concern in treating horses with salmonellosis. Depending on the severity of the disease, fluid losses may be minimal or massive. Fluid and electrolytes can be administered orally or IV. Some horses with mild to moderate diarrhea may maintain hydration and electrolyte balance by consuming water and electrolytes voluntarily. Fresh water and water containing electrolytes should be available in all cases. In many instances, periodic nasogastric intubation and administration of water and electrolytes through the tube may be sufficient to maintain hydration.[740] IV administration of fluids is preferred in cases requiring significant quantities of fluid to replace and maintain hydration and electrolyte balance.[357] It is not unusual for patients with severe diarrhea to require large volumes (50–100 L/day) of IV fluids to maintain hydration. Monitoring of packed cell volume, serum electrolyte concentration, venous blood gases, blood urea nitrogen and creatinine, urine protein and cytology, and body weight is important to evaluate hydration, electrolyte and acid-base balance, and renal function.

Isotonic sodium chloride or lactated Ringer's solution is frequently used to restore and maintain fluid and electrolyte balance. Potassium chloride can be added to the fluids and administered at a rate up to 0.5 to 1.0 mEq/kg/h. Generally, a rate of less than 0.5 mEq/kg/h is used. Hypertonic NaCl solutions (1–2 L of 5% or 7.5% NaCl) have been used in horses with acute, severe hyponatremia (<120 mEq/dL) and those with hemodynamic shock resulting from sepsis. The beneficial effects of hypertonic NaCl are short-lived (30–60 minutes). Isotonic solutions should be administered concurrently or immediately after administration of hypertonic NaCl solutions. Isotonic (1.3%) or hypertonic (5.0%) sodium bicarbonate solutions are used to correct metabolic acidosis. Prolonged administration of sodium-containing fluids may promote diuresis and renal water loss or accumulation of peripheral edema and should be used conservatively when a relative free water loss is noted. Administration of isotonic dextrose (5%) or 2.5% dextrose/0.45% NaCl solutions may be beneficial when free water loss (relative sodium excess) is evident.

Many horses with acute colitis are concurrently hypoproteinemic because of gastrointestinal losses and are absorbing bacterial products that induce a systemic inflammatory response. Plasma oncotic pressures are abnormally low in the face of increased vascular permeability. Interstitial edema formation is a clinical problem in these patients, which contributes to organ dysfunction. Crystalloid fluids, although critical for replacing water and electrolyte losses resulting from diarrhea, may actually contribute to a decline in plasma oncotic pressure as a result of hemodilution.[436,439] Administration of colloid solutions is helpful for volume expansion and maintenance of plasma oncotic pressures. Discussion of colloid replacement is included in the section Endotoxemia.

Antiinflammatory Therapy

Control of colonic inflammation and secretion is a difficult and poorly studied aspect of equine acute colitis. The role of inflammation and mediators such as prostaglandins as a cause of fluid loss is well known during *S. enterica* and clostridial infection.[103,142,198,552,571,637,638,741] COX inhibitors (NSAIDs) have antisecretory effects on the inflamed intestinal tract,[91,169] including in the equine colon.[742,743] NSAIDs are commonly administered to horses with colitis to reduce inflammation-associated fluid secretion. However, prostaglandins such as PGE_2 and PGI_2 also have cytoprotective effects on gastrointestinal mucosa and are critical for mucosal repair.[91,701] NSAIDs used pharmacologically to inhibit colonic inflammation and secretion may be detrimental to mucosal integrity and healing if not used judiciously. NSAIDs exacerbate colonic inflammation in humans with inflammatory colitis, impede mucosal healing in several models of mucosal injury, and have well-documented detrimental effects on colonic mucosa in horses.[91,690,698,701] In addition to toxicity to the colonic mucosa, gastric ulceration is not unusual in horses with enterocolitis and may be related to treatment with NSAIDs. Although the use of COX-2–selective NSAIDs should, in theory, be less likely to harm or impede repair of the colonic mucosal in patients with colonic inflammation causing diarrhea, the safety and efficacy of these medications has not been evaluated in this application. The prothrombotic adverse effects of COX-2–selective drugs[744] suggest that very cautious use of highly COX-2–selective medications is warranted in patients with systemic inflammation at risk for thrombosis (e.g., endotoxemia and sepsis).

In addition to NSAIDs, other drugs are occasionally used as antiinflammatory and antisecretory therapy. Bismuth subsalicylate is commonly used in adults and foals with diarrhea. The volume required for any effect in adults with colitis is quite high (1–4 L by nasogastric tube every 4–8 hours), which often precludes its use. Metronidazole has beneficial effects in experimental models of gastrointestinal inflammation, including NSAID toxicity,[706] and may be useful for treating horses with colitis; however, evidence supporting its use is lacking.

Mucosal Repair and Protection

Sucralfate (20 mg/kg by mouth every 6 hours) has been advocated to aid in healing of the colonic mucosa in patients with NSAID toxicity. There is evidence in experimental phenylbutazone toxicosis in foals suggesting that sucralfate administration lessens ulceration and other histopathologic lesions throughout the alimentary tract and lessens protein loss.[745] There is no evidence supporting the use of sucralfate to treat colonic ulceration in adult horses.

Misoprostol (5 μg/kg by mouth every 12 hours or 2 μg/kg by mouth every 6–8 hours) and other synthetic PGE analogs enhance mucosal healing in the intestine and promote recovery in experimental models of colitis.[746] Misoprostol may be particularly useful for treating NSAID toxicity, either the generalized form or RDUC. However, the efficacy of misoprostol in hastening mucosal healing is clinically unproven in equine colitis. The primary drawbacks of prostaglandin analogs such as misoprostol are the adverse effects of the drug, including abdominal cramping, diarrhea, sweating, and abortion in pregnant mares.

Psyllium mucilloid can be added to the diet (5 tablespoons every 12–24 hours) to increase the production of SCFAs in the colon. Amylase-resistant fermentable fiber such as psyllium is hydrolyzed by colonic bacteria to SCFAs such as butyrate, which represent a major energy source for colonocytes. Butyrate and other SCFAs hasten epithelial maturation and stimulate salt (and thus fluid) absorption in the colon, improve the clinical course of ulcerative colitis, and hasten colon healing.[747] Psyllium is itself a source of butyrate in the colon and also promotes the movement of amylase-sensitive carbohydrates into the distal colon, which are then fermented to SCFAs. Psyllium is thought to be clinically useful for promoting mucosal healing in colitis.

Pain Control

Many horses with colitis have mild to severe signs of abdominal pain. Analgesia can be accomplished with NSAIDs such as flunixin, but the potential for worsening mucosal injury or nephrotoxicity may prevent the use of analgesic doses, especially in horses with suspected NSAID toxicity. Relative COX-2–selective inhibitors may spare the gastrointestinal mucosa. For example, meloxicam and firocoxib have analgesic properties in horses in experimental models of ischemic colic and spare the intestinal mucosa from the detrimental effects associated with nonselective COX inhibitor treatment.[695,748]

Xylazine, detomidine, or butorphanol (bolus dosing) may provide temporary analgesia. Constant rate infusions of butorphanol, lidocaine, and/or ketamine (alone or in combination) may provide more profound analgesia and can be useful if one is certain that an obstructive or infarctive process is not present. Pain management is discussed in Chapter 3 of this text.

Antimicrobial Therapy

Broad-spectrum antibiotic treatment is often recommended in neutropenic horses or horses with signs of sepsis. Broad-spectrum antibiotics lessen septic complications in human patients. Evidence supporting this principle in horses with colitis is lacking.

Treatment with antibiotics is not thought to alter the course of the enterocolitis, but it may lessen dissemination and severity of disease. Treatment with antibiotics directly targeted at *S. enterica* is often reserved for patients with sepsis. Lipid-soluble antibiotics are ideally suited for *S. enterica* infections because the bacteria persist intracellularly. Enrofloxacin is often preferred, but antimicrobial sensitivity of the isolate should dictate directed therapy.

In patients in which the likelihood of *N. risticii* infection is high, treatment with directed antimicrobial therapy is often indicated before a definitive diagnosis. Lipid-soluble drugs are preferred because the organism can live within cells. Oxytetracycline (6.6 mg/kg/day IV), often followed by doxycycline (10 mg/kg PO every 12 hours), appear to be the most effective antibiotics for treatment of Potomac horse fever and are considered the treatment of choice. Treatment is most successful if initiated before the onset of diarrhea.[584,749]

If antibiotics are being administered at the onset of enterocolitis, they should be discontinued if possible. Specific treatment with metronidazole is effective for treating clostridiosis in humans and appears to be effective in horses.[624,750] Metronidazole resistance in clinical isolates of *C. difficile* has been reported in one outbreak but appears to be rare in most human and equine cases.[751] Metronidazole-resistant isolates were sensitive to vancomycin, which may be effective for treating clinical cases if metronidazole resistance is suspected. Vancomycin use is not recommended in horses because of concerns for antimicrobial stewardship. *C. perfringens* type C antitoxin has been recommended for treatment of neonatal clostridiosis, but there is no evidence of its efficacy.[752] Antitoxin preparations are not advocated for use in adult horses with clostridiosis.

Anticoagulation

Hypercoagulability is a common complication of enterocolitis, associated with systemic inflammation resulting from endotoxemia. Administration of heparin (20–80 IU/kg SC or IV every 6–12 hours) may prevent thrombosis in these patients, provided antithrombin III concentrations are adequate in the plasma. Concentrated sources of antithrombin III are not available for use in horses, but whole plasma may provide an important source. Treatment with heparin is thought to decrease thrombosis, especially of the jugular vein, which is a serious complication of salmonellosis. Low-dose aspirin treatment (10 mg/kg PO every 24–48 hours) in conjunction with heparin treatment may provide added benefit by irreversibly inhibiting platelet function.[753] Heparin and aspirin may have protective effects on the digital lamina.[753,754] Heparin may enhance the phagocytic activity of the reticuloendothelial system by enhancing the efficiency of opsonins, such as fibronectin and immunoglobulin, stimulating phagocytosis of products of coagulation and possibly other particles, including bacteria.[755,756]

Probiotics

Maintenance of the bacterial flora in the gastrointestinal tract is an important defense mechanisms preventing colonization by pathogenic bacteria. Little work has been done to investigate the efficacy of these products in horses. Preparations of *Saccharomyces boulardii* were shown to decrease the duration and severity of disease in horses with colitis.[757] In foals, probiotic use was associated with increased risk of diarrhea and, especially, diarrhea requiring clinical intervention. In humans, fecal transplantation (known to most veterinarians as transfaunation) has been proven effective for intestinal clostridiosis. As the intestinal microbiota are extremely diverse,[756] restoration of the overall flora is more likely to be effective than restoration of single bacterial species.

Absorbent Powders and Mineral Oil

Absorbent powders are often used to reduce the bioactivity and absorption of bacterial toxins produced by bacterial or toxic metabolites produced by bacteria and other microorganisms (e.g., lactic acid in grain overload). In a rat model of cantharidin toxicosis, mineral oil, which is often used in equine cantharidin toxicosis, increased cantharidin absorption and also increased morbidity and mortality.[758] In that study, rats treated with charcoal or DTO smectite had improved survival. DTO smectite powder (Bio-Sponge, Platinum Performance, Buellton, California) binds *C. difficile* and *C. perfringens* exotoxins in vitro[343,759] and may be useful for treating intestinal clostridiosis, or unspecified colitis, in horses. DTO smectite is available as a powder or paste and should be administered according to the manufacturer's instructions for 3 to 5 days.

Nutrition

Good nursing care and adequate nutrition are vital to the treatment of horses with colitis. Normal intake of roughage to provide energy may be inadequate; however, feeding of grains should be avoided to prevent delivery of highly fermentable carbohydrate to the colon. Dietary management usually consists of restricting or eliminating long-stem roughage (hay) from the diet and feeding exclusively a complete pelleted diet (at least 30% dietary fiber). The rationale behind this recommendation is to reduce the mechanical and physiologic load on the colon. Frequent meals (4–6 times a day) are recommended. Corn oil (1 cup every 12–24 hours) can be added to the pellets to increase the caloric intake without adding roughage or grain. It is important to note that if a horse with colitis refuses to eat pelleted feed, then high-quality grass hay should be fed. In anorectic or severely catabolic patients, enteral and parenteral nutrition (total and partial) has been used successfully to provide calories and nutritional support.

Specific Therapies

Strongylosis

Treatment of *S. vulgaris* infection requires treatment of the migrating parasite larvae and the lesions produced by the parasite. Fenbendazole (10 mg/kg PO every 24 hours for 3 days or 10 mg/kg PO every 24 hours for 5 days) and ivermectin (200 mg/kg, PO) are effective in killing fourth-stage larvae.[647] Other anthelmintics may also be effective when given at higher doses than those required to kill adult worms. The efficacy of these anthelmintics against larvae within thrombi is not known.

Thrombolytic and antithrombotic therapy has been advocated in horses with suspected strongylosis.[647,654] Heparin (20–80 IU IV or SC every 6–12 hours) may be administered as an anticoagulant. High-molecular-weight heparin causes anemia by inducing aggregation of red blood cells, which is an undesirable effect in sepsis. Low-molecular-weight heparins appear to be less likely to have this effect in horses. Aspirin (10–30 mg/kg PO every 12–48 hours) is usually combined with heparin to inhibit platelet adhesion. Aspirin may also inhibit release of platelet products, such as thromboxane, which affect the motility of the large intestine. Low-molecular-weight dextrans have been advocated as antithrombotics that act by inhibiting platelet function and coagulation.[654] The clinical efficacy of dextran administration appears to be good, but no controlled studies have been performed.

Cyathostomiasis

Anthelmintic administration is usually the only treatment necessary for mild to moderate cases of cyathostomiasis treated early in the course of the disease (within 1–3 weeks of onset). Fenbendazole is effective against many larval stages, but resistance is high in some populations. Although the reported efficacy of ivermectin is variable against certain stages,[760] one study reported an overall efficacy of 75%.[761] Currently, fenbendazole (7.5–10 mg/kg PO every 24 hours for 5 days) followed on day 6 by ivermectin (200 mg/kg PO) is the most commonly advocated treatment regimen.[659,762] Moxidectin (400 µg/kg PO every 24 hours) may also be effective against adult organisms and L_3 and L_4 larval stages[763] and may be useful for treating cyathostomiasis. Antiinflammatory therapy may be beneficial, especially in severe or refractory cases or before treatment with larvicidal medications. Pretreatment with dexamethasone or prednisolone is indicated before anthelmintic administration if heavy larval loads are suspected to prevent an acute exacerbation of the disease by rapid death of encysted larvae. Larvicidal treatment with moxidectin appears to be less likely than fenbendazole to result in tissue inflammation resulting from larval death.[764] NSAID administration may have limited value, but dexamethasone appears to be efficacious in refractory cases when used in conjunction with larvicidal anthelmintics.[659,662] Bismuth subsalicylate is often administered orally as an antisecretory agent in young animals. Supportive care may be necessary in severe cases, particularly if hypoproteinemia is severe. Administration of IV crystalloid fluids and plasma or other colloids is occasionally required. Proper nutritional support is also important.

Arsenic Toxicity

Reduction of arsenic absorption by administration of cathartics such as activated charcoal should be initiated immediately.[718] Chelation therapy with sodium thiosulfate (20–30 g in 300 mL of water, administered orally) and dimercaprol (British anti-Lewisite; 3 mg/kg PO every 4 hours) is indicated.[718] Dimercaprol is a specific antidote for trivalent arsenicals, but its efficacy in horses is questionable.

Intestinal Anaphylaxis

Treatment of intestinal anaphylaxis is in principle similar to treatment of other forms of colitis, but it is often unsuccessful because of the rapidly progressive nature of the syndrome.[162,722,727] Early treatment with prednisolone sodium succinate (10–20 mg/kg IV) or dexamethasone (0.1–0.2 mg/kg IV) may be essential for successful treatment.[722]

Peritonitis

STRUCTURE AND FUNCTION

The peritoneum consists of a single layer of mesothelial cells. The mesothelial lining of the diaphragm, abdominal walls, and pelvic cavity is termed the *parietal peritoneum*. The visceral peritoneum forms the serosal surfaces of the intraabdominal organs. Caudally, the peritoneum reflects over the surfaces of the pelvic organs (portions of the urogenital tract and rectum), excluding them from the peritoneal space, and defining the retroperitoneal space. The peritoneal space communicates with the lumen of the uterus (and thus the external environment) via the fallopian tubes in females. In males the peritoneum forms a true blind sac. The vascular supply and nervous innervation of the visceral peritoneum are supplied by the splanchnic vessels and visceral autonomic nerves, respectively. Branches of the intercostal, lumbar, and iliac arteries supply the parietal peritoneum, and the phrenic and intercostal nerves provide nervous innervation. Inflammation of the parietal peritoneum is perceived as somatic pain, resulting in a splinted abdominal wall, pain on external palpation, and reluctance to move.

The peritoneal lining functions as a semipermeable barrier to the diffusion of water and low-molecular-weight solutes between the blood and the abdominal cavity.[765] The peritoneum secretes a serous fluid that lubricates the abdominal cavity, inhibits adhesion formation, and has minor antibacterial properties.[765,766] Macrophages, mast cells, mesothelial cells, and lymphocytes provide immune function within the peritoneum.[767] The peritoneal surface maintains a high level of fibrinolytic activity through the production of plasminogen activators by mesothelial cells. This function, together with the lubricant properties of the PF, helps to maintain gliding surfaces within the peritoneum and prevent adhesion formation. PF produced by the mesothelium tends to move ventrally and cranially, aided largely by diaphragmatic movement. PF, waste products, and foreign material exit the peritoneal cavity to enter the lymphatic system through diffusely distributed subendothelial pores or via the large diaphragmatic stomata, depending on particle size. Large molecules and particles greater than approximately 40,000 MW (such as bacteria) exit through the diaphragmatic stomata and ultimately enter the thoracic duct. The term *peritonitis* refers to inflammation of the mesothelial lining of the peritoneal cavity.

ETIOPATHOGENESIS

Peritonitis can occur in association with any insult—mechanical, chemical, or infectious—that results in disruption or irritation of the peritoneal lining, inflammation or infection of abdominal organs, or compromise of the intestinal wall.[765] Common mechanical injuries include blunt or perforating trauma to the abdominal wall, breeding and foaling accidents, and abdominal surgery. A variety of iatrogenic insults can cause peritonitis, such as abdominocentesis, enterocentesis, splenic puncture, bowel trocarization, liver biopsy, uterine biopsy, castration, and rectal tear. Chemical insults of endogenous origin include blood, urine, pancreatic enzymes, bile, gastric juice, chyme, and chyle. Talc, contrast agents, antibiotics, and lavage solutions are additional examples of chemical insults. Traumatic events often involve bacterial contamination at the time of injury, and mechanical and chemical injuries can become infected secondarily.

The most common manifestation of peritonitis is acute, diffuse, septic peritonitis following inflammation, vascular insult, perforation, or surgical manipulation (enterotomy, resection, and anastomosis) of the gastrointestinal tract. The septic process in such cases involves mixed bacteria of gastrointestinal origin. Penetrating abdominal wounds also result in mixed infections. Less commonly, singular bacterial forms gain access to the peritoneum through hematogenous spread, extension from a contiguous organ, or through the female genital tract. Primary monomicrobial infections have been reported involving *Streptococcus equi* subsp. *equi,*[768] *S. equi* subsp. *zooepidemicus,*[769] *Rhodococcus equi,*[770] and *Corynebacterium pseudotuberculosis.*[771] Several case series involving peritonitis associated with *Actinobacillus equuli* have been reported.[772-775] Sepsis, septic omphalitis, ascending urinary tract infections, and uterine infections are additional causes of monomicrobial infection.

Most cases of trauma or intestinal perforation result in contamination of the peritoneum with large numbers of many types of bacteria. The intestinal tract contains a mixed population of bacteria, and the quantity of bacteria and prevalence of anaerobic species increase in the distal segments. Not surprisingly, mortality associated with contamination from the lower bowel is high. Hirsch and Jang[776] reported isolation of an infective agent from exudative equine PF in approximately 25% of attempts. Obligate anaerobic bacteria were cultured most frequently, followed by members of the Enterobacteriaceae family (predominantly *E. coli*). Penicillin-resistant *Bacteroides fragilis* was isolated from 10% to 20% of cases. In another study in which bacteria were identified in equine abdominal fluid by cytologic examination or culture, *E. coli* was the organism most commonly isolated.[777] In human beings and laboratory animals, despite the variety of organisms initially introduced by polymicrobial contamination, established infections are characterized by only a few types of bacteria, which are often gram-negative aerobes and anaerobic bacteria. This selectivity occurs through the processes of selective reduction of bacterial populations and bacterial synergism. An example of synergism in human beings and laboratory animals is peritonitis involving *E. coli* and *B. fragilis.* The presence of each organism is beneficial to the survival of the other, and each is important in the overall pathogenesis of the disease. *E. coli* is associated with septicemia and early mortality, whereas *B. fragilis* infection tends to result in chronic abscessation with delayed morbidity and mortality.[766]

Other causes of equine peritonitis include parasites, viral disorders (influenza, equine viral arteritis, equine infectious anemia, African horse sickness), and neoplasia. Verminous arteritis caused by strongylosis can lead to vascular damage (thromboembolism and infarction) to the intestine. The activities of strongyles, ascarids, and tapeworms can result in perforation of the bowel and damage to other abdominal organs. There has been one report of septic peritonitis caused by colonic perforation associated with aberrant migration of *Gasterophilus intestinalis* larva.[778]

Biologic events resulting from contamination of the abdomen or injury to the mesothelial cells include release of catecholamines, histamine, and serotonin from peritoneal mast cells; vasodilation and hyperemia; an increase in peritoneal vascular permeability; secretion of protein-rich fluid into the peritoneum; transformation of mesothelial cells into macrophages; and influx of PMN cells, humoral opsonins, natural antibodies, and serum complement into the peritoneal cavity.[765] Other possible events include depression of the peritoneal fibrinolytic activity, fibrin deposits on the peritoneal surface, and inflammatory-mediated and sympathetic-mediated ileus. These processes serve to confine contamination and infection and, with clean, minimally invasive procedures such as enterocentesis or trocarization, are effective. However, with greater severity of peritoneal contamination or irritation, these processes are magnified and become deleterious. Consequences include hypovolemia, hypoproteinemia, gastrointestinal ileus, ischemia of the bowel wall with subsequent absorption of bacteria and toxins, and ultimately adhesion and abscess formation. Equine peritoneal macrophages release a multitude of inflammatory mediators when exposed to bacterial LPS, and endotoxemia contributes the clinical picture. A complete review of endotoxemia is presented in Endotoxemia.

A complete pathologic description of peritonitis includes origin (primary or secondary), onset (peracute, acute, and chronic), distribution (localized versus diffuse), and presence of bacteria (septic versus nonseptic). Clinically, viewing the pathogenesis of peritonitis as a series of stages is useful. The contamination stage, lasting 3 to 6 hours, involves introduction of bacteria into the peritoneum and initiation of the acute inflammatory response previously described. If the organisms are not eliminated, the process evolves to the stage of acute diffuse peritonitis as, regardless of the location of the initial contamination, bacteria spread throughout the peritoneum within several hours. The stage of acute diffuse peritonitis lasts up to 5 days. The inflammatory response persists and escalates with continued exudation of proteinaceous fluid and influx of inflammatory cells. Offending organisms are delivered to the lymphatic system and may be eliminated by the immune system. Alternatively, organisms can gain access to the systemic circulation in sufficient numbers to result in clinically relevant bacteremia. This stage of the disease process has the highest mortality because of the effects of severe peritoneal inflammation, endotoxemia, and sepsis. If the animal survives this stage but fails to eliminate infection from the peritoneal cavity, the disease enters a transitional phase referred to as the acute adhesive (or localizing) stage, typically occurring 4 to 10 days after the initial insult. Neutrophils are still active, macrophages are increasing in numbers, and fibrin aggregates are being organized or lysed. If infection persists beyond this point, organization of fibrin proceeds and organisms become isolated from host

defenses. At this point, the disease process enters the stage of chronic abscessation. This stage can begin as early as 8 days after inoculation and persist indefinitely.

Clinical Signs

Clinical signs of peritonitis depend on the primary disease process, the duration of the problem, and the extent of peritoneal inflammation. Localized peritonitis may have few or no systemic manifestations, whereas severe localized or generalized peritonitis often is accompanied by severe toxemia, sepsis, or both. Septic peritonitis usually causes more severe clinical signs because of the systemic inflammatory response and endotoxemia. Most clinical signs are nonspecific and include fever, lethargy, inappetence, decreased borborygmi, and dehydration. Additional signs include colic, ileus, weight loss, and diarrhea.[768]

Horses with peracute peritonitis, as occurs with rupture of the bowel or rectal tear, have clinical signs associated with severe endotoxemia, weakness, lethargy, colic, and/or circulatory failure. Fever may not be present depending on the degree of shock. Parietal pain, characterized by reluctance to move, splinting of the abdominal wall, and sensitivity to external abdominal pressure occur in some acute cases. With extensive abdominal fecal contamination, rectal examination may reveal a gritty feeling of the serosal and parietal surface of the peritoneum because of fibrin deposition.

In horses with more chronic peritonitis, clinical signs include intermittent colic, lethargy, anorexia, weight loss, intermittent fever, ventral edema, exercise intolerance, decreased or absent intestinal sounds, and mild dehydration. Heart and respiratory rates may be normal. Fecal output may be normal; however, horses with chronic diarrhea and weight loss have been reported. Rectal examination findings can include pain on palpation of fibrinous or fibrous adhesions, intestinal distention, an abdominal mass, or an impression of bowel floating in fluid. In many cases, rectal examination does not reveal significant abnormalities.[768]

In cases of *A. equuli* peritonitis, clinical signs in most horses included lethargy, inappetence, and mild to moderate abdominal pain if acute or weight loss if chronic.[773,774] Postpartum mares with peritonitis secondary to a uterine perforation typically present with fever and depression, with or without abdominal pain. Septic tenosynovitis of the tarsal sheath secondary to bacterial peritonitis from gastrointestinal perforation has been reported.[779] Foals with peritonitis usually exhibit signs of colic (acute or chronic) and are febrile, depressed, and inappetent. In young foals, peritonitis can cause rapid metabolic deterioration, and determination and correction of the primary problem require immediate attention. In older foals, peritonitis may occur insidiously in association with *S. equi* subsp. *equi* or *R. equi* infections.

Clinicopathologic Findings

Clinicopathologic abnormalities vary depending on the time of onset and severity. In the acute stage, leukopenia, hemoconcentration, metabolic acidosis, and azotemia predominate. After several days, leukocytosis and hyperfibrinogenemia are more typical. In chronic peritonitis, hyperproteinemia with hyperglobulinemia may be present. SAA is also increased.[254] Neonates with uroperitoneum tend to develop azotemia, hyponatremia, hypochloremia, hyperkalemia, and acidemia.

PF analysis is principal to the diagnosis of peritonitis. Detailed description of PF analysis is provided in the Examination section of this chapter. PF WBC counts in acute peritonitis are reportedly higher than those in chronic peritonitis,[768] but this is not always the case, and the WBC count does not always correlate with disease severity or prognosis. The PF WBC count can be greater than 100,000/μL following enterocentesis, with no clinical signs or problems.[780] Conversely, peritoneal WBC counts of fewer than 100,000/μL may be found in foals or horses with intraabdominal abscesses.[781] The peritoneal WBC count can increase to greater than 150,000/μL following celiotomy[782] and can be higher if an enterotomy is done. Postoperatively, the WBC count normally continues to decline and returns to near normal by 5 to 7 days. Failure of the WBC count to decrease suggests peritonitis resulting from a postoperative complication. Finally, PF WBC counts greater than 500,000/μL indicate severe focal or generalized peritoneal sepsis. With acute peritonitis, PMN cells typically increase to a greater degree than mononuclear cells, but this depends on the cause. In horses with gastrointestinal disease and endotoxemia, the number of peritoneal mononuclear cells increases, as does transformation of mesothelial cells to macrophages. In chronic cases, one easily may mistake transforming mesothelial cells for neoplastic cells, which can make diagnosis difficult.

The presence of free and phagocytosed bacteria in PF indicates generalized suppuration, abscessation, or compromised bowel. If one observes numerous microorganisms of mixed types free in the PF, especially in conjunction with plant material, bowel rupture likely has occurred. The presence of toxic or degenerate neutrophils and bacteria within PMN cells helps to distinguish PF from intestinal contents in such horses. Fluid obtained from enterocentesis is largely devoid of WBCs but is discolored and contains mixed microorganisms and plant material. Bacterial contamination of a sample can occur during collection of the sample, and iatrogenic contamination of a sample can result in free and intracellular bacteria, particularly if processing is delayed. In such cases the bacterial numbers are few and the neutrophils appear healthy. In some cases of gastrointestinal perforation the luminal material, inflammatory cells, and protein may be sequestered by the omentum and further contained by fibrinous adhesions. Abdominal fluid obtained via standard ventral paracentesis may have low cellularity and protein content but large numbers of mixed bacteria, indicating bowel rupture.[768] Examples include gastric rupture along the greater curvature of the stomach between the omental layers (omental bursa) and perforated gastric or duodenal ulcers in foals. Correlating all cytologic findings with clinical and clinicopathologic findings is important for interpreting the results of PF cytologic examination.

Biochemical analysis of PF may be useful in detecting sepsis when cytologic examination and culture are negative or otherwise unavailable. PF pH and glucose concentrations from horses with septic peritonitis were significantly lower than in horses with nonseptic peritonitis and healthy horses.[783] PF pH less than 7.3, glucose less than 30 mg/dL, and fibrinogen concentration greater than 200 mg/dL were considered highly predictive of septic peritonitis. Serum to peritoneal glucose concentration differences of greater than 50 mg/dL were considered the most diagnostically useful test for septic peritonitis in the study. PF lactate concentration is also increased because of septic peritonitis, and a blood-to-peritoneal lactate concentration difference may have diagnostic significance. In a small study in dogs, a blood-to-fluid lactate difference of ≤2.0 mmol/L was shown to be 100% sensitive and specific for a diagnosis of septic peritonitis.[784]

PF samples should be submitted for aerobic and anaerobic cultures in appropriate media (i.e., BBL Port-A-Cul tubes, Becton, Dickinson & Co., Franklin Lakes, NJ, in an attempt to identify the pathogenic organism(s). To enhance recovery of bacteria, PF may be inoculated into blood culture medium (i.e., Septi-Chek Columbia, Hoffmann-LaRoche Inc., Nutley, NJ). If the horse has received prior antimicrobial treatment, the fluid sample should be passed through an antimicrobial removal device before culture (i.e., A.R.D., Becton Dickinson & Co., Franklin Lakes, NJ).

Treatment

Early and aggressive therapy is important for a successful outcome. Treatment goals are to resolve the primary problem, minimize inflammation, and prevent long-term complications. In the acute phase, analgesia and therapy as described for endotoxemia are important. Flunixin meglumine is advocated for its local and systemic antiinflammatory effects and may be effective in retarding adhesion formation.[785]

Initial antimicrobial selection should be broad spectrum based on the presumption that mixed infection is present. IV administration is preferred over oral or intramuscular routes in acute, diffuse, septic peritonitis because more reliable concentrations of drugs are achieved in the tissues and PF.[786] The combination of a β-lactam antibiotic with an aminoglycoside, such as potassium penicillin (22,000–44,000 IU/kg IV every 6 hours) combined with gentamicin (6.6 mg/kg every 24 hours), is appropriate in most circumstances. Metronidazole (25 mg/kg orally every 12 hours) can be added to the regimen given the strong possibility of infection involving penicillin-resistant *B. fragilis*. This regimen can be modified when culture and antimicrobial sensitivity results become available. Peritonitis caused by *A. equuli* generally responds well to therapy with penicillin either alone or in combination with gentamicin. Aminoglycosides and NSAIDs have the potential to induce acute renal tubular damage, particularly with concurrent dehydration and decreased renal perfusion. Therefore adequate restoration and maintenance of hydration and monitoring of renal function are important. Monitoring of the WBC count, plasma fibrinogen, SAA, and abdominal fluid analysis often guide duration of therapy. Horses with abdominal abscessation resulting from monomicrobial infections typically require weeks to months of therapy, whereas polymicrobial infection may require many months of antibiotic treatment. Further details regarding principles of antimicrobial therapy are provided elsewhere in this text (Chapter 2).

Abdominal drainage and lavage can help remove excess fluid, foreign materials, fibrin, and bacterial products from horses with peritonitis. Postoperative lavage decreases the incidence of experimentally induced abdominal adhesions.[787] Open surgical exploration provides the most effective and thorough examination of all peritoneal surfaces and is recommended if gastrointestinal perforation or ischemia is suspected or in any other case in which correction of a primary lesion is indicated. A ventral abdominal drain can either be placed at the time of surgery or in the standing horse with sedation and local anesthesia. Techniques have been described in detail elsewhere.[788,789]

Peritoneal lavage is typically performed with 10 to 20 L of a balanced isotonic electrolyte solution twice a day for 3 to 5 days, until the lavage solution becomes clear or until the catheter becomes clogged with fibrin or omentum. Hypertonic solutions should be avoided because they can result in fluid shifts into the peritoneum. The addition of povidone iodine to a balanced solution should be avoided because concentrations as low as 3% can induce peritoneal inflammation.[790] Other agents such as antibiotics or heparin have also been suggested as components of the lavage solution, but data demonstrating their benefit are not currently available. Active (or closed suction) abdominal drains have also been advocated, with similar benefits and potential complications to other methods.[789] Lavage with a plain isotonic solution did not alter the pharmacokinetics of gentamicin administered systemically.[791] Thus alteration of antimicrobial dosing does not appear necessary if lavage with plain solutions is part of the therapeutic regimen. Complications associated with the use of abdominal drains or repeated peritoneal drainage include retrograde infection, local irritation, pneumoperitoneum, and SC seepage around the drain and resultant cellulitis. If the patient is hypovolemic or hypoproteinemic, volume replacement and administration of plasma or synthetic colloids should be considered before removing large quantities of fluid from the abdomen.

In horses with suspected parasitic involvement, one should give larvicidal doses of an anthelmintic once the condition of the horse is stabilized. Ivermectin, fenbendazole, and thiabendazole have been recommended as larvicidal therapies.

The decision to perform surgical versus medical treatment is controversial in horses with peritonitis and needs to be made on a case-by-case basis. Although surgical exploration may allow diagnosis and resolution of the inciting cause and more thorough lavage of the abdomen, the risks of anesthesia, possible additional cost of surgery, and potential for a prolonged return to performance may be significant. In a recent study of horses and foals with peritonitis, survival to discharge without surgery was associated with lack of signs of abdominal pain, normal or improved rectal temperature, normal or improved borborygmi, normal fecal production, no abnormal findings on palpation per rectum, no nasogastric reflux, and yellow/orange PF.[792] A retrospective comparison of medical and surgical treatment of postpartum mares with peritonitis secondary to a uterine tear found no significant difference between admission variables, survival rate, hospital bill, duration of hospital stay, and likelihood to foal following discharge.[793] A limitation of this study is the ability to definitively diagnose a uterine tear in those mares treated medically; if a tear was not palpable, the diagnosis was based on the exclusion of other causes.

Prognosis

The prognosis is grave for peritonitis associated with gastrointestinal rupture. Reported survival rates for horses with peritonitis vary but can be as high as 78%.[792,794] In one study with a 78% overall survival rate, 68% of horses treated medically survived[792]; in another, 93% of those treated medically survived.[795] Some of the variability in reported survival percentages can be related to inclusion criteria, mainly whether or not horses with gastrointestinal rupture were included. Septic peritonitis following abdominal surgery was associated with high mortality (56%) in some reports,[777] although no difference in short-term survival was seen in another study.[796] Peritonitis associated with *A. equuli* carries a very favorable prognosis, and all horses in these reports responded to medical therapy if attempted.[773,774]

Pathophysiology of Mucosal Injury and Repair

MUCOSAL BARRIER FUNCTION

To gain an appreciation of the mechanisms in which the mucosa is injured and subsequently repaired, it is important to understand how the integrity of the mucosa is physiologically regulated. Regulation of mucosal integrity is referred to as mucosal barrier function, which is vital because it prevents bacteria and associated toxins from gaining access to subepithelial tissues and the circulation. However, the mucosa has two conflicting functions: it must serve as a protective barrier while continuing to absorb solutes necessary to maintain the well-being of the host. This conflict is most notable at the intercellular (paracellular) space, which allows passage of select solutes and water[797-800] but does not admit large molecules, including bacterial toxins.[801] The paracellular space is almost exclusively regulated by the tight junction,[574] which is the interepithelial junction at the apical-most aspect of the paracellular space. Although these tight junctions were originally viewed as inert cellular adhesion sites, it has become clear in recent years that tight junction permeability is dependent on tissue-specific molecular structure and regulated by a complex array of intracellular proteins and the cytoskeleton. Tight junctions consist of a group of transmembrane proteins that interdigitate from adjacent cells. Although occludin was originally thought to be the predominant tight junction transmembrane protein, a group of proteins termed *claudins* appear to fine-tune the function of the tight junction. For example, select claudins are responsible for the relative porosity of the barrier to select electrolytes based on their charge within the paracellular space.[802] These transmembrane proteins interact with the cytoskeleton via a series of intracellular proteins, including zonula occludens (ZO)-1, ZO-2, ZO-3, cingulin, and others.[803] In addition, local regulatory proteins such as the small GTPase Rho are critical to tight junction function. Generally, the relative contractile state of the actin cytoskeleton determines the degree to which tight junctions are open or closed, but the complexities of regulation of this process are poorly understood.[804,805]

The most sensitive measure of mucosal barrier function is transepithelial electrical resistance, which is measured by mounting mucosa in an in vitro system called an Ussing chamber, because this measurement is largely a reflection of the permeability of mucosa to ions.[806,807] There are two routes ions it may follow when traversing the epithelium: transcellular and paracellular.[801] Because cell membranes have a resistance to the passive flow of ions 1.5 to 3 log units greater than that of the epithelium as a whole, measurements of transepithelial resistance largely reflect the resistance of the paracellular space, particularly the tight junctions that regulate the paracellular flow of ions.[807] Because tight junctions differ in structure from different portions of the mucosa,[808] measurements of transepithelial resistance reflect the net resistance of the epithelium of variable permeability within a given tissue. For example, tight junctions in the intestinal glandular structures called crypts are leakier than those in the surface epithelium because of fewer and less organized tight junction strands.[806,809] Conversely, surface epithelium has a greater number of well-organized tight junction strands that result in epithelium with a relatively high resistance.[806] This correlates well with the absorptive function of epithelium located on the mucosal surface and the secretory function of crypt epithelium. The structure of tight junctions also varies with the segment of intestine. For example, tight junctions have more strands in the ileum than the jejunum, which is reflected by a higher transepithelial resistance in the ileum.[810] In addition, cells are more closely apposed at the level of the tight junction within the colon. This is in keeping with the largely absorptive role of the colon and is advantageous given the hostile microbial environment of the colon.

Gastric Mucosal Barrier Function

There are four regions of the stomach based on the type of mucosal lining (in an orad to aborad order): nonglandular stratified squamous epithelium, cardiac epithelium, proper gastric mucosa, and pyloric mucosa.[811] Stratified squamous epithelium has distinct differences in terms of barrier function compared with the remainder of the gastrointestinal tract. This epithelium has baseline transepithelial resistance measurements of approximately 2 to 3000 $\Omega \cdot cm^2$, which is an order of magnitude higher than the adjacent cardiac mucosa.[812,813] Thus the stratified squamous mucosa is exceptionally impermeable. This is the only mechanism this mucosa has to defend itself against injury. The stratified squamous epithelium consists of four layers: the outer *stratum corneum, stratum transitionale, stratum spinosum,* and the basal *stratum germinativum.* However, not all layers contribute equally to barrier function, which is largely composed of interepithelial tight junctions in the *stratum corneum* and mucosubstances secreted by the *stratum spinosum.*[812,814] The relative impermeability of stratified squamous mucosa can be demonstrated by the effects of HCl on this type of epithelium in vitro, which has very little effect until it reaches a pH of 2.5 or below.[813] Although the majority of the literature on equine ulceration pertains to the effects of HCl and inhibitors of HCl secretion,[815-818] other factors may be critical to the development of gastric ulcer disease.

The site of HCl secretion (proper gastric mucosa) is protected from so-called back diffusion of H^+ ions by a relatively high transepithelial electrical resistance (compared with cardiac mucosa), but there are also a number of other critical mechanisms to prevent acid injury. The gastric mucosa secretes both mucus and bicarbonate, which together form an HCO_3^- containing gel that titrates acid before it reaches the lumen.[819,820] The mucous layer is principally formed by glycoproteins (mucins) secreted by goblet cells but also includes other gastric secretions and sloughed epithelial cells. Mucins consist of core peptides with a series of densely packed *O*-linked polysaccharide side chains that, once secreted, become hydrated and form a viscoelastic gel. The mucous layer, however, does not form an absolute barrier to back diffusion of acid. For acid that does back diffuse into the gastric mucosa, epithelial Na^+/H^+ exchangers are capable of expelling H^+ once the cell reaches a critical pH.[820]

Recent studies have renewed interest in the protective mechanisms of mucus because of the discovery of a group of compounds secreted by goblet cells called the trefoil peptides. The name of these peptides is derived from a highly conserved cloverleaf structural motif, which confers substantial resistance to degradation by proteases including pepsin. There are three known members of this group, pS2, SP, and intestinal trefoil factor (ITF), the latter of which is solely secreted by

goblet cells in the small and large intestine. Both pS2 and SP are secreted by goblet cells within the stomach and are believed to intercalate with mucous glycoproteins, possibly contributing to the barrier properties of mucus.[821] These peptides also play a critical role in repair of injured mucosa.

An additional mucosal function that serves to reduce the level of injury is adaptive cytoprotection, in which application of topical irritants to gastric mucosa results in subsequent protection of mucosa in response to repeated exposure to damaging agents. For example, pretreatment with 10% ethanol protected against mucosal damage in response to subsequent application of absolute ethanol, and this effect was abolished by treatment with the COX inhibitor indomethacin.[822] The cytoprotective effects of prostaglandins have been demonstrated directly in studies in which preadministration of prostaglandins protected gastric mucosa from damage by agents such as concentrated hydrochloric acid and hypertonic saline.[823] Prostaglandins appear to be cytoprotective in the stomach at doses less than those used to inhibit gastric acid secretion, ruling out a simple antacid mechanism.[824] Although not fully characterized, cytoprotection has been attributed in part to prostaglandin-stimulated mucous production.[825] An associated beneficial effect of prostaglandins is the increased production of bicarbonate, which is trapped within mucus on the surface of the mucosa.[826,827] Interestingly, PGE_2 appears to lose its cytoprotective activity in the presence of the mucolytic agent *N*-acetylcysteine. Attention has also been directed at enhanced mucosal blood flow as a potential mechanism for prostaglandin-mediated cytoprotection—for example, pretreatment with PGI_2 protected against ethanol-induced mucosal damage as a result of increased mucosal blood flow.[828] In addition PGE_2, which is also cytoprotective despite the fact that it does not increase blood flow,[829] prevents vascular stasis associated with irritant-induced vascular damage by inhibiting neutrophil adherence to damaged endothelium.[830]

Sensory nerves distributed throughout gastrointestinal mucosa have also been implicated in cytoprotective mechanisms. As an example of their importance in mucosal cytoprotection, pretreatment of newborn rats with capsaicin (which dose dependently destroys sensory nerves) renders the mature rats more susceptible to gastric injury.[831] Alternatively, the use of a low dose of capsaicin, which stimulates rather than destroys sensory nerves, protects gastric mucosa against injurious agents.[832,833] Sensory nerves contain neuropeptides such as calcitonin-gene–related peptide (CGRP) and substance P, which may play a protective role via vascular mechanisms. For instance, CGRP stimulates increased gastric blood flow, which is theorized to reduce injury similarly to prostaglandins. Recent studies suggest that the roles of prostaglandins and CGRP in gastric cytoprotection are intimately intertwined. In particular, PGI_2 is believed to sensitize sensory nerves following treatment with a mild irritant, with resultant increases in CGRP release and mucosal flow. Similar studies have shown that antagonists of CGRP inhibit the cytoprotective action of PGE_2.[834] Another neural mediator, nitric oxide, has also been implicated in adaptive cytoprotection. Interestingly, nitric oxide has a number of actions that are similar to those of prostaglandins, including maintenance of mucosal blood flow.[835]

Intestinal Barrier Function

Regulation of barrier function in the intestine is not as well characterized as that of the stomach, although mechanisms of barrier function, including secretion of mucus and regulation of mucosal blood flow, are presumed to be similar. The proximal duodenum also has to protect itself from acid damage as it receives gastric contents, and this involves secretion of mucus and bicarbonate similarly to the stomach. One other mechanism that helps both the stomach and the intestine to maintain mucosal barrier function is the speed with which the mucosa repairs. For a defect to develop in the mucosal barrier, injurious factors have to outpace mucosal recovery. Such recovery initially involves epithelial migration across denuded regions of basement membrane (restitution).[821] This process is so rapid that epithelial defects may be resurfaced within minutes. For example, in bile salt–injured equine colon, denuded surface mucosa was completely covered by restituting epithelium within 180 minutes.[207] In the small intestine, intestinal villi greatly amplify the surface area of the mucosal luminal surface, which in turn takes far longer to resurface with restituting epithelium once it has become denuded.[836] Intestinal villi, however, are able to dramatically reduce the denuded surface area by contracting.[837]

MECHANISMS OF GASTRIC INJURY

Although the stratified squamous epithelium is relatively impermeable to HCl, there are a number of factors that can dramatically enhance the damaging effects of HCl in this epithelium. In particular, bile salts and SCFAs are capable of breaking down the squamous epithelial barrier at an acid pH, exposing deep layers to HCl, with subsequent development of ulceration.[813,838] Relatively high concentrations of SCFA normally exist within the equine stomach as a result of microbial fermentation.[812] These weak acids penetrate squamous mucosa and appear to damage Na^+ transport activity principally located in the stratum germinativum. Bile salts may also be present in the proximal stomach as a result of reflux from the duodenum. Although such reflux has a relatively high pH, it appears that bile salts adhere to stratified squamous epithelium, becoming lipid soluble and triggering damage once the pH falls below 4.[839] Diet and management (e.g., periods of fasting) also play crucial roles in the development of conditions conducive to gastric ulceration. Typically, there is a pH gradation in horses from proximal to distal compartments of the stomach, with the lowest pH values in the distal stomach.[840] During periods of fasting, this stratification is disrupted such that low pH values may be recorded in the proximal stomach.[841] Fasting conditions also increase the concentration of duodenal contents within the proximal stomach, particularly bile.[839]

Proper gastric mucosa is exposed to injurious agents, including pepsin, bile, and acid. The latter is constantly secreted by parietal cells in the horse as an adaptation to near-continuous intake of roughage,[811] but it is tightly regulated by enterochromaffin (ECL)-like cells within the proper gastric mucosa and G and D cells, which are present within the pyloric mucosa. Acid secretion is amplified by ECL-released histamine, which interacts with H_2 receptors on parietal cells, and G cells, which release the prosecretory hormone gastrin. A combination of histamine and gastrin can have a synergistic effect on parietal cell gastric secretion, because these mediators have distinct receptors and second messengers. On the other hand, D cells are sensitive to an acidic environment and release somatostatin, which inhibits acid secretion.[842] Nonetheless, gastric mucosa may be exposed to acid for prolonged periods of time, particularly in horses that are extensively meal

fed and do not have the benefit of roughage, which tends to buffer stomach contents.[839,842]

Aside from peptic ulceration, induced by combinations of acid and pepsin, research in the human field has revealed the tremendous importance of *Helicobacter pylori* in inducing ulceration. Infection with this organism has the effect of raising gastric pH because of disruption of gastric glands but also induces an inflammatory reaction that causes damage.[843] However, there is very little evidence that this organism is involved in gastric ulcers in horses. In the absence of a known role for infectious agents in gastric ulceration in animals, ulceration likely develops from injurious factors similar to those found in the proximal stomach, including gastric acid and bile. Some factors that are important to the induction of squamous epithelial ulceration may not be important in the development of proper gastric mucosal ulceration. For example, feed deprivation and intensive training reproducibly induce squamous epithelial ulceration in horses but have little effect on proper gastric mucosa in horses.[844] Gastric acid likely plays a key role, whereas other factors such as NSAIDs serve to reduce gastric defense mechanisms. In particular, inhibition of prostaglandin production would reduce mucous and bicarbonate secretion while reducing gastric mucosal blood flow.[845] Some of the NSAIDs also have a topical irritant effect, although this appears to be of minor significance, because the route of administration (oral or parenteral) seems to have little influence on the development of ulceration.[846]

The source of prostaglandins responsible for gastric protection was originally assumed to be COX-1, because this COX isoform is constitutively expressed in gastric mucosa, whereas COX-2 is not expressed in the stomach unless it is induced by inflammatory mediators. However, mice in which the COX-1 gene has been knocked out fail to develop spontaneous gastric lesions,[847] possibly because of compensatory increases in prostaglandin production by COX-2.[848] This concept agrees with recent data indicating that inhibition of both COX isoforms is required to induce gastric ulceration.[849] From a clinical perspective, these data indicate that drugs selective for either COX-1 or COX-2 may be less ulcerogenic in the horse because they allow the uninhibited COX isoforms to continue to produce protective prostanoids. Because COX-2 elaborates prostaglandins induced by inflammatory stimuli, preferential or selective inhibitors of COX-2 may be particularly useful because of their ability to serve as antiinflammatory agents that are less ulcerogenic.[392]

INTESTINAL ISCHEMIA/ REPERFUSION INJURY

The most notable cause of intestinal mucosal injury in horses, particularly those suffering from colic, is ischemia. Initially, it seems intuitive that by reducing gastrointestinal blood supply, the mucosa becomes injured. The anatomy of the gastrointestinal tract, and the differing structure of the intestinal mucosa at various anatomic locations, has a significant influence on the extent of mucosal injury. Ischemic injury may be induced by several different mechanisms, including occlusion of arterial supply by a thrombus, strangulation of intestinal vasculature, and generalized reduction in blood flow associated with various shock states. A number of seemingly distinct mechanisms of intestinal injury, such as intestinal distention, also trigger mucosal injury via an ischemic mechanism. Reperfusion injury may also influence the extent of mucosal injury following an ischemic episode and has been proposed as a potential site of therapeutic intervention.[850,851] It is critical that the mechanisms of ischemia/reperfusion injury be understood to develop an understanding of the severity of various clinical conditions, and begin to formulate a therapeutic approach to diseases characterized by this devastating form of injury.

Regulation of Intestinal Blood Flow

The intestinal circulation is capable of closely regulating blood flow during periods of low systemic perfusion pressure.[852,853] In particular, local regulation of resistance vessels within the microvasculature is particularly prominent; metabolic end products of ATP result in continued dilatation of resistance vessels despite reductions in systemic arterial pressure. This results in continued perfusion of gastrointestinal tissues during the early stages of shock, while other organs such as skeletal muscle undergo massive shunting of blood as a result of marked increases in the resistance of resistance vessels. The reasons for these differences in regulation are not entirely clear but may relate to the relatively high level of energy required to fuel the intestinal mucosa and the serious systemic effects of breaches in the mucosal barrier. When blood flow falls below a critical level, regulatory systems are no longer effective and oxygen uptake by the gastrointestinal tissue decreases, culminating in tissue damage.[852]

The villous tip is the most susceptible region affected by hypoxia in the equine small intestine, largely because of the countercurrent exchange mechanism of blood flow in the small intestinal villus.[852] This countercurrent exchange mechanism is attributable to the vascular architecture, which consists of a central arteriole that courses up the core villus, arborizes at the tip, and is drained by venules coursing down the periphery of the villus.[854] As oxygenated blood flows into the central arteriole, oxygen tends to diffuse across to the adjacent venules, which are flowing in the opposite direction. This series of events takes place along the length of the villus, resulting in a villous tip that is relatively hypoxic even under normal conditions. Furthermore, when blood flow is reduced, as occurs in hypovolemic or septic shock, the countercurrent exchange of oxygen is enhanced, and the tip becomes absolutely hypoxic.[852] This mechanism might explain why the small intestinal mucosa is more susceptible to ischemic injury, compared with the colon, which has no villi. The duration of ischemia required to produce severe morphologic damage to the equine colon is approximately 25% longer than the small intestine.[855]

Ischemic Epithelial Injury

Intestinal mucosal epithelium is very susceptible to hypoxia because of the relatively high level of energy required to fuel the Na^+/K^+-ATPase that directly or indirectly regulates ion and nutrient flux. The first biochemical event to occur during hypoxia is a loss of oxidative phosphorylation. The resulting diminished ATP concentration causes failure of the energy-dependent Na^+/K^+-ATPase and the accumulation of sodium and subsequently intracellular water. The pH of the cytosol drops as lactic acid and inorganic phosphates accumulate from anaerobic glycolysis. The falling pH damages cell membranes, including lysosomal membranes, resulting in the release and activation of lysosomal enzymes into the cytosol, further damaging cellular membranes. Damage to the cell membrane allows the accumulation of high concentrations of calcium in

TABLE **12.6 Grading System for Ischemia/Reperfusion Injury in Small Intestinal Mucosa**

Grade	Description
1	Separation of epithelium at the tip of the villus, creating a small space between the epithelium and basement membrane called Grüenhagen's space
2	Loss of epithelium from the tip of the villus
3	Loss of epithelium from the upper third of the villus
4	Complete loss of villous epithelium
5	Injury or loss of epithelium within the crypt in addition to complete loss of villous epithelium

Adapted from Chiu CJ, McArdle AH, Brown R, et al. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg*. 1970; 101:478-483.

the cytosol, which activates calcium-dependent degradative enzymes.[856] These events result in cytoplasmic blebbing of the basal membrane, with subsequent detachment of cells from the underlying basement membrane.

Recent studies on epithelial injury during ischemia suggest that the majority of epithelial cells undergo programmed cell death (apoptosis) during ischemia and reperfusion rather than necrosis, allowing retention of reusable components of irreversibly injured cells.[857] In one study, 80% of detached epithelium during small intestinal ischemia/reperfusion underwent apoptosis.[858] Although the most obvious result of apoptosis is loss of surface epithelium, a number of cells on the lower portion of the villus (in the small intestine) and cells within the crypts may also undergo apoptosis that only becomes evident up to 24 hours following reperfusion of ischemic tissue.[859]

Morphologic changes observed in ischemic-injured small intestinal mucosa follow a similar sequence regardless of whether injury is induced by ischemia alone or ischemia/reperfusion (Table 12.6).[860] Initially, epithelium separates from the underlying basement membrane, forming a fluid-filled space termed *Grüenhagen's space* (Fig. 12.7). The mechanism of fluid accumulation in this space is not entirely understood but may result from continued epithelial absorption of NaCl and water before it has fully detached from neighboring epithelial cells. This fluid accumulation likely exacerbates epithelial separation from the basement membrane. Subsequently, epithelium progressively sloughs from the tip of the villus toward the crypts, which are the last components of the intestinal mucosa to become injured.[163,406,861] This likely relates to the vascular architecture, because crypts receive a blood supply that is separate from the vasculature involved in the villous countercurrent exchange mechanism. The early morphologic changes observed in the equine large colon during ischemia are somewhat different from those described in the equine small intestine because of the lack of intestinal villi. As might be expected, the more superficially located surface cells are sloughed before those in crypts.[855,862] The orderly progression of tissue injury has been used by one group of investigators to accurately predict survival in horses with large colon volvulus. Biopsies were taken from the pelvic flexure, which has been previously shown to accurately reflect mucosal changes along the length of the colon,[863] and histologically examined for the width of the crypts and intercrypt interstitial space. The latter measurements were expressed as an interstitium: crypt width (I:C) ratio. Nonviable colon was defined as that which had

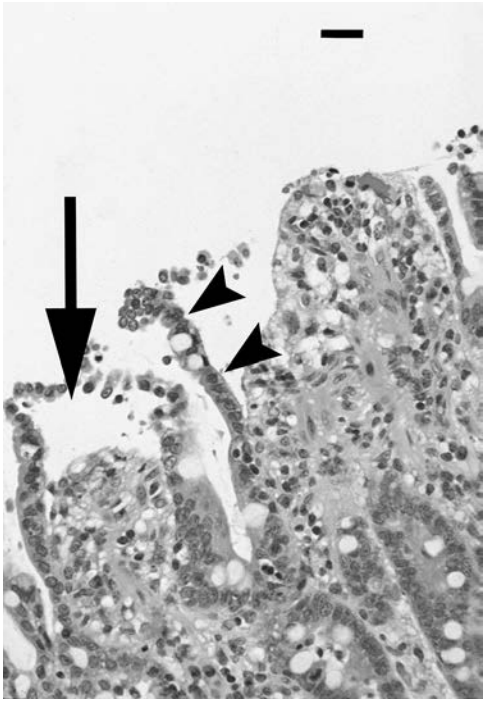


FIG. **12.7** Histologic appearance of Grüenhagen's space in ischemic injured ileal mucosa. Note separation of epithelium at the tip of the villus from its basement membrane, creating a space *(arrows)*. Epithelium subsequently sloughs into the lumen *(arrowheads)*. 1-cm bar = 100 μm.

>60% loss of crypt and an I:C ratio >3. Using this methodology, survival was correctly predicted in 94% of horses.[864]

Strangulating Obstruction

Since the dramatic decline in *S. vulgaris*–induced colic, which was frequently associated with infarction of intestinal arterial blood supply,[865] the vast majority of ischemic lesions are associated with strangulating obstruction. Therefore it is important to consider mechanisms of ischemic injury in horses with naturally occurring strangulating lesions. The majority of experimental work has either assessed complete ischemia (complete occlusion of the arterial blood supply)[855] or low-flow ischemia (reduction of arterial blood flow).[866,867] During intestinal strangulation, however, a disparity between the degree of occlusion of the veins and arteries occurs in which veins are occluded before arteries because of differences in the compliance of vascular walls. Strangulating lesions are typically hemorrhagic (hemorrhagic strangulating obstruction), because the arteries continue to supply blood to tissues that have little or no venous drainage. This results in ischemic injury, as previously outlined, but also in tremendous congestion of the tissues. Such hemorrhagic congestion has two opposing effects: it disrupts tissue architecture, including the mucosa and its epithelium, but it continues to provide oxygenated blood to the tissues during much of the ischemic episode. In contrast, when strangulation results in sudden cessation of arterial blood flow (ischemic strangulating obstruction), tissues appear pale, and the mucosa rapidly degenerates because of a complete lack of oxygenated blood.[163] From a clinical standpoint, this makes it difficult to assess the degree of mucosal injury in horses with strangulating injuries, because intestine that may look nonviable (dark red) may in fact have less mucosal injury than that of ischemic strangulated intestine.[868]

An additional consideration in clinical strangulating obstruction is the degree of ischemia that may be induced by intestinal distention. For example, experimental distention (18 cm of H_2O for 2 hours) and decompression (2 hours) of the jejunum resulted in a significant increase in microvascular permeability and a significant decrease in tissue oxygenation similar to what would be expected with low-flow ischemia.[869,870] In particular, microscopic evaluation of vasculature revealed capillary endothelial cell damage and local edema formation.[871] These data suggest that distended intestine proximal to an obstruction may undergo mucosal injury despite its relatively normal appearance. Indeed, in one study, intraluminal pressures greater than 15 cm H_2O in naturally occurring cases of colic correlated with a poor prognosis for survival.[872]

Reperfusion Injury

Although it has recently been taken for granted that reperfusion of ischemic tissues results in exacerbation of mucosal injury, it should be remembered that mechanisms underlying intestinal reperfusion injury have been largely defined in laboratory animals under specific conditions.[104,410,873-875] On the other hand, studies on reperfusion injury in horses have had some conflicting results.[861,867,876] This may be attributed to the way in which the studies have been performed. In particular, the type of ischemia used in most laboratory animal studies has been "low-flow ischemia" (in which the blood flow is typically reduced to 20% of baseline flow), whereas studies in horses have used a number of different ischemic models, including various types of strangulating obstruction. Although strangulating obstruction is of great clinical relevance, this type of ischemic insult is less likely to develop reperfusion injury.[861,877,878] Conversely, low-flow ischemia appears to prime tissues for subsequent injury once the tissue is reperfused, and there is considerable evidence to support the presence of reperfusion injury in horses following low-flow ischemia.[866,867,871,879] Nonetheless, low-flow ischemia may not be a common clinical entity.

In addition to the type of ischemia, there are other factors involved in priming tissues for reperfusion injury, including species and anatomic-specific variation in oxidant enzyme and neutrophil levels. For example, the foal appears to have very low levels of small intestinal xanthine oxidase, an enzyme that plays a critical role in triggering reperfusion injury in laboratory animals,[410,875,880] whereas adult levels are much greater, particularly in the proximal small intestine.[881] In addition, horses appear to have low numbers of resident neutrophils in the intestinal mucosa,[882] and it is this population of neutrophils (rather than those recruited from the circulation) that appear to be most critical for induction of reperfusion injury.[104] Studies demonstrating reperfusion injury in the equine colon following low-flow ischemia have shown significant accumulation of neutrophils within the mucosa.[866] A complete understanding of the mechanisms of neutrophilic infiltration and the mechanisms by which they damage tissue will require further study.

Reperfusion injury is initiated during ischemia when the enzyme xanthine dehydrogenase is converted to xanthine oxidase, and its substrate, hypoxanthine, accumulates simultaneously because of ATP utilization (Fig. 12.8).[850,883] There is little xanthine oxidase activity during ischemia, because oxygen is required as an electron acceptor. During reperfusion, xanthine oxidase rapidly degrades hypoxanthine in the presence of oxygen, producing the superoxide radical as a byproduct.[850] The superoxide radical contributes to oxidative tissue damage and, most importantly, activates neutrophil chemoattractants.[410,875] Inhibition of xanthine oxidase in feline studies of intestinal ischemia/reperfusion injury prevents infiltration of neutrophils and subsequent mucosal injury.[410,874] Inhibition of xanthine oxidase has had no effect on ischemia/reperfusion injury in equine small intestine[876] and colon,[407] suggesting that either reperfusion injury is simply a continuation of injury initiated during ischemia, as suggested in some equine studies,[856] or that the classic reperfusion injury pathway is activated by alternate sources of ROMs. The latter has been suggested by studies in feline models of ischemia/reperfusion injury, in which the source of a significant proportion of ROMs is unknown, and independent of xanthine oxidase and neutrophils.[874]

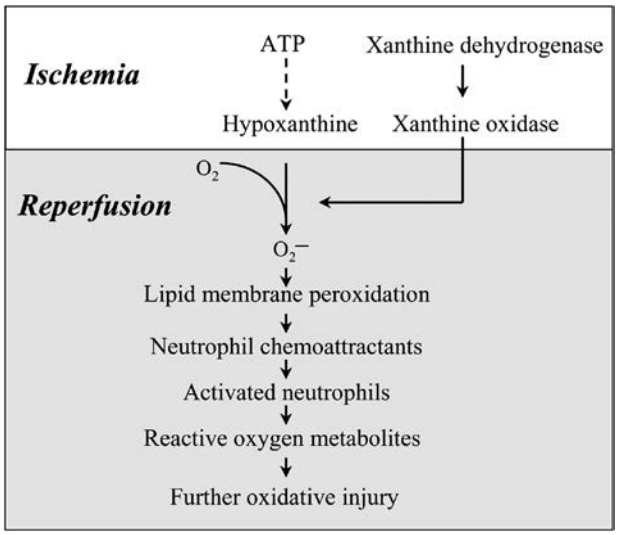


FIG. **12.8** Intestinal reperfusion injury cascade. Reperfusion injury is initiated by elaboration of superoxide by metabolism of hypoxanthine by xanthine oxidase and subsequent infiltration of neutrophils.

In a veterinary review of the pathogenesis of intestinal reperfusion injury in the horse, the concept of a therapeutic window in which treatment of reperfusion injury would be beneficial was suggested.[850] The basis of this concept is that there are certain conditions under which ischemic injury is minimal and that tissues are severely damaged during reperfusion.[877] Thus under conditions of low-flow ischemia, very little injury is demonstrated during 3 hours of ischemia, but remarkable injury occurs during 1 hour of reperfusion.[410,874,875] A therapeutic window may not exist under conditions of strangulating obstruction in which severe injury occurs during ischemia and minimal injury occurs during reperfusion.[884] This in turn greatly reduces clinicians' abilities to ameliorate ischemia/reperfusion injury with treatments such as antioxidants at the time of reperfusion.

MECHANISMS OF GASTROINTESTINAL MUCOSAL REPAIR

Gastric Reparative Mechanisms

Mechanisms of gastric repair are highly dependent on the extent of injury. For instance, superficial erosions can be rapidly covered by migration of epithelium adjacent to the wound, a process termed *epithelial restitution*. Ulceration

(full-thickness disruption of mucosa and penetration of the muscularis mucosa), however, requires repair of submucosal vasculature and ECM. This is initiated by the formation of granulation tissue, which supplies connective tissue elements and microvasculature necessary for mucosal reconstruction. Connective tissue elements include proliferating fibroblasts that accompany newly produced capillaries that form from proliferating endothelium. Recent studies indicate that nitric oxide is critical to both of these processes,[835,885] which likely explains the reparative properties of it in the stomach.[886]

Once an adequate granulation bed has been formed, newly proliferated epithelium at the edge of the wound begins to migrate across the wound. Gastric glands at the base of the ulcer begin to bud and migrate across the granulation bed in a tubular fashion.[887] Epidermal growth factor (EGF) is expressed by repairing epithelium and appears to facilitate these processes.[888] These events are facilitated by a mucoid cap, which retains reparative factors and serum adjacent to the wound bed.[845] Once the ulcer crater has been filled with granulation tissue, and the wound has been reepithelialized, the subepithelial tissue remodels by altering the type and amount of collagen. Despite the remodeling process, ulcers tend to recur at sites of previous ulceration, and there is concern that this remodeling can result in excessive deposition of collagen and fibrosis.[821]

Intestinal Reparative Mechanisms

Reparative mechanisms are similar in the intestine, except that in the small intestine, mucosal villi contribute to mucosal repair. Once intestinal epithelium is disrupted, there are two events that occur almost immediately to reduce the size of the denuded portion of the villus: contraction of the villus and epithelial restitution (Fig. 12.9). For example, in porcine ileum subjected to 2 hours of ischemia, villi were 60% of their former height, and 50% of the denuded villous surface area was covered in flattened epithelium within 6 hours.[836] Villous contraction appears to be regulated by enteric nerves, because inhibition of enteric nerve conduction prevents villous shortening following injury. The contractile component of the villus is a network of myofibroblasts distributed throughout the lamina propria of the villus and along the central lacteal. Inhibition of villous contraction results in retarded epithelial repair because of the larger denuded surface that remains to be covered by migrating epithelium compared with similarly injured villi that have contracted.[837] PGE_2 has also been implicated in regulating villous contraction, because application of PGE_2 resulted in villous contraction when perfused through normal rat ileum.[889] As villi contract, assuming there is an intact basement membrane, epithelium from the margins of the wound migrates in a centripetal direction to resurface toward the tip of the villus.[837] The process of restitution is similar in denuded colonic mucosa, except that it may proceed more rapidly because of the lack of villi.[207] Epithelial restitution is solely a migratory event that does not depend on provision of new enterocytes by proliferation. Cellular migration is initiated by extension of cellular lamellipodia that receive signals from the basement membrane via integrins. Intracellular signaling converges on the actin cytoskeleton, which is responsible for movement of lamellipodia. Specific components of the basement membrane appear to be critical to the migratory process. For example, application of antibodies to collagen types III and IV, which are important components of intestinal mucosal basement membrane, impeded epithelial restitution.[890,891] Other elements of the basement membrane, including proteoglycans, hyaluronic acid, and noncollagenous proteins, such as fibronectin and laminin, may also provide important signals.[892] The subepithelial matrix components that facilitate restitution may form the basis for clinical treatments designed to speed up the repair process, analogous to the administration of matrix components to horses with articular cartilage damage.

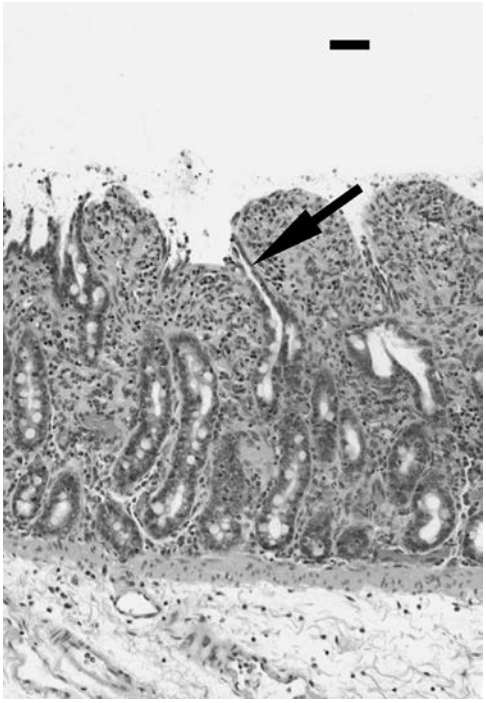


FIG. **12.9** Histologic appearance of repairing intestinal mucosa 6 hours after a 2-hour ischemic episode. Note blunting of the villus, attributable to villous contraction, and evidence of epithelial restitution *(arrows)*. 1-cm bar = 100 μm.

Although epithelial restitution results in gross closure of previously denuded regions of gastrointestinal mucosa, the closure of interepithelial spaces is ultimately required to restore normal epithelial barrier resistance.[893] Because the tight junction is principally responsible for regulating the permeability of the interepithelial space, it is likely that repair and closure of this structure is critical to restore intestinal barrier function. Recent research indicates that prostaglandins play a vital role in the recovery of tight junction resistance,[893] indicating that the administration of nonselective COX inhibitors to horses with colic, particularly those recovering from strangulating obstruction, may be deleterious. Judicious use of NSAIDs is appropriate until more selective drugs that allow continued production of reparative prostaglandins are available for use in horses. Recent studies have shown that NSAIDs preferential for COX-2 allow for optimal repair of injured intestine compared with traditional nonselective NSAIDs.[392]

Once the epithelial barrier has been restored, normal mucosal architecture must be reestablished to allow normal gut absorptive and digestive function. In porcine ileum subjected to 2 hours of ischemia, the epithelial barrier was restored within 18 hours, but villi were contracted and covered in epithelium with a squamous appearance. Restoration of normal villous architecture required a further 4 days.[836] The flattened villous epithelium that characterizes restitution

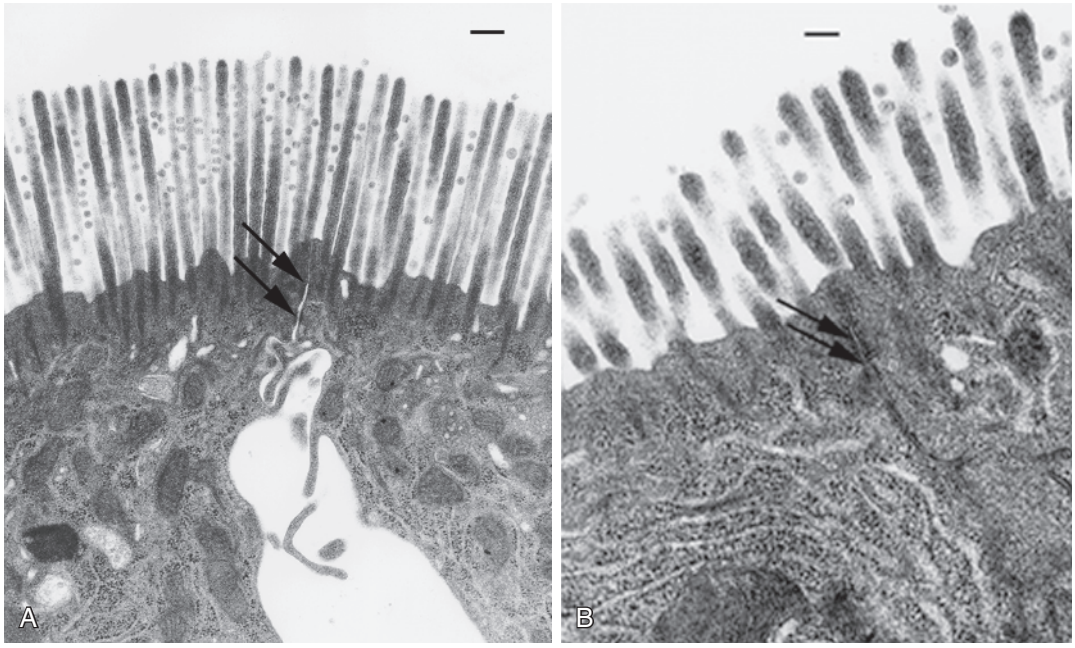


FIG. **12.10** Ultrastructural appearance of repairing ischemic-injured mucosa. (A) Restituting epithelium 2 hours after a 1-hour ischemic episode in the presence of the nonselective cyclooxygenase inhibitor indomethacin. Note dilation of the interepithelial space and the apical tight junction *(arrows),* which correlates with a "leaky" intestinal barrier. (B) Similar restituting epithelium that has been additionally treated with PGE_2 and PGI_2. Note the close apposition of the tight junction *(arrows)* and the interepithelial space correlated with normalization of intestinal barrier function. 1-cm bar = 6 μm.

is replaced by newly proliferated crypt epithelium. Under normal circumstances, new enterocytes are formed by the division of stem cells, of which there are approximately four at the base of each mucosal crypt. Newly divided enterocytes migrate from the crypt onto the villus.[894] During migration, enterocytes differentiate and acquire specific absorptive and digestive functions. Fully differentiated enterocytes reside on the upper third of the villus for 2 to 3 days and are then sloughed into the intestinal lumen.[895] This process is accelerated during mucosal repair, which requires increased proliferative rates. Increased proliferation may be stimulated within 12 to 18 hours by a variety of locally available gut-derived factors, including luminal nutrients, polyamines, and growth factors.[836] The return of the normal leaf-like shape of the villus occurs subsequent to the appearance of normal columnar epithelium.

MEDIATORS OF REPAIR

Prostaglandins

Although prostaglandins have been strongly implicated in mucosal cytoprotective function, relatively few studies have assessed their importance in mucosal repair. One study implicated prostaglandins in growth factor–stimulated restitution,[896] but a more prominent role of prostaglandins in mucosal repair is their ability to close interepithelial tight junctions.[893,897,898] For instance, ischemic-injured small intestine rapidly recovered barrier function (as measured in vitro as transepithelial resistance) in the presence of prostaglandins I_2 and E_2, despite the fact that these prostanoids had relatively little effect on villous contraction and epithelial restitution. However, electron microscopic examination of tissues reveals dilation of tight junctions in tissues treated with NSAIDs,[898] whereas those additionally treated with prostaglandins have closely apposed tight junctions (Fig. 12.10). Prostaglandins stimulate closure of tight junctions via the second messengers cAMP and Ca^{2+},[893] which interestingly were among the first mediators found to modulate tight junction permeability.[899,900] Such tight junction closure is of considerable importance to patients with intestinal injury that are treated with nonsteroidal antiinflammatory agents, because reduced prostaglandin levels may result in increased intestinal permeability. For example, in a study on ischemic-injured porcine ileum, treatment with the NSAID indomethacin resulted in a significant increase in intestinal permeability to inulin and lipopolysaccharide compared with tissues that were additionally treated with PGI_2 and PGE_2.[893]

Polyamines

The process of restitution is absolutely dependent on a group of compounds called polyamines.[901,902] The rate-limiting enzyme in the formation of the polyamines spermine, spermidine, and putrescine is ornithine decarboxylase (ODC). In rats with stress-induced duodenal ulcers, systemic administration of the ODC inhibitor DL-alpha-difluoromethyl ornithine significantly reduced polyamine levels and markedly reduced epithelial restitution. Intragastric treatment of these same rats with putrescine, spermidine, and spermine prevented the delayed mucosal repair induced by DL-alpha-difluoromethylornithine.[901] Interestingly, gastric tissue levels of ODC were increased in rats with stress-induced gastric ulcers, suggesting that polyamine production is enhanced during tissue injury and may contribute to the normal rapid rate of epithelial restitution.[903]

The mechanisms by which polyamines stimulate epithelial restitution are not clear. McCormack et al. hypothesized that polyamines increased transglutaminase activity, an enzyme that catalyzes the cross-linking of cytoskeletal and basement membrane proteins.[904] Further investigation of the role of polyamines in IEC-6 cell migration showed that depletion of polyamines resulted in disruption of the cytoskeleton and reduced the physical extension of lamellipodia.[905] More recent studies have clarified this pathway. Polyamines regulate cytoskeletal cellular migration via activation of the small GTPase Rho-A by elevating intracellular Ca^{2+} levels. These elevations in Ca^{2+} result from polyamine regulation of expression of voltage-gated K^+ channels and altered membrane electrical potential.[906]

Polyamines also play a role in the normal physiologic regulation of crypt cell proliferation and differentiation.[907,908] They are produced by fully differentiated enterocytes at the villous tip and may reach the crypt either within sloughed luminal epithelium or via local villous circulation.[909] Following intestinal injury, polyamines appear to stimulate enhanced proliferation by increasing the expression of protooncogenes, which control the cell cycle.[910] The mechanism by which polyamines influence gene expression likely relates to the cationic nature of these compounds, which may influence the tertiary structure of negatively charged DNA and RNA.[901]

Growth Factors

Locally produced growth factors, including EGF, TGF-α, TGF-β, and hepatocyte growth factor, have the ability to modulate mucosal recovery. The most important of these growth factors in early mucosal repair events is TGF-β, which is a potent stimulus of epithelial restitution and modulator of the ECM.[821] Neutralization of TGF-β retards epithelial migration in vitro, and it appears that TGF-β may serve as a point of convergence for mediators of restitution, because neutralizing TGF-β also inhibits the effects of other peptides. However, TGF-β paradoxically inhibits epithelial proliferation, reducing the supply of new enterocytes for mucosal repair. Conversely, EGF, produced by the salivary glands and duodenal Brunner's glands, and the related TGF-α, produced by small intestinal enterocytes, are potent stimulants of enterocyte proliferation. These growth factors share approximately 30% of their amino acid structure, bind to the same receptor on the basolateral surface of enterocytes, and are not related to TGF-β.[911] The physiologic role of EGF is somewhat difficult to discern because it is present in the intestinal lumen, with no apparent access to its basally located receptor.[912] It has been proposed that EGF acts as a "surveillance agent" that gains access to its receptor during epithelial injury (when the EGF receptor would likely be exposed) to stimulate proliferation.[912] TGF-α presumably has a similar role, but it is present in greater concentrations in the small intestine because it is produced by differentiated villous enterocytes. The mature peptide is cleaved from the extracellular component of the transmembrane TGF-α precursor and released into the lumen.[911]

Trefoil Peptides

Another group of proreparative peptides that are locally produced within the gastrointestinal tract are the trefoil peptides. Under physiologic conditions, trefoil peptides are secreted by mucus-producing cells at distinct anatomic sites. For example, the trefoil peptide pS2 is produced by gastric epithelium, whereas ITF is produced by small and large intestinal mucosa.[701] However, any of the trefoil peptides may be upregulated within repairing epithelium regardless of the anatomic site.[821,913] In addition, trefoil peptides have the ability to induce their own expression, amplifying the level of these reparative factors at sites of mucosal repair.[914] Trefoil peptides are the most potent stimulants of epithelial migration in vitro, and their effects are independent of growth factors, including TGF-β.[915] Recent evidence suggests that EGF receptor activation is required for the induction of pS2 and another of the trefoil peptides called spasmolytic peptide in gastric epithelium in vitro. The importance of trefoil peptides to the mucosal repair response in vivo is illustrated by gene knockout studies, in which mice deficient in ITF have a dramatically reduced ability to repair intestinal injury.[916] Detergent-induced mucosal injury was lethal because of a lack of restitution compared with wild-type mice that fully recovered from similar mucosal injury. The fact that restitution was restored by administration of ITF has important therapeutic implications. The mechanism by which trefoil peptides stimulate epithelial migration has yet to be fully characterized, but it appears to involve translocation of the adherens junction protein E-cadherin, allowing cells to become untethered from neighboring cells.[821]

Intestinal Nutrients

The principal metabolic fuel of enterocytes is glutamine, and for colonocytes it is butyrate. However, recent studies suggest that glutamine and butyrate have more specific proliferative actions aside from their role as nutrients. For example, in the piglet IPEC-J2 enterocyte cell line, glutamine enhanced gene transcription by increasing MAPK activity.[917,918] Similarly, butyrate stimulated mucosal growth following colonic infusion in the rat.[919] Because of such growth-promoting actions, glutamine was shown to prevent the intestinal mucosal atrophy and dysfunction that accompany starvation[920,921] and long-term total parenteral nutrition.[922,923] Glutamine improves the function of transplanted small intestine[924,925] and protects intestinal mucosa from injury if administered before chemotherapy[926] and radiation.[927,928] Intestinal nutrients may also synergize with other proliferative agents. For example, administration of glutamine and TGF-α to porcine ileum that had been subjected to 2 hours of ischemia resulted in a synergistic increase in MAPK activity, enterocyte proliferation, and villous surface area.[836] Although there has been a concern that such early return to normal surface area may result in dysfunctional mucosal digestive and absorptive function because of resurfacing denuded mucosa with immature epithelium, nutrients and growth factors also appear to promote early differentiation. In the case of glutamine and TGF-α restoration of postischemic small intestine, rapid recovery of digestive enzymes was also documented.[929]

Gastrointestinal Ileus

Effective gastrointestinal motility involves a complex interaction between the ENS, muscular wall, and luminal contents. Additional factors that influence the net transit of digesta include gravity, the volume and viscosity of the contents, and pressure gradients created by simultaneous contraction and relaxation of adjacent segments of bowel. Casual use of the term *intestinal motility* in veterinary medicine often underestimates the complexity of the processes involved in the transit of intestinal contents. This is particularly true when the term is used to describe the frequency and or intensity of intestinal sounds, or borborygmi. The existence of borborygmi does not always equate with progressive movement of intestinal contents.

Disruption to normal motility occurs commonly in horses for a variety of reasons. Examples of diseases in which altered motility may be present include grass sickness, gastroduodenal ulceration, intraluminal obstruction or impaction, excessive wall distention, strangulating obstruction, peritonitis, and inflammatory disorders such as duodenitis proximal jejunitis or colitis. Ineffective intestinal motility is also a feature of several neonatal diseases, including prematurity, systemic sepsis, and perinatal asphyxia. Certain parasitic infections, electrolyte derangements, and endotoxemia can modify digesta transit in horses of all ages. General anesthesia and specific sedatives, such as xylazine, romifidine, or detomidine, also disturb motility.

MANIFESTATIONS OF ILEUS

The inhibition of propulsive bowel activity usually is referred to as ileus. Ileus is ascribed most frequently to the condition that occurs after laparotomy and is termed *simple* or *uncomplicated POI*. The term *complicated* or *paralytic ileus* describes intestinal motility disturbed for longer periods after surgery. POI in horses is associated most commonly with surgery of the small intestine, particularly after resection and anastomosis,[930,931] and can have a negative effect on short-term postoperative survival.[932-935] Motility dysfunction likely is present in all horses after laparotomy, but many are affected subclinically and require minimal or no specific intervention. In symptomatic animals, clinical signs are apparent shortly after recovery and include colic, tachycardia, dehydration, decreased borborygmi and fecal output, and sequestration of fluid within the stomach. Rectal examination and ultrasound reveal small intestinal distention with rare or absent wall movement. The severity and duration of intestinal stasis varies, lasting from minutes to days.

A specific motility disorder involving the cecum or ileocecocolic region occurs sporadically in horses.[936-938] The condition most commonly occurs after general anesthesia and extraabdominal surgery, particularly orthopedic and upper airway procedures, and often is categorized as a form of POI. Other cases occur spontaneously, often in animals with painful primary conditions such as uveitis or septic tenosynovitis. In a study of 114 horses diagnosed with cecal impaction, 12 were hospitalized for a condition other than colic at the time of diagnosis, and 9 others were being treated with phenylbutazone, most for a musculoskeletal injury.[939] Eight of the 114 horses had undergone general anesthesia in the 8 days preceding diagnosis. The syndrome is frustrating in that clinical signs are often subtle unless cecal perforation has occurred. In horses with a cecal emptying defect after anesthesia, overt signs are usually apparent 3 to 5 days after the procedure. The earliest detectable signs include depression and a reduction in feed intake and fecal output. Ineffective emptying results in overfilling of the cecum with moist contents, which is manifested by signs of mild to moderate colic. If the condition is recognized late or untreated, the cecum may rupture and result in fatal peritonitis.

PHYSIOLOGY

Current understanding of motility throughout the equine gastrointestinal tract is remarkably limited, and much of our presumptive knowledge comes from work in other species. The ENS is involved in all aspects of motility, either directly via neurotransmitters or indirectly via interstitial cells of Cajal (ICC) or immune or endocrine regulation. The inherent rhythmicity of electric activity in the intestine is controlled by the ICC, which is specialized cells that are electrically coupled to myocytes via gap junctions.[940] These cells are responsible for generating and propagating slow-wave activity; hence, they are deemed the pacemaker cells of the intestine. A decrease in ICC density has been observed in horses with obstructive disorders of the large intestine[941] and in the ileum and pelvic flexure of horses diagnosed with equine grass sickness (dysautonomia),[942] although such a decrease was not evident in a horse with dysautonomia that recovered.[943] This alteration in ICC infrastructure appears to result in reduced slow-wave activity in vitro.[944]

The ENS primarily controls and coordinates intestinal contraction. A combination of central and autonomic innervation influences events, but contraction does not require external neural input. The parasympathetic supply to the gastrointestinal tract is via the vagus and pelvic nerves, and the sympathetic supply is through postganglionic fibers of the cranial and caudal mesenteric plexuses. A complex network of interneurons within each plexus integrates and amplifies neural input; the intensity and frequency of resultant smooth muscle contractions are proportional to the amount of sympathetic and parasympathetic input. Additional binding sites for a number of other endogenous chemicals, including dopamine, motilin, and serotonin, exist within the ENS and on smooth muscle cells.[945] Acetylcholine is the dominant excitatory neurotransmitter in the gastrointestinal tract and exerts its action through muscarinic type 2 receptors on smooth muscle cells. Sympathetic fibers innervating the gastrointestinal tract are adrenergic, postganglionic fibers with cell bodies located in the prevertebral ganglia. Activation of α_2-adrenergic receptors on cholinergic neurons within enteric ganglia inhibits the release of acetylcholine, reducing intestinal contraction. β_1-, β_2-, and β-atypical receptors are directly inhibitory to the intestinal smooth muscle.[946] Inhibitory nonadrenergic, noncholinergic neurotransmitters include adenosine triphosphate, vasoactive intestinal peptide, and nitric oxide.[947,948] These neurotransmitters are critical for mediating descending inhibition during peristalsis and receptive relaxation. Substance P is a nonadrenergic, noncholinergic neurotransmitter that may be involved in contraction of the large colon.[949,950]

The rate and force of intestinal contractions along the small intestine and large colon of the horse are important determinants of intestinal motility; of even greater importance to the net propulsion of digesta are the cyclical patterns of contractile activity. These patterns are known as the small intestinal and colonic migrating motility (or myoelectric) complexes (MMCs).[951,952] The colonic complex usually originates in the right ventral colon and variably traverses the ascending and descending colons. Many of these complexes are related temporally to a specialized motility event of the ileum, the migrating action potential complex.[953]

PATHOPHYSIOLOGY

Inflammation

Local inflammation within the intestinal muscularis and inhibitory neural events are important initiators of intestinal ileus.[954,955] Intestinal inflammation not only is important in primary intestinal diseases in horses, such as DPJ and colitis, but also is induced after simple intestinal handling during laparotomy. In rodents, simple intestinal manipulation causes

a cascade of inflammation within the muscularis, resulting in leukocyte infiltration and subsequent suppression of muscle contractility. Similar inflammatory effects were evident with mechanical manipulation of the equine jejunum.[956] The inflammatory response to bowel manipulation is not limited to the affected tissue, but it can also result in global inflammation and ileus throughout the gastrointestinal tract.[957]

The associated inflammatory events are extremely complex, involving a milieu of proinflammatory cytokines, prostaglandins, and leukocytes. Depletion or inactivation of muscularis macrophage function can prevent inflammation associated with intestinal manipulation and associated decreased contractility.[958] Mast cell activation is involved in intestinal manipulation-associated POI in humans.[959] Inflammation associated with colonic manipulation may involve gut-derived bacterial products.[960] Another factor in the development of intestinal stasis after inflammation is the local overproduction of nitric oxide caused by the upregulation of iNOS by resident macrophages.[961] The iNOS upregulation was important for initiation of the inflammatory response and inhibition of motility. Nitric oxide is a key inhibitory neurotransmitter of the nonadrenergic, noncholinergic system.[948]

In the horse, significant neutrophilic inflammation is apparent in the jejunum from clinical cases necessitating resection and following a period of recovery in the jejunum subjected to 1 or 2 hours of ischemia.[962] The ischemic tissue also had evidence of leukocyte activation, as demonstrated by calprotectin-positive cells in associated tissue histologically.

Pharmacologic Alteration

The inhibitory effects of α_2-adrenergic agonists such as xylazine and detomidine on duodenal, cecal, and large colon motility are well described, because these drugs activate presynaptic receptors within the ENS.[4,963,964-968] IV-administered xylazine inhibits cecal and large colon motility for 20 to 30 minutes without seriously disrupting small intestinal myoelectric activity, and detomidine can reduce large intestinal myoelectric activity for up to 3 hours. Detomidine decreases duodenal motility in a dose-dependent fashion.[969] The α_2-antagonist yohimbine has a weak but positive effect on cecal emptying in normal ponies, suggesting that normal motility is under constant α_2-adrenergic tone.[4]

Several opioid agonists also have documented inhibitory effects on equine gastrointestinal motility at both a central and peripheral level. Morphine administration decreased frequency of defecation and fecal moisture content while increasing gastrointestinal transit time in normal horses at a dose of 0.5 mg/kg twice daily for 6 days.[970] Single doses of fentanyl or morphine decrease jejunal and colonic MMC activity in ponies, whereas the antagonist naloxone elicited increased propulsive activity in the colon.[971] Fentanyl administered as an IV constant rate infusion did not have an apparent deleterious effect on duodenal motility.[972] Butorphanol, an opioid agonist-antagonist, decreases myoelectrical activity in the jejunum but not pelvic flexure.[973] In another series of experiments, butorphanol alone did not decrease gastric or duodenal motility,[974] but administration in combination with xylazine resulted in a synergistic inhibitory effect, which was more pronounced than that obtained by administration of xylazine alone.[968] Administration of butorphanol as a constant rate infusion appears to have a minimal to no effect on global gastrointestinal[470,975] or duodenal motility.[976] When lidocaine, ketamine, and butorphanol were administered alone or in combination as constant rate infusions to healthy horses, combinations containing butorphanol (butorphanol/lidocaine, or a combination of all three drugs) delayed total gastrointestinal transit time.[977]

N-Butylscopolammonium bromide has a profound but very short-lived negative effect on duodenal motility, but this effect was not significant between groups.[976] Atropine is a postganglionic blocking agent that binds to muscarinic receptors. When administered at 0.04 mg/kg, atropine inhibits individual small intestinal, cecal, and colonic contractions for about 120 minutes but suppresses small intestinal and colonic migrating complexes for up to 8 hours.[978]

Neural Reflexes

Neural reflexes may mediate inhibition of motility associated with peritoneal inflammation.[979,980] The afferent segment is composed partly of capsaicin-sensitive visceral afferent C fibers that terminate in the dorsal horn of the spinal cord, in which they can activate inhibitory sympathetic fibers or synapse directly on the sympathetic ganglia. Consequently, the efferent limb of the reflex expresses increased sympathetic outflow, primarily mediated through stimulation of α_2-adrenoreceptors, and inhibition of acetylcholine release, which provides the rationale for α_2-blockade in treating ileus. Intraluminal infusion of capsaicin before abdominal surgery ameliorated the severity of POI in experimental rats. This finding highlights the importance of visceral afferent fibers in the development of POI.[981]

Distention

Ileus also can occur in association with intestinal obstruction or displacement. Mild to moderate distention of the bowel, such as that occurring in the early stages of an intraluminal obstruction, evokes an increase in local contractile activity.[982,983] Excessive distention results in the inhibition of motility within the distended segment of bowel. Intestinal stasis is not always detrimental and under certain conditions may be protective. Repeated distention for determination of nociceptive threshold results in an overall decrease in duodenal motility over time, irrespective of other interventions.[972,976]

Endotoxin

Endotoxemia is a clinical feature of many diseases of the equine gastrointestinal tract, and endotoxins independently can exert a negative effect on intestinal motility and transit.[214] A variety of mediators likely are involved, but the activation of α_2-adrenoreceptors and production of prostanoids appear to be important, because pretreatment with yohimbine or NSAIDs (phenylbutazone or flunixin), respectively, ameliorates the inhibitory effects of experimental endotoxin infusion.[201,984-986] Pretreatment with metoclopramide or cisapride had a similar effect.[71,987] Endotoxin infusion induced an inflammatory response in the intestine of rats that mimicked the response induced by handling during laparotomy.[988] The similarity of the responses was highlighted in a recent study that demonstrated that prior exposure of the muscularis to endotoxin protected the intestine from the effects of manipulation.[989] In rats, colonic manipulation alone causes transference of intraluminal LPS to the muscularis, which likely contributes to the global gastrointestinal inflammatory response and decrease in contractility associated with tissue manipulation.[960] In response to endotoxin alone, the inflammatory response within the jejunal muscularis is predominantly monocytic, whereas the response to polymicrobial sepsis is predominantly neutrophilic.[990]

Other Effects

Temperature also appears to affect in vitro slow-wave activity in the horse. In a recent study, the slow-wave frequency was approximately linearly related to temperature in the range studied (27–41°C) and was highly temperature sensitive in isolated equine ileal segments.[991]

The pathophysiology of cecal emptying defect is not known. This syndrome may best mimic POI in human beings, which is generally considered a large intestinal disorder. An important difference in horses is that laparotomy is a rare predisposing factor, and most cases occur in horses undergoing routine extraabdominal surgical procedures. The disorder, therefore, is probably not appropriately considered as a form of POI in horses. General anesthesia itself is a potent inhibitor of gastrointestinal motility in horses, but these effects are short-lived and reversible within hours of anesthetic withdrawal.[952] The return of normal motility in horses after experimental ileus was most delayed in the cecum, suggesting that this may be a common site of ileus in horses.[992] A link between routine postoperative medications, such as phenylbutazone and aminoglycoside antibiotics, has been suspected but not established. An inhibitory effect of NSAIDs on large colon contractility has been demonstrated using in vitro techniques.[993] Primary sympathetic overstimulation could be involved, because many of the affected animals are young, male horses or animals with painful diseases.

The duration of surgery influences the development of small intestinal POI but not cecal emptying dysfunction.[938,994] Technique may have a weak influence on small intestinal POI after jejunojejunostomy. The duration of intestinal ileus was shorter in animals that received a side-to-side stapled anastomosis than those that had a hand-sewn end-to-end procedure.[932] The duration of ileus after stapled end-to-end anastomosis was not different from that after either procedure. Jejunocecostomy more commonly results in POI than other types of small intestinal resection and anastomosis, whether related to diseases necessitating this procedure or the procedure itself.[995]

Other reported risk factors for the development of POI include age (>10 years), small intestinal resection and anastomosis, breed (Arabians had a greater risk than other breeds), and duration of surgery.[994] A prospective study found small intestinal lesion, high packed cell volume, and duration of anesthesia to increase the risk of POI, whereas performance of a pelvic flexure enterotomy and intraoperative administration of lidocaine may have a modest protective effect against POI.[996]

DIAGNOSIS

The diagnosis of ileus is based on history and physical examination findings. Case inclusion criteria for clinical studies of POI have varied.[994-997] Recent surveys of the Diplomates of the European Colleges of Equine Internal Medicine and Veterinary Surgery and the American Colleges of Veterinary Surgery, Veterinary Internal Medicine, and Veterinary Emergency and Critical Care revealed that the presence of reflux on passage of a nasogastric tube, evidence of multiple fluid-distended loops of small intestine on ultrasonographic examination, and the presence of multiple fluid-distended loops of small intestine on rectal palpation were the features most commonly identified as "extremely important" for a diagnosis of POI.[998,999] One can palpate cecal distention with digesta in horses with advanced cecal dysfunction.

Distinguishing functional ileus from mechanical obstruction is important and can be difficult, but horses with mechanical obstruction typically have sustained high volumes of gastric reflux that vary little over time and abdominal pain, which is typically not relieved by gastric decompression. Abdominal ultrasound in horses with ileus typically reveals mild to moderately fluid-filled hypomotile to immotile small intestine, without alteration in the amount or character of PF or small intestinal wall thickness. Horses with reflux from other causes (peritonitis and mechanical obstruction) will have changes reflective of their disease process. This differentiation is important for appropriate case management, because horses with a mechanical obstruction often need either primary or repeat laparotomy, which should not be delayed.

TREATMENT

The management of intestinal ileus depends on the segment of gastrointestinal tract involved. Therapy for ileus of the proximal gastrointestinal tract involves a combination of gastric decompression, fluid and electrolyte therapy, and antiinflammatory therapy. Electrolyte therapy is critical, particularly for maintaining adequate extracellular concentrations of potassium, calcium, and magnesium. Calculation of the volume of fluid to be administered should include maintenance requirements plus an estimate of losses, especially those lost through gastric decompression. Parenteral provision of calories should be considered when feed has been withheld for more than 96 hours, particularly after surgery. Hand walking also may provide some benefit to these animals but is not likely to have a direct effect on intestinal motility.

Drugs that can have an inhibitory effect on motility should be avoided or used sparingly. Horses with primary cecal impaction or impaction caused by an emptying defect may require surgery to prevent fatal rupture. The surgical management of these cases is controversial and may include typhlotomy alone, typhlotomy with a bypass procedure such as ileocolic or jejunocolic anastomosis, or a bypass without typhlotomy.[1000] Most horses that undergo simple typhlotomy have an uneventful recovery.[1001] In a large retrospective study, 44/54 horses treated medically survived to discharge, whereas 37 of 49 horses treated surgically were allowed to recover, 35 of which survived to discharge.[939] Ileocolostomy was only performed in two of the 37 horses treated surgically (one of which survived to discharge), with the remainder receiving typhlotomy without bypass. Survival to 1 year was not statistically different between horses treated medically (18/19) or surgically (25/28), although 6 horses had a recurrence of cecal impaction.[939]

Experimental and anecdotal evidence provides a strong rationale for using antiinflammatory drugs to prevent and treat gastrointestinal ileus, particularly in animals that may have endotoxemia.[1002] Flunixin meglumine is used widely in equine practice as an analgesic and antiinflammatory agent, and it ameliorates many of the adverse systemic effects of endotoxin, particularly those on the cardiovascular system. A potential negative effect of NSAIDs on large intestinal contractility has been suggested. A differential effect on contractility between selective and nonselective COX inhibitors is currently unknown. Broad-spectrum antimicrobials are indicated when one suspects sepsis or in cases of profound neutropenia. High concentrations of aminoglycoside antimicrobials inhibited intestinal contractions in exposed sections of intestine in vitro, but this inhibitory effect is unlikely to occur at clinically relevant doses.[1003]

Motility-enhancing drugs have been advocated to treat gastrointestinal ileus. Unfortunately, information directly pertinent to horses is limited and must be extrapolated cautiously from that of other species because of the differences in intestinal anatomy and physiology. Prokinetic drugs potentially can shorten the length of hospitalization, reducing the cost of treatment and the number of potential complications such as weight loss, thrombophlebitis, and laminitis. Experimental evidence indicates that prokinetic drugs can minimize the development of postoperative abdominal adhesions.[1004] Most prokinetic drugs require a healthy gut wall to enhance intestinal contraction, and downregulation of motilin receptors has been demonstrated in the inflamed equine jejunum.[473] Therefore one should not assume that many of these drugs would be effective in the presence of an inflammatory injury such as that which can occur after intestinal manipulation at surgery or that associated with DPJ.

Cholinomimetics

Bethanechol is a parasympathomimetic agent that acts at the level of the myenteric plexus and directly on intestinal smooth cells through muscarinic receptors. In the horse, this effect is mediated predominantly by the M3 receptor, but the M2 receptor may also play a role.[1005] Bethanechol is a synthetic ester of acetylcholine and is not degraded by anticholinesterase. Bethanechol has cholinergic side effects, including abdominal discomfort, sweating, and salivation, although these are minimal when the drug is administered at 0.025 mg/kg body mass SC or orally. Bethanechol has efficacy in diseases that involve abnormal gastric emptying and delayed small intestinal transit and increases gastric contractility and hastens the emptying of liquid and solid phase markers from the stomach of normal horses.[1006] Bethanechol also increases the strength and duration of wall contractions in the cecum and right ventral colon, consequently speeding up cecal emptying.[4]

Neostigmine increases receptor concentration of acetylcholine by inhibiting cholinesterase. The drug (0.022–0.025 mg/kg IV) promotes cecal and colonic contractile activity and hastens the emptying of radiolabeled markers from the cecum.[4] Neostigmine has been used to manage small intestinal ileus, but it significantly delayed the emptying of 6-mm beads from the stomach of normal adult horses.[1007]

Benzamides and Dopamine Antagonists

Metoclopramide acts principally as a 5-hydroxytryptamine 4-receptor (5HT-4) agonist and 5HT-3–receptor antagonist. In contrast to newer generation benzamides, metoclopramide is also an antagonist at dopamine 1 (DA1) and dopamine 2 (DA2) receptors. Antagonism of prejunctional DA2 receptors facilitates acetylcholine release and smooth muscle contraction. Metoclopramide crosses the blood-brain barrier, where its antagonist properties on central DA2 receptors can result in extrapyramidal signs, including seizure. Metoclopramide increased contractility of muscle strips in vitro in the pyloric antrum, proximal duodenum, and midjejunum.[1008] These in vitro data support previous work in which metoclopramide administration restored gastroduodenal coordination of motility in a model of POI.[1009] In another study, metoclopramide had no effect on jejunal or pelvic flexure myoelectrical activity.[973] Constant IV infusion (0.04 mg/kg/h) of metoclopramide was well tolerated in a population of postoperative horses and significantly decreased the volume and duration of gastric reflux over control and intermittent drug infusion groups.[1010]

Cisapride is a second-generation benzamide that acts as a 5HT-4 agonist and 5HT-3–receptor antagonist but is without antidopaminergic action. Stimulation of 5HT-4 receptors within the ENS enhances release of acetylcholine from the myenteric plexus. Several reports suggest the efficacy of cisapride in managing intestinal disease in horses, including the resolution of persistent large colon impaction, treatment of equine grass sickness, and as a preventative for POI in horses after small intestinal surgery (0.1 mg/kg body mass intramuscularly during the postoperative period).[1011-1014] The horse erratically absorbs tablets administered rectally, but a method for preparing a parenteral form of the drug from tablets has been described.[1015] Cisapride has the potential to cause adverse cardiac side effects mediated through blockage of the rapid component of the delayed rectifier potassium current that include lengthening of the QT interval and development of torsades de pointes, a potentially fatal arrhythmia.[1016] These adverse effects have resulted in withdrawal of the drug in the United States but have not been reported in the horse.

Tegaserod, a 5HT-4 agonist, increases pelvic flexure smooth muscle contractility (0.27 mg/kg PO)[1017] and hastens gastrointestinal transit time (0.02 mg/kg IV) in healthy horses.[1018] It has not, to the author's knowledge, been objectively evaluated in abnormal horses, but it may prove useful. In humans, this drug was marketed for women with constipation-predominant or mixed symptom irritable bowel syndrome and demonstrated clear benefits in quality of life and gastrointestinal symptoms but is currently only available in a restricted fashion because of an association with ischemic colitis and cardiovascular disease.[1019]

Domperidone acts as a competitive antagonist at peripheral DA2 receptors. The drug is a therapeutic agent (1.1 mg/kg/day) for mares grazing endophyte-infected tall fescue, principally because of drug-enhanced prolactin release. Modest efficacy of domperidone (0.2 mg/kg IV) has been demonstrated in experimental ileus in ponies.[1011] In another study, a much higher oral dosage (5 mg/kg) was required to increase gastric emptying; 1.1 mg/kg orally had limited effects on the gastrointestinal tract in healthy horses.[1020]

Antimicrobials

Erythromycin, a macrolide antibiotic, is a direct motilin receptor agonist on smooth muscle cells and may act within the ENS to facilitate the release of acetylcholine and motilin. Erythromycin displaces motilin from its receptor in the equine duodenum, jejunum, cecum, and pelvic flexure.[476] Erythromycin enhances gastric emptying in normal horses but has a more pronounced effect on the hindgut.[1006,1021] Erythromycin lactobionate (1.0 mg/kg IV) hastens cecal emptying in normal animals and induces colonic MMC-like activity across the colon. Administration often is associated with defecation and abdominal discomfort. The prokinetic effect of erythromycin apparent in the ileum, cecum, and pelvic flexure documented in normal horses was reduced in the immediate postoperative period.[686] Luminal distention and decompression resulted in inflammation and a decreased response to erythromycin.[1022] A decrease in motilin receptors in response to luminal distention has been documented in the equine jejunum,[473] and this may explain the difference in response between normal and clinically affected horses. Repeated dosing can cause downregulation of motilin receptors in other species.[1023] Erythromycin can induce diarrhea in adults; therefore, one should avoid dosing over many days.

Potassium penicillin (20 million IU IV to adult horses) can stimulate defecation and increase myoelectrical activity in the

cecum and pelvic flexure, and these effects are not produced by an equimolar amount of potassium ion given IV as potassium chloride.[1024]

Opioid and α_2-Adrenoreceptor Antagonists

Naloxone (0.05 mg/kg IV) induces contractile activity in the cecum and left colon.[971] Defecation commonly follows administration of naloxone within 15 to 20 minutes. *N*-methylnaltrexone increases jejunal and pelvic flexure contractility in vitro[1025] and prevents the negative effects of morphine on fecal output and intestinal transit time when administered concurrently.[1026]

α_2-Adrenoreceptor antagonists such as yohimbine or tolazoline counteract increased sympathetic outflow in response to nociceptive stimulation. Yohimbine infusion (75 μg/kg) also may attenuate the negative effects of endotoxin on motility.[201,985]

Local Anesthetics

The use of IV lidocaine as a prokinetic has gained tremendous popularity and was reported to be the agent most commonly used by equine surgeons for treatment of POI.[1027] In more recent surveys, flunixin meglumine and lidocaine were used most frequently for the treatment of POI by specialists in Europe and North America.[998,999] Lidocaine may exert prokinetic effects by suppressing primary afferent neurons, limiting reflex efferent inhibition of motility.[1028] Other proposed mechanisms of action include antiinflammatory properties, potentially through NF-κB signaling[1029] or improving mucosal repair.[413] IV lidocaine also has analgesic effects, although it was shown to alter somatic but not visceral antinociception in clinically normal horses in one study.[1030] Lidocaine increased contractile activity in isolated strips of proximal duodenum in vitro.[1008] The most commonly cited dosage is a 1.3-mg/kg bolus, typically over 15 minutes, followed by a 0.05-mg/kg/min constant rate infusion. This dosage did not alter MMC duration or spiking activity, reset the MMC in the jejunum in clinically normal horses[1031] or significantly alter a variety of indicators of POI after colic surgery.[1032] Significantly more horses with POI stopped refluxing within 30 hours following the institution of lidocaine infusion, relative to saline infusion.[475] Lidocaine administration did not affect the prevalence of POI, duration or volume of reflux, or survival in horses requiring surgery for treatment of small intestinal colic.[1033] Lidocaine infusion can be associated with reversible side effects that include muscle fasciculations, ataxia, and seizure. Consequently, the rate of infusion requires close monitoring. Prolonged infusion of lidocaine in the horse appears safe, although accumulation of the GX metabolite has been documented.[1034]

Ischemic Disorders of the Intestinal Tract[g]

PATHOPHYSIOLOGY OF STRANGULATING OBSTRUCTION

Strangulating obstruction of the intestine is characterized by simultaneous occlusion of the intestinal lumen and its blood supply. Although strangulation of the intestinal lumen results in clinical signs similar to those of simple obstruction, occlusion of the blood supply results in a more rapid deterioration of the intestinal mucosa and subsequent onset of sepsis. Although there has been a great deal of interest in the relevance and treatment of intestinal reperfusion injury,[851,861,882] the lesion that develops during strangulation is often severe, leaving little viable bowel for further injury during reperfusion.[882] Although extensive lengths of strangulated small intestine may be resected, strangulation of the large colon presents a much greater treatment dilemma because strangulated intestine usually extends beyond the limits of surgical resection.[1035] Therefore horses with large intestinal strangulation are often recovered with extensive intestinal injury left in place. As a result, subtle degrees of reperfusion injury may be very important in horses with large colon disease, warranting further work in this area in an attempt to reduce mortality.[851]

Strangulating obstruction may be divided into hemorrhagic and ischemic forms.[163] In hemorrhagic strangulating obstruction, which is most common, the veins become occluded before the arteries because of the greater stiffness of arterial walls. This lesion is noted by a darkened appearance in affected bowel and increased thickness as blood is pumped into the lesion. Ischemic strangulating obstruction occurs if the intestine is twisted tightly enough to simultaneously occlude both arteries and veins. In the case of the colon, some researchers suggest that this may be determined by how much ingesta is in the colon, because intestinal contents may prevent the intestine from twisting tightly.[1036] Tissue involved in ischemic strangulating obstruction appears pale and of normal or reduced thickness because of a complete lack of blood flow (Fig. 12.11). Bowel peripheral to strangulating lesions may also become injured as a result of distention, which reduces mural blood flow once it reaches critical levels. As this intestine is decompressed, it may also undergo reperfusion injury.[879,1037,1038]

SMALL INTESTINAL STRANGULATION

Clinical Signs

Horses with small intestinal strangulating obstruction typically have moderate to severe signs of abdominal pain that is only intermittently responsive to analgesic medications. During the latter stages of the disease process, horses may not experience much pain but rather become profoundly depressed as affected intestine undergoes necrosis. Affected horses have progressive signs of sepsis, including congested mucous membranes, delayed capillary refill time, and an elevated heart rate (>60 beats/min in most cases). Reflux is typically obtained after passage of a stomach tube, and loops of distended small intestine are usually detected on rectal palpation of the abdomen. These latter findings are variable, depending on the duration and location of the obstruction. For example, horses with ileal obstructions tend to reflux later in the course of the disease process than horses with jejunal obstructions. A horse that has an entrapment of small intestine in the epiploic foramen or a rent in the proximal small intestinal mesentery may not have palpable loops of small intestine because of the cranial location of these structures.[1039] Abdominocentesis can provide critical information on the integrity of the intestine and is indicated in horses with suspected strangulation of the small intestine. Affected horses typically have serosanguineous abdominal fluid with an elevated protein concentration

[g]Previous version by Anthony T. Blikslager.

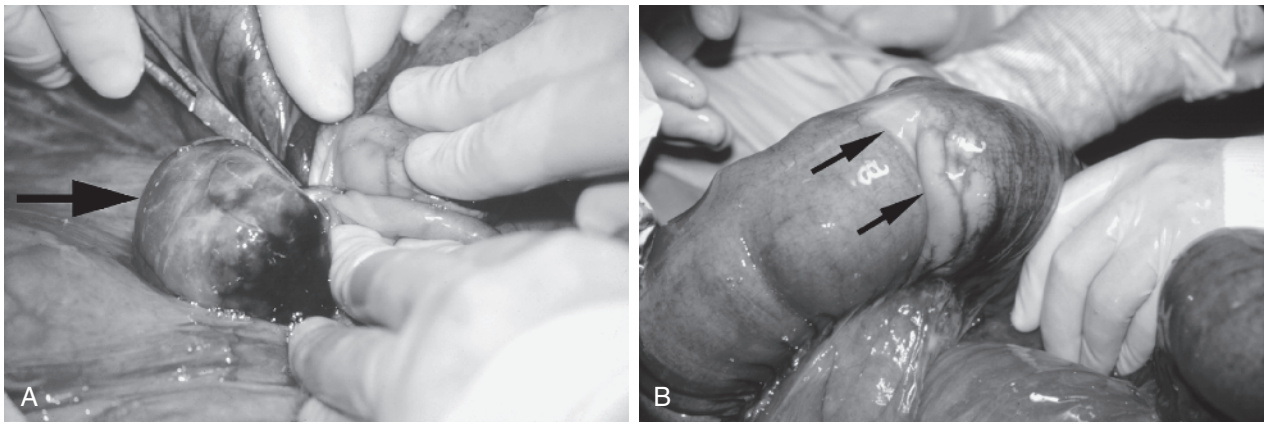


FIG. **12.11** Ischemic strangulating obstruction of the small colon by a mesenteric lipoma. (A) Note the lipoma *(arrow),* which has tightly encircled a segment of small colon. (B) After resection of the lipoma, a pale area of strangulated small colon is clearly demarcated *(arrows),* the appearance of which is consistent with ischemic strangulating obstruction.

(>2.5 mg/dL) and an increase in PF:plasma lactate, although these cases must be differentiated from proximal enteritis.[11,22,783] When an initial sample is inconclusive, repeated measurement of PF lactate may provide additional support for a diagnosis of a strangulating lesion.[27] Generally, horses with small intestinal strangulation show continued signs of abdominal pain, whereas horses with proximal enteritis (discussed in Duodenitis-Proximal Jejunitis previously in this chapter) tend to be depressed after initial episodes of mild abdominal pain.[1040] In addition, horses with small intestinal strangulation continue to deteriorate clinically despite appropriate medical therapy and begin to show elevated WBC counts (>10,000 cells/μL) in the abdominal fluid as the duration of strangulation increases. There are horses in which small intestinal strangulation and proximal enteritis cannot be not readily distinguished, at which point surgery may be elected rather than prolonging the decision to perform abdominal exploration on a horse with a potential strangulating lesion.[1040]

Prognosis

The prognosis for survival in horses with small intestinal strangulating lesions is generally lower than for most forms of colic,[995] but some reports indicate that more than 80% of horses with small intestinal strangulating lesions will survive to discharge from the hospital.[1041] Owners should be warned that long-term survival rates are less than 70%,[1042] in part because of long-term complications such as adhesions.[1043,1044] The prognosis is particularly poor for some forms of strangulation, including entrapment of small intestine within a mesenteric rent.[1045] Some horses with small intestinal strangulation can be managed surgically without resection, with favorable short- and long-term prognosis.[1046] Geriatric horses have a higher likelihood of small intestinal strangulation, relative to mature counterparts undergoing colic surgery, but carry a similar prognosis.[1047] In one hospital, horses with gastrosplenic ligament entrapment (GLE) had higher short-term survival (72.7%), relative to horses with other small intestinal strangulating lesions (50%),[1048] whereas short-term survival was similar between horses with GLE (88%) and EFE (85%) at another hospital.[1049] Short-term survival in horses with EFE was higher (95%) than for other causes of small intestinal strangulation in another report.[1050]

Epiploic Foramen Entrapment

The epiploic foramen is a potential opening (because the walls of the foramen are usually in contact) to the omental bursa located within the right cranial quadrant of the abdomen. It is bounded dorsally by the caudate process of the liver and caudal vena cava and ventrally by the pancreas, the hepatoduodenal ligament, and the portal vein.[1039] Intestine may enter the foramen from the visceral surface of the liver toward the right body wall or the opposite direction. Studies differ as to which is the most common form. In the case of entrapments that enter the foramen in a left-to-right direction, the omental bursa is ruptured as the intestine migrates through the epiploic foramen, which may contribute to the intraabdominal hemorrhage often seen with this condition. Clinical signs include acute onset of severe colic with examination findings compatible with small intestinal obstruction. The stereotypic behavior of crib biting is a significant risk factor for EFE,[1051] possibly because of changes in abdominal pressure as the horse prepares the esophagus to ingest air. Other risk factors include increased height of the horse and previous colic surgery.[1051] The condition was once believed to be more prevalent in older horses,[1039] but this has been refuted.[1051] The disorder has also been recognized in foals as young as 4 months of age.[1052] The diagnosis is definitively made at surgery, although ultrasonographic findings of distended loops of edematous small intestine adjacent to the right middle body wall are suggestive of EFE.[1039] Generally, thickened, immotile intestine on ultrasonographic examination is highly predictive for small intestinal strangulating obstruction.[1053,1054] Small intestine entrapped in the epiploic foramen may be limited to a portion of the intestinal wall (parietal hernia),[1055] and the large colon may become entrapped within the epiploic foramen.[1051] In treating EFE, the epiploic foramen must not be enlarged either by blunt force or with a sharp instrument, because rupture of the vena cava or portal vein and fatal hemorrhage may occur. Prognosis has substantially improved over the past decade, with current short-term survival rates (discharge from the hospital) ranging from 74% to 95%.[1039,1049-1051,1056] Survival to 1 year (50.6%) and 2 years (34.3%) postoperatively declines substantially, with a median survival time of 397 days in one report.[1051] Preoperative abdominocentesis,[1039,1056] packed cell volume, length of small intestine resected, and POI[1051] have been associated with postoperative survival.

Strangulation by Pedunculated Mesenteric Lipoma

As horses age, lipomas form between the leaves of the mesentery and develop mesenteric stalks as the weight of the lipoma tugs on the mesentery. The stalk of the lipoma may subsequently wrap around a loop of small intestine or small colon, causing strangulation. Strangulating lipomas should be suspected in aged (>15 years) geldings with acute colic referable to the small intestinal tract.[1057-1059] Ponies also appear to be at risk for developing disease,[1059] suggesting alterations in fat metabolism may predispose certain horses to the development of mesenteric lipomas. The diagnosis is usually made at surgery, although on rare occasions a lipoma can be palpated rectally. Treatment involves surgical resection of the lipoma and strangulated bowel, although strangulated intestine is not always nonviable.[1059] Studies indicate that approximately 50%[1058] to 78%[1059] of horses are discharged from the hospital after surgical treatment.

Small Intestinal Volvulus

A volvulus is a twist along the axis of the mesentery, whereas torsion is a twist along the longitudinal axis of the intestine. Small intestinal volvulus is theoretically initiated by a change in local peristalsis or the occurrence of a lesion around which the intestine and its mesentery may twist (e.g., an ascarid impaction).[1060] It is reportedly one of the most commonly diagnosed causes of small intestinal obstruction in foals.[1061,1062] It has been theorized that young foals may be at risk for small intestinal volvulus because of changing feed habits and adaptation to a bulkier adult diet. Onset of acute, severe colic; a distended abdomen; and radiographic evidence of multiple loops of distended small intestine in a young foal would be suggestive of small intestine volvulus. However, it is not possible to differentiate volvulus from other causes of small intestinal obstruction preoperatively. In adult horses volvulus frequently occurs in association with another disease process, during which small intestinal obstruction results in distention and subsequent rotation of the small intestinal around the root of the mesentery. Although any segment of the small intestine may be involved, the distal jejunum and ileum are most frequently affected because of their relatively longer mesenteries.[1060] The diagnosis is made at surgery by palpating a twist at the origin of the cranial mesenteric artery. Treatment includes resection of devitalized bowel, which may not be an option because of the extent of small intestinal involvement (similar to large colon volvulus). Prognosis is based on the extent of small intestine involved and its appearance after surgical correction of the lesion. Generally, horses in which more than 50% of the small intestine is devitalized are considered to have a grave prognosis.[1063]

Strangulation by Way of Mesenteric or Ligamentous Rents

There are a number of structures that, when torn, may incarcerate a segment of intestine (typically the small intestine), including intestinal mesentery,[1045] the gastrosplenic ligament,[1048,1049,1064] the broad ligament,[1065] and the cecocolic ligament.[1066] Horses with such incarcerations present signs typical of a horse with strangulating small intestine. The prognosis for many of these cases appears to be worse than for horses with other types of small intestinal strangulations. Of horses with small intestine entrapped in a mesenteric rent, only 7 of 15 horses were discharged from the hospital, and only 2 of 5 horses for which follow-up information was available survived long term (>5 months).[1045] Poor outcome may result from the difficulty in releasing the incarcerated intestine, the degree of hemorrhage, and the length of intestine affected.

Inguinal Hernia

Inguinal hernias are more common in Standardbred and Tennessee Walking Horses that tend to have congenitally large inguinal canals.[1060] Inguinal hernias may also occur in neonatal foals but differ from hernias in mature horses in that they are typically nonstrangulating. The nature of the hernia (direct versus indirect) is determined on the basis of the integrity of the parietal vaginal tunic. In horses in which the bowel remains within the parietal vaginal tunic, the hernia is referred to as *indirect* because, strictly speaking, the bowel remains within the peritoneal cavity. Direct hernias are those in which strangulated bowel ruptures through the parietal vaginal tunic and occupies an SC location. These direct hernias most commonly occur in foals and should be suspected when a congenital inguinal hernia is associated with colic, swelling that extends from the inguinal region of the prepuce, and intestine that may be palpated SC.[1067,1068] Although most congenital indirect inguinal hernias resolve with repeated manual reduction or application of a diaper, surgical intervention is recommended for congenital direct hernias.[1068]

Historical findings in horses with strangulating inguinal hernias include acute onset of colic in a stallion that had recently been used for breeding. A cardinal sign of inguinal herniation is a cool, enlarged testicle on one side of the scrotum.[1069-1071] However, inguinal hernias, including of the large colon, have also been reported in geldings.[1072,1073] Inguinal hernias can be detected on rectal palpation, and manipulation of herniated bowel per rectum has been used to reduce a hernia, but this procedure is not recommended because of the risk of rectal tears. In many horses the short segment of herniated intestine will markedly improve in appearance once it has been reduced and does not always have to be resected.[1074] The affected testicle will be congested because of vascular compromise within the spermatic cord, and although it may remain viable, it is generally recommended that it be resected.[1074] The prognosis in adult horses is good, with up to 75% of horses surviving to 6 months of age.[1069-1071] Horses that have been treated for inguinal hernias may be used for breeding. In these horses the remaining testicle will have increased sperm production, although an increased number of sperm abnormalities will be noticed after surgery because of edema and increased temperature of the scrotum.

Strangulating Umbilical Hernias

Although umbilical hernias are common in foals, strangulation of herniated bowel is rare. In one study, 6 of 147 (4%) horses with umbilical hernias had incarcerated intestine.[1075] Clinical signs include a warm, swollen, firm, and painful hernia sac associated with signs of colic. The affected segment of bowel is usually small intestine, but herniation of cecum or large colon has also been reported.[1076] In rare cases a hernia that involves only part of the intestinal wall may be found; this is termed a *Richter's hernia.* In foals that have a Richter's hernia, an enterocutaneous fistula may develop.[1076] In one study, 13 of 13 foals with strangulating umbilical hernias survived to discharge, although at least 3 were lost to long-term complications.[1076]



FIG. **12.12** Jejunojejunal intussusception in a horse with colic. Note the intussusceptum, which has become ischemic as a result of invagination of intestine and its mesenteric blood supply into the intussuscipiens.

Intussusceptions

An intussusception involves a segment of bowel (intussusceptum) that invaginates into an adjacent aboral segment of bowel (intussuscipiens). The reason for such invagination is not always clear, but it may involve a lesion at the leading edge of the intussusception, including small masses, foreign bodies, or parasites. In particular, tapeworms *(Anoplocephala perfoliata)* have been implicated.[1077] Ileocecal intussusceptions are the most common intestinal intussusceptions in the horse and typically affect young animals. In one study evaluating 26 cases of ileocecal intussusception, the median age of affected horses was 1 year.[1078] Acute ileocecal intussusceptions are those in which the horses have a duration of colic of less than 24 hours and involve variable lengths of intestine, which ranged in one study from 6 cm to 457 cm in length. In acute cases the involved segment of ileum typically has a compromised blood supply. Chronic ileocecal intussusceptions typically involve short segments of ileum (up to 10 cm in length), and the ileal blood supply is frequently intact.[1078] Abdominocentesis results are variable because strangulated bowel is contained within the adjacent bowel. There is often evidence of obstruction of the small intestine, including nasogastric reflux and multiple distended loops of small intestine on rectal palpation. Horses with chronic ileocecal intussusceptions have mild, intermittent colic, often without evidence of small intestinal obstruction. In one study a mass was palpated in the region of the cecal base in approximately 50% of affected horses.[1078] Transabdominal ultrasound may be helpful in discerning the nature of the mass. The intussusception has a characteristic target appearance on cross section[1079-1081] and has been reported as a frequent incidental finding in Standardbred foals.[1082] Other segments of the small intestine may also be intussuscepted, including the jejunum (Fig. 12.12). In one study of 11 jejunojejunal intussusceptions, the length of bowel involved ranged between 0.4 and 9.1 m.[1083] Attempts at reducing intussusceptions at surgery are usually futile because of intramural swelling of affected bowel. Jejunojejunal intussusceptions should be resected.

For acute ileocecal intussusceptions the small intestine should be transected as far distally as possible, and a jejunocecal anastomosis should be performed. In cases with particularly long intussusceptions (up to 10 m has been reported), an intracecal resection may be attempted.[1084] For horses with chronic ileocecal intussusceptions, a jejunocecal bypass without small intestinal transection should be performed. The prognosis is good for horses with chronic ileocecal intussusceptions and guarded to poor for horses with acute ileocecal intussusceptions, depending on the length of bowel involved.[1078]

Diaphragmatic Hernias

Herniation of intestine through a rent in the diaphragm is rare in the horse. Any segment of bowel may be involved, although small intestine is most frequently herniated.[1085] Diaphragmatic rents may be congenital or acquired, but acquired hernias are more common.[1085] Congenital rents may result from incomplete fusion of any of the four embryonic components of the diaphragm: pleuroperitoneal membranes, transverse septum, body wall, and esophageal mesentery.[1085] Abdominal compression of the foal at parturition may result in a congenital hernia.[1085] Acquired hernias are presumed to result from trauma to the chest or a sudden increase in intraabdominal pressure, such as might occur during parturition, distention of the abdomen, a sudden fall, and strenuous exercise.[1086] Hernias have been described in a number of different locations; large congenital hernias are typically present at the ventralmost aspect of the diaphragm, and most acquired hernias are located at the junction of the muscular and tendinous portions of the diaphragm.[1085] A peritoneopericardial hernia has been documented in at least one horse.[1087]

The clinical signs are usually associated with intestinal obstruction rather than respiratory distress.[1086] Careful auscultation may reveal an area of decreased lung sounds associated with obstructed intestine and increased fluid within the chest cavity.[1088] Such signs may prompt thoracic radiography or ultrasound, both of which can be used to make a diagnosis. Auscultation may also reveal thoracic intestinal sounds, but it is typically not possible to differentiate these from sounds referred from the abdomen. In one report, two of three horses diagnosed with small intestinal strangulation by diaphragmatic hernia had respiratory acidemia, attributable to decreased ventilation.[1089] Treatment of horses with diaphragmatic hernia is fraught with complications because of the need to reduce and resect strangulated bowel and the need to repair the defect in the diaphragm; prognosis has not appeared to improve over time.[1089-1092] Because dorsal defects in the diaphragm are among the most common forms of diaphragmatic defect, it may not be possible to close the diaphragmatic hernia by way of the approach used for abdominal exploratory. Because herniation is likely to recur, it is appropriate to schedule a second surgery using an appropriate approach to resolve the diaphragmatic defect.

LARGE COLON VOLVULUS

Clinical Signs

Horses with large colon volvulus have rapid onset of severe, unrelenting abdominal pain. Postpartum broodmares appear to be at risk for this form of colic.[1035] Once the large colon is strangulated (>270-degree volvulus), gas distention is marked, leading to gross distention of the abdomen, compromised respiration as the distended bowel presses up against the diaphragm, and visceral pooling of blood as the caudal

vena cava is compressed. Horses with this condition are frequently refractory to even the most potent of analgesics. These horses may prefer to lie in dorsal recumbency, presumably to take weight off the strangulated colon. An abbreviated physical examination is warranted in these cases because the time from the onset of strangulation to surgical correction is critical. Under experimental conditions the colon is irreversibly damaged within 3 to 4 hours of a 360-degree volvulus of the entire colon.[855] Despite severe pain and hypovolemia, horses may have a paradoxically low heart rate, possibly related to increased vagal tone. Results of abdominocentesis often do not indicate the degree of colonic compromise,[1035,1093] and in many cases it is not worth attempting to obtain abdominal fluid because of extreme colonic distention.[1093] Rectal palpation reveals severe gas distention of the large colon, frequently associated with colonic bands traversing the abdomen. Severe colonic distention may restrict access to the abdomen beyond the pelvic brim. One study has shown that plasma lactate levels below 6.0 mmol/L had a sensitivity of 84% and a specificity of 83% in predicting survival in horses with large colon volvulus.[1094]

Surgical Findings

At surgery the volvulus is typically located at the mesenteric attachment of the colon to the dorsal body wall, and the most common direction of the twist is dorsomedial when the right ventral colon is used as a reference point.[1035] However, the colon may twist in the opposite direction, twist greater than 360 degrees (up to 720 degrees has been reported), or twist at the level of the diaphragmatic and sternal flexures.[1035] In all cases the colon should be decompressed as much as possible, and in many cases a colonic evacuation by way of a pelvic flexure enterotomy will greatly aid correction of the volvulus. After correction of the volvulus, a determination must be made as to whether the colon has been irreversibly injured. This should be based on mucosal color and bleeding (if an enterotomy has been performed), palpation of a pulse in the colonic arteries, serosal color, and appearance of colonic motility.[1036] If the colon is judged to be irreversibly damaged, the feasibility of a large colon resection can be considered. Although 95% of the colon can be resected (that part of the colon distal to the level of the cecocolic fold), damage from the volvulus may exceed that which can be resected. In these cases surgeons may elect to resect as much damaged bowel as possible or advise euthanasia.[1036]

Prognosis

Although early reports of short-term survival were low (35%),[1093] survival can vary with degree of volvulus. Short-term survival was reported as 36% for horses with 360-degree volvulus compared with 71% for horses with 270-degree volvulus.[1035] More recent studies report higher short-term survival (88%), with duration of disease as a major factor associated with survival.[1095] Impression of improved prognosis was echoed in a recent survey of the American Colleges of Veterinary Surgery Diplomates, who also note time to surgery as the most important factor associated with survival.[1096] Another study reports much higher short-term survival (74%) with large colon resection, along with positive survival rates at 1 year (67.8%), 2 years (66%), and 3 years (63.5%) postoperatively.[1097] Postoperative complications include hypovolemic and endotoxemic shock, extensive loss of circulating protein, DIC, diarrhea, and laminitis. Large colon volvulus may have a propensity to recur. Although one study documented a recurrence rate of less than 5%,[1093] some authors believe recurrence may be as high as 50%.[1036] Methods to prevent recurrence may be considered in patients at risk for recurrence, particularly broodmares that tend to suffer from the disease recurrently during the foaling season.[1098]

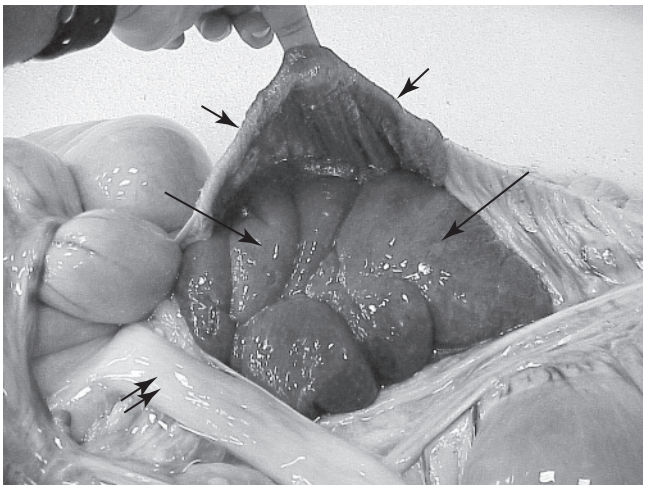


FIG. **12.13** Cecocolic intussusception in a horse with colic. An enterotomy has been made in the right ventral colon *(short arrows)* to reveal an intussuscepted cecum *(arrows)*. Although this picture was taken at necropsy, an enterotomy such as the one shown in this figure can be used to exteriorize and resect the majority of the compromised cecum. Note the ileum adjacent to the colon *(double arrow)*.

Intussusceptions

The most common intussusceptions of the large intestine are cecocecal and cecocolic.[1099] Both are likely attributable to the same disease process, with variable inversion of the cecum. These conditions tend to occur in young horses (63% were younger than 3 years old in one study) and may be associated with intestinal tapeworms.[1099] Clinical signs are variable, including acute, severe colic, intermittent pain over a number of days, and chronic weight loss.[1099] These variable presentations likely relate to the degree to which the cecum has intussuscepted. Initially, the cecal tip inverts, creating a cecocecal intussusception, which does not obstruct the flow of ingesta. As the intussusception progresses, the cecum inverts into the right ventral colon (cecocolic intussusception), which obstructs the flow of ingesta and often causes severe colic (Fig. 12.13). The cause of abdominal pain is often difficult to differentiate in these cases, although it is sometimes possible to detect a mass on the right side of the abdomen by either rectal palpation or ultrasound examination.[1099] Treatment involves manual surgical reduction by retracting the intussusceptum directly or by way of an enterotomy in the right ventral colon.[1099,1100] Sometimes the cecum cannot be readily reduced because of severe thickening, and in other cases surgical procedures result in fatal contamination. In one report 8 of 11 horses were euthanized in the perioperative period because of complications,[1101] and in another report 12 of 30 horses were euthanized either before or during surgery. The latter included all of the horses with chronic disease because of irreversible

TABLE **12.7 Classification of Rectal Prolapse**

Grade	Description	Prognosis
I	Prolapse of rectal mucosa	Good
II	Prolapse of full-thickness rectum	Fair
III	Grade 2 prolapse with additional protrusion of small colon	Guarded
IV	Intussusception of rectum and small colon through the anus	Poor

changes to the cecum.[1099] One report on cecocolic intussusceptions indicated that 7 of 8 horses that underwent right ventral colon enterotomy and cecal resection survived long term,[1100] suggesting that continued improvements in surgical techniques may improve the prognosis.

Colocolic intussusceptions are exceptionally rare but have reportedly affected the pelvic flexure and the left colon.[1102-1104] Although the condition is reportedly more common in young horses,[1102,1104] older horses may be affected.[1103] Clinical findings may include a palpable mass on the left side of the abdomen.[1102] Ultrasonography may also be useful. Treatment requires manual reduction of the intussusception at surgery or resection of affected bowel. Because the left colons can be extensively exteriorized and manipulated at surgery, the prognosis is fair.[1102-1104]

RECTAL PROLAPSE

Rectal prolapse may occur secondary to any disease that causes tenesmus, including diarrhea, rectal neoplasia, and parasitism,[1105] or it can occur secondary to elevations in intraabdominal pressure during parturition or episodes of coughing.[1106] Rectal prolapse is classified into four categories (Table 12.7) depending on the extent of prolapsed tissue and the level of severity.[1107] Type I rectal prolapse is most common and is characterized by a doughnut-shaped prolapse of rectal mucosa and submucosa (Fig. 12.14). Type II prolapse involves full-thickness rectal tissue, and type III prolapse additionally has invagination of small colon into the rectum. Type IV prolapse involves intussusception of proximal rectum or small colon through the anus in the absence of prolapse of tissue at the mucocutaneous junction at the anus.[1107] These can be differentiated from other forms of prolapse by their appearance and a palpable trench between prolapsed tissue and the anus.

Type I prolapses are most frequently seen in horses with diarrhea, and the rectal mucosa becomes irritated and protrudes intermittently during episodes of tenesmus. If tenesmus persists, rectal mucosa can remain prolapsed. Rectal mucosa rapidly becomes congested and edematous under these conditions and should be treated with osmotic agents such as glycerin or magnesium sulfate and by massaging and reducing the prolapse.[1108] A purse-string suture may be necessary to keep the mucosa inside the rectum. Topical application of lidocaine solution or jelly, epidural anesthesia, and sedation may help reduce tenesmus that incites and exacerbates rectal prolapse. Similar treatments can be applied with type II rectal prolapses. However, these more severe prolapses may not be reducible without surgical resection of mucosa and submucosa from the prolapsed bowel.[1105,1106]



FIG. **12.14** Type I rectal prolapse in a horse. Note circumferential protrusion of partial-thickness rectal tissue *(arrows)* that is becoming congested as a result of pressure from the surrounding anus.

Types III and IV rectal prolapses are more serious injuries because the small colon is involved.[1109] In horses with type III prolapse, an abdominocentesis should be performed to determine whether the injury to the small colon has resulted in peritonitis. The small colon component should be reduced manually if possible, whereas prolapsed rectal tissue typically requires mucosal or submucosal resection. Surgical exploration of the abdomen should be performed to determine the status of the small colon, although serial abdominocenteses can be used in lieu of surgery to detect progressive necrosis of the bowel. Type IV prolapses are seen most commonly in horses with dystocia.[1106] This type of prolapse is almost always fatal because of stretching and tearing of mesenteric vasculature, with subsequent infarction of affected bowel. Euthanasia is often warranted on the basis of physical examination findings. Confirmation of severe small colon injury requires abdominal exploration using either a midline approach or laparoscopy.[1110] It is conceivable that a horse with a compromised small colon could undergo a colostomy of the proximal small colon, but the compromised small colon will typically necrose beyond that which can be resected using a midline abdominal approach.[1108]

NONSTRANGULATING INFARCTION

Nonstrangulating infarction occurs secondary to cranial mesenteric arteritis caused by migration of *S. vulgaris*[1111] and has become a relatively rare disorder since the advent of broad-spectrum anthelmintics. Although thromboemboli have been implicated in the pathogenesis of this disease, careful dissection of naturally occurring lesions has not revealed the presence of thrombi at the site of intestinal infarctions in most cases.[1111] These findings suggest that vasospasm plays an important role in this disease.[1060] Clinical signs are highly variable, depending on the extent to which arterial flow is reduced and the segment of intestine affected. Any segment of intestine supplied by the cranial mesenteric artery or one of its major branches may be affected, but the distal small intestine and large colon are more commonly involved.[1111] There are no clinical variables that can be used to reliably differentiate this disease from strangulating obstruction.[1099] In some cases massive infarction results

in acute, severe colic.[1111] Occasionally, an abnormal mass and fremitus may be detected on rectal palpation of the root of the cranial mesenteric artery. This disease should be considered a differential diagnosis in horses with a history of inadequate anthelmintic treatment and the presence of intermittent colic that is difficult to localize. Although fecal parasite egg counts should be performed, they are not indicative of the degree of parasitic infestation.

In addition to routine treatment of colic, dehydration, and endotoxemia, medical treatment may include aspirin (20 mg/kg/day) to decrease thrombosis.[11] Definitive diagnosis requires surgical exploration. Affected horses can be difficult to treat because of the patchy distribution of the lesions and the possibility of lesions extending beyond the limits of surgical resection. Further infarction may occur after surgery. The prognosis is fair for horses with intermittent mild episodes of colic that may be amenable to medical therapy but poor in horses that require surgical intervention.[1111]

Obstructive Disorders of the Gastrointestinal Tract

Examination and approach to the horse with colic are discussed in Chapter 7; pain management is discussed in Chapter 3. These are critical factors in the diagnosis and treatment of a horse with obstructive disease.

SMALL INTESTINAL SIMPLE OBSTRUCTION

Simple obstruction involves intestinal obstruction of the lumen without obstruction of vascular flow. Because there is a tremendous volume of fluid that enters the small intestinal lumen on a daily basis, the obstructed intestine tends to become distended, which in turn may cause reduced mural blood flow.[879] Ultimately, such distention can result in necrosis of tissues, particularly in the immediate vicinity of the obstruction.[1112] There are relatively few causes of simple obstruction in the small intestine, and the incidence of these types of obstructions is low (approximately 3% of all referred horses in one large hospital-based study).[1041] In some geographic regions this type of obstruction has a higher prevalence. For example, in the southeastern United States, ileal impactions are relatively common.[1113-115]

Ascarid Impaction

Impactions caused by *Parascaris equorum* typically occur in foals younger than 6 months of age that have been on a poor deworming program and have a heavy parasite burden. Products that cause sudden ascarid death, including organophosphates, ivermectin, and pyrantel pamoate, have been incriminated in triggering acute intestinal obstruction by dead parasites.[1116] This is a particular problem with ascarids because of the relatively large size of the adult parasite. Clinical signs include acute onset of colic after administration of an anthelmintic and signs compatible with small intestinal obstruction, including nasogastric reflux. Occasionally, dead parasites are present in the reflux. The onset of the disease varies according to the degree of obstruction.[1116] A tentative diagnosis may be made on the basis of the history and signs referable to small intestinal obstruction. Abdominal ultrasound may indicate the presence of multiple loops of distended small intestine and can be used to estimate ascarid burden within the small intestine.[1117] Initial medical treatment should include pain management and supportive care. Surgical treatment may involve an enterotomy removal of ascarids, although manual reduction without enterotomy was associated with improved survival in one report.[1118] The prognosis is fair in cases that are rapidly addressed but poor in foals with evidence of hypovolemia and septic shock. In one study, long-term survival of 25 affected horses was 33%.[1116] Another reported 80% short-term survival and 60% survival to 1 year.[1118]

Ileal Impaction

Ileal impactions occur most commonly in adult horses in the southeastern United States. Although feeding of coastal Bermuda hay has been implicated in this regional distribution,[1115] it has been difficult to separate geographic location from regional hay sources as risk factors.[1119] Nonetheless, it is likely that feeding suboptimal quality coastal Bermuda hay puts horses at risk for ileal impaction, possibly because this type of hay may have a high fiber content and thin strands that can lead to premature swallowing. The relationship between fiber content and eating patterns is theoretical and remains to be proved. Sudden changes in feed from an alternative type of hay to coastal Bermuda hay likely put a horse at risk for ileal impaction.[1119] Studies in the United Kingdom have revealed tapeworm infection as an important risk factor for ileal impaction. Based on risk analysis, the data suggested that more than 80% of the ileal impaction cases studied were associated with serologic or fecal evidence of tapeworm infection.[1120] Because of the poor sensitivity of fecal analysis for tapeworms, a serologic test (ELISA) has been developed by Proudman et al. with a sensitivity of approximately 70% and a specificity of 95%.[1121,1122]

Clinical signs of horses with ileal impaction are typical for a horse with small intestinal obstruction, including onset of moderate to severe colic and rectally palpable loops of distended small intestine as the condition progresses. Because the ileum is the distal-most aspect of the small intestinal tract, nasogastric reflux may take a considerable time to develop and is found in only approximately 50% to 60% of horses requiring surgical correction of impacted ileum[1113,1123]; reflux is more likely in horses with ileal impaction taken to surgery.[1113] A definitive diagnosis is usually made at surgery, although an impacted ileum may on occasion be palpated rectally.[1114] Multiple loops of distended small intestine make the impaction difficult to palpate. More than 50% of ileal impactions may resolve with medical treatment.[1113] Most reports indicate a good to excellent prognosis for short-term survival[1113, 1115, 1123] with 1-year survival of 91% to 92% in horses treated surgically or medically.[1113]

Ileal Hypertrophy

Ileal hypertrophy is a disorder in which the muscular layers (both circular and longitudinal) of the ileum thicken for unknown reasons (idiopathic) or secondary to an incomplete or functional obstruction. A proposed mechanism for idiopathic ileal hypertrophy is parasympathetic neural dysfunction resulting in chronically increased muscle tone and subsequent

hypertrophy of the muscular layers of the ileal wall. Such neural dysfunction possibly results from parasite migration.[1124] Alternative hypotheses include chronic increases in the muscular tone of the ileocecal valve, leading to muscular hypertrophy of the ileum as it contracts against a partially occluded ileocecal valve. The jejunum may also be hypertrophied, either alone or in combination with the ileum.[1124] Clinical signs include chronic intermittent colic as the ileum hypertrophies and gradually occludes the lumen. Partial anorexia and chronic weight loss (1–6 months) were documented in 45% of affected horses, most likely because of intermittent colic and reduced appetite.[1124] Because the ileal mucosa is not affected by this condition, there is no reason to believe that these horses experience malabsorption of nutrients. The diagnosis is usually made at surgery, although the hypertrophied ileum may be palpated rectally in some cases.[1124] An ileocecal or jejunocecal anastomosis to bypass the hypertrophied ileum is usually performed in affected horses. Without surgical bypass intermittent colic persists, and the thickened ileum may ultimately rupture.[1124] The prognosis is fair with surgical treatment.[1125]

Secondary ileal hypertrophy is most commonly noted in horses that have previously had colic surgery and that may have a partial or functional obstruction at an anastomotic site. For example, in one case report a horse developed ileal hypertrophy after surgical correction of an ileocecal intussusception.[1126] Ileal hypertrophy was also observed in a horse in which an ileocolic anastomosis was incorrectly oriented during surgical treatment of a cecal impaction.[1000] Horses are typically reexamined for recurrence of colic in these cases. Surgical therapy is directed at addressing the cause of small intestinal obstruction and resecting hypertrophied intestine.

Meckel's Diverticulum

Meckel's diverticulum is an embryonic remnant of the vitelloumbilical duct, which fails to completely atrophy and becomes a blind pouch projecting from the antimesenteric border of the ileum.[1127,1128] Similar diverticula have also been noted in the jejunum.[1129] These diverticula may become impacted, resulting in partial luminal obstruction, or may wrap around an adjacent segment of intestine, causing strangulation.[1128] Occasionally, an associated mesodiverticular band may course from the diverticulum to the umbilical remnant and serve as a point around which small intestine may become strangulated. Mesodiverticular bands may also originate from the embryonic ventral mesentery and attach to the antimesenteric surface of the bowel, forming a potential space within which intestine may become entrapped.[1038] Clinical signs range from chronic colic, for an impacted Meckel's diverticulum, to acute, severe colic if a mesodiverticular band strangulates intestine. The diagnosis is made at surgery, and treatment requires resection of the diverticulum and any associated bands.[1038] The prognosis is good for horses with simple impaction of a Meckel's diverticulum and guarded for horses with an associated small intestinal strangulation.[1038]

Adhesions

Adhesions of one segment of bowel to another or of a segment of intestine to other organs and the body wall typically occur after abdominal surgery and may be clinically silent, cause chronic colic attributable to partial obstruction, or result in acute obstruction. These differing clinical syndromes are attributable to the type of adhesions that develop. For example, a fibrous adhesion that does not by itself obstruct the intestinal lumen might serve as the pivot point for a volvulus, whereas an adhesion between adjacent segments of the intestinal tract may create a hairpin turn that causes chronic partial obstruction.[1130] The number of adhesions that develop may also vary dramatically from horse to horse. Some horses may develop a single adhesion adjacent to an anastomotic site or a discrete segment of injured intestine, whereas other horses may develop diffuse adhesions involving multiple segments of intestine, likely because of widespread inflammatory disease at the time of the original surgery.

The mechanisms by which adhesions develop are complex but likely involve injury to the serosa initiated by intestinal ischemia, reperfusion injury, and luminal distention.[1037] Such injury involves infiltration of neutrophils into the serosa, accompanied by loss of mesothelial cells. In one study assessing the margins of resected small intestine, extensive neutrophil infiltration was documented in the serosa, particularly in the proximal resection margin that had been distended before correction of a variety of strangulating lesions.[868] Regions of serosal injury and inflammation subsequently undergo reparative events similar to those of any wound, including local production of fibrin, de novo synthesis of collagen by infiltrating fibroblasts, and ultimately maturation and remodeling of fibrous tissue. Unfortunately, during this process fibrin may result in injured intestinal surfaces adhering to adjacent injured bowel or an adjacent organ. Once a fibrinous adhesion has developed, new collagen synthesis may result in a permanent fibrous adhesion. Alternatively, fibrinous exudate may be lysed by proteases released by local phagocytes, reversing the adhesive process. Formation of adhesions may be viewed as an imbalance of fibrin deposition and fibrinolysis.[1131]

Prevention of adhesions relies on inhibition of the mechanisms involved in adhesion formation, including reduction of serosal injury with early intervention and good surgical technique, reduction of inflammation by administration of antiinflammatory medications, physical separation of inflamed serosal surfaces (e.g., carboxymethylcellulose, hyaluronan),[1132-1134] and pharmacologic modulation of fibrinous adhesion formation (e.g., heparin[1135]). Early return of motility in the small intestine after surgery may reduce contact time between inflamed surfaces of intestine, reducing the chances of adhesion formation.[1131]

Horses at greatest risk of developing adhesions after colic surgery appear to be those that have small intestinal disease.[1130] In one study of horses undergoing surgical correction of small intestinal obstruction, 22% developed a surgical lesion associated with adhesions. Foals appear to have an increased incidence of adhesions compared with mature horses, regardless of the nature of the abdominal surgery.[1130] One study indicated that 17% of foals developed lesions attributable to adhesions regardless of the type of the initial surgery.[1136] Studies conflict as to whether the degree of surgical intervention influences adhesion formation,[1130] but horses that require enterotomy or resection and anastomosis were at greatest risk of developing adhesions in one study.[1137] Adhesions are among the most important reasons for repeat laparotomy in postoperative colic patients.[1137,1138]

Clinical signs in horses with adhesions are highly variable, depending on whether the adhesion is causing partial obstruction, complete luminal obstruction, or involvement of intestinal vasculature. Adhesions are an important differential for intermittent colic in the postoperative period, particularly if such colic was not relieved by nasogastric decompression

of the stomach. Continued intermittent colic should prompt abdominocentesis to determine whether there is evidence of septic peritonitis, which may contribute to adhesion formation. If postoperative colic persists, repeat laparotomy or laparoscopy may be elected. In one study on adhesions, 70% of repeat laparotomies were performed within 60 days, suggesting that surgical colic attributable to adhesions typically occurs within 2 months of an initial surgical procedure. Unfortunately, the prognosis for horses with colic attributable to adhesions is poor, with only 16% of horses surviving adhesion-induced colic in one study.[1130]

LARGE INTESTINAL SIMPLE OBSTRUCTION

Simple obstructions of the large intestine, such as impaction, tend to have a more gradual onset than those of the small intestine, although horses may experience acute and severe pain with some forms of colon displacement. In fact, some of these cases mimic and may progress toward large colon volvulus. Medical therapy is frequently successful in correcting large colon impactions. However, cecal impactions present much more of a dilemma because of the greater propensity of this organ to rupture, the relative difficulty of surgically manipulating the cecum, and the onset of cecal dysfunction that may prevent the cecum from emptying after surgical resolution of impaction.

Cecal Impaction

Cecal impaction may be divided into two syndromes: primary cecal impactions that result from excessive accumulation of ingesta in the cecum and secondary cecal impactions that develop while a horse is being treated for a separate problem.[936,1139] Although primary impactions typically consist of impacted, relatively dry fecal material and secondary cecal impactions tend to have very fluid contents, there is considerable overlap between the two syndromes. In horses with primary cecal impactions, there is a gradual onset of abdominal pain over a number of days reminiscent of the development of a large colon impaction. Cecal impactions should be differentiated from large colon impactions on the basis of rectal palpation findings. These impactions have a propensity to rupture before the development of severe abdominal pain or systemic deterioration and therefore must be closely monitored.[1139] Secondary cecal impactions typically develop after unrelated surgical procedures that result in postoperative pain (particularly orthopedic surgeries).[1140] Secondary cecal impactions may be even more difficult to detect because postoperative depression and decreased fecal output may be attributed to the operative procedure rather than colic. By the time horses with secondary cecal impactions show noticeable signs of colic, the cecum may be close to rupture. In many cases there will be no signs of impending rupture.[1140] Therefore the feed intake and manure production of all horses that undergo surgery in which considerable postoperative pain may develop should be closely monitored. A recent study indicated that horses producing less than three piles of manure daily in the postoperative period are at risk of developing a large intestinal impaction. Horses that underwent prolonged (>1 hour) orthopedic surgery that received inadequate treatment with phenylbutazone were at considerable risk of reduced postoperative fecal output.[1141]

A diagnosis of cecal impaction is based on rectal palpation of a firm, impacted or fluid-filled cecum. In some cases, cecal impactions may be difficult to differentiate from large colon impactions. Careful palpation, however, will reveal the inability to move the hand completely dorsal to the impacted viscus because of the cecum's attachment to the dorsal body wall.

Treatment may include initial medical therapy with IV or oral fluids and analgesics, or surgical intervention with typhlotomy alone or jejunocecostomy. In two recent larger scale retrospective studies, the prognosis appeared to vary significantly by geographic region.[1139,939] In one, a very favorable short-term outcome was achieved with either medical (81%) or surgical (95%) management.[939] In the majority of surgical cases, typhlotomy alone was performed. In another, short-term outcome was less favorable with either medical (61%) or surgical (82%) management.[1139] Many (68%) cases in the latter report appeared to have secondary cecal impactions as they had recent illness or surgery unrelated to gastrointestinal disease. This report underscores the importance of prompt diagnosis and supports the benefit of early surgical intervention.

Large Colon Impaction

Ingesta impactions of the large colon occur at sites of anatomic reductions in luminal diameter, particularly the pelvic flexure and the right dorsal colon.[1142] Although there are a number of reported risk factors, most have not been proved. A sudden restriction in exercise associated with musculoskeletal injury appears to be frequently associated with onset of impaction.[1143] Twice-daily feeding of concentrate results in large fluxes of fluid into and out of the colon, associated with readily fermentable carbohydrate in the colon and subsequent increases in serum aldosterone, respectively.[1144] These fluid fluxes, which may cause dehydration of ingesta during aldosterone-stimulated net fluid flux out of the colon, may be prevented with frequent small feedings.

Impaction of the ascending colon can be induced by the drug amitraz, an acaricide associated with clinical cases of colon impaction,[1145,1146] providing some clues as to the pathogenesis of large colon impaction. Amitraz appears to alter pelvic flexure pacemaker activity, resulting in uncoordinated motility patterns between the left ventral and left dorsal colon and excessive retention of ingesta. Absorption of water from the ingesta increases with retention time, dehydrating the contents of the colon and resulting in impaction. It is conceivable that parasite migration in the region of a pacemaker may have a similar action.[653] Other factors implicated in large colon impaction include limited exercise, poor dentition, coarse roughage, and dehydration.

Clinical signs of large colon impaction include slow onset of mild colic. Fecal production is reduced, and the feces are often hard, dry, and covered with mucus because of delayed transit time. The heart rate may be mildly elevated during episodes of pain but is often normal. Signs of abdominal pain are typically well controlled with administration of analgesics but become increasingly more severe and refractory if the impaction does not resolve. The diagnosis is based on rectal palpation of a firm mass in the large colon. The extent of the impaction may be underestimated by rectal palpation alone because much of the colon remains out of reach.[1142] Adjacent colon may be distended if the impaction has resulted in complete obstruction. Initial medical treatment should include pain management and enteral fluid therapy if reflux is not obtained on nasogastric intubation. Hydration of colonic contents is superior with

enteral fluid therapy compared with IV fluid therapy.[1147,1148] Saline cathartics such as magnesium sulfate (0.1 mg/kg in 2–4 L by nasogastric tube) may also be useful. Access to feed should not be permitted. If the impaction remains unresolved, the horse's pain becomes uncontrollable, or extensive gas distention of the colon occurs, surgery is indicated. At surgery the contents of the colon are evacuated by way of a pelvic flexure enterotomy. Long-term survival is good with medical management (95%) and fair (58%) in horses that require surgical intervention.[1143]

Enteroliths

Enteroliths are mineralized masses typically composed of ammonium magnesium phosphate (struvite).[37] However, magnesium vivianite has also been identified in enteroliths, along with variable quantities of Na, S, K, and Ca. The formation of Mg-based minerals is puzzling because of the relative abundance of Ca in colonic fluids, which would favor the formation of Ca phosphates (apatite) rather than struvite. Elevated dietary intake of magnesium and protein may play a role. Feeding of alfalfa hay and decreased dietary proportions of grass hay and pasture grass have been consistently identified as risk factors in horses with enterolithiasis.[1149-1151] Alfalfa hay has a concentration of magnesium approximately six times the daily requirements of the horse.[1152] The high protein concentration in alfalfa hay may contribute to calculus formation by increasing the ammonia nitrogen load in the large intestine. Enteroliths most commonly form around a nucleus of silicon dioxide (a flintlike stone), but nidi have included nails, rope, and hair that have been ingested.[1153] Enteroliths are usually found in the right dorsal and transverse colons.[1152] Although enterolithiasis has a wide geographic distribution, horses in California have the highest incidence. In one California study, horses with enterolithiasis represented 28% of the surgical colic population, and Arabians, Morgans, American Saddlebreds, and donkeys were at greatest risk of this disease.[37] In a study of enterolithiasis in Texas, risk factors also included feeding of alfalfa hay and the Arabian breed. In that study, Miniature Horses were also found to be at risk.[1149] Horses with enteroliths are rarely younger than 4 years old, with a median age of 11 in one report[37]; an enterolith has been reported in an 11-month-old Miniature Horse.[1154]

The most common reported clinical signs are episodic mild to moderate abdominal pain.[37,1152] Enteroliths may be diagnosed by abdominal radiography or at surgery.[40,1155] On rare occasions an enterolith may be palpated rectally, particularly if it is present in the distal small colon.

Generally, surgery is required, although there are reports of enteroliths being retrieved rectally. In one study 14% of horses that required treatment of enterolithiasis had a history of passing an enterolith in the feces.[37] Enteroliths are typically located in the right dorsal colon, transverse colon, or small colon. After removal of an enterolith, further exploration must be conducted to determine whether other enteroliths are present. Solitary enteroliths are usually round, whereas multiple enteroliths have flat sides. The prognosis is good (92% 1-year survival in 900 horses), unless the colon is ruptured during removal of an enterolith, which was reported in 15% of cases.[37]

Sand Impaction of the Large Colon

Sand impactions are common in horses with access to sandy soils, particularly horses whose feed is placed on the ground. Some horses, especially foals, deliberately eat sand. Fine sand tends to accumulate in the ventral colon, whereas coarse sand may accumulate in the dorsal colon.[737,1156] Individual differences in colonic function may contribute to accumulation of sand because some horses can clear consumed sand, whereas others cannot. Distention resulting from the impaction itself, or gas proximal to the impaction, causes abdominal pain. Sand may also trigger diarrhea, presumably as a result of irritation of the colonic mucosa.[738] In horses with sand impactions, clinical signs are similar to those of horses with large colon impactions. Sand may be found in the feces, and auscultation of the ventral abdomen may reveal sounds of sand moving within the large colon.[1157] The diagnosis is made via radiography or surgery but may be tentatively based on clinical signs compatible with a large colon impaction and evidence of sand in the feces. Sand sedimentation is performed by mixing feces with water in a rectal palpation sleeve or other container. Abdominal radiographs can be used to detect mineral opacity within the ventral colon.[38,1158] Ultrasonography can also be used to support the diagnosis but is not as accurate as radiography.[1159] Abdominal paracentesis is not recommended in suspected cases, because large quantities of sand in the ventral colon make inadvertent perforation of the colon more likely.[737]

Medical management typically offers a good prognosis.[1160] Administration of psyllium hydrophilic mucilloid (0.25–0.5 kg/500 kg in 2 L mineral oil by stomach tube) may facilitate passage of sand. If mixed with water, it should be administered rapidly because of formation of a viscous gel. The psyllium leaves the oil phase and mixes with the water, forming a gel within the stomach. Psyllium is thought to act by stimulating motility or agglutinating the sand. However, one experimental study failed to show a benefit of this treatment.[1161] If a severe impaction is present, then psyllium should not be given until the impaction is softened by administering IV or oral fluids and other laxatives. Perforation is a potential complication in horses with sand impactions because the sand stretches and irritates the intestinal wall and causes inflammation. If colic becomes intractable, surgical evacuation of the large colon should be performed. The prognosis is generally regarded as good.[737,1156]

Large Colon Displacement

Displacement of the ascending colon is a common cause of large intestinal obstruction. The ascending colon is freely movable except for the right dorsal and ventral colons. Contact with adjacent viscera and the abdominal wall tends to inhibit movement of the ascending colon from a normal position; accumulation of gas and fluid or ingesta, however, may cause the colon to migrate.[1162] Feeding behavior, including feeding of large concentrate meals, likely plays a role in initiating displacement of the large colon. Large concentrate meals increase the rate of passage of ingesta, allowing a greater percentage of soluble carbohydrates to reach the large intestine.[1163] This in turn increases the rate of fermentation and the amount of gas and volatile fatty acids that are produced. The production of large amounts of volatile fatty acids stimulates the secretion of large volumes of fluid into the colon.[2] The association between feeding concentrate and development of displacements of the large colon is illustrated by studies indicating that ascending colon displacement is more prevalent in horses fed a high-concentrate, low-roughage diet.[1164] Abnormal motility patterns of the ascending colon may also contribute to the development of colonic displacement. Feeding stimulates colonic motility by way of the gastrocolic reflex, but large meals may alter normal

motility patterns and concurrently allow rapid accumulation of gas and fluid resulting from fermentation.[1163,1165] Migration of parasite larvae (strongyles) through the intestinal wall also alters colonic motility patterns.[650] *S. vulgaris* infection results in reduced blood flow to segments of the large intestine without necessarily causing infarction. Electrical activity of the colon and cecocolic junction increases after infection with *S. vulgaris* and cyathostome larvae, probably reflecting a direct effect of migration through the intestine and an early response to reduced blood flow.[650]

Displacements of the ascending colon are generally divided into three types: left dorsal displacement, right dorsal displacement, and retroflexion.[1162,1166] Left dorsal displacement is characterized by entrapment of the ascending colon in the renosplenic space. The colon is often twisted 180 degrees, such that the left ventral colon is situated in a dorsal position relative to the left dorsal colon. The entrapped portion may be only the pelvic flexure or may involve a large portion of the ascending colon, with the pelvic flexure situated near the diaphragm. The colon may become entrapped by migrating dorsally between the left abdominal wall and the spleen or may migrate in a caudodorsal direction over the nephrosplenic ligament.[1166] Occasionally, the ascending colon can be palpated between the spleen and abdominal wall, lending support to the first mechanism of displacement. Gastric distention is thought to predispose horses to left dorsal displacement of the ascending colon by displacing the spleen medially, allowing the colon room to migrate along the abdominal wall.[1166] Right dorsal displacement begins by movement of the colon cranially, either medial (medial flexion) or lateral (lateral flexion) to the cecum. According to one author, the proportion of right dorsal displacements with medial versus lateral flexion is approximately 1:15.[1166] In either case the pelvic flexure ends up adjacent to the diaphragm. Retroflexion of the ascending colon occurs by movement of the pelvic flexure cranially without movement of the sternal or diaphragmatic flexures.

Displacement of the ascending colon partially obstructs the lumen, resulting in accumulation of gas or ingesta and causing distention. The distention may be exacerbated by the secretion of fluid in response to the distention. Tension and stretch of the visceral wall are important sources of the pain associated with colonic displacement. Tension on mesenteric attachments and the root of the mesentery by the enlarged colon may also cause pain.[1162] Ischemia is rarely associated with nonstrangulating displacement of the colon. Congestion and edema, however, are often seen in the displaced segments of colon because of increased hydrostatic pressure from reduced venous outflow. Morphologic damage to tissues is usually minor.

Clinically, displacement of the ascending colon is often characterized by intermittent signs of mild to moderate abdominal pain of acute onset, but an insidious onset may also be noted.[1166] Dehydration may occur if the duration of the displacement is prolonged. The heart rate may be increased in conjunction with abdominal pain but is often normal. Abdominal distention may be present, and fecal production is reduced. Left dorsal displacements are often diagnosed by rectal palpation, because the ascending colon can be traced to the nephrosplenic space, and the spleen may be displaced medially. Alternatively, a tentative diagnosis can be reached using abdominal ultrasonography.[1167] The spleen can be imaged on the left side of the abdomen, but the left kidney will be obscured by gas-distended bowel. Evaluation of this technique indicates that there are very few instances of false-positive results, although false-negative results may occasionally occur.[1167] A definitive diagnosis may require surgery. Right dorsal displacements are characterized by the presence of the distended ventral colon running across the pelvic inlet and may be felt between the cecum and the body wall if a lateral flexion is present. The pelvic flexure is usually not palpable. Retroflexion of the ascending colon may produce a palpable kink in the colon. If the displaced colons are not distended by gas in the instance of right dorsal displacement and retroflexion, the ascending colon may not be palpable and is conspicuous by its absence from a normal position. Horses with right dorsal displacement often have an increased GGT, relative to horses with left dorsal displacement, which is presumptively caused by compression of the bile duct and temporary extrahepatic obstruction.[17]

For nephrosplenic entrapment of the large colon, medical management is often effective.[1167-1169] Medical therapy with phenylephrine and rolling under anesthesia (84%) was significantly more effective than phenylephrine (3–6 μg/kg/min over 15 minutes) and exercise (63.2%) in a recent report.[1169] There are reports of fatal internal hemorrhage caused by rupture of large blood vessels after treatment of older horses with phenylephrine, and the drug should probably be used with caution in horses older than 15 years.[1170]

Medical therapy has also been reported effective (64%) in horses with right dorsal displacement,[1171] although definitive diagnosis of that condition is challenging without surgical confirmation. If medical management is not successful, horses should undergo surgery promptly. Surgical prognosis for horses with large colon displacement is good. A number of horses will suffer recurrence of nephrosplenic entrapment of the colon. Currently, the least invasive method of preventing this complication is laparoscopic closure of the nephrosplenic space.[78,1172,1173]

Foreign Body and Fecalith Obstruction

Foreign material such as bedding, rope, plastic, fence material, and feedbags can cause obstruction and may be ingested, particularly by young horses. These foreign bodies may result in impaction with ingesta and distention of the intestine, typically in the transverse or descending colon. Young horses are usually affected. In one study the obstructing mass could be rectally palpated in three of six horses.[1174] Fecaliths are common in ponies, miniature horses, and foals.[1175] Older horses with poor dentition may also be predisposed to fecaliths because of the inability to fully masticate fibrous feed material. Fecaliths commonly cause obstruction in the descending colon and may cause tenesmus.[1174] Other clinical signs are similar to those of enterolithiasis. Abdominal radiography may be useful in smaller patients to identify the obstruction, especially if gas distention around the foreign body or fecalith provides contrast. Surgical treatment is usually required.

Mural Masses and Strictures

Mural masses such as abscesses, tumors (adenocarcinoma and lymphosarcoma), granulomas, and hematomas can cause luminal obstruction and impaction, typically in older horses. Impaction may result from obstruction of the lumen or impaired motility in the segment of intestine with the mass. Abscesses may originate from the lumen of the intestine or may extend from the mesentery or mesenteric lymph nodes. Intramural hematomas form most commonly in the descending colon and cause acute abdominal pain.[1176] Once the acute

pain caused by the hematoma subsides, impaction proximal to the hematoma develops as a result of impaired motility through the affected portion of the colon. Trauma, ulceration of the mucosa, and parasitic damage are speculated causes of intramural hematomas.[1176,1177] Stricture of the large intestine occurs when fibrous tissue forms in a circular pattern around or within the intestine, reducing the luminal diameter and the ability of the wall to stretch. Strictures may be congenital or secondary to peritonitis, previous abdominal surgery, or inflammatory bowel disease. In a report of 11 horses with inflammatory bowel disease, 6 horses had strictures, 4 of which were in the small intestine and 2 of which were in the large colon.[517]

Clinical signs vary according to the degree of luminal obstruction. Partial obstruction and impaction tend to produce mild to moderate abdominal pain of insidious onset. Mural hematomas tend to produce signs of acute abdominal pain.[1176,1177] Rectal palpation of the abdomen may reveal the presence of a mass or simply the impacted segment, without the mass itself being felt. Fever, weight loss, and anorexia may be noted if an abscess or tumor is the cause. An elevated WBC count; hyperfibrinogenemia; hyperglobulinemia; or normocytic, normochromic anemia may be seen with abscesses or tumors. PF may reflect the cause of the mass. Tumor cells may infrequently be seen. Evidence of inflammation with bacteria may be noted if the cause of colic is an abscess or granuloma, in which case the fluid should be cultured. Hematomas may cause hemorrhage into the PF. Treatment usually requires surgical resection of the mass. Abscesses may be treated with appropriate antibiotics if the impaction can be resolved medically with oral or IV analgesics and laxatives. *Streptococcus* spp., *Actinomyces pyogenes, C. pseudotuberculosis, R. equi,* anaerobic bacteria, and gram-negative enteric organisms are commonly involved in abscesses.

Small Colon Impaction

Small colon impaction is distinct from other forms of impaction in its predispositions and clinical appearance. In one study the key risk factor for impaction of this segment of the intestine was diarrhea.[1178] This paradoxic finding may be explained by edema of the colonic mucosa associated with proinflammatory causes of diarrheal disease that is usually noted in the ascending colon but may extend into the transverse and small colons. Once diarrheal disease is initiated, large volumes of ingesta are rapidly expelled from the ascending colon into the small colon, which has a far smaller diameter, especially if it is edematous. This may result in the initial appearance of diarrhea, followed by intermittent episodes of colic that may be explained by impaction. Diagnosis is by rectal examination, during which the rectum typically feels edematous and gritty. The most important point to remember is that horses should be closely assessed for impaction even if diarrheal disease is present. Other parameters that are typically helpful for assessing the severity of colic, such as heart rate, are not predictive of obstruction in horses with small colon impaction.[1178] Horses may be treated medically during the early stages with fluids, laxatives, and analgesics. The key clinical sign that indicates the need for surgery appears to be abdominal distention, associated with distention of the large colon. Other clinical signs, such as elevations in heart rate and refractory colic, are less pronounced in this disease. Postdiarrheal disease is not the only form of small colon impaction. These impactions can be formed as simple collections of ingesta or in response to luminal narrowing.

Atresia Coli

Atresia of a segment of the colon is a rare congenital abnormality in horses.[1179] The heritability and causes of the condition are unknown. One potential mechanism for development of the lesion is intestinal ischemia during fetal life, which secondarily results in necrosis of a segment of intestine.[1179] Clinical signs include a failure to pass meconium and colic within the first 12 to 24 hours of life. Secondary abdominal distention results from complete intestinal obstruction, and abdominal radiographs may reveal a gas-distended colon. The diagnosis is made at surgery. Any portion of the colon may be absent, but the distal segment of the large colon or the proximal small colon (or both) is usually most severely affected. If sufficient tissue is present, anastomosis to the proximal blind end of the colon may be attempted.[1179] The prognosis depends on the segment of the colon that is absent but is usually poor because of an absence of distal colon.

Ileocolonic Aganglionosis

Ileocolonic aganglionosis, commonly known as *lethal white foal syndrome* (LWFS), occurs in white foals with overo-spotted parents. Affected foals are either completely white or have very little pigmented hair around the muzzle, base of the tail, or hooves. They are homozygous for an abnormal endothelin receptor B (EDNRB) gene that results in altered neural crest cell migration or survival, which affects progenitor cells for melanocytes and intestinal ganglia.[1180,1181] The EDNRB genotype is highly correlated with white patterning; frame overo, highly white calico overo, and frame blend overo have the highest incidence of heterozygotes.[1182] Rarely, an LWFS-affected foal may be born to a solid-colored mare. Homozygous foals have aganglionosis of the submucosal and myenteric ganglia of the distal portion of the ileum and large intestine, and extrinsic innervation of the ileum and pelvic flexure has been identified.[1183,1184] This results in a foal that appears normal at birth but develops signs of intestinal ileus and colic within 12 to 24 hours. The eyes are blue, and the skin is pink. A genetic test is available to identify horses that are heterozygous for the defective EDNRB gene.

Diseases of the Oral Cavity, Esophagus, and Stomach

ORAL CAVITY

The mouth is bounded laterally by the cheeks, dorsally by the palate, and ventrally by the body of the mandible and by the mylohyoideus muscles. The caudal margin is the soft palate. The mouth of the horse is long and cylindric, and when the lips are closed, the contained structures almost fill the cavity. A small space remains between the root of the tongue and the epiglottis and is termed the *oropharynx*. The cavity of the mouth is subdivided into sections by the teeth. The space external to the teeth and enclosed by the lips is termed the *vesicle* of the mouth, and in the resting state the lateral margins of the vesicle, that is, the buccal mucosa, are in close contact with the cheek teeth. Caudally, the external space communicates with

the pharynx through the aditus pharyngis. The mucous membrane of the mouth is continuous at the margin of the lips with the skin and during life is chiefly pink but can be more or less pigmented, depending on the skin color and the breed type.

Morphology and Function

The lips are two muscular membranous folds that unite at angles close to the first cheek teeth. Each lip presents an outer and an inner surface. The upper lip has a shallow median furrow (philtrum); the lower lip has a rounded prominence or chin (mentum). The internal surface is covered with a thick mucous membrane that contains small, pitted surfaces that are the openings of the ducts of the labial glands. Small folds of the mucous membrane called the frenulum labii pass from the lips to the gum.

The free border of the lip is dense and bears short, stiff hairs. The arteries of the mouth are derived from the maxillary, mandibular, labial, and sphenopalatine arteries of the major palatine artery. The veins drain chiefly to the lingual facial vein. Sensory nerves originate from the trigeminal nerve (cranial nerve V) and the motor nerves from the facial nerve (cranial nerve VII). The cheeks spread back from the lips and form both sides of the mouth and are attached to the alveolar borders of the bones of the jaws. The cheeks are composed of skin and muscular and glandular layers and then the internal mucous membrane. The skin is thin and pliable. In contrast, the oral mucous membrane is dense and in many areas of the oral cavity is attached firmly to the periosteum so that construction of oral mucosal flaps can be achieved only by horizontal division of the periosteal attachment. Such a feature is important in reconstructive techniques applied to the oral cavity. The blood supply to the cheeks comes from the facial and buccal arteries and the sensory nerves from the trigeminal and motor nerves from the facial nerve.

The hard palate (palatum durum) is bounded rostrally and laterally by the alveolar arches and is continuous with the soft palate caudally. The hard palate has a central raphe that divides the surface into two equal portions. From the line of the rostral cheek tooth, the hard palate is concave to the line of the caudal cheek tooth. Paired transverse ridges (about 18) traverse the concavity and have their free edges directed caudally. The incisive duct is a small tube of mucous membrane that extends obliquely through the palatine fissure. The dorsal component communicates by a slitlike opening in the rostral portion of the ventral nasal meatus, and its palatine end is blind and lies in the submucosa of the palate. When stallions display their flehmen response, watery secretions enter the nose from the glands of the vomeronasal duct. To what extent these secretions aid in pheromone reception is not known.[1185]

That portion of the palatine mucosa immediately behind the incisor teeth frequently is swollen (lampas) during eruption of the permanent teeth. This swelling is physiologic, not pathologic.

The tongue is situated on the floor of the mouth between the bodies of the mandible and is supported by the sling formed by the mylohyoideus muscles. The root of the tongue is attached to the hyoid bone, soft palate, and pharynx. The upper surface and the rostral portion of the tongue are free; the body of the tongue has three surfaces. The apex of the tongue is spatulate and has a rounded border. The mucous membrane adheres intimately to the adjacent structure and on the dorsum is dense and thick. From the lower surface of the free part of the tongue, a fold of mucous membrane passes to the floor of the mouth, forming the lingual frenulum. Caudally, a fold passes on each side of the dorsum to join the soft palate, forming the palatoglossal arch. Dorsally from the soft palate the palatopharyngeal arch attaches and circumvents the aditus laryngis and attaches to the roof of the nasopharynx. The mucous membrane of the tongue presents four kinds of papillae:

1. Filiform papillae are fine threadlike projections across the dorsum of the tongue. They are absent on the root of the tongue and are small on the rostral portion of the tongue.
2. The fungiform papillae are larger and easily seen at the rounded free end. They occur principally on the lateral portion of the tongue.
3. Vallate papillae are usually two or three in number and are found on the caudal portion of the dorsum of the tongue. The free surface bears numerous small, round secondary papillae.
4. Foliate papillae are situated rostral to the palatoglossal arches of the soft palate on which they form a rounded eminence about 2 or 3 cm in length marked by transverse fissures.

Foliate, vallate, and fungiform papillae are covered with taste buds and secondary papillae. The lingual and sublingual arteries supply the tongue from the linguofacial trunk and matching veins. The linguofacial trunk drains into the linguofacial vein. The lingual muscles are innervated by the hypoglossal nerve (cranial nerve XII), and the sensory supply is from the lingual and glossopharyngeal (cranial nerve IX) nerves.

TEETH

The formula for the deciduous teeth of the horse is 2 times I3-3 C0-0 P3-3 for a total of 24. The permanent dental formula is 2 times I3-3 C1-1 P3-3 or P4-3 M3-3 for a total of 40 or 42. In the mare the canine teeth are usually small or do not erupt, reducing the number to 36 or 38. The first premolar tooth (wolf tooth) is often absent and has been reported as occurring in only 20% of the upper dentition of Thoroughbred horses.[1186] The teeth of the horse are complex in shape and are compounded of different materials (dentin, cementum, and enamel). They function as grinding blades to masticate and macerate cellulose food in the important first stage of the digestive process. The cheek teeth in the horse are a well-documented feature of the evolution of *Equus caballus.*

The first deciduous incisor is present at birth or the first week of life. The second incisor erupts at 4 to 6 weeks of age; the third incisor, at 6 to 9 months of age; the first and second premolars, at birth to 2 weeks of age; and the third premolar, at 3 months of age.

The eruption times for the permanent teeth are as follows: first incisor, 2½ years of age; second incisor, 3½ years of age; third incisor, 4½ years of age; the canine tooth, 4 to 5 years of age; the first premolar (wolf tooth), 5 to 6 months of age; the second premolar, 2½ years of age; the third premolar, 3 years of age; the fourth premolar, 4 years of age; the first molar, 10 to 12 months of age; the second molar, 2 years of age; and the third molar, 3½ to 4 years of age. This eruption sequence clearly indicates that the eruption of the second and third permanent premolar teeth have the potential for dental impaction.

The modern horse has six incisor teeth in each jaw that are placed close together so that the labile edges form a semicircle. The occlusal surface has a deep enamel invagination

(infundibulum) filled only partially with cementum. As the incisor teeth wear, a characteristic pattern forms in which the infundibulum is surrounded by rings of enamel, dentin, enamel, and crown cementum in a concentric pattern. Each incisor tooth tapers from a broad crown to a narrow root so as the midportion of the incisor is exposed to wear, the cross-sectional diameters are about equal; that is, at 14 years of age, the central incisor tooth of the horse has an occlusal surface that is an equilateral triangle. Observations on the state of eruption, the angles of incidence of the incisor teeth, and the pattern of the occlusal surfaces are used as guides for the aging of horses. The canine teeth are simple teeth without complex crowns and are curved. The crown is compressed and is smooth on its labial aspect but carries two ridges on its lingual aspect. No occlusal contact occurs between the upper and lower canine teeth.

When erupted, the six cheek teeth of the horse function as a single unit in the mastication of food. Each arcade consists of three premolar and three molar teeth. The maxillary arcade is slightly curved, and the teeth have a square occlusal surface. The occlusal surfaces of the mandibular teeth are more oblong, and each arcade is straighter. The horse is anisognathic—that is, the distance between the mandibular teeth is narrower (one-third) than the distance between the upper cheek teeth. This anatomic arrangement affects the inclination of the dental arcade as the jaws slide across each other in the food preparation process. The unworn upper cheek tooth presents a surface with two undulating and narrow ridges, one of which is lateral and the other medial. On the rostral and lingual side of the medial style is an extra hillock. The central portion of these surfaces is indented by two depressions that are comparable with, but much deeper than, the infundibula of the incisor teeth. When the teeth have been subjected to wear, the enamel that closed the ridges is worn through and the underlying dentin appears on the surface. After a time the chewing surface displays a complicated pattern that may be likened to the outline of an ornate letter B, and the upright stroke of the B is on the lingual aspect. Dentin supports the enamel internally, cementum supports the enamel lakes, and the peripheral cementum fills in the spaces between the teeth so that all six teeth may function as a single unit—that is, the dental arcade. Transverse ridges cross each tooth so that the entire maxillary arcade consists of a serrated edge. The serrations are formed so that a valley is present at the area of contact with adjacent teeth. These serrations match fitting serrations on the mandibular arcade. One should note that the mediolateral mandibular motion while chewing pellets does not provide full occlusal contact as it does when chewing hay.[1187]

The true roots of the cheek teeth are short compared with the total length of the tooth. Cheek teeth have three roots: two small lateral roots and one large medial root. That portion of the crown embedded within the dental alveolus is referred to as the reserve crown, and the term *root* is confined to that area of the tooth that is comparatively short and enamel free. Wear on the tooth gradually exposes the reserve crown, and the roots lengthen. In an adult 1000-lb horse the maxillary cheek teeth are between 8.0 and 8.5 cm in length. Dental wear accounts for erosion and loss of tooth substance at a rate of 2 mm/year. The pulp chambers of the teeth are also complex. The incisors and canines have a single pulp chamber. The mandibular cheek teeth have two roots and two separate pulp chambers. The maxillary cheek teeth, although they have three roots, have five pulp chambers.

As occlusal wear proceeds, deposition of secondary dentin within the pulp chambers protects the chambers (e.g., the dental star, medial to the infundibulum on the incisor teeth). In the mandibular cheek teeth the transverse folding of the enamel anlage (during morphogenesis of the tooth) does not take place, and the occlusal surface is a simple surface of central dentin surrounded by enamel. Each tooth then is conformed to a single arcade by the presence of peripheral crown cementum.

Examination of the Oral Cavity

The oral cavity and oropharynx are subject to a variety of diseases, many of which produce the same clinical signs, regardless of their cause. The classic signs of dental disease in the horse include difficulty and slowness in feeding, together with a progressive unthriftiness and loss of body condition. In some instances, the horse may quid (drop food while chewing), and halitosis may be obvious. Additional problems reported by owners include bitting and riding problems and headshaking or head shyness. Facial or mandibular swelling, nasal discharge (dental disease associated with maxillary sinus), and mandibular fistulae (lower cheek tooth apical infections) are also possible. Some correlation exists between the age of the animal and clinical signs.

One can examine a considerable portion of the mouth and teeth from the outside by palpation of the structures through the folds of the cheek. Most horses allow a cursory oral examination without sedation or the use of an oral speculum. In many horses, however, a detailed oral examination is best achieved after sedation and with the use of an oral speculum and a light source. The mouth should be irrigated to remove retained food material so as to be able to inspect and palpate the lips, cheeks, teeth, and gums.

Ancillary aids for a complete examination of the oral cavity of the horse may include radiology, endoscopic examination, fluoroscopy, biopsy, and culture. Oral endoscopy should be performed with either sedation and an oral speculum or under general anesthesia to prevent inadvertent mastication of the endoscope. CT allows detailed imaging of the oral cavity and sinuses.

Dysphagia

The lips of the horse are mobile and prehensile. Consequently, loss of motor function (e.g., facial palsy) affects the efficiency of the prehensile system. The lips grasp food in grazing or browsing, and the incisor teeth section the food. With mastication and lubrication with saliva, the bolus of food forms and is manipulated from side to side across the mouth, assisted by the tight cheeks of the horse and the palatine ridges. Swallowing begins as the food bolus contacts the base of the tongue and the pharyngeal walls. During swallowing, the soft palate elevates to close the nasopharynx, the base of the tongue elevates, and the hyoid bone and the larynx move rostrally following contraction of the hyoid muscles. During this process, the rima glottidis closes and the epiglottis tilts dorsally and caudally to protect the airway so that food is swept through lateral food channels around the sides of the larynx into the laryngoesophagus. Fluoroscopic studies in nursing foals in the dorsoventral view showed that contact occurs between the lateral food channels in the midline so that in outline the food bolus achieves a bow-tie shape.[1188]

Dysphagia is defined as a difficulty or inability to swallow. Anatomic classifications for dysphagia include prepharyngeal,

pharyngeal, and esophageal (postpharyngeal) dysphagias. The site of the cause for dysphagia influences the clinical signs. Prepharyngeal dysphagia is characterized by dropping food (quidding) or water from the mouth, reluctance to chew, hypersalivation, or abnormalities in prehension. Pharyngeal and esophageal dysphagias are characterized by coughing; nasal discharge containing saliva, water, or food material; gagging; anxiousness; and neck extension during attempts to swallow. The following section describes esophageal dysphagia in more detail. Causes of dysphagia can be divided into four types: painful, muscular, neurologic, or obstructive (Table 12.8). Pain and obstruction cause dysphagia by interfering with the mechanics of prehension, bolus formation and transfer to the pharynx, and deglutition. Muscular and neurologic causes of dysphagia impede prehension and swallowing by affecting the motor function of the lingual or buccal musculature, muscles of mastication (temporal and masseters), and pharyngeal and cranial esophageal muscles. Sensory loss to the lips, buccal mucous membranes, pharynx, or tongue also may cause dysphagia. Neurologic causes of dysphagia may affect the forebrain, brainstem, or peripheral nerves that control prehension (cranial nerves Vm, Vs, VII, and XII), transfer of the food bolus to the pharynx (cranial nerves Vs and XII), and swallowing (cranial nerves IX and X). The latter point was classical thinking, but recent evidence suggests that, while stimulation of cranial nerve IX stimulates swallowing, bilateral blockade of that nerve does not prevent normal swallowing of either liquid or solid material.[1189]

Diagnosis of the cause of dysphagia is based on physical examination including a careful oral examination; neurologic examination; clinical signs; and endoscopy of the pharynx, esophagus, and guttural pouches. Radiology may be useful to assess the bony structures of the head and throat. Ultrasonography is valuable for examining the retropharyngeal space and esophagus to detect and evaluate masses. Pharyngeal or esophageal causes of dysphagia may be detected with routine endoscopic examination or with contrast radiography. Endoscopy may also be used to assess deglutition, but this function may be adversely affected by sedation of the horse. Deglutition may also be assessed using fluoroscopy[1190] or manometry,[1191] but these techniques require specialized equipment. Specific diagnostic procedures for nonalimentary causes of dysphagia are covered elsewhere in this text (see Chapter 7).

TABLE **12.8 Differential Diagnoses for Dysphagia**

Class of Dysphagia	Differential Diagnoses
Painful	Tooth root abscess or periodontal disease
	Broken teeth
	Abnormal dentition or wear
	Stomatitis, glossitis, or pharyngitis
	Nonsteroidal antiinflammatory drug toxicity
	Chemical irritation
	Thrush (candidiasis)
	Influenza
	Streptococcus equi subsp. *equi*
	Vesicular stomatitis virus
	Actinobacillus lignieresii
	Buccal, gingival, or glossal trauma (bits or chains)
	Foreign bodies
	Retropharyngeal lymphadenopathy or abscess
	Mandibular trauma
	Temporohyoid osteoarthropathy
	Temporomandibular osteopathy
Muscular	Hyperkalemic periodic paralysis
	Nutritional myopathy (white muscle disease)
	Polysaccharide storage disease
	Glycogen branching enzyme deficiency
	Masseter myositis
	Hypocalcemia tetany or eclampsia
	Myotonia
	Rectus capitis ventralis rupture
	White snakeroot toxicity
	Megaesophagus
Obstructive	Retropharyngeal abscess and lymphadenopathy
	Oral, pharyngeal, retropharyngeal, laryngeal, or esophageal malformations, injury, edema, or neoplasia
	Pharyngeal or epiglottic cysts
	Pharyngeal abscess or foreign body
	Dorsal displacement of the soft palate
	Cleft palate

TABLE **12.8 Differential Diagnoses for Dysphagia—cont'd**

Class of Dysphagia	Differential Diagnoses
Neurologic forebrain disease; generalized neuropathy; disorders of cranial nerves V, VII, IX, X, or XII	Guttural pouch tympany or empyema Follicular pharyngitis Esophageal obstruction Pharyngeal cicatrix Retropharyngeal abscess or neoplasia Guttural pouch empyema, mycosis, or neoplasia Stylohyoid osteopathy Lead poisoning Petrous temporal bone osteomyelitis or fracture Retropharyngeal abscess Botulism Yellow star thistle toxicity Viral encephalitis Cerebral edema Cerebral or brainstem hemorrhage Intracranial masses (hematoma, neoplasia, and abscess) Meningitis Verminous encephalitis Equine protozoal myeloencephalitis Equine herpesvirus 1 Equine dysautonomia Hepatoencephalopathy Tetanus Polyneuritis equi

Specific treatments aimed at resolving the underlying disorder causing dysphagia are discussed in detail elsewhere. Most horses with dysphagia should not be fed roughage with long fiber length (hay or grass). Dietary modifications that promote swallowing, such as feeding slurries made from complete pelleted feeds, may be sufficient to manage some cases of partial dysphagia. Aspiration pneumonia is a potential complication in horses with pharyngeal or esophageal dysphagia. Foals may be managed by feeding mare's milk or a suitable substitute via nasogastric tube. Pellet slurries or formulated liquid diets may be fed via nasogastric tube to older horses. Prolonged nutritional management of dysphagic horses may require extraoral feeding using a tube placed through an esophagostomy.[1192]

Formulated pelleted diets are often easy to administer through a tube as slurry and are balanced to meet the nutritional requirements for healthy horses. Sufficient quantities must be fed to deliver adequate calories (16–17 Mcal/day for a 500-kg horse). Adjustments may be necessary for horses that are cachectic or have extra metabolic demand (such as pregnancy). Adding corn oil to the ration (1 cup every 12 or 24 hours) is a common method of increasing fed calories. Equine-specific enteral formulations are also available (Well-Gel, Land O'Lakes Purina Feed LLC, Arden Hills, MN). Regardless of the method of nutritional management, salivary losses of electrolytes should be monitored and electrolyte replacement therapy provided as needed. Saliva contains high concentrations of Na, K, and Cl. A group of ponies with experimental esophagostomies[1193] and a horse with esophageal squamous cell carcinoma[1194] were fed a complete pelleted diet through esophagostomy tubes but developed metabolic acidosis, hyponatremia, and hypochloremia apparently because of salivary losses. Surprisingly, salivary losses of potassium did not result in hypokalemia in these cases, presumably because of replacement in the diet. Electrolyte replacement may be accomplished by adding NaCl and KCl to the diet. Horses can be maintained for months with frequent feedings through an esophagostomy tube.[1194] Parenteral nutrition (total or partial) may be useful in the short term but is not often feasible for long-term management.

DENTAL DISEASES

Eruption Disorders

Tooth eruption is a complex phenomenon involving the interplay of dental morphogenesis and those vascular forces responsible for creating the eruption pathway. These changes are responsible for osteitis and bone remodeling within the maxilla and mandible. Young horses frequently show symmetric bony swelling resulting from these eruption cysts. In some cases, additional clinical signs of nasal obstruction with respiratory stridor or nasal discharges may be apparent.

Pathologic problems associated with maleruption include a variety of dental diseases.[1195] Oral trauma can displace or damage erupting teeth or the permanent tooth buds. As a result, teeth may be displaced and erupt in abnormal positions or may have abnormal shapes. Supernumerary teeth, incisors and molars, can develop, as well as palatal displacement of impacted teeth (maxillary P3-3, or third cheek tooth). In almost all of these conditions some form of surgical treatment is necessary, but depending on the number and location, conservative therapy can be successful.[1196]

Dental impaction is a major cause of dental disease in the horse. In a series of 142 extracted teeth, 63 were P3-3 or P4-4 (cheek tooth 2 or 3, respectively).[1197] Early observations had indicated that the first molar (M1, or cheek tooth 4) was the most commonly diseased tooth, and an "open infundibulum" in this tooth has been suggested as the cause.[1198] In a later study, the mandibular cheek teeth 2 and 3 were the most commonly affected, whereas cheek teeth 2 and 4 were most commonly affected in the maxillary arcade, which was the more commonly affected arcade.[1199] Studies on cementogenesis of the maxillary cheek teeth have shown, however, that most maxillary cheek teeth have a greater or lesser degree of hypoplasia of cementum within the enamel lakes and that this "lesion" rarely expands into the pulp. The central infundibular hole is the site of its vascular supply to the unerupted cement lake. On those occasions in which caries of cementum occurs—that is, secondary inflammatory disease and acid necrosis of the cementum—apical osteitis may develop.

Dental Decay

Pulpitis is key to the pathogenesis of dental decay in the horse. The initiation of inflammatory pulp changes may be a sequela to dental impaction or dental caries or may result from fracture of a tooth. If the onset of the inflammatory process is slow, then formation of secondary dentin within the pulp chambers may protect the pulp and the tooth. Secondary dentin formation occurs from stimulation of odontoblasts within the pulp chamber. Such changes are the normal process of protection during dental wear and attrition as crown substances wear away and the reserve crown comes into wear. In acute disease, however, this defense mechanism is ineffective, and the changes that occur and that are sequelae to pulpitis reflect the location of each affected tooth. For example, pulpitis and apical osteitis of the third mandibular cheek tooth most commonly results in the development of a mandibular dental fistula. Pulpitis of the third maxillary cheek tooth, however, results in an inflammatory disease within the rostral maxillary sinus and in development of chronic maxillary sinus empyema.

Oblique radiographs greatly assist the diagnosis of dental decay by demonstrating sinus tract formation, sequestration of bone, mandibular osteitis, hyperplasia of cementum, and new bone formation (so-called alveolar periostitis).[1200] Nuclear scintigraphy and CT can aid in an accurate diagosis.[49,53] The management of dental decay in the horse usually involves surgical extraction of the diseased tooth. In some horses, apicoectomy and retrograde endodontic techniques may be used to save the diseased tooth. Care must be taken, however, in selection of patients. In most cases of apical osteitis in the horse that result from dental impaction, immature root structures make achieving an apical seal of the exposed pulp difficult.

Periodontal Disease

Gingival hyperemia and inflammation occur during the eruption of the permanent teeth and are common causes of a sore mouth in young horses (particularly 3-year-olds as the first dental caps loosen). Such periodontal changes usually resolve as the permanent dental arcade is established. During normal mastication, the shearing forces generated by the occlusal contact of the cheek teeth essentially clean the teeth of plaque and effectively inhibit deposition of dental calculus. Wherever occlusal contact is ineffective, periodontal changes and calculus buildup occur; for example, the deposition of calculus on the canine teeth of mature geldings and stallions is common. Routine dental prophylaxis forms an important component of maintaining normal occlusal contact, and for this reason arcade irregularities that result in enamel point formation on the buccal edges of the maxillary cheek teeth and the lingual edges of the mandibular cheek teeth should be corrected. These edges may be smoothed annually in horses that are at grass and twice yearly in young horses, aged horses, and stabled horses. Horses at grass have a greater range of occlusal contact and therefore better periodontal hygiene than stabled horses. In stabled horses the range of occlusal contact is narrower, and the formation of enamel points occurs more frequently with subsequent buccal ulceration and the initiation of a cycle of altered occlusal contact and, hence, irregular arcade formation. This process leads to severe forms of periodontal disease and wave mouth formation.

Periodontal disease occurs with abnormal occlusal contact and initiation of the cycle of irregular wear and abnormal contact. Such changes progress to loss of alveolar bone, gross periodontal sepsis, and loss of tooth support. In this sense periodontal disease truly is the scourge of the equine mouth and results in tooth loss.[1201]

CONGENITAL AND DEVELOPMENTAL ABNORMALITIES

Cleft Palate

Palatine clefts may result from an inherited defect and are caused by failure of the transverse palatal folds to fuse in the oral cavity. Harelip accompanies few palatine clefts in the horse. The degree of palatine clefting depends on the stage at which interruption in the fusion of the palatopalatal folds occurs. Toxic or teratogenic effects are documented in other species, but few data are available in the horse.

Treatment for repair of uncomplicated palatine defects has been recommended, but prognosis is generally poor because of the considerable nursing care required and the high incidence of surgical failures. Early surgery and the use of mandibular symphysiotomy in affording surgical exposure should be emphasized. The combination of mandibular symphysiotomy and trans-hyoid pharyngotomy to approach the caudal margins of the soft palate affords surgical access, and mucosal flaps can be constructed to repair the defects. The incidence of breakdown in the surgical repair is high, and healing by first intention is the exception rather than the rule. A surgical report documented the successful closure of a median cleft of the lower lip and mandible in a donkey.[1202]

Campylorhinus Lateralis

Foals born with a severely deviated premaxilla and palate have a wry nose. One can achieve a good functional and cosmetic outcome with surgical correction.[1203] Circumstantial evidence indicates that such a defect has a genetic cause, and the defect occurs most frequently in the Arabian breed.

Cysts

Subepiglottic cysts are congenital abnormalities resulting from cystic distortion of remnants of the thyroglossal duct, which may cause dyspnea and choking in foals. Surgical removal of these cysts results in normal function.

Brachygnathism

The most significant developmental defect of dental origin is a maxilla that is longer than the mandible (parrot mouth). An overbite of 2 cm in the incisor arcade may be present in a horse with a mismatch of less than 1 cm between the first upper and lower cheek teeth. Parrot mouth and monkey or sow mouth are thought to be inherited conditions. Some correction of minor incisor malocclusion occurs up to 5 years of age. Recognition and detection of parrot mouth are important in the examination of potential breeding stock. Surgical attempts to inhibit overgrowth of the premaxilla by wiring or by the application of dental bite plate procedures have been documented.[1204]

Oral Wounds

Wounds of the lips, incisive bone, and the mandibular incisor area occur commonly in the horse and usually result from the horse getting the lips, jaw, or teeth caught in feeding buckets, in fence posts, or in halters or having a segment of tongue encircled with hair in tail chewing. As the horse panics and pulls away from its oral entrapment, considerable trauma can occur to the lips, teeth, and gums. Most wounds repair satisfactorily, provided they are identified early after the injury and basic principles of wound hygiene, excision of necrotic tissue, and wound closure are observed. Oral mucosal defects should be closed and effective oral seals made before external wounds are closed. In some horses, offering specially constructed diets or feeding the horse by nasogastric tube or esophagostomy during the healing processes may be necessary.

Stomatitis and Glossitis

Foreign body penetration of the tongue, cheek, or palate has been reported in grazing and browsing horses and in particular in horses that have certain hay sources that contain desiccated barley awns or yellow bristle grass.[1205] Other plant material and grass awns occasionally may penetrate the tongue, gingiva, or cheek, causing inflammation or abscesses. Metallic foreign bodies have been reported in the tongue, and a history of feeding hay or the use of cable-framed tractor tires was often reported as part of the history.[1206] Ulcerative stomatitis may occur as the result of phenylbutazone toxicity.[1207] Vesicular stomatitis is a highly contagious viral blistering disease of horses and other animals. Treatment of glossitis and stomatitis primarily aims at removing the inciting cause. *A. lignieresii,* the causative agent of actinobacillosis, has been isolated and identified from ulcers on the free border of the soft palate and oral and laryngeal granulomata. The bacterium also was reported in a sublingual caruncle in a horse with a greatly swollen tongue.[1208] Therapy with 150 mL of 20% sodium iodide and 5 g of ampicillin every 8 to 12 hours effected a clinical cure.

Salivary Glands

Saliva is important for lubricating and softening food material. The horse has paired parotid, mandibular, and polystomatic sublingual salivary glands. The parotid gland is the largest of the salivary glands in the horse and is situated in the space between the ramus of the mandible and the wing of the atlas. The parotid duct is formed at the ventral part of the gland near the facial crest by the union of three or four smaller ducts. The duct leaves the gland above the linguofacial vein, crosses the tendon of the sternocephalicus muscle, and enters the mouth obliquely in the cheek opposite the third upper cheek tooth. The parotic duct orifice is small, but some dilation of the duct and a circular mucous fold (the parotid papillae) exist at this point. The mandibular gland is smaller than the parotid gland and extends from the atlantal fossa to the basihyoid bone. For the most part, the mandibular gland is covered by the parotid gland and by the lower jaw. The mandibular duct is formed by the union of a number of small duct radicles that emerge along the concave edge of the gland and run rostral to the border of the mouth opposite the canine tooth.

The orifice is at the end of a sublingual caruncle. The mandibular gland possesses serous, mucous, and mixed alveolar glandular components. The parotid gland is a compound alveolar serous gland. The parotid salivary gland can secrete saliva to yield rates of 50 mL/min, and a total daily parotid secretion can be as much as 12 L in a 500-kg horse. Parotid secretion only occurs during mastication, and administration of atropine or anesthesia of the oral mucosa can block secretion. Parotid saliva is hypotonic compared with plasma, but at high rates of flow, concentrations of sodium, chloride, and bicarbonate ions increase.

Parotid saliva of the horse has a high concentration of calcium, and occasionally calculi (sialoliths) form within the duct radicles of the parotid salivary gland.[1209] Congenital parotid duct atresia, acquired stricture from trauma to the duct, or obstruction by plant material (sticks or foxtails and other seeds) also may occur. The clinical signs of sialolithiasis or other forms of ductule obstruction include a fluid swelling in the form of a mucocele proximal to the stone and occasionally inflammation of the parotid gland. Ultrasonography is useful to diagnose salivary mucoceles and to detect foreign bodies or sialoliths. Measurement of electrolyte concentrations in aspirates from suspected mucoceles might be helpful to distinguish them from hematomas. Salivary potassium and calcium concentrations are higher than plasma. Treatment may require surgical removal of the stone or plant material in the case of sialolithiasis or foreign body obstructions. Other causes of obstruction may require resection of the affected portion of the duct or chemical ablation of the gland.[1210]

Primary sialadenitis is unusual but can occur in one or both glands. The condition is painful and may be associated with a fever and anorexia. Secondary sialadenitis is more common and usually is associated with trauma. Infectious sialadenitis from *C. pseudotuberculosis*[1211] or other bacterial pathogens also may occur. Diagnosis is by physical examination and by finding an enlarged edematous parotid gland tissue on ultrasonographic examination. Culture and cytologic examination of aspirates may be useful for diagnostic purposes. Treatment in usually palliative, consisting of NSAIDs. Appropriate antibiotic therapy is indicated as directed by culture and sensitivity results.

Chemical irritation, glossitis, stomatitis, or other causes of prepharyngeal dysphagia cause ptyalism or excessive salivation in horses. Specific therapy for the ptyalism usually is not required as long as salivary losses are not excessive, resulting in dehydration and electrolyte imbalances. Ingestion of the fungal toxin slaframine also causes hypersalivation in horses.[1212] The fungus *Rhizoctonia leguminicola,* which produces slaframine, causes black patch disease in red clover. Slaframine is a parasympathomimetic compound that stimulates exocrine secretion in the parotid gland.

Slaframine toxicosis most commonly occurs in the spring or early summer and rarely requires treatment other than removal from the pasture. Mowing removes the source in most cases because regrowth in pastures often has less fungal contamination.[1213]

ESOPHAGUS

The esophagus is a musculomembranous tube that originates from the pharynx dorsal to the larynx and terminates at the cardia of the stomach.[1214] In adult Thoroughbred horses the esophagus is approximately 120 cm long. The cervical portion is approximately 70 cm long; the thoracic portion, approximately 50 cm long; and the short abdominal portion, only approximately 2 cm long. The cervical esophagus generally lies dorsal and to the left of the trachea in the cervical region. In the thorax the esophagus courses through the mediastinum lying dorsal to the trachea and crosses to the right of the aortic arch dorsal to the heart base.

The esophagus has no digestive or absorptive functions and serves as a conduit to the stomach for food, water, and salivary secretions. The esophageal mucosa is a keratinized stratified squamous epithelium.[1214] The submucosa contains elastic fibers that contribute to the longitudinal folds of the esophagus and confer elasticity to the esophageal wall. A transition occurs in the muscle type composing the tunica muscularis from striated skeletal muscle in the proximal two thirds of the esophagus to smooth muscle in the distal third. In the proximal esophagus the skeletal muscle layers spiral across one another at angles. Within the smooth muscle layers of the distal esophagus the outer layer becomes more longitudinal, whereas the inner layer thickens and becomes circular. The wall of the terminal esophagus can be 1 to 2 cm thick. Deep cervical fascia, pleura, and peritoneum contribute to the thin fibrous tunica adventitia of the esophagus. Motor innervation to the striated skeletal muscle of the esophagus includes the pharyngeal and esophageal branches of the vagus nerve, which originate in the nucleus ambiguus of the medulla oblongata. Parasympathetic fibers of the vagus nerve supply the smooth muscle of the distal esophagus. Sympathetic innervation of the esophagus is minimal.

Passage of ingesta through the esophagus can be considered part of the swallowing process, which consists of oral, pharyngeal, and esophageal stages. The oral stage is voluntary and involves transport of the food bolus from the mouth into the oropharynx. During the involuntary pharyngeal stage the food bolus is forced through the momentarily relaxed upper esophageal sphincter by simultaneous contractions of the pharyngeal muscles. In the esophageal phase of swallowing the upper esophageal sphincter closes immediately, the lower esophageal sphincter opens, and esophageal peristalsis propels the bolus into the stomach.[1215] Unlike a food bolus, liquids do not require peristalsis to reach the lower esophageal sphincter and may precede the food bolus during swallowing.

The upper esophageal sphincter prevents esophagopharyngeal reflux during swallowing and air distention of the esophagus during inspiration. Upper esophageal pressure increases in response to pressure from a food bolus and to increased intraluminal acidity, as would occur with gastroesophageal reflux. The lower esophageal sphincter is a smooth muscle located at the gastroesophageal junction that is morphologically ill defined but forms an effective functional barrier.[1215] Normally the lower esophageal sphincter is closed in response to gastric distention to restrict gastroesophageal reflux. Relaxation of the lower esophageal sphincter permits passage of ingested material from the esophagus to the stomach. Distention of the stomach with ingesta mechanically constricts the lower esophageal sphincter. Gastric distention also triggers a vagal reflex that increases lower esophageal sphincter tone, which is a safety mechanism against gastroesophageal reflux. The mechanical and vagal mechanisms that promote lower esophageal sphincter tone prevent spontaneous decompression of the stomach, which along with a lack of a vomiting reflex in the horse, increases the risk of gastric rupture during episodes of severe distention.

A wide variety of congenital and acquired disorders of the esophagus have been described in horses. These are summarized in Table 12.9 and discussed in detail in the following section.

Esophageal Obstruction

Esophageal obstruction has many causes (Table 12.10) and most often is manifested clinically by impaction of food material and resulting esophageal dysphagia. Esophageal obstruction may be caused by primary impactions (simple choke) of roughage, particularly leafy alfalfa hay; coarse grass hay; bedding; and even grass.[1216] Prior esophageal trauma or poor mastication caused by dental abnormalities may predispose horses to primary esophageal impaction.[1217] "Wolfing" or gulping food may precipitate primary impactions, particularly if the horse is exhausted or mildly dehydrated after a long ride or is weakened from chronic debilitation. Impactions also may result from disorders that physically impede the passage of food material and fluid by narrowing the luminal diameter, reduce the compliance of the esophageal wall, or alter the conformation of the esophageal wall such that food material accumulates in a pocket or diverticulum. Foreign bodies, intramural or extramural masses, or acquired or congenital anomalies cause these so-called secondary impactions. Intramural causes of esophageal obstruction include tumors (squamous cell carcinoma [SCC]), strictures, diverticula, and cysts.[1218-1226] Mediastinal or cervical masses (tumors or abscesses) may cause extramural obstructions. Congenital anomalies are covered in detail later.

Clinical Signs and Diagnosis

A thorough physical examination, including complete oral and neurologic examination, can help rule out causes of dysphagia and nasal discharge other than esophageal obstruction. Clinical signs associated with esophageal obstructions are related primarily to regurgitation of food, water, and saliva caused by esophageal (postpharyngeal) dysphagia.[1227] Horses with esophageal obstruction are often anxious and stand with their neck extended. Gagging or retching may be observed, particularly with acute proximal obstructions. Bilateral frothy nasal discharge containing saliva, water, and food material; coughing; odynophagia; and ptyalism are characteristic clinical signs, the severity of which varies with the degree and location of the obstruction. Distention in the jugular furrow may be evident at the site of obstruction. Other clinical signs related to regurgitation of saliva, water, and food material, such as dehydration, electrolyte, or

TABLE **12.9** **Esophageal Disorders of Horses**

Disorder	Presenting Complaints	Diagnosis	Treatment	Selected References
ACQUIRED DISORDERS				
Choke	Nasal discharge of saliva and food; retching; excessive salivation; cough; sweating; extension of head and neck	Passage of a nasogastric tube; endoscopy	Medical and surgical treatment options as described in text	1187–1189
Foreign bodies	Acute or recurrent choke	Endoscopy; radiography	Manual retrieval or removal; endoscopic removal; surgery	1190–1194
External compression	Acute or recurrent choke	Endoscopy; radiography; ultrasound	Removal of the obstructive mass	1195
Muscular hypertrophy	No clinical signs observed in most affected horses; may predispose to esophageal diverticula	Incidental finding at necropsy	None	1196
Gastroesophageal reflux disease	Inappetence; bruxism; ptyalism; colic; gastric reflux; weight loss; exercise intolerance	Esophageal and gastric endoscopy	Correct primary problem; decrease gastric acidity; gastric protectants; surgery	1197–1199
Stricture	Recurrent choke; weight loss	Endoscopy; contrast radiography	Bougienage; surgery	49, 53, 1200, 1201–1207
Diverticula[a]	Recurrent choke; weight loss	Endoscopy; contrast radiography	Surgery	1206, 1208–1211
Perforation, trauma	Salivation; bruxism; cough; nasal discharge; sepsis	Endoscopy	Enteral feeding; supportive care	1212, 1213
Megaesophagus[a]	Recurrent choke; intermittent food and saliva from nares; pneumonia; weight loss; colic	Endoscopy; contrast radiography	Nutritional modification as described in text	1214, 1215
Neoplasia	Recurrent choke; weight loss	Endoscopy; biopsy	Surgical resection	1216, 1217
Granulation tissue	Recurrent choke; swelling in the region of the cervical esophagus	Endoscopy; biopsy	Laser surgical resection	1218
CONGENITAL DISORDERS				
Tubular duplication of the esophagus	Young horse; mass caudal to mandible; dyspnea; dysphagia; nasal regurgitation of food and saliva	Radiography and ultrasonography of mass; endoscopy; contrast radiography	Surgical excision	1219, 1220
Cystic duplication of the esophagus	Young horse; mass in cervical or throatlatch area; recurrent choke; bruxism; excessive salivation; nasal regurgitation of saliva and feed; weight loss	Endoscopy; ultrasonography; aspiration of cyst; contrast radiography	Surgical excision; marsupialization of cyst	1224–1231
Vascular ring anomaly	Cervical swelling after introduction to solid feed; chronic respiratory disease	Endoscopy; contrast radiography; computed tomography; magnetic resonance imaging	Surgical correction	1224–1231
Congenital stenosis	Nasal regurgitation of milk; cough	Endoscopy; contrast radiography	Dietary management as described in text	36
Ectasia	Nasal regurgitation of milk	Histologic evaluation	None described	1232, 1233

[a]May occur as congenital or acquired lesions.

TABLE **12.10 Causes of Complete or Partial Esophageal Obstruction in the Horse**

Category	Differential	Examples
Intraluminal	Foreign body	Apples, potatoes
	Feed material	
Extramural	Neoplasia	Squamous cell carcinoma, lymphoma
	Vascular ring anomaly	Persistent right aortic arch
	Granuloma	
Intramural	Esophageal abscess	
	Granuloma	
	Neoplasia	Squamous cell carcinoma, leiomyosarcoma
	Cysts	Intramural cysts, duplication cysts
	Diverticula	
	Stenosis	
Functional disorders	Dehydration	
	Exhaustion	
	Pharmacologic	Acepromazine, detomidine
	Primary megaesophagus	Congenital ectasia
	Esophagitis	
	Autonomic dysautonomia	
	Vagal neuropathies	

acid-base imbalances; weight loss; and aspiration pneumonia may be observed. In extreme cases, pressure necrosis from the impaction or trauma to the esophagus may cause esophageal rupture. If the rupture is in the cervical esophagus, crepitus or cellulitis may be evident along with signs of systemic inflammation. Thoracic auscultation is important to determine whether aspiration pneumonia is present. Intrathoracic esophageal rupture may result in pleuritis and its associated clinical signs.

Passage of a nasogastric tube is an effective way to detect and localize an obstruction but provides little information about the nature of the obstruction or the condition of the esophagus. The most direct method for diagnosis of esophageal obstructions is endoscopic examination. Esophageal obstruction occurs most commonly at sites of natural narrowing of the esophageal lumen, such as the cervical esophagus, the thoracic inlet, base of the heart, or the terminal esophagus; an endoscope greater than 1 m in length is necessary for complete evaluation. Endoscopic evaluation is useful before relief of an impaction to localize the obstruction and to investigate the nature of the impaction if one suspects a foreign body. Foreign bodies may be retrievable via transendoscopic tethering.[1228] Critical diagnostic and prognostic information is also obtained via endoscopy after resolution of the impaction. Assessing the affected esophagus for mucosal ulceration, rupture, masses, strictures, diverticula, and signs of functional abnormalities is important (Figs. 12.15 and 12.16).

Ultrasonography of the cervical region is useful not only to confirm a cervical esophageal impaction but also to provide critical information about the location and extent of the impaction and esophageal wall thickness and integrity. Ultrasonography may provide information about the cause.[1194] Radiographic assessment of the esophagus can confirm the presence of esophageal obstruction in cases in which one cannot view the affected area adequately using endoscopy. Impacted food material in the esophagus may be visualized as a typical granular pattern with gas accumulation proximal to the obstruction. Air or barium contrast radiographic studies are most useful for evaluating the esophagus following relief of the impaction if one suspects a stricture. Esophageal dilation, diverticula, rupture, functional disorder (megaesophagus), or luminal narrowing



FIG. **12.15** Endoscopic view of the cervical esophagus in an adult horse 6 months after an episode of choke that caused circumferential ulceration of the esophageal mucosa. The area of luminal narrowing (stricture) is at the upper right of the image, and the proximal dilation forms an outpouching of the esophageal wall. A contrast esophagram revealed that the outpouching was a pulsion diverticulum.

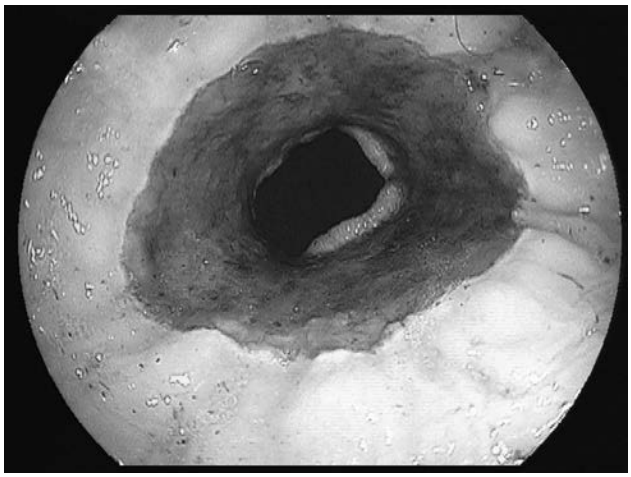


FIG. **12.16** Endoscopic view of the proximal esophagus in a yearling filly with recurrent esophageal obstruction. Circumferential mucosal ulceration is evident proximal to incomplete stricture formation.

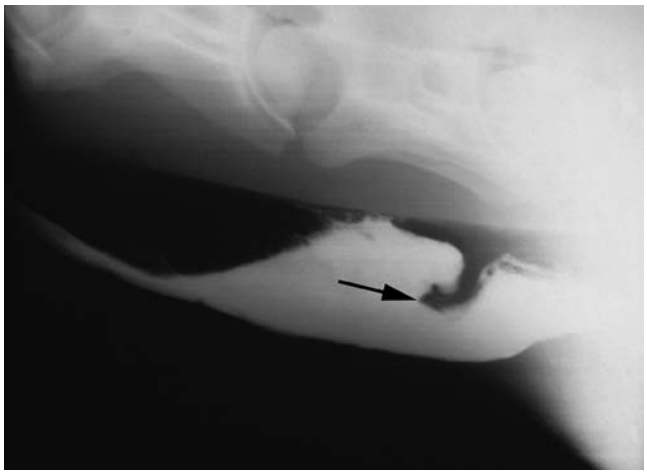


FIG. **12.17** Contrast esophagram in a horse with circumferential esophageal stricture *(arrow)* and a pulsion diverticulum proximal to the stricture.

caused by extraluminal compression are detected more easily using contrast radiographic studies than endoscopy (Fig. 12.17).[1229-1231] Radiographic studies of the esophagus should be interpreted cautiously in sedated horses, particularly after passage of a nasogastric tube or other esophageal manipulations that may contribute to esophageal dilation.[36]

Treatment

The primary goal of treatment for esophageal impaction is to relieve the obstruction. A variety of approaches have been described, ranging from minimal conservative therapy to aggressive intervention to relieving the obstruction. Parenteral administration of acepromazine (0.05 mg/kg IV), xylazine (0.25–0.5 mg/kg IV) or detomidine (0.01–0.02 mg/kg IV), oxytocin (0.11–0.22 IU/kg intramuscularly), and/or esophageal instillation of lidocaine (30–60 mL of 1% lidocaine) may reduce esophageal spasms caused by pain or may decrease esophageal tone.[36,1232,1233] *N*-Butylscopolammonium bromide may reduce smooth muscle tone and has been used to help resolve some obstructions.

In many horses with esophageal obstruction secondary to a feed impaction, the problem may be resolved with conservative management. To facilitate examination, relieve anxiety, and relax the esophagus, one should begin by sedating the horse. A nasogastric tube is then passed to confirm the diagnosis. When an obstruction is encountered, gentle pressure is applied in an attempt to dislodge and move distally the offending feed material. If the choke is not easily dislodged with gentle pressure, the tube is removed. Appropriate sedative, antiinflammatory/analgesic, and smooth muscle–relaxing drugs are administered. The horse is moved to an unbedded stall with absolutely no food or water within reach and muzzled if necessary. The horse is left alone in the stall for several hours. If there is evidence of dehydration, appropriate IV fluid therapy is provided. If there is evidence of aspiration pneumonia, appropriate IV antimicrobial therapy is provided. On reexamination, a stomach tube is passed and gentle pressure is again applied to the area of obstruction. In many horses, the impaction will have softened and can be easily dislodged, if it has not resolved already, with minimal pressure from the stomach tube.

Some clinicians prefer a more interventional approach to the resolution of an impaction, and some impactions are severe enough to require physical dispersal of the material.[1232] A nasogastric tube can be used to displace the impacted material along with external massage if the obstruction is in the cervical region. Careful lavage of the esophagus with water via an uncuffed or a cuffed nasogastric tube while the head is lowered may be a useful aid in breaking up the impaction. Some clinicians advocate a dual tube method in which a tube is placed through each nasal passage into the esophagus for ingress and egress of the lavage fluid. Because of the risk of aspiration of water and food material, esophageal lavage sometimes is done under general anesthesia with a cuffed nasotracheal tube. In refractory cases, IV administration of polyionic fluids provides hydration and alleviates any electrolyte or acid-base imbalances resulting from salivary losses of chloride, sodium, and potassium.[1193] Oxytocin may or may not provide a direct effect for the resolution of an esophageal obstruction. Oxytocin reduced the area under the curve for esophageal smooth muscle strip contractions but had no effect on skeletal muscle strips in vitro.[1234] In one in vivo report, oxytocin administration resulted in decreased esophageal tone in the proximal esophagus (aborad to the larynx and thoracic inlet).[1233] In a separate study, oxytocin administration did not affect esophageal manometric recordings.[1235] Rarely, esophageal obstruction ultimately may require esophagotomy to relieve the impaction. Strict restriction of food and water, including access to bedding material, must be enforced until the obstruction is resolved and the esophagus has regained function. Surgical removal of esophageal foreign bodies can be considered if the size and/or orientation of the object is such that transendoscopic retrieval is considered unlikely to be successful.[1226,1236] In one report, an intraluminal mass comprised of exuberant granulation tissue was removed via serial transendoscopic Nd:YAG laser ablation.[1237]

Systemic effects of dysphagia associated with esophageal impaction include dehydration, hyponatremia, hypochloremia, and metabolic alkalosis from prolonged loss of salivary free water and electrolytes.[1193]

Esophageal endoscopy should be performed after relief of the impaction to determine whether any complications of the

impaction have developed or if a primary cause of the obstruction is present. Endoscopic examination is critical to determine the postobstruction treatment plan and for follow-up evaluation of esophageal healing. The horse should be reevaluated every 2 to 4 weeks following resolution of the impaction if esophageal dilation or mucosal injury is observed. In one report, male sex, age >15 years, and need for general anesthesia to relieve the obstruction were associated with an increased risk of complications.[1238]

Dilation proximal to the site of obstruction, mucosal injury from trauma, stricture formation, formation of a diverticulum, megaesophagus, and esophagitis are sequelae to esophageal obstruction that predispose patients to reobstruction. Underlying functional or morphologic abnormalities were much more likely in cases of recurrent, relative to first time, obstruction in a retrospective study.[1216]

The rate of reobstruction may be as high as 37%. Depending on the duration of the obstruction and the degree of trauma or dilation, the risk of reobstruction is high for 24 to 48 hours or longer. Food should be withheld for at least 24 to 48 hours after resolution of the obstruction. Sucralfate (20 mg/kg orally every 6 hours) may hasten healing if esophageal ulceration is evident, but the efficacy of sucralfate for this purpose is not established. Some clinicians suggest that administration of an NSAID may reduce the development of strictures, although judicious use of NSAIDs is recommended to prevent worsening of esophageal mucosal injury. Orally administered NSAIDs should be avoided if esophagitis is present. After 48 to 72 hours or when the esophageal mucosa has recovered as assessed by endoscopy, the horse may be fed soft food (moistened pellets and bran mashes) then transitioned gradually to a high-quality roughage diet over 7 to 21 days, depending on the degree of esophageal damage induced by the impaction and the nature of any underlying disease. The prognosis for survival is good (78–88%), but some horses may require permanent dietary modification if persistent chronic obstruction is a problem.[1216,1220,1238]

Aspiration pneumonia is a potential complication in every case of esophageal obstruction, and perforation is possible with severe or prolonged obstruction. In one report, duration of obstruction before presentation was a good predictor of pneumonia, whereas endoscopic evidence of tracheal food contamination was not.[1216] Administration of broad-spectrum antibiotics that are effective against gram-positive and gram-negative organisms, including metronidazole for anaerobes, is highly recommended if the duration of obstruction is either unknown or prolonged or if aspiration is suspected.

Esophagitis

Esophagitis refers to a clinical syndrome of esophageal inflammation that may or may not be ulcerative. The major protective mechanisms of the esophageal mucosa include salivary and food material buffers, normal peristaltic motility, and the barrier formed by the gastroesophageal sphincter. Reflux esophagitis is caused by repeated episodes of gastric fluid regurgitation into the distal esophagus and subsequent chemical injury to the mucosa.[1239] Esophageal mucosal ulceration also can occur if the clearance of gastric fluid from the esophagus is delayed, such as in functional disorders of the esophagus. Like ulceration of the squamous portion of the stomach in horses, gastric acid and bile salt chemical injury is a major mechanism of esophageal squamous epithelial ulceration.[838,1239] Reflux esophagitis may occur along with gastric ulcer disease, motility disorders, increased gastric volume from gastric outflow obstructions, gastric paresis, intestinal ileus, or impaired lower esophageal sphincter function.[1222,1239] Other causes of esophagitis in horses include trauma (foreign bodies, food impactions, and nasogastric tubes), infection (mural abscesses), or chemical injury (pharmaceuticals and cantharidin; Fig. 12.18).[1236,1240,1241]

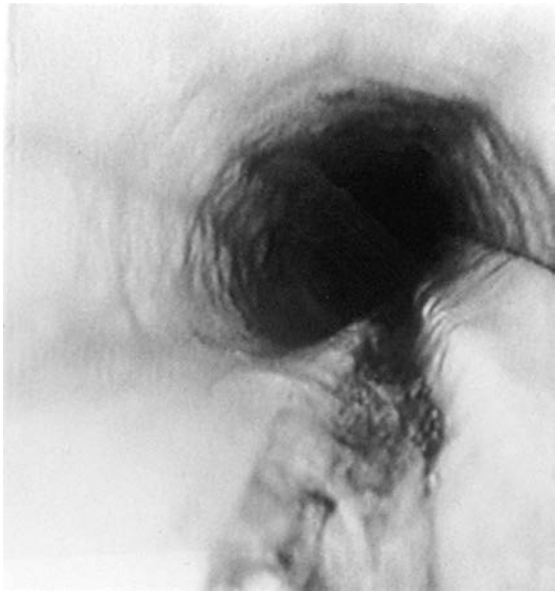


FIG. **12.18** Endoscopic view of the cervical esophagus of a horse that had repeated passages of a stiff nasogastric tube. The deep, linear ulceration of the esophageal mucosa is notable.

Clinical Signs and Diagnosis

The clinical signs of esophagitis are nonspecific and similar to esophageal obstruction and gastric ulceration. Gagging or discomfort when swallowing may be evident, along with hypersalivation and bruxism. Esophageal (postpharyngeal) dysphagia may be evident. Partial or complete anorexia with resultant weight loss may be observed in affected horses. Esophageal hypomotility dysfunction caused by the inflammatory process may result in esophageal impaction. Clinical signs of underlying diseases that predispose to esophagitis may predominate or mask the signs of esophagitis. Horses with gastrointestinal motility disorders such as proximal enteritis or gastric outflow obstruction are at a high risk of developing reflux esophagitis because of the presence of gastric acid and bile salts in the fluid reflux. Foals with obstructive gastroduodenal ulcer disease (GDUD) commonly have reflux esophagitis.

Diagnosis requires endoscopic examination, with diffuse, patchy, linear, or coalescing erosion or ulcerations or significant edema or hyperemia. Determining whether an underlying disease, such as infection, neoplasia, esophageal strictures, or diverticula, is present is important. Evaluation of the stomach is important to rule out gastric outflow obstruction. Contrast radiography may be helpful to assess esophageal motility and transit time.[1230]

Treatment

The principles of therapy for reflux esophagitis include control of gastric acidity, mucosal protection, and correction of any underlying disorder contributing to gastroesophageal reflux. Reduction of gastric acid production with proton pump antagonists or H_2 receptor antagonists is critical for resolution. Some clinicians advocate using sucralfate to promote healing of ulcerated esophageal mucosa. The ability of sucralfate to bind ulcerated esophageal mucosa or hasten ulcer healing, however, is not proven in the horse.

If a primary gastric outflow obstruction is present, surgical intervention is warranted. Horses with delayed gastric outflow without obstruction may benefit from prokinetics such as metoclopramide (0.02–0.1 mg/kg SC every 4–12 hours) or bethanechol (0.025–0.035 mg/kg SC every 4–24 hours). For esophagitis from trauma or pressure injury after esophageal impaction, judicious use of NSAIDs may be warranted to reduce inflammation and pain.

Dietary modification may be necessary for patients with esophagitis, depending on the degree of ulceration or if motility is impaired. Horses with mild esophagitis should be fed frequent small meals of moistened pellets and fresh grass. Severe esophagitis may necessitate withholding food and complete esophageal rest for several days. Although the prognosis for esophagitis is good in the absence of underlying disease, the risk of stricture formation is high if severe circumferential or coalescing ulcerations are present. Esophagitis from severe trauma or infection may be prone to stricture formation.

Motility Disorders

Motility dysfunction of the equine esophagus is most often manifest as hypomotility resulting in esophageal dilation (ectasia) or megaesophagus. Although megaesophagus in horses most commonly is acquired, reports indicate idiopathic megaesophagus in young horses may be congenital.[1191,1242-1245] Acquired megaesophagus in horses may be a consequence of chronic or recurrent esophageal obstruction.[1220,1222] Esophageal impactions of a short duration cause a proximal dilation of the esophagus that is generally reversible.[1230] If the duration of the obstruction is long enough, the motility of the esophagus proximal to the site of obstruction may be impaired permanently. Other causes of acquired megaesophagus include extraesophageal obstruction by tumors or abscesses, pleuropneumonia, and vascular ring anomalies.[1220,1223] A retrospective report of horses with megaesophagus reveals an overrepresentation of Friesian horses (14 of 18 cases) suggesting the possibility of a predisposition for esophageal disorders in this breed.[1214] The authors state that preliminary pedigree analyses suggest the possibility of a recessive pattern of inheritance.[1214]

Megaesophagus also may result from neurologic, neuromuscular, and muscular disorders. Neurologic diseases that cause vagal neuropathy, such as equine protozoal myeloencephalitis, equine herpesvirus myeloencephalitis, and idiopathic vagal neuropathy, have been associated with megaesophagus in horses. Pleuropneumonia may be associated with a vagal neuropathy resulting in megaesophagus. Megaesophagus is an early sign of equine dysautonomia[1246] and may be observable in patients with botulism. Myasthenia gravis is a well-known cause of megaesophagus in nonequine species but has not been reported in horses. Also in other species, electrolyte disorders, cachexia, primary myopathies, myositis, and Addison's disease may affect esophageal motility but have not been associated with megaesophagus in horses. Iatrogenic megaesophagus can be induced by the α_2-adrenergic agonist detomidine but is transient and reversible.[36,1247] Acepromazine, detomidine, and a combination of xylazine and butorphanol can alter proximal esophageal motility by disrupting coordinated peristalsis and decreasing spontaneous swallowing, but only acepromazine affected the manometric profile of the distal esophagus.[1235] None of these drugs altered contractility of isolated esophageal smooth or striated muscle strips in vitro.[1234] Nonetheless, the use of these drugs may complicate clinical evaluation of esophageal motility. Esophageal disorders including megaesophagus, esophageal diverticulum, and esophageal rupture appear to be more common in young Friesian horses, suggesting the possibility of a genetic predisposition in this breed.

Esophageal inflammation, particularly reflux esophagitis, may affect motility and cause megaesophagus. Because esophageal hypomotility affects the tone and function of the lower esophageal sphincter, reflux esophagitis also may be a complication of a primary functional disorder. Assessing esophageal motility in horses with esophagitis that is not responding appropriately to treatment is important.

Clinical Signs and Diagnosis

Thorough neurologic examination is important to help rule out primary neurologic causes of megaesophagus. Because esophageal hypomotility is a functional obstruction, the clinical signs are similar to esophageal obstruction, although the onset is typically insidious. The clinical signs include those associated with esophageal dysphagia.[1191,1222,1223,1243-1245] The cervical esophagus may be dilated enough to be evident externally, and weight loss is common.

Diagnosis of esophageal hypomotility requires evaluation of transit via fluoroscopy or contrast radiography.[1230,1246] Endoscopy may reveal a dilated esophagus and an absence of peristaltic waves[1191,1222] or evidence of underlying disease causing obstruction or esophageal dilation.[1220,1222] Esophageal manometry may be useful to document abnormal postdeglutition contraction pressures, contraction time, and propagation times but is not often available for routine clinical application.[1248] If neurologic or neuromuscular disease is suspected, cerebrospinal fluid analysis or electromyography may be indicated.

Treatment

Treatment of an underlying cause should be initiated, if applicable. Dietary modification should include a soft/slurry diet and feeding from an elevated position to promote transit by gravity flow. Metoclopramide or bethanechol may benefit patients with reflux esophagitis associated with megaesophagus. The prognosis depends on the underlying cause and the degree of dilation. Although many cases of megaesophagus associated with reflux esophagitis respond well to treatment, many other forms of megaesophagus including congenital megaesophagus have a poor prognosis.

Esophageal Stricture

Strictures most commonly are caused by pressure necrosis from esophageal impactions that induce circumferential erosion or ulceration of the esophageal mucosa, although

esophageal injury caused by oral administration of corrosive medicinal agents and trauma to the neck may also result in stricture formation.[1249] Congenital strictures also have been reported.[1250] Strictures caused by mucosal and submucosal trauma are termed *esophageal webs* or *rings*. Strictures may also originate in the muscular layers and adventitia of the esophagus (mural strictures) or in all of the layers of the esophagus (annular stenosis).[1217,1250] Horses with these lesions have a presentation similar to those with simple obstructions, because strictures result in partial obstruction and impaction of food material in the lumen. Esophageal webs or rings may be detected with endoscopy (see Figs. 12.15 and 12.16), whereas identification of mural strictures or annular stenosis may require a double-contrast esophagram (see Fig. 12.17). In a retrospective study of horses with esophageal stricture following simple obstruction, maximal reduction in esophageal lumen diameter occurred within 30 days of the esophageal obstruction. Although surgery has been used to relieve such strictures, initial medical management is warranted because strictures may resolve with conservative therapy, and the esophagus continues to remodel for up to 60 days following ulceration. In one report, seven horses with esophageal obstruction–induced stricture were treated conservatively by feeding a slurry diet and administering antiinflammatory and antimicrobial medications, and five of seven were clinically normal within 60 days.[1249] One of the five successfully treated horses had a 10-cm area of circumferential ulceration, suggesting that the potential exists for extensive mucosal injury to resolve without permanent stricture formation.

If resolution of strictures within 60 days is insufficient, other methods to increase esophageal diameter should be investigated. Balloon dilation has been used successfully in several reports.[1251-1256] Commercial balloon dilators have been adapted for use in the horse, although it is important to note that repeated dilation is necessary.[1251,1252,1257] Corticosteroid injection was used successfully in conjunction with this procedure in foals.[1251] Alternatively, a number of surgical techniques have been used to resolve strictures, including resection and anastomosis,[1258,1259] temporary esophagostomy with fenestration of the stricture,[1217] esophagomyotomy for strictures of the muscularis and adventitia,[1260,1261] or patch grafting with local musculature.[1262] Such surgeries, however, are fraught with complications, largely because of the propensity of the traumatized esophagus to restricture.[1220,1249] The esophagus lacks a serosal layer and does not rapidly form a fibrin seal as does the remainder of the intestinal tract, so anastomoses tend to leak.[1259] In addition, tension on the esophagus during swallowing and movement of the neck impairs healing of anastomoses.[1217,1258] In spite of these difficulties, the long-term prognosis for horses with chronic esophageal strictures treated surgically is better than for those treated nonsurgically.[1220] Two recent reviews describe surgical approaches to the esophagus in detail.[1263,1264]

Esophageal Diverticula

Esophageal diverticula may be classified as traction (true) diverticula and pulsion (false) diverticula. Traction diverticula result from wounding and subsequent contraction of periesophageal tissues, with resultant tenting of the wall of the esophagus. Pulsion diverticula arise from protrusion of esophageal mucosa through defects in the muscular wall of the esophagus and usually result from trauma or acute changes in intraluminal pressure.[1250] Traction diverticula appear as a dilation with a broad neck on contrast esophagography, whereas pulsion diverticula typically have a flask shape with a small neck on an esophagram (see Fig. 12.17).[1225,1265] Although traction diverticula are usually asymptomatic and of little clinical significance, pulsion diverticula may fill with feed material, ultimately leading to esophageal obstruction and rupture.[1265-1267]

A movable mass in the midcervical region may be noticeable before onset of complete obstruction.[1250] Pulsion diverticula may be corrected surgically by inverting or resecting prolapsed mucosa and closing the defect in the wall of the esophagus.[1225,1265,1266] Inversion of excessive mucosa may reduce the diameter of the esophageal lumen and predispose horses to esophageal obstruction; therefore, it should be reserved for small diverticula.[1225]

Congenital Disorders

Congenital disorders of the esophagus are rare. Reported congenital abnormalities include congenital stenosis,[1268] persistent right aortic arch,[1223,1269-1273] other vascular anomalies,[1274] esophageal duplication cysts,[1275-1277] intramural inclusion cysts,[1224,1278] and idiopathic megaesophagus.[1243-1245] In the one report of congenital stenosis, double-contrast radiography revealed concentric narrowing of the thoracic esophagus in the absence of any vascular abnormalities at the base of the heart. Successful treatment included having the foal stand with the forelimbs elevated off the ground following each feeding.[1268]

Persistent right aortic arch is a congenital anomaly in which the right fourth aortic arch becomes the definitive aorta instead of the left aortic arch, which results in constriction of the esophagus by the ligamentum arteriosum as it extends between the anomalous right aorta and the left pulmonary artery.[1279] Clinical signs may include those associated with esophageal (postpharyngeal) dysphagia, drooling, and distention of the cervical esophagus resulting from partial obstruction of the thoracic esophagus.[1223,1269] Endoscopic examination typically reveals dilation of the esophagus cranial to the obstruction with evidence of diffuse esophagitis. Surgical treatment of persistent right aortic arch has been reported in foals, with varying degrees of success.[1269,1271,1273] In one report, preoperative CT was used to identify the exact anatomic location of the offending lesion and guide the surgical approach.[1273]

Esophageal duplication cysts and intramural inclusion cysts cause typical signs of esophageal obstruction, including salivation, esophageal dysphagia, and swelling of the cervical esophagus as the cysts enlarge.[1275,1277,1278] Such signs can make them difficult to differentiate from other forms of esophageal obstruction. Endoscopic examination may reveal compression of the esophageal lumen and communication with the esophageal lumen if it exists.

Ultrasonographic examination may be the most useful method of antemortem diagnosis if the cyst is in the cervical esophagus. Examination of an aspirate of the mass may aid in the diagnosis by revealing the presence of keratinized squamous cells.[1275,1278] Surgical treatments have included complete surgical resection and surgical marsupialization.[1275,1277,1278] The latter appears to be more successful and results in fewer complications.[1277,1280] Possible complications of surgical resection include laryngeal hemiplegia following surgical trauma to the recurrent laryngeal nerve in the region of the esophagus and esophageal fistula formation.[1278]

Esophageal Perforation

Esophageal perforation typically occurs in the cervical region in response to external trauma, necrosis of the esophageal wall caused by a food impaction, or rupture of an esophageal lesion such as an impacted diverticulum. The esophagus is particularly vulnerable to external trauma in the distal third of the neck because only a thin layer of muscle covers it at this point.[1281] Iatrogenic perforation may occur in response to excessive force with a stomach tube against an obstruction or a compromised region of the esophagus.[1240] Esophageal perforations may be open or closed and tend to cause extensive cellulitis and necrosis of tissues surrounding the wound because of drainage of saliva and feed material within fascial planes. Systemic inflammation from septic cellulitis may occur. Closed perforations of the esophagus are particularly troublesome because food material, water, saliva, and air may migrate to the mediastinum and pleural space via fascial planes.[1240,1281] Because of the leakage of air into the tissues surrounding the rupture, extensive SC and fascial emphysema frequently develops and is usually evident clinically and on cervical radiographs. Pneumomediastinum and pneumothorax are potentially fatal complications of esophageal ruptures. If the esophagus ruptures into the mediastinum, horses most commonly present with signs of acute SIRS that may be mistaken for colic.

Treatment should include converting closed perforations to open perforations if possible,[1282] extensive debridement and lavage of affected tissues, broad-spectrum antibiotics, tetanus prophylaxis, and esophageal rest. The clinician may achieve the latter by placing a feeding tube into the esophagus via the wound. Alternatively, a small nasogastric tube (12-Fr diameter) may be placed.[1240] For open perforations, once the wound has granulated and contracted to a small size, oral feeding may be attempted.[1281] Extensive loss of saliva via esophageal wounds may lead to hyponatremia and hypochloremia. Transient metabolic acidosis may occur because of salivary bicarbonate loss, followed by progressive metabolic alkalosis.[1193] Although reports of esophageal wounds healing well by second intention exist, healing takes a prolonged time.[1283] In addition, some perforations never completely heal and form permanent esophagocutaneous fistulae that may require surgical correction. The development of esophageal strictures is not common because wounds are usually linear and not circumferential. However, traction diverticula may develop. Other potential complications of esophageal wounds include Horner's syndrome and left laryngeal hemiplegia.[1281]

In a retrospective study on esophageal disorders, only 2 of 11 horses with esophageal perforations survived long term,[1220] and in a report of esophageal trauma following nasogastric intubation, 4 of 5 horses were euthanized.[1240] The prognosis is poor in horses with esophageal perforations, largely because of the extent of cellulitis, tissue necrosis, shock, and local wound complications.

DISEASES OF THE STOMACH

Gastroduodenal Ulceration

Pathophysiology of mucosal injury and repair is detailed previously in this chapter in Mucosal Injury and Repair. Of importance, ulceration can occur in either the stratified squamous or glandular epithelium, although different clinical syndromes and pathophysiologic mechanisms apply. As a result, the broad term "*equine gastric ulcer syndrome*" (EGUS) has been used to encompass the wide array of associated clinical syndromes,[1284] although separation into equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD) has recently been proposed.[1285] GDUD primarily affects weanling/suckling foals and is typically considered a separate entity.[1286] Regardless of terminology, EGUS in its various forms is arguably the most clinically and economically important clinical entity of the equine stomach.

Prevalence

The prevalence of gastric ulceration has been reported for a variety of breeds and usages. The prevalence of squamous ulceration in horses in race training varies from 70% to 95%[1287-1295] and can be as high as 100% when limited to animals actively racing.[1291] Horses performing in other disciplines have also been evaluated, including active show horses (58% prevalence),[1296] endurance horses (67% overall[1297]; 48% interseason and 93% during competition season[1298]), Western performance horse (40%),[1299] Thoroughbred broodmares (67% pregnant; 77% nonpregnant),[1300] and nonracing performance horses (17% precompetition; 56% postcompetition).[1301] In one large retrospective study (3715 adult horses from the years 1924–1996) evaluating incidences of gastric ulceration identified at necropsy, an overall prevalence of 10.3% was found, with the highest prevalence in Thoroughbreds (including Arabians) and Standardbred trotters (19%).[1302] Horses in a university riding program demonstrated a low squamous ulceration prevalence (11%).[1303] Approximately 49% of horses presented to a referral hospital for colic had evidence of gastric ulceration.[1304] The reported prevalence of gastric ulceration in foals varies from 25% to 57%.[1305-1307]

Many earlier studies investigating prevalence of gastric ulceration do not differentiate between nonglandular and glandular lesions, and many evaluate only the nonglandular region of the stomach, but this trend is changing. In 1 of 162 horses in a hospital setting, 58% had antral or pyloric erosions or ulcerations, 58% had squamous mucosal lesions, and 8% had lesions involving the glandular body.[1308] In other studies, 56% of Thoroughbreds had EGGD,[1293] and 47% of racehorses (Thoroughbred and Standardbred) had EGGD.[1295] In the former,[1293] all horses with glandular lesions also had squamous disease, whereas such an association was not seen in the latter study.[1295] In one report of endurance horses with a 67% overall lesion prevalence, 27% had EGGD.[1297] In another, prevalence varied by season, with 16% EGGD prevalence out of competition and 33% during competition season.[1298] In Danish horses, the prevalence of EGGD was 57% in two separate reports.[1309,1310] In a postmortem evaluation, lesions were most commonly located in the squamous mucosa along the margo plicatus, followed by the glandular body, proximal squamous mucosa, and antrum.[1302] Overall, glandular lesions tend to occur near the pylorus.

Pathophysiology

Because the pathophysiology of mucosal injury and repair was documented earlier, it will not be reviewed here. Acid secretion clearly plays a role in squamous mucosal ulceration, and key components of acid regulation will be summarized. Horses secrete acid continuously, and measured pH of equine gastric

contents is variable from less than 2 to greater than 6 depending on the horse's dietary state (fed/fasted).[818,1311] A protocol of repeated 24-hour periods of fasting and feeding has been shown to induce squamous erosion and ulceration.[841] Because this protocol results in periods of prolonged gastric acidity (pH <2.0) and concurrent administration of the H_2 receptor antagonist ranitidine reduces lesion severity, it supports the role of acid exposure in the pathogenesis of squamous ulcer disease. This effect was also noted as pH of the proximal, but not ventral, stomach decreased in early morning hours, corresponding to periods of decreased hay intake.[1312]

Several peptides can stimulate or inhibit the secretion of acid by the parietal cell. The predominant stimuli to hydrochloric acid secretion are gastrin, histamine, and acetylcholine via the vagus nerve.[1313] Gastrin is released by G cells within the antral mucosa, whereas histamine is released by mast cells and ECL-like cells in the gastric gland. Histamine binds to type 2 receptors on the parietal cell membrane, causing an increase in adenosine monophosphate (cAMP), resulting in phosphorylation of enzymes that activate the proton pump. Gastrin and acetylcholine can act via calcium-mediated intracellular pathways and also stimulate histamine release directly.[1314] Isolated equine parietal cells respond maximally to histamine stimulation and only minimally to carbachol and pentagastrin.[1315] In vivo, histamine or pentagastrin infusion can stimulate similar maximal acid output.[1316] Interestingly, pentagastrin stimulation also induces a marked duodenal secretion of a sodium chloride–rich fluid, which can reflux back into the stomach under fasting conditions.[1316] Gastrin release is primarily controlled by gastrin-releasing peptide, which is stimulated by gastric distention and increased luminal pH, but the interaction between gastrin and histamine has not been fully elucidated in the horse.

Gastric acid secretion by parietal cells is primarily inhibited by somatostatin, which is released by fundic and antral D cells. The inhibitory effect of somatostatin is primarily paracrine, but plasma levels of somatostatin negatively correlate with gastric luminal acidity.[1317] Gastric acid secretion also is inhibited by EGF, a peptide produced in saliva.[1318]

Foals can produce significant amounts of gastric acid by the second day of life, with consistent periods of acidity (pH <2.0) in clinically normal animals.[1319,1320] In one study, foals tended to have a high gastric pH at day 1 of age,[1319] but in a study of critically ill foals, some foals demonstrated periods of gastric acidity on the first day of life.[1321] Suckling was associated with an immediate rise in gastric pH, whereas periods of rest in which foals did not suck for more than 20 minutes were associated with prolonged periods of acidity.[1320] Premature human infants are capable of gastric acid production at 28 weeks of gestation.[1322] Only 1 of 7 premature foals demonstrated an acidic pH recording in a study of gastric pH profiles in critically ill foals.[1321] Although multiple factors were likely involved in those foals, the true ontogeny of gastric acid production in foals is currently unknown.

Equine squamous mucosa is very thin at birth but becomes hyperplastic and parakeratotic within days (Fig. 12.19).[814] The parallel between decreasing pH and proliferation of squamous epithelium correlates with that seen in other species.[1323] The combination of a relatively thin gastric epithelium with a high acid output may leave neonatal foals susceptible to ulcer formation at a very young age. The difference in the normal appearance of the squamous mucosa should be considered when interpreting gastric endoscopy in a neonatal population.

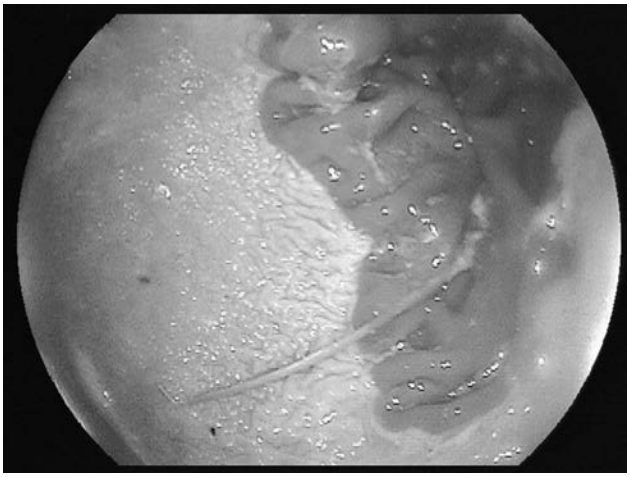


FIG. **12.19** Endoscopic image of the greater curvature of the stomach in a 3-day-old Thoroughbred colt.

Overall, acid remains the major contributing factor to nonglandular mucosal damage, although other factors such as pepsin and bile salts may play an important role as well, either in the initiation or perpetuation of disease.

Risk Factors

Many aspects of diet and management are associated with the development of nonglandular ulceration in adult horses, although some data are conflicting. Risk factors associated with ESGD are clearer than are those associated with EGGD.

Horses in race training have a high incidence of ESGD and are frequently fed high-concentrate, low-roughage diets. A diet high in concentrate and either high or low in forage, in conjunction with stall confinement but no exercise, induce ESGD within 2 weeks.[1324] In one study, higher volatile fatty acid concentrations, higher gastric juice pH, and lower number and severity of ESGD were documented after feeding an alfalfa hay–grain diet compared with a bromegrass hay diet.[1325] Many factors differed between the diets, however, such as digestible energy, bulk, crude protein, and mineral content (especially calcium). These findings were supported by a study in which an alfalfa hay/pelleted concentrate diet significantly reduced ESGD severity scores and/or prevented ulcer development relative to a coastal hay/concentrate diet in horses managed with dry lot housing and regular exercise.[1326] Intermittent feeding has clearly been shown to induce ESGD and is a consistent model of ulcer induction.[841,1311,1327] Similarly, quality of forage (straw versus other types) and increased time between forage meals was associated with higher incidence of ESGD in one report.[1328]

The pathophysiologic correlation between exercise and ESGD has not yet been defined despite the high prevalence of disease in performance horses. In one large epidemiologic study, there was a significant association between ESGD and individual trainer, urban versus rural area, lack of direct contact with other horses, solid barriers, and talk versus music radio.[1329] ESGD can develop within 8 days of exercise varying from light halter to active race training,[1330] and time in work was found to be a risk factor for ESGD in racing Thoroughbreds.[1331] Elevations in postfeeding serum gastrin concentration have been demonstrated after treadmill exercise.[1332]

During treadmill exercise at gaits faster than a walk, proximal gastric size decreases in conjunction with an increase in intraabdominal pressure, resulting in a simultaneous decrease in proximal gastric pH.[1333] Both proximal gastric size and pH return to baseline levels as soon as the horse returned to a walk, and the resultant theory was that gastric contracture could result in increased acid exposure to the squamous mucosa by raising the level of liquid gastric contents.

Stall confinement[1327] and transport[1334] are associated with ESGD, but a distinct mechanism for these associations has not been definitively determined. A recent study did not detect a difference in pH in the proximal or ventral stomach in response to three different environmental situations (stall confinement alone in a barn, stall confinement with a horse in an adjacent stall, and paddock turnout with a companion horse), each for 24 hours.[1312] This work suggests that increased acid exposure to the proximal stomach alone is not causative.

Several studies have failed to document a correlation between NSAID administration and naturally occurring ulcer disease.[1287,1288,1290,1291,1302] NSAID administration, however, is a well-known cause of gastric ulceration under experimental conditions.[393,691,692,1335,1336] NSAID-related ulceration is typically described as predominantly glandular in nature, although nonglandular ulceration can also occur by a mechanism that has not yet been fully characterized. NSAIDs cause a decrease in PGE_2 synthesis caused by inhibition of the COX pathway. A resultant decrease in glandular mucosal protection, most notably via decreased mucosal blood flow and mucous production, is the most likely mechanism of action. In one study, however, phenylbutazone administration resulted in ulceration of the glandular mucosa at the pyloric antrum but did not significantly alter mucosal PGE_2 concentration.[1335]

Other risk factors associated with gastric ulceration include gender and age, and the reported prevalence of gastric ulcers has increased over time. In one study, the frequency of gastric ulceration increased from <6% before 1945 to approximately 18% after 1975.[1302] The association between sex or age and ulceration has not been consistent among studies.[1291,1293,1302,1337] Crib biting has also been discussed as a risk factor for ESGD.[1331,1338] In foals, risk factors are less clearly defined and will be discussed along with the clinical syndrome in each age group. Limited access to water has been associated with ESGD.[1328]

Clinical Syndrome: Neonatal Foals

Clinical signs typically associated with gastric ulceration in foals include poor appetite, diarrhea, and colic. Many foals probably never exhibit clinical signs, and some do not exhibit clinical signs until ulceration is severe or fatal perforation has occurred. Glandular ulceration is typically considered the most clinically significant type of disease in this population.

The physiologic stress of a concurrent illness has been associated with gastric ulceration in foals. Retrospectively, 14 (23%) of 61 foals up to 85 days of age with a clinical disorder had lesions in the gastric glandular mucosa,[1307] and prospectively 8 (40%) of 20 foals up to 30 days of age with a clinical disorder had glandular ulceration.[1339] In contrast, only 4% to 9% of clinically normal foals examined in endoscopic surveys had lesions in the gastric glandular mucosa.[1306,1340]

Critically ill neonatal foals can have a markedly different pH profile compared with that seen in clinically normal foals, potentially caused by alterations in gastric motility and acid secretion.[1321] Gastric ulceration was not identified in any animals at necropsy in that study; however, ulceration has been documented in a similar population.[1305] Thus factors other than acid exposure, most notably mucosal perfusion, may play an important role in the "stress"-related ulceration seen in neonates. Gastric ulceration and rupture in the hospitalized neonatal population appears to occur less commonly now than in previous reports, despite a decline in the use of ulcer prophylaxis in one hospital.[1341] Advances in overall neonatal care, especially supportive care, have likely contributed to this decline.



FIG. **12.20** Endoscopic image of the lesser curvature in a 3-month-old foal diagnosed with gastroduodenal ulcer disease and a gastric outflow obstruction.

Gastroduodenal Ulcer Disease

GDUD occurs almost exclusively in suckling and early weanling foals. Clinical signs of duodenal ulceration are similar to those described for gastric ulceration (bruxism, colic, ptyalism, and diarrhea), but the consequences are often more severe. Lesions occur primarily in the proximal duodenum, ranging from diffuse inflammation to severe ulceration, but affected foals typically have severe squamous and/or glandular ulceration as well (Fig. 12.20). Foals with duodenal ulceration often have delayed gastric emptying and may have gastroesophageal reflux. Complications can include gastric or duodenal rupture, pyloric or duodenal stricture (Fig. 12.21), and ascending cholangitis. Severe squamous and esophageal ulceration and aspiration pneumonia can occur secondary to gastroesophageal reflux.[1222,1342-1345]

GDUD syndrome can occur in outbreaks and is most commonly identified in intensive breeding operations. The cause of duodenal lesions in foals is not known. One theory is that the problem begins with diffuse duodenal inflammation that can coalesce down to a focal area of ulceration (G.D. Lester and A.M. Merritt, personal communication). A temporal relationship between GDUD and rotaviral diarrhea has been suggested, but an infectious etiology remains unproven. Although lesion location and severity associated with rotaviral infection varies among species, duodenal ulceration has not been reported.[1346]

Clinical Signs: Adults

Reported clinical signs attributable to EGUS in adult horses are variable and classically include anorexia and intermittent or recurrent postprandial colic of varying severity.[1347] Overall,

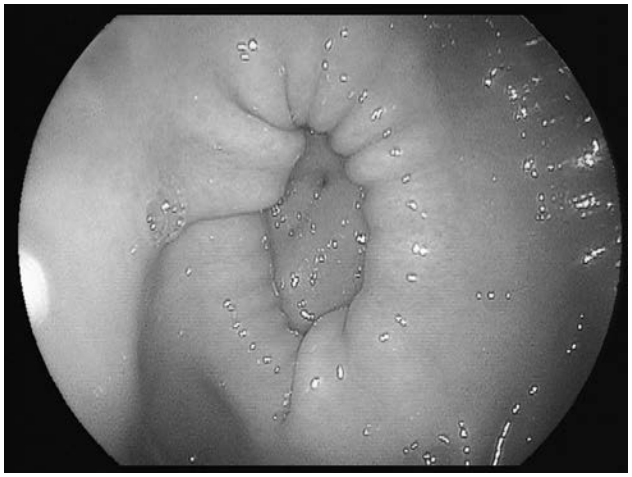


FIG. **12.21** Endoscopic image of the pylorus in the foal depicted in Fig. 2, 2 months after surgery. Note the extremely small pyloric opening, which had formed a mechanical obstruction to gastric outflow.

clinical signs are nonspecific and poorly associated with the presence of EGUS.[1285] Many horses with endoscopic evidence of disease may appear to be clinically normal. Vague signs including decreased consumption of concentrates, postprandial episodes of colic, poor performance or failure to train up to expectations, poor quality hair coat, and diminished condition or failure to thrive have been reported. Diarrhea is not typically associated with gastric ulceration in adult horses, although ulceration can occur concurrently with other causes of diarrhea. Bleeding from ulcers in the gastric squamous mucosa is typically not associated with anemia or hypoproteinemia.

Diagnosis

Gastroscopy is the only reliable method for diagnosis of EGUS in the live animal.[1285] Evaluation of the entire stomach and, preferably, proximal duodenum is critical to a diagnosis, because there is no relationship between the presence of squamous and glandular ulceration.[1295,1308,1309] Urine[1348] and blood[1349] sucrose absorption testing or fecal albumin or hemoglobin[31] is not reliable. Various ulcer scoring systems have been promoted, but the system proposed by the Equine Gastric Ulcer Council[1284] (Table 12.11) has been recommended because of its repeatability and ease of use.[1285,1350]

Duodenal ulceration can be difficult to confirm, and duodenoscopy is the most specific means of diagnosis, although the procedure is more difficult than gastroscopy. Diffuse reddening or inflammation may be the only recognizable lesion in foals with early duodenal disease. In older foals with GDUD, detection of gastric outflow obstruction is critical to the therapeutic plan and appropriate prognosis. A distended stomach is typically evident with ultrasound. Abdominal radiography without contrast in foals with outflow obstruction typically reveals a distinctly enlarged, gas-filled stomach. Liquid barium contrast will either have markedly delayed (with incomplete obstruction) or no (complete obstruction) outflow. Clinically, foals with outflow obstruction will develop reflux after suckling, or marked reflux even with limited to no suckling if the duodenal obstruction is distal to the common bile duct.

TABLE **12.11** **Equine Gastric Ulcer Syndrome Lesion Scoring System**[1284]

Lesion Grade	Description
0	Intact epithelium with no appearance of hyperemia or hyperkeratosis
1	Intact mucosa with areas of reddening or hyperkeratosis (squamous)
2	Small single or multifocal lesions
3	Large single or multifocal lesions or extensive superficial lesions
4	Extensive lesions with areas of deep ulceration

Adapted from Chiu CJ, McArdle AH, Brown R, et al. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg*. 1970; 101:478-483; Baker GJ. Diseases of the teeth. In: Colohan PT, Mayhew IG, Merritt AM, et al., eds. *Equine medicine and surgery,* 4th ed., Vol. 1, Goleta, CA: American Veterinary Publications; 1991.

Treatment

Multiple pharmacologic treatments have been suggested for the treatment of EGUS, and these can vary for ESGD, EGGD, and GDUD. Because acid has been implicated as the most important pathophysiologic component of ESGD, acid suppression is critical. The principal therapeutic options for ulcer treatment include H_2 antagonists (cimetidine, ranitidine, famotidine, and nizatidine), proton pump inhibitors (PPIs; omeprazole, pantoprazole, rabeprazole, and esomeprazole), the mucosal adherent sucralfate, and antacids.

The H_2 antagonists suppress hydrochloric acid secretion through competitive inhibition of the parietal cell histamine receptor that can be partially overcome with exogenous pentagastrin.[1351] Use of H_2 antagonists has been successful in raising gastric pH and resolving gastric lesions in both foals and adult horses.[1320,1344,1352] Clinical and experimental evidence has demonstrated greater individual variability with lower dosages of H_2 antagonists.[1339] Thus dosage recommendations are based on levels necessary to increase gastric pH and promote ulcer healing in a majority of horses. Commonly recommended ranitidine dosages are 6.6 mg/kg orally every 8 hours or 1.5 to 2 mg/kg IV every 6 hours. Although clinically normal foals respond predictably to ranitidine,[1320] sick neonates have shown variability in pH response to IV ranitidine, with a much shorter duration of action and, in some cases, no noticeable response.[1321]

PPIs block secretion of H^+ at the parietal cell membrane by irreversibly binding to the H^+, K^+-ATPase proton pump. The powder form of omeprazole is rapidly degraded in an acidic environment; an enteric-coated capsule or a specially formulated paste must be used to allow delivery of the active drug to the small intestine for absorption. An increase in gastric pH and a decrease in acid output are evident 5 to 8 hours after omeprazole paste administration.[1353] Omeprazole (GastroGard, Merial, Ltd., Duluth, GA) is the only currently FDA-approved agent for the treatment of EGUS in the United States; other preparations, including enteric-coated granules and buffered formulations, are available elsewhere.[1354-1356] In the United States, compounded preparations have shown limited to no efficacy in pharmacodynamic and clinical trials,[1357,1358] and GastroGard was the only product (including buffers, H_2-receptor antagonists, sucralfate, and compounded preparations) that decreased the odds of gastric ulceration in a

population of racehorses.[1359] Several studies have documented the safety of oral omeprazole in foals and adult horses.[1360,1361] Omeprazole appears more effective for ulcer healing than either ranitidine or cimetidine.[1362,1363] Omeprazole (4 mg/kg PO q 24 h) has consistently demonstrated efficacy of (70%–80% healing) for ESGD, including Thoroughbreds maintained in race training.[816,815,1362,1364-1366]

Treatment with 1 mg/kg, 2 mg/kg, or 4 mg/kg orally every 24 hours decreases or prevents disease or the recurrence of disease in horses maintained in training.[815,1330,1367,1368] Other preparations have shown efficacy for ESGD healing at lower dosages (1 mg/kg or 2 mg/kg PO q 24 h) in clinical trials,[1354,1369,1370] although a lower dosage is not yet recommended for treatment. Preexercise versus postexercise administration did not have a significant effect on healing of ESGD or EGGD in a recent trial.[1371] Duration of therapy for ESGD has been recommended for 28 days, although most healing likely occurs within 21 days.[1364] In summary, the primary current recommendation for treatment of ESGD is omeprazole, 4 mg/kg PO q 24 h for 21 to 28 days. In locations in which the enteric-coated granule formation is available, 1 mg/kg may be substituted. Alternate recommendations include ranitidine or lower dosages of omeprazole.[1285]

Lower healing rates (25%) have recently been reported following omeprazole treatment for EGGD.[1354,1370,1371]

Omeprazole (4 mg/kg) also has demonstrated efficacy for raising intragastric pH in clinically normal[1372] or critically ill[1373] foals and for ulcer healing in foals.[1366] The definitive reason for this is unknown but may be related to timing or duration of acid suppression. A bacterial role in EGGD has not been established, despite solid efforts, and the addition of trimethoprim-sulfa did not have apparent benefit in ulcer healing.[1374] Because these factors and basic principles of responsible antimicrobial use, antimicrobial therapy is not recommended for treatment of EGUS in general or EGGD specifically.[1285]

Sucralfate is effective in the treatment of peptic ulcers and prevention of stress-induced ulcers in humans, likely via adherence to ulcerated mucosa, stimulation of mucous secretion, enhanced PGE synthesis, and concentration of growth factor at the site of ulceration.[1375] These are all factors relevant to glandular mucosa; thus, its use seems reasonable for treatment of EGGD. In one study, sucralfate did not promote subclinical ulcer healing in foals, relative to corn syrup.[1376] In a recent study, sucralfate (12 mg/kg PO q 12 h) combined with omeprazole (4 mg/kg PO q 24 h) resulted in a 67.5% EGGD healing rate.[1377] Current recommendations for EGGD include omeprazole plus sucralfate at the previously mentioned dosages for a minimum of 4 weeks, followed by repeat gastroscopy.[1285] Therapy for at least 8 weeks should be pursued before the addition of adjunctive therapy.

The use of synthetic PGE_1 analogs, such as misoprostol, has been effective in the treatment of gastric and duodenal ulcers in humans, and the proposed mechanism of action involves both inhibition of gastric acid secretion and mucosal cytoprotection.[1378] In horses, misoprostol (5 μg/kg) increases gastric pH[1379] and ameliorates deleterious effects of flunixin on mucosal recovery after ischemic injury in vitro,[391] but data supporting its use in clinical trials are currently lacking. Misoprostol is contraindicated in pregnant mares.

Prokinetic drugs should be considered in foals with duodenal disease, gastroesophageal reflux, and when delayed gastric emptying without a physical obstruction is suspected. Bethanechol and erythromycin have been shown to increase the rate of gastric emptying in horses.[1006] In foals with acute gastric atony, bethanechol 0.025 to 0.030 mg/kg SC every 3 to 4 hours has been effective in promoting gastric motility and emptying, followed by oral maintenance dosages of 0.35 to 0.45 mg/kg three to four times daily. Adverse effects can include diarrhea, inappetence, salivation, and colic, but at the dosages stated, adverse effects have been infrequent and mild.

For foals with severe GDUD that have developed duodenal stricture, surgical therapy is necessary.[1343,1380] These animals require a serious financial commitment because intensive perioperative medical therapy is critical for a successful outcome. Prognosis in two recent abstracts has improved over that previously reported. In one, 98% of surgically treated foals survived to discharge from the hospital, with 68% survival 8 months following discharge from the hospital.[1381] In that study, of the surviving Thoroughbreds of racing age, 71% had started a race. In another, short-term survival was reported as 80% for those foals treated surgically and 50% for those treated medically.[976]

A compounded IV preparation of omeprazole (0.5 mg/kg) increases gastric juice pH and decrease the number of nonglandular lesions in horses.[1382] From a regulatory standpoint, omeprazole (4 mg/kg PO q 24 h) does not appear to affect quantitative measurements of performance in Standardbreds.[1383]

Prophylaxsis

Because gastric perforation caused by glandular ulcer disease has been reported in hospitalized neonates, many clinicians routinely use prophylactic antiulcer therapy in this population. Because some critically ill foals have a predominantly alkaline gastric pH profile, and because gastric acidity may be protective against bacterial translocation in neonates, the need for prophylactic ulcer therapy is controversial. In critically ill human neonates, although IV ranitidine therapy increases gastric pH and gastric bacterial colonization, it does not increase the risk of sepsis.[1384] In a retrospective study of 85 hospitalized foals <30 days of age, no difference in the frequency of gastric ulceration at necropsy was found between those foals that received prophylactic treatment for gastric ulcers and those that did not; none died as a result of gastric ulcer disease.[1341] In a multicenter retrospective study, acid prophylaxis was associated with an increased incidence of diarrhea but not with alteration in clinical outcome.[1385] Many clinicians no longer recommend routine ulcer prophylaxis in all ill neonates. Exceptions could potentially include those foals requiring significant doses of NSAIDs for painful orthopedic disorders.

In adults, dietary and environmental management can help prevent gastric ulceration. Pasture turnout and continuous access to good quality forage, especially alfalfa, are currently recommended. For horses at high risk, the best proven pharmacologic approach to prevention involves administration of omeprazole 1 to 2 mg/kg orally every 24 hours.[815,1363,1367,1368] Further work on these recommendations will likely be forthcoming.

Neutraceuticals and Antacids

Although appealing, most agents have not yet been shown to promote healing of EGUS. Some products—including those containing sea buckthorn berries[1386]; organic acids and B vitamins[1387]; and a combination of yeast, magnesium hydroxide, and Apolectol[1388]—have shown promise for prevention of EGUS. Various other products, including a pectin-lecithin complex, have not demonstrated efficacy despite initial promise.[1389]

The use of antacids in the treatment of gastric ulcers has not been critically examined in the horse. Research in horses has shown that 30 g aluminum hydroxide/15 g magnesium hydroxide will result in an increase in gastric pH above 4 for approximately 2 hours.[1390] Although antacids may be useful for the treatment of ulcers in horses, a dose of approximately 180 to 200 mL every 2 to 4 hours is necessary for a standard adult horse, and the use of antacids is not justified or recommended.

Other Disorders of the Stomach

Pyloric Obstruction and Delayed Gastric Emptying

Pyloric stenosis is a structural resistance to gastric outflow. Congenital pyloric stenosis has been reported in foals and one yearling and results from hypertrophy of the pyloric musculature.[1061,1391,1392] Acquired pyloric stenosis can result from neoplasia or duodenal ulceration.[1393-1396] Clinical signs are dependent on the degree of obstruction but include abdominal pain, salivation, and teeth grinding. Complete or near complete obstruction can result in gastric reflux and reflux esophagitis. In foals with congenital pyloric hypertrophy, clinical signs may begin with the consumption of solid feed. In foals, a presumptive diagnosis can be made via gastric endoscopy and radiography (plain and contrast studies). Depending on the cause and severity of disease, gastric endoscopy may provide a presumptive diagnosis in the adult horse. Measurement of gastric emptying can aid the diagnosis. Several methods of measurement are currently available, including nuclear scintigraphy, acetaminophen absorption, and postconsumption [^{13}C]octanoic acid blood or breath testing.[986,1006,1397] During an exploratory laparotomy, a distended stomach and thickened pylorus are accompanied by a relatively empty intestinal tract.

If complete obstruction is not present, medical therapy with a prokinetic such as bethanechol can increase the rate of gastric emptying.[1006] Phenylbutazone and cisapride have also been shown to attenuate the delay in gastric emptying caused by endotoxin administration.[986,987] Surgical repair is necessary for definitive treatment of complete or near-complete obstruction and consists of either gastroenterostomy or pyloroplasty.[1075,1380] Pyloric-duodenal intussusception has been reported in an adult horse presenting for colic.[1398]

Gastric Dilatation and Rupture

Gastric dilatation can be classified as primary, secondary, or idiopathic. Causes of primary gastric dilatation include gastric impaction, grain engorgement, excessive water intake after exercise, aerophagia, and parasitism.[1396,1399] Secondary gastric dilatation occurs more commonly and can result from primary intestinal ileus or small or large intestinal obstruction. Time to development of gastric reflux is proportional to the distance to the intestinal segment involved, with duodenal obstruction resulting in reflux within 4 hours.[1400] Clinical signs of gastric dilatation include those associated with acute colic and, in severe cases, ingesta appearing at the nares. Associated laboratory abnormalities include hemoconcentration, hypokalemia, and hypochloremia.[1396]

The most common cause of gastric rupture in horses varies among reports. In a retrospective study of 54 horses, gastric rupture occurred most commonly as a secondary phenomenon (65%), usually caused by small intestinal obstruction, with primary gastric dilatation and idiopathic rupture occurring almost equally (15% and 17%, respectively).[1399] In another retrospective study of 50 horses in combination with a search of the Veterinary Medical Database (VMDB), 60% of the gastric rupture cases were classified as idiopathic.[1401] Risk factors for gastric rupture include feeding grass hay, not feeding grain, gelding, and a nonautomatic water source.[1399,1401] Nasogastric intubation does not preclude the possibility of gastric rupture, and the amount of reflux obtained before rupture is highly variable.[1399] Because of the retrospective nature of these reports, one cannot rule out confounding factors with certainty.

Regardless of the initiating cause, gastric rupture usually occurs along the greater curvature. In horses with rupture caused by gastric dilatation, tears in the seromuscular layer are frequently larger than the corresponding tears in the mucosal layer, indicating that the seromuscularis likely weakens and tears before the mucosa.[1399,1401] In contrast, horses with gastric rupture secondary to gastric ulceration usually demonstrate full-thickness tears of equal size in all layers. Gastric rupture is usually fatal because of widespread contamination of the peritoneal cavity, septic peritonitis, and septic shock. Initial clinical signs vary with the primary disease; however, when rupture occurs, an animal previously in pain can exhibit signs of relief. Subsequent signs are consistent with peritonitis and shock, including tachypnea, tachycardia, sweating, and muscle fasciculations. Surgical repair is difficult but has been reported for partial-thickness tears,[1402] and, in one case of a combined tear of the mucosa and muscularis with only a focal serosal tear, a full-thickness repair was performed with a favorable outcome.[1403]

Gastric Impaction

Gastric impaction can result in either acute or chronic signs of colic in the horse. Although a specific cause is not always evident, ingestion of coarse roughage (straw bedding and poor-quality forage), foreign objects (rubber fencing material), and feed that may swell after ingestion or improper mastication (persimmon seeds, mesquite beans, wheat, barley, and sugar beet pulp) has been implicated.[1404-1407] Possible predisposing factors include poor dentition, poor mastication and rapid consumption of feedstuffs, and inadequate water consumption. Clinical signs can vary from anorexia and weight loss to those consistent with severe abdominal pain. In severe cases, spontaneous reflux may occur, with gastric contents visible at the nares. In cases presenting with acute severe abdominal pain, a diagnosis is often made during exploratory celiotomy. In animals not exhibiting signs of colic warranting surgical intervention, an endoscopic finding of a full stomach after a normally adequate fast (18–24 hours) can often confirm the diagnosis. Abdominal radiographs are reserved for smaller horses and ponies. In addition to pain management, specific treatment consists of gastric lavage via nasogastric intubation or massage and injection of fluid to soften the impaction during laparotomy.[1404-1406]

Miscellaneous Causes of Gastritis

Nonulcerative gastritis rarely appears to be a clinical problem in the horse, but it has been reported at necropsy in a large retrospective study.[1302] Emphysematous gastritis caused by *C. perfringens*[1408] and *C. septicum*[1409] has been reported.

Neoplasia of the Alimentary Tract

Neoplasia in the alimentary tract of the horse is uncommon.[1410] Primary and metastatic neoplasia can affect multiple locations within the oral cavity and gastrointestinal tract, and occurrence is not isolated to geriatric horses. The alimentary form of lymphoma occurs commonly in horses less than 5 years of age.[1411] In one study, Arabian horses were 4.5 times more likely to have intestinal neoplasia relative to other breeds.[1412] Identification of tumor type is important for formulating a prognosis and therapeutic plan.

CLINICAL SIGNS

The clinical signs associated with alimentary neoplasia are related to the tumor location. For example, oral tumors can cause cutaneous enlargement; tumors of the tongue can cause quidding, dysphagia, and halitosis[1413]; tumors of the esophagus cause dysphagia, ptyalism, intermittent colic, weight loss, and halitosis[1219,1414,1415]; and gastric neoplasia is associated with anorexia, weight loss, abdominal distention, and intermittent fever.[1416] Abdominal neoplasia has been implicated in 4% of horses with intermittent or chronic colic.[1417,1418] Weight loss is the most common clinical sign of horses with intestinal neoplasia.[1412] Acute signs of colic can occur with intestinal obstructions from malignant or benign tumors. Paraneoplastic syndromes can occur in the horse. These include weight loss despite adequate caloric intake (cancer cachexia), ectopic hormone production, anemia, leukocytosis, thrombocytopenia, hypergammaglobulinemia, fever, and neurologic abnormalities.[1419]

Diagnostic Evaluation

Diagnosis of alimentary neoplasia can be challenging. Data collected from a complete blood count, biochemistry panel and urinalysis are rarely confirmatory. Normocytic normochromic anemia of chronic disease is the most common red cell abnormality associated with abdominal neoplasia; blood loss and hemolytic anemia occur less frequently.[1420] Peripheral eosinophilia and MEED have been reported with lymphoma.[1421] Leukocytosis and hyperfibrinogenemia are common. The most common biochemical abnormalities include hypoalbuminemia, hyperglobulinemia, and hypercalcemia.[1422,1423] Hypoglycemia can occur with pancreatic or hepatic neoplasia.[1423]

Rectal examination may detect an abdominal mass, thickening of the intestinal wall, or lymph node enlargement. Rectal biopsy can detect diffuse lymphoma in some cases.[489] Some tumors can exfoliate into the peritoneal cavity, allowing diagnosis via abdominocentesis.[1414,1424-1426] Collecting a large volume of fluid followed by Cytospin and subsequent cytologic evaluation may improve the diagnostic potential; characterization as an inflammatory exudate or modified transudate without any neoplastic cells present is common. Neoplasia was the apparent cause of hemoperitoneum in 13% of cases in one report of 54 cases.[1427] Abdominocentesis accurately predicted neoplasia in 11 of 25 cases in another study.[769]

Specific immunoglobulin testing for IgM deficiency may aid in the diagnosis of lymphoma.[1428] DNA cell cycle analysis of suspect neoplastic cells has been used to detect lymphoma in equine patients confirmed with the disease. This method of evaluating fluid or tissue aspirates may increase the accuracy for diagnosing neoplasia in the future.[1429]

Previously mentioned diagnostics, such as endoscopy, contrast radiography, and cross-sectional imaging may be helpful depending on tumor location. For abdominal neoplasia, transcutaneous ultrasonography is often most useful. An experienced sonographer and thorough examination are likely to improve diagnostic yield.[46] Laparoscopy or exploratory laparotomy may be required to obtain a final diagnosis.

Specific Tumors

Lymphoma is the most common neoplasia in the horse and has been divided into four categories[1412]; only the intestinal/alimentary form will be discussed. The term *lymphoma* is preferred over lymphosarcoma because there is no benign form of this disease.[1423] Lymphoma originates from lymphoid tissue and predominantly affects intestinal lymph nodes. Chronic weight loss, intermittent colic, and fever are the most common clinical findings[502,1430]; chronic diarrhea,[1431] pruritus, and alopecia[1432] have been reported. Peripheral lymphadenopathy is not generally noted, but enlargement of mesenteric lymphadenopathy may be palpable rectally. Therapeutic large colon resection has been reported in two horses with lymphoma.[1433]

SCC is the second most common neoplasia in the horse and is the most common tumor of the proximal gastrointestinal tract.[1434] In the oral cavity SCC may affect the lips, tongue, hard palate, pharynx, and oral mucosa.[1435,1436] Metastasis beyond the regional lymph nodes is rare for oral, esophageal, and SCC, although it is possible. Abnormal masses were palpable rectally in 4 of 5 cases of gastric SCC.[1416] Most discussions of SCC treatment about the head are based on dermal, ocular, and adnexal SCC, which are more common. Available treatment includes surgical resection, iridium-192 brachytherapy, 5-fluorouracil, and intralesional cisplatin or carboplatin.[1437-1441] The prognosis for survival is good if complete resection is possible, although it typically is not for gastric SCC.[1423] Local recurrence is possible. One report of recurring SCC on the lip with metastasis to the lymph nodes was successfully treated with piroxicam over 58 months.[1442] Hypertrophic osteopathy secondary to gastric SCC has been documented in one horse.[1443]

Adenocarcinoma has been reported in the small intestine, cecum, and large colon.[1412,1434,1444] The tumor arises from the glandular crypts of the gastrointestinal tract and has been reported in middle-aged and older horses. Metastasis to the lymph nodes, liver, and lungs is possible; osseous metaplasia has also been reported.[1445] In a retrospective study of intestinal neoplasia, adenocarcinoma represented 32% of cases, 82% of which involved the small intestine.[1412] The short-term prognosis for resectable adenocarcinoma is fair; long-term prognosis remains poor.[1412,1446]

Leiomyosarcoma and leiomyoma are malignant and benign, respectively, tumors of the smooth muscle lining the gastrointestinal tract. Both have been reported in the stomach, small intestine, and small colon/rectum; leiomyoma has also been reported in the omentum.[1434,1447-1453] Clinical signs are consistent with intestinal obstruction, and prognosis is favorable with resection of the affected intestinal segment.

Lipoma is a benign tumor that occurs in older horses arising from mesenteric adipocytes. Clinical signs do not typically occur unless the tumor stalk results in intestinal obstruction.

Oral cavity neoplasia may involve the dental tissue (odontogenic tumors), bone (osteogenic tumors), or soft

tissues. Ameloblastoma occurs in horses >10 years old, and it mainly affects the mandible. Ameloblastic odontoma affects younger horses and usually involves the maxilla. Both are benign but locally invasive. Radiographs may distinguish between an ameloblastoma (radiolucent lesion) and ameloblastic odontoma (radiolucent lesion with partially mineralized density). The best treatment option is surgical resection and/or radiation therapy regardless of the type.[1454]

Juvenile mandibular ossifying fibroma occurs in the rostral mandible of young horses between the age of 2 months and 2 years. It may cause significant distortion of the bone. Surgical excision of the mass has a good prognosis if diagnosed early.[1455]

Melanomas, sarcoids, SCC, and papilloma can occur on the mouth and lips. Melanomas are commonly found in the commissure of the lips and parotid salivary glands and can metastasize to regional lymph nodes. Sarcoids can cause local ulcerations of the buccal mucosa, which are difficult to treat. Intralesional cisplatin, cryosurgery, radiation, and laser excision have been tried with limited success. Equine papilloma virus is responsible for the common skin wart that is found on the lips and muzzle of young horses. These lesions are typically self-limiting, but cryosurgery or excision can be used for removal, if desired.

REFERENCES

1. Sellers A, Lowe J. Visualization of auscultation sounds of the large intestine. *Proc Am Assoc Equine Pract*. 1983;29:359–364.
2. Argenzio RA. Functions of the equine large intestine and their interrelationship in disease. *Cornell Vet*. 1975;65:303–330.
3. Adams SB. Equine intestinal motility: an overview of normal activity, changes in disease, and effects of drug administration. *Proc Am Assoc Equine Pract*. 1987;33:539–553.
4. Lester GD, Merritt AM, Neuwirth L, et al. Effect of alpha 2-adrenergic, cholinergic, and nonsteroidal anti-inflammatory drugs on myoelectric activity of ileum, cecum, and right ventral colon and on cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res*. 1998;59:320–327.
5. Naylor JM, Poirier KL, Hamilton DL, et al. The effects of feeding and fasting on gastrointestinal sounds in adult horses. *J Vet Intern Med*. 2006;20:1408–1413.
6. Parry BW, Anderson GA, Gay CC. Prognosis in Equine Colic—A Study of Individual Variables Used in Case Assessment. *Equine Vet J*. 1983;15:337–344.
7. King JN, Gerring EL. Observations on the colic motor complex in a pony with a small intestinal obstruction. *Equine Vet J Suppl*. 1989:43–45.
8. Blikslager AT, Roberts MC. Accuracy of clinicians in predicting site and type of lesion as well as outcome in horses with colic. *J Am Vet Med Assoc*. 1995;207:1444–1447.
9. Navarro M, Monreal L, Segura D, et al. A comparison of traditional and quantitative analysis of acid-base and electrolyte imbalances in horses with gastrointestinal disorders. *J Vet Intern Med*. 2005;19:871–877.
10. Grosche A, Morton AJ, Graham AS, et al. Effect of large colon ischemia and reperfusion on concentrations of calprotectin and other clinicopathologic variables in jugular and colonic venous blood in horses. *Am J Vet Res*. 2013;74:1281–1290.
11. Delesalle C, Dewulf J, Lefebvre RA, et al. Determination of lactate concentrations in blood plasma and peritoneal fluid in horses with colic by an Accusport analyzer. *J Vet Intern Med*. 2007;21:293–301.
12. Tennent-Brown BS, Wilkins PA, Lindborg S, et al. Sequential plasma lactate concentrations as prognostic indicators in adult equine emergencies. *J Vet Intern Med*. 2010;24:198–205.
13. Nieto JE, Dechant JE, le Jeune SS, et al. Evaluation of 3 handheld portable analyzers for measurement of L-lactate concentrations in blood and peritoneal fluid of horses with colic. *Vet Surg*. 2015;44:366–372.
14. Saulez MN, Cebra CK, Dailey M. Comparative biochemical analyses of venous blood and peritoneal fluid from horses with colic using a portable analyser and an in-house analyser. *Vet Rec*. 2005;157:217–223.
15. Gossett KA, Cleghorn B, Adams R, et al. Contribution of whole blood L-lactate, pyruvate, D-lactate, acetoacetate, and 3-hydroxybutyrate concentrations to the plasma anion gap in horses with intestinal disorders. *Am J Vet Res*. 1987;48:72–75.
16. Gossett KA, Cleghorn B, Martin GS, et al. Correlation between anion gap, blood L lactate concentration and survival in horses. *Equine Vet J*. 1987;19:29–30.
17. Gardner RB, Nydam DV, Mohammed HO, et al. Serum gamma glutamyl transferase activity in horses with right or left dorsal displacements of the large colon. *J Vet Intern Med*. 2005;19:761–764.
18. Andrews FM, Hamlin RL, Stalnaker PS. Blood viscosity in horses with colic. *J Vet Intern Med*. 1990;4:183–186.
19. Johnston JK, Morris DD. Comparison of Duodenitis Proximal Jejunitis and Small Intestinal-Obstruction in Horses—68 Cases (1977-1985). *J Am Vet Med Assoc*. 1987;191:849–854.
20. Hunt E, Tennant B, Whitlock RH. Interpretation of peritoneal fluid erythrocyte counts in horses with abdominal disease. *Proc Equine Colic Res Symp*. 1986;2:168–174.
21. Estepa JC, Lopez I, Mayer-Valor R, et al. The influence of anticoagulants on the measurement of total protein concentration in equine peritoneal fluid. *Res Vet Sci*. 2006;80:5–10.
22. Latson KM, Nieto JE, Beldomenico PM, et al. Evaluation of peritoneal fluid lactate as a marker of intestinal ischaemia in equine colic. *Equine Vet J*. 2005;37:342–346.
23. Turner A, McIlwraith C, Trotter G, et al. Biochemical analysis of serum and peritoneal fluid in experimental colonic infarction in horses. *Proc Equine Colic Res Symp*. 1984:79–87.
24. Arden WA, Stick JA. Serum and peritoneal fluid phosphate concentrations as predictors of major intestinal injury associated with equine colic. *J Am Vet Med Assoc*. 1988;193:927–931.
25. Parry BW. Use of clinical pathology in evaluation of horses with colic. *Vet Clin North Am Equine Pract*. 1987;3:529–542.
26. Yamout SZ, Nieto JE, Beldomenico PM, et al. Peritoneal and Plasma D-lactate Concentrations in Horses with Colic. *Vet Surg*. 2011;40:817–824.
27. Peloso JG, Cohen ND. Use of serial measurements of peritoneal fluid lactate concentration to identify strangulating intestinal lesions in referred horses with signs of colic. *J Am Vet Med Assoc*. 2012;240:1208–1217.
28. Bach LG, Ricketts SW. Paracentesis as an aid to the diagnosis of abdominal disease in the horse. *Equine Vet J*. 1974;6:116–121.
29. Morris DD, Whitlock RH, Palmer JE. Fecal Leukocytes and Epithelial—Cells in Horses with Diarrhea. *Cornell Vet*. 1983;73:265–274.
30. Pellegrini FL. Results of a large-scale necroscopic study of equine colonic ulcers. *J Equine Vet Sci*. 2005;25:113–117.
31. Sykes BJJ, Hallowell GD. Evaluation of a commercial faecal blood test for the diagnosis of gastric ulceration in Thoroughbred racehorses: a preliminary report. *Proc Equine Colic Res Symp*. 2014;11:4.
32. Burgess BA, Weller CB, Pabilonia KL, et al. Detection of different serotypes of Salmonella enterica in experimentally inoculated equine fecal samples by commercially available rapid tests. *J Vet Intern Med*. 2014;28:1853–1859.
33. Ekiri AB, Long MT, Hernandez JA. Diagnostic performance and application of a real-time PCR assay for the detection of Salmonella in fecal samples collected from hospitalized horses with or without signs of gastrointestinal tract disease. *Vet J*. 2016;208:28–32.

34. Pusterla N, Byrne BA, Hodzic E, et al. Use of quantitative real-time PCR for the detection of Salmonella spp in fecal samples from horses at a veterinary teaching hospital. *Vet J*. 2010;186:252–255.
35. Ward MP, Alinovi CA, Couetil LL, et al. Evaluation of a PCR to detect Salmonella in fecal samples of horses admitted to a veterinary teaching hospital. *J Vet Diagn Invest*. 2005;17:118–123.
36. King JN, Davies JV, Gerring EL. Contrast radiography of the equine oesophagus: effect of spasmolytic agents and passage of a nasogastric tube. *Equine Vet J*. 1990;22:133–135.
37. Hassel DM, Langer DL, Snyder JR, et al. Evaluation of enterolithiasis in equids: 900 cases (1973-1996). *J Am Vet Med Assoc*. 1999;214:233–237.
38. Keppie NJ, Rosenstein DS, Holcombe SJ, et al. Objective radiographic assessment of abdominal sand accumulation in horses. *Vet Radiol Ultrasound*. 2008;49:122–128.
39. Ruohoniemi M, Kaikkonen R, Raekallio M, et al. Abdominal radiography in monitoring the resolution of sand accumulations from the large colon of horses treated medically. *Equine Vet J*. 2001;33:59–64.
40. Yarbrough TB, Langer DL, Snyder JR, et al. Abdominal radiography for diagnosis of enterolithiasis in horses: 141 cases (1990-1992). *J Am Vet Med Assoc*. 1994;205:592–595.
41. Campbell ML, Ackerman N, Peyton LC. Radiographic Gastrointestinal Anatomy of the Foal. *Veterinary Radiol*. 1984;25:194–204.
42. Fischer AT, Kerr LY, Obrien TR. Radiographic Diagnosis of Gastrointestinal Disorders in the Foal. *Veterinary Radiol*. 1987;28:42–48.
43. Fischer AT, Yarbrough TY. Retrograde contrast radiography of the distal portions of the intestinal tract in foals. *J Am Vet Med Assoc*. 1995;207:734–737.
44. Lester GD, Lester NV. Abdominal and thoracic radiography in the neonate. *Vet Clin North Am Equine Pract*. 2001;17:19–46. v.
45. Busoni V, De Busscher V, Lopez D, et al. Evaluation of a protocol for fast localised abdominal sonography of horses (FLASH) admitted for colic. *Vet J*. 2011;188:77–82.
46. le Jeune S, Whitcomb MB. Ultrasound of the Equine Acute Abdomen. *Vet Clin North Am Equine Pract*. 2014;30:353–381.
47. Korolainen R, Kaikkonen R, Ruohoniemi M. Ultrasonography in monitoring the resolution of intestinal sand accumulations in the horse. *Equine Vet Educ*. 2003;15:331–336.
48. Weller R, Cauvin ER, Bowen IM, et al. Comparison of radiography, scintigraphy and ultrasonography in the diagnosis of a case of temporomandibular joint arthropathy in a horse. *Vet Rec*. 1999;144:377–379.
49. Weller R, Livesey L, Maierl J, et al. Comparison of radiography and scintigraphy in the diagnosis of dental disorders in the horse. *Equine Vet J*. 2001;33:49–58.
50. East LM, Trumble TN, Steyn PF, et al. The application of technetium-99m hexamethylpropyleneamine oxime (99mTc-HMPAO) labeled white blood cells for the diagnosis of right dorsal ulcerative colitis in two horses. *Vet Radiol Ultrasound*. 2000;41:360–364.
51. Lohmann KL, Roussel AJ, Cohen ND, et al. Comparison of nuclear scintigraphy and acetaminophen absorption as a means of studying gastric emptying in horses. *Am J Vet Res*. 2000;61:310–315.
52. Morrow KL, Park RD, Spurgeon TL, et al. Computed tomographic imaging of the equine head. *Vet Radiol Ultrasound*. 2000;41:491–497.
53. Tietje S, Becker M, Bockenhoff G. Computed tomographic evaluation of head diseases in the horse: 15 cases. *Equine Vet J*. 1996;28:98–105.
54. Carmalt JL, Kneissl S, Rawlinson JE, et al. Computed tomographic appearance of the temporomandibular joint in 1018 Asymptomatic Horses: A Multi-Institution Study. *Vet Radiol Ultrasound*. 2016;57:237–245.
55. Manso-Diaz G, Dyson SJ, Dennis R, et al. Magnetic resonance imaging characteristics of equine head disorders: 84 cases (2000-2013). *Vet Radiol Ultrasound*. 2015;56:176–187.
56. Beccati F, Cercone M, Angeli G, et al. Imaging diagnosis—use of multiphase computed tomographic urography in the diagnosis of ureteral tear in a 6-day-old foal. *Vet Radiol Ultrasound*. 2016;57:E10–E15.
57. Breukink HJ. Oral mono- and disaccharide tolerance tests in ponies. *Am J Vet Res*. 1974;35:1523–1527.
58. Fintl C, Ihler CF. The effect of sedation on D(+)-xylose absorption tests in 6 normal horses. *Equine Vet J Suppl*. 2011:149–152.
59. Mair TS, Hillyer MH, Taylor FG, et al. Small intestinal malabsorption in the horse: an assessment of the specificity of the oral glucose tolerance test. *Equine Vet J*. 1991;23:344–346.
60. Murphy D, Reid SW, Love S. The effect of age and diet on the oral glucose tolerance test in ponies. *Equine Vet J*. 1997;29:467–470.
61. Jacobs KA, Norman P, Hodgson DRG, et al. Effect of Diet on the Oral D-Xylose Absorption Test in the Horse. *Am J Vet Res*. 1982;43:1856–1858.
62. Pratt SE, Geor RJ, McCutcheon LJ. Effects of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in Standardbred horses. *Equine Vet J Suppl*. 2006:579–584.
63. De La Corte FD, Valberg SJ, MacLeay JM, et al. Glucose uptake in horses with polysaccharide storage myopathy. *Am J Vet Res*. 1999;60:458–462.
64. Freeman DE, Ferrante PL, Kronfeld DS, et al. Effect of food deprivation on d-xylose absorption test results in mares. *Am J Vet Res*. 1989;50:1609–1612.
65. Bolton JR, Merritt AM, Cimprich RE, et al. Normal and abnormal xylose absorption in the horse. *Cornell Vet*. 1976;66:183–197.
66. Ferrante PL, Freeman DE, Ramberg CF, et al. Kinetic analysis of d-xylose absorption after its intragastric administration to mares deprived of food. *Am J Vet Res*. 1993;54:2110–2114.
67. Ferrante PL, Freeman DE, Ramberg CF, et al. Kinetic-Analysis of d-Xylose Distribution After Intravenous Administration to Mares. *Am J Vet Res*. 1993;54:147–151.
68. Merritt T, Mallonee PG, Merritt AM. d-xylose absorption in the growing foal. *Equine Vet J*. 1986;18:298–300.
69. Roberts MC. Carbohydrate digestion and absorption studies in the horse. *Res Vet Sci*. 1975;18:64–69.
70. Sutton DG, Bahr A, Preston T, et al. Validation of the 13C-octanoic acid breath test for measurement of equine gastric emptying rate of solids using radioscintigraphy. *Equine Vet J*. 2003;35:27–33.
71. Doherty TJ, Andrews FM, Abraha TW, et al. Metoclopramide ameliorates the effects of endotoxin on gastric emptying of acetaminophen in horses. *Can J Vet Res*. 1999;63:37–40.
72. Lohmann KL, Bahr A, Cohen ND, et al. Evaluation of acetaminophen absorption in horses with experimentally induced delayed gastric emptying. *Am J Vet Res*. 2002;63:170–174.
73. Clements JA, Heading RC, Nimmo WS, et al. Kinetics of acetaminophen absorption and gastric emptying in man. *Clin Pharmacol Ther*. 1978;24:420–431.
74. Wyse CA, Murphy DM, Preston T, et al. The(13)C-octanoic acid breath test for detection of effects of meal composition on the rate of solid-phase gastric emptying in ponies. *Res Vet Sci*. 2001;71:81–83.
75. Day MJ, Bilzer T, Mansell J, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. *J Comp Pathol*. 2008;138(suppl 1):S1–S43.
76. Hendrickson DA, Wilson DG. Instrumentation and techniques for laparoscopic and thoracoscopic surgery in the horse. *Vet Clin North Am Equine Pract*. 1996;12:235–259.

77. Trostle S. Gastrointestinal endoscopic surgery. *Vet Clin North Am Equine Pract*. 2000;16:329–341.
78. Albanese V, Hanson RR, McMaster MA, et al. Use of a Barbed Knotless Suture for Laparoscopic Ablation of the Nephrosplenic Space in 8 Horses. *Vet Surg*. 2016;45:824–830.
79. Bracamonte JL, Duke-Novakovski T. A pilot study evaluating laparoscopic closure of the nephrosplenic space using an endoscopic suturing device in standing horses. *Can Vet J*. 2016;57:651–654.
80. van Bergen T, Wiemer P, Schauvliege S, et al. Laparoscopic Evaluation of the Epiploic Foramen after Celiotomy for Epiploic Foramen Entrapment in the Horse. *Vet Surg*. 2016;45:596–601.
81. Kagnoff MF, Eckmann L. Epithelial cells as sensors for microbial infection. *J Clin Invest*. 1997;100:6–10.
82. Huber AR, Kunkel SL, Todd 3rd RF, et al. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science*. 1991;254:99–102.
83. McCormick BA, Colgan SP, Delp-Archer C, et al. Salmonella typhimurium attachment to human intestinal epithelial monolayers: transcellular signalling to subepithelial neutrophils. *J Cell Biol*. 1993;123:895–907.
84. McCormick BA, Miller SI, Carnes D, et al. Transepithelial signaling to neutrophils by salmonellae: a novel virulence mechanism for gastroenteritis. *Infect Immun*. 1995;63:2302–2309.
85. Jung HC, Eckmann L, Yang SK, et al. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J Clin Invest*. 1995;95:55–65.
86. Akira S. Toll-like receptors and innate immunity. *Adv Immunol*. 2001;78:1–56.
87. Malaviya R, Abraham SN. Mast cell modulation of immune responses to bacteria. *Immunol Rev*. 2001;179:16–24.
88. Pothoulakis C, Castagliuolo I, LaMont JT, et al. CP-96,345, a substance P antagonist, inhibits rat intestinal responses to Clostridium difficile toxin A but not cholera toxin. *Proc Natl Acad Sci U S A*. 1994;91:947–951.
89. Castagliuolo I, LaMont JT, Letourneau R, et al. Neuronal involvement in the intestinal effects of Clostridium difficile toxin A and Vibrio cholerae enterotoxin in rat ileum. *Gastroenterology*. 1994;107:657–665.
90. Bogdan C, Rollinghoff M, Diefenbach A. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Curr Opin Immunol*. 2000;12:64–76.
91. Blikslager AT, Moeser AJ, Gookin JL, et al. Restoration of barrier function in injured intestinal mucosa. *Physiol Rev*. 2007;87:545–564.
92. Joris I, Cuenoud HF, Doern GV, et al. Capillary leakage in inflammation. A study by vascular labeling. *Am J Pathol*. 1990;137:1353–1363.
93. Joris I, Majno G, Corey EJ, et al. The mechanism of vascular leakage induced by leukotriene E4. Endothelial contraction. *Am J Pathol*. 1987;126:19–24.
94. Brett J, Gerlach H, Nawroth P, et al. Tumor necrosis factor/cachectin increases permeability of endothelial cell monolayers by a mechanism involving regulatory G proteins. *J Exp Med*. 1989;169:1977–1991.
95. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell*. 1994;76:301–314.
96. Harlan JM, Killen PD, Harker LA, et al. Neutrophil-mediated endothelial injury in vitro mechanisms of cell detachment. *J Clin Invest*. 1981;68:1394–1403.
97. Rosengren S, Olofsson AM, von Andrian UH, et al. Leukotriene B4-induced neutrophil-mediated endothelial leakage in vitro and in vivo. *J Appl Physiol*. 1985;1991(71):1322–1330.
98. Hernandez LA, Grisham MB, Twohig B, et al. Role of neutrophils in ischemia-reperfusion-induced microvascular injury. *Am J Physiol*. 1987;253:H699–H703.
99. Coe DA, Freischlag JA, Johnson D, et al. Pentoxifylline prevents endothelial damage due to ischemia and reperfusion injury. *J Surg Res*. 1997;67:21–25.
100. Nakagawa K, Miller FN, Knott AW, et al. Pentoxifylline inhibits FMLP-induced macromolecular leakage. *Am J Physiol*. 1995;269:H239–H245.
101. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol*. 2006;6:173–182.
102. Dallegri F, Ottonello L. Tissue injury in neutrophilic inflammation. *Inflamm Res*. 1997;46:382–391.
103. Giannella RA. Importance of the intestinal inflammatory reaction in salmonella-mediated intestinal secretion. *Infect Immun*. 1979;23:140–145.
104. Kubes P, Hunter J, Granger DN. Ischemia/reperfusion-induced feline intestinal dysfunction: importance of granulocyte recruitment. *Gastroenterology*. 1992;103:807–812.
105. Elliott E, Li Z, Bell C, et al. Modulation of host response to Escherichia coli o157: H7 infection by anti-CD18 antibody in rabbits. *Gastroenterology*. 1994;106:1554–1561.
106. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol*. 1990;259:G462–G467.
107. Ley K, Tedder TF. Leukocyte interactions with vascular endothelium. New insights into selectin-mediated attachment and rolling. *J Immunol*. 1995;155:525–528.
108. Brown EJ, Lindberg FP. Leucocyte adhesion molecules in host defence against infection. *Ann Med*. 1996;28:201–208.
109. Nagahata H, Kehrli Jr ME, Murata H, et al. Neutrophil function and pathologic findings in Holstein calves with leukocyte adhesion deficiency. *Am J Vet Res*. 1994;55:40–48.
110. Anderson DC, Springer TA. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med*. 1987;38:175–194.
111. Issekutz AC, Issekutz TB. Monocyte migration to arthritis in the rat utilizes both CD11/CD18 and very late activation antigen 4 integrin mechanisms. *J Exp Med*. 1995;181:1197–1203.
112. Shuster DE, Kehrli Jr ME, Ackermann MR. Neutrophilia in mice that lack the murine IL-8 receptor homolog. *Science*. 1995;269:1590–1591.
113. Huang AJ, Furie MB, Nicholson SC, et al. Effects of human neutrophil chemotaxis across human endothelial cell monolayers on the permeability of these monolayers to ions and macromolecules. *J Cell Physiol*. 1988;135:355–366.
114. Berton G, Yan SR, Fumagalli L, et al. Neutrophil activation by adhesion: mechanisms and pathophysiological implications. *Int J Clin Lab Res*. 1996;26:160–177.
115. Rosales C, O'Brien V, Kornberg L, et al. Signal transduction by cell adhesion receptors. *Biochim Biophys Acta*. 1995;1242:77–98.
116. Wershil BK. Lessons from genetically engineered animal models—IX. Mast cell-deficient mice and intestinal biology. *Am J Phys Gastrointest Liver Physiol*. 2000;278:G343–G348.
117. Araki Y, Andoh A, Fujiyama Y, et al. Development of dextran sulphate sodium-induced experimental colitis is suppressed in genetically mast cell-deficient Ws/Ws rats. *Clin Exp Immunol*. 2000;119:264–269.
118. Stein J, Ries J, Barrett KE. Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. *Am J Physiol*. 1998;274:G203–G209.
119. Andoh A, Kimura T, Fukuda M, et al. Rapid intestinal ischaemia-reperfusion injury is suppressed in genetically mast cell-deficient Ws/Ws rats. *Clin Exp Immunol*. 1999;116:90–93.
120. Kimura T, Fujiyama Y, Sasaki M, et al. The role of mucosal mast cell degranulation and free-radical generation in intestinal ischaemia-reperfusion injury in rats. *Eur J Gastroenterol Hepatol*. 1998;10:659–666.
121. Yang PC, Berin MC, Yu L, et al. Mucosal pathophysiology and inflammatory changes in the late phase of the intestinal allergic reaction in the rat. *Am J Pathol*. 2001;158:681–690.

122. Galli SJ, Maurer M, Lantz CS. Mast cells as sentinels of innate immunity. *Curr Opin Immunol*. 1999;11:53–59.
123. Castro GA, Harari Y, Russell D. Mediators of anaphylaxis-induced ion transport changes in small intestine. *Am J Physiol*. 1987;253:G540–G548.
124. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev*. 1997;77:1033–1079.
125. Kawabata A, Matsunami M, Sekiguchi F. Gastrointestinal roles for proteinase-activated receptors in health and disease. *Br J Pharmacol*. 2008;153(suppl 1):S230–S240.
126. Bueno L, Fioramonti J. Protease-activated receptor 2 and gut permeability: a review. *Neurogastroenterol Motil*. 2008;20:580–587.
127. Malaviya R, Abraham SN. Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J Leukoc Biol*. 2000;67:841–846.
128. Malaviya R, Ikeda T, Ross E, et al. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature*. 1996;381:77–80.
129. McLachlan JB, Shelburne CP, Hart JP, et al. Mast cell activators: a new class of highly effective vaccine adjuvants. *Nat Med*. 2008;14:536–541.
130. Goldstein IM. Complement: biologically active products. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: Basic Principles and Clinical Correlates*. New York: Raven Press; 2008:63.
131. Brown EJ. Complement receptors and phagocytosis. *Curr Opin Immunol*. 1991;3:76–82.
132. Williams JP, Pechet TT, Weiser MR, et al. Intestinal reperfusion injury is mediated by IgM and complement. *J Appl Physiol (1985)*. 1999;86:938–942.
133. Wada K, Montalto MC, Stahl GL. Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat. *Gastroenterology*. 2001;120:126–133.
134. Eror AT, Stojadinovic A, Starnes BW, et al. Antiinflammatory effects of soluble complement receptor type 1 promote rapid recovery of ischemia/reperfusion injury in rat small intestine. *Clin Immunol*. 1999;90:266–275.
135. Austen Jr WG, Kyriakides C, Favuzza J, et al. Intestinal ischemia-reperfusion injury is mediated by the membrane attack complex. *Surgery*. 1999;126:343–348.
136. Cochrane CG, Griffin JH. The biochemistry and pathophysiology of the contact system of plasma. *Adv Immunol*. 1982;33:241–306.
137. Bockmann S, Paegelow I. Kinins and kinin receptors: importance for the activation of leukocytes. *J Leukoc Biol*. 2000;68:587–592.
138. Stadnicki A, Sartor RB, Janardham R, et al. Specific inhibition of plasma kallikrein modulates chronic granulomatous intestinal and systemic inflammation in genetically susceptible rats. *FASEB J*. 1998;12:325–333.
139. Stadnicki A, Sartor RB, Janardham R, et al. Kallikrein-kininogen system activation and bradykinin (B2) receptors in indomethacin induced enterocolitis in genetically susceptible Lewis rats. *Gut*. 1998;43:365–374.
140. Arai Y, Takanashi H, Kitagawa H, et al. Effect of icatibant, a bradykinin B2 receptor antagonist, on the development of experimental ulcerative colitis in mice. *Dig Dis Sci*. 1999;44:845–851.
141. Thiagarajan RR, Winn RK, Harlan JM. The role of leukocyte and endothelial adhesion molecules in ischemia-reperfusion injury. *Thromb Haemost*. 1997;78:310–314.
142. Mashimo H, Goyal RK. Lessons from genetically engineered animal models. IV. Nitric oxide synthase gene knockout mice. *Am J Physiol*. 1999;277:G745–750.
143. Kelly CP, Becker S, Linevsky JK, et al. Neutrophil recruitment in Clostridium difficile toxin A enteritis in the rabbit. *J Clin Invest*. 1994;93:1257–1265.
144. Brown E. Neutrophil adhesion and the therapy of inflammation. *Semin Hematol*. 1997;34:319–326.
145. Edens HA, Parkos CA. Modulation of epithelial and endothelial paracellular permeability by leukocytes. *Adv Drug Deliv Rev*. 2000;41:315–328.
146. Suzuki M, Asako H, Kubes P, et al. Neutrophil-derived oxidants promote leukocyte adherence in postcapillary venules. *Microvasc Res*. 1991;42:125–138.
147. Scudamore CL, Jepson MA, Hirst BH, et al. The rat mucosal mast cell chymase, RMCP-II, alters epithelial cell monolayer permeability in association with altered distribution of the tight junction proteins ZO-1 and occludin. *Eur J Cell Biol*. 1998;75:321–330.
148. Scudamore CL, Thornton EM, McMillan L, et al. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med*. 1995;182:1871–1881.
149. Colgan SP, Resnick MB, Parkos CA, et al. IL-4 directly modulates function of a model human intestinal epithelium. *J Immunol*. 1994;153:2122–2129.
150. Mullin JM, Snock KV. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. *Cancer Res*. 1990;50:2172–2176.
151. Whitlock RH. Colitis: differential diagnosis and treatment. *Equine Vet J*. 1986;18:278–283.
152. Merritt AM, Bolton JR, Cimprich R. Differential diagnosis of diarrhoea in horses over six months of age. *J S Afr Vet Assoc*. 1975;46:73–76.
153. Johnson CM, Cullen JM, Roberts MC. Morphologic Characterization of Castor Oil-Induced Colitis in Ponies. *Vet Pathol*. 1993;30:248–255.
154. Roberts MC, Clarke LL, Johnson CM. Castor-oil induced diarrhoea in ponies: a model for acute colitis. *Equine Vet J Suppl*. 1989:60–67.
155. Rikihisa Y, Johnson GC, Wang YZ, et al. Loss of absorptive-capacity for sodium and chloride in the colon causes diarrhea in potomac horse fever. *Res Vet Sci*. 1992;52:353–362.
156. Cordes DO, Perry BD, Rikihisa Y, et al. Enterocolitis Caused by Ehrlichia Sp in the Horse (Potomac Horse Fever). *Vet Pathol*. 1986;23:471–477.
157. Umemura T, Ohishi H, Ikemoto Y, et al. Histopathology of colitis X in the horse. *Nippon Juigaku Zasshi*. 1982;44:717–724.
158. Ochoa R, Kern SR. The effects of Clostridium perfringens type A enterotoxin in Shetland ponies—clinical, morphologic and clinicopathologic changes. *Vet Pathol*. 1980;17:738–747.
159. Lees P, Higgins AJ. Effects of a phenylbutazone paste in ponies: model of acute nonimmune inflammation. *Am J Vet Res*. 1986;47:2359–2363.
160. Keshavarzian A, Morgan G, Sedghi S, et al. Role of reactive oxygen metabolites in experimental colitis. *Gut*. 1990;31:786–790.
161. Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med*. 1989;320:365–376.
162. Rooney JR, Bryans JT, Prickett ME, et al. Exhaustion shock in the horse. *Cornell Vet*. 1966;56:220–235.
163. Meschter CL, Tyler DE, White NA, et al. Histologic findings in the gastrointestinal tract of horses with colic. *Am J Vet Res*. 1986;47:598–606.
164. Guerrant RL, Bobak DA. Bacterial and protozoal gastroenteritis. *N Engl J Med*. 1991;325:327–340.
165. Argenzio RA. Pathophysiology of diarrhea. In: Anderson NV, ed. *Veterinary Gastroenterology*. Philadelphia, PA: Lea & Febiger; 1992.
166. Field M, Rao MC, Chang EB. Intestinal electrolyte transport and diarrheal disease (1). *N Engl J Med*. 1989;321:800–806.
167. Field M, Rao MC, Chang EB. Intestinal electrolyte transport and diarrheal disease (2). *N Engl J Med*. 1989;321:879–883.
168. Musch MW, Kachur JF, Miller RJ, et al. Bradykinin-stimulated electrolyte secretion in rabbit and guinea pig intestine. Involvement of arachidonic acid metabolites. *J Clin Invest*. 1983;71:1073–1083.

169. Perdue MH, McKay DM. Integrative immunophysiology in the intestinal mucosa. *Am J Physiol*. 1994;267:G151–G165.
170. Powell DW. Immunophysiology of intestinal electrolyte transport. In: Field M, Frizzell RA, eds. *Handbook of Physiology: the Gastrointestinal System*. Rockville, MD: American Physiology Society; 1991.
171. Argenzio RA. Physiology of diarrhea—large intestine. *J Am Vet Med Assoc*. 1978;173:667–672.
172. Field M, Graf Jr LH, Laird WJ, et al. Heat-stable enterotoxin of Escherichia coli: in vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. *Proc Natl Acad Sci USA*. 1978;75:2800–2804.
173. Mantyh CR, Pappas TN, Lapp JA, et al. Substance P activation of enteric neurons in response to intraluminal Clostridium difficile toxin A in the rat ileum. *Gastroenterology*. 1996;111:1272–1280.
174. Racusen LC, Binder HJ. Effect of prostaglandin on ion transport across isolated colonic mucosa. *Dig Dis Sci*. 1980;25:900–904.
175. Weymer A, Huott P, Liu W, et al. Chloride secretory mechanism induced by prostaglandin E1 in a colonic epithelial cell line. *J Clin Invest*. 1985;76:1828–1836.
176. Jett MF, Marshall P, Fondacaro JD, et al. Action of peptidoleukotrienes on ion transport in rabbit distal colon in vitro. *J Pharmacol Exp Ther*. 1991;257:698–705.
177. Hanglow AC, Bienenstock J, Perdue MH. Effects of platelet-activating factor on ion transport in isolated rat jejunum. *Am J Physiol*. 1989;257:G845–G850.
178. Chang EB, Musch MW, Mayer L. Interleukins 1 and 3 stimulate anion secretion in chicken intestine. *Gastroenterology*. 1990;98:1518–1524.
179. Grisham MB, Gaginella TS, von Ritter C, et al. Effects of neutrophil-derived oxidants on intestinal permeability, electrolyte transport, and epithelial cell viability. *Inflammation*. 1990;14:531–542.
180. Berschneider HM, Powell DW. Fibroblasts modulate intestinal secretory responses to inflammatory mediators. *J Clin Invest*. 1992;89:484–489.
181. Tamai H, Kachur JF, Baron DA, et al. Monochloramine, a neutrophil-derived oxidant, stimulates rat colonic secretion. *J Pharmacol Exp Ther*. 1991;257:887–894.
182. Tamai H, Gaginella TS, Kachur JF, et al. Ca-mediated stimulation of Cl secretion by reactive oxygen metabolites in human colonic T84 cells. *J Clin Invest*. 1992;89:301–307.
183. Wilson KT, Xie Y, Musch MW, et al. Sodium nitroprusside stimulates anion secretion and inhibits sodium chloride absorption in rat colon. *J Pharmacol Exp Ther*. 1993;266:224–230.
184. Perdue MH, Masson S, Wershil BK, et al. Role of mast cells in ion transport abnormalities associated with intestinal anaphylaxis. Correction of the diminished secretory response in genetically mast cell-deficient W/Wv mice by bone marrow transplantation. *J Clin Invest*. 1991;87:687–693.
185. Verspaget HW, Mulder TP, van dSV, et al. Reactive oxygen metabolites and colitis: a disturbed balance between damage and protection. A selective review. *Scand J Gastroenterol Suppl*. 1991;188:44–51.
186. Buell MG, Berin MC. Neutrophil-independence of the initiation of colonic injury. Comparison of results from three models of experimental colitis in the rat. *Dig Dis Sci*. 1994;39:2575–2588.
187. Petrone WF, English DK, Wong K, et al. Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proc Natl Acad Sci USA*. 1980;77:1159–1163.
188. Granger RN, Rutili G. Neutrophil-mediated mucosal injury: role of reactive oxygen metabolites. *Dig Dis Sci*. 1988;33:S6–S15.
189. Miller MJ, Zhang XJ, Barkemeyer B, et al. Rabbit gut permeability in response to histamine chloramines and chemotactic peptide. *Gastroenterology*. 1992;103:1537–1546.
190. Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology*. 1984;87:1344–1350.
191. Wardle TD, Hall L, Turnberg LA. Inter-relationships between inflammatory mediators released from colonic mucosa in ulcerative colitis and their effects on colonic secretion. *Gut*. 1993;34:503–508.
192. McConnico RS, Roberts MC, Poston MB. The interrelationship between arachidonic acid and reactive oxygen metabolites with neutrophilic infiltration in the large intestine in a pony model of acute colitis. *Proc ACVIM Forum*. 1994;12:A1016.
193. Grisham MB, Benoit JN, Granger DN. Assessment of leukocyte involvement during ischemia and reperfusion of intestine. *Methods Enzymol*. 1990;186:729–742.
194. Moore JN, Morris DD. Endotoxemia and septicemia in horses: experimental and clinical correlates. *J Am Vet Med Assoc*. 1992;200:1903–1914.
195. Hinterleitner TA, Powell DW. Immune system control of intestinal ion transport. *Proc Soc Exp Biol Med*. 1991;197:249–260.
196. Karayalcin SS, Sturbaum CW, Wachsman JT, et al. Hydrogen peroxide stimulates rat colonic prostaglandin production and alters electrolyte transport. *J Clin Invest*. 1990;86:60–68.
197. Bern MJ, Sturbaum CW, Karayalcin SS, et al. Immune system control of rat and rabbit colonic electrolyte transport. Role of prostaglandins and enteric nervous system. *J Clin Invest*. 1989;83:1810–1820.
198. Clarke LL, Argenzio RA. NaCl transport across equine proximal colon and the effect of endogenous prostanoids. *Am J Physiol*. 1990;259:G62–G69.
199. van Deventer SJ, ten Cate JW, Tytgat GN. Intestinal endotoxemia. Clinical significance. *Gastroenterology*. 1988;94:825–831.
200. Ciancio MJ, Vitiritti L, Dhar A, et al. Endotoxin-induced alterations in rat colonic water and electrolyte transport. *Gastroenterology*. 1992;103:1437–1443.
201. Eades SC, Moore JN. Blockade of endotoxin-induced cecal hypoperfusion and ileus with an alpha 2 antagonist in horses. *Am J Vet Res*. 1993;54:586–590.
202. Katz AJ, Rosen FS. Gastro Intestinal Complications of Immuno Deficiency Syndromes. *Ciba Found Symp*. 1977:243–261.
203. Mair TS, Taylor FG, Harbour DA, et al. Concurrent cryptosporidium and coronavirus infections in an Arabian foal with combined immunodeficiency syndrome. *Vet Rec*. 1990;126:127–130.
204. Bjorneby JM, Leach DR, Perryman LE. Persistent cryptosporidiosis in horses with severe combined immunodeficiency. *Infect Immun*. 1991;59:3823–3826.
205. MacLeay JM, Ames TR, Hayden DW, et al. Acquired B lymphocyte deficiency and chronic enterocolitis in a 3-year-old quarter horse. *Vet Immunol Immunopathol*. 1997;57:49–57.
206. Chandra RK, Kumari S. Nutrition and Immunity—An Overview. *J Nutr*. 1994;124:S1433–S1435.
207. Chandra RK. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr*. 2002;56:S73–S76.
208. Costa MC, Arroyo LG, Allen-Vercoe E, et al. Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. *PLoS One*. 2012;7:e41484.
209. Weese JS, Holcombe SJ, Embertson RM, et al. Changes in the faecal microbiota of mares precede the development of post partum colic. *Equine Vet J*. 2015;47:641–649.
210. Costa MC, Stampfli HR, Arroyo LG, et al. Changes in the equine fecal microbiota associated with the use of systemic antimicrobial drugs. *BMC Vet Res*. 2015;11:19.
211. Schoster A, Mosing M, Jalali M, et al. Effects of transport, fasting and anaesthesia on the faecal microbiota of healthy adult horses. *Equine Vet J*. 2016;48:595–602.

212. Harlow BE, Lawrence LM, Hayes SH, et al. Effect of Dietary Starch Source and Concentration on Equine Fecal Microbiota. *PLoS One*. 2016;11:e0154037.
213. O'Loughlin EV, Scott RB, Gall DG. Pathophysiology of infectious diarrhea: changes in intestinal structure and function. *J Pediatr Gastroenterol Nutr*. 1991;12:5–20.
214. King JN, Gerring EL. The action of low dose endotoxin on equine bowel motility. *Equine Vet J*. 1991;23:11–17.
215. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20(6):864–874.
216. Vincent J-L. Dear SIRS, I'm sorry to say that I don't like you. *Crit Care Med*. 1997;25:372–374.
217. Opal SM. The uncertain value of the definition for SIRS. Systemic inflammatory response syndrome. *Chest*. 1998;113: 1442–1443.
218. Pfeiffer R. Untersuchungen ueber das Choleragift. *Z Hyg*. 1892;11:393–412.
219. Vaara M. Lipopolysaccharide and the permeability of the bacterial outer membrane. In: Brade H, Opal SM, Vogel SN, et al., eds. *Endotoxin in Health and Disease*. New York: Marcel Dekker; 1999.
220. Rietschel ET, Brade H, Holst O, et al. Bacterial endotoxin: chemical constitution, biological recognition, host response, and immunological detoxification. In: Rietschel ET, Wagner H, eds. *Pathology of Septic Shock*. Berlin: Springer; 1996.
221. Jansson P-E. The chemistry of O-polysaccharide chains in bacterial lipopolysaccharides. In: Brade H, Opal SM, Vogel SN, et al., eds. *Endotoxin in Health and Disease*. New York: Marcel Dekker; 1999.
222. Zahringer U, Lindner B, Rietschel ET. Molecular structure of lipid A, the endotoxic center of bacterial lipopolysaccharides. *Adv Carbohydr Chem Biochem*. 1994;50:211–276.
223. Holst O. Chemical structure of the core region of lipopolysaccharides. In: Brade H, Opal SM, Vogel SN, et al., eds. *Endotoxin in Health and Disease*. New York: Marcel Dekker; 1999.
224. Poxton IR. Antibodies to lipopolysaccharide. *J Immunol Methods*. 1995;186(1):1–15.
225. Galanos C, Luderitz O, Rietschel ET, et al. Synthetic and natural Escherichia coli free lipid A express identical endotoxic activities. *Eur J Biochem*. 1985;148(1):1–5.
226. Bradley SG. Cellular and molecular mechanisms of action of bacterial endotoxins. *Annu Rev Microbiol*. 1979;33:67–94.
227. Barton MH, Morris DD, Norton N, et al. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. *J Vet Intern Med*. 1998;12(1):26–35.
228. Alexander JW, Boyce ST, Babcock GF, et al. The process of microbial translocation. *Ann Surg*. 1990;212(4):496–510.
229. Moore JN, Garner HE, Berg JN, et al. Intracecal endotoxin and lactate during the onset of equine laminitis: a preliminary report. *Am J Vet Res*. 1979;40(5):722–723.
230. Triger DR, Boyer TD, Levin J. Portal and systemic bacteraemia and endotoxaemia in liver disease. *Gut*. 1978;19:935–939.
231. Barton MH, Collatos C. Tumor necrosis factor and interleukin-6 activity and endotoxin concentration in peritoneal fluid and blood of horses with acute abdominal disease. *J Vet Intern Med*. 1999;13(5):457–464.
232. Steverink PJGM, Sturk A, Rutten VPMG, et al. Endotoxin, interleukin-6 and tumor necrosis factor concentrations in equine acute abdominal disease: relation to clinical outcome. *J Endotoxin Res*. 1995;2:289–299.
233. Henry MM, Moore JN. Whole blood re-calcification time in equine colic. *Equine Vet J*. 1991;23(4):303–308.
234. Morris DD. Endotoxemia in horses: a review of cellular and humoral mediators involved in its pathogenesis. *J Vet Intern Med*. 1991;5(3):167–181.
235. Schlag G, Redl H, Dinges HP, et al. Bacterial translocation in a baboon model of hypovolemic-traumatic shock. In: Schlag G, Redl H, Siegel JH, et al., eds. *Shock, Sepsis and Organ Failure*. Berlin: Springer; 1991.
236. Deitch EA, Maejima K, Berg R. Effect of oral antibiotics and bacterial overgrowth on the translocation of the GI tract microflora in burned rats. *J Trauma*. 1985;25(5):385–392.
237. Deitch EA, Winterton J, Berg R. Effect of starvation, malnutrition, and trauma on the gastrointestinal tract flora and bacterial translocation. *Arch Surg*. 1987;122(9):1019–1024.
238. Deitch EA, Berg R, Specian R. Endotoxin promotes the translocation of bacteria from the gut. *Arch Surg*. 1987;122(2):185–190.
239. Baker B, Gaffin SL, Wells M, et al. Endotoxaemia in racehorses following exertion. *J S Afr Vet Assoc*. 1988;59(2):63–66.
240. Barton MH, Williamson L, Jacks S, Norton N. Effects on plasma endotoxin and eicosanoid concentrations and serum cytokine activities in horses competing in a 48-, 83-, or 159-km endurance ride under similar terrain and weather conditions. *Am J Vet Res*. 2003;64(6):754–761.
241. Donovan DC, Jackson CA, Colahan PT, et al. Assessment of exercise-induced alterations in neutrophil function in horses. *Am J Vet Res*. 2007;68(11):1198–1204.
242. Tobias PS, Soldau K, Ulevitch RJ. Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *J Exp Med*. 1986;164(3):777–793.
243. Ramadori G, Meyer zum Buschenfelde KH, Tobias PS, et al. Biosynthesis of lipopolysaccharide-binding protein in rabbit hepatocytes. *Pathobiology*. 1990;58(2):89–94.
244. Schumann RR, Leong SR, Flaggs GW, et al. Structure and function of lipopolysaccharide binding protein. *Science*. 1990; 249(4975):1429–1431.
245. Zweigner J, Schumann RR, Weber JR. The role of lipopolysaccharide-binding protein in modulating the innate immune response. *Microbes Infect*. 2006;8:946–952.
246. Jack RS, Fan X, Bernheiden M, et al. Lipopolysaccharide-binding protein is required to combat a murine gram-negative bacterial infection. *Nature*. 1997;389(6652):742–745.
247. Wright SD, Ramos RA, Tobias PS, et al. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*. 1990;249(4975):1431–1433.
248. Wright SD, Tobias PS, Ulevitch RJ, et al. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *J Exp Med*. 1989;170(4):1231–1241.
249. Schiff DE, Kline L, Soldau K, et al. Phagocytosis of gram-negative bacteria by a unique CD14-dependent mechanism. *J Leukoc Biol*. 1997;62(6):786–794.
250. Grunwald U, Fan X, Jack RS, et al. Monocytes can phagocytose gram-negative bacteria by a CD14-dependent mechanism. *J Immunol*. 1996;157(9):4119–4125.
251. Tobias PS. Lipopolysaccharide-binding protein. In: Brade H, Opal SM, Vogel SN, et al., eds. *Endotoxin in Health and Disease*. New York: Marcel Dekker; 1999.
252. Wurfel MM, Hailman E, Wright SD. Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. *J Exp Med*. 1995;181(5):1743–1754.
253. Lamping N, Dettmer R, Schroder NW, et al. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *J Clin Invest*. 1998;101(10):2065–2071.
254. Vanderplas ML, Moore JN, Barton MH, et al. Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. *Am J Vet Res*. 2005;66(9):1509–1516.
255. Chow JC, Young DW, Golenbock DT, et al. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem*. 1999;274(16):10689–10692.
256. Janeway Jr CA. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol Today*. 1992;13(1):11–16.

257. Haziot A, Chen S, Ferrero E, et al. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. *J Immunol*. 1988;141(2):547–552.
258. Stelter F. Structure/function relationships of CD14. *Chem Immunol*. 2000;74:25–41.
259. Durieux JJ, Vita N, Popescu O, et al. The two soluble forms of the lipopolysaccharide receptor, CD14: characterization and release by normal human monocytes. *Eur J Immunol*. 1994;24(9):2006–2012.
260. Jack RS. Introduction: hunting devils. *Chem Immunol*. 2000;74:1–4.
261. Shimazu R, Akashi S, Ogata H, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*. 1999;189(11):1777–1782.
262. Poltorak A, He X, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*. 1998;282(5396):2085–2088.
263. Qureshi ST, Lariviere L, Leveque G, et al. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J Exp Med*. 1999;189(4):615–625.
264. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet*. 2000;25(2):187–191.
265. Lu Y-C, Yeh W-C, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine*. 2008;42:145–151.
266. Maniatis T. Catalysis by a multiprotein IkappaB kinase complex. *Science*. 1997;278(5339):818–819.
267. Tizard IR. Cytokines and the immune system. In: *Veterinary Immunology: An Introduction*. 5th ed. Philadelphia: WB Saunders; 1996.
268. Beutler B, Cerami A. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature*. 1986;320(6063):584–588.
269. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science*. 1985;229(4716):869–871.
270. Le J, Vilcek J. Biology of disease; tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. *Lab Invest*. 1987;56:234–248.
271. Le JM, Vilcek J. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. *Lab Invest*. 1989;61(6):588–602.
272. MacKay RJ. Treatment of endotoxemia and SIRS. *Proc Am Coll Vet Int Med*. 2001;11:283–285.
273. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med*. 1996;24(7):1125–1128.
274. Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continnuum in sepsis and predict mortality. *J Immunol*. 2006;177:1967–1974.
275. Schade U, Flach R, Hirsch T, et al. Endotoxin as an inducer of cytokines. In: Redl H, Schlag G, eds. *Cytokines in Severe Sepsis and Septic Shock*. Basel: Birkhauser Verlag; 1999.
276. Mengozzi M, Ghezzi P. Cytokine down-regulation in endotoxin tolerance. *Eur Cytokine Netw*. 1993;4(2):89–98.
277. Cavaillon J-M, Adib-Conquy M: Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit Care* 10(5):233
278. Nomura F, Akashi S, Sakao Y, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface Toll-like receptor 4 expression. *J Immunol*. 2000;164(7):3476–3479.
279. Heagy W, Hansen C, Nieman K, et al. Impaired mitogen-activated protein kinase activation and altered cytokine secretion in endotoxin-tolerant human monocytes. *J Trauma*. 2000;49(5):806–814.
280. Barton MH, Collatos C, Moore JN. Endotoxin induced expression of tumour necrosis factor, tissue factor and plasminogen activator inhibitor activity by peritoneal macrophages. *Equine Vet J*. 1996;28(5):382–389.
281. Allen GK, Campbell-Beggs C, Robinson JA, et al. Induction of early-phase endotoxin tolerance in horses. *Equine Vet J*. 1996;28(4):269–274.
282. Elsbach P. Bactericidal/permeability-increasing protein, p15s and phospholipases A2, endogenous antibiotics in host defense against bacterial infections. In: Brade H, Opal SM, Vogel SN, et al., eds. *Endotoxin in Health and Disease*. New York: Marcel Dekker; 1999.
283. Lentsch AB, Ward PA. Regulation of inflammatory vascular damage. *J Pathol*. 2000;190(3):343–348.
284. Klabunde RE, Anderson DE. Role of NO and ROS in platelet activating factor-induced microvascular leakage. *FASEB J*. 2001;15:A47.
285. Turek JJ, Templeton CB, Bottoms GD, et al. Flunixin meglumine attenuation of endotoxin-induced damage to the cardiopulmonary vascular endothelium of the pony. *Am J Vet Res*. 1985;46(3):591–596.
286. Barton MH. Endotoxemia. In: White NA, Moore JN, eds. *Current Techniques in Equine Surgery and Lameness*. 2nd ed. Philadelphia: WB Saunders; 1998.
287. Morris DD, Crowe N, Moore JN. Correlation of clinical and laboratory data with serum tumor necrosis factor activity in horses with experimentally induced endotoxemia. *Am J Vet Res*. 1990;51(12):1935–1940.
288. Andonegui G, Goyert SM, Kubes P. Lipopolysaccharide-induced leukocyte-endothelial cell interactions: a role for CD14 versus toll-like receptor 4 within microvessels. *J Immunol*. 2002;169:2111–2119.
289. Tizard IR. Innate immunity: inflammation. In: *Veterinary Immunology: An Introduction*. 6th ed. Philadelphia: WB Saunders; 2000.
290. Meager A. Cytokine regulation of cellular adhesion molecule expression in inflammation. *Cytokine Growth Factor Rev*. 1999;10(1):27–39.
291. Wagner JG, Roth RA. Neutrophil migration during endotoxemia. *J Leukocyte Biol*. 1999;66:10–24.
292. Wagner JG, Harkema JR, Roth RA. Temporal relationship between intravenous and intratracheal administrations of lipopolysaccharide (LPS) on pulmonary neutrophil (PMN) recruitment. *Am J Respir Crit Care Med*. 1996;153:A837 (abstract).
293. Gardner AB, Nydam DV, Luna JA, et al. Serum opsonization capacity, phagocytosis, and oxidative burst activity in neonatal foals in the intensive care unit. *J Vet Intern Med*. 2007;21(4):797–805.
294. McTaggart C, Penhale J, Raidala SL. Effect of plasma transfusion on neutrophil function in healthy and septic foals. *Aust Vet J*. 2005;83(8):499–505.
295. Johnstone IB, Crane S. Hemostatic abnormalities in equine colic. *Am J Vet Res*. 1986;47(2):356–358.
296. Prasse KW, Topper MJ, Moore JN, et al. Analysis of hemostasis in horses with colic. *J Am Vet Med Assoc*. 1993;203(5):685–693.
297. Levi M, de Jonge E, van der Poll T. Sepsis and disseminated intravascular coagulation. *J Throm Thrombolysis*. 2003;16:43–47.
298. Amaral A, Opal SM, Vincent JL. Coagulation in sepsis. *Intensive Care Med*. 2004;30:1032–1040.
299. Morris DD. Recognition and management of disseminated intravascular coagulation in horses. *Vet Clin North Am Equine Pract*. 1988;4(1):115–143.
300. Hack CE. Cytokines, coagulation and fibrinolysis. In: Redl H, Schlag G, eds. *Cytokines in Severe Sepsis and Septic Shock*. Basel: Birkhauser Verlag; 1999.
301. Kalter ES, Daha MR, ten Cate JW, et al. Activation and inhibition of Hageman factor-dependent pathways and the complement system in uncomplicated bacteremia or bacterial shock. *J Infect Dis*. 1985;151(6):1019–1027.
302. Lyberg T. Clinical significance of increased thromboplastin activity on the monocyte surface: a brief review. *Haemostasis*. 1984;14(5):430–439.

303. Drake TA, Cheng J, Chang A, et al. Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal *Escherichia coli* sepsis. *Am J Pathol*. 1993;142(5):1458–1470.
304. Henry MM, Moore JN. Clinical relevance of monocyte procoagulant activity in horses with colic. *J Am Vet Med Assoc*. 1991;198(5):843–848.
305. Welles EG, Prasse KW, Moore JN. Use of newly developed assays for protein C and plasminogen in horses with signs of colic. *Am J Vet Res*. 1991;52(2):345–351.
306. Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med*. 1986;163(3):740–745.
307. Pober JS, Gimbrone Jr MA, Lapierre LA, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J Immunol*. 1986;137(6):1893–1896.
308. Hack CE, Zeerleder S. The endothelium in sepsis: source of and a target for inflammation. *Crit Care Med*. 2001;29(suppl 7):S21–S27.
309. Kruithof EK. Plasminogen activator inhibitors: a review. *Enzyme*. 1988;40(2-3):113–121.
310. Travis J, Salvesen GS. Human plasma proteinase inhibitors. *Annu Rev Biochem*. 1983;52:655–709.
311. Krishnamurti C, Barr CF, Hassett MA, et al. Plasminogen activator inhibitor: a regulator of ancrod-induced fibrin deposition in rabbits. *Blood*. 1987;69(3):798–803.
312. Suffredini AF, Harpel PC, Parrillo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. *N Engl J Med*. 1989;320(18):1165–1172.
313. Collatos C, Barton MH, Schleef R, et al. Regulation of equine fibrinolysis in blood and peritoneal fluid based on a study of colic cases and induced endotoxaemia. *Equine Vet J*. 1994;26(6):474–481.
314. Collatos C, Barton MH, Prasse KW, et al. Intravascular and peritoneal coagulation and fibrinolysis in horses with acute gastrointestinal tract diseases. *J Am Vet Med Assoc*. 1995;207(4):465–470.
315. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med*. 2001;344:699–709.
316. Ramadori G, Christ B. Cytokines and the hepatic acute-phase response. *Semin Liver Dis*. 1999;19(2):141–155.
317. Tizard IR. Inflammation. In: *Veterinary Immunology: An Introduction*. 5th ed. Philadelphia: WB Saunders; 1996.
318. Topper MJ, Prasse KW. Analysis of coagulation proteins as acute-phase reactants in horses with colic. *Am J Vet Res*. 1998;59(5):542–545.
319. Nunokawa Y, Fujunaga T, Taira T, et al. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci*. 1993;55(6):1011–6.
320. Hultén C, Demmers S. Serum amyloid A (SAA) as an aid in the management of infectious disease in the foal: comparison with total leucocyte count, neutrophil count and fibrinogen. *Equine Vet J*. 2002;34(7):693–8.
321. Jacobsen S, Kjelgaard-Hansen M, Hagbard Petersen H, Jensen AL. Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J*. 2006;172(2):315–319.
322. Muir WW. Shock. *Compend Cont Educ Pract Vet*. 1998;20(5):549–566.
323. Thiemermann CTDW. Nitric oxide and endothelin-1 in circulatory shock involving cytokines. In: Redl H, Schlag G, eds. *Cytokines in Severe Sepsis and Septic Shock*. Basel: Birkhauser Verlag; 1999.
324. Burrows GE. Escherichia coli endotoxemia in the conscious pony. *Am J Vet Res*. 1971;32(2):243–248.
325. Clark ES, Collatos C. Hypoperfusion of the small intestine during slow infusion of a low dosage of endotoxin in anesthetized horses. *Cornell Vet*. 1990;80(2):163–172.
326. Armstrong GP. Cellular and humoral immunity in the horseshoe crab. In: Gupta AP, ed. *Limulus polyphemus: Immunology of Insects and Other Arthropods*. Boca-Raton: CRC Press; 1991.
327. Shuster R, Traub-Dargatz J, Baxter G. Survey of diplomates of the American College of Veterinary Internal Medicine and the American College of Veterinary Surgeons regarding clinical aspects and treatment of endotoxemia in horses. *J Am Vet Med Assoc*. 1997;210(1):87–92.
328. Dinarello CA, Cannon JG, Wolff SM, et al. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med*. 1986;163(6):1433–1450.
329. Coceani F, Bishai I, Dinarello CA, et al. Prostaglandin E2 and thromboxane B2 in cerebrospinal fluid of afebrile and febrile cat. *Am J Physiol*. 1983;244(6):R785–R793.
330. Parsons CS, Orsini JA, Krafty R, et al. Risk factors for development of acute laminitis in horses during hospitalization: 73 cases (1997-2004). *J Am Vet Med Assoc*. 2007;230(6):885–889.
331. Daels PF, Stabenfeldt GH, Hughes JP, et al. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. *Am J Vet Res*. 1991;52(2):282–288.
332. Daels PF, Stabenfeldt GH, Hughes JP, et al. Effects of flunixin meglumine on endotoxin-induced prostaglandin F2 alpha secretion during early pregnancy in mares. *Am J Vet Res*. 1991;52(2):276–281.
333. Ingle-Fehr JE, Baxter GM. Evaluation of digital and laminar blood flow in horses given a low dose of endotoxin. *Am J Vet Res*. 1998;59(2):192–196.
334. Menzies-Gow NJ, Bailey SR, Katz LM, et al. Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Equine Vet J*. 2004;36(3):273–278.
335. Zerpa H, Verga FM, Vasquez J, et al. Effect of sublethal endotoxemia on in vitro digital vascular reactivity in horses. *J Vet Med A*. 2005;52:67–73.
336. Menzies-Gow NJ, Bailey SR, Berhane Y, et al. Evaluation of the induction of vasoactive mediators from equine digital vein endothelial cells by endotoxin. *Am J Vet Res*. 2008;69(3):349–355.
337. Zaloga GP, Chernow B. The multifactorial basis for hypocalcemia during sepsis: studies of the parathyroid hormone-vitamin D axis. *Ann Intern Med*. 1987;107(1):36–41.
338. Dart AJ, Snyder JR, Spier SJ, et al. Ionized calcium concentration in horses with surgically managed gastrointestinal disease: 147 cases (1988-1990). *J Am Vet Med Assoc*. 1992;201(8):1244–1248.
339. Lavoie JP, Madigan JE, Cullor JS, et al. Haemodynamic, pathological, haematological and behavioural changes during endotoxin infusion in equine neonates. *Equine Vet J*. 1990;22(1):23–29.
340. Welch RD, Watkins JP, Taylor TS, et al. Disseminated intravascular coagulation associated with colic in 23 horses (1984-1989). *J Vet Intern Med*. 1992;6(1):29–35.
341. Olson NC. Effects of endotoxin on lung water, hemodynamics, and gas exchange in anesthetized ponies. *Am J Vet Res*. 1985;46(11):2288–2293.
342. Moore JN, Barton MH. An update on endotoxemia part 2: treatment and the way ahead. *Equine Vet Educ*. 1999;11(1):30–34.
343. Weese JS, Cote NM, DeGannes RVG. Evaluation of in vitro properties of di-tri-octahedral smectite on clostridial toxins and growth. *Equine Vet J*. 2003;35(7):638–641.
344. Bentley AP, Barton MH, Norton N, et al. Antimicrobial-induced endotoxin and cytokine activity in an in vitro model of septicemia in foals. *Am J Vet Res*. 2002;63(5):660–668.
345. Sprouse RF, Garner HE, Lager K. Protection of ponies from heterologous and homologous endotoxin challenges via *Salmonella typhimurium* bacterin-toxoid. *Equine Pract*. 1989;11(2):34–40.
346. Garner HE, Sprouse RF, Green EM. Active and passive immunization for blockade of endotoxemia. *Proc Amer Assoc Equine Pract*. 1985;31:525–532.

347. Ziegler EJ, Fisher Jr CJ, Sprung CL, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, doubleblind, placebo-controlled trial, The HA-1A Sepsis Study Group *N Engl J Med*. 1991;324(7):429–436.
348. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med*. 1982;307(20):1225–1230.
349. Sakulramrung R, Domingue GJ. Cross-reactive immunoprotective antibodies to *Escherichia coli* O111 rough mutant J5. *J Infect Dis*. 1985;151(6):995–1004.
350. Garner HE, Sprouse RF, Lager K. Cross-protection of ponies from sublethal Escherichia coli endotoxemia by *Salmonella typhimurium* antiserum. *Equine Pract*. 1988;10(4):10–17.
351. Spier SJ, Lavoie JP, Cullor JS, et al. Protection against clinical endotoxemia in horses by using plasma containing antibody to an Rc mutant *E. coli* (J5). *Circ Shock*. 1989;28(3):235–248.
352. Gaffin SL, Baker B, DuPreez J, et al. Prophylaxis and therapy with anti-endotoxin hyperimmune serum against gastroenteritis and endotoxemia in horses. *Proc Amer Assoc Equine Pract*. 1982;28:335–340.
353. Peek SF, Semrad S, McGuirk SM, et al. Prognostic value of clinicopathologic variables obtained at admission and effect of antiendotoxin plasma on survival in septic and critically ill foals. *J Vet Int Med*. 2006;20:569–574.
354. Durando MM, MacKay RJ, Linda S, et al. Effects of polymyxin B and *Salmonella typhimurium* antiserum on horses given endotoxin intravenously. *Am J Vet Res*. 1994;55(7):921–927.
355. Morris DD, Whitlock RH, Corbeil LB. Endotoxemia in horses: protection provided by antiserum to core lipopolysaccharide. *Am J Vet Res*. 1986;47(3):544–550.
356. Morris DD, Whitlock RH. Therapy of suspected septicemia in neonatal foals using plasma-containing antibodies to core lipopolysaccharide (LPS). *J Vet Intern Med*. 1987;1(4):175–182.
357. Cohen ND, Divers T. Acute colitis in horses. 2. Initial management. *Compend Cont Educ Pract Vet*. 1998;20:228–234.
358. Coyne CP, Fenwick BW. Inhibition of lipopolysaccharide-induced macrophage tumor necrosis factor-alpha synthesis by polymyxin B sulfate. *Am J Vet Res*. 1993;54(2):305–314.
359. Parviainen AK, Barton MH, Norton NN. Evaluation of polymyxin B in an ex vivo model of endotoxemia in horses. *Am J Vet Res*. 2001;62(1):72–76.
360. Barton MH. Use of polymyxin B for treatment of endotoxemia in horses. *Compend Cont Educ Pract Vet*. 2000;11:1056–1059.
361. Raisbeck MF, Garner HE, Osweiler GD. Effects of polymyxin B on selected features of equine carbohydrate overload. *Vet Hum Toxicol*. 1989;31(5):422–426.
362. Barton MH, Parviainen AK. Use of polymyxin B for equine endotoxemia. *Proceedings of the American College of Veterinary Internal Medicine Forum*. Seattle: Wash; May 25-28, 2000.
363. Morresey PR, MacKay RJ. Endotoxin-neutralizing activity of polymyxin B in blood after IV administration in horses. *Am J Vet Res*. 2006;67:642–647.
364. Coyne CP, Moritz JT, Fenwick BW. Inhibition of lipopolysaccharide-induced TNF-alpha production by semisynthetic polymyxin-B conjugated dextran. *Biotechnol Ther*. 1994;5(3-4):137–162.
365. MacKay RJ, Clark CK, Logdberg L, et al. Effect of a conjugate of polymyxin B-dextran 70 in horses with experimentally induced endotoxemia. *Am J Vet Res*. 1999;60(1):68–75.
366. Hellman J, Warren HS. Antiendotoxin strategies. *Infect Dis Clin North Am*. 1999;13(2):371–386.
367. Weiss J, Olsson I. Cellular and subcellular localization of the bactericidal/permeability-increasing protein of neutrophils. *Blood*. 1987;69(2):652–659.
368. Weersink AJ, van Kessel KP, van den Tol ME, et al. Human granulocytes express a 55-kDa lipopolysaccharide-binding protein on the cell surface that is identical to the bactericidal/permeability-increasing protein. *J Immunol*. 1993;150(1):253–263.
369. Abrahamson SL, Wu HM, Williams RE, et al. Biochemical characterization of recombinant fusions of lipopolysaccharide binding protein and bactericidal/permeability-increasing protein: implications in biological activity. *J Biol Chem*. 1997;272(4):2149–2155.
370. Vaara M. Lipid A: target for antibacterial drugs. *Science*. 1996;274(5289):939–940.
371. Levin M, Quint PA, Goldstein B, et al. Recombinant bactericidal/permeability-increasing protein (rBPI21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial, rBPI21 Meningococcal Sepsis Study Group. *Lancet*. 2000;356(9234):961–967.
372. Winchell WW, Hardy J, Levine DM, et al. Effect of administration of a phospholipid emulsion on the initial response of horses administered endotoxin. *Am J Vet Res*. 2002;63(10):1370–1378.
373. Moore JN, Norton N, Barton MH, et al. Rapid infusion of a phospholipid emulsion attenuates the effects of endotoxaemia in horses. *Equine Vet J*. 2007;39(3):243–248.
374. Lei MG, Qureshi N, Morrison DC. Lipopolysaccharide (LPS) binding to 73-kDa and 38-kDa surface proteins on lymphoreticular cells: preferential inhibition of LPS binding to the former by *Rhodopseudomonas sphaeroides* lipid A. *Immunol Lett*. 1993;36(3):245–250.
375. Golenbock DT, Hampton RY, Qureshi N, et al. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. *J Biol Chem*. 1991;266(29):19490–19498.
376. Zuckerman SH, Qureshi N. In vivo inhibition of lipopolysaccharide-induced lethality and tumor necrosis factor synthesis by *Rhodobacter sphaeroides* diphosphoryl lipid A is dependent on corticosterone induction. *Infect Immun*. 1992;60(7):2581–2587.
377. Christ WJ, Asano O, Robidoux AL, et al. E5531, a pure endotoxin antagonist of high potency. *Science*. 1995;268(5207):80–83.
378. Bunnell E, Lynn M, Habet K, et al. A lipid A analog, E5531, blocks the endotoxin response in human volunteers with experimental endotoxemia. *Crit Care Med*. 2000;28(8):2713–2720.
379. Rossignol DP, Lynn M. Antagonism of in vivo and ex vivo response to endotoxin by E5564, a synthetic lipid A analogue. *J Endotoxin Res*. 2002;8(6):483–488.
380. Lohmann KL, Vandenplas M, Barton MH, Moore JN. Lipopolysaccharide from Rhodobacter sphaeroides is an agonist in equine cells. *J Endotoxin Res*. 2003;9(1):33–7.
381. Bryant CE, Oullette A, Lohmann K, et al. The cellular Toll-like receptor 4 antagonist E5531 can act as an agonist in horse whole blood. *Vet Immunol Immunopathol*. 2007;116(3-4):182–9.
382. Lohmann KL, Vandenplas ML, Barton MH, et al. The equine TLR4/MD-2 complex mediates recognition of lipopolysaccharide from *Rhodobacter sphaeroides* as an agonist. *J Endotoxin Res*. 2007;13(4):235–42.
383. Fink MP. Eicosanoids and platelet activating factor in the pathogenesis of sepsis and organ dysfunction. In: Williams JG, ed. *Multiple Organ Dysfunction Syndrome: Examining the Role of Eicosanoids and Procoagulants*. Austin, TX: RG Landes; 1996.
384. Ewert KM, Fessler JF, Templeton CB, et al. Endotoxin-induced hematologic and blood chemical changes in ponies: effects of flunixin meglumine, dexamethasone, and prednisolone. *Am J Vet Res*. 1985;46(1):24–30.
385. Moore JN, Garner HE, Shapland JE, et al. Prevention of endotoxin-induced arterial hypoxaemia and lactic acidosis with flunixin meglumine in the conscious pony. *Equine Vet J*. 1981;13(2):95–98.
386. Moore JN, Hardee MM, Hardee GE. Modulation of arachidonic acid metabolism in endotoxic horses: comparison of flunixin meglumine, phenylbutazone, and a selective thromboxane synthetase inhibitor. *Am J Vet Res*. 1986;47(1):110–113.

387. Gerdemann R, Deegen E, Kietzmann M, et al. Effect of flunixin meglumine on plasma prostanoid concentrations in horses with colic in the perioperative period. *Dtsch Tierarztl Wochenschr*. 1997;104(9):365–368.
388. Templeton CB, Bottoms GD, Fessler JF, et al. Effects of repeated endotoxin injections on prostanoids, hemodynamics, endothelial cells, and survival in ponies. *Circ Shock*. 1985;16(3):253–264.
389. Bottoms GD, Fessler JF, Roesel OF, et al. Endotoxin-induced hemodynamic changes in ponies: effects of flunixin meglumine. *Am J Vet Res*. 1981;42(9):1514–1518.
390. Fessler JF, Bottoms GD, Roesel OF, et al. Endotoxin-induced change in hemograms, plasma enzymes, and blood chemical values in anesthetized ponies: effects of flunixin meglumine. *Am J Vet Res*. 1982;43(1):140–144.
391. Tomlinson JE, Blikslager AT. Effects of cyclooxygenase inhibitors flunixin and deracoxib on permeability of ischaemic-injured equine jejunum. *Equine Vet J*. 2005;37(1):75–80.
392. Little D, Brown SA, Campbell NB, et al. Effects of the cyclooxygenase inhibitor meloxicam on recovery of ischemia-injured equine jejunum. *Am J Vet Res*. 2007;68(6):614–624.
393. MacAllister CG, Morgan SJ, Borne AT, et al. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc*. 1993;202(1):71–77.
394. Semrad SD, Hardee GE, Hardee MM, et al. Low dose flunixin meglumine: effects on eicosanoid production and clinical signs induced by experimental endotoxaemia in horses. *Equine Vet J*. 1987;19(3):201–206.
395. Jackman BR, Moore JN, Barton MH, et al. Comparison of the effects of ketoprofen and flunixin meglumine on the in vitro response of equine peripheral blood monocytes to bacterial endotoxin. *Can J Vet Res*. 1994;58(2):138–143.
396. MacKay RJ, Daniels CA, Bleyaert HF, et al. Effect of eltenac in horses with induced endotoxaemia. *Equine Vet J Suppl*. 2000;(32):26–31.
397. Breuhaus BA, DeGraves FJ, Honore EK, et al. Pharmacokinetics of ibuprofen after intravenous and oral administration and assessment of safety of administration to healthy foals. *Am J Vet Res*. 1999;60(9):1066–1073.
398. Morris DD, Moore JN, Crowe N, et al. Dexamethasone reduces endotoxin-induced tumor necrosis factor activity production in vitro by equine peritoneal macrophages. *Cornell Vet*. 1991;81(3):267–276.
399. Frauenfelder HC, Fessler JF, Moore AB, et al. Effects of dexamethasone on endotoxin shock in the anesthetized pony: hematologic, blood gas, and coagulation changes. *Am J Vet Res*. 1982;43(3):405–411.
400. Annane D. Corticosteroids for septic shock. *Crit Care Med*. 2001;29(suppl 7):S117–S120.
401. Gold JR, Divers TJ, Barton MH, et al. Plasma adrenocorticotropin, cortisol, and adrenocorticotropin/cortisol ratios in septic and normal-term foals. *J Vet Intern Med*. 2007; 21(4):791–6.
402. Barton MH, Moore JN. Pentoxifylline inhibits mediator synthesis in an equine in vitro whole blood model of endotoxemia. *Circ Shock*. 1994;44(4):216–220.
403. Barton MH, Moore JN, Norton N. Effects of pentoxifylline infusion on response of horses to in vivo challenge exposure with endotoxin. *Am J Vet Res*. 1997;58(11):1300–1307.
404. Baskett A, Barton MH, Norton N, et al. Effect of pentoxifylline, flunixin meglumine, and their combination on a model of endotoxemia in horses. *Am J Vet Res*. 1997;58(11):1291–1299.
405. Ingle-Fehr JE, Baxter GM. The effect of oral isoxsuprine and pentoxifylline on digital and laminar blood flow in healthy horses. *Vet Surg*. 1999;28(3):154–60.
406. Arden WA, Slocombe RF, Stick JA, et al. Morphologic and ultrastructural evaluation of effect of ischemia and dimethyl sulfoxide on equine jejunum. *Am J Vet Res*. 1990;51(11):1784–1791.
407. Moore RM, Muir WW, Bertone AL, et al. Effects of dimethyl sulfoxide, allopurinol, 21-aminosteroid U-74389G, and manganese chloride on low-flow ischemia and reperfusion of the large colon in horses. *Am J Vet Res*. 1995;56(5):671–687.
408. Kelmer G, Doherty TJ, Elliott S, et al. Evaluation of dimethyl sulphoxide effects on initial response to endotoxin in the horse. *Equine Vet J*. 2008;40(4):358–363.
409. Weisiger RA. Oxygen radicals and ischemic tissue injury. *Gastroenterology*. 1986;90(2):494–496.
410. Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am J Physiol*. 1986;251(4 Pt 1):G567–G574.
411. Lochner F, Sangiah S, Burrows G, et al. Effects of allopurinol in experimental endotoxin shock in horses. *Res Vet Sci*. 1989;47(2):178–184.
412. Taniguchi T, Shibata K, Yamamoto K, et al. Effects of lidocaine administration on hemodynamics and cytokine responses to endotoxemia in rabbits. *Crit Care Med*. 2000;28(3):755–759.
413. Cook VL, Jones Shults J, McDowell M, et al. Attenuation of ischaemic injury in the equine jejunum by administration of systemic lidocaine. *Equine Vet J*. 2008;40(4):353–357.
414. Carrick JB, McCann ME. The effect of short-term administration of omega 3 fatty acids on endotoxemia. In: *Proceedings of the American College of Veterinary Internal Medicine Forum*. Lake Buena Vista, FL; 1997.
415. Chu AJ, Walton MA, Prasad JK, et al. Blockade by polyunsaturated n-3 fatty acids of endotoxin-induced monocytic tissue factor activation is mediated by the depressed receptor expression in THP-1 cells. *J Surg Res*. 1999;87(2):217–224.
416. Morris DD, Henry MM, Moore JN, et al. Effect of dietary alpha-linolenic acid on endotoxin-induced production of tumor necrosis factor by peritoneal macrophages in horses. *Am J Vet Res*. 1991;52(4):528–532.
417. Henry MM, Moore JN, Feldman EB, et al. Effect of dietary alpha-linolenic acid on equine monocyte procoagulant activity and eicosanoid synthesis. *Circ Shock*. 1990;32(3):173–188.
418. Henry MM, Moore JN, Fischer JK. Influence of an omega-3 fatty acid-enriched ration on in vivo responses of horses to endotoxin. *Am J Vet Res*. 1991;52(4):523–527.
419. McCann ME, Moore JN, Carrick JB, et al. Effect of intravenous infusion of omega-3 and omega-6 lipid emulsions on equine monocyte fatty acid composition and inflammatory mediator production in vitro. *Shock*. 2000;14(2):222–228.
420. Cargile JL, MacKay RJ, Dankert JR, et al. Effects of tumor necrosis factor blockade on interleukin 6, lactate, thromboxane, and prostacyclin responses in miniature horses given endotoxin. *Am J Vet Res*. 1995;56(11):1445–1450.
421. Cargile JL, MacKay RJ, Dankert JR, et al. Effect of treatment with a monoclonal antibody against equine tumor necrosis factor (TNF) on clinical, hematologic, and circulating TNF responses of miniature horses given endotoxin. *Am J Vet Res*. 1995;56(11):1451–1459.
422. Barton MH, Bruce EH, Moore JN, et al. Effect of tumor necrosis factor antibody given to horses during early experimentally induced endotoxemia. *Am J Vet Res*. 1998;59(6):792–797.
423. MacKay RJ, Socher SH. Anti-equine tumor necrosis factor (TNF) activity of antisera raised against human TNF-alpha and peptide segments of human TNF-alpha. *Am J Vet Res*. 1992;53(6):921–924.
424. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome: a randomized, controlled, double-blind, multicenter clinical trial, TNF-alpha MAb Sepsis Study Group. *J Am Med Assoc*. 1995;273(12):934–941.
425. Fisher Jr CJ, Opal SM, Dhainaut JF, et al. Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis, The CB0006 Sepsis Syndrome Study Group. *Crit Care Med*. 1993;21(3):318–327.

426. Wilson DV, Eberhart SW, Robinson NE, et al. Cardiovascular responses to exogenous platelet-activating factor (PAF) in anesthetized ponies, and the effects of a PAF antagonist, WEB 2086. *Am J Vet Res*. 1993;54(2):274–279.
427. Jarvis GE, Evans RJ. Platelet-activating factor and not thromboxane A2 is an important mediator of endotoxin-induced platelet aggregation in equine heparinised whole blood in vitro. *Blood Coagul Fibrinolysis*. 1996;7(2):194–198.
428. King JN, Gerring EL. Antagonism of endotoxin-induced disruption of equine gastrointestinal motility with the platelet-activating factor antagonist WEB 2086. *J Vet Pharmacol Ther*. 1990;13(4):333–339.
429. Mills PC, Ng JC, Seawright AA, et al. Kinetics, dose response, tachyphylaxis and cross-tachyphylaxis of vascular leakage induced by endotoxin, zymosan-activated plasma and platelet-activating factor in the horse. *J Vet Pharmacol Ther*. 1995;18(3):204–209.
430. Dawson J, Lees P, Sedgwick AD. Platelet activating factor as a mediator of equine cell locomotion. *Vet Res Commun*. 1988;12(2-3):101–107.
431. Foster AP, Lees P, Cunningham FM. Platelet activating factor is a mediator of equine neutrophil and eosinophil migration in vitro. *Res Vet Sci*. 1992;53(2):223–229.
432. Carrick JB, Morris DD, Moore JN. Administration of a receptor antagonist for platelet-activating factor during equine endotoxaemia. *Equine Vet J*. 1993;25(2):152–157.
433. Bertone JJ, Gossett KA, Shoemaker KE, et al. Effect of hypertonic vs isotonic saline solution on responses to sublethal *Escherichia coli* endotoxemia in horses. *Am J Vet Res*. 1990;51(7):999–1007.
434. Pantaleon LG, Furr MO, McKenzie 2nd HC, Donaldson L. Cardiovascular and pulmonary effects of hetastarch plus hypertonic saline solutions during experimental endotoxemia in anesthetized horses. *J Vet Int Med*. 2006;20(6):1422–1428.
435. McFarlane D. Hetastarch: a synthetic colloid with potential in equine patients. *Compend Cont Educ Pract Vet*. 1999;21(9):867–877.
436. Jones PA, Bain FT, Byars TD, et al. Effect of hydroxyethyl starch infusion on colloid oncotic pressure in hypoproteinemic horses. *J Am Vet Med Assoc*. 2001;218(7):1130–1135.
437. Cohen ND, Divers T. Equine colitis. In: *Proceedings of the Fifteenth American College of Veterinary Internal Medicine Forum*. Lake Buena Vista, Fla; 1997.
438. Turkan H, Ural A, Beyan C, et al. Effects of hydroxyethyl starch on blood coagulation profile. *Eur J Anaesthesiol*. 1999;16(3):156–159.
439. Jones PA, Tomasic M, Gentry PA. Oncotic, hemodilutional, and hemostatic effects of isotonic saline and hydroxyethyl starch solutions in clinically normal ponies. *Am J Vet Res*. 1997;58(5):541–548.
440. Meister D, Hermann M, Mathis GA. Kinetics of hydroxyethyl starch in horses. *Schweiz Arch Tierheilkd*. 1992;134(7):329–339.
441. Moore JN, Garner HE, Shapland JE, et al. Lactic acidosis and arterial hypoxemia during sublethal endotoxemia in conscious ponies. *Am J Vet Res*. 1980;41(10):1696–1698.
442. Hosgood G. Pharmacologic features and physiologic effects of dopamine. *J Am Vet Med Assoc*. 1990;197(9):1209–1211.
443. Corley KT, McKenzie HC, Amoroso LM, et al. Initial experience with norepinephrine infusion in hypotensive critically ill foals. *J Vet Emerg Crit Care*. 2000;10(4):267–276.
444. Moore BR, Hinchcliff KW. Heparin: a review of its pharmacology and therapeutic use in horses. *J Vet Intern Med*. 1994;8(1):26–35.
445. Mahaffey EA, Moore JN. Erythrocyte agglutination associated with heparin treatment in three horses. *J Am Vet Med Assoc*. 1986;189(11):1478–1480.
446. Moore JN, Mahaffey EA, Zboran M. Heparin-induced agglutination of erythrocytes in horses. *Am J Vet Res*. 1987;48(1):68–71.
447. Monreal L, Villatoro AJ, Monreal M, et al. Comparison of the effects of low-molecular-weight and unfractioned heparin in horses. *Am J Vet Res*. 1995;56(10):1281–1285.
448. Weiss DJ, Evanson OA, McClenahan D, et al. Evaluation of platelet activation and platelet-neutrophil aggregates in ponies with alimentary laminitis. *Am J Vet Res*. 1997;58(12):1376–1380.
449. Jarvis GE, Evans RJ. Endotoxin-induced platelet aggregation in heparinised equine whole blood in vitro. *Res Vet Sci*. 1994;57(3):317–324.
450. Daels PF, Starr M, Kindahl H, et al. Effect of Salmonella typhimurium endotoxin on PGF-2 alpha release and fetal death in the mare. *J Reprod Fertil Suppl*. 1987;35:485–492.
451. Immegart HM. Abnormalities of pregnancy. In: Youngquist RS, ed. *Current Therapy in Large Animal Theriogenology*. Philadelphia: WB Saunders; 1997.
452. Baxter GM. Alterations of endothelium-dependent digital vascular responses in horses given low-dose endotoxin. *Vet Surg*. 1995;24(2):87–96.
453. Pollitt CC, van Eps AW. Prolonged, continuous distal limb cryotherapy in the horse. *Equine Vet J*. 2004;36:216–220.
454. van Eps AW. Progress towards effective prevention and therapy for laminitis. *Equine Vet J*. 2012;44:746–748.
455. Van Eps AW, Pollitt CC. Equine laminitis model: cryotherapy reduces the severity of lesions evaluated seven days after induction with oligofructose. *Equine Vet J*. 2009;41:741–746.
456. van Eps AW, Pollitt CC, Underwood C, et al. Continuous digital hypothermia initiated after the onset of lameness prevents lamellar failure in the oligofructose laminitis model. *Equine Vet J*. 2014;46:625–630.
457. Kullmann A, Holcombe SJ, Hurcombe SD, et al. Prophylactic digital cryotherapy is associated with decreased incidence of laminitis in horses diagnosed with colitis. *Equine Vet J*. 2014;46:554–559.
458. van Eps AW, Orsini JA. A comparison of seven methods for continuous therapeutic cooling of the equine digit. *Equine Vet J*. 2016;48:120–124.
459. Blackwell RB, White NA. Duodenitis-proximal jejunitis in the horse. *Proc Equine Colic Res Symp*. 1982;1:106.
460. Seahorn TL, Cornick JL, Cohen ND. Prognostic indicators for horses with duodenitis-proximal jejunitis—75 Horses (1985–1989). *J Vet Intern Med*. 1992;6:307–311.
461. White NA, Tyler DE, Blackwell RB, et al. Hemorrhagic fibrinonecrotic duodenitis-proximal jejunitis in horses: 20 cases (1977-1984). *J Am Vet Med Assoc*. 1987;190:311–315.
462. Davis JL, Blikslager AT, Catto K, et al. A retrospective analysis of hepatic injury in horses with proximal enteritis (1984–2002). *J Vet Intern Med*. 2003;17:896–901.
463. Arroyo LG, Staempfli H, Rousseau JD, et al. Culture evaluation of Clostridium spp. in the nasogastric reflux of horses with duodenitis proximal jejunitis. *Proc Equine Colic Res Symp*. 2005;8:51–52.
464. Schumacher J, Mullen J, Shelby R, et al. An investigation of the role of *Fusarium moniliforme* in duodenitis/proximal jejunitis of horses. *Vet Hum Toxicol*. 1995;37:39–45.
465. Cohen ND, Toby E, Roussel AJ, et al. Are feeding practices associated with duodenitis-proximal jejunitis? *Equine Vet J*. 2006;38:526–531.
466. Freeman DE. Duodenitis-proximal jejunitis. *Equine Vet Educ*. 2000;12:322–332.
467. Morris DD, Johnston JK. Peritoneal fluid constituents in horses with colic due to small intestinal disease. *Proc Equine Colic Res Symp*. 1986;2.
468. Desrochers AM. Abdominal ultrasonography of normal and colicky adult horses. *Proc AAEP Focus on Colic*. 2005:20–26.

469. Lammers TW, Roussel AJ, Boothe DM, et al. Effect of an indwelling nasogastric tube on gastric emptying rates of liquids in horses. *Am J Vet Res*. 2005;66:642–645.
470. Sellon DC, Roberts MC, Blikslager AT, et al. Effects of continuous rate intravenous infusion of butorphanol on physiologic and outcome variables in horses after celiotomy. *J Vet Intern Med*. 2004;18:555–563.
471. Durham AE, Phillips TJ, Walmsley JP, et al. Nutritional and clinicopathological effects of post operative parenteral nutrition following small intestinal resection and anastomosis in the mature horse. *Equine Vet J*. 2004;36:390–396.
472. Durham AE, Phillips TJ, Walmsley JP, et al. Study of the clinical effects of postoperative parenteral nutrition in 15 horses. *Vet Rec*. 2003;153:493–498.
473. Koenig JB, Sawhney S, Cote N, et al. Effect of intraluminal distension or ischemic strangulation obstruction of the equine jejunum on jejunal motilin receptors and binding of erythromycin lactobionate. *Am J Vet Res*. 2006;67:815–820.
474. Cook VL, Blikslager AT. Use of systemically administered lidocaine in horses with gastrointestinal tract disease. *J Am Vet Med Assoc*. 2008;232:1144–1148.
475. Malone E, Ensink J, Turner T, et al. Intravenous continuous infusion of lidocaine for treatment of equine ileus. *Vet Surg*. 2006;35:60–66.
476. Koenig JB, Cote N, LaMarre J, et al. Binding of radiolabeled porcine motilin and erythromycin lactobionate to smooth muscle membranes in various segments of the equine gastrointestinal tract. *Am J Vet Res*. 2002;63:1545–1550.
477. Cohen ND, Faber NA, Brumbaugh GW. Use of bethanechol and metoclopramide in horses with duodenitis proximal jejunitis: 13 cases (1987–1993). *J Equine Vet Sci*. 1995;15:492–494.
478. Leeth B, Robertson JT. A retrospective comparison of surgical to medical management of proximal enteritis in the horse. *Proc Am Assoc Equine Pract*. 1989;34:69–79.
479. Edwards GB. Duodenitis-proximal jejunitis (anterior enteritis) as a surgical problem. *Equine Vet Educ*. 2000;12:318–321.
480. Oue Y, Ishihara R, Edamatsu H, et al. Isolation of an equine coronavirus from adult horses with pyrogenic and enteric disease and its antigenic and genomic characterization in comparison with the NC99 strain. *Vet Microbiol*. 2011;150:41–48.
481. Oue Y, Morita Y, Kondo T, et al. Epidemic of equine coronavirus at Obihiro Racecourse, Hokkaido, Japan in 2012. *J Vet Med Sci*. 2013;75:1261–1265.
482. Pusterla N, Mapes S, Wademan C, et al. Emerging outbreaks associated with equine coronavirus in adult horses. *Vet Microbiol*. 2013;162:228–231.
483. Davis E, Rush BR, Cox J, et al. Neonatal enterocolitis associated with coronavirus infection in a foal: a case report. *J Vet Diagn Invest*. 2000;12:153–156.
484. Guy JS, Breslin JJ, Breuhaus B, et al. Characterization of a coronavirus isolated from a diarrheic foal. *J Clin Microbiol*. 2000;38:4523–4526.
485. Giannitti F, Diab S, Mete A, et al. Necrotizing enteritis and hyperammonemic encephalopathy associated with equine coronavirus infection in equids. *Vet Pathol*. 2015;52:1148–1156.
486. Fielding CL, Higgins JK, Higgins JC, et al. Disease associated with equine coronavirus infection and high case fatality rate. *J Vet Intern Med*. 2015;29:307–310.
487. Roberts MC, Kidder DE, Hill FW. Small intestinal beta-galactosidase activity in the horse. *Gut*. 1973;14:535–540.
488. Roberts MC. Malabsorption-syndromes in the horse. *Comp Contin Educ Pract Vet*. 1985;7:S637–S646.
489. Lindberg R, Nygren A, Persson SG. Rectal biopsy diagnosis in horses with clinical signs of intestinal disorders: a retrospective study of 116 cases. *Equine Vet J*. 1996;28:275–284.
490. Kemper DL, Perkins GA, Schumacher J, et al. Equine lymphocytic-plasmacytic enterocolitis: a retrospective study of 14 cases. *Equine Vet J Suppl*. 2000:108–112.
491. Gibson KT, Alders RG. Eosinophilic enterocolitis and dermatitis in two horses. *Equine Vet J*. 1987;19:247–252.
492. Church S, Middleton DJ. Transient glucose malabsorption in two horses—fact or artefact? *Aus Vet J*. 1997;75:716–718.
493. Love S, Mair TS, Hillyer MH. Chronic diarrhea in adult horses—a review of 51 referred cases. *Vet Rec*. 1992;130:217–219.
494. Freeman DE. In vitro concentrative accumulation of D-xylose by jejunum from horses and rabbits. *Am J Vet Res*. 1993;54:965–969.
495. Brown CM. The diagnostic value of the d-xylose absorption test in horses with unexplained chronic weight loss. *Brit Vet Jl*. 1992;148:41–44.
496. Roberts MC. Small intestinal malabsorption in horses. *Equine Vet Educ*. 2000;12:214–219.
497. Haven M, Roberts M, Argenzio R, et al. Intestinal adaptation following 70% small bowel resection in the horse. *Pferdeheilkunde*. 1992:86–87.
498. Tate Jr LP, Ralston SL, Koch CM, et al. Effects of extensive resection of the small intestine in the pony. *Am J Vet Res*. 1983;44:1187–1191.
499. Lindberg R, Persson SG, Jones B, et al. Clinical and pathophysiological features of granulomatous enteritis and eosinophilic granulomatosis in the horse. *Zentralbl Veterinarmed A*. 1985;32:526–539.
500. Duryea JH, Ainsworth DM, Mauldin EA, et al. Clinical remission of granulomatous enteritis in a Standardbred gelding following long-term dexamethasone administration. *Equine Vet J*. 1997;29:164–167.
501. Roberts MC, Pinsent PJ. Malabsorption in the horse associated with alimentary lymphosarcoma. *Equine Vet J*. 1975;7:166–172.
502. van den Hoven R, Franken P. Clinical aspects of lymphosarcoma in the horse: a clinical report of 16 cases. *Equine Vet J*. 1983;15:49–53.
503. Platt H. Alimentary lymphomas in the horse. *J Comp Pathol*. 1987;97:1–10.
504. Cimprich RE. Equine granulomatous enteritis. *Vet Pathol*. 1974;11:535–547.
505. Schumacher J, Edwards JF, Cohen ND. Chronic idiopathic inflammatory bowel diseases of the horse. *J Vet Intern Med*. 2000;14:258–265.
506. Lindberg R. Pathology of equine granulomatous enteritis. *J Comp Pathol*. 1984;94:233–247.
507. Merritt AM, Cimprich RE, Beech J. Granulomatous enteritis in nine horses. *J Am Vet Med Assoc*. 1976;169:603–609.
508. Fogarty U, Perl D, Good P, et al. A cluster of equine granulomatous enteritis cases: the link with aluminium. *Vet Hum Toxicol*. 1998;40:297–305.
509. Collery P, McElroy M, Sammin D, et al. Equine granulomatous enteritis linked with aluminum? *Vet Hum Toxicol*. 1999;41:49–50.
510. Meuten DJ, Butler DG, Thomson GW, et al. Chronic enteritis associated with malabsorption and protein-losing enteropathy in the horse. *J Am Vet Med Assoc*. 1978;172:326–333.
511. Pass DA, Bolton JR. Chronic eosinophilic gastroenteritis in the horse. *Vet Pathol*. 1982;19:486–496.
512. Nimmo Wilkie JS, Yager JA, Nation PN, et al. Chronic eosinophilic dermatitis: a manifestation of a multisystemic, eosinophilic, epitheliotropic disease in five horses. *Vet Pathol*. 1985;22:297–305.
513. Pass DA, Bolton JR, Mills JN. Basophilic enterocolitis in a horse. *Vet Pathol*. 1984;21:362–364.
514. Lindberg R, Karlsson L. Topography and enterocyte morphology of the small bowel mucosal surface in equine granulomatous enteritis. *J Comp Pathol*. 1985;95:65–78.
515. Platt H. Chronic inflammatory and lymphoproliferative lesions of the equine small intestine. *J Comp Pathol*. 1986;96:671–684.
516. Edwards GB, Kelly DF, Proudman CJ. Segmental eosinophilic colitis: a review of 22 cases. *Equine Vet J Suppl*. 2000:86–93.

517. Scott EA, Heidel JR, Snyder SP, et al. Inflammatory bowel disease in horses: 11 cases (1988–1998). *J Am Vet Med Assoc*. 1999;214:1527–1530.
518. Lavoie JP, Drolet R, Parsons D, et al. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine Vet J*. 2000;32:418–425.
519. Frazer M. *Lawsonia intracellularis* infection in horses: 2005-2007. *J Vet Intern Med*. 2008;22:1243–1248.
520. McClintock SA, Collins AM. *Lawsonia intracellularis* proliferative enteropathy in a weanling foal in Australia. *Aust Vet J*. 2004;82:750–752.
521. McGurrin MK, Vengust M, Arroyo LG, et al. An outbreak of *Lawsonia intracellularis* infection in a standardbred herd in Ontario. *Can Vet J*. 2007;48:927–930.
522. Pusterla N, Gebhart C. *Lawsonia intracellularis* infection and proliferative enteropathy in foals. *Vet Microbiol*. 2013.
523. Wilson JH, Gebhart CJ. *Lawsonia* proliferative enteropathy in foals: clinical features and piglet parallels. *Proc AAEP Focus on the First Year of Life*. 2008.
524. Williams NM, Harrison LR, Gebhart CJ. Proliferative enteropathy in a foal caused by *Lawsonia intracellularis*-like bacterium. *J Vet Diagn Invest*. 1996;8:254–256.
525. Cooper DM, Swanson DL, Gebhart CJ. Diagnosis of proliferative enteritis in frozen and formalin-fixed, paraffin-embedded tissues from a hamster, horse, deer and ostrich using a *Lawsonia intracellularis*-specific multiplex PCR assay. *Vet Microbiol*. 1997;54:47–62.
526. Page AE, Stills Jr HF, Horohov DW. The effect of passively acquired antibodies on *Lawsonia intracellularis* infection and immunity in the horse. *Equine Vet J*. 2015;47:655–661.
527. Sampieri F, Hinchcliff KW, Toribio RE. Tetracycline therapy of *Lawsonia intracellularis* enteropathy in foals. *Equine Vet J*. 2006;38:89–92.
528. Jacobson M, Aspan A, Königsson MH, et al. Routine diagnostics of *Lawsonia intracellularis* performed by PCR, serological and post mortem examination, with special emphasis on sample preparation methods for PCR. *Vet Microbiol*. 2004;102:189–201.
529. Page AE, Slovis NM, Horohov DW. *Lawsonia intracellularis* and equine proliferative enteropathy. *Vet Clin North Am Equine Pract*. 2014;30:641–658.
530. Dauvillier J, Picandet V, Harel J, et al. Diagnostic and epidemiological features of *Lawsonia intracellularis* enteropathy in 2 foals. *Can Vet J*. 2006;47:689–691.
531. Pusterla N, Vannucci FA, Mapes SM, et al. Efficacy of an avirulent live vaccine against *Lawsonia intracellularis* in the prevention of proliferative enteropathy in experimentally infected weanling foals. *Am J Vet Res*. 2012;73:741–746.
532. Hayden DW, Johnson KH, Wolf CB, et al. AA amyloid-associated gastroenteropathy in a horse. *J Comp Pathol*. 1988;98:195–204.
533. MacKay RJ, Iverson WO, Merritt AM. Exuberant granulation tissue in the stomach of a horse. *Equine Vet J*. 1981;13:119–122.
534. Smith BP. Salmonella infection in horses. *Comp Cont Ed Pract Vet*. 1981;3:S4–S17.
535. Smith BP, Reina-Guerra M, Hardy AJ. Prevalence and epizootiology of equine salmonellosis. *J Am Vet Med Assoc*. 1978;172:353.
536. Donahue JM. Emergence of antibiotic-resistant *Salmonella agona* in horses in Kentucky. *J Am Vet Med Assoc*. 1986;188:592.
537. Traub-Dargatz JL, Garber LP, Fedorka-Cray PJ, et al. Fecal shedding of *Salmonella* spp by horses in the United States during 1998 and 1999 and detection of *Salmonella* spp in grain and concentrate sources on equine operations. *J Am Vet Med Assoc*. 2000;217:226.
538. Traub-Dargatz JL, Salman MD, Jones RL. Epidemiologic study of salmonellae shedding in the feces of horses and potential risk factors for development of the infection in hospitalized horses. *J Am Vet Med Assoc*. 1990;196:1617.
539. House JK, Mainar-Jaime RC, Smith BP, et al. Risk factors for nosocomial *Salmonella* infection among hospitalized horses. *J Am Vet Med Assoc*. 1999;214:1511.
540. Schott HC, Ewart SL, Walker RD, R.M, et al. An outbreak of salmonellosis among horses at a veterinary teaching hospital. *J Am Vet Med Assoc*. 2001;218:1152.
541. Tillotson K, Savage CJ, Salman MD, et al. Outbreak of *Salmonella infantis* infection in a large animal veterinary teaching hospital. *J Am Vet Med Assoc*. 1997;211:1554.
542. Mainar-Jaime RC, House JK, Smith BP, et al. Influence of fecal shedding of *Salmonella* organisms on mortality in hospitalized horses. *J Am Vet Med Assoc*. 1998;213:1162.
543. Hartmann FA, Callan RJ, McGuirk SM, et al. Control of an outbreak of salmonellosis caused by drug-resistant *Salmonella anatum* in horses at a veterinary hospital and measures to prevent future infections. *J Am Vet Med Assoc*. 1996;209:629.
544. Bucknell DG, Gasser RB, Irving A, Whithear K. Antimicrobial resistance in Salmonella and Escherichia coli isolated from horses. *Aust Vet J*. 1997;75:355.
545. Hartmann FA, West SE. Utilization of both phenotypic and molecular analyses to investigate an outbreak of multidrug-resistant in horses. *Can J Vet Res*. 1997;61:173.
546. Dargatz DA, Traub-Dargatz JL. Multidrug-resistant *Salmonella* and nosocomial infections. *Vet Clin North Am Equine Pract*. 2004;20:587.
547. Smith BP. Understanding the role of endotoxins in gram-negative sepsis. *Vet Med*. 1986;12:1148.
548. Carter JD, Hird DW, Farver TB, Hjerpe CA. Salmonellosis in hospitalized horses: seasonality and case fatality rates. *J Am Vet Med Assoc*. 1986;188:163.
549. Morse EV, Duncan MA, Page EA, Fessler JF. Salmonellosis in Equidae: a study of 23 cases. *Cornell Vet*. 1976;66:198.
550. Ernst NS, Hernandez JA, MacKay RJ, et al. Risk factors associated with fecal Salmonella shedding among hospitalized horses with signs of gastrointestinal tract disease. *J Am Vet Med Assoc*. 2004;225:275.
551. Kim LM, Morley PS, Traub-Dargatz JL, Salman MD, Gentry-Weeks C. Factors associated with Salmonella shedding among equine colic patients at a veterinary teaching hospital. *J Am Vet Med Assoc*. 2001;218:740.
552. Giannella RA. Pathogenesis of acute bacterial diarrheal disorders. *Annu Rev Med*. 1981;32:341.
553. Hirsh DC. The alimentary canal as a microbial habitat. In: Biberstein EL, Zee YC, eds. *Review of Veterinary Microbiology*. Boston: Blackwell Scientific; 1990:93.
554. Selsted ME, Miller SI, Henschen AH, Ouellette AJ. Enteric defensins: antibiotic peptide components of intestinal host defense. *J Cell Biol*. 1992;118:929.
555. Brandtzaeg P, Baekkevold ES, Farstad IN, et al. Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today*. 1999;20:141.
556. Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis. *Annu Rev Med*. 2001;52:259.
557. Clarke RC, Gyles CL. Salmonella. In: Gyles CL, Thoen CO, eds. *Pathogenesis of Bacterial Infections in Animals*. Ames, IA: Iowa State University Press; 2001:95.
558. Hirsh DC. Salmonella. In: Biberstein EL, Zee YC, eds. *Review of veterinary microbiology*. Boston: Blackwell Scientific; 2001:110.
559. Smith BP, Hardy AJ, Reina-Guerra M. A preliminary evaluation of some preparations of Salmonella typhimurium vaccines in horses. In: Moore JN, White NA, Becht JL, eds. *Proceedings of the first Equine Colic Symposium*. Lawrenceville, NJ: Veterinary Learning Systems; 1982:211.
560. Smith BP, Reina-Guerra M, Hoiseth SK, et al. Aromatic-dependent Salmonella typhimurium as modified live vaccines for calves. *Am J Vet Res*. 1984;45:59.
561. Sheoran AS, Timoney JF, Tinge SA, Sundaram P, Curtiss R. Intranasal immunogenicity of a Delta cya Delta crp-pabA mutant of Salmonella enterica serotype Typhimurium for the horse. *Vaccine*. 2001;19:3787.

562. Sansonetti PJ, Phalipon A. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin Immunol*. 1999;11:193.
563. Galan JE, Collmer A. Type III secretion machines: bacterial devices for protein delivery into host cells. *Science*. 1999;284:1322.
564. Vazquez-Torres A, Jones-Carson J, Baumler AJ, et al. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. *Nature*. 1999;401:804.
565. Vazquez-Torres A, Fang FC. Oxygen-dependent anti-Salmonella activity of macrophages. *Trends Microbiol*. 2001;9:29.
566. Koo FC, Peterson JW, Houston CW, Molina NC. Pathogenesis of experimental salmonellosis: inhibition of protein synthesis by cytotoxin. *Infect Immun*. 1984;43:93.
567. Giannella RA, Gots RE, Charney AN, Greenough WB, Formal SB. Pathogenesis of Salmonella-mediated intestinal fluid secretion. Activation of adenylate cyclase and inhibition by indomethacin. *Gastroenterology*. 1975;69:1238.
568. Peterson JW, Molina NC, Houston CW, Fader RC. Elevated cAMP in intestinal epithelial cells during experimental cholera and salmonellosis. *Toxicon*. 1983;21:761.
569. Chopra AK, Huang JH, Xu X, et al. Role of Salmonella enterotoxin in overall virulence of the organism. *Microb Pathog*. 1999;27:155.
570. Watson PR, Galyov EE, Paulin SM, et al. Mutation of invH, but not stn, reduces Salmonella-induced enteritis in cattle. *Infect Immun*. 1432;66.
571. Murray MJ. Enterotoxin activity of a Salmonella typhimurium of equine origin in vivo in rabbits and the effect of Salmonella culture lysates and cholera toxin on equine colonic mucosa in vitro. *Am J Vet Res*. 1986;47:769.
572. Murray MJ. Digestive physiology of the large intestine in adult horses. Part II: pathophysiology of colitis. *Comp Cont Ed Pract Vet*. 1988;10:1309.
573. Powell DW. Neuroimmunophysiology of the gastrointestinal mucosa: implications for inflammatory diseases. *Trans Am Clin Climatol Assoc*. 1994;106:124.
574. Madara JL. Review article: pathobiology of neutrophil interactions with intestinal epithelia. *Aliment Pharmacol Ther*. 2000;11(suppl 3):57.
575. Smith BP, Reina-Guerra M, Hardy AJ, Habasha F. Equine salmonellosis: experimental production of four syndromes. *Am J Vet Res*. 1979;40:1072.
576. Cohen ND, Woods AM. Characteristics and risk factors for failure to survive of horses with acute diarrhea: 122 cases (1990-1996). *J Am Vet Med Assoc*. 1999;214:382.
577. van Duijkeren E, Flemming C, van Oldruitenborgh-Oosterbaan MS, H, et al. Diagnosing salmonellosis in horses. Culturing of multiple versus single faecal samples. *Vet Q*. 1995;17:63.
578. Palmer JE, Whitlock RH, Benson CE, et al. Comparison of rectal mucosal cultures and fecal cultures in detecting Salmonella infection in horses and cattle. *Am J Vet Res*. 1985;46:697.
579. Cohen ND, Martin LJ, Simpson RB, et al. Comparison of polymerase chain reaction and microbiological culture for detection of salmonellae in equine feces and environmental samples. *Am J Vet Res*. 1996;57:780.
580. Amavisit P, Browning GF, Lightfoot D, et al. Rapid PCR detection of Salmonella in horse faecal samples. *Vet Microbiol*. 2001;79:63.
581. Kurowski PB, Traub-Dargatz JL, Morley PS, Gentry-Weeks CR. Detection of Salmonella spp in fecal specimens by use of real-time polymerase chain reaction assay. *Am J Vet Res*. 2002;63:1265.
582. Dumler JS, Barbet AF, Bekker CP, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila. *Int J Syst Evol Microbiol*. 2001;51:2145.
583. Palmer JE. Potomac horse fever. *Vet Clin North Am Equine Pract*. 1993;9:399.
584. Mulville P. Equine monocytic ehrlichiosis (Potomac horse fever): a review. *Equine Vet J*. 1991;23:400.
585. Dutta SK, Myrup AC, Rice RM, et al. Experimental reproduction of Potomac horse fever in horses with a newly isolated Ehrlichia organism. *J Clin Microbiol*. 1985;22:265.
586. Rikihisa Y, Perry BD. Causative ehrlichial organisms in Potomac horse fever. *Infect Immun*. 1985;49:513.
587. Madigan JE, Pusterla N. Ehrlichial diseases. *Vet Clin North Am Equine Pract*. 2000;16:487. ix.
588. Levine JF, Levy MG, Nicholson WL, Gager RB. Attempted Ehrlichia risticii transmission with Dermacentor variabilis (Acari: Ixodidae). *J Med Entomol*. 1990;27:931.
589. Burg JG, Roberts AW, Williams NM, et al. Attempted transmission of Ehrlichia risticii (Rickettsiaceae) with Stomoxys calcitrans (Diptera: Muscidae). *J Med Entomol*. 1990;27:874.
590. Reubel GH, Barlough JE, Madigan JE. Production and characterization of Ehrlichia risticii, the agent of Potomac horse fever, from snails (Pleuroceridae: Juga spp.) in aquarium culture and genetic comparison to equine strains. *J Clin Microbiol*. 1998;36:1501.
591. Barlough JE, Reubel GH, Madigan JE, et al. Detection of Ehrlichia risticii, the agent of Potomac horse fever, in freshwater stream snails (Pleuroceridae: Juga spp.) from northern California. *Appl Environ Microbiol*. 1998;64:2888.
592. Kanter M, Mott J, Ohashi N, et al. Analysis of 16S rRNA and 51-kilodalton antigen gene and transmission in mice of Ehrlichia risticii in virgulate trematodes from Elimia livescens snails in Ohio. *J Clin Microbiol*. 2000;38:3349.
593. Mott J, Muramatsu Y, Seaton E, et al. Molecular analysis of Neorickettsia risticii in adult aquatic insects in Pennsylvania, in horses infected by ingestion of insects, and isolated in cell culture. *J Clin Microbiol*. 2002;40:690.
594. Park BK, Kim MJ, Kim EH, et al. Identification of trematode cercariae carrying Neorickettsia risticii in freshwater stream snails. *Ann N Y Acad Sci*. 2003;990:239–247.
595. Pusterla N, Johnson EM, Chae JS, Madigan JE. Digenetic trematodes, Acanthatrium sp. and Lecithodendrium sp., as vectors of Neorickettsia risticii, the agent of Potomac horse fever. *J Helminthol*. 2003;77:335.
596. Gibson KE, Rikihisa Y, Zhang C, Martin C. Neorickettsia risticii is vertically transmitted in the trematode Acanthatrium oregonense and horizontally transmitted to bats. *Environ Microbiol*. 2005;7:203.
597. Chae JS, Pusterla N, Johnson E, et al. Infection of aquatic insects with trematode metacercariae carrying Ehrlichia risticii, the cause of Potomac horse fever. *J Med Entomol*. 2000;37:619.
598. Pusterla N, Madigan JE, Chae JS, et al. Helminthic transmission and isolation of Ehrlichia risticii, the causative agent of Potomac horse fever, by using trematode stages from freshwater stream snails. *J Clin Microbiol*. 2000;38:1293.
599. Pusterla N, Johnson E, Chae J, et al. Infection rate of Ehrlichia risticii, the agent of Potomac horse fever, in freshwater stream snails (Juga yrekaensis) from northern California. *Vet Parasitol*. 2000;92:151.
600. Madigan JE, Pusterla N, Johnson E, et al. Transmission of Ehrlichia risticii, the agent of Potomac horse fever, using naturally infected aquatic insects and helminth vectors: preliminary report. *Equine Vet J*. 2000;32:275.
601. Rikihisa Y, Perry BD, Cordes DO. Ultrastructural study of ehrlichial organisms in the large colons of ponies infected with Potomac horse fever. *Infect Immun*. 1985;49:505.
602. Rikihisa Y. Growth of Ehrlichia risticii in human colonic epithelial cells. *Ann N Y Acad Sci*. 1990;590:104.
603. Williams NM, Cross RJ, Timoney PJ. Respiratory burst activity associated with phagocytosis of Ehrlichia risticii by mouse peritoneal macrophages. *Res Vet Sci*. 1994;57:194.
604. Williams NM, Timoney PJ. In vitro killing of Ehrlichia risticii by activated and immune mouse peritoneal macrophages. *Infect Immun*. 1993;61:861.

605. Wells MY, Rikihisa Y. Lack of lysosomal fusion with phagosomes containing Ehrlichia risticii in P388D1 cells: abrogation of inhibition with oxytetracycline. *Infect Immun*. 1988;56:3209.
606. Rikihisa Y, Johnson GC, Cooke HJ. Pathophysiological changes in the large colon of horses infected with Ehrlichia risticii. In: Moore JN, White S, Morris DD, eds. *Proceedings of the third equine colic symposium*. Lawrenceville NJ: Veterinary Learning Systems; 1988:93.
607. Dutta SK, Penney BE, Myrup AC, et al. Disease features in horses with induced equine monocytic ehrlichiosis (Potomac horse fever). *Am J Vet Res*. 1988;49:1747.
608. Ziemer EL, Whitlock RH, Palmer JE, Spencer PA. Clinical and hematologic variables in ponies with experimentally induced equine ehrlichial colitis (Potomac horse fever). *Am J Vet Res*. 1987;48:63.
609. Long MT, Goetz TE, Kakoma I, et al. Evaluation of fetal infection and abortion in pregnant ponies experimentally infected with Ehrlichia risticii. *Am J Vet Res*. 1995;56:1307.
610. Long MT, Goetz TE, Whiteley HE, et al. Identification of Ehrlichia risticii as the causative agent of two equine abortions following natural maternal infection. *J Vet Diagn Invest*. 1995;7:201.
611. Morris DD, Messick J, Whitlock RH, et al. Effect of equine ehrlichial colitis on the hemostatic system in ponies. *Am J Vet Res*. 1988;49:1030.
612. Dutta SK, Rice RM, Hughes TD, et al. Detection of serum antibodies against Ehrlichia risticii in Potomac horse fever by enzyme-linked immunosorbent assay. *Vet Immunol Immunopathol*. 1987;14:85.
613. Madigan JE, Rikihisa Y, Palmer JE, et al. Evidence for a high rate of false-positive results with the indirect fluorescent antibody test for Ehrlichia risticii antibody in horses. *J Am Vet Med Assoc*. 1995;207:1448.
614. Pusterla N, Leutenegger CM, Sigrist B, et al. Detection and quantitation of Ehrlichia risticii genomic DNA in infected horses and snails by real-time PCR. *Vet Parasitol*. 2000;90:129.
615. Mott J, Rikihisa Y, Zhang Y, et al. Comparison of PCR and culture to the indirect fluorescent-antibody test for diagnosis of Potomac horse fever. *J Clin Microbiol*. 1997;35:2215.
616. Biswas B, Mukherjee D, Mattingly-Napier BL, Dutta SK. Diagnostic application of polymerase chain reaction for detection of Ehrlichia risticii in equine monocytic ehrlichiosis (Potomac horse fever). *J Clin Microbiol*. 1991;29:2228.
617. Atwill ER, Mohammed HO. Evaluation of vaccination of horses as a strategy to control equine monocytic ehrlichiosis. *J Am Vet Med Assoc*. 1996;208:1290.
618. Dutta SK, Vemulapalli R, Biswas B. Association of deficiency in antibody response to vaccine and heterogeneity of Ehrlichia risticii strains with Potomac horse fever vaccine failure in horses. *J Clin Microbiol*. 1998;36:506.
619. Wierup M. Equine intestinal clostridiosis. An acute disease in horses associated with high intestinal counts of Clostridium perfringens type A. *Acta Vet Scand suppl*. 1977;1.
620. Prescott JF, Staempfli HR, Barker IK, et al. A method for reproducing fatal idiopathic colitis (colitis X) in ponies and isolation of a clostridium as a possible agent. *Equine Vet J*. 1988;20:417.
621. Jones RL, Adney WS, Alexander AF, et al. Hemorrhagic necrotizing enterocolitis associated with Clostridium difficile infection in four foals. *J Am Vet Med Assoc*. 1988;193:76.
622. Madewell BR, Tang YJ, Jang S, et al. Apparent outbreaks of Clostridium difficile-associated diarrhea in horses in a veterinary medical teaching hospital. *J Vet Diagn Invest*. 1995;7:343.
623. Weese JS, Staempfli HR, Prescott JF. A prospective study of the roles of clostridium difficile and enterotoxigenic Clostridium perfringens in equine diarrhoea. *Equine Vet J*. 2001;33:403.
624. Jones RL. Clostridial enterocolitis. *Vet Clin North Am Equine Pract*. 2000;16:471.
625. Donaldson MT, Palmer JE. Prevalence of Clostridium perfringens enterotoxin and Clostridium difficile toxin A in feces of horses with diarrhea and colic. *J Am Vet Med Assoc*. 1999;215:358.
626. Baverud V, Gustaffsson A, Franklin A, et al. Clostridium difficile associated with acute colitis in mature horses treated with antibiotics. *Equine vet j*. 1997;29:279. v.
627. Linerode PA, Goode RL. The effect of colic on the microbial activity of the equine intestine. *Proc Am Assoc Eq Pract*. 1970;16:219.
628. White G, Prior SD. Comparative effects of oral administration of trimethoprim/sulphadiazine or oxytetracycline on the faecal flora of horses. *Vet Rec*. 1982;111:316.
629. Baverud V, Franklin A, Gunnarsson A, et al. Clostridium difficile associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for Rhodococcus equi pneumonia. *Equine Vet J*. 1998;30:482.
630. Staempfli HR, Prescott JF, Brash ML. Lincomycin-induced severe colitis in ponies: association with Clostridium cadaveris. *Can J Vet Res*. 1992;56:168.
631. Samuel SC, Hancock P, Leigh DA. An investigation into Clostridium perfringens enterotoxin-associated diarrhoea. *J Hosp Infect*. 1991;18:219.
632. Niilo L. Enterotoxigenic Clostridium perfringens. In: Gyles CL, Thoen CO, eds. *Pathogenesis of bacterial infections in animals*. Ames, IA: Iowa State University Press; 1986:321.
633. Gibert M, Jolivet-Reynaud C, Popoff MR, Jolivet-Renaud C. Beta2 toxin, a novel toxin produced by Clostridium perfringens. *Gene*. 1997;203:65.
634. Herholz C, Miserez R, Nicolet J, et al. Prevalence of beta2-toxigenic Clostridium perfringens in horses with intestinal disorders. *J Clin Microbiol*. 1999;37:358.
635. McClane BA. An overview of Clostridium perfringens enterotoxin. *Toxicon*. 1996;34:1335.
636. Sarker MR, Singh U, McClane BA. An update on Clostridium perfringens enterotoxin. *J Nat Toxins*. 2000;9:251.
637. Kelly CP, LaMont JT. Clostridium difficile infection. *Annual Review of Medicine*. 1998;49:375.
638. Wershil BK, Castagliuolo I, Pothoulakis C. Direct evidence of mast cell involvement in Clostridium difficile toxin A-induced enteritis in mice. *Gastroenterology*. 1998;114:956.
639. Castagliuolo I, Keates AC, Qiu B, et al. Increased substance P responses in dorsal root ganglia and intestinal macrophages during Clostridium difficile toxin A enteritis in rats. In: *Proceedings of the National Academy of Sciences of the United States of America*. 1997;94:4788.
640. Wierup M, DiPietro JA. Bacteriologic examination of equine fecal flora as a diagnostic tool for equine intestinal clostridiosis. *Am J Vet Res*. 1981;42:2167.
641. Daube G, Simon P, Limbourg B, et al. Hybridization of 2,659 Clostridium perfringens isolates with gene probes for seven toxins (alpha, beta, epsilon, iota, theta, mu, and enterotoxin) and for sialidase. *Am J Vet Res*. 1996;57:496.
642. Netherwood T, Wood JL, Mumford JA, Chanter N. Molecular analysis of the virulence determinants of Clostridium perfringens associated with foal diarrhoea. *Vet J*. 1998;155:289.
643. Meer RR, Songer JG. Multiplex polymerase chain reaction assay for genotyping Clostridium perfringens. *Am J Vet Res*. 1997;58:702.
644. Weese JS, Staempfli HR, Prescott JF. Survival of Clostridium difficile and its toxins in equine feces: implications for diagnostic test selection and interpretation. *J Vet Diagn Invest*. 2000;12:332.
645. Jones RL. Diagnostic procedures for isolation and characterization of Clostridium difficile associated with enterocolitis in foals. *J Vet Diagn Invest*. 1989;1:84.
646. Marler LM, Siders JA, Wolters LC, et al. Comparison of five cultural procedures for isolation of Clostridium difficile from stools. *J Clin Microbiol*. 1992;30:514.

647. Drudge JH. Clinical aspects of Strongylus vulgaris infection in the horse. Emphasis on diagnosis, chemotherapy, and prophylaxis. *Vet Clin North Am Large Anim Pract*. 1979;1:251.
648. Owen J, Slocombe D. Pathogenesis of helminths in equines. *Vet Parasitol*. 1985;18:139.
649. Bueno L, Ruckebusch Y, Dorchies P. Disturbances of digestive motility in horses associated with strongyle infection. *Vet Parasitol*. 1979;5:253.
650. Lester GD, Bolton JR, Cambridge H, Thurgate S. The effect of Strongylus vulgaris larvae on equine intestinal myoelectrical activity. *Equine Vet J Suppl*. 1989:8–13.
651. White NA. Intestinal infarction associated with mesenteric vascular thrombotic disease in the horse. *J Am Vet Med Assoc*. 1981;178:259.
652. Becht JL. The role of parasites in colic. *Proc Am Assoc Eq Pract*. 1987;33:301.
653. Sellers AF, Lowe JE, Drost CJ, et al. Retropulsion-propulsion in equine large colon. *Am J Vet Res*. 1982;43:390.
654. Greatorex JC. Diarrhoea in horses associated with ulceration of the colon and caecum resulting from S vulgaris larval migration. *Vet Rec*. 1975;97:221.
655. Patton S, Drudge JH. Clinical response of pony foals experimentally infected with Strongylus vulgaris. *Am J Vet Res*. 1977;38:2059.
656. Amborski GF, Bello TR, Torbert BJ. Host response to experimentally induced infections of Strongylus vulgaris in parasite-free and naturally infected ponies. *Am J Vet Res*. 1974;35:1181.
657. Klei TR, Torbert BJ, Ochoa R, Bello TR. Morphologic and clinicopathologic changes following Strongylus vulgaris infections of immune and nonimmune ponies. *Am J Vet Res*. 1982;43:1300.
658. Patton S, Mock RE, Drudge JH, Morgan D. Increase of immunoglobulin T concentration in ponies as a response to experimental infection with the nematode Strongylus vulgaris. *Am J Vet Res*. 1978;39:19.
659. Lyons ET, Drudge JH, Tolliver SC. Larval cyathostomiasis. *Vet Clin North Am Equine Pract*. 2000;16:501.
660. Chiejina SN, Mason JA. Immature stages of Trichonema spp as a cause of diarrhoea in adult horses in spring. *Vet Rec*. 1977;100:360.
661. Giles CJ, Urquhart KA, Longstaffe JA. Larval cyathostomiasis (immature trichonema-induced enteropathy): a report of 15 clinical cases. *Equine Vet J*. 1985;17:196.
662. Church S, Kelly DF, Obwolo MJ. Diagnosis and successful treatment of diarrhoea in horses caused by immature small strongyles apparently insusceptible to anthelmintics. *Equine Vet J*. 1986;18:401.
663. Mair TS. Recurrent diarrhoea in aged ponies associated with larval cyathostomiasis. *Equine Vet J*. 1993;25:161.
664. Mair TS. Outbreak of larval cyathostomiasis among a group of yearling and two-year-old horses. *Vet Rec*. 1994;135:598.
665. Love S, Murphy D, Mellor D. Pathogenicity of cyathostome infection. *Vet Parasitol*. 1999;85:113.
666. Mair TS, de Westerlaken LV, Cripps PJ, Love S. Diarrhoea in adult horses: a survey of clinical cases and an assessment of some prognostic indices. *Vet Rec*. 1990;126:479.
667. Murphy D, Love S. The pathogenic effects of experimental cyathostome infections in ponies. *Vet Parasitol*. 1997;70:99.
668. Murphy D, Reid SW, Graham PA, Love S. Fructosamine measurement in ponies: validation and response following experimental cyathostome infection. *Res Vet Sci*. 1997;63:113.
669. Klei TR, Rehbein S, Visser M, et al. Re-evaluation of ivermectin efficacy against equine gastrointestinal parasites. *Vet Parasitol*. 2001;98:315.
670. Tarigo-Martinie JL, Wyatt AR, Kaplan RM. Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses. *J Am Vet Med Assoc*. 2001;218:1957.
671. Jacobs DE, Hutchinson MJ, Parker L, Gibbons LM. Equine cyathostome infection: suppression of faecal egg output with moxidectin. *Vet Rec*. 1995;137:545.
672. Monahan CM, Chapman MR, Taylor HW, et al. Experimental cyathostome challenge of ponies maintained with or without benefit of daily pyrantel tartrate feed additive: comparison of parasite burdens, immunity and colonic pathology. *Vet Parasitol*. 1998;74:229.
673. Chapman MR, French DD, Monahan CM, Klei TR. Identification and characterization of a pyrantel pamoate resistant cyathostome population. *Vet Parasitol*. 1996;66:205.
674. Little D, Flowers JR, Hammerberg BH, Gardner SY. Management of drug-resistant cyathostominosis on a breeding farm in central North Carolina. *Equine Vet J*. 2003;35:246.
675. Andersson G, Ekman L, Mansson I, et al. Lethal complications following administration of oxytetracycline in the horse. *Nord Vet Med*. 1971;23:2.
676. Raisbeck MF, Holt GR, Osweiler GD. Lincomycin-associated colitis in horses. *J Am Vet Med Assoc*. 1981;179:362.
677. Stratton-Phelps M, Wilson WD, Gardner IA. Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases (1986–1996). *J Am Vet Med Assoc*. 2000;217:68.
678. Wilson DA, MacFadden KE, Green EM, et al. Case control and historical cohort study of diarrhea associated with administration of trimethoprim-potentiated sulphonamides to horses and ponies. *J Vet Intern Med*. 1996;10:258.
679. Gustafsson A, Baverud V, Gunnarsson A, et al. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. *Equine Vet J*. 1997;29:314–318.
680. Owen RA, Fullerton J, Barnum DA. Effects of transportation, surgery, and antibiotic therapy in ponies infected with Salmonella. *Am J Vet Res*. 1983;44:46.
681. Borriello SP. The influence of the normal flora on Clostridium difficile colonisation of the gut. *Ann Med*. 1990;22:61.
682. Owen R, Fullerton JN, Tizard IR, et al. Studies on experimental enteric salmonellosis in ponies. *Can J Comp Med*. 1979;43:247.
683. Rao SS, Edwards CA, Austen CJ, et al. Impaired colonic fermentation of carbohydrate after ampicillin. *Gastroenterology*. 1988;94:928.
684. Clausen MR, Bonnen H, Tvede M, Mortensen PB. Colonic fermentation to short-chain fatty acids is decreased in antibiotic-associated diarrhea. *Gastroenterology*. 1991;101:1497.
685. Grossman RF. The relationship of absorption characteristics and gastrointestinal side effects of oral antimicrobial agents. *Clin Ther*. 1991;13:189.
686. Roussel AJ, Hooper RN, Cohen ND, et al. Prokinetic effects of erythromycin on the ileum, cecum, and pelvic flexure of horses during the postoperative period. *Am J Vet Res*. 2000;61:420.
687. Lakritz J, Madigan J, Carlson GP. Hypovolemic hyponatremia and signs of neurologic disease associated with diarrhea in a foal. *J Am Vet Med Assoc*. 1992;200:1114.
688. Kore AM. Toxicology of nonsteroidal antiinflammatory drugs. *Vet Clin North Am Small Anim Pract*. 1990;20:419.
689. Gibson GR, Whitacre EB, Ricotti CA. Colitis induced by nonsteroidal anti-inflammatory drugs. Report of four cases and review of the literature. *Arch Intern Med*. 1992;152:625.
690. Meschter CL, Gilbert M, Krook L, et al. The effects of phenylbutazone on the intestinal mucosa of the horse: a morphological, ultrastructural and biochemical study. *Equine Vet J*. 1990;22:255.
691. Collins LG, Tyler DE. Experimentally induced phenylbutazone toxicosis in ponies: description of the syndrome and its prevention with synthetic prostaglandin E2. *Am J Vet Res*. 1985;46:1605.
692. Collins LG, Tyler DE. Phenylbutazone toxicosis in the horse: a clinical study. *J Am Vet Med Assoc*. 1984;184:699.
693. Karcher LF, Dill SG, Anderson WI, King JM. Right dorsal colitis. *J Vet Intern Med*. 1990;4:247.
694. Hough ME, Steel CM, Bolton JR, Yovich JV. Ulceration and stricture of the right dorsal colon after phenylbutazone administration in four horses. *Aust Vet J*. 1999;77:785.

695. Cook, V. L., C. T. Meyers, N. B. Campbell, and A. T. Blikslager. 2008. Effect of firocoxib or flunixin meglumine on recovery of ischemic-injured equine jejunum. 70:992–1000.
696. Murray MJ. Phenylbutazone toxicity in a horse. *Comp Cont Ed Pract Vet*. 1985;7:S389–S394.
697. Lees P, Creed RF, Gerring EE, et al. Biochemical and haematological effects of phenylbutazone in horses. *Equine Vet J*. 1983;15:158.
698. Cohen ND, Carter GK, Mealey RH, Taylor TS. Medical management of right dorsal colitis in 5 horses: a retrospective study (1987–1993). *J Vet Intern Med*. 1995;9:272.
699. Kaufmann HJ, Taubin HL. Nonsteroidal anti-inflammatory drugs activate quiescent inflammatory bowel disease. *Ann Intern Med*. 1987;107:513.
700. Hovde O, Farup PG. NSAID-induced irreversible exacerbation of ulcerative colitis. *J Clin Gastroenterol*. 1992;15:160.
701. Blikslager AT, Roberts MC. Mechanisms of intestinal mucosal repair. *J Am Vet Med Assoc*. 1998;211:1437.
702. Semble EL, Wu WC. Prostaglandins in the gut and their relationship to non-steroidal anti-inflammatory drugs. *Baillieres Clin Rheumatol*. 1989;3:247.
703. Campbell NB, Jones SL, Blikslager AT. The effects of cyclooxygenase inhibitors on bile-injured and normal equine colon. *Equine Vet J*. 2002;34:493.
704. Jones SL, Blikslager AT. The future of antiinflammatory therapy. *Vet Clin North Am Equine Pract*. 2001;17:245.
705. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91.
706. Yamada T, Deitch E, Specian RD, et al. Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. *Inflammation*. 1993;17:641.
707. Beck PL, Xavier R, Lu N, et al. Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. *Gastroenterology*. 2000;119:699.
708. Wallace JL, Granger DN. Pathogenesis of NSAID gastropathy: are neutrophils the culprits? *Trends Pharm Sci*. 1992;13:129.
709. Morise Z, Komatsu S, Fuseler JW, et al. ICAM-1 and P-selectin expression in a model of NSAID-induced gastropathy. *Am J Physiol Cell Physiol*. 1998;274:G246–G252.
710. Brune K, Beck WS. Towards safer nonsteroidal anti-inflammatory drugs. *Agents Actions Suppl*. 1991;32:13–25.
711. Davis JL, Gardner SY, Jones SL, Schwabenton BA, Papich MG. Pharmacokinetics of azithromycin in foals after I.V. and oral dose and disposition into phagocytes. *J Vet Pharmacol Ther*. 2002;25:99.
712. Cohen ND, Mealey RH, Chaffin MK, Carter GK. The recognition and medical management of right dorsal colitis in horses. *Vet Med*. 1995;9:687.
713. Schmitz DG. Cantharidin toxicosis in horses. *J Vet Intern Med*. 1989;3:208.
714. Shawley RV, Rolf LLJ. Experimental cantharidiasis in the horse. *Am J Vet Res*. 1984;45:2261.
715. Schoeb TR, Panciera RJ. Pathology of blister beetle (Epicauta) poisoning in horses. *Vet Pathol*. 1979;16:18.
716. Ray AC, Kyle AL, Murphy MJ, Reagor JC. Etiologic agents, incidence, and improved diagnostic methods of cantharidin toxicosis in horses. *Am J Vet Res*. 1989;50:187.
717. Helman RG, Edwards WC. Clinical features of blister beetle poisoning in equids: 70 cases (1983-1996). *J Am Vet Med Assoc*. 1997;211:1018.
718. Osweiler GD, Carron JL, Buck WB. *Clinical and diagnostic veterinary toxicology*. Dubuque, IA: Kendal Hunt; 1985:253.
719. Tamaki S, Frankenberger WTJ. Environmental biochemistry of arsenic. *Rev Environ Contam Toxicol*. 1992;124:79.
720. Louria DB. Trace metal poisoning. In: Wyndgaarden JB, Smith LH, eds. *Cecil Textbook of Medicine*. Philadelphia: WB Saunders; 1988:2385.
721. Mack RB. Gee, honey, why does the iced tea have a garlic taste? Arsenic intoxication. *N C Med J*. 1983;44:753.
722. Olson NE. Acute diarrheal disease in the horse. *J Am Vet Med Assoc*. 1966;148:418.
723. Wershil BK, Walker WA. The mucosal barrier, IgE-mediated gastrointestinal events, and eosinophilic gastroenteritis. *Gastroenterol. Clin North Am*. 1992;21:387.
724. Strobel S. IgE-mediated (and food-induced) intestinal disease. *Clin Exp Allergy*. 1995;25(suppl 1):3.
725. Ohtsuka Y, Naito K, Yamashiro Y, et al. Induction of anaphylaxis in mouse intestine by orally administered antigen and its prevention with soluble high affinity receptor for IgE. *Pediatr Res*. 1999;45:300.
726. Zimmel DN, Blikslager AT, Jones SL, et al. Vaccine-associated anaphylactic-like reaction in a horse. *Comp Cont Ed Pract Vet*. 2000;1:81.
727. Mansmann RA. Equine anaphylaxis. *J Am Vet Med Assoc*. 1972;161:438.
728. McGavin MD, Gronwall RR, Mia AS. Pathologic changes in experimental equine anaphylaxis. *J Am Vet Med Assoc*. 1972;160:1632.
729. Stenton GR, Vliagoftis H, Befus AD. Role of intestinal mast cells in modulating gastrointestinal pathophysiology. *Ann Allergy Asthma Immunol*. 1998;81:1.
730. Mourad FH, O'Donnell LJ, Ogutu E, et al. Role of 5-hydroxytryptamine in intestinal water and electrolyte movement during gut anaphylaxis. *Gut*. 1995;36:553.
731. Catto-Smith AG, Patrick MK, Hardin JA, Gall DG. Intestinal anaphylaxis in the rat: mediators responsible for the ion transport abnormalities. *Agents Actions*. 1989;28:185.
732. Scott RB, Diamant SC, Gall DG. Motility effects of intestinal anaphylaxis in the rat. *Am J Physiol*. 1988;255:G505–G511.
733. Baron DA, Baird AW, Cuthbert AW, Margolius HS. Intestinal anaphylaxis: rapid changes in mucosal ion transport and morphology. *Am J Physiol*. 1988;254:G307–G314.
734. Garner HE, Hutcheson DP, Coffman JR, et al. Lactic acidosis: a factor associated with equine laminitis. *J Anim Sci*. 1977;45:1037.
735. Garner HE, Moore JN, Johnson JH, et al. Changes in the caecal flora associated with the onset of laminitis. *Equine Vet J*. 1978;10:249.
736. Sprouse RF, Garner HE, Green EM. Plasma endotoxin levels in horses subjected to carbohydrate induced laminitis. *Equine Vet J*. 1987;19:25.
737. Ragle CA, Meagher DM, Lacroix CA, Honnas CM. Surgical treatment of sand colic. Results in 40 horses. *Vet Surg*. 1989;18:48.
738. Bertone JJ, Traub-Dargatz JL, Wrigley RW, et al. Diarrhea associated with sand in the gastrointestinal tract of horses. *J Am Vet Med Assoc*. 1988;193:1409.
739. Ramey DW, Reinertson EL. Sand-induced diarrhea in a foal. *J Am Vet Med Assoc*. 1984;185:537.
740. McGuinness SG, Mansmann RA, Breuhaus BA. Nasogastric electrolyte replacement in horses. *Comp Cont Ed Pract Vet*. 1996;18:942.
741. Duebbert IE, Peterson JW. Enterotoxin-induced fluid accumulation during experimental salmonellosis and cholera: involvement of prostaglandin synthesis by intestinal cells. *Toxicon*. 1985;23:157.
742. Freeman DE, Inoue OJ, Eurell TE. Effects of flunixin meglumine on short circuit current in equine colonic mucosa in vitro. *Am J Vet Res*. 1997;58:915.
743. Richter RA, Freeman DE, Wallig M, et al. In vitro anion transport alterations and apoptosis induced by phenylbutazone in the right dorsal colon of ponies. *Am J Vet Res*. 2002;63:934.
744. Joshi GP, Gertler R, Fricker R. Cardiovascular thromboembolic adverse effects associated with cyclooxygenase-2 selective inhibitors and nonselective antiinflammatory drugs. *Anesth Analg*. 2007;105:1793.

745. Geor RJ, Petrie L, Papich MG, Rousseaux C. The protective effects of sucralfate and ranitidine in foals experimentally intoxicated with phenylbutazone. *Can J Vet Res*. 1989;53:231.
746. Fedorak RN, Empey LR, MacArthur C, Jewell LD. Misoprostol provides a colonic mucosal protective effect during acetic acid-induced colitis in rats. *Gastroenterology*. 1990;98:615.
747. Wachtershauser A, Stein J. Rationale for the luminal provision of butyrate in intestinal diseases. *Eur J Nutr*. 2000;39:164.
748. Rhoads JM, Chen W, Gookin J, et al. Arginine stimulates intestinal cell migration through a focal adhesion kinase dependent mechanism. *Gut*. 2004;53:514.
749. Palmer JE, Benson CE, Whitlock RH. Effect of treatment with oxytetracycline during the acute stages of experimentally induced equine ehrlichial colitis in ponies. *Am J Vet Res*. 1992;53:2300.
750. McGorum BC, Dixon PM, Smith DG. Use of metronidazole in equine acute idiopathic toxaemic colitis. *Vet Rec*. 1998;142:635.
751. Jang SS, Hansen LM, Breher JE, et al. Antimicrobial susceptibilities of equine isolates of Clostridium difficile and molecular characterization of metronidazole-resistant strains. *Clin Infect Dis*. 1997;25(suppl 2):S266–S267.
752. MacKay RJ. Equine neonatal clostridiosis: treatment and prevention. *Comp Cont Ed Pract Vet*. 2001;23:280.
753. Cambridge H, Lees P, Hooke RE, Russell CS. Antithrombotic actions of aspirin in the horse. *Equine Vet J*. 1991;23:123.
754. Belknap JK, Moore JN. Evaluation of heparin for prophylaxis of equine laminitis: 71 cases (1980–1986). *J Am Vet Med Assoc*. 1989;195:505.
755. Fuller R. Probiotics in man and animals. *J Appl Bacteriol*. 1989;66:365.
756. Schoster A, Staempfli HR, Abrahams M, et al. Effect of a probiotic on prevention of diarrhea and Clostridium difficile and Clostridium perfringens shedding in foals. *J Vet Intern Med*. 2015;29:925–931.
757. Desrochers AM, Dolente BA, Roy MF, et al. Efficacy of Saccharomyces boulardii for treatment of horses with acute enterocolitis. *J Am Vet Med Assoc*. 2005;227:954.
758. Qualls HJ, Holbrook TC, Gilliam LL, et al. Evaluation of efficacy of mineral oil, charcoal, and smectite in a rat model of equine cantharidin toxicosis. *J Vet Intern Med*. 2013;27:1179–1184.
759. Lawler JB, Hassel DM, Magnuson RJ, et al. Adsorptive effects of di-tri-octahedral smectite on Clostridium perfringens alpha, beta, and beta-2 exotoxins and equine colostral antibodies. *Am J Vet Res*. 2008;69:233.
760. Xiao L, Herd RP, Majewski GA. Comparative efficacy of moxidectin and ivermectin against hypobiotic and encysted cyathostomes and other equine parasites. *Vet Parasitol*. 1994;53:83.
761. Love S, Duncan JL, Parry JM, Grimshaw WT. Efficacy of oral ivermectin paste against mucosal stages of cyathostomes. *Vet Rec*. 1995;136:18.
762. Duncan JL, Bairden K, Abbott EM. Elimination of mucosal cyathostome larvae by five daily treatments with fenbendazole. *Vet Rec*. 1998;142:268.
763. Hutchens DE, Paul AJ. Moxidectin: spectrum of activity and uses in an equine anthelmintic program. *Comp Cont Ed Pract Vet*. 2000;22:373.
764. Steinbach T, Bauer C, Sasse H, et al. Small strongyle infection: consequences of larvicidal treatment of horses with fenbendazole and moxidectin. *Vet Parasitol*. 2006;139:115.
765. Peritonitis Hosgood G. 1. A Review of the Pathophysiology and Diagnosis. *Aust Vet Pract*. 1986;16:184–190.
766. Trent AM. The peritoneum and peritoneal cavity. In: Kobluk CN, Ames TR, Geor RJ, eds. *The horse: diseases and clinical management*. Philadelphia: WB Saunders; 1995:373–404.
767. Dabareiner RM. Peritonitis in horses. In: Smith BP, ed. *Large Animal Internal Medicine*. St. Louis, MO: Mosby; 1996:742–749.
768. Dyson S. Review of 30 cases of peritonitis in the horse. *Equine Vet J*. 1983;15:25–30.
769. Zicker SC, Wilson WD, Medearis I. Differentiation between intra-abdominal neoplasms and abscesses in horses, using clinical and laboratory data: 40 cases (1973–1988). *J Am Vet Med Assoc*. 1990;196:1130–1134.
770. Reuss SM, Chaffin MK, Cohen ND. Extrapulmonary disorders associated with Rhodococcus equi infection in foals: 150 cases (1987–2007). *J Am Vet Med Assoc*. 2009;235:855–863.
771. Pratt SM, Spier SJ, Carroll SP, et al. Evaluation of clinical characteristics, diagnostic test results, and outcome in horses with internal infection caused by Corynebacterium pseudotuberculosis: 30 cases (1995–2003). *J Am Vet Med Assoc*. 2005;227:441–448.
772. Gay CC, Lording PM. Peritonitis in horses associated with Actinobacillus equuli. *Aust Vet J*. 1980;56:296–300.
773. Golland LC, Hodgson DR, Hodgson JL, et al. Peritonitis associated with Actinobacillus equuli in horses: 15 cases (1982–1992). *J Am Vet Med Assoc*. 1994;205:340–343.
774. Matthews S, Dart AJ, Dowling BA, et al. Peritonitis associated with Actinobacillus equuli in horses: 51 cases. *Aust Vet J*. 2001;79:536–539.
775. Watts A, Johnson A, Felippe M, et al. Recurrent Actinobacillus peritonitis in an otherwise healthy Thoroughbred horse. *Aust Vet J*. 2011;89:143–146.
776. Hirsh DC, Jang SS. Antimicrobic susceptibility of bacterial pathogens from horses. *Vet Clin North Am Equine Pract*. 1987;3:181–190.
777. Hawkins JF, Bowman KF, Roberts MC, et al. Peritonitis in horses: 67 cases (1985–1990). *J Am Vet Med Assoc*. 1993;203:284–288.
778. Lapointe JM, Celeste C, Villeneuve A. Septic peritonitis due to colonic perforation associated with aberrant migration of a Gasterophilus intestinalis larva in a horse. *Vet Pathol*. 2003;40:338–339.
779. Archer DC, Clegg PD, Edwards GB. Septic tenosynovitis of the tarsal sheath of an Arab gelding and suspected sepsis of the lateral digital flexor tendon subsequent to bacterial peritonitis. *Vet Rec*. 2004;155:485–489.
780. Schumacher J, Spano JS, Moll HD. Effects of enterocentesis on peritoneal fluid constituents in the horse. *J Am Vet Med Assoc*. 1985;186:1301–1303.
781. Rumbaugh GE, Smith BP, Carlson GP. Internal abdominal abscesses in the horse: a study of 25 cases. *J Am Vet Med Assoc*. 1978;172:304–309.
782. Blackford JT, Schneiter HL, Van Steenehouse JL. Equine peritoneal fluid analysis following celiotomy. *Proc Equine Colic Res Symp*. 1986;3:130.
783. Van Hoogmoed L, Rodger LD, Spier SJ, et al. Evaluation of peritoneal fluid pH, glucose concentration, and lactate dehydrogenase activity for detection of septic peritonitis in horses. *J Am Vet Med Assoc*. 1999;214:1032–1036.
784. Bonczynski JJ, Ludwig LL, Barton LJ, et al. Comparison of peritoneal fluid and peripheral blood pH, bicarbonate, glucose, and lactate concentration as a diagnostic tool for septic peritonitis in dogs and cats. *Vet Surg*. 2003;32:161–166.
785. Sullins KE, White NA, Lundin CS, et al. Prevention of ischaemia-induced small intestinal adhesions in foals. *Equine Vet J*. 2004;36:370–375.
786. Kunesh JP. Therapeutic strategies involving antimicrobial treatment of large animals with peritonitis. *J Am Vet Med Assoc*. 1984;185:1222–1225.
787. Hague BA, Honnas CM, Berridge BR, et al. Evaluation of postoperative peritoneal lavage in standing horses for prevention of experimentally induced abdominal adhesions. *Vet Surg*. 1998;27:122–126.
788. Davis JL. Treatment of peritonitis. *Vet Clin North Am Equine Pract*. 2003;19:765–778.

789. Nieto JE, Snyder JR, Vatistas NJ, et al. Use of an active intra-abdominal drain in 67 horses. *Vet Surg*. 2003;32:1–7.
790. Schneider RK, Meyer DJ, Embertson RM, et al. Response of pony peritoneum to four peritoneal lavage solutions. *Am J Vet Res*. 1988;49:889–894.
791. Easter JL, Hague BA, Brumbaugh GW, et al. Effects of postoperative peritoneal lavage on pharmacokinetics of gentamicin in horses after celiotomy. *Am J Vet Res*. 1997;58:1166–1170.
792. Southwood LL, Russell G. The use of clinical findings in the identification of equine peritonitis cases that respond favorably to medical therapy. *J Vet Emerg Crit Care*. 2007;17:382–390.
793. Javsicas LH, Giguere S, Freeman DE, et al. Comparison of Surgical and Medical Treatment of 49 Postpartum Mares with Presumptive or Confirmed Uterine Tears. *Veterinary Surgery*. 2010;39:254–260.
794. Nogradi N, Slovis NM, Gebhart CJ, et al. Evaluation of the field efficacy of an avirulent live Lawsonia intracellularis vaccine in foals. *Vet J*. 2012;192:511–513.
795. Nogradi N, Toth B, Macgillivray KC. Peritonitis in horses: 55 cases (2004–2007). *Acta Vet Hung*. 2011;59:181–193.
796. Mair TS, Smith LJ. Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 4: early (acute) relaparotomy. *Equine Vet J*. 2005;37:315–318.
797. Pappenheimer JR. Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: relation to body size. *Am J Physiol*. 1990;259:G290–G299.
798. Pappenheimer JR. Physiological regulation of epithelial junctions in intestinal epithelia. *Acta Physiol Scand Suppl*. 1988;571:43–51.
799. Pappenheimer JR. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. *J Membr Biol*. 1987;100:137–148.
800. Pappenheimer JR, Reiss KZ. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J Membr Biol*. 1987;100:123–136.
801. Madara JL. Warner-Lambert/Parke-Davis Award lecture. Pathobiology of the intestinal epithelial barrier. *Am J Pathol*. 1990;137:1273–1281.
802. Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol*. 2006;68. 403–29.
803. Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol*. 1999;147:1351–1363.
804. Karczewski J, Groot J. Molecular physiology and pathophysiology of tight junctions III. Tight junction regulation by intracellular messengers: differences in response within and between epithelia. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G660–G665.
805. Mitic LL, Van Itallie CM, Anderson JM. Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G250–G254.
806. Madara JL, Trier JS. The functional morphology of the mucosa of the small intestine. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*. New York: Raven Press; 1994:1577–1622.
807. Madara JL. Loosening tight junctions. Lessons from the intestine. *J Clin Invest*. 1989;83:1089–1094.
808. Madara JL, Marcial MA. Structural correlates of intestinal tight-junction permeability. *Kroc Found Ser*. 1984;17:77–100.
809. Tice LW, Carter RL, Cahill MB. Changes in tight junctions of rat intestinal crypt cells associated with changes in their mitotic activity. *Tissue Cell*. 1979;11:293–316.
810. Marcial MA, Carlson SL, Madara JL. Partitioning of paracellular conductance along the ileal crypt-villus axis: a hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. *J Membr Biol*. 1984;80:59–70.
811. Stevens CE, Hume ID. *Comparative Physiology of the Vertebrate Digestive System*. New York: Cambridge University Press; 1995.
812. Argenzio RA. Comparative pathophysiology of nonglandular ulcer disease: a review of experimental studies. *Equine Vet J Suppl*. 1999;29:19–23.
813. Argenzio RA. Mechanisms of acid injury in porcine gastroesophageal mucosa. *Am J Vet Res*. 1996;57:564–573.
814. Murray MJ, Mahaffey EA. Age-related characteristics of gastric squamous epithelial mucosa in foals. *Equine Vet J*. 1993;25:514–517.
815. Andrews FM, Sifferman RL, Bernard W, et al. Efficacy of omeprazole paste in the treatment and prevention of gastric ulcers in horses. *Equine Vet J Suppl*. 1999;29:81–86.
816. Vatistas NJ, Snyder JR, Nieto J, et al. Acceptability of a paste formulation and efficacy of high dose omeprazole in healing gastric ulcers in horses maintained in race training. *Equine Vet J Suppl*. 1999;29:71–76.
817. Murray MJ. Suppression of gastric acidity in horses. *J Am Vet Med Assoc*. 1997;211:37–40.
818. Campbell-Thompson ML, Merritt AM. Basal and pentagastrin-stimulated gastric secretion in young horses. *Am J Physiol*. 1990;259:R1259–R1266.
819. Schreiber S, Nguyen TH, Stuben M, Scheid P. Demonstration of a pH gradient in the gastric gland of the acid-secreting guinea pig mucosa. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G597–G604.
820. Flemstrom G. Gastric and duodenal mucosal secretion of bicarbonate. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*. New York: Raven Press; 1994:1285–1309.
821. Podolsky DK. Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. *Am J Physiol*. 1999;277:G495–G499.
822. Robert A, Nezamis JE, Lancaster C, et al. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. *Am J Physiol*. 1983;245:G113–G121.
823. Robert A. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology*. 1979;77:433–443.
824. Robert A. Prostaglandins: effects on the gastrointestinal tract. *Clin Physiol Biochem*. 1984;2:61–69.
825. Ruppin H, Person B, Robert A, Domschke W. Gastric cytoprotection in man by prostaglandin E_2. *Scand J Gastroenterol*. 1981;16:647–652.
826. Mutoh H, Ota S, Hiraishi H, et al. Adaptive cytoprotection in cultured rat gastric mucus-producing cells. Role of mucus and prostaglandin synthesis. *Dig Dis Sci*. 1995;40:872–878.
827. Wallace JL. Increased resistance of the rat gastric mucosa to hemorrhagic damage after exposure to an irritant. Role of the "mucoid cap" and prostaglandin synthesis. *Gastroenterology*. 1988;94:22–32.
828. Konturek SJ, Robert A. Cytoprotection of canine gastric mucosa by prostacyclin: possible mediation by increased mucosal blood flow. *Digestion*. 1982;25:155–163.
829. Leung FW, Robert A, Guth PH. Gastric mucosal blood flow in rats after administration of 16,16-dimethyl prostaglandin E2 at a cytoprotective dose. *Gastroenterology*. 1985;88:1948–1953.
830. Asako H, Kubes P, Wallace J, et al. Modulation of leukocyte adhesion in rat mesenteric venules by aspirin and salicylate. *Gastroenterology*. 1992;103:146–152.
831. Holzer P, Sametz W. Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. *Gastroenterology*. 1986;91:975–981.
832. Holzer P, Pabst MA, Lippe IT, et al. Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. *Gastroenterology*. 1990;98:838–848.
833. Holzer P, Pabst MA, Lippe IT. Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. *Gastroenterology*. 1989;96:1425–1433.

834. Merchant NB, Dempsey DT, Grabowski MW, et al. Capsaicin-induced gastric mucosal hyperemia and protection: the role of calcitonin gene-related peptide. *Surgery*. 1994;116:419–425.
835. Wallace JL, Miller MJ. Nitric oxide in mucosal defense: a little goes a long way. *Gastroenterology*. 2000;119:512–520.
836. Blikslager AT, Rhoads JM, Bristol DG, et al. Glutamine and transforming growth factor-alpha stimulate extracellular regulated kinases and enhance recovery of villous surface area in porcine ischemic-injured intestine. *Surgery*. 1999;125:186–194.
837. Moore R, Carlson S, Madara JL. Villus contraction aids repair of intestinal epithelium after injury. *Am J Physiol*. 1989;257:G274–G283.
838. Lang J, Blikslager A, Regina D, et al. Synergistic effect of hydrochloric acid and bile acids on the pars esophageal mucosa of the porcine stomach. *Am J Vet Res*. 1998;59:1170–1176.
839. Berschneider HM, Blikslager AT, Roberts MC. Role of duodenal reflux in nonglandular gastric ulcer disease of the mature horse. *Equine Vet J Suppl*. 1999;29:24–29.
840. Baker SJ, Gerring EL. Technique for prolonged, minimally invasive monitoring of intragastric pH in ponies. *Am J Vet Res*. 1993;54:1725–1734.
841. Murray MJ. Equine model of inducing ulceration in alimentary squamous epithelial mucosa. *Dig Dis Sci*. 1994;39:2530–2535.
842. Merritt AM. Normal equine gastroduodenal secretion and motility. *Equine Vet J Suppl*. 1999;29:7–13.
843. Peek RMJ IV. Helicobacter pylori strain-specific activation of signal transduction cascades related to gastric inflammation. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G525–G530.
844. Murray MJ. Pathophysiology of peptic disorders in foals and horses: a review. *Equine Vet J Suppl*. 1999;29:14–18.
845. Wallace JL. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology*. 1997;112:1000–1016.
846. Henry D, Dobson A, Turner C. Variability in the risk of major gastrointestinal complications from nonaspirin nonsteroidal anti-inflammatory drugs. *Gastroenterology*. 1993;105:1078–1088.
847. Langenbach R, Morham SG, Tiano HF, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*. 1995;83:483–492.
848. Smith WL, Langenbach R. Why there are two cyclooxygenase isozymes. *J Clin Invest*. 2001;107:1491–1495.
849. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-Induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology*. 2000;119:706–714.
850. Moore RM, Muir WW, Granger DN. Mechanisms of gastrointestinal ischemia-reperfusion injury and potential therapeutic interventions: a review and its implications in the horse. *J Vet Intern Med*. 1995;9:115–132.
851. Moore RM. Clinical relevance of intestinal reperfusion injury in horses. *J Am Vet Med Assoc*. 1997;211:1362–1366.
852. Shepherd AP, Granger DN. Metabolic regulation of intestinal circulation. In: Shepherd AP, Granger DN, eds. *Physiology of Intestinal Circulation*. New York: Raven Press; 2001.
853. Bulkley GB, Kvietys PR, Parks DA, et al. Relationship of blood flow and oxygen consumption to ischemic injury in the canine small intestine. *Gastroenterology*. 1985;89:852–857.
854. Dart AJ, Snyder JR, Julian D, Hinds DM. Microvascular circulation of the small intestine in horses. *Am J Vet Res*. 1992;53:995–1000.
855. Snyder JR, Olander HJ, Pascoe JR, et al. Morphologic alterations observed during experimental ischemia of the equine large colon. *Am J Vet Res*. 1988;49:801–809.
856. McAnulty JF, Stone WC, Darien BJ. The effects of ischemia and reperfusion on mucosal respiratory function, adenosine triphosphate, electrolyte, and water content in the ascending colon of ponies. *Vet Surg*. 1997;26:172–181.
857. Noda T, Iwakiri R, Fujimoto K, et al. Programmed cell death induced by ischemia-reperfusion in rat intestinal mucosa. *Am J Physiol*. 1998;274:G270–G276.
858. Ikeda H, Suzuki Y, Suzuki M, et al. Apoptosis is a major mode of cell death caused by ischaemia and ischaemia/reperfusion injury to the rat intestinal epithelium. *Gut*. 1998;42:530–537.
859. Coopersmith CM, O'Donnell D, Gordon JI. Bcl-2 inhibits ischemia-reperfusion-induced apoptosis in the intestinal epithelium of transgenic mice. *Am J Physiol*. 1999;276:G677–G686.
860. Chiu CJ, McArdle AH, Brown R, et al. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg*. 1970;101:478–483.
861. Laws EG, Freeman DE. Significance of reperfusion injury after venous strangulation obstruction of equine jejunum. *J Invest Surg*. 1995;8:263–270.
862. Meschter CL, Craig D, Hackett R. Histopathological and ultrastructural changes in simulated large colonic torsion and reperfusion in ponies. *Equine Vet J*. 1991;23:426–433.
863. van Hoogmoed L, Snyder JR, Pascoe JR, Olander HJ. Evaluation of uniformity of morphological injury of the large colon following severe colonic torsion. *Equine Vet J Suppl*. 2000;32:98–100.
864. van Hoogmoed L, Snyder JR, Pascoe JR, Olander H. Use of pelvic flexure biopsies to predict survival after large colon torsion in horses. *Vet Surg*. 2000;29:572–577.
865. White NA, Moore JN, Douglas M. SEM study of Strongylus vulgaris larva-induced arteritis in the pony. *Equine Vet J*. 1983;15:349–353.
866. Moore RM, Bertone AL, Bailey MQ, et al. Neutrophil accumulation in the large colon of horses during low-flow ischemia and reperfusion. *Am J Vet Res*. 1994;55:1454–1463.
867. Moore RM, Bertone AL, Muir WW, et al. Histopathologic evidence of reperfusion injury in the large colon of horses after low-flow ischemia. *Am J Vet Res*. 1994;55:1434–1443.
868. Gerard MP, Blikslager AT, Roberts MC, et al. The characteristics of intestinal injury peripheral to strangulating obstruction lesions in the equine small intestine. *Equine Vet J*. 1999;31:331–335.
869. Dabareiner RM, White NA, Donaldson LL. Effects of intraluminal distention and decompression on microvascular permeability and hemodynamics of the equine jejunum. *Am J Vet Res*. 2001;62:225–236.
870. Dabareiner RM, Sullins KE, Snyder JR, et al. Evaluation of the microcirculation of the equine small intestine after intraluminal distention and subsequent decompression. *Am J Vet Res*. 1993;54:1673–1682.
871. Dabareiner RM, Snyder JR, White NA, et al. Microvascular permeability and endothelial cell morphology associated with low-flow ischemia/reperfusion injury in the equine jejunum. *Am J Vet Res*. 1995;56:639–648.
872. Allen DJ, White NA, Tyler DE. Factors for prognostic use in equine obstructive small intestinal disease. *J Am Vet Med Assoc*. 1986;189:777–780.
873. Schoenberg MH, Poch B, Younes M, et al. Involvement of neutrophils in postischaemic damage to the small intestine. *Gut*. 1991;32:905–912.
874. Nilsson UA, Schoenberg MH, Aneman A, et al. Free radicals and pathogenesis during ischemia and reperfusion of the cat small intestine. *Gastroenterology*. 1994;106:629–636.
875. Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am J Physiol*. 1988;255:H1269–H1275.
876. Horne MM, Pascoe PJ, Ducharme NG, et al. Attempts to modify reperfusion injury of equine jejunal mucosa using dimethylsulfoxide, allopurinol, and intraluminal oxygen. *Vet Surg*. 1994;23:241–249.
877. Park PO, Haglund U, Bulkley GB, Falt K. The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion. *Surgery*. 1990;107:574–580.

878. Haglund U. Gut ischaemia. *Gut*. 1994;35:S73–S76.
879. Dabareiner RM, Snyder JR, Sullins KE, White NA, Gardner IA. Evaluation of the microcirculation of the equine jejunum and ascending colon after ischemia and reperfusion. *Am J Vet Res*. 1993;54:1683–1692.
880. Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Dig Dis Sci*. 1988;33:6S–15S.
881. Prichard M, Ducharme NG, Wilkins PA, et al. Xanthine oxidase formation during experimental ischemia of the equine small intestine. *Can J Vet Res*. 1991;55:310–314.
882. Blikslager AT, Roberts MC, Gerard MP, Argenzio RA. How important is intestinal reperfusion injury in horses? *J Am Vet Med Assoc*. 1997;211:1387–1389.
883. Parks DA, Williams TK, Beckman JS. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a reevaluation. *Am J Physiol*. 1988;254:G768–G774.
884. Blikslager AT, Roberts MC, Rhoads JM, Argenzio RA. Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia? *Surgery*. 1997;121:526–534.
885. Schaffer MR, Efron PA, Thornton FJ, et al. Nitric oxide, an autocrine regulator of wound fibroblast synthetic function. *J Immunol*. 1997;158:2375–2381.
886. Konturek SJ, Brzozowski T, Majka J, et al. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur J Pharmacol*. 1993;239:215–217.
887. Tarnawski A, Tanoue K, Santos AM, Sarfeh IJ. Cellular and molecular mechanisms of gastric ulcer healing. Is the quality of mucosal scar affected by treatment? *Scand J Gastroenterol Suppl*. 1995;210:9–14.
888. Tarnawski A, Stachura J, Durbin T, et al. Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. *Gastroenterology*. 1992;102:695–698.
889. Erickson RA. 16,16-Dimethyl prostaglandin E_2 induces villus contraction in rats without affecting intestinal restitution. *Gastroenterology*. 1990;99:708–716.
890. Moore R, Madara JL, MacLeod RJ. Enterocytes adhere preferentially to collagen IV in a differentially regulated divalent cation-dependent manner. *Am J Physiol*. 1994;266:G1099–G1107.
891. Moore R, Madri J, Carlson S, Madara JL. Collagens facilitate epithelial migration in restitution of native guinea pig intestinal epithelium. *Gastroenterology*. 1992;102:119–130.
892. McCormack SA, Viar MJ, Johnson LR. Migration of IEC-6 cells: a model for mucosal healing. *Am J Physiol*. 1992;263:G426–G435.
893. Blikslager AT, Roberts MC, Rhoads JM, Argenzio RA. Prostaglandins I_2 and E_2 have a synergistic role in rescuing epithelial barrier function in porcine ileum. *J Clin Invest*. 1997;100:1928–1933.
894. Bjerknes M, Cheng H. Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology*. 1999;116:7–14.
895. Jankowski JA, Goodlad RA, Wright NA. Maintenance of normal intestinal mucosa: function, structure, and adaptation. *Gut*. 1994;35:S1–S4.
896. Zushi S. Role of prostaglandins in intestinal epithelial restitution stimulated by growth factors. *Am J Physiol*. 1996;270:G757–G762.
897. Blikslager AT, Roberts MC, Young KM, et al. Genistein augments prostaglandin-induced recovery of barrier function in ischemia-injured porcine ileum. *Am J Physiol Gastrointest Liver Physiol*. 2000;278:G207–G216.
898. Moeser AJ, Haskell MM, Shifflett DE, et al. ClC-2 chloride secretion mediates prostaglandin-induced recovery of barrier function in ischemia-injured porcine ileum. *Gastroenterology*. 2004;127:802–815.
899. Duffey ME, Hainau B, Ho S, Bentzel CJ. Regulation of epithelial tight junction permeability by cyclic AMP. *Nature*. 1981;294:451–453.
900. Palant CE, Duffey ME, Mookerjee BK, et al. Ca^{2+} regulation of tight-junction permeability and structure in Necturus gallbladder. *Am J Physiol*. 1983;245:C203–C212.
901. Wang JY, Johnson LR. Luminal polyamines substitute for tissue polyamines in duodenal mucosal repair after stress in rats. *Gastroenterology*. 1992;102:1109–1117.
902. Wang JY, Johnson LR. Polyamines and ornithine decarboxylase during repair of duodenal mucosa after stress in rats. *Gastroenterology*. 1991;100:333–343.
903. Wang JY, Johnson LR. Role of ornithine decarboxylase in repair of gastric mucosal stress ulcers. *Am J Physiol*. 1990;258:G78–G85.
904. McCormack SA, Wang JY, Viar MJ, et al. Polyamines influence transglutaminase activity and cell migration in two cell lines. *Am J Physiol*. 1994;267:C706–C714.
905. McCormack SA, Wang JY, Johnson LR. Polyamine deficiency causes reorganization of F-actin and tropomyosin in IEC-6 cells. *Am J Physiol*. 1994;267:C715–C722.
906. Rao JN, Li L, Golovina VA, et al. Ca2+-RhoA signaling pathway required for polyamine-dependent intestinal epithelial cell migration. *Am J Physiol Cell Physiol*. 2001;280:C993–1007.
907. Caco-2 cells by different mechanisms. *Am J Physiol Gastrointest Liver Physiol*. 2001;281:G37–G43.
908. Ray RM, Zimmerman BJ, McCormack SA, et al. Polyamine depletion arrests cell cycle and induces inhibitors p21(Waf1/Cip1), p27(Kip1), and p53 in IEC-6 cells. *Am J Physiol*. 1999;276:C684–C691.
909. Johnson LR, Tseng CC, Wang P, et al. Mucosal ornithine decarboxylase in the small intestine: localization and stimulation. *Am J Physiol*. 1989;256:G624–G630.
910. Wang JY, Johnson LR. Expression of protooncogenes c-fos and c-myc in healing of gastric mucosal stress ulcers. *Am J Physiol*. 1994;266:G878–G886.
911. Barnard JA, Beauchamp RD, Russell WE, et al. Epidermal growth factor-related peptides and their relevance to gastrointestinal pathophysiology. *Gastroenterology*. 1995;108:564–580.
912. Playford RJ, Wright NA. Why is epidermal growth factor present in the gut lumen? *Gut*. 1996;38:303–305.
913. Khulusi S, Hanby AM, Marrero JM, et al. Expression of trefoil peptides pS2 and human spasmolytic polypeptide in gastric metaplasia at the margin of duodenal ulcers. *Gut*. 1995;37:205–209.
914. Taupin D, Wu DC, Jeon WK, et al. The trefoil gene family are coordinately expressed immediate-early genes: EGF receptor- and MAP kinase-dependent interregulation. *J Clin Invest*. 1999;103:R31–R38.
915. Goke M, Zuk A, Podolsky DK. Regulation and function of extracellular matrix intestinal epithelial restitution in vitro. *Am J Physiol*. 1996;271:G729–G740.
916. Mashimo H, Wu DC, Podolsky DK, Fishman MC. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science*. 1996;274:262–265.
917. Rhoads JM, Argenzio RA, Chen W, et al. Glutamine metabolism stimulates intestinal cell MAPKs by a cAMP-inhibitable, Raf-independent mechanism. *Gastroenterology*. 2000;118:90–100.
918. Rhoads JM, Argenzio RA, Chen W, et al. l-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. *Am J Physiol*. 1997;272:G943–G953.
919. Kripke SA, Fox AD, Berman JM, et al. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. *J Parenter Enteral Nutr*. 1989;13:109–116.
920. Inoue Y, Grant JP, Snyder PJ. Effect of glutamine-supplemented total parenteral nutrition on recovery of the small intestine after starvation atrophy. *J Parenter Enteral Nutr*. 1993;17:165–170.
921. Souba WW, Herskowitz K, Salloum RM, et al. Gut glutamine metabolism. *J Parenter Enteral Nutr*. 1990;14:45S–50S.
922. Platell C, McCauley R, McCulloch R, Hall J. The influence of parenteral glutamine and branched-chain amino acids on total parenteral nutrition-induced atrophy of the gut. *J Parenter Enteral Nutr*. 1993;17:348–354.

923. Tremel H, Kienle B, Weilemann LS, et al. Glutamine dipeptide-supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology*. 1994;107:1595–1601.
924. Frankel WL, Zhang W, Afonso J, et al. Glutamine enhancement of structure and function in transplanted small intestine in the rat. *J Parenter Enteral Nutr*. 1993;17:47–55.
925. Zhang W, Frankel WL, Singh A, et al. Improvement of structure and function in orthotopic small bowel transplantation in the rat by glutamine. *Transplantation*. 1993;56:512–517.
926. Fox AD, Kripke SA, De Paula J, et al. Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis. *J Parenter Enteral Nutr*. 1988;12:325–331.
927. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. *Arch Surg*. 1990;125:1040–1045.
928. Klimberg VS, Souba WW, Dolson DJ, et al. Prophylactic glutamine protects the intestinal mucosa from radiation injury. *Cancer*. 1990;66:62–68.
929. Ahdieh N, Blikslager AT, Bhat BG, et al. l-glutamine and transforming growth factor-alpha enhance recovery of monoacylglycerol acyltransferase and diacylglycerol acyltransferase activity in porcine postischemic ileum. *Pediatr Res*. 1998;43:227–233.
930. Adams SB. Recognition and management of ileus. *Vet Clin North Am Equine Pract*. 1988;4:91–104.
931. Becht JL, Richardson DW. Ileus in the horse: clinical significance and management. *Proc Am Assoc Equine Pract*. 1981;27:291–297.
932. Semevolos SA, Ducharme NG, Hackett RP. Clinical assessment and outcome of three techniques for jejunal resection and anastomosis in horses: 59 cases (1989–2000). *J Am Vet Med Assoc*. 2002;220:215–218.
933. van den Boom R, van der Velden MA. Short-and long-term evaluation of surgical treatment of strangulating obstructions of the small intestine in horses: a review of 224 cases. *Vet Q*. 2001;23:109–115.
934. Morton AJ, Blikslager AT. Surgical and postoperative factors influencing short-term survival of horses following small intestinal resection: 92 cases (1994–2001). *Equine Vet J*. 2002;34:450–454.
935. Mair TS, Smith LJ. Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 2: short-term complications. *Equine Vet J*. 2005;37:303–309.
936. Campbell ML, Colahan PC, Brown MP, et al. Cecal impaction in the horse. *J Am Vet Med Assoc*. 1984;184:950–952.
937. Ross MW, Martin BB, Donawick WJ. Cecal perforation in the horse. *J Am Vet Med Assoc*. 1985;187:249–253.
938. Hilbert BJ, Little CB, Bolton JR, et al. Caecal overload and rupture in the horse. *Aust Vet J*. 1987;64:85–86.
939. Plummer AE, Rakestraw PC, Hardy J, et al. Outcome of medical and surgical treatment of cecal impaction in horses: 114 cases (1994–2004). *J Am Vet Med Assoc*. 2007;231:1378–1385.
940. Horowitz B, Ward SM, Sanders KM. Cellular and molecular basis for electrical rhythmicity in gastrointestinal muscles. *Annu Rev Physiol*. 1999;61:19–43.
941. Fintl C, Hudson NP, Mayhew IG, et al. Interstitial cells of Cajal (ICC) in equine colic: an immunohistochemical study of horses with obstructive disorders of the small and large intestines. *Equine Vet J*. 2004;36:474–479.
942. Hudson N, Mayhew I, Pearson G. A reduction in interstitial cells of Cajal in horses with equine dysautonomia (grass sickness). *Auton Neurosci*. 2001;92:37–44.
943. Milne EM, Fintl C, Hudson NP, et al. Observations on the interstitial cells of Cajal and neurons in a recovered case of equine dysautonomia (grass sickness). *J Comp Pathol*. 2005;133:33–40.
944. Hudson N, Mayhew I, Pearson G. Presence of in vitro electrical activity in the ileum of horses with enteric nervous system pathology: equine dysautonomia (grass sickness). *Auton Neurosci*. 2002;99:119–126.
945. Bertaccini G, Coruzzi G. Receptors in the gastrointestinal tract. *Pharmacol Res Commun*. 1987;19:87–118.
946. Re G, Belloli C, Badino P, et al. Identification of beta-adrenergic receptor subtypes mediating relaxation in isolated equine ileum. *Am J Vet Res*. 1997;58:621–625.
947. Malone ED, Kannan MS, Brown DR, et al. Adrenergic, cholinergic, and nonadrenergic-noncholinergic intrinsic innervation of the jejunum in horses. *Am J Vet Res*. 1999;60:898–904.
948. Rakestraw PC, Snyder JR, Woliner MJ, et al. Involvement of nitric oxide in inhibitory neuromuscular transmission in equine jejunum. *Am J Vet Res*. 1996;57:1206–1213.
949. Sellers AF, Lowe JE, Cummings JF. Trials of serotonin, substance P and alpha 2-adrenergic receptor effects on the equine large colon. *Cornell Vet*. 1985;75:319–323.
950. Sonea IM, Wilson DV, Bowker RM, et al. Tachykinin receptors in the equine pelvic flexure. *Equine Vet J*. 1997;29:306–312.
951. Merritt AM, Panzer RB, Lester GD, et al. Equine pelvic flexure myoelectric activity during fed and fasted states. *Am J Physiol*. 1995;269:G262–G268.
952. Lester GD, Bolton JR, Cullen LK, et al. Effects of general anesthesia on myoelectric activity of the intestine in horses. *Am J Vet Res*. 1992;53:1553–1557.
953. Ross MW, Cullen KK, Rutkowski JA. Myoelectric activity of the ileum, cecum, and right ventral colon in ponies during interdigestive, nonfeeding, and digestive periods. *Am J Vet Res*. 1990;51:561–566.
954. Kalff JC, Carlos TM, Schraut WH, et al. Surgically induced leukocytic infiltrates within the rat intestinal muscularis mediate postoperative ileus. *Gastroenterology*. 1999;117:378–387.
955. Turler A, Moore BA, Pezzone MA, et al. Colonic postoperative inflammatory ileus in the rat. *Ann Surg*. 2002;236:56–66.
956. Hopster-Iversen CC, Hopster K, Staszyk C, et al. Effects of experimental mechanical manipulations on local inflammation in the jejunum of horses. *Am J Vet Res*. 2014;75:385–391.
957. Schwarz NT, Kalff JC, Turler A, et al. Selective jejunal manipulation causes postoperative pan-enteric inflammation and dysmotility. *Gastroenterology*. 2004;126:159–169.
958. Wehner S, Behrendt FF, Lyutenski BN, et al. Inhibition of macrophage function prevents intestinal inflammation and postoperative ileus in rodents. *Gut*. 2007;56:176–185.
959. The FO, Bennink RJ, Ankum WM, et al. Intestinal handling-induced mast cell activation and inflammation in human postoperative ileus. *Gut*. 2008;57:33–40.
960. Turler A, Schnurr C, Nakao A, et al. Endogenous endotoxin participates in causing a panenteric inflammatory ileus after colonic surgery. *Ann Surg*. 2007;245:734–744.
961. Kalff JC, Schraut WH, Billiar TR, et al. Role of inducible nitric oxide synthase in postoperative intestinal smooth muscle dysfunction in rodents. *Gastroenterology*. 2000;118:316–327.
962. Little D, Tomlinson JE, Blikslager AT. Post operative neutrophilic inflammation in equine small intestine after manipulation and ischaemia. *Equine Vet J*. 2005;37:329–335.
963. Roger T, Ruckebusch Y. Colonic alpha 2-adrenoceptor-mediated responses in the pony. *J Vet Pharmacol Ther*. 1987;10:310–318.
964. Merritt AM, Campbell-Thompson ML, Lowrey S. Effect of xylazine treatment on equine proximal gastrointestinal tract myoelectrical activity. *Am J Vet Res*. 1989;50:945–949.
965. Clark ES, Thompson SA, Becht JL, et al. Effects of xylazine on cecal mechanical activity and cecal blood flow in healthy horses. *Am J Vet Res*. 1988;49:720–723.
966. Adams SB, Lamar CH, Masty J. Motility of the distal portion of the jejunum and pelvic flexure in ponies: effects of six drugs. *Am J Vet Res*. 1984;45:795–799.

967. Rutkowski JA, Ross MW, Cullen K. Effects of xylazine and/or butorphanol or neostigmine on myoelectric activity of the cecum and right ventral colon in female ponies. *Am J Vet Res*. 1989;50:1096–1101.
968. Merritt AM, Burrow JA, Hartless CS. Effect of xylazine, detomidine, and a combination of xylazine and butorphanol on equine duodenal motility. *Am J Vet Res*. 1998;59:619–623.
969. Elfenbein JR, Sanchez LC, Robertson SA, et al. Effect of detomidine on visceral and somatic nociception and duodenal motility in conscious adult horses. *Vet Anaesth Analg*. 2009;36:162–172.
970. Boscan P, Van Hoogmoed LM, Farver TB, et al. Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. *Am J Vet Res*. 2006;67:992–997.
971. Roger T, Bardon T, Ruckebusch Y. Colonic motor responses in the pony: relevance of colonic stimulation by opiate antagonists. *Am J Vet Res*. 1985;46:31–35.
972. Sanchez LC, Robertson SA, Maxwell LK, et al. Effect of fentanyl on visceral and somatic nociception in conscious horses. *J Vet Intern Med*. 2007;21:1067–1075.
973. Sojka JE, Adams SB, Lamar CH, et al. Effect of butorphanol, pentazocine, meperidine, or metoclopramide on intestinal motility in female ponies. *Am J Vet Res*. 1988;49:527–529.
974. Merritt AM, Campbell-Thompson ML, Lowrey S. Effect of butorphanol on equine antroduodenal motility. *Equine Vet J Suppl*. 1989:21–23.
975. Sellon DC, Monroe VL, Roberts MC, et al. Pharmacokinetics and adverse effects of butorphanol administered by single intravenous injection or continuous intravenous infusion in horses. *Am J Vet Res*. 2001;62:183–189.
976. Sanchez LC, Elfenbein JR, Robertson SA. Effect of acepromazine, butorphanol, or N-butylscopolammonium bromide on visceral and somatic nociception and duodenal motility in conscious horses. *Am J Vet Res*. 2008;69:579–585.
977. Elfenbein JR, Robertson SA, MacKay RJ, et al. Systemic and anti-nociceptive effects of prolonged lidocaine, ketamine, and butorphanol infusions alone and in combination in healthy horses. *BMC Vet Res*. 2014;10(suppl 1):S6.
978. Lester GD. *The development and application of a computer system for the recording and analysis of intestinal myoelectrical activity in the horse*. Perth, Australia: Murdoch University; 1990.
979. Sjoqvist A, Hallerback B, Glise H. Reflex adrenergic inhibition of colonic motility in anesthetized rat caused by nociceptive stimuli of peritoneum. An alpha 2-adrenoceptor-mediated response. *Dig Dis Sci*. 1985;30:749–754.
980. Pairet M, Ruckebusch Y. On the relevance of non-steroidal anti-inflammatory drugs in the prevention of paralytic ileus in rodents. *J Pharm Pharmacol*. 1989;41:757–761.
981. Zittel TT, Meile T, Huge A, et al. Preoperative intraluminal application of capsaicin increases postoperative gastric and colonic motility in rats. *J Gastrointest Surg*. 2001;5:503–513.
982. Lowe JE, Sellers AF, Brondum J. Equine pelvic flexure impaction. A model used to evaluate motor events and compare drug response. *Cornell Vet*. 1980;70:401–412.
983. MacHarg MA, Adams SB, Lamar CH, et al. Electromyographic, myomechanical, and intraluminal pressure changes associated with acute extraluminal obstruction of the jejunum in conscious ponies. *Am J Vet Res*. 1986;47:7–11.
984. King JN, Gerring EL. Antagonism of endotoxin-induced disruption of equine bowel motility by flunixin and phenylbutazone. *Equine Vet J Suppl*. 1989:38–42.
985. Meisler SD, Doherty TJ, Andrews FM, et al. Yohimbine ameliorates the effects of endotoxin on gastric emptying of the liquid marker acetaminophen in horses. *Can J Vet Res*. 2000;64:208–211.
986. Valk N, Doherty TJ, Blackford JT, et al. Phenylbutazone prevents the endotoxin-induced delay in gastric emptying in horses. *Can J Vet Res*. 1998;62:214–217.
987. Valk N, Doherty TJ, Blackford JT, et al. Effect of cisapride on gastric emptying in horses following endotoxin treatment. *Equine Vet J*. 1998;30:344–348.
988. Eskandari MK, Kalff JC, Billiar TR, et al. Lipopolysaccharide activates the muscularis macrophage network and suppresses circular smooth muscle activity. *Am J Physiol*. 1997;273:G727–G734.
989. Schwarz NT, Engel B, Eskandari MK, et al. Lipopolysaccharide preconditioning and cross-tolerance: the induction of protective mechanisms for rat intestinal ileus. *Gastroenterology*. 2002;123:586–598.
990. Overhaus M, Togel S, Pezzone MA, et al. Mechanisms of polymicrobial sepsis-induced ileus. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G685–G694.
991. Fintl C, Hudson NP, Handel I, et al. The effect of temperature changes on in vitro slow wave activity in the equine ileum. *Equine Vet J*. 2016;48:218–223.
992. Hooper RN, Roussel AJ, Cohen ND. Erythromycin stimulates myoelectric activity in the ileum and pelvic flexure of horses in the post-operative period. *Proc Equine Colic Res Symp*. 1998;6:42.
993. Van Hoogmoed L, Rakestraw PC, Snyder JR, et al. In vitro effects of nonsteroidal anti-inflammatory agents and prostaglandins I2, E2, and F2alpha on contractility of taenia of the large colon of horses. *Am J Vet Res*. 1999;60:1004–1009.
994. Roussel Jr AJ, Cohen ND, Hooper RN, et al. Risk factors associated with development of postoperative ileus in horses. *J Am Vet Med Assoc*. 2001;219:72–78.
995. Freeman DE, Hammock P, Baker GJ, et al. Short- and long-term survival and prevalence of postoperative ileus after small intestinal surgery in the horse. *Equine Vet J Suppl*. 2000:42–51.
996. Cohen ND, Lester GD, Sanchez LC, et al. Evaluation of risk factors associated with development of postoperative ileus in horses. *J Am Vet Med Assoc*. 2004;225:1070–1078.
997. Blikslager AT, Bowman KF, Levine JF, et al. Evaluation of factors associated with postoperative ileus in horses: 31 cases (1990–1992). *J Am Vet Med Assoc*. 1994;205:1748–1752.
998. Lefebvre D, Hudson NP, Elce YA, et al. Clinical features and management of equine post operative ileus (POI): Survey of Diplomates of the American Colleges of Veterinary Internal Medicine (ACVIM), Veterinary Surgeons (ACVS) and Veterinary Emergency and Critical Care (ACVECC). *Equine Vet J*. 2016;48:714–719.
999. Lefebvre D, Pirie RS, Handel IG, et al. Clinical features and management of equine post operative ileus: survey of diplomates of the European Colleges of Equine Internal Medicine (ECEIM) and Veterinary Surgeons (ECVS). *Equine Vet J*. 2016;48:182–187.
1000. Gerard MP, Bowman KF, Blikslager AT, et al. Jejunocolostomy or ileocolostomy for treatment of cecal impaction in horses: nine cases (1985–1995). *J Am Vet Med Assoc*. 1996;209:1287–1290.
1001. Roberts CT, Slone DE. Caecal impactions managed surgically by typhlotomy in 10 cases (1988-1998). *Equine Vet J Suppl*. 2000:74–76.
1002. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. *Gastroenterology*. 1996;111:1683–1699.
1003. Paradelis AG. Inhibition of the pendular movements of the intestine by aminoglycoside antibiotics. *Methods Find Exp Clin Pharmacol*. 1981;3:173–177.
1004. Sparnon AL, Spitz L. Pharmacological manipulation of postoperative intestinal adhesions. *Aust N Z J Surg*. 1989;59:725–729.
1005. Marti M, Mevissen M, Althaus H, et al. In vitro effects of bethanechol on equine gastrointestinal contractility and functional characterization of involved muscarinic receptor subtypes. *J Vet Pharmacol Ther*. 2005;28:565–574.

1006. Ringger NC, Lester GD, Neuwirth L, et al. Effect of bethanechol or erythromycin on gastric emptying in horses. *Am J Vet Res*. 1996;57:1771–1775.
1007. Adams SB, MacHarg MA. Neostigmine methylsulfate delays gastric emptying of particulate markers in horses. *Am J Vet Res*. 1985;46:2498–2499.
1008. Nieto JE, Rakestraw PC, Snyder JR, et al. In vitro effects of erythromycin, lidocaine, and metoclopramide on smooth muscle from the pyloric antrum, proximal portion of the duodenum, and middle portion of the jejunum of horses. *Am J Vet Res*. 2000;61:413–419.
1009. Gerring EE, Hunt JM. Pathophysiology of equine postoperative ileus: effect of adrenergic blockade, parasympathetic stimulation and metoclopramide in an experimental model. *Equine Vet J*. 1986;18:249–255.
1010. Dart AJ, Peauroi JR, Hodgson DR, et al. Efficacy of metoclopramide for treatment of ileus in horses following small intestinal surgery: 70 cases (1989–1992). *Aust Vet J*. 1996;74:280–284.
1011. Gerring EL, King JN. Cisapride in the prophylaxis of equine post operative ileus. *Equine Vet J Suppl*. 1989:52–55.
1012. Milne EM, Doxey DL, Woodman MP, et al. An evaluation of the use of cisapride in horses with chronic grass sickness (equine dysautonomia). *Br Vet J*. 1996;152:537–549.
1013. Steinebach MA, Cole D. Use of cisapride in the resolution of pelvic flexure impaction in a horse. *Can Vet J*. 1995;36:624–625.
1014. Valden MA, Klein WR. The effects of cisapride on the restoration of gut motility after surgery of the small intestine in horses: a clinical trial. *Vet Q*. 1993;15:175–179.
1015. Cable CS, Ball MA, Schwark WS, et al. Preparation of a parenteral formulation of cisapride from propulsid tablets and pharmacokinetic analysis after its intravenous administration. *J Equine Vet Sci*. 1999;18:616–621.
1016. Cubeddu LX. QT prolongation and fatal arrhythmias: a review of clinical implications and effects of drugs. *Am J Ther*. 2003;10:452–457.
1017. Delco ML, Nieto JE, Craigmill AL, et al. Pharmacokinetics and in vitro effects of tegaserod, a serotonin 5-hydroxytryptamine 4 (5-HT4) receptor agonist with prokinetic activity in horses. *Vet Ther*. 2007;8:77–87.
1018. Lippold BS, Hildebrand J, Straub R. Tegaserod (HTF 919) stimulates gut motility in normal horses. *Equine Vet J*. 2004;36:622–627.
1019. Hammerle CW, Surawicz CM. Updates on treatment of irritable bowel syndrome. *World J Gastroenterol*. 2008;14:2639–2649.
1020. Nieto JE, Maher O, Stanley SD, et al. In vivo and in vitro evaluation of the effects of domperidone on the gastrointestinal tract of healthy horses. *Am J Vet Res*. 2013;74:1103–1110.
1021. Lester GD, Merritt AM, Neuwirth L, et al. Effect of erythromycin lactobionate on myoelectric activity of ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res*. 1998;59:328–334.
1022. Nieto JE, Van Hoogmoed LM, Spier SJ, et al. Use of an extracorporeal circuit to evaluate effects of intraluminal distention and decompression on the equine jejunum. *Am J Vet Res*. 2002;63:267–275.
1023. Bologna SD, Hasler WL, Owyang C. Down-regulation of motilin receptors on rabbit colon myocytes by chronic oral erythromycin. *J Pharmacol Exp Ther*. 1993;266:852–856.
1024. Roussel AJ, Hooper RN, Cohen ND, et al. Evaluation of the effects of penicillin G potassium and potassium chloride on the motility of the large intestine in horses. *Am J Vet Res*. 2003;64:1360–1363.
1025. Van Hoogmoed LM, Boscan PL. In vitro evaluation of the effect of the opioid antagonist N-methylnaltrexone on motility of the equine jejunum and pelvic flexure. *Equine Vet J*. 2005;37:325–328.
1026. Boscan P, Van Hoogmoed LM, Pypendop BH, et al. Pharmacokinetics of the opioid antagonist N-methylnaltrexone and evaluation of its effects on gastrointestinal tract function in horses treated or not treated with morphine. *Am J Vet Res*. 2006;67:998–1004.
1027. Van Hoogmoed LM, Nieto JE, Snyder JR, et al. Survey of prokinetic use in horses with gastrointestinal injury. *Vet Surg*. 2004;33:279–285.
1028. Rimback G, Cassuto J, Tollesson PO. Treatment of postoperative paralytic ileus by intravenous lidocaine infusion. *Anesthesia Analgesia*. 1990;70:414–419.
1029. Lahat A, Horin SB, Lang A, et al. Lidocaine down-regulates nuclear factor-kappaB signalling and inhibits cytokine production and T cell proliferation. *Clin Exp Immunol*. 2008;152:320–327.
1030. Robertson SA, Sanchez LC, Merritt AM, et al. Effect of systemic lidocaine on visceral and somatic nociception in conscious horses. *Equine Vet J*. 2005;37:122–127.
1031. Milligan M, Beard W, Kukanich B, et al. The effect of lidocaine on postoperative jejunal motility in normal horses. *Vet Surg*. 2007;36:214–220.
1032. Brianceau P, Chevalier H, Karas A, et al. Intravenous lidocaine and small-intestinal size, abdominal fluid, and outcome after colic surgery in horses. *J Vet Intern Med*. 2002;16:736–741.
1033. Salem SE, Proudman CJ, Archer DC. Has intravenous lidocaine improved the outcome in horses following surgical management of small intestinal lesions in a UK hospital population? *BMC Vet Res*. 2016;12:157.
1034. Dickey EJ, McKenzie 3rd HC, Brown JA, et al. Serum concentrations of lidocaine and its metabolites after prolonged infusion in healthy horses. *Equine Vet J*. 2008.
1035. Snyder JR, Pascoe JR, Olander HJ, et al. Strangulating volvulus of the ascending colon in horses. *J Am Vet Med Assoc*. 1989;195:757–764.
1036. Hughes FE, Slone Jr DE. Large colon resection. *Vet Clin North Am Equine Pract*. 1997;13:341–350.
1037. Lundin C, Sullins KE, White NA, et al. Induction of peritoneal adhesions with small intestinal ischaemia and distention in the foal. *Equine Vet J*. 1989;21:451–458.
1038. Freeman DE, Koch DB, Boles CL. Mesodiverticular bands as a cause of small intestinal strangulation and volvulus in the horse. *J Am Vet Med Assoc*. 1979;175:1089–1094.
1039. Vachon AM, Fischer AT. Small intestinal herniation through the epiploic foramen: 53 cases (1987–1993). *Equine Vet J*. 1995;27:373–380.
1040. White NA, Lessard P. Determining the diagnosis and prognosis of the acute abdomen. In: White NA, ed. *The Equine Acute Abdomen*. Philadelphia, PA: Lea & Febiger; 1990:101–152.
1041. White NA, Lessard P. Risk factors and clinical signs associated with cases of equine colic. *Proc Am Assoc Equine Pract*. 1986:637–644.
1042. Mair TS, Smith LJ. Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 1: short-term survival following a single laparotomy. *Equine Vet J*. 2005;37:296–302.
1043. MacDonald MH, Pascoe JR, Stover SM, et al. Survival after small intestine resection and anastomosis in horses. *Vet Surg*. 1989;18:415–423.
1044. Proudman CJ, Edwards GB, Barnes J, et al. Factors affecting long-term survival of horses recovering from surgery of the small intestine. *Equine Vet J*. 2005;37:360–365.
1045. Gayle JM, Blikslager AT, Bowman KF. Mesenteric rents as a source of small intestinal strangulation in horses: 15 cases (1990–1997). *J Am Vet Med Assoc*. 2000;216:1446–1449.
1046. Freeman DE, Schaeffer DJ, Cleary OB. Long-term survival in horses with strangulating obstruction of the small intestine managed without resection. *Equine Vet J*. 2014;46:711–717.

1047. Gazzerro DM, Southwood LL, Lindborg S. Short-term complications after colic surgery in geriatric versus mature nongeriatric horses. *Vet Surg*. 2015;44:256–264.
1048. Bergren AL, Credille BC, Epstein KL, et al. Retrospective Comparison of Gastrosplenic Entrapment of the Small Intestine to Other Strangulating Small Intestinal Lesions in Adult Horses. *Vet Surg*. 2015;44:535–539.
1049. Kilcoyne I, Dechant JE, Nieto JE. Comparison of clinical findings and short-term survival between horses with intestinal entrapment in the gastrosplenic ligament and horses with intestinal entrapment in the epiploic foramen. *J Am Vet Med Assoc*. 2016;249:660–667.
1050. Freeman DE, Schaeffer DJ. Short-term survival after surgery for epiploic foramen entrapment compared with other strangulating diseases of the small intestine in horses. *Equine Vet J*. 2005;37:292–295.
1051. Archer DC, Pinchbeck GL, Proudman CJ. Factors associated with survival of epiploic foramen entrapment colic: a multicentre, international study. *Equine Vet J Suppl*. 2011;39:56–62.
1052. Freeman DE, Schaeffer DJ. Age distributions of horses with strangulation of the small intestine by a lipoma or in the epiploic foramen: 46 cases (1994–2000). *J Am Vet Med Assoc*. 2001;219:87–89.
1053. Beccati F, Pepe M, Gialletti R, et al. Is there a statistical correlation between ultrasonographic findings and definitive diagnosis in horses with acute abdominal pain? *Equine Vet J Suppl*. 2011:98–105.
1054. Klohnen A, Vachon AM, Fischer AT. Use of diagnostic ultrasonography in horses with signs of acute abdominal pain. *J Am Vet Med Assoc*. 1996;209:1597–1601.
1055. Hammock PD, Freeman DE, Magid JH, et al. Parietal hernia of the small intestine into the epiploic foramen of a horse. *J Am Vet Med Assoc*. 1999;214:1354–1356. 1334-1355.
1056. Engelbert TA, Tate Jr LP, Bowman KF, et al. Incarceration of the small intestine in the epiploic foramen. Report of 19 cases (1983-1992). *Vet Surg*. 1993;22:57–61.
1057. Garcia-Seco E, Wilson DA, Kramer J, et al. Prevalence and risk factors associated with outcome of surgical removal of pedunculated lipomas in horses: 102 cases (1987–2002). *J Am Vet Med Assoc*. 2005;226:1529–1537.
1058. Edwards GB, Proudman CJ. An analysis of 75 cases of intestinal obstruction caused by pedunculated lipomas. *Equine Vet J*. 1994;26:18–21.
1059. Blikslager AT, Bowman KF, Haven ML, et al. Pedunculated lipomas as a cause of intestinal obstruction in horses: 17 cases (1983–1990). *J Am Vet Med Assoc*. 1992;201:1249–1252.
1060. Robertson JT. Diseases of the small intestine. In: White NA, ed. *The Equine Acute Abdomen*. Philadelphia, PA: Lea & Febiger; 1990:347–368.
1061. Crowhurst RC, Simpson DJ, McEnery RJ, et al. Intestinal surgery in the foal. *J S Afr Vet Assoc*. 1975;46:59–67.
1062. Orsini JA. Abdominal surgery in foals. *Vet Clin North Am Equine Pract*. 1997;13:393–413.
1063. Tate Jr LP, Ralston SL, Koch CM, et al. Effects of extensive resection of the small intestine in the pony. *Am J Vet Res*. 1983;44:1187–1191.
1064. Jenei TM, Garcia-Lopez JM, Provost PJ, et al. Surgical management of small intestinal incarceration through the gastrosplenic ligament: 14 cases (1994–2006). *J Am Vet Med Assoc*. 2007;231:1221–1224.
1065. Becht JL, McIlwraith CW. Jejunal displacement through the mesometrium in a pregnant mare. *J Am Vet Med Assoc*. 1980;177:436.
1066. Gayle JM, Macharg MA, Smallwood JE. Strangulating obstruction caused by intestinal herniation through the proximal aspect of the cecocolic fold in 9 horses. *Vet Surg*. 2001;30:40–43.
1067. Spurlock GH, Robertson JT. Congenital inguinal hernias associated with a rent in the common vaginal tunic in five foals. *J Am Vet Med Assoc*. 1988;193:1087–1088.
1068. van der Velden MA. Ruptured inguinal hernia in new-born colt foals: a review of 14 cases. *Equine Vet J*. 1988;20:178–181.
1069. Schneider RK, Milne DW, Kohn CW. Acquired inguinal hernia in the horse: a review of 27 cases. *J Am Vet Med Assoc*. 1982;180:317–320.
1070. van der Velden MA. Surgical treatment of acquired inguinal hernia in the horse: a review of 51 cases. *Equine Vet J*. 1988;20:173–177.
1071. Weaver AD. Acquired incarcerated inguinal hernia: a review of 13 horses. *Can Vet J*. 1987;28:195–199.
1072. Ivens PA, Piercy RJ, Eliashar E. Inguinal herniation of the large colon in a cob gelding four weeks after castration. *Vet Rec*. 2009;165:380–381.
1073. van der Velden MA, Stolk PW. Different types of inguinal herniation in two stallions and a gelding. *Vet Q*. 1990;12:46–50.
1074. Freeman DE. Surgery of the small intestine. *Vet Clin North Am Equine Pract*. 1997;13:261–301.
1075. Freeman DE, Orsini JA, Harrison IW, et al. Complications of umbilical hernias in horses: 13 cases (1972–1986). *J Am Vet Med Assoc*. 1988;192:804–807.
1076. Markel MD, Pascoe JR, Sams AE. Strangulated umbilical hernias in horses: 13 cases (1974–1985). *J Am Vet Med Assoc*. 1987;190:692–694.
1077. Edwards GB. Surgical management of intussusception in the horse. *Equine Vet J*. 1986;18:313–321.
1078. Ford TS, Freeman DE, Ross MW, et al. Ileocecal intussusception in horses: 26 cases (1981–1988). *J Am Vet Med Assoc*. 1990;196:121–126.
1079. Bernard WV, Reef VB, Reimer JM, et al. Ultrasonographic diagnosis of small-intestinal intussusception in three foals. *J Am Vet Med Assoc*. 1989;194:395–397.
1089. Edens LM, White NA, Dabareiner RM, et al. Transrectal ultrasonographic diagnosis of an ileocaecal intussusception in a horse. *Equine Vet J*. 1996;28:81–83.
1081. Fontaine-Rodgerson G, Rodgerson DH. Diagnosis of small intestinal intussusception by transabdominal ultrasonography in 2 adult horses. *Can Vet J*. 2001;42:378–380.
1082. Abraham M, Reef VB, Sweeney RW, et al. Gastrointestinal ultrasonography of normal Standardbred neonates and frequency of asymptomatic intussusceptions. *J Vet Intern Med*. 2014;28:1580–1586.
1083. Gift LJ, Gaughan EM, DeBowes RM, et al. Jejunal intussusception in adult horses: 11 cases (1981–1991). *J Am Vet Med Assoc*. 1993;202:110–112.
1084. Beard WL, Byrne BA, Henninger RW. Ileocecal intussusception corrected by resection within the cecum in two horses. *J Am Vet Med Assoc*. 1992;200:1978–1980.
1085. Bristol DG. Diaphragmatic hernias in horses and cattle. *Comp Contin Ed Pract Vet*. 1986;8:S407–S411.
1086. Wimberly HC, Andrews EJ, Haschek WM. Diaphragmatic hernias in the horse: a review of the literature and an analysis of six additional cases. *J Am Vet Med Assoc*. 1977;170:1404–1407.
1087. Orsini JA, Koch C, Stewart B. Peritoneopericardial hernia in a horse. *J Am Vet Med Assoc*. 1981;179:907–910.
1088. Everett KA, Chaffin MK, Brinsko SP. Diaphragmatic herniation as a cause of lethargy and exercise intolerance in a mare. *Cornell Vet*. 1992;82:217–223.
1089. Santschi EM, Juzwiak JS, Moll HD, et al. Diaphragmatic hernia repair in three young horses. *Vet Surg*. 1997;26:242–245.
1090. Dabareiner RM, White NA. Surgical repair of a diaphragmatic hernia in a racehorse. *J Am Vet Med Assoc*. 1999;214:1517–1518. 1496.
1091. Hart SK, Brown JA. Diaphragmatic hernia in horses: 44 cases (1986–2006). *J Vet Emerg Crit Care (San Antonio)*. 2009;19:357–362.
1092. Romero AE, Rodgerson DH. Diaphragmatic herniation in the horse: 31 cases from 2001–2006. *Can Vet J*. 2010;51:1247–1250.

1093. Harrison IW. Equine large intestinal volvulus. A review of 124 cases. *Vet Surg*. 1988;17:77–81.
1094. Johnston K, Holcombe SJ, Hauptman JG. Plasma lactate as a predictor of colonic viability and survival after 360 degrees volvulus of the ascending colon in horses. *Vet Surg*. 2007;36:563–567.
1095. Hackett ES, Embertson RM, Hopper SA, et al. Duration of disease influences survival to discharge of Thoroughbred mares with surgically treated large colon volvulus. *Equine Vet J*. 2015;47:650–654.
1096. Fiege JK, Hackett ES, Rao S, et al. Current treatment of ascending colon volvulus in horses: a survey of ACVS Diplomates. *Vet Surg*. 2015;44:398–401.
1097. Ellis CM, Lynch TM, Slone DE, et al. Survival and complications after large colon resection and end-to-end anastomosis for strangulating large colon volvulus in seventy-three horses. *Vet Surg*. 2008;37:786–790.
1098. Hance SR, Embertson RM. Colopexy in broodmares: 44 cases (1986-1990). *J Am Vet Med Assoc*. 1992;201:782–787.
1099. Martin Jr BB, Freeman DE, Ross MW, et al. Cecocolic and cecocecal intussusception in horses: 30 cases (1976–1996). *J Am Vet Med Assoc*. 1999;214:80–84.
1100. Hubert JD, Hardy J, Holcombe SJ, et al. Cecal amputation within the right ventral colon for surgical treatment of nonreducible cecocolic intussusception in 8 horses. *Vet Surg*. 2000;29:317–325.
1101. Gaughan EM, Hackett RP. Cecocolic intussusception in horses: 11 cases (1979–1989). *J Am Vet Med Assoc*. 1990;197:1373–1375.
1102. Dyson S, Orsini J. Intussusception of the large colon in a horse. *J Am Vet Med Assoc*. 1983;182:720.
1103. Robertson JT, Tate Jr LP. Resection of intussuscepted large colon in a horse. *J Am Vet Med Assoc*. 1982;181:927–928.
1104. Wilson DG, Wilson WD, Reinertson EL. Intussusception of the left dorsal colon in a horse. *J Am Vet Med Assoc*. 1983;183:464–465.
1105. Turner TA, Fessler JF. Rectal prolapse in the horse. *J Am Vet Med Assoc*. 1980;177:1028–1032.
1106. Blythman WG. Rectal prolapse in a foaling mare. *Vet Rec*. 1988;122:471–472.
1107. Rick MC. Management of rectal injuries. *Vet Clin North Am Equine Pract*. 1989;5:407–428.
1108. Freeman DE. Rectum and anus. In: Auer JA, Stick JA, eds. *Equine Surgery*. Philadelphia, PA: WB Saunders; 2005:286–293.
1109. Jacobs KA, Barber SM, Leach DH. Disruption of the blood supply to the small colon following rectal prolapse and small colon intussusception in a mare. *Can Vet J*. 1982;23:132–134.
1110. Ragle CA, Southwood LL, Galuppo LD, et al. Laparoscopic diagnosis of ischemic necrosis of the descending colon after rectal prolapse and rupture of the mesocolon in two postpartum mares. *J Am Vet Med Assoc*. 1997;210:1646–1648.
1111. White 2nd NA. Intestinal infarction associated with mesenteric vascular thrombotic disease in the horse. *J Am Vet Med Assoc*. 1981;178:259–262.
1112. Allen Jr D, White 2nd NA, Tyler DE. Morphologic effects of experimental distention of equine small intestine. *Vet Surg*. 1988;17:10–14.
1113. Fleming K, Mueller PO. Ileal impaction in 245 horses: 1995-2007. *Can Vet J*. 2011;52:759–763.
1114. Hanson RR, Schumacher J, Humburg J, et al. Medical treatment of horses with ileal impactions: 10 cases (1990–1994). *J Am Vet Med Assoc*. 1996;208:898–900.
1115. Little D, Blikslager AT. Factors associated with development of ileal impaction in horses with surgical colic: 78 cases (1986–2000). *Equine Vet J*. 2002;34:464–468.
1116. Cribb NC, Cote NM, Boure LP, et al. Acute small intestinal obstruction associated with Parascaris equorum infection in young horses: 25 cases (1985–2004). *N Z Vet J*. 2006;54:338–343.
1117. Nielsen MK, Donoghue EM, Stephens ML, et al. An ultrasonographic scoring method for transabdominal monitoring of ascarid burdens in foals. *Equine Vet J*. 2016;48:380–386.
1118. Tatz AJ, Segev G, Steinman A, et al. Surgical treatment for acute small intestinal obstruction caused by Parascaris equorum infection in 15 horses (2002–2011). *Equine Vet J Suppl*. 2012:111–114.
1119. Parks AH, Allen D. The purported role of coastal Bermuda hay in the etiology of ileal impactions: results of a questionnaire (abstract). *Equine Colic Res Symp*. 1988;37.
1120. Proudman CJ, French NP, Trees AJ. Tapeworm infection is a significant risk factor for spasmodic colic and ileal impaction colic in the horse. *Equine Vet J*. 1998;30:194–199.
1121. Proudman CJ, Trees AJ. Correlation of antigen specific IgG and IgG(T) responses with Anoplocephala perfoliata infection intensity in the horse. *Parasite Immunol*. 1996;18:499–506.
1122. Proudman CJ, Trees AJ. Use of excretory/secretory antigens for the serodiagnosis of Anoplocephala perfoliata cestodosis. *Vet Parasitol*. 1996;61:239–247.
1123. Hanson RR, Wright JC, Schumacher J, et al. Surgical reduction of ileal impactions in the horse: 28 cases. *Vet Surg*. 1998;27:555–560.
1124. Chaffin MK, Fuenteabla IC, Schumacher J, et al. Idiopathic muscular hypertrophy of the equine small intestine: 11 cases (1980–1991). *Equine Vet J*. 1992;24:372–378.
1125. Edwards GB. Obstruction of the ileum in the horse: a report of 27 clinical cases. *Equine Vet J*. 1981;13:158–166.
1126. Mair TS, Lucke VM. Ileal muscular hypertrophy and rupture in a pony three years after surgery for ileocaecal intussusception. *Vet Rec*. 2000;146:472–473.
1127. Grant BD, Tennant B. Volvulus associated with Meckel's diverticulum in the horse. *J Am Vet Med Assoc*. 1973;162:550–551.
1128. Hooper RN. Small intestinal strangulation caused by Meckel's diverticulum in a horse. *J Am Vet Med Assoc*. 1989;194:943–944.
1129. Yovich JV, Horney FD. Congenital jejunal diverticulum in a foal. *J Am Vet Med Assoc*. 1983;183:1092.
1130. Baxter GM, Broome TE, Moore JN. Abdominal adhesions after small intestinal surgery in the horse. *Vet Surg*. 1989;18:409–414.
1131. Southwood LL, Baxter GM. Current concepts in management of abdominal adhesions. *Vet Clin North Am Equine Pract*. 1997;13:415–435.
1132. Hay WP, Mueller PO, Harmon B, et al. One percent sodium carboxymethylcellulose prevents experimentally induced abdominal adhesions in horses. *Vet Surg*. 2001;30:223–227.
1133. Mueller PO, Harmon BG, Hay WP, et al. Effect of carboxymethylcellulose and a hyaluronate-carboxymethylcellulose membrane on healing of intestinal anastomoses in horses. *Am J Vet Res*. 2000;61:369–374.
1134. Mueller PO, Hunt RJ, Allen D, et al. Intraperitoneal use of sodium carboxymethylcellulose in horses undergoing exploratory celiotomy. *Vet Surg*. 1995;24:112–117.
1135. Parker JE, Fubini SL, Car BD, et al. Prevention of intraabdominal adhesions in ponies by low-dose heparin therapy. *Vet Surg*. 1987;16:459–462.
1136. Vatistas NJ, Snyder JR, Wilson WD, et al. Surgical treatment for colic in the foal (67 cases): 1980–1992. *Equine Vet J*. 1996;28:139–145.
1137. Phillips TJ, Walmsley JP. Retrospective analysis of the results of 151 exploratory laparotomies in horses with gastrointestinal disease. *Equine Vet J*. 1993;25:427–431.
1138. Parker JE, Fubini SL, Todhunter RJ. Retrospective evaluation of repeat celiotomy in 53 horses with acute gastrointestinal disease. *Vet Surg*. 1989;18:424–431.
1139. Aitken MR, Southwood LL, Ross BM, et al. Outcome of Surgical and Medical Management of Cecal Impaction in 150 Horses (1991–2011). *Vet Surg*. 2015;44:540–546.

1140. Dart AJ, Dowling BA, Hodgson DR. Caecal disease. *Equine Vet Educ*. 1999;1:21–28.
1141. Little D, Redding WR, Blikslager AT. Risk factors for reduced postoperative fecal output in horses: 37 cases (1997–1998). *J Am Vet Med Assoc*. 2001;218:414–420.
1142. White 2nd NA, Dabareiner RM. Treatment of impaction colics. *Vet Clin North Am Equine Pract*. 1997;13:243–259.
1143. Dabareiner RM, White NA. Large colon impaction in horses: 147 cases (1985–1991). *J Am Vet Med Assoc*. 1995;206:679–685.
1144. Clarke LL, Argenzio RA, Roberts MC. Effect of meal feeding on plasma volume and urinary electrolyte clearance in ponies. *Am J Vet Res*. 1990;51:571–576.
1145. Roberts MC, Argenzio A. Effects of amitraz, several opiate derivatives and anticholinergic agents on intestinal transit in ponies. *Equine Vet J*. 1986;18:256–260.
1146. Roberts MC, Seawright AA. Amitraz induced large intestinal impaction in the horse. *Aust Vet J*. 1979;55:553–554.
1147. Lopes MA, Walker BL, White 2nd NA, et al. Treatments to promote colonic hydration: enteral fluid therapy versus intravenous fluid therapy and magnesium sulphate. *Equine Vet J*. 2002;34:505–509.
1148. Lopes MA, White 2nd NA, Donaldson L, et al. Effects of enteral and intravenous fluid therapy, magnesium sulfate, and sodium sulfate on colonic contents and feces in horses. *Am J Vet Res*. 2004;65:695–704.
1149. Cohen ND, Vontur CA, Rakestraw PC. Risk factors for enterolithiasis among horses in Texas. *J Am Vet Med Assoc*. 2000;216:1787–1794.
1150. Hassel DM, Aldridge BM, Drake CM, et al. Evaluation of dietary and management risk factors for enterolithiasis among horses in California. *Res Vet Sci*. 2008;85:476–480.
1151. Hassel DM, Rakestraw PC, Gardner IA, et al. Dietary risk factors and colonic pH and mineral concentrations in horses with enterolithiasis. *J Vet Intern Med*. 2004;18:346–349.
1152. Lloyd K, Hintz HF, Wheat JD, et al. Enteroliths in horses. *Cornell Vet*. 1987;77:172–186.
1153. Blue MG, Wittkopp RW. Clinical and structural features of equine enteroliths. *J Am Vet Med Assoc*. 1981;179:79–82.
1154. Peloso JG, Coatney RW, Caron JP, et al. Obstructive enterolith in an 11-month-old miniature horse. *J Am Vet Med Assoc*. 1992;201:1745–1746.
1155. Maher O, Puchalski SM, Drake C, et al. Abdominal computed radiography for the diagnosis of enterolithiasis in horses: 142 cases (2003–2007). *J Am Vet Med Assoc*. 2011;239:1483–1485.
1156. Specht TE, Colahan PT. Surgical treatment of sand colic in equids: 48 cases (1978–1985). *J Am Vet Med Assoc*. 1988;193:1560–1564.
1157. Ragle CA, Meagher DM, Schrader JL, et al. Abdominal auscultation in the detection of experimentally induced gastrointestinal sand accumulation. *J Vet Intern Med*. 1989;3:12–14.
1158. Kendall A, Ley C, Egenvall A, et al. Radiographic parameters for diagnosing sand colic in horses. *Acta Vet Scand*. 2008;50:17.
1159. Korolainen R, Ruohoniemi M. Reliability of ultrasonography compared to radiography in revealing intestinal sand accumulations in horses. *Equine Vet J*. 2002;34:499–504.
1160. Hart KA, Linnenkohl W, Mayer JR, et al. Medical management of sand enteropathy in 62 horses. *Equine Vet J*. 2013;45:465–469.
1161. Hammock PD, Freeman DE, Baker GJ. Failure of psyllium mucilloid to hasten evaluation of sand from the equine large intestine. *Vet Surg*. 1998;27:547–554.
1162. Hackett RP. Nonstrangulated colonic displacement in horses. *J Am Vet Med Assoc*. 1983;182:235–240.
1163. Clarke LL, Roberts MC, Argenzio RA. Feeding and digestive problems in horses. Physiologic responses to a concentrated meal. *Vet Clin North Am Equine Pract*. 1990;6:433–450.
1164. Morris DD, Moore JN, Ward S. Comparison of age, sex, breed, history and management in 229 horses with colic. *Equine Vet J Suppl*. 1989:129–132.
1165. Ruckebusch Y. Motor functions of the intestine. *Adv Vet Sci Comp Med*. 1981;25:345–369.
1166. Huskamp B. Displacement of the large colon. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. Philadelphia, PA: W.B. Saunders; 1987:60–65.
1167. Santschi EM, Slone Jr DE, Frank WM. Use of ultrasound in horses for diagnosis of left dorsal displacement of the large colon and monitoring its nonsurgical correction. *Vet Surg*. 1993;22:281–284.
1168. Baker WT, Frederick J, Giguere S, et al. Reevaluation of the effect of phenylephrine on resolution of nephrosplenic entrapment by the rolling procedure in 87 horses. *Vet Surg*. 2011;40:825–829.
1169. Fultz LE, Peloso JG, Giguere S, et al. Comparison of phenylephrine administration and exercise versus phenylephrine administration and a rolling procedure for the correction of nephrosplenic entrapment of the large colon in horses: 88 cases (2004–2010). *J Am Vet Med Assoc*. 2013;242:1146–1151.
1170. Frederick J, Giguere S, Butterworth K, et al. Severe phenylephrine-associated hemorrhage in five aged horses. *J Am Vet Med Assoc*. 2010;237:830–834.
1171. McGovern KF, Bladon BM, Fraser BS, et al. Attempted medical management of suspected ascending colon displacement in horses. *Vet Surg*. 2012;41:399–403.
1172. Rocken M, Schubert C, Mosel G, et al. Indications, surgical technique, and long-term experience with laparoscopic closure of the nephrosplenic space in standing horses. *Vet Surg*. 2005;34:637–641.
1173. Zekas LJ, Ramirez S, Brown MP. Ablation of the nephrosplenic space for treatment of recurring left dorsal displacement of the large colon in a racehorse. *J Am Vet Med Assoc*. 1999;214:1361–1363. 1335.
1174. Gay CC, Speirs VC, Christie BA, et al. Foreign body obstruction of the small colon in six horses. *Equine Vet J*. 1979;11:60–63.
1175. McClure JT, Kobluk C, Voller K, et al. Fecalith impaction in four miniature foals. *J Am Vet Med Assoc*. 1992;200:205–207.
1176. Speirs VC, van Veenendaal JC, Christie BA, et al. Obstruction of the small colon by intramural haematoma in three horses. *Aust Vet J*. 1981;57:88–90.
1177. Pearson H, Waterman AE. Submucosal haematoma as a cause of obstruction of the small colon in the horse: a review of four cases. *Equine Vet J*. 1986;18:340–341.
1178. Frederico LM, Jones SL, Blikslager AT. Predisposing factors for small colon impaction in horses and outcome of medical and surgical treatment: 44 cases (1999–2004). *J Am Vet Med Assoc*. 2006;229:1612–1616.
1179. Benamou AE, Blikslager AT, Sellon DC. Intestinal atresia in foals. *Compend Contin Educ Pract Vet*. 1995;17:1510.
1180. McCabe L, Griffin LD, Kinzer A, et al. Overo lethal white foal syndrome: equine model of aganglionic megacolon (Hirschsprung disease). *Am J Med Genet*. 1990;36:336–340.
1181. Metallinos DL, Bowling AT, Rine J. A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschsprung disease. *Mamm Genome*. 1998;9:426–431.
1182. Santschi EM, Vrotsos PD, Purdy AK, et al. Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white-patterned horses. *Am J Vet Res*. 2001;62:97–103.
1183. Santschi EM, Purdy AK, Valberg SJ, et al. Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. *Mamm Genome*. 1998;9:306–309.
1184. Giancola F, Gentilini F, Romagnoli N, et al. Extrinsic innervation of ileum and pelvic flexure of foals with ileocolonic aganglionosis. *Cell Tissue Res*. 2016;366:13–22.
1185. Lindsay FE, Burton FL. Observational study of "urine testing" in the horse and donkey stallion. *Equine Vet J*. 1983;15:330–336.

1186. Baker GJ. Oral examination and diagnosis: management of oral disease. In: Harvey CE, ed. *Veterinary dentistry*. Philadelphia, PA: WB Saunders; 1985.
1187. Bonin SJ, Clayton HM, Lanovaz JL, et al. Comparison of mandibular motion in horses chewing hay and pellets. *Equine Vet J*. 2007;39:258–262.
1188. Baker GJ. Fluroscopic investigations of swallowing in the horse. *Vet Radiol*. 1982;23:84–88.
1189. Klebe EA, Holcombe SJ, Rosenstein D, et al. The effect of bilateral glossopharyngeal nerve anaesthesia on swallowing in horses. *Equine Vet J*. 2005;37:65–69.
1190. Heffron CJ, Baker GJ, Lee R. Fluoroscopic Investigation of Pharyngeal Function in the Horse. *Equine Veterinary Journal*. 1979;11:148–152.
1191. Clark ES, Morris DD, Whitlock RH. Esophageal dysfunction in a weanling thoroughbred. *Cornell Vet*. 1987;77:151–160.
1192. Freeman DE, Naylor JM. Cervical esophagostomy to permit extraoral feeding of the horse. *J Am Vet Med Assoc*. 1978;172:314–320.
1193. Stick JA, Robinson NE, Krehbiel JD. Acid-base and electrolyte alterations associated with salivary loss in the pony. *Am J Vet Res*. 1981;42:733–737.
1194. Jones SL, Zimmel D, Tate LP, et al. Case presentation—Dysphagia caused by squamous cell carcinoma in two horses. *Compend Contin Educ Pract Vet*. 2001;23:1020–1024.
1195. Dixon PM, Tremaine WH, Pickles K, et al. Equine dental disease part 2: a long-term study of 400 cases: disorders of development and eruption and variations in position of the cheek teeth. *Equine Vet J*. 1999;31:519–528.
1196. Quinn GC, Tremaine WH, Lane JG. Supernumerary cheek teeth (n = 24): clinical features, diagnosis, treatment and outcome in 15 horses. *Equine Vet J*. 2005;37:505–509.
1197. Baker GJ. Diseases of the teeth. In: Colahan PC, Mayhew IG, Merrit AM, et al., eds. *Equine medicine and surgery*. Goleta, CA: American Veterinary Publications; 1991.
1198. Hormeyr CB. Comparative dental pathology (with particular reference to caries and paradental disease in the horse and the dog). *J S Afr Vet Med Assoc*. 1960;29:471–475.
1199. Dixon PM, Tremaine WH, Pickles K, et al. Equine dental disease part 4: a long-term study of 400 cases: apical infections of cheek teeth. *Equine Vet J*. 2000;32:182–194.
1200. Baker GJ. Some aspects of equine dental radiology. *Equine Vet J*. 1971;3:46–51.
1201. Baker GJ. Some aspects of equine dental decay. *Equine Vet J*. 1974;6:127–130.
1202. Farmand M, Stohler T. The median cleft of the lower lip and mandible and its surgical correction in a donkey. *Equine Vet J*. 1990;22:298–301.
1203. Schumacher J, Brink P, Easley J, et al. Surgical correction of wry nose in four horses. *Vet Surg*. 2008;37:142–148.
1204. Gift LJ, DeBowes RM, Clem MF, et al. Brachygnathia in horses: 20 cases (1979–1989). *J Am Vet Med Assoc*. 1992;200:715–719.
1205. Bankowski RA, Wichmann RW, Stuart EE. Stomatitis of cattle and horses due to yellow bristle grass (Setaria lutescens). *J Am Vet Med Assoc*. 1956;129:149–152.
1206. Pusterla N, Latson KM, Wilson WD, et al. Metallic foreign bodies in the tongues of 16 horses. *Vet Rec*. 2006;159:485–488.
1207. Snow DH, Bogan JA, Douglas TA, et al. Phenylbutazone toxicity in ponies. *Vet Rec*. 1979;105:26–30.
1208. Baum KH, Shin SJ, Rebhun WC, et al. Isolation of Actinobacillus-Lignieresii from Enlarged Tongue of A Horse. *J Am Vet Med Assoc*. 1984;185:792–793.
1209. Freestone JF, Seahorn TL. Miscellaneous conditions of the equine head. *Vet ClinNorth Am Equine Pract*. 1993;9:235–242.
1210. Schmotzer WB, Hultgren BD, Huber MJ, et al. Chemical involution of the equine parotid salivary gland. *Vet Surg*. 1991;20:128–132.
1211. Aleman M, Spier SJ, Wilson WD, et al. Corynebacterium pseudotuberculosis infection in horses: 538 cases (1982–1993). *J Am Vet Med Assoc*. 1996;209:804–809.
1212. Sockett DC, Baker JC, Stowe CM. Slaframine (Rhizoctonia leguminicola) intoxication in horses. *J Am Vet Med Assoc*. 1982;181:606.
1213. Plumlee KH, Galey FD. Neurotoxic mycotoxins: a review of fungal toxins that cause neurological disease in large animals. *J Vet InternMed*. 1994;8:49–54.
1214. Sisson S. Equine digestive system. In: Getty R, ed. *Sisson and Grossman's the anatomy of domestic animals*. Philadelphia, PA: WB Saunders; 1975.
1215. Green EM, MacFadden KE. Esophageal disorders of the horse. In: Smith BP, ed. *Large animal internal medicine*. St. Louis, MO: Mosby; 1996:698–709.
1216. Feige K, Schwarzwald C, Furst A, et al. Esophageal obstruction in horses: a retrospective study of 34 cases. *Can Vet J*. 2000;41:207–210.
1217. Craig D, Todhunter R. Surgical repair of an esophageal stricture in a horse. *Vet Surg*. 1987;16:251–254.
1218. Roberts MC, Kelly WR. Squamous cell carcinoma of the lower cervical oesophagus in a pony. *Equine Vet J*. 1979;11:199–201.
1219. Moore JN, Kintner LD. Recurrent esophageal obstruction due to squamous cell carcinoma in a horse. *Cornell Vet*. 1976;66:590–597.
1220. Craig DR, Shivy DR, Pankowski RL, et al. Esophageal disorders in 61 horses. Results of nonsurgical and surgical management. *Vet Surg*. 1989;18:432–438.
1221. Green S, Green EM, Aronson E. Squamous-Cell Carcinoma—An Unusual Cause of Choke in A Horse. *Modern Veterinary Practice*. 1986;67:870–875.
1222. Murray MJ, Ball MM, Parker GA. Megaesophagus and aspiration pneumonia secondary to gastric ulceration in a foal. *J Am Vet Med Assoc*. 1988;192:381–383.
1223. Butt TD, MacDonald DG, Crawford WH, et al. Persistent right aortic arch in a yearling horse. *Can Vet J*. 1998;39:714–715.
1224. Scott EA, Snoy P, Prasse KW, et al. Intramural esophageal cyst in a horse. *J Am Vet Med Assoc*. 1977;171:652–654.
1225. Hackett RP, Dyer RM, Hoffer RE. Surgical correction of esophageal diverticulum in a horse. *J Am Vet Med Assoc*. 1978;173:998–1000.
1226. Jansson N. Foreign body obstruction of the esophagus in a foal. *Comp Contin Educ Vet Equine*. 2006;1:147–150.
1227. MacKay RJ. On the true definition of dysphagia. *Compend Contin Educ Pract Vet*. 2001;23:1024–1028.
1228. Traver DS, Egger E, Moore JN. Retrieval of an esophageal foreign body in a horse. *Vet Med Small Anim Clin*. 1978;73:783–785.
1229. Alexander JE. Radiologic findings in equine choke. *J Am Vet Med Assoc*. 1967;151:47–53.
1230. Greet TR. Observations on the potential role of oesophageal radiography in the horse. *Equine Vet J*. 1982;14:73–79.
1231. Quick CB, Rendano VT. Equine radiology: the esophagus. *Mod Vet Pract*. 1978;59:625–631.
1232. Hillyer M. Management of oesophageal obstruction ('choke') in horses. *In Practice*. 1995;17:450–457.
1233. Meyer GA, Rashmir-Raven A, Helms RJ, et al. The effect of oxytocin on contractility of the equine oesophagus: a potential treatment for oesophageal obstruction. *Equine Vet J*. 2000;32:151–155.
1234. Wooldridge AA, Eades SC, Hosgood GL, et al. In vitro effects of oxytocin, acepromazine, detomidine, xylazine, butorphanol, terbutaline, isoproterenol, and dantrolene on smooth and skeletal muscles of the equine esophagus. *Am J Vet Res*. 2002;63:1732–1737.
1235. Wooldridge AA, Eades SC, Hosgood GL, et al. Effects of treatment with oxytocin, xylazine butorphanol, guaifenesin, acepromazine, and detomidine on esophageal manometric pressure in conscious horses. *Am J Vet Res*. 2002;63:1738–1744.

1236. Meagher DM, Spier S. Foreign-Body Obstruction in the Cervical Esophagus of the Horse—A Case-Report. *J Equine V Sci*. 1989;9:137–140.
1237. Erkert RS, MacAllister CG, Higbee R, et al. Use of a neodymium:yttrium-aluminum-garnet laser to remove exuberant granulation tissue from the esophagus of a horse. *J Am Vet Med Assoc*. 2002;221:403–407. 368.
1238. Chiavaccini L, Hassel DM. Clinical features and prognostic variables in 109 horses with esophageal obstruction (1992-2009). *J Vet Intern Med*. 2010;24:1147–1152.
1239. Crawford JM. The gastrointestinal tract: esophagus. In: Contran RS, Kumar V, Robbins SL, eds. *Pathologic basis of disease*. Philadelphia, PA: WB Saunders; 1994.
1240. Hardy J, Stewart RH, Beard WL, et al. Complications of nasogastric intubation in horses: nine cases (1987–1989). *J Am Vet Med Assoc*. 1992;201:483–486.
1241. Appt SA, Moll HD, Scarratt WK, et al. Esophageal foreign body obstruction in a Mustang. *Equine Practice*. 1996;18:8–11.
1242. Broekman LE, Kuiper D. Megaesophagus in the horse. A short review of the literature and 18 own cases. *Vet Q*. 2002;24:199–202.
1243. Bowman KF, Vaughan JT, Quick CB, et al. Megaesophagus in a colt. *J Am Vet Med Assoc*. 1978;172:334–337.
1244. Barber SM, McLaughlin BG, Fretz PB. Esophageal Ectasia in A Quarterhorse Colt. *Can Vet J Rev Vet Canadienne*. 1983;24:46–49.
1245. Rohrbach BW. Congenital esophageal ectasia in a thoroughbred foal. *J Am Vet Med Assoc*. 1980;177:65–67.
1246. Greet TR, Whitwell KE. Barium swallow as an aid to the diagnosis of grass sickness. *Equine Vet J*. 1986;18:294–297.
1247. Watson TDG, Sullivan M. Effects of Detomidine on Equine Esophageal Function As Studied by Contrast Radiography. *Veterinary Record*. 1991;129:67–69.
1248. Clark ES, Morris DD, Whitlock RH. Esophageal manometry in horses, cows, and sheep during deglutition. *Am J Vet Res*. 1987;48:547–551.
1249. Todhunter RJ, Stick JA, Trotter GW, et al. Medical management of esophageal stricture in seven horses. *J Am Vet Med Assoc*. 1984;185:784–787.
1250. Fubini SL, Starrack GS, Freeman DE. Esophagus. In: Auer JA, Stick JA, eds. *Equine surgery*. Philadelphia: WB Saunders; 1999.
1251. Chidlow HB, Robbins EG, Slovis NM. Balloon dilation to treat oesophageal strictures in five foals. *Equine Vet Educ*. 2015.
1252. Prutton JS, Marks SL, Aleman M. Endoscopic Balloon Dilation of Esophageal Strictures in 9 Horses. *J Vet Intern Med*. 2015;29:1105–1111.
1253. Reichelt U, Hamann J, Lischer C. Balloon dilation of oesophageal strictures in two horses. *Equine Vet Educ*. 2012;24:379–384.
1254. Tillotson K, Traub-Dargatz JL, Twedt D. Balloon dilation of an oesophageal stricture in a one-month-old Appaloosa colt. *Equine Vet Educ*. 2003;15:67–71.
1255. Borchers-Collyer P, Wong D, Sponseller B, et al. Esophageal strictures. *Compendium Equine*. 2007;2:144–156.
1256. Knottenbelt DC, Harrison LJ, Peacock PJ. Conservative treatment of oesophageal stricture in five foals. *Vet Rec*. 1992;131:27–30.
1257. Hawkins JF. Balloon dilation of oesophageal strictures. *Equine Vet Educ*. 2012;24:385–386.
1258. Suann CJ. Oesophageal resection and anastomosis as a treatment for oesophageal stricture in the horse. *Equine Vet J*. 1982;14:163–164.
1259. Gideon L. Esophageal anastomosis in two foals. *J Am Vet Med Assoc*. 1984;184:1146–1148.
1260. Nixon AJ, Aanes WA, Nelson AW, et al. Esophagomyotomy for relief of an intrathoracic esophageal stricture in a horse. *J Am Vet Med Assoc*. 1983;183:794–796.
1261. Wagner PC, Rantanen NW. Myotomy As A Treatment for Esophageal Stricture in A Horse. *Equine Practice*. 1980;2:40–45.
1262. Hoffer RE, Barber SM, Kallfelz FA, et al. Esophageal patch grafting as a treatment for esophageal stricture in a horse. *J Am Vet Med Assoc*. 1977;171:350–354.
1263. Freeman DE. Surgery for obstruction of the equine oesophagus and trachea. *Equine Vet Educ*. 2005;17:135–141.
1264. Waguespack RW, Bolt DM, Hubert JD. Esophageal strictures and diverticula. *Compendium Equine*. 2007;2:194–206.
1265. Ford TS, Schumacher J, Chaffin MK, et al. Surgical repair of an intrathoracic esophageal pulsion diverticulum in a horse. *Vet Surg*. 1991;20:316–319.
1266. Frauenfelder HC, Adams SB. Esophageal diverticulectomy in a horse. *J Am Vet Med Assoc*. 1982;180:771–772.
1267. MacDonald MH, Richardson DW, Morse CC. Esophageal phytobezoar in a horse. *J Am Vet Med Assoc*. 1987;191:1455–1456.
1268. Clabough DL, Roberts MC, Robertson I. Probable congenital esophageal stenosis in a thoroughbred foal. *J Am Vet MedAssoc*. 1991;199:483–485.
1269. Mackey VS, Large SM, Breznock EM, et al. Surgical-Correction of A Persistent Right Aortic-Arch in A Foal. *Vet Surg*. 1986;15:325–328.
1270. Bartels JE, Vaughan JT. Persistent right aortic arch in the horse. *J Am Vet Med Assoc*. 1969;154:406–409.
1271. Petrick SW, Roos CJ, van Niekerk J. Persistent right aortic arch in a horse. *J S Afr Vet Assoc*. 1978;49:355–358.
1272. van der Linde-Sipman JS, Goedegebuure SA, Kroneman J. Persistent right aortic arch associated with a persistent left ductus arteriosus and an interventricular septal defect in a horse. *Tijdschr Diergeneeskd*. 1979;104(suppl 4):189–194.
1273. Bauer S, Livesey MA, Bjorling DE, et al. Computed tomography assisted surgical correction of persistent right aortic arch in a neonatal foal. *Equine VetEduc*. 2006;8:40–46.
1274. Smith TR. Unusual vascular ring anomaly in a foal. *CanVet J*. 2004;45:1016–1018.
1275. Orsini JA, Sepesy L, Donawick WJ, et al. Esophageal duplication cyst as a cause of choke in the horse. *J Am Vet Med Assoc*. 1988;193:474–476.
1276. Peek SF, De Lahunta A, Hackett RP. Combined oesophageal and tracheal duplication cyst in an Arabian filly. *Equine Vet J*. 1995;27:475–478.
1277. Gaughan EM, Gift LJ, Frank RK. Tubular duplication of the cervical portion of the esophagus in a foal. *J Am Vet Med Assoc*. 1992;201:748–750.
1278. Sams AE, Weldon AD, Rakestraw P. Surgical treatment of intramural esophageal inclusion cysts in three horses. *Vet Surg*. 1993;22:135–139.
1279. Grevemeyer B, Kainer RA, Morgan JP. Persistent right aortic arch in foals. *Compendium Equine*. 2008;3:95–102.
1280. Wilcke JR, Crisman MV, Sams RA, et al. Pharmacokinetics of phenylbutazone in neonatal foals. *AmJVetRes*. 1993;54:2064–2067.
1281. Freeman DE. Wounds of the esophagus and trachea. *Vet Clin North Am Equine Pract*. 1989;5:683–693.
1282. Digby NJ, Burguez PN. Traumatic oesophageal rupture in the horse. *Equine Vet J*. 1982;14:169–170.
1283. Lunn DP, Peel JE. Successful treatment of traumatic oesophageal rupture with severe cellulitis in a mare. *Vet Rec*. 1985;116:544–545.
1284. Andrews FM, Bernard WV, Byars TD, et al. Recommendations for the diagnosis and treatment of equine gastric ulcer syndrome (EGUS). *Equine Vet Educ*. 1999;1:122–134.
1285. Sykes BW, Hewetson M, Hepburn RJ, et al. European College of Equine Internal Medicine Consensus Statement—Equine Gastric Ulcer Syndrome in Adult Horses. *J Vet Intern Med*. 2015;29:1288–1299.
1286. Murray MJ. Gastroduodenal ulceration in foals. *Equine Vet Educ*. 1999;1:43–52.

1287. Hammond CJ, Mason DK, Watkins KL. Gastric ulceration in mature thoroughbred horses. *Equine Vet J*. 1986;18:284–287.
1288. Vatistas NJ, Snyder JR, Carlson G, et al. Epidemiological study of gastric ulceration in the thoroughbred racehorse: 202 horses 1992–1993. *Proc Am Assoc Equine Pract*. 1994;40:125–126.
1289. Vatistas NJ, Snyder JR, Carlson G, et al. Cross-sectional study of gastric ulcers of the squamous mucosa in thoroughbred racehorses. *Equine Vet J Suppl*. 1999:34–39.
1290. Murray MJ, Grodinsky C, Anderson CW, et al. Gastric ulcers in horses: a comparison of endoscopic findings in horses with and without clinical signs. *Equine Vet J Suppl*. 1989:68–72.
1291. Murray MJ, Schusser GF, Pipers FS, et al. Factors associated with gastric lesions in thoroughbred racehorses. *Equine Vet J*. 1996;28:368–374.
1292. Orsini JA, Pipers FS. Endoscopic evaluation of the relationship between training, racing, and gastric ulcers. *Vet Surg*. 1997;26:424.
1293. Bell RJW, Kingston JK, Mogg TD, et al. The prevalence of gastric ulceration in racehorses in New Zealand. *N Z Vet J*. 2007;55:13–18.
1294. Jonsson H, Egenvall A. Prevalence of gastric ulceration in Swedish Standardbreds in race training. *Equine Vet J*. 2006;38:209–213.
1295. Begg LM, O'Sullivan CB. The prevalence and distribution of gastric ulceration in 345 racehorses. *Aust Vet J*. 2003;81:199–201.
1296. McClure SR, Glickman LT, Glickman NW. Prevalence of gastric ulcers in show horses. *J Am Vet Med Assoc*. 1999;215:1130–1133.
1297. Nieto JE, Snyder JR, Beldomenico P, et al. Prevalence of gastric ulcers in endurance horses—a preliminary report. *Vet J*. 2004;167:33–37.
1298. Tamzali Y, Marguet C, Priymenko N, et al. Prevalence of gastric ulcer syndrome in high-level endurance horses. *Equine Vet J*. 2011;43:141–144.
1299. Bertone J. Prevalence of gastric ulcers in elite, heavy use western performance horses. *J Vet Intern Med*. 2000;14:366.
1300. Jeune S, Nieto J, Dechant J, et al. Prevalence of gastric ulcers in Thoroughbred broodmares in pasture. *Vet J*. 2009;181:251–255.
1301. Hartmann AM, Frankeny RL. A preliminary investigation into the association between competition and gastric ulcer formation in non-racing performance horses. *J Equine Vet Sci*. 2003;23:560–561.
1302. Sandin A, Skidell J, Haggstrom J, et al. Postmortem findings of gastric ulcers in Swedish horses older than age one year: a retrospective study of 3715 horses (1924–1996). *Equine Vet J*. 2000;32:36–42.
1303. Chameroy KA, Nadeau JA, Bushmich SL, et al. Prevalence of non-glandular gastric ulcers in horses involved in a university riding program. *J Equine Vet Sci*. 2006;26:207–211.
1304. Dukti SA, Perkins S, Murphy J, et al. Prevalence of gastric squamous ulceration in horses with abdominal pain. *Equine Vet J*. 2006;38:347–349.
1305. Wilson JH. Gastric and duodenal ulcers in foals: a retrospective study. *Proc 2nd Symp Equine Colic Res*. 1985:126–128.
1306. Murray MJ, Grodinsky C, Cowles RR, et al. Endoscopic evaluation of changes in gastric lesions of Thoroughbred foals. *J Am Vet Med Assoc*. 1990;196:1623–1627.
1307. Murray MJ. Endoscopic appearance of gastric lesions in foals: 94 cases (1987–1988). *J Am Vet Med Assoc*. 1989;195:1135–1141.
1308. Murray MJ, Nout YS, Ward DL. Endoscopic findings of the gastric antrum and pylorus in horses: 162 cases (1996–2000). *J Vet Intern Med*. 2001;15:401–406.
1309. Luthersson N, Nielsen KH, Harris P, et al. The prevalence and anatomical distribution of equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. *Equine Vet J*. 2009;41:619–624.
1310. Husted L, Jensen TK, Olsen SN, et al. Examination of equine glandular stomach lesions for bacteria, including Helicobacter spp by fluorescence in situ hybridisation. *BMC Microbiol*. 2010;10:84.
1311. Murray MJ, Schusser GF. Measurement of 24-h gastric pH using an indwelling pH electrode in horses unfed, fed and treated with ranitidine. *Equine Vet J*. 1993;25:417–421.
1312. Husted L, Sanchez LC, Olsen SN, et al. Effect of paddock vs. stall housing on 24 hour gastric pH within the proximal and ventral equine stomach. *Equine Vet J*. 2008.
1313. Mertz HR, Walsh JH. Peptic ulcer pathophysiology. *Med Clin North Am*. 1991;75:799–814.
1314. Wolfe MM, Soll AH. The physiology of gastric acid secretion. *N Engl J Med*. 1988;319:1707–1715.
1315. Campbell-Thompson M. Secretagogue-induced [14C]aminopyrine uptake in isolated equine parietal cells. *Am J Ve tRes*. 1994;55:132–137.
1316. Kitchen DL, Burrow JA, Heartless CS, et al. Effect of pyloric blockade and infusion of histamine or pentagastrin on gastric secretion in horses. *Am J Vet Res*. 2000;61:1133–1139.
1317. Schubert ML, Edwards NF, Makhlouf GM. Regulation of gastric somatostatin secretion in the mouse by luminal acidity: a local feedback mechanism. *Gastroenterology*. 1988;94:317–322.
1318. Lewis JJ, Goldenring JR, Asher VA, et al. Effects of epidermal growth factor on signal transduction in rabbit parietal cells. *Am J Physiol*. 1990;258:G476–G483.
1319. Baker SJ, Gerring EL. Gastric pH monitoring in healthy, suckling pony foals. *Am J Vet Res*. 1993;54:959–964.
1320. Sanchez LC, Lester GD, Merritt AM. Effect of ranitidine on intragastric pH in clinically normal neonatal foals. *J Am Vet Med Assoc*. 1998;212:1407–1412.
1321. Sanchez LC, Lester GD, Merritt AM. Intragastric pH in critically ill neonatal foals and the effect of ranitidine. *J Am Vet Med Assoc*. 2001;218:907–911.
1322. Kuusela AL. Long-term gastric pH monitoring for determining optimal dose of ranitidine for critically ill preterm and term neonates. *Arch Dis Child Fetal Neonatal Ed*. 1998;78:F151–F153.
1323. De Backer A, Haentjens P, Willems G. Hydrochloric acid. A trigger of cell proliferation in the esophagus of dogs. *Dig Dis Sci*. 1985;30:884–890.
1324. McGowan CM, McGowan TW, Andrews FM, et al. Induction and recovery of dietary induced gastric ulcers in horses. *J Vet Intern Med*. 2007;21. 603–603.
1325. Nadeau JA, Andrews FM, Mathew AG, et al. Evaluation of diet as a cause of gastric ulcers in horses. *Am J Vet Res*. 2000;61:784–790.
1326. Lybbert T, Gibbs P, Cohen N, et al. Feeding alfalfa hay to exercising horses reduces the severity of gastric squamous mucosal ulceration. *Proc Am Assoc Equine Pract*. 2007;53:525–526.
1327. Murray MJ, Eichorn ES. Effects of intermittent feed deprivation, intermittent feed deprivation with ranitidine administration, and stall confinement with ad libitum access to hay on gastric ulceration in horses. *Am J Vet Res*. 1996;57:1599–1603.
1328. Luthersson N, Nielsen KH, Harris P, et al. Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. *Equine Vet J*. 2009;41:625–630.
1329. Lester GD, Robinson I, Secombe C. Risk factors for gastric ulceration in Thoroughbred racehorses. In: *Canberra: Australian Government: Rural industries Research and Development Corporation.* ; 2008:1–42.
1330. White G, McClure SR, Sifferman R, et al. Effects of short-term light to heavy exercise on gastric ulcer development in horses and efficacy of omeprazole paste in preventing gastric ulceration. *J Am Vet Med Assoc*. 2007;230:1680–1682.
1331. Lester GD, Robertson ID, Secombe C. Risk factors for gastric ulceration in thoroughbred racehorses. *Proc Am Assoc Equine Pract*. 2007;53:529.

1332. Furr M, Taylor L, Kronfeld D. The effects of exercise training on serum gastrin responses in the horse. *Cornell Vet*. 1994;84:41–45.
1333. Lorenzo-Figueras M, Merritt AM. Effects of exercise on gastric volume and pH in the proximal portion of the stomach of horses. *Am J Vet Res*. 2002;63:1481–1487.
1334. McClure SR, Carithers DS, Gross SJ, et al. Gastric ulcer development in horses in a simulated show or training environment. *J Am Vet Med Assoc*. 2005;227:775–777.
1335. Meschter CL, Gilbert M, Krook L, et al. The effects of phenylbutazone on the morphology and prostaglandin concentrations of the pyloric mucosa of the equine stomach. *Vet Pathol*. 1990;27:244–253.
1336. MacKay RJ, French TW, Nguyen HT, et al. Effects of large doses of phenylbutazone administration to horses. *Am J Vet Res*. 1983;44:774–780.
1337. Roy MA, Vrins A, Beauchamp G, et al. Prevalence of ulcers of the squamous gastric mucosa in Standardbred horses. *J Vet Intern Med*. 2005;19:744–750.
1338. Nicol CJ, Davidson HPD, Harris PA, et al. Study of crib-biting and gastric inflammation and ulceration in young horses. *Vet Rec*. 2002;151:658–662.
1339. Furr MO, Murray MJ, Ferguson DC. The effects of stress on gastric ulceration, T3, T4, reverse T3 and cortisol in neonatal foals. *Equine Vet J*. 1992;24:37–40.
1340. Murray MJ, Murray CM, Sweeney HJ, et al. Prevalence of gastric lesions in foals without signs of gastric disease: an endoscopic survey. *Equine Vet J*. 1990;22:6–8.
1341. Barr BS, Wilkins PA, Del Piero F, et al. Is prophylaxis for gastric ulcers necessary in critically ill equine neonates?: a retrospective study of necropsy cases 1989–1999. *JVetInternMed*. 2000;14:328.
1342. Nappert G, Vrins A, Larybyere M. Gastroduodenal ulceration in foals. *Comp Contin Ed*. 1989;11:338–345.
1343. Orsini JA, Donawick WJ. Surgical treatment of gastroduodenal obstructions in foals. *Vet Surg*. 1986;15:205–213.
1344. Becht JL, Byars TD. Gastroduodenal ulceration in foals. *Equine Vet J*. 1986;18:307–312.
1345. Campbell-Thompson ML, Merritt AM. Gastroduodenal ulceration in foals. *Proc Am Assoc Equine Pract*. 1987;33:29–40.
1346. Morris AP, Estes MK. Microbes and microbial toxins: paradigms for microbial-mucosal interactions. VIII. Pathological consequences of rotavirus infection and its enterotoxin. *Am J Physiol Gastrointest Liver Physiol*. 2001;281:G303–G310.
1347. Murray MJ. Gastric ulceration in horses: 91 cases (1987–1990). *J Am Vet Med Assoc*. 1992;201:117–120.
1348. O'Conner MS, Steiner JM, Roussel AJ, et al. Evaluation of urine sucrose concentration for detection of gastric ulcers in horses. *AmJ Vet Res*. 2004;65:31–39.
1349. Hewetson M, Cohen ND, Love S, et al. Sucrose concentration in blood: a new method for assessment of gastric permeability in horses with gastric ulceration. *J Vet Intern Med*. 2006;20:388–394.
1350. Bell RJW, Kingston JK, Mogg TD. A comparison of two scoring systems for endoscopic grading of gastric ulceration in horses. *N Z Vet J*. 2007;55:19–22.
1351. Campbell-Thompson ML, Merritt AM. Effect of ranitidine on gastric acid secretion in young male horses. *Am J Vet Res*. 1987;48:1511–1515.
1352. Furr MO, Murray MJ. Treatment of gastric ulcers in horses with histamine type 2 receptor antagonists. *Equine Vet J Suppl*. 1989:77–79.
1353. Daurio CP, Holste JE, Andrews FM, et al. Effect of omeprazole paste on gastric acid secretion in horses. *Equine Vet J Suppl*. 1999:59–62.
1354. Sykes BW, Sykes KM, Hallowell GD. A comparison of three doses of omeprazole in the treatment of equine gastric ulcer syndrome: a blinded, randomised, dose-response clinical trial. *Equine Vet J*. 2015;47:285–290.
1355. Sykes BW, Underwood C, Greer R, et al. Pharmacokinetics and bioequivalence testing of five commercial formulations of omeprazole in the horse. *J Vet Pharmacol Ther*. 2016;39:78–83.
1356. Sykes BW, Underwood C, McGowan CM, et al. The effect of feeding on the pharmacokinetic variables of two commercially available formulations of omeprazole. *J Vet Pharmacol Ther*. 2015;38:500–503.
1357. Merritt AM, Sanchez LC, Burrow JA, et al. Effect of GastroGard and three compounded oral omeprazole preparations on 24 h intragastric pH in gastrically cannulated mature horses. *Equine Vet J*. 2003;35:691–695.
1358. Nieto JE, Spier S, Pipers FS, et al. Comparison of paste and suspension formulations of omeprazole in the healing of gastric ulcers in racehorses in active training. *J Am Vet Med Assoc*. 2002;221:1139–1143.
1359. Orsini JA, Haddock M, Stine L, et al. Odds of moderate or severe gastric ulceration in racehorses receiving antiulcer medications. *J Am Vet Med Assoc*. 2003;223:336–339.
1360. Plue RE, Wall HG, Daurio C, et al. Safety of omeprazole paste in foals and mature horses. *Equine Vet J Suppl*. 1999:63–66.
1361. Murray MJ, Eichorn ES, Holste JE, et al. Safety, acceptability and endoscopic findings in foals and yearling horses treated with a paste formulation of omeprazole for twenty-eight days. *Equine Vet J Suppl*. 1999:67–70.
1362. Lester GD, Smith RL, Robertson ID. Effects of treatment with omeprazole or ranitidine on gastric squamous ulceration in racing Thoroughbreds. *J Am Vet Med Assoc*. 2005;227:1636–1639.
1363. Nieto JE, Spier SJ, Van Hoogmoed L, et al. Comparison of omeprazole and cimetidine in healing of gastric ulcers and prevention of recurrence in horses. *Equine Vet Educ*. 2001;13:260–264.
1364. Murray MJ, Haven ML, Eichorn ES, et al. Effects of omeprazole on healing of naturally-occurring gastric ulcers in thoroughbred racehorses. *Equine Vet J*. 1997;29:425–429.
1365. Doucet MY, Vrins AA, Dionne R, et al. Efficacy of a paste formulation of omeprazole for the treatment of naturally occurring gastric ulcers in training standardbred racehorses in Canada. *Can Vet J*. 2003;44:581–585.
1366. MacAllister CG, Sifferman RL, McClure SR, et al. Effects of omeprazole paste on healing of spontaneous gastric ulcers in horses and foals: a field trial. *Equine Vet J Suppl*. 1999:77–80.
1367. McClure SR, White GW, Sifferman RL, et al. Efficacy of omeprazole paste for prevention of recurrence of gastric ulcers in horses in race training. *J Am Vet Med Assoc*. 2005;226:1685–1688.
1368. McClure SR, White GW, Sifferman RL, et al. Efficacy of omeprazole paste for prevention of gastric ulcers in horses in race training. *J Am Vet Med Assoc*. 2005;226:1681–1684.
1369. Sykes BW, Sykes K, Hallowell GD. Comparison of the effect of two doses of omeprazole on the squamous gastric mucosa in thoroughbred racehorses. *Vet Rec*. 2014;175:249.
1370. Sykes BW, Sykes KM, Hallowell GD. A comparison of two doses of omeprazole in the treatment of equine gastric ulcer syndrome: a blinded, randomised, clinical trial. *Equine Vet J*. 2014;46:416–421.
1371. Sykes BW, Sykes KM, Hallowell GD. A comparison between pre- and post exercise administration of omeprazole in the treatment of equine gastric ulcer syndrome: a blinded, randomised, clinical trial. *Equine Veterinary Journal*. 2014;46:422–426.
1372. Sanchez LC, Murray MJ, Merritt AM. Effect of omeprazole paste on intragastric pH in clinically normal neonatal foals. *Am J Vet Res*. 2004;65:1039–1041.
1373. Javsicas LH, Sanchez LC. The effect of omeprazole paste on intragastric pH in clinically ill neonatal foals. *Equine Vet J*. 2008;40:41–44.
1374. Sykes BW, Sykes KM, Hallowell GD. Administration of trimethoprim-sulphadimidine does not improve healing of glandular gastric ulceration in horses receiving omeprazole: a randomised, blinded, clinical study. *BMC Veterinary Research*. 2014;10.

1375. Ogihara Y, Okabe S. Effect and mechanism of sucralfate on healing of acetic acid-induced gastric ulcers in rats. *J Physiol Pharmacol*. 1993;44:109–118.
1376. Borne AT, MacAllister CG. Effect of sucralfate on healing of subclinical gastric ulcers in foals. *J Am Vet Med Assoc*. 1993;202:1465–1468.
1377. Hepburn RJ, Proudman CJ. Treatment of ulceration of the gastric glandular mucosa: retrospective evaluation of omeprazole and sucralfate combination therapy in 204 sport and leisure horses [abstract]. *International Equine Colic Research Symposium*. 2014:108.
1378. Leandro G, Pilotto A, Franceschi M, et al. Prevention of acute NSAID-related gastroduodenal damage: a meta- analysis of controlled clinical trials. *Dig Dis Sci*. 2001;46:1924–1936.
1379. Sangiah S, MacAllister CC, Amouzadeh HR. Effects of misoprostol and omeprazole on basal gastric pH and free acid content in horses. *Res Vet Sci*. 1989;47:350–354.
1380. Campbell-Thompson ML, Brown MP, Slone DE, et al. Gastroenterostomy for treatment of gastroduodenal ulcer disease in 14 foals. *J Am Vet Med Assoc*. 1986;188:840–844.
1381. Zedler ST, Embertson RM, Bernard WV, et al. Surgical treatment of gastric outflow obstruction in 40 foals. *Vet Surg*. 2009;38:623–630.
1382. Andrews FM, Frank N, Sommardahl CS, et al. Effects of intravenously administrated omeprazole on gastric juice pH and gastric ulcer scores in adult horses. *J Vet Intern Med*. 2006;20:1202–1206.
1383. McKeever JM, McKeever KH, Albeirci JM, et al. Effect of omeprazole on markers of performance in gastric ulcer-free standardbred horses. *Equine Vet J Suppl*. 2006:668–671.
1384. Cothran DS, Borowitz SM, Sutphen JL, et al. Alteration of normal gastric flora in neonates receiving ranitidine. *JPerinatol*. 1997;17:383–388.
1385. Furr M, Cohen ND, Axon JE, et al. Treatment with histamine-type 2 receptor antagonists and omeprazole increase the risk of diarrhoea in neonatal foals treated in intensive care units. *Equine Vet J*. 2012;44:80–86.
1386. Huff NK, Auer AD, Garza Jr F, et al. Effect of sea buckthorn berries and pulp in a liquid emulsion on gastric ulcer scores and gastric juice pH in horses. *J Vet Intern Med*. 2012;26:1186–1191.
1387. Hellings IR, Larsen S. ImproWin® in the treatment of gastric ulceration of the squamous mucosa in trotting racehorses. *Acta Vet Scand*. 2014;56:13.
1388. Sykes BW, Sykes KM, Hallowell GD. Efficacy of a Combination of Apolectol, Live Yeast (Saccharomyces cerevisiae [CNCM I-1077]), and Magnesium Hydroxide in the Management of Equine Gastric Ulcer Syndrome in Thoroughbred Racehorses: A Blinded, Randomized, Placebo-Controlled Clinical Trial. *J Equine Vet Sci*. 2014;34:1274–1278.
1389. Venner M, Lauffs S, Deegen E. Treatment of gastric lesions in horses with pectin-lecithin complex. *Equine Vet J Suppl*. 1999:91–96.
1390. Clark CK, Merritt AM, Burrow JA, et al. Effect of aluminum hydroxide/magnesium hydroxide antacid and bismuth subsalicylate on gastric pH in horses. *J Am Vet Med Assoc*. 1996;208:1687–1691.
1391. Munroe GA. Pyloric stenosis in a yearling with an incidental finding of Capillaria hepatica in the liver. *Equine Vet J*. 1984;16:221–222.
1392. Barth AD, Barber SM, McKenzie NT. Pyloric stenosis in a foal. *CanVet J*. 1980;21:234–236.
1393. Church S, Baker JR, May SA. Gastric retention associated with acquired pyloric stenosis in a gelding. *Equine Vet J*. 1986;18:332–334.
1394. McGill CA, Bolton JR. Gastric retention associated with a pyloric mass in two horses. *Aust Vet J*. 1984;61:190–191.
1395. Laing JA, Hutchins DR. Acquired pyloric stenosis and gastric retention in a mare. *Aust Vet J*. 1992;69:68–69.
1396. Campbell-Thompson ML, Merritt AM. Alimentary System: diseases of the stomach. In: Colahan PT, Mayhew IG, Merritt AM, et al., eds. *Equine Medicine and Surgery*. St. Louis, MO: Mosby; 1999:699–715.
1397. Wyse C, Murphey D, Preston T, et al. Use of the [13 C]octanoic acid breath test for assessment of gastric emptying in ponies: a preliminary study. *Proc 6th Equine Colic Res Symp*. 1998;40.
1398. Buchanan BR, Sommardahl CS, Moore RR, et al. What is your diagnosis? Pyloric-duodenal intussusception. *J Am Vet Med Assoc*. 2006;228:1339–1340.
1399. Todhunter RJ, Erb HN, Roth L. Gastric rupture in horses: a review of 54 cases. *Equine Vet J*. 1986;18:288–293.
1400. Puotunen-Reinert A, Huskamp B. Experimantal duodenal obstruction in the horse. *Vet Surg*. 1986;15:420–428.
1401. Kiper ML, Traub-Dargatz J, Curtis CR. Gastric rupture in horses: 50 cases (1979–1987). *JAm VetMed Assoc*. 1990;196:333–336.
1402. Steenhaut M, Vlaminck K, Gasthuys F. Surgical repair of a partial gastric rupture in a horse. *Equine Vet J*. 1986;18:331–332.
1403. Hogan PM, Bramlage LR, Pierce SW. Repair of a full-thickness gastric rupture in a horse. *J Am Vet Med Assoc*. 1995;207:338–340.
1404. Owen RA, Jagger DW, Jagger F. Two cases of equine primary gastric impaction. *Vet Rec*. 1987;121:102–105.
1405. Barclay WP, Foerner JJ, Phillips TN, et al. Primary gastric impaction in the horse. *J Am Vet Med Assoc*. 1982;181:682–683.
1406. Honnas CM, Schumacher J. Primary gastric impaction in a pony. *J Am Vet Med Assoc*. 1985;187:501–502.
1407. Kellam LL, Johnson PJ, Kramer J, et al. Gastric impaction and obstruction of the small intestine associated with persimmon phytobezoar in a horse. *J Am Vet Med Assoc*. 2000;216:1279–1281.
1408. Weldon AD, Rowland PH, Rebhun WC. Emphysematous gastritis in a horse. *Cornell Vet*. 1991;81:51–58.
1409. Delesalle C, Deprez P, Vanbrantegem L, et al. Emphysematous gastritis associated with Clostridium septicum in a horse. *J Vet Intern Med*. 2003;17:115–118.
1410. Pascoe RR, Summers PM. Clinical survey of tumours and tumour-like lesions in horses in south east Queensland. *Equine Vet J*. 1981;13:235–239.
1411. Carlson GP. Lymphosarcoma in horses. *Leukemia*. 1995;9(suppl 1):S101.
1412. Taylor SD, Pusterla N, Vaughan B, et al. Intestinal neoplasia in horses. *J Vet Intern Med*. 2006;20:1429–1436.
1413. Rhind SM, Hawe C, Dixon PM, et al. Oral metastasis of renal cell carcinoma in a horse. *J Comp Pathol*. 1999;120:97–103.
1414. McKenzie EC, Mills JN, Bolton JR. Gastric squamous cell carcinoma in three horses. *Aust Vet J*. 1997;75:480–483.
1415. Ford TS, Vaala WE, Sweeney CR, et al. Pleuroscopic diagnosis of gastroesophageal squamous cell carcinoma in a horse. *J Am Vet Med Assoc*. 1987;190:1556–1558.
1416. Olsen SN. Squamous cell carcinoma of the equine stomach: a report of five cases. *Vet Rec*. 1992;131:170–173.
1417. Mair TS, Hillyer MH. Chronic colic in the mature horse: a retrospective review of 106 cases. *Equine Vet J*. 1997;29:415–420.
1418. Hillyer MH, Mair TS. Recurrent colic in the mature horse: a retrospective review of 58 cases. *Equine Vet J*. 1997;29:421–424.
1419. Ogilvie GK. Paraneoplastic syndromes. *Vet Clin North Am Equine Pract*. 1998;14:439–449. v.
1420. Reef VB, Dyson SS, Beech J. Lymphosarcoma and associated immune-mediated hemolytic anemia and thrombocytopenia in horses. *J Am Vet Med Assoc*. 1984;184:313–317.
1421. La Perle KM, Piercy RJ, Long JF, et al. Multisystemic, eosinophilic, epitheliotropic disease with intestinal lymphosarcoma in a horse. *Vet Pathol*. 1998;35:144–146.
1422. McCoy DJ, Beasley R. Hypercalcemia associated with malignancy in a horse. *J Am Vet Med Assoc*. 1986;189:87–89.

1423. East LM, Savage CJ. Abdominal neoplasia (excluding urogenital tract). *Vet Clin North Am Equine Pract*. 1998;14:475–493. v-vi.
1424. Harps O, Brumhard J, Bartmann CP, et al. Ascites as a result of peritoneal mesotheliomas in a horse. *Tierarztl Prax*. 1996;24:270–274.
1425. Ricketts SW, Peace CK. A case of peritoneal mesothelioma in a thoroughbred mare. *Equine Vet J*. 1976;8:78–80.
1426. Fulton IC, Brown CM, Yamini B. Adenocarcinoma of intestinal origin in a horse: diagnosis by abdominocentesis and laparoscopy. *Equine Vet J*. 1990;22:447–448.
1427. Conwell RC, Hillyer MH, Mair TS, et al. Haemoperitoneum in horses: a retrospective review of 54 cases. *Vet Rec*. 2010;167:514–518.
1428. Perkins GA, Nydam DV, Flaminio MJ, et al. Serum IgM concentrations in normal, fit horses and horses with lymphoma or other medical conditions. *J Vet Intern Med*. 2003;17:337–342.
1429. Davis EG, Wilkerson MJ, Rush BR. Flow cytometry: clinical applications in equine medicine. *J Vet Intern Med*. 2002;16:404–410.
1430. Rebhun WC, Bertone A. Equine lymphosarcoma. *J Am Vet Med Assoc*. 1984;184:720–721.
1431. Wiseman A, Petrie L, Murray M. Diarrhoea in the horse as a result of alimentary lymphosarcoma. *Vet Rec*. 1974;95:454–457.
1432. Finley MR, Rebhun WC, Dee A, et al. Paraneoplastic pruritus and alopecia in a horse with diffuse lymphoma. *J Am Vet Med Assoc*. 1998;213:102–104.
1433. Dabareiner RM, Sullins KE, Goodrich LR. Large colon resection for treatment of lymphosarcoma in two horses. *J Am Vet Med Assoc*. 1996;208:895–897.
1434. Taylor SD, Haldorson GJ, Vaughan B, et al. Gastric neoplasia in horses. *J Vet Intern Med*. 2009;23:1097–1102.
1435. Schuh JC. Squamous cell carcinoma of the oral, pharyngeal and nasal mucosa in the horse. *Vet Pathol*. 1986;23:205–207.
1436. Tuckey JC, Hilbert BJ, Beetson S, et al. Squamous cell carcinoma of the pharyngeal wall in a horse. *Aust Vet J*. 1995;72:227.
1437. Hewes CA, Sullins KE. Use of cisplatin-containing biodegradable beads for treatment of cutaneous neoplasia in equidae: 59 cases (2000–2004). *J Am Vet Med Assoc*. 2006;229:1617–1622.
1438. Theon AP, Wilson WD, Magdesian KG, et al. Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995–2004). *J Am Vet Med Assoc*. 2007;230:1506–1513.
1439. Mosunic CB, Moore PA, Carmichael KP, et al. Effects of treatment with and without adjuvant radiation therapy on recurrence of ocular and adnexal squamous cell carcinoma in horses: 157 cases (1985–2002). *J Am Vet Med Assoc*. 2004;225:1733–1738.
1440. Theon AP, Pascoe JR, Galuppo LD, et al. Comparison of perioperative versus postoperative intratumoral administration of cisplatin for treatment of cutaneous sarcoids and squamous cell carcinomas in horses. *J Am Vet Med Assoc*. 1999;215:1655–1660.
1441. Scherrer NM, Lassaline-Utter M, McKenna BC. Characterization and outcome following excision of masses in the nictitating membranes of horses: 50 cases (1998–2012). *J Am Vet Med Assoc*. 2014;245:812–815.
1442. Moore AS, Beam SL, Rassnick KM, et al. Long-term control of mucocutaneous squamous cell carcinoma and metastases in a horse using piroxicam. *Equine Vet J*. 2003;35:715–718.
1443. Leach MW, Pool RR. Hypertrophic osteopathy in a Shetland pony attributable to pulmonary squamous cell carcinoma metastases. *Equine Vet J*. 1992;24:247–249.
1444. Moran JA, Lemberger K, Cadore JL, et al. Small intestine adenocarcinoma in conjunction with multiple adenomas causing acute colic in a horse. *J Vet Diagn Invest*. 2008;20:121–124.
1445. Kirchhof N, Steinhauer D, Fey K. Equine adenocarcinomas of the large intestine with osseous metaplasia. *J Comp Pathol*. 1996;114:451–456.
1446. Roy MF, Parente EJ, Donaldson MT, et al. Successful treatment of a colonic adenocarcinoma in a horse. *Equine Vet J*. 2002;34:102–104.
1447. Mair TS, Davies EV, Lucke VM. Small colon intussusception associated with an intralumenal leiomyoma in a pony. *Vet Rec*. 1992;130:403–404.
1448. Schaudien D, Muller JM, Baumgartner W. Omental leiomyoma in a male adult horse. *Vet Pathol*. 2007;44:722–726.
1449. Hanes GE, Robertson JT. Leiomyoma of the small intestine in a horse. *J Am Vet Med Assoc*. 1983;182:1398.
1450. Collier MA, Trent AM. Jejunal intussusception associated with leiomyoma in an aged horse. *J Am Vet Med Assoc*. 1983;182:819–821.
1451. Watt BC, Trostle SS, Cooley AJ. Intraluminal leiomyoma colon polyp in a mare. *Equine Vet J*. 2001;33:326–328.
1452. Kasper C, Doran R. Duodenal leiomyoma associated with colic in a two-year-old horse. *J Am Vet Med Assoc*. 1993;202:769–770.
1453. Johnson PJ, Wilson DA, Turk JR, et al. Disseminated peritoneal leiomyomatosis in a horse. *J Am Vet Med Assoc*. 1994;205:725–728.
1454. Pirie RS, Tremaine WH. Neoplasia of the mouth and surrounding structures. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. Philadelphia, PA: WB Saunders; 1997:153–155.
1455. Morse CC, Saik JE, Richardson DW, et al. Equine juvenile mandibular ossifying fibroma. *Vet Pathol*. 1988;25:415–421.

CHAPTER 13

Disorders of the Liver

Thomas J. Divers and Michelle Henry Barton

NORMAL LIVER

Anatomy

The liver is the second largest single organ in the horse (skin is first), accounting for approximately 1.5% of body weight.[1] In the adult horse, the liver is positioned obliquely in the cranial abdomen, with the left lobes located ventrally near the ninth to tenth intercostal spaces and the right lobes positioned more dorsally and opposite the ninth to sixteenth intercostal spaces. The smaller ventral quadrate and right dorsal caudate lobes are located midabdomen.[2] The adult horse liver is completely contained within the rib cage and does not contact the ventral abdominal floor, although in disease processes resulting in hepatomegaly, the liver may extend beyond the caudal border of the last rib. In the fetus and newborn foal, the liver is relatively much larger, and on fetal ultrasound the liver is the easiest organ to identify.[1] The liver on the left encompasses approximately one third of the liver mass and is often adjacent to the more caudally located spleen; this provides an opportunity, when visualized with ultrasound, to compare the relative echogenicity of each organ. The liver should be hypoechoic compared to the spleen, and with hepatic fibrosis the liver and spleen appear more similar in echogenicity.[3] An unusual anatomic variant in horses is atrophy of the right lobe of the liver that occurs in some middle-aged to older horses. In this condition, there is a loss of hepatocytes with condensation of the hepatic stroma, and a thick, wrinkled hepatic capsule develops.[4] This atrophy may occasionally prevent ultrasound visualization of the right liver. The cause of this atrophy is somewhat unique to the horse and is not believed to be a result of divergence of arterial or venous blood flow. Instead, it is believed to result from compression of this portion of the liver by the right dorsal colon and cecum.[4]

The equine liver consists of two surfaces, diaphragmatic and visceral. The visceral surface of the liver in situ is malleable and contains impressions of the organs with which it is in contact. The visceral surface also contains the hilum, or *porta* (door), of the liver, through which blood vessels, lymphatics, and nerves enter, and the hepatic duct exits. In the horse, six ligaments secure the liver in the abdominal cavity.[2] The *coronary ligament* has two laminae, right and left, which attach the diaphragmatic surface of the liver to the caudal vena cava and the abdominal esophagus. The two laminae of the coronary ligament unite ventrally to form the *falciform ligament.* The falciform ligament, a remnant of the fetal ventral mesentery that extends from the diaphragm to the umbilicus, attaches the quadrate and left lobes to the sternal diaphragm and ventral abdominal floor. The *round ligament,* the remnant of the fetal umbilical vein, is contained within the free border of the falciform ligament. The right and left *triangular ligaments* attach the dorsal right lobe to the right costal diaphragm and the dorsal left lobe to the tendinous center of the diaphragm. The *hepatorenal ligament* connects the caudate process of the quadrate lobe to the right kidney and the base of the cecum.

Seventy percent of blood flow to the liver is deoxygenated blood from the hepatic portal vein.[5] The hepatic portal vein receives blood from the stomach, spleen, pancreas, small intestines, cecum, and large colon. The portal vein does not receive blood from the most distal colon and rectum, kidneys, reproductive organs, or mammary gland.[6] The other 30% of blood to the liver is oxygenated, provided by the accompanying hepatic artery.[5,7] Approximately 10% of the total blood volume at any one time is found in the liver.[5]

Bile ducts from each lobe join and eventually form the left and right hepatic ducts. These unite to form the common bile duct. Both the bile duct and pancreatic duct empty into the duodenum at the major duodenal papilla, located on a slightly raised mucosal ridge.[1] This ridge can be seen on endoscopic examination of the duodenum, just before the cranial flexure of the duodenum at the beginning of the sigmoid loop. Opposite the major duodenal papilla is a smaller projection into the bowel, the minor duodenal papilla, which contains the opening of the accessory pancreatic duct.

Histology

At the hilum of the liver, a tree of connective tissue consisting of collagen and fibroblasts enters the hepatic parenchyma. The parenchymal cells, or hepatocytes, compose approximately 50% to 60% of the mass of the liver and are epithelial cells.[8,9]

At the light microscopic level, the liver can be divided into structural arranged units called *hepatic lobules,* portal lobules, or acinus[1,5,6] (Fig. 13.1A). The classic hepatic lobule is a polygonal histologic unit composed of numerous plates of liver cells (hepatocytes), radiating centrifugally toward a *central vein.* Situated around the perimeter, at each "corner" of the hepatic lobule, are branches of the hepatic artery, hepatic portal vein, and bile duct (together called the *hepatic*

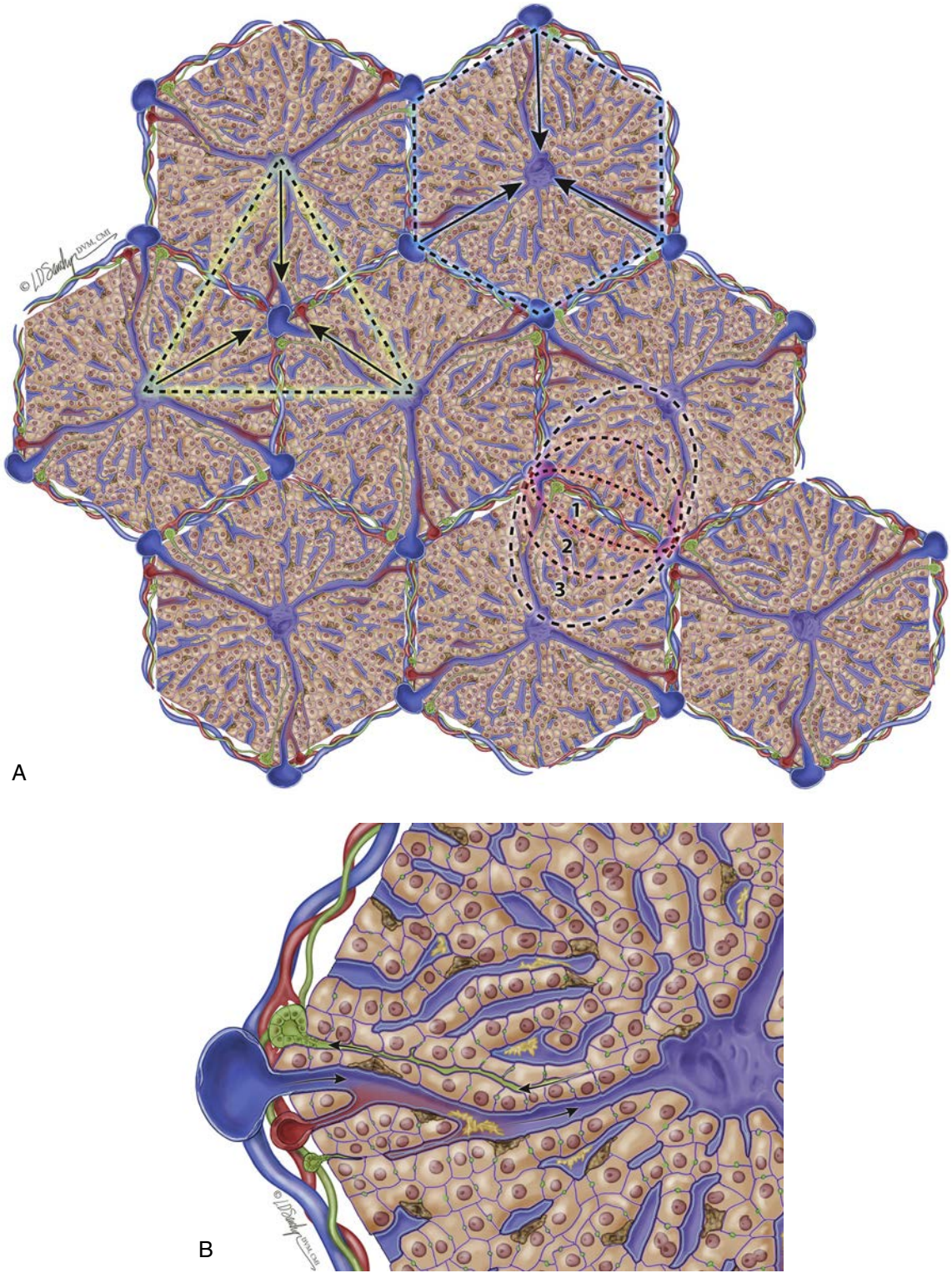


FIG. **13.1** (A) Anatomic and functional divisions of the liver. *Top right:* Classic hepatic lobule (emphasizes blood flow); cross-sectional view with portal triads centered around a single central vein. *Top left:* Portal lobule (emphasizes exocrine function/bile flow). Three central veins centered around a portal tract. *Lower right:* Hepatic acinus (emphasizes hepatic oxygen concentration to liver)—blood flowing from two hepatic triads (hepatic artery and portal vein) toward a central vein. Zone 1 has the highest oxygen content and zone 3 the lowest. (B) Basic liver structures. Hepatic arteries and portal veins perfuse the liver via the liver sinusoids that run between adjacent hepatic cords. The sinusoidal blood flows toward and drains into the central vein. Kupffer cells (depicted in the illustration as "shaggy"-appearing structures) are phagocytic cells of the mononuclear phagocyte series found on the luminal surface of the hepatic sinusoids. The space of Disse is located between the hepatocytes and the endothelium of the sinusoids, where blood components can be absorbed by the hepatocytes. Triangular-shaped cells in the illustration depict hepatic stellate cells (Ito cells), which are located in the space of Disse. On the opposite side of the hepatic cord from the sinusoid is the bile canaliculus draining bile toward the bile duct at the portal triad. The bile flow is opposite direction of blood flow *(arrows).* (Illustration courtesy of Dr. Lauren D. Sawchyn.)

triad), plus lymphatics. Blood from hepatic arterioles and portal venules, comprising approximately one third of the cardiac output, mixes together in *hepatic sinusoids* that surround the plates of hepatocytes, thus bathing each hepatocyte in a mix of arterial blood and venous blood. Sinusoidal blood then travels along the plates of hepatocytes toward the central vein, eventually entering larger hepatic veins that converge near the diaphragm to enter the caudal vena cava. Hepatic sinusoids (Fig. 13.1B) are larger than capillaries and are lined with endothelial cells and Kupffer cells. Kupffer cells are tissue-fixed macrophages and are estimated to make up 20% of the mass of the liver.[9] The endothelial cells make up approximately 20% of the mass of the liver.[9] A cleft, called the *space of Disse,* lies between the hepatocytes and the cells lining the sinusoids. The space of Disse contains fluid similar to the composition of blood but does not contain erythrocytes. The space of Disse also contains Ito cells, also called *hepatic stellate cells*, which store fat or fat-soluble vitamins (e.g., vitamin A).[5]

The space between contiguous hepatocytes in a cord forms a bile canaliculus through which bile excreted by the hepatocytes drains into bile ductules and ducts. The bile canaliculi thus are formed solely by the cell membranes of the hepatocytes. The bile ductules and ducts are lined with cuboidal and columnar epithelial cells, respectively, that make up approximately 7% of the mass of the liver.[9] The bile ducts run in the connective tissue tree, adjacent to branches of the portal vein and hepatic artery, to form a distinct portal tract, radicle, canal, or triad (see Fig. 13.1). The bile ducts converge at the hilum to form the *hepatic duct,* which drains into the duodenum just distal to the pylorus. Because the horse does not have a gallbladder or a sphincter at the entry site of the hepatic duct into the intestine, the bile is unconcentrated and flows almost continuously in a direction opposite that of the blood flow in the portal vein and hepatic artery.[2]

In contrast to the structural units, *hepatic lobules,* the portal lobule is viewed as a triangular (three central veins centered on a portal triad) oriented functional unit describing the exocrine (bile secretion) duties of the liver. The acinus lobules describe the vascular supply to the hepatic parenchyma, divided according to the tissue oxygen content. Zone I (periportal zone) of the acinus lobule is located immediately adjacent to branching hepatic arteries and portal veins, is the most metabolically active zone, and receives the best oxygen supply. Zone 1 hepatocytes are therefore especially suited for oxidative liver functions such as gluconeogenesis and β-oxidation of fatty acids. Zone 1 hepatocytes are the primary site for deposition of hemosiderin, which can be especially pronounced even in normal horses. Zone III is located adjacent to central veins, has the highest cytochrome P-450 mixed-function oxidase activity, is least favorably situated with respect to oxygen content, and thus is most susceptible to toxins that are metabolized in the liver and hypoxic damage.[10] Zone II is situated between zones I and III, and toxins such as creeping indigo *(Indigofera spicata)* may have a predominant zone II distribution pattern of disease.[11]

Physiology

The liver is both anatomically and functionally arranged such that it is the most important organ for assimilation and metabolism of nutrients, orally administered drugs, and gut-derived toxins or bacteria. The liver is the main organ involved in the regulation of nutrient distribution.[12] The majority of nutrients absorbed from the gastrointestinal tract pass directly to the liver via the portal circulation. The incoming nutrients are metabolized for energy, transformed to other nutrient classes, packaged and exported to peripheral tissues, or stored by the liver. The liver is capable of adjusting to the carbohydrate, protein, and lipid load from the gastrointestinal tract, as well as maintaining consistent blood levels of nutrients between feedings and in response to special needs. In addition to its role in nutrient metabolism and homeostasis, the liver is involved in excretion (bile), detoxification and metabolism of endogenous and exogenous substances, and hematopoiesis.[9]

Protein Metabolism

Amino acids, which are transported to the liver via the portal or hepatic blood, may be used in the biosynthesis of intrinsic hepatocellular proteins, plasma proteins, porphyrins, polyamines, purines, and pyrimidines.[12] The liver synthesizes 90% of the plasma proteins, including albumin, many factors involved in coagulation and fibrinolysis (fibrinogen and factors II, V, VII–XIII; antithrombin III; protein C; plasminogen; plasminogen activator inhibitor; α_2-antiplasmin; α_2-macroglobulin; and α_1-antitrypsin), transport proteins (haptoglobin, transferrin, ceruloplasmin, hormone transport proteins), and acute phase reactant proteins (α- and β-globulins).[9] The liver is the only site of synthesis of albumin, fibrinogen, and C-reactive protein and is a predominant site for production of amyloid A and hepcidin.[13] Hepcidin is a peptide hormone synthesized mainly in the liver. It reduces dietary iron absorption by reducing iron transport across the gut mucosa (enterocytes) and reduces iron exit from macrophages and the liver, the main sites of iron storage. In horses and many other species, endotoxemia and systemic inflammation therefore cause a rapid decline in serum iron.[14]

The liver is also capable of transamination, or the reversible transfer of an amino group on one amino acid to an α-keto acid, thus forming a new amino acid and a new keto acid. If the liver receives an excess of amino acids or if carbohydrates are unavailable as an energy source, the liver deaminates the amino acids and converts them to pyruvate, acetoacetate, and intermediates of the tricarboxylic acid cycle[12] (Fig. 13.2). These intermediates may be oxidized for energy or used as precursors in *gluconeogenesis,* the synthesis of glucose from noncarbohydrate precursors. Endogenous and exogenous glucocorticoids, glucagon, and thyroid hormone act directly on the liver to increase gluconeogenesis (Fig. 13.3). Simultaneously, glucocorticoids indirectly influence liver gluconeogenesis by promoting peripheral protein catabolism, thus increasing the availability of amino acids. Insulin inhibits gluconeogenesis in the liver.[15]

In addition to protein synthesis and gluconeogenesis, the liver plays an important role in eliminating the major toxic byproduct of amino acid catabolism, *ammonia.*[12,16] Tissues and intestinal microflora generate ammonia, which is subsequently released into the circulation. One method by which the liver, as well as certain peripheral tissues, eliminates ammonia is by synthesizing nonessential amino acids from α-keto acids and ammonia in a reversal of deamination. A fundamental reaction in the synthesis of nonessential amino acids is the formation of *glutamate* from α-ketoglutarate and ammonia

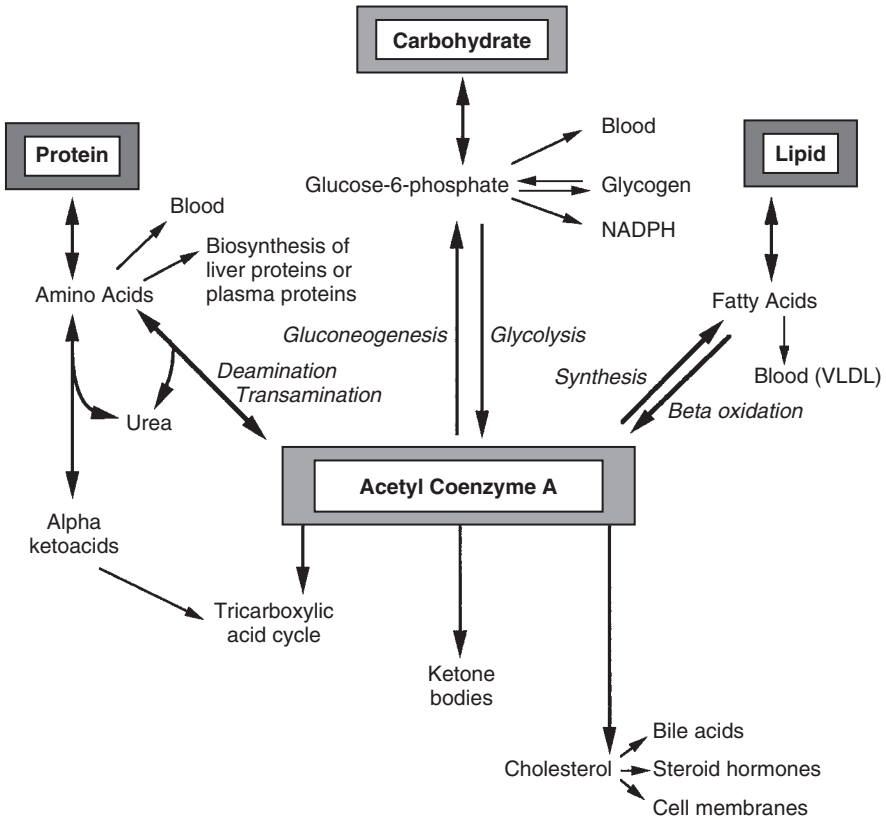


FIG. **13.2** Role of the liver in the metabolism of nutrients. *NADPH,* Nicotinamide adenine dinucleotide phosphate; *VLDL,* very low-density lipoprotein.

(Fig. 13.4). Subsequently, glutamate is used in transamination reactions to form other amino acids. Glutamate also participates in the conversion of cytotoxic free ammonia into a nontoxic (except in neuronal glial cells) transport form, *glutamine.* Glutamine may be delivered to the kidney, converted back to free ammonia and excreted, or delivered to the liver for urea synthesis. Excessive glutamine in the brain, mostly as a result of glial cell metabolism of excessive ammonia, may cause cerebral edema.[17]

The liver has sole responsibility for converting free ammonia or glutamine into *urea,* the principal form of amino group nitrogen excretion by mammals.[9] Urea is formed by the irreversible condensation of two ammonia molecules with carbon dioxide (see Fig. 13.4). The reaction takes place in the hepatocyte mitochondria via the Krebs-Henseleit cycle.[12] The newly formed urea is released from the hepatocyte, secreted into the sinusoidal blood, and transported to the kidney as *blood urea nitrogen* (BUN) for excretion.

Carbohydrate Metabolism

The liver is responsible for the synthesis, storage, and release of glucose.[12] Most soluble carbohydrates such as starches and sugars are readily broken down to glucose or other monosaccharides in the small intestine, absorbed and delivered via portal blood to the liver. In the hepatocyte the majority of glucose is phosphorylated to *glucose-6-phosphate* by the enzyme hexokinase (see Fig. 13.2). The remaining glucose is released into the systemic circulation. Other monosaccharides (fructose, galactose) are phosphorylated and converted in the liver to glucose-6-phosphate. The majority of glucose-6-phosphate is converted to glycogen for storage. A small amount of glucose-6-phosphate is oxidized to form adenosine triphosphate, although the major source of adenosine triphosphate in the liver is amino acid and fatty acid oxidation. Approximately half of the liver glucose enters the phosphogluconate pathway for generation of nicotinamide adenine dinucleotide phosphate, which is required as a reducing agent in the biosynthesis of fatty acids and cholesterol. Glucocorticoids, catecholamines, glucagon, and thyroid hormone increase gluconeogenesis and glycogenolysis in the liver, whereas insulin inhibits gluconeogenesis[15] (see Fig. 13.3). Structural carbohydrates are converted to volatile free fatty acids (VFAs) in the large bowel of the horse and are an important source of energy. Some of the VFAs (e.g., acetate) may be used directly for energy whereas others (e.g., propionate and lactate) undergo hepatic gluconeogenesis.[18,19]

Lipid Metabolism

Short-chain fatty acids (fewer than 10 carbon atoms) can be absorbed directly from the gastrointestinal tract, bound to albumin, and delivered to the liver via the portal circulation.[12] However, the majority of short-chain fatty acids are incorporated into phospholipid or triglyceride by the intestinal epithelium and transported to the liver via the portal blood. The remaining small percentage of fatty acids are absorbed from the gastrointestinal tract and are transported as triglyceride in *chylomicrons.* After formation in the intestinal epithelial cells and absorption into lymphatics, chylomicrons enter the systemic circulation via the thoracic duct and subsequently are delivered to the liver. The liver also may take up albumin-bound fatty acids released from adipose tissue.

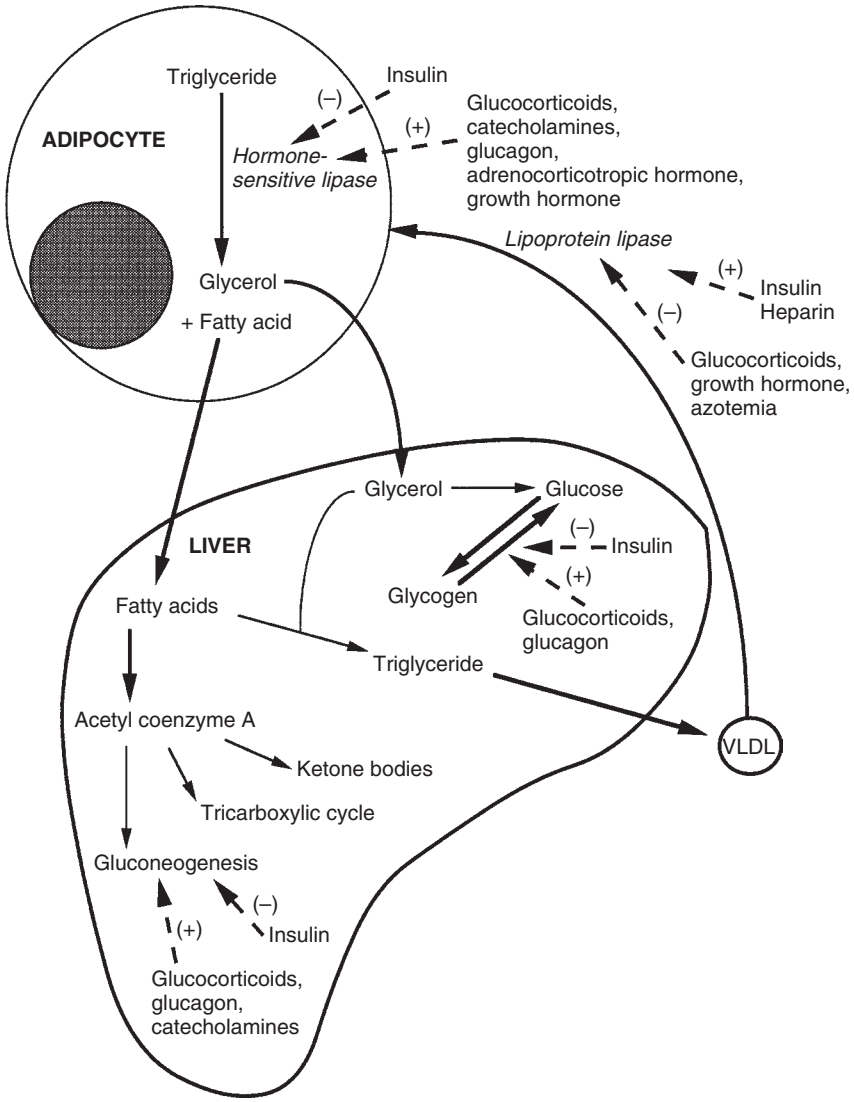


FIG. **13.3** Hormonal control of metabolism. –, Inhibitory effect; +, stimulatory effect.

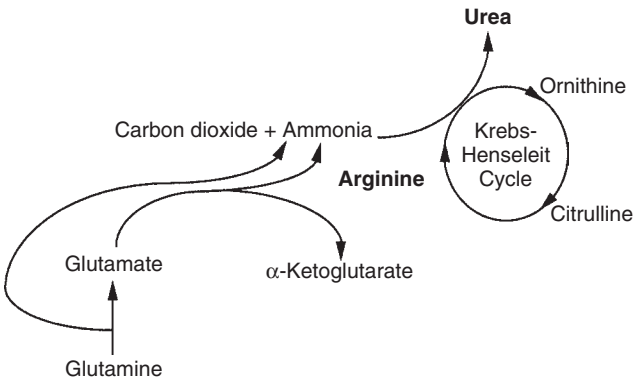


FIG. **13.4** The urea cycle.

The fate of fatty acids in the liver depends on the state of energy demand, the rate of fatty acid delivery, and hormonal influences. One of the primary roles of the liver in lipid metabolism is to esterify free fatty acids into triglycerides for export to other tissues[12] (see Fig. 13.3). The triglycerides are packaged with protein, carbohydrate, and cholesterol in the endoplasmic reticulum of the hepatocyte into *very low-density lipoproteins* (VLDLs), which primarily contain triglyceride, and *high-density lipoproteins* (HDLs), which primarily contain protein and phospholipid.[20] The VLDLs and HDLs are released into the hepatic sinusoids. Once the VLDLs are in the systemic circulation, the adipose tissue takes them up or endothelial cell lipases alter their composition by removing triglyceride, forming *intermediate-* and *low-density lipoproteins.*

In addition to exporting plasma lipoprotein, the liver can oxidize free fatty acids for energy to acetyl coenzyme A (acetyl CoA), a fundamental compound in the tricarboxylic acid cycle (see Fig. 13.2). The acetyl CoA thus formed also may be used in the synthesis of other fatty acids, cholesterol, steroids, and ketone bodies, acetoacetate, and β-hydroxybutyrate.[12] Furthermore, through the synthesis of acetyl CoA from glucose and most amino acids, the liver is capable of converting carbohydrates and proteins into lipids. Ketone bodies may occur from incomplete oxidation of free fatty acids in the liver and in other species are used for energy by peripheral tissues, especially the brain, when glucose is deficient. However, in the horse this does not appear to be a significant route for free fatty acid metabolism.

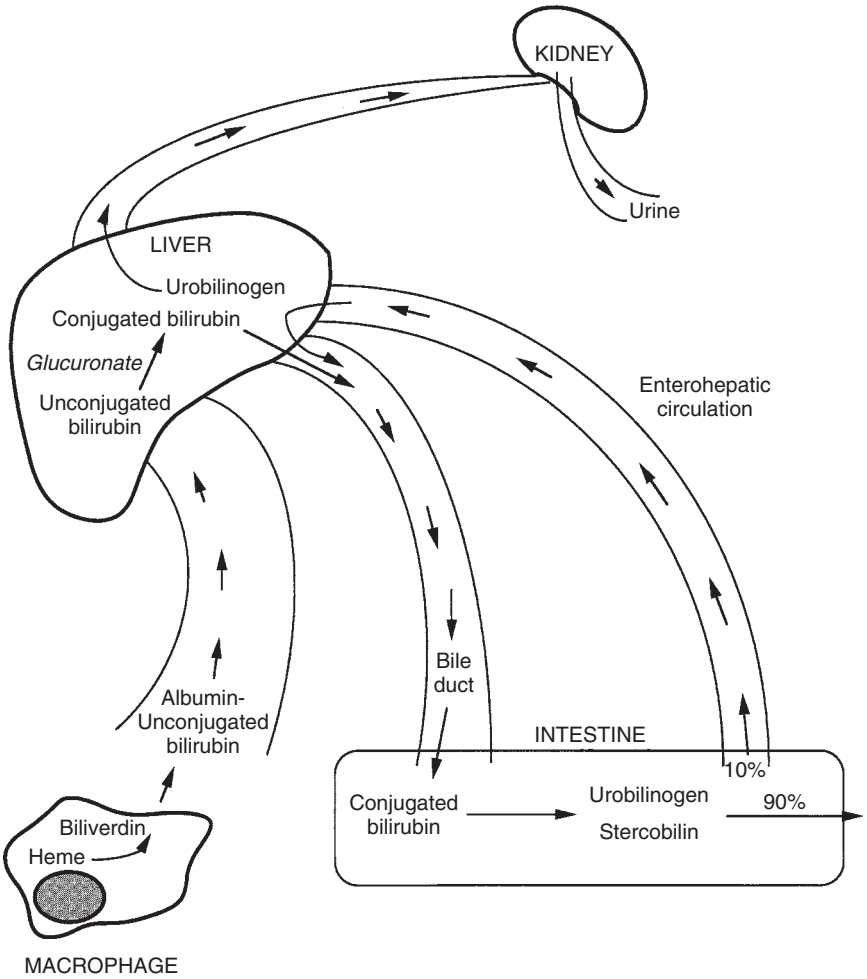


FIG. **13.5** Metabolism and excretion of bile.

Insulin and glucocorticoids closely regulate lipid metabolism[15] (see Fig. 13.3). Glucocorticoids function primarily to increase fatty acid mobilization from the periphery, whereas insulin decreases adipose tissue release of fatty acids by activating lipoprotein lipase and inhibiting hormone-sensitive lipase. Insulin acts on the liver to increase fatty acid synthesis from glucose.

Excretion of Bile

Bile consists of several components, including conjugated bilirubin, bile acids, cholesterol, lecithin, water, and electrolytes.[9] Bile is released by hepatocytes into the bile canaliculi where water diffuses passively. Bile then is transported by large bile ducts and the hepatic duct to the intestine. Water and electrolyte exchange takes place between the bile and the bile duct epithelium; however, isotonicity is maintained. Because the horse does not have a gallbladder or a sphincter at the site of entry of the hepatic duct into the duodenum, the bile is unconcentrated and flow is nearly continuous.[2]

Primary bile acids are produced in the liver, mostly from cholesterol, and compose 90% of the organic portion of bile.[21] Bile acids are amphoteric molecules that act as detergents. These detergents facilitate the excretion of cholesterol and phospholipid from the liver into bile and facilitate the digestion and absorption of lipids and lipid-soluble compounds (vitamins A, D, E, and K) from the intestinal tract. The principal *primary bile acids* (i.e., nondegraded) in the horse are *cholate* and *chenodeoxycholate,* both of which are conjugated with taurine.[21] Once secreted into the lumen of the intestinal tract, cholate and chenodeoxycholate may be reabsorbed or degraded by bacteria, forming the *secondary bile acids, deoxycholate* or *lithocholate,* respectively. More than 90% of the conjugated bile acids excreted in bile and released into the intestinal lumen are reabsorbed by the jejunum and ileum and returned to the liver via the *enterohepatic circulation.* Deoxycholate acts as a normal bile acid and can undergo enterohepatic circulation, whereas lithocholate is only reabsorbed once. Bile acids are estimated to be recycled at least 38 times a day in healthy ponies.[21]

Bilirubin is the breakdown product of tetrapyrroles that function as electron transport pigments.[4] The majority of bilirubin is formed from hemoglobin and myoglobin, but nonheme pigments such as the cytochromes also serve as a source of bilirubin. Macrophages in the spleen, bone marrow, and liver (Kupffer cells) engulf the pigments first, convert it to biliverdin (Fig. 13.5), and then convert biliverdin to bilirubin and release it from the cell as free, insoluble bilirubin. This form of bilirubin also is referred to as *indirect-reacting* or *unconjugated bilirubin.* Unconjugated bilirubin is bound with albumin in the plasma to decrease its hydrophobicity and is delivered to the liver. At the surface of the hepatocyte, the bilirubin is transferred from albumin to *ligandin,*

an intrahepatic transport and storage protein.[9,21] Within the hepatocyte, the bilirubin is conjugated with glucuronide in the endoplasmic reticulum. *Conjugated bilirubin,* also called *direct-reacting bilirubin,* is water soluble and is excreted into the bile canaliculi. Under normal circumstances, little conjugated bilirubin escapes into the general circulation. With severe liver disease increased amounts of conjugated bilirubin will escape into circulation and be freely filtered in the urine.

Microflora in the intestinal tract reduce conjugated bilirubin to *urobilinogen* and *stercobilin* (see Fig. 13.5), which impart a yellow-brown color to feces. In herbivores, the presence of chlorophyll pigments in the feces masks the color of urobilinogen.[22] Only in the neonatal herbivore receiving a milk diet are the feces yellow. Urobilinogen is absorbed by the intestinal mucosa and transported back to the liver via the enterohepatic circulation. A small amount of conjugated bilirubin in the intestinal lumen is hydrolyzed to unconjugated bilirubin and subsequently is reabsorbed. The liver extracts most of the urobilinogen; however, a small amount spills over into the urine. Urobilinogen is concentrated in the normally alkaline urine of horses and thus is detectable.[22]

Detoxification

The liver is responsible for the *biotransformation* of numerous endogenous and exogenous compounds. Biotransformation involves a series of enzymatic reactions that alter the physical properties or activity of compounds. Biotransformation occurs in two phases.[23] In phase 1, polar groups are added to the compound or existing polar groups are exposed by oxidation, hydroxylation, deamination, or reduction. In phase 2, the product of phase 1 is conjugated, usually with glucuronate or sulfate. Substrates for detoxification usually are water insoluble, and biotransformation renders them more susceptible to renal or biliary excretion.[9,24] Examples of endogenous substances biotransformed by the liver include ammonia, bilirubin, and steroid hormones (estrogen, cortisol, aldosterone). The liver biotransforms countless exogenous substances (xenobiotics), including many drugs, plant toxins such as pyrrolizidine alkaloids, insecticides, and mercaptans.

Phase 1 of biotransformation occurs primarily on the enzyme-bound systems of the endoplasmic reticulum, called *microsomes.*[9,24] Most of these enzymes are iron-containing enzymes of the P-450 system, thus named because they absorb light at 450 nm. The P-450 enzymes are also called *mixed function oxidases.* Some substrates, referred to as *inducers,* are capable of saturating the enzymes involved in biotransformation. Enzyme saturation and induction causes hypertrophy of the endoplasmic reticulum and all contained enzymes, thus accelerating substance removal rates. Inducers not only accelerate their own removal rate but also may accelerate the biotransformation of other endogenous and exogenous substances. Examples of enzyme inducers are the barbiturates, phenylbutazone, and chlorinated hydrocarbons. Other agents presented for biotransformation, including chloramphenicol, cimetidine, organophosphates, morphine, and quinidine, may inhibit microsomal enzymes, thus prolonging the effect of other substrates. Hepatic biotransformation sometimes results in the formation of a toxic metabolite from a nontoxic parent compound, examples being aspirin and halothane.[24] Compared to adult horses, young horses may have less ability to metabolize aromatic hydrocarbons and less conjugative ability and may be at greater risk when exposed to xenobiotics requiring metabolism by these systems.[25] This might explain why we have seen more markedly increased hepatic enzymes in foals versus adult horses exposed to the same toxic pasture containing red fescue (authors' experience).

Mononuclear Phagocyte System

Hepatic macrophages, or Kupffer cells, make up a major portion of the mononuclear phagocyte system. Cells of the mononuclear phagocyte system are derived from bone marrow myeloid progenitors and serve two main functions: (1) phagocytosis and (2) to act as antigen-processing cells for lymphocytes. Kupffer cells respond to opsonins and synthesize a vast array of inflammatory mediators, including interleukins, tumor necrosis factor (TNF), and eicosanoids. Unlike other macrophages in the mononuclear phagocyte system, Kupffer cells function mainly in phagocytosis and are located strategically along the hepatic sinusoids, where portal blood can be cleansed, for example, of bacterial endotoxin, before exposure to the hepatocytes and subsequently the systemic circulation.[5] Kupffer cells also help cleanse systemic blood entering via the hepatic artery by removing *fibrin degradation products (FDPs),* tissue plasminogen activators, hemoglobin, microbes, foreign antigens, and other particulate debris. They also help in recycling iron from senescent or injured red blood cells, and, as a result, Kupffer cells accumulate hemosiderin and this can be pronounced even in disease-free horses.[26]

Miscellaneous Functions

The liver serves as a storage site for several vitamins and trace minerals, including vitamins A, D, and B_{12}; copper; zinc; and iron. Vitamin D is first converted in the liver to 25-hydroxycholecalciferol and exported to the kidney, where it is transformed into 1,25-dihydroxycholecalciferol, the active form of the vitamin.[15] In the fetus the liver is involved in *hematopoiesis.*[9] In the adult the bone marrow serves as the primary site for hematopoiesis; however, the liver may serve as an extramedullary site of hematopoiesis under intense conditions of erythrocyte regeneration or if a large portion of the bone marrow is destroyed.

HEPATIC DISEASE AND HEPATIC INSUFFICIENCY

Definition

Hepatic disease is any type of damage (mild to severe and focal to diffuse) to the liver. Disease is best detected by increases in the serum of liver derived enzymes. *Hepatic insufficiency* or failure refers to the inability of the liver to perform its normal functions properly and is detected by both clinical signs and laboratory test of hepatic dysfunction. Because the liver is involved in such a diverse array of physiologic activities, any pathologic process may hinder one or several functions without impeding others. Furthermore, most hepatic functions are not impaired until greater than 80% of the hepatic mass is lost.[9,20,22] The liver also has the capability to regenerate under certain conditions. Although hepatocytes are fully differentiated cells, they are capable of active proliferation by self-duplication in response to acute external stimulation and upregulation of humoral factors, all without any contribution

from hepatic stem or progenitor cells.[27-29] However, in the chronically injured liver hepatocytes do not proliferate as well or as uniformly, and biliary cells commonly proliferate and form ductules around the portal veins. This biliary cell proliferation associated with chronic injury may contain progenitor cells, which can give rise to both hepatocyte and biliary cells in an attempt to repair the liver.[27,30]

If hepatocyte loss is gradual and regeneration parallels destruction, hepatic failure does not necessarily ensue. Thus hepatic disease may be present without accompanying hepatic failure. Consequently, hepatic disease does not always manifest clinically.

Patterns and Pathology of Hepatic Injury

The severity of the accompanying clinical signs and the course of hepatic disease vary depending on the pattern, location, rate, and extent of hepatic damage. The disease may be predominantly biliary or hepatocellular in origin or may affect both parts of the functional unit equally. This determination can sometimes be detected by degree of elevation in liver-derived enzymes in the serum. Hepatocyte injury may be reversible (fatty degeneration, cloudy swelling) or irreversible (necrosis), focal or zonal, generalized, acute, chronic, inflammatory, anatomic, or functional.

Acute Focal or Multifocal Hepatic Injury

Focal hepatic injury occurs with uniform damage to one small area of the liver. Examples of focal hepatic injury include hepatic abscesses, solitary infarctions, and neoplastic growths. Because adequate hepatic reserve exists in the unaffected regions, clinical signs of hepatic failure rarely accompany focal hepatic injury, although evidence of hepatic disease may be demonstrable.[23] Acute multifocal hepatic injury is more likely to result in clinically significant hepatic disease. Acute hepatic injury may be degenerative, infiltrative (fat or neoplasia), necrotizing, apoptotic, or inflammatory. Hepatic *degeneration* refers to a toxic or immunologic insult that causes hepatocytes to swell and take on an edematous appearance.[31] *Ballooning degeneration* is used to describe irregularly clumped cytoplasm with large clear areas.[14] If biliary material has been retained, the hepatocytes appear foamy and swollen *(foamy degeneration). Ischemic coagulative necrosis* refers to poorly stained and mummified hepatocytes with lysed nuclei, whereas *lytic necrosis* describes osmotically swollen and ruptured cells.[31] Necrosis of contiguous hepatocytes that spans adjacent lobules in a portal to portal, portal to central, or central to central fashion is called *bridging necrosis.*[14] In apoptosis, there is no disintegration of the cell membrane (wall); rather there is shrinkage of cellular contents along with clumping of the membrane. This leads to formation of condensed bodies known as *apoptotic bodies*. Necrotic and apoptotic modes of cell death are often intermingled as a continuous process during liver injury.[32]

Hepatic injury may be zonal—that is, affecting certain zones of the liver uniformly throughout the entire organ.[23] The liver often appears pale with an enhanced lobular pattern on the cut surface. The two most common types of zonal hepatic injuries are centrilobular and periacinar. In *centrilobular zonal injury,* the area adjacent to the central veins (zone III) is uniformly affected, whereas in *periacinar* or *(paracentral) zonal injury,* cellular degeneration involves only a wedge around the central vein (see Fig. 13.1). Hepatocytes in these locations are most susceptible to anoxic damage, because the normal oxygen tension is lowest and mixed function oxidase activity is the greatest in these areas. Examples of disease states resulting in centrilobular injury are severe acute anemia, passive congestion caused by congestive heart failure *(nutmeg liver),* and some toxic hepatopathies (e.g., pyrrolizidine alkaloids). Periportal (zone I acinar lobular) injury may occur with infarction of hepatic vessels, as may occur during verminous arteritis, or exposure to gut-derived toxins that do not require metabolism by mixed function oxidases (e.g., phosphorus).

Acute Generalized Hepatic Injury

Acute generalized hepatic injury is often accompanied by clinical signs of hepatic failure, with the extent of damage dictating the severity of the clinical signs.[23] Typically, the liver appears pale and enlarged and is often friable. Acute generalized hepatic injury may be the result of infection, necrosis, apoptosis, inflammation, or hepatotoxic agents.[9] Bacterial or viral infections, parasitic infestations, or immune disorders may cause acute generalized necrosis or inflammation. Despite its cause, any process that results in an inflammatory response in the hepatic parenchyma is referred to as *hepatitis.* Acute inflammation most commonly accompanies necrosis and is characterized by the presence of neutrophils and lymphocytes in the areas of cell death or surrounding portal triads. An inflammatory process primarily involving the biliary system is called *cholangitis,* usually the result of ascending infection from the intestinal tract or after cholestasis.

Chronic Generalized Hepatic Injury

Chronic hepatic injury is accompanied by clinical signs of hepatic failure when greater than 80% of the hepatic mass is destroyed or replaced by fibrosis.[9,20] Fibrosis, the presence of collagen and fibroblasts, occurs when the rate of ongoing necrosis exceeds the rate of regeneration. Following injury, hepatic fibrosis is believed to be promoted predominantly by hepatic stellate cells (also known as perisinusoidal or Ito cells).[5,7,27] These stellate-shaped cells are normally quiescent and are present in relatively small numbers containing intracellular lipids that serve to store vitamin A as retinal ester. However, when the liver is damaged, nearby Kupffer cells produce tumor necrosis factor–α (TNF-α), causing the stellate cells to contract individually but proliferate in numbers. These activated cells become chemotactic, decrease their vitamin A stores, secrete collagen (thus causing fibrosis), and eventually become senescent.[10,27]

Typically, the liver appears smaller than normal although on occasion it may be enlarged. Fibrosis commonly follows conditions resulting in chronic hypoxia, chronic inflammation, chronic cholangitis or cholestasis, metastatic neoplasia, trauma, or ingestion of antimitotic agents such as plants containing pyrrolizidine alkaloids. *Cirrhosis,* or an *end-stage liver disease,* refers to chronic hepatic disease characterized by the presence of widespread fibrosis, nodular regeneration, and biliary hyperplasia.[23] *Nodular regeneration,* or islands of hepatocytes, occurs when the normal architecture and blood supply of the liver are disrupted or destroyed by the presence of fibrosis. *Bridging fibrosis* implies fibrosis that extends from one portal area to another or from portal areas to central areas.[31] The cause of biliary hyperplasia during chronic liver disease is unknown. One form of chronic hepatic disease, called *chronic active hepatitis* (CAH), is characterized by the presence of cirrhosis plus an acute inflammatory response.[23]

BOX 13.1 Clinical Signs of Liver Disease

COMMON SIGNS

Depression
Anorexia
Colic
Hepatic encephalopathy (HE)
Weight loss
Icterus
Pigmenturia (yellow-brown with bilirubinuria; red-brown with hemoglobinuria)

LESS COMMON SIGNS

Photosensitization
Diarrhea
Bilateral laryngeal paralysis
Bleeding
Ascites
Dependent edema

RARE SIGNS

Steatorrhea
Tenesmus
Generalized seborrhea
Pruritus
Endotoxic shock
Polydipsia

Anatomic or Functional Injury

Anatomic or functional shunts cause liver injury by anoxic damage. Additionally, if blood habitually bypasses the liver, the liver cannot perform its normal metabolic regulatory or detoxifying functions; thus clinical signs of hepatic failure become imminent. Anatomic shunts can be either congenital or acquired, intrahepatic or extrahepatic.

Clinical Signs of Hepatic Insufficiency

The clinical signs of hepatic insufficiency are highly variable, are nonspecific, and depend on the extent and duration of hepatic disease (Box 13.1). Usually, greater than 80% of the liver mass must be lost or obstruction of the common bile duct occurs before clinical signs become apparent, regardless of the cause of hepatic disease. Thus despite the duration of hepatic disease, the onset of clinical signs is often abrupt. The most common clinical signs of hepatic insufficiency in horses are depression, anorexia, colic, hepatic encephalopathy (HE), weight loss, orange-colored urine, and icterus.[24,32-34] Less commonly reported clinical signs include hepatogenic photosensitization, diarrhea, abdominal pain, bilateral laryngeal paralysis, and hemorrhagic diathesis. Rarely reported clinical signs of hepatic insufficiency in horses are ascites, dependent abdominal edema, steatorrhea, tenesmus, generalized seborrhea, pruritus, endotoxic shock, polydipsia, and hemolysis. The appearance of specific clinical signs of hepatic disease often reflects the type of hepatic function (or functions) that is altered.

Hepatic Encephalopathy

HE is a complex clinical syndrome characterized by abnormal mental status that accompanies severe hepatic insufficiency.[35-37] Clinical signs are widely variable but represent manifestations of augmented neuronal inhibition. This syndrome occurs in patients with advanced decompensated liver disease of all types or hepatic shunts and may be a feature of acute, subacute, or chronic hepatocellular disease. HE generally is considered to be a potentially reversible metabolic encephalopathy.[36] Whether multiple episodes of HE could lead to irreversible neuronal damage is uncertain.

TABLE 13.1 Clinical Stages of Hepatic Encephalopathy

Stage	Mental Status
I	Mild confusion, decreased attention, slowed ability to perform mental tasks, irritability
II	Drowsiness, lethargy, obvious personality changes, inappropriate behavior, disorientation
III	Somnolent but rousable, marked confusion, amnesia, occasional aggressive uncontrolled behavior
IV	Coma

Adapted from Gammel SH, Jones EA: Hepatic encephalopathy, *Med Clin North Am*. 1989;73:793–813.

Clinical Signs. No specific features of HE allow this syndrome to be distinguished from other causes of cerebral dysfunction. The clinical signs of HE in horses may be mild with only depression, anorexia, and frequent yawning. In other cases the signs can be fulminant and include head pressing, blindness, circling, and coma. Ataxia can be noticeable in some cases but absent in others. Rarely, ataxia may precede HE signs or icterus in acute liver failure.[38,39] Clinical examination may reveal decreased muscle tone of the lower lips, delayed or absent response to touching the inner nares, and cortical blindness often accompanied by mydriasis. These signs are all a result of the cortical disease that occurs with HE. Laryngeal paralysis or dysphagia (presumably due to HE and functional disturbance in the nucleus ambiguous) can be signs of HE.[40] The earliest phase of HE is probably missed in most equine patients because it represents minimal behavioral changes with subtle impairment of intellect because of bilateral forebrain dysfunction[41] (stage I; Table 13.1). In humans, these early signs are more apparent to close friends and family members than to a physician. As encephalopathy progresses, motor function, intellectual abilities, and consciousness become impaired; generally at this stage (corresponding to stage II) horses become obviously affected. Clinical signs include depression, head pressing, circling, mild ataxia, aimless walking, persistent yawning, and other manifestations of inappropriate behavior. Somnolence develops. Next, the horse is rousable but responds minimally or excessively to the usual stimuli. At this stage (III) the horse often manifests aggressive or violent behavior interspersed with periods of stupor. Finally, consciousness fades, the horse becomes recumbent, and coma ensues. Occasionally seizures occur during the later stages of HE, but in general they are atypical. The severity of encephalopathy corresponds to the degree of hepatic dysfunction; however, neither of these parameters correlates with type or reversibility of the underlying hepatic disease.

Cause and Pathophysiology. By definition, the cause of HE is insufficient hepatocellular function, irrespective of the cause of the liver disease. That is, an adequately functioning liver is necessary to maintain normal brain neuron and astrocyte function. With acute liver disease, HE is associated with astrocyte swelling, acute cytotoxic cerebral edema, and intracranial hypertension. In chronic hepatic disease, HE may develop more insidiously or may have peracute onset.

Astrocytes, the star-shaped glial cells in the brain and spinal cord, are swollen but also show evidence of Alzheimer type II changes, which include enlarged nuclei and lack of cytoplasm.[41] In the equine, Alzheimer type II cells can develop rapidly (less than 2 days). The pathophysiology of HE is complex and likely involves several gut-derived neurotoxins, cerebral and systemic inflammation, cerebrovascular dysfunction, and neuroendocrine abnormalities.[42,43] The following mechanisms have been suggested for the development of HE, and any or all may be involved to greater or lesser degree:

1. Gastrointestinal-derived neurotoxins. High concentrations of blood and cerebrospinal fluid (CSF) ammonia have been most commonly incriminated as causing the pathophysiologic events of HE.[44]
2. Augmented activity of γ-aminobutyric acid (GABA) in the brain.
3. Altered expression of benzodiazepine receptors.
4. Increased neurosteroid synthesis.
5. False neurotransmitter accumulation after plasma amino acid imbalance.
6. Increased blood manganese concentrations.
7. Increased inflammatory mediator expression, principally cytokines.
8. Increased permeability of the blood-brain barrier and cerebral hypertension
9. Impaired central nervous system (CNS) energy metabolism.

Perhaps the oldest and most "predominant" hypothesis for HE involves the accumulation of toxic materials in the blood (derived from the metabolism of nitrogenous substrates in the gastrointestinal tract) that bypass the liver through functional or anatomic shunts.[35,41,45] Accordingly, HE may be caused primarily by failure of the liver to adequately remove certain substances from the blood that have the direct or indirect ability to modulate function of the CNS. Ammonia, produced after the degradation of amino acids, amines, and purines by enteric bacteria, has been supported widely as the major neurotoxin of hepatic disease.[45-47] In patients with liver failure ammonia is insufficiently metabolized through the urea cycle; thus plasma concentrations increase and ammonia enters the CNS, where it may cause encephalopathy.[45,48] Evidence also points to zinc deficiency, which is important for the urea cycle, in the pathogenesis of HE.[49]

Ammonia (NH_3, unionized and a weak base) is toxic at high concentrations and believed to easily pass the blood-brain barrier by diffusion while the relatively nontoxic ionized ammonia (NH_4^+) is limited to a transcellular route for translocation.[44] Ammonia has a toxic effect on cell membrane neurons by inhibition of the Na,K-dependent adenosine triphosphatase activity in nerve cell membranes, causing depletion of adenosine triphosphate.[47,49] Hyperammonemia also is associated with a disturbance in CNS energy production caused by alterations in the tricarboxylic acid cycle that result in a decrease in α-ketoglutarate formation and increased synthesis of glutamine.[50] Astrocytes in the brain also detoxify ammonia by synthesizing glutamine through amidation of glutamate. Glutamine accumulation in astrocytes is a major cause of cell swelling and generation of cerebral edema in acute fulminate hepatic failure.[46,49] Another effect of prolonged neuronal exposure to ammonia is alteration of glutamatergic function. Extracellular glutamate may be increased as a result of increased release at the neuronal presynaptic junction or because of decreased uptake in the glutamine and ammonia-saturated glial cells. Because glutamate is the major excitatory neurotransmitter in the mammalian brain, in acute liver failure increased synaptic release of glutamate results in overactivation of glutamate receptors with resultant hyperexcitatory clinical signs. In chronic hepatic failure, downregulation of glutamate receptor activity or increase in GABA activity (see later discussion) likely contributes to the decreased excitatory transmission in HE. Hyperammonemia also induces nitric oxide and reactive oxygen generation, which leads to accumulation of peroxides, oxidative stress, and nerve cell damage. These cumulative effects of ammonia on neural tissue play an important role in the pathogenesis of HE. Experimentally, ammonia can induce encephalopathy[51]; because of congenital enzyme deficiencies, children with hyperammonemia have encephalopathy and horses with primary hyperammonemia (without liver dysfunction) have encephalopathy.[52-54] Furthermore, therapy aimed at reducing the absorption of ammonia from the intestine tends to ameliorate HE.[41] Although older clinical studies argued against the role of ammonia in the pathogenesis of HE because plasma ammonia concentrations correlated poorly with the severity of HE,[36,55] some more recent reviews demonstrate that blood ammonia levels correlate strongly with the severity of HE.[42-44] Thus the actions of ammonia on the CNS are complex, and ammonia likely is involved in, but is not solely responsible for, HE.

The synergistic neurotoxin hypothesis for the pathogenesis of HE implicates not only ammonia but also other gut-derived neurotoxins, specifically mercaptans, short-chain fatty acids, oxindole, and phenols.[41] Members of each of these classes of substances are increased in the blood of patients with hepatic failure in concentrations that alone are insufficient to induce encephalopathy. However, the combination of some or all of them may induce encephalopathy by their synergistic actions and by augmenting endogenous metabolic abnormalities,[41] mostly centering around inhibition of brain Na^+,K^+-ATPase with subsequent impaired neurotransmission.[36] As with ammonia, blood and brain concentrations of mercaptans correlate poorly with the stage of HE.[56]

Another popular theory regarding the pathogenesis of HE involves augmented activity of inhibitory neurotransmitter systems, GABA-benzodiazepine and serotonin, and depression of the function of the excitatory glutamatergic system.[49] Hyperammonemia has been shown to increase GABAergic tone in patients with liver disease.[46] When released from presynaptic neurons, GABA binds to specific receptors on postsynaptic neurons, resulting in increased chloride ion conductance across the postsynaptic neural membrane, membrane hyperpolarization, and generation of an inhibitory postsynaptic potential.[57,58] The GABA receptor is a chloride ionophore supramolecular complex that has interactive binding sites for three classes of synergistic ligands: (1) GABA and agonists, (2) benzodiazepines, and (3) barbiturates.[36,59] The binding of benzodiazepine or a barbiturate on the binding site of the GABA receptor potentiates GABA-induced sedation. The GABA hypothesis of HE originally was based on a series of observations using a model of HE in rabbits in which increased GABA-like activity was identified in the serum and CSF along with an increased density of GABA receptors.[57]

Increased brain levels of natural benzodiazepines probably constitute one mechanism for the increased GABAergic tone in HE.[49] Ammonia, which acts synergistically with natural

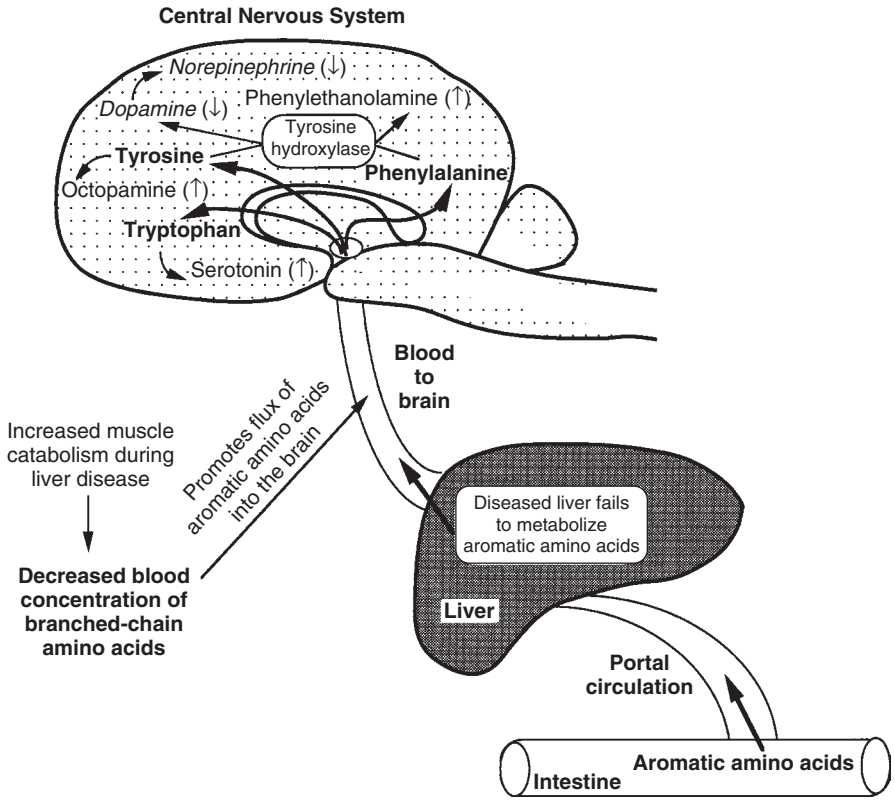


FIG. **13.6** Role of aromatic amino acids in the brain in hepatic encephalopathy. Aromatic amino acids (tryptophan, tyrosine, phenylalanine) enter the central nervous system, where they are metabolized, altering the balance of neurotransmitters. True neurotransmitters (dopamine, norepinephrine) decrease *(arrow down),* and "false" neurotransmitters (octopamine, phenylethanolamine) increase *(arrow up).* The net effect is increased neuronal inhibition and reduced neuronal excitation.

benzodiazepines, also may enhance GABAergic neurotransmission. Agonists of benzodiazepine increase the frequency of GABA-induced chloride channel openings, and barbiturates lengthen the average time that channels are open.[60] Results of studies using electrophysiologic and in vitro techniques to study an animal model of HE provide strong evidence for a functional increase in GABAergic tone that is mediated allosterically through the benzodiazepine receptor by an endogenous diazepam-like substance.[61,62] Clinical studies that showed improved consciousness and reduced electroencephalogram changes of HE in patients treated with the benzodiazepine receptor antagonist flumazenil support this suggestion.[63,64] Neurosteroids also may affect GABAergic tone through benzodiazepine receptors. Activation of benzodiazepam receptors in liver failure facilitates the entry of cholesterol into astrocyte mitochondria, where synthesis of neurosteroids such as tetrahydroprogesterone and tetrahydrodeoxycorticosterone occurs. These substances are potent positive allosteric modulators of GABA receptors that can induce profound sedation and behavioral changes.[65]

A separate and perhaps outdated hypothesis holds that during liver failure, true neurotransmitters in the CNS such as norepinephrine and dopamine become depleted and that false neurotransmitters, especially octopamine and phenylethanolamine, increase.[37,66] The net neurophysiologic effect of such changes is reduced neuronal excitation and increased neural inhibition. The mechanism of this effect is related to the increased serum concentrations of aromatic amino acids (AAAs; phenylalanine, tyrosine, tryptophan) and decreased concentrations of branched-chain amino acids (BCAAs; valine, leucine, isoleucine) that occur in liver failure.[67,68] Serum glucagon increases in hepatic failure, leading to muscle catabolism and release of amino acids. However, hepatic metabolism of AAAs is reduced, and because BCAAs are metabolized by muscle and adipose tissue, a relative increase in AAAs and a decrease in BCAAs occurs. The decreased plasma BCAA-to-AAA ratio during liver failure and increased brain glutamine concentration (presumably a consequence of increased ammonia metabolism) are considered to promote an influx of AAA into the brain and an efflux of glutamine from the brain by exchange transport processes at the blood-brain barrier.[36] Phenylalanine can compete with tyrosine for tyrosine hydroxylase, resulting in decreased production of dopamine[68] (Fig. 13.6). The displaced tyrosine may be decarboxylated to tyramine and then converted to the false neurotransmitter octopamine. Accumulated tyrosine also competes for dopamine β-oxidase and reduces the formation of norepinephrine. Phenylalanine and tryptophan in the CNS are ultimately converted to phenylethanolamine and serotonin, a false neurotransmitter and a neuroinhibitor, respectively. Tryptophan is also metabolized to serotonin and oxindole, which has a strong sedative effect.[49]

Consistent with this theory are the observations of increased serum concentrations of AAAs accompanied by increased CSF concentrations of octopamine, serotonin, and phenylethanolamine in patients with HE.[66] However,

octopamine alone cannot induce encephalopathy, and the plasma BCAA-to-AAA ratio is expected in most cases of severe liver disease and correlates poorly with HE in human beings.[69] Controlled clinical trials of oral or intravenous BCAA therapy demonstrate inconsistent amelioration of signs of HE.[68,70-72]

Manganese is a trace element that is excreted by the liver. Patients with chronic liver disease have increased blood and brain concentrations of manganese. Chronic exposure to manganese causes neuronal and glial loss and Alzheimer type II changes in astrocytes.[73]

A recent hypothesis on the pathophysiology of HE centers on the role of inflammatory mediators in brain injury. Central to this hypothesis is the fact that human patients with acute liver failure are more likely to progress to severe HE if they concurrently have evidence of a systemic inflammatory response.[38] A variety of stimuli are capable of inducing astrocytes and microglial cells in the brain, as well as peripherally located mononuclear cells, to release inflammatory mediators. Breakdown products of injured hepatocytes, as well as increases in systemic inflammatory cytokine production, free radicals, and metalloproteinases in liver failure, have severe systemic effects in addition to increasing permeability of the BBB.[38] The toxic effect of these events on the brain with HE is believed to result in both cytotoxic (intracellular swelling without increased permeability of BBB) and vasogenic (increased permeability of BBB with net gain of fluid) brain edema.[42,74,75] Several studies have demonstrated significantly increased levels of the cytokine TNF-α in patients with both acute and chronic liver disease. Furthermore, TNF-α concentrations correlate with the severity of HE and prognosis, and therapeutic strategies aimed at reducing TNF-α levels ameliorate HE.[76] Several key mechanisms whereby increased TNF-α concentrations have been shown to be involved in the pathogenesis of HE include increasing ammonia diffusion into the brain, inhibition of astrocyte uptake of glutamate and inhibition of glutamate synthetase, increased expression of benzodiazepine receptors in the brain, and increasing brain capillary fluid leakage and cerebral edema formation. It has also been demonstrated that excess manganese potentiates the production of TNF-α in microglia.[76]

Finally, alterations in cerebral blood flow and blood-brain barrier permeability leading to cerebral hypertension and edema and changes in cerebral metabolism are present in patients with HE. Although the mechanisms responsible for these changes are not fully known, changes in astrocyte function, either directly or indirectly by increased blood ammonia concentrations, are likely to be involved in the cytotoxic edema.[46]

Diagnosis. The diagnosis of HE is based on the presence of neurologic signs of cerebral dysfunction in a horse, with physical examination and laboratory findings compatible with liver disease (see Diagnosis and Laboratory Findings of Hepatic Disease and Insufficiency). Other possible causes for the neurologic signs should be excluded because no specific features of HE allow one to distinguish this syndrome definitively from other encephalopathies. A partial list of conditions to be ruled out includes primary hyperammonemia, trauma, viral encephalomyelitis, rabies, moldy corn toxicity (leukoencephalomalacia), brain abscess, equine protozoal myeloencephalitis, parasite larval migrans, blister beetle toxicosis, organophosphate toxicity, nigropallidal encephalomalacia, botulism, fluphenazine or other sedative overdose, uremic encephalopathy, and heavy metal toxicosis. Many of these conditions have other characteristic clinical signs, the absence of which would exclude them from the differential diagnosis. Access to potential toxins or drugs should be carefully gleaned from the history.

Serum electrolyte analysis, calcium and creatinine values, and a complete blood count may aid in ruling out other encephalopathies. Serology for the viral encephalitides and toxicologic screening for organophosphates and heavy metals may be appropriate. CSF analysis may be indicated if other causes of encephalopathy are highly suspect. The CSF is normal in horses with HE. In human beings, electroencephalogram changes of symmetric generalized slowing of cerebral electrical activity are sensitive indicators of HE; however, they are not specific for this disorder because other metabolic encephalopathies can cause similar abnormalities.[35,47] Visual evoked potentials are superior to conventional electroencephalogram in terms of specificity and ease of quantitation.[77] An average visual evoked potential reflects the pattern and magnitude of postsynaptic neuronal activity evoked by a visual afferent stimulus. Hepatoencephalopathy in humans is attended by a distinctively abnormal visual evoked potential trace; however, this testing would be technically difficult in horses and has not been explored. As a general rule, the brain may not show definite light or electron microscopic structural changes, although horses with hepatic failure and portosystemic shunts frequently have an increase in the number and size of astrocytes (Alzheimer type II) in the gray matter of the cerebrum and cerebellum.[36] These changes appear to be reversible and specific for portosystemic shunting of blood. The relevance of these changes, if any, to behavioral dysfunction of HE is unknown.

Icterus

Icterus, or *jaundice,* is caused by *hyperbilirubinemia* with subsequent deposition of the pigment in tissues causing yellow discoloration. Icterus is most apparent in nonpigmented skin such as mucous membranes, especially the vulvar mucosa and the sclerae. Approximately 10% to 15% of horses normally have slightly yellow sclerae.[24] Disease states that result in hyperbilirubinemia can be categorized as follows: increased production of bilirubin, impaired hepatic uptake or conjugation of bilirubin, and impaired excretion of bilirubin.[20] Increased production of bilirubin occurs with hemolysis, both intravascular and extravascular, and after reabsorption of erythrocytes after massive intracorporeal hemorrhage. This form of hyperbilirubinemia, often called *hemolytic* or *prehepatic icterus,* occurs despite a normally functioning liver, because the rate of bilirubin production by the reticuloendothelial system temporarily exceeds the ability of the liver to conjugate and excrete. Classically, this form of icterus is caused by the increased concentration of unconjugated bilirubin in the blood. However, on occasion, the concentration of conjugated bilirubin also mildly increases in the blood because of hepatic spillover when the liver processes the excessive bilirubin or the enterohepatic circulation reabsorbs it. The rate and extent of erythrocyte destruction and the rate of uptake of bilirubin determine the presence and intensity of hemolytic icterus by the liver.

Impaired uptake and conjugation of bilirubin also result in increased blood levels of unconjugated bilirubin with subsequent icterus. This form of icterus is referred to as *retention* or *hepatic icterus* and is the most common form in horses with liver disease, usually the result of acute hepatocellular

disease.[20,22] In horses the presence of icterus is highly inconsistent with chronic hepatocellular disease.[21,34] In addition to hepatocellular disease, certain drugs, anorexia, or prematurity can impede bilirubin uptake and conjugation by hepatocytes, despite an otherwise normally functioning liver.[9,20,22] Steroids can inhibit bilirubin uptake in all species. Heparin administration to horses sometimes results in icterus and is believed to be caused in part by impaired uptake of bilirubin by hepatocytes.[21] Anorexia in horses causes variable degrees of hyperbilirubinemia and may be related to the half-life of ligandin.[21,22] Ligandin is the intrahepatic protein responsible for extracting unconjugated bilirubin from albumin in the sinusoidal blood. The half-life of ligandin is relatively short (days), and starvation in other species reduces the store of ligandin in the hepatocytes, thus impeding bilirubin uptake.[9] Premature and neonatal foals are also more susceptible to retention icterus, in the absence of hepatic disease. The cause of icterus in equine neonates is presumably a result of lower hepatocellular ligandin concentrations, compared with the adult.[78] In human beings, inherited or congenital deficiencies in enzymes responsible for conjugation (bilirubin-uridine diphosphate glucuronyl transferase) may result in intermittent or persistent icterus (Gilbert's syndrome or Crigler-Najjar syndrome type II) that often is not recognized until the patient is several years of age. The icterus occurs without other clinical or laboratory evidence of liver disease.[22] Persistent hyperbilirubinemia without anorexia, hemolysis, or evidence of acquired liver disease was reported in an otherwise healthy 4-year-old Thoroughbred gelding and has been seen by the authors in several other horses.[79] Conjugated bilirubin concentrations were normal, but total serum bilirubin concentrations ranged from 9.0 to 12.3 mg/dL during a 2.5-year monitoring period. The case most closely resembled Gilbert's syndrome.[79]

If the excretion of conjugated bilirubin into the biliary tract is impeded, *regurgitation icterus* occurs.[20] Blockage of bile flow with resultant regurgitation icterus can accompany cholangitis, hepatitis, obstructive cholelithiasis, neoplastic infiltration, fibrosis, physical obstruction of the biliary tract associated with colon displacements or duodenal ulcers, or hyperplasia of the biliary tract. Because conjugated bilirubin is water soluble, this form of icterus may be accompanied by bilirubinuria and produce "greenish"-orange discoloration of the urine.

In hepatocellular disease, icterus most often is the result of a combined increase in both unconjugated and conjugated bilirubin.[20] Of the two fractions, the majority of the increase in the total bilirubin is from unconjugated bilirubin. Increases in the conjugated fraction less than 25% of the total are usually indicative of predominant hepatocellular disease, and increases greater than 30% are usually indicative of cholestasis.[22]

Weight Loss

Significant weight loss and failure to thrive are most consistently present during chronic hepatic insufficiency. However, chronic liver disease may be present without apparent weight loss. Weight loss is due to anorexia and the loss of normal hepatocellular metabolic activities.

Hepatogenic Photosensitization

Photosensitization refers to abnormally heightened reactivity of the skin to ultraviolet sunlight because of the increased blood concentration of a photodynamic agent. In the case of hepatogenic photosensitization, the photodynamic agent is *phylloerythrin*. Phylloerythrin is normally formed in the gastrointestinal tract as a result of bacterial degradation of chlorophyll and is absorbed into the general circulation, conjugated, and excreted by the liver. During hepatic insufficiency, the blood concentrations of this photodynamic agent are increased. Subsequent exposure of phylloerythrin to ultraviolet light causes activation of electrons within the molecule to an excited state, with resultant free radical formation. The local production of free radicals causes cell membrane damage and necrosis. Unpigmented areas most efficiently absorb ultraviolet light; thus the lesions of photosensitization are restricted to white skin. The skin first appears erythematous and edematous. Pruritus, pain, vesiculation, ulceration, necrosis, and sloughing may ensue. Mucous membranes may also be affected.[80]

Colic, Diarrhea, Tenesmus, Ascites, Steatorrhea

Abdominal pain associated with acute hepatocellular disease may be a result of an acute change in hepatic size (most commonly hepatic swelling) or biliary obstruction (cholelithiasis).[9,81] Signs of anterior abdominal pain include anorexia, bruxism, sitting like a dog, recumbency, and rolling up onto the dorsum. Palpation over the last few ribs (especially on the right) or immediately caudal to the last rib may elicit a pain response. Alternations in intestinal motility also may cause concurrent signs of colic with liver disease. McGorum et al.[34] reported that 10 of 25 horses with liver disease and signs of colic had clinically significant gastric impactions. Of the horses with gastric impactions, seven also had signs of HE; none of these horses survived.

Diarrhea infrequently may accompany chronic hepatic insufficiency in horses. Alterations in the intestinal microflora, portal hypertension, intestinal hyperemia, and deficiency of bile acids may be involved in the pathogenesis.[9] Though uncommon in horses, portal hypertension can lead to increased hydrostatic and oncotic pressure in the intestinal mucosa, with resultant water and protein loss into the lumen of the bowel and the peritoneal cavity (ascites). Tenesmus may be the result of constipation but has been rarely reported to be a sign of hepatoencephalopathy in horses.[34]

Decreased excretion of bile may result in lipid malabsorption and excessive amounts of fat in the feces, or *steatorrhea,*[9] which subsequently may cause osmotic diarrhea. Because the normal equine diet is low in fat, steatorrhea is rare in horses. Chronic cholestasis may cause clay-colored feces because of lack of fecal urobilinogen and stercobilin. This coloration rarely is observed in adult herbivores, because the normal fecal color is generated primarily by plant chlorophylls and not by bilirubin metabolites.[22]

Hemorrhagic Diathesis

Because the liver is responsible for the synthesis of numerous factors involved in coagulation and fibrinolysis, abnormal hemostasis may be a sequela to hepatic insufficiency. Clinical signs may vary from petechial or ecchymotic hemorrhages to hemorrhage after trauma or venipuncture to spontaneous hemorrhage (epistaxis, melena, hemoptysis, hematuria, or hematomas).[9,20,22,41,82] Especially sensitive to hepatic disease is the synthesis of fibrinogen and the vitamin K–dependent factors (II, VII, IX, X, and protein C), which have short half-lives. Factor VII has a half-life of only 4 to 5 hours. Other vitamin K–dependent factors and fibrinogen have half-lives

in the range of 4 to 5 days. Because vitamin K is fat soluble and requires bile acids for proper absorption from the intestinal tract, vitamin K–dependent factors are affected particularly during hepatic insufficiency when bile excretion is decreased.

During hepatic insufficiency, the synthesis of protein C and antithrombin III may be altered. Decreased plasma concentrations of these two anticoagulants would result in uncontrolled clot formation and consumption of other coagulation factors. In chronic hepatic disease the plasma concentration of protein C is normal or decreased; however, antithrombin III may be normal, increased, or decreased.[41] Pregnant women with fatty livers have decreased antithrombin III activity, but patients with biliary cirrhosis or biliary obstruction have increased antithrombin III activity.[41] Horses with liver disease have increased antithrombin III activity and theoretically should tend to bleed.[83] Alterations in the factors controlling fibrinolysis vary in chronic liver disease.[41] Conditions that promote fibrinolysis—such as increased plasminogen and plasminogen activator, or decreased plasminogen activator inhibitor, α_2-antiplasmin, and α_2-macroglobulin—result in bleeding tendencies. Conditions that aid thrombus formation, such as decreased plasminogen, further promote consumptive coagulopathy. The fibrinolytic factors have not been evaluated in horses with liver disease.

Finally, the liver plays an important role in balancing normal hemostasis by Kupffer cell removal of activated coagulation factors and FDPs from the general circulation.[41] Failure to remove activated coagulation factors further promotes coagulation, and FDPs interfere with platelet function and fibrin clot formation.

Fever

Horses with hepatic abscesses, acute bacterial hepatitis, CAH, obstructive cholelithiasis, fatty liver failure, or neoplasia may have constant or intermittent fevers.[81,84-89]

Hemolysis

Hemolysis is a rarely seen but grave prognostic indicator of fulminant hepatic failure in horses.[32] The exact cause of hemolysis is not known but is believed to be the result of increased erythrocyte fragility or destruction of erythrocytes as they pass through the damaged liver.

Pruritus and Seborrhea

Retention of bile acids and accumulation in the skin may cause pruritus and seborrhea. This finding is rarely reported in horses.[32] A hepatocutaneous syndrome affecting the hoof wall and coronary bands has been discussed in horses, but documentation is rare.[90]

Edema

Hypoalbuminemia and water retention can occur with chronic liver failure and may result in dependent edema. Because the half-life of albumin is long (19 to 20 days) in the horse, edema is a rare clinical sign.[91] Ponies with hyperlipemia may develop dependent abdominal edema after vascular thrombosis[88] or as a result of increased blood flow and hydrostatic pressure in the subcutaneous abdominal vein caused by partial obstruction of the caudal vena cava by the rapidly enlarged liver. Dependent abdominal edema also may form if significant portal hypertension and ascites exist.

Endotoxemia

Kupffer cells are specialized macrophages that are part of the reticuloendothelial system. The Kupffer cell plays an important role in removing bacterial endotoxin that is normally absorbed from the lumen of the intestinal tract and carried to the liver via the portal circulation.[9] Failure of Kupffer cell phagocytosis of endotoxin may result in clinical and laboratory evidence of endotoxemia. Kupffer cells also help in recycling iron from senescent or injured red blood cells and, as a result, they accumulate hemosiderin, and this can be pronounced even in disease-free horses.[22]

Polydipsia, Polyuria, and the Hepatorenal Syndrome

Alterations in renal function, including deranged sodium concentrations, impaired water excretion, and urine-concentrating ability, may accompany severe liver disease.[9,41] Sodium retention results from increased blood aldosterone concentrations because of failure of hepatic biotransformation and a decrease in the effective circulating blood volume as a result of portal hypertension and hypoalbuminemia. Sodium retention raises the osmolality of the extracellular fluid, thereby stimulating the thirst center. Polydipsia has been reported in horses with chronic liver disease.[92,93] Despite the potential increase in exchangeable sodium, the serum sodium concentration is usually normal or decreased, as a result of superimposed water retention. The mechanism for water retention is multifactorial, but increased antidiuretic hormone, reduced effective circulating volume, and altered renal prostaglandin synthesis are most likely involved.[41] Sometimes the urine-concentrating ability is impaired because of reduced medullary interstitial urea, the net effect being polyuria, isosthenuria, or both.

Hepatorenal syndrome is characterized by acute azotemia and anuria and may occur in ponies with hyperlipemia and hepatic lipidosis (see Hyperlipemia and Hepatic Lipidosis later in this chapter). The pathogenesis is obscure, but speculative causes include reduced effective circulating volume, decreased hepatic inactivation of renin, and endotoxemia.[41]

Diagnosis and Laboratory Findings of Hepatic Disease and Insufficiency

Historical information (as discussed under Specific Hepatic Diseases later in this chapter) may be useful in the diagnosis of certain types of hepatic insufficiencies. Nonspecific clinical signs and variable laboratory findings confound the definitive diagnosis of hepatic disease in horses. Hepatic testing can also be divided into disease test (enzymes) and function test (bile acids, conjugated bilirubin, ammonia, partial thromboplastin time, prothrombin time). Hepatic-derived enzymes measured in serum or plasma can be divided into those that leak from hepatocytes (sorbitol dehydrogenase, aspartate aminotransferase, glutamate dehydrogenase) and those that come from biliary epithelium (γ-glutamyl transferase). Comparison of elevations in hepatocellular enzyme with biliary-derived enzymes can often be helpful in anatomic and etiologic diagnosis of liver disease. Paramount to the laboratory diagnosis of hepatic insufficiency in horses is knowledge of the sensitivity and specificity of the tests (Table 13.2). The magnitude of elevation of hepatic-derived enzymes may not correspond to functional abnormalities and should therefore be considered *tests that measure disease and not tests that*

TABLE **13.2 Clinical Pathology of Liver Disease in Horses**

Test	Aberration	Normal Value
SPECIFIC INDICATORS OF LIVER DISEASE		
Serum sorbitol dehydrogenase (SDH)	Increases	<8 U/L
Serum γ-glutamyl-transferase (GGT)	Increases	<25 U/L
Serum bile acids concentration	Increases	<15 μmol/L
Arginase (ARG)	Increases	
Glutamate dehydrogenase (GLDH)	Increases	<11 U/L
Direct bilirubin	>25% of total	0–0.4 mg/dL (0-6.8 μmol/L)
Ammonia	Increases	(Laboratory dependent)
Bromsulphthalein (BSP) half-life	Prolonged	2.8 ± 0.5 min
Branched chain: aromatic amino acid ratio	Decreases	3.5–4.5
Urine bilirubin	Increases	
NONSPECIFIC INDICATORS OF LIVER DISEASE		
Total bilirubin	Increases	
Indirect bilirubin	Increases	
Lactate dehydrogenase-5 (LDH-5)	Increases	
Aspartate aminotransferase (AST)	Increases	
Alanine aminotransferase (ALT)	Increases	
Alkaline phosphatase (ALP)	Increases	
Blood urea nitrogen (BUN)	Decreases	
Globulins	Increase	
Albumin	Decreases	
Glucose	Decreases	
Prothrombin time (PT)	Prolonged	
Activated partial thromboplastin time (APTT)	Prolonged	
Triglyceride	Increases	
White blood cell count	Increases with infection or inflammation; decreases with endotoxemia	

measure function. Because massive hepatic disease must be present before alterations are seen with some function tests, and because different liver functions are variably altered by disease, the specificity of the laboratory diagnosis of hepatic disease increases with the magnitude of abnormal findings. Laboratory findings also may be useful for therapeutic and prognostic considerations.

Evaluation of Bilirubin

Serum bilirubin concentration is not a sensitive indicator of liver disease in horses. In one report on serum biochemical changes in horses with liver disease, only one fourth of the cases had increased serum bilirubin concentrations.[34] The *total bilirubin* concentration in the blood, as determined by the van den Bergh test, is a combination of both unconjugated and conjugated bilirubin. Because the diagnostic value of the bilirubin concentration, when used for evaluation of hepatic disease, depends on which subfraction is increased, the concentrations of both unconjugated and conjugated bilirubin must be determined.

The serum bilirubin concentration is stable for several days if the sample is protected against sunlight.[22] The total bilirubin concentration is determined first in a chromogenic assay by reaction for 30 minutes with a diazo reagent (sulfanilic acid and sodium nitrite) and methyl alcohol.[22] *Conjugated* or *direct-reacting bilirubin* is similarly determined over 5 minutes, without the addition of methyl alcohol. The amount of *unconjugated bilirubin* can then be determined by the difference between the total bilirubin concentration and the direct-reacting bilirubin. Because the unconjugated fraction is determined arithmetically, it is appropriately called *indirect-reacting bilirubin.* In normal horses, the total bilirubin concentration is in the range of 0.2 to 5.0 mg/dL (3.4–85.5 μmol/L) with conjugated bilirubin in the range from 0.0 to 0.4 mg/dL (0–6.8 μmol/L).[20] As discussed previously, increases in the unconjugated bilirubin fraction may occur without hepatic disease. Hemolysis, anorexia, intestinal obstruction, cardiac insufficiency, Gilbert's syndrome, and the administration of certain drugs (steroids, heparin, halothane) may cause an increase in the unconjugated bilirubin concentration.[21] If the unconjugated bilirubin concentration is increased, the erythron should be concurrently evaluated to rule out hemolysis as the causative factor. Hemolysis may cause the unconjugated bilirubin concentration to rise as high as 80 mg/dL (1368 μmol/L).[22] Complete anorexia can cause an increase in the unconjugated bilirubin concentration within 12 hours; however, it is unlikely to rise greater than 6 to 8 mg/dL (102.6–136.8 μmol/L) in horses suffering purely from anorexia,[22] although values as high as 10.5 mg/dL have been reported in anorexic horses.[94] In experimental horses withheld from feed the bilirubin increase did not occur for 72 hours.[30] Finally, the age of the horse and concurrent drug therapy must be considered. Neonates normally have more unconjugated bilirubin than adults. The higher bilirubin concentration in foals most likely is caused by the turnover of fetal hemoglobin to adult hemoglobin and the deficiency of liver-binding and conjugating enzymes compared with the adult.[78] In the absence of liver disease, prematurity or illness in foals can further increase the unconjugated bilirubin fraction.

Keeping the foregoing limitations of interpretation in mind, increases in the unconjugated bilirubin fraction in horses with hepatic disease are most likely to occur with acute hepatocellular disease.[20-22] Rarely does the unconjugated bilirubin fraction in acute hepatic disease exceed 25 mg/dL (427.5 μmol/L).[22] An increase in the bilirubin concentration may be indicative of hepatic disease and dysfunction but not necessarily hepatic failure. Furthermore, a normal bilirubin value, as commonly occurs in chronic hepatic disease, does not necessarily preclude the diagnosis of hepatic insufficiency.

An increase in the conjugated bilirubin fraction in horses more reliably is indicative of hepatic disease.[20-22] If the conjugated bilirubin concentration is less than 25% of the total bilirubin value, hepatocellular disease should be suspected. If the conjugated bilirubin concentration is greater than 30% of the total value, cholestasis should be suspected.[22] Conjugated bilirubin is water soluble and detectable in the urine of horses only if blood concentrations become sufficiently increased to surpass the renal threshold.[22] Therefore when urine tests positive for the presence of bilirubin, cholestatic disease should be suspected. Urine dipstick analysis is less sensitive than analysis with a diazo tablet.[22] A small number of healthy horses will have a positive reading for bilirubin on urine dipstick analysis. Urobilinogen may be detected by dipstick analysis of normal horse urine, and its presence indicates a patent bile duct.[22] Because urobilinogen is highly unstable, it must be determined on a fresh urine sample. Dilute or acidic urine may interfere with accurate determination of urobilinogen. Reagent strips are not sensitive enough to detect the absence of urobilinogen; therefore Ehrlich's reagent must be used. The absence of urobilinogen does not necessarily indicate liver disease, but it may be compatible with failure of excretion of bilirubin into the intestine, biliary obstruction, failure of intestinal bacterial reduction (diarrhea, overuse of oral antimicrobials), or failure to reabsorb it from the ileum.[9] Increased concentrations of urobilinogen in the urine may be caused by the increased production of urobilinogen by intestinal bacteria, failure of the liver to remove it from the enterohepatic circulation, portosystemic shunting, or spillover after severe hemolysis.[9]

Serum Bile Acid Concentration

The enterohepatic circulation normally removes greater than 90% of bile acids. Thus the blood concentration of bile acids may be increased with liver disease, and quantitation provides an excellent screen of liver failure and has essentially replaced foreign dye clearance (e.g., bromosulphthalein) as a functional test of the liver.[21,93,94] Serum bile acids are stable for at least 1 month if stored at −20°C and are measured by radioimmunoassay or by an enzymatic colorimetric method. The concentration of total serum bile acids is not affected by short-term fasting (less than 14 hours) but may be increased by more prolonged fasting.[95] The major serum bile acids in horses include ursodeoxycholic acid, chenodeoxycholic acid, and deoxycholic acid, but the exact composition of serum bile acids varies markedly among normal horses.[96] Reported normal mean values for horses and ponies as determined by radioimmunoassay are 8.2 ± 1.6 μmol/L (n = 9)[93] and 5.3 ± 6.5 μmol/L (n = 51)[94] and 5.0 ± 28.0 μmol/L[96] for the colorimetric method. Increased serum bile acid concentrations greater than 25 μmol/L are highly specific for the presence of liver disease or dysfunction (may increase within 24–48 hours after onset of hepatic disease) but are not specific for the type of liver disease.[21,97] Because bile acids are 90% restricted to the enterohepatic circulation, increases in the blood may occur as a result of shunting or decreased blood flow to the liver (first-pass effect), failure of the liver to remove bile acids from the enterohepatic circulation, failure of the hepatocytes to conjugate the bile acids for excretion, or failure of excretion with subsequent regurgitation of the bile acids into the blood (biliary obstruction). Increases in the primary (nondegraded) bile acids cholate and chenodeoxycholate account for most of the increase in total serum bile acid concentration in horses with liver disease.[96] In relation to the severity of liver disease, a significant shift in composition of serum bile acid profile from taurocholate to free unconjugated cholate also occurs. This finding suggests that hepatocellular excretion, and not resorption, is the most sensitive step in the enterohepatic circulation of bile acids in horses.[96]

Fasting for longer than 3 days in mature horses caused an increase in the serum bile acid concentration of 3 times over baseline values.[95] Ligation of the bile duct caused a sixfold increase in serum bile acid concentration compared with fasted horses.[95] Carbon tetrachloride toxicity resulted in a threefold increase compared with fasted horses.[95] Concentrations of serum bile acids greater than 50 μmol/L in horses with pyrrolizidine toxicosis were associated with a grave prognosis.[93] Increases in serum bile acids may occur with either acute or chronic disease and dysfunction. Levels above 25 μmol/L have been shown to be a poor prognostic indicator for some chronic diseases and failure,[98] but this does not apply to acute disease. A value less than 20 μmol/L appears to be a good predictor in ruling out significant functional liver disease and should be included in the evaluation of horses suspected to have hepatic disease.[94] It is important to note that, compared with mature horses, serum bile acid concentrations are often higher in healthy neonatal foals during the first month of life and frequently exceed 20 μmol/L.[99] Bile acid concentrations are highest in biliary obstructive diseases and portosystemic shunts.

Tests of Protein Synthesis

The blood concentrations of protein or amino acids relate not only to the rate of synthesis by the liver but also to their half-life in the circulation. The half-life of albumin in horses is long (19–20 days); thus a decrease in the albumin concentration is rarely detectable until greater than 80% of the liver mass is lost for more than 3 weeks.[20,22,34,91] It is unusual for the total serum protein concentration to decrease to less than 5 g/dL (50 g/L) in chronic liver disease.[100] Hypoalbuminemia is a nonspecific finding in chronic liver disease, because it may occur secondary to endoparasitism, nephrosis, malnutrition, malabsorption, circulatory failure, and many other chronic diseases.[22,91]

The globulin fraction often is increased in chronic hepatic disease as a result of decreased Kupffer cell mass. Loss of Kupffer cell function may result in wider dissemination of enteric-derived foreign antigens. Plasma cells respond to the general increased antigen load, resulting in polyclonal gammopathy.[9,20,22] Although the globulin fraction may be increased, it is a nonspecific finding with chronic hepatic disease, because polyclonal gammopathy can occur after numerous chronic diseases.[101] The α- and β-globulins may also increase with chronic liver disease. A decreased serum albumin concentration concurrent with an increased globulin concentration in chronic hepatic disease causes the total plasma protein or serum protein to appear normal. Thus serum protein fractionation is paramount. Protein electrophoresis most accurately determines fractionation of the components making up the total serum protein concentration.[20]

The blood concentration of amino acids may be increased after acute hepatocellular necrosis or during protein catabolic states such as illness or starvation, in response to insulin or glucagon.[9] Fractionation of the blood amino acids and

determination of the BCAA-to-AAA ratio is rarely done in clinical practice but is more useful than evaluating either fraction separately (see Hepatic Encephalopathy earlier in this chapter). Decreases in this ratio indicate hepatic insufficiency.[67] The risk of clinical signs of HE may be projected from the BCAA-to-AAA (phenylalanine, tyrosine) ratio. A normal ratio falls between 3.5 and 4.5. The risk of HE is low, medium, or high if the ratio is 3.0 to 3.5, 2.5 to 3.0, and less than 2.5, respectively.[67]

Because the liver is primarily responsible for removing *ammonia* from the circulation and converting it to urea for renal excretion, increases in the blood ammonia concentration or a decrease in the BUN concentration (less than 9 mg/dL; 6.43 mmol/L) may be indicative of subacute to chronic hepatocellular disease.[20,34,100] Daily blood ammonia levels vary widely in normal horses.[33] The compounding effects of ammonia-generating and urea-using bacteria in the gastrointestinal tract, ammonia generation with blood storage, and the effects of the ration may partly account for these fluctuations. In ethylenediaminetetraacetic acid (EDTA), whole blood can be refrigerated anaerobically for up to 6 hours without a significant increase in ammonia content.[102] The effect of the ration and handling can be evaluated by concurrently determining the blood ammonia concentration of a stable mate receiving a similar ration. When a longer period of time will occur before measurement, there should be anaerobic collection, prompt removal of the red blood cells, and the plasma sample frozen until measurement. Oral ammonia challenge tests have not been fully evaluated in horses; however, the sensitivity of the oral challenge test may be decreased because of the effect of enteric bacteria. Normal values for ammonia for horses vary among laboratories but have been reported in the range of 13 to 108 μg/dL (7.63–63.42 μmol/L).[32]

No correlation exists between the blood ammonia concentration and the severity of liver disease in horses, but increased blood ammonia concentration is significantly correlated with the presence of liver dysfunction, hepatoencephalopathy, or both.[33,34] Although hyperammonemia appears to be a sensitive indicator of liver dysfunction in the horse, it is not specific for liver disease. Encephalopathy associated with hyperammonemia without concurrent evidence of liver disease has been reported in horses with acute gastrointestinal disease.[103,104] Hyperammonemia has also been reported as a fatal heritable disorder in Morgan foals and is believed to be caused by a defective mitochondrial transporter protein involved in urea synthesis.[105] The syndrome may be accompanied by mild to moderate increases in serum liver enzyme activities.

Because the liver is also responsible for the synthesis of certain coagulation factors, evaluation of hemostatic function may be useful. Changes in hemostatic function values are not specific for liver disease and must be evaluated in light of the other laboratory findings. The vitamin K–dependent factor with the shortest half-life is factor VII; thus abnormalities are frequently first observed in the *prothrombin time (PT).*[33] However, adequate evaluation of hemostatic function necessitates determination of the *activated partial thromboplastin time* (APTT), the *fibrinogen* and FDP concentrations, and a platelet count. In one retrospective clinical review, almost half of the horses with liver disease had either an abnormally prolonged PT or APTT.[34] Typically, a 50% to 70% decrease in the blood concentration of the coagulation factors is necessary before a change in these clotting time–based assays is detectable.[34] Daily variation in the normal values for clotting times also hinders accurate detection of the abnormal. The clotting times may be standardized more appropriately if one concurrently determines a clotting assay on a normal horse. If the ratio of clotting time (PT or APTT) of the patient with possible hepatic disease to the normal horse's value is greater than 1.3, the test may be interpreted as abnormal.[106] The sensitivity of coagulation factor deficiency during hepatic insufficiency may be increased by diluting the plasma[107] or by determining the concentration of specific factors, by either clot-based, chromogenic, or radioimmunologic assays. These later assays are not widely available.

Hypofibrinogenemia cannot be accurately detected by the heat block precipitation method, the most common method for determination of the plasma fibrinogen concentration.[20] Clotting assays using thrombin more accurately determine the fibrinogen concentration. Fibrinogen concentrations less than 100 mg/dL (1 g/L) indicate either decreased production or increased consumption of fibrinogen. The FDP concentration, as determined by latex agglutination, may be increased during hepatic insufficiency owing to decreased removal by Kupffer cells. Concentrations of FDPs greater than 16 μg/dL are indicative of increased production or reduced removal. The plasma concentration of several factors acting as anticoagulants or involved in fibrinolysis also may be altered in chronic liver failure (see Hemorrhagic Diathesis earlier in this chapter). Tests for these factors are primarily limited to academic and research institutions.

Thrombocytopenia with associated petechiae or nasal mucosal bleeding has been reported in horses with liver disease; therefore in possible cases of liver disease, platelet counts should also be evaluated before invasive diagnostic or therapeutic techniques.[33]

Tests of Carbohydrate Metabolism

Changes in the blood glucose concentration rarely occur in adult horses with liver insufficiency.[34] Hyperglycemia may occur with stress-associated catecholamine and glucocorticoid release. Hypoglycemia (glucose less than 60 mg/dL; 3.33 mmol/L) may occur in adult horses with acute massive hepatic failure but is more likely in chronic liver disease as anorexia progresses, glycogen stores are depleted, and gluconeogenesis and glycolysis are impaired by increased glucagon concentrations. If glucose is administered, blood glucose levels often remain abnormally increased, indicating tissue insulin resistance.[37] Insulin receptor number and binding affinity for insulin are diminished in human beings with chronic liver disease. Changes in the blood glucose concentration are not specific for liver disease and must be evaluated in the light of other laboratory findings. Hypoglycemia is common in foals with hepatic failure.[108]

Tests of Lipid Metabolism

The concentration of blood triglycerides may become increased during hepatic insufficiency because of increased mobilization from adipose tissue to support energy-requiring processes and a decreased clearance by the liver.[9,22] In contrast, the VLDL and esterified cholesterol blood concentrations may be decreased because of failure of liver synthesis. Compared with other species, equids are thought to possess a greater clearance capacity for triglycerides and a greater hepatic exporting capacity for VLDL. Thus changes in the

blood VLDL, esterified cholesterol, or triglyceride concentrations rarely occur in horses.[22] An exception to this is the marked increase in blood triglyceride levels that occurs with hyperlipidemia syndrome in ponies, donkeys, and Miniature Horses (see Hyperlipemia and Hepatic Lipidosis later in this chapter). Because nonhepatic diseases may alter blood lipid concentrations, these tests are neither sensitive nor specific for liver disease in horses.[22] The blood cholesterol and triglyceride concentrations are normally higher in neonates than in adults.[78]

Increased mobilization of triglycerides and fatty acid oxidation by the liver may result in increased production of *ketone bodies,* acetoacetate, and β-hydroxybutyric acid.[12] Although peripheral tissues may use ketone bodies for energy, these compounds are weak acids and increased levels in the blood may result in ketoacidosis. The pathway for ketone formation is poorly developed in horses; thus ketoacidosis is not common.[22] Ketoacidosis could be considered in horses that are acidemic and have an abnormally high anion gap. Ketones may be quantitated in the blood or urine. Because the renal threshold of ketone bodies is low, ketonuria usually precedes marked ketonemia. Routine urine dipsticks only detect acetoacetate.[22]

Liver Enzymes

Acute hepatocellular necrosis or changes in hepatocyte membrane permeability result in the release of soluble cytosolic and mitochondrial enzymes into the sinusoidal blood. Thus increased blood activity of these enzymes may be indicative of active hepatocellular disease. Caution must be exercised when evaluating increases in these enzymes because not all are liver specific. Furthermore, some of these hepatocellular enzymes may be increased because of induction by drugs. Most of these enzymes are quantitated colorimetrically; thus hemolysis or lipemia may interfere with accurate evaluation. Furthermore, wide variation in values can exist because of age differences, stage of hepatic disease, and laboratory methodology. In horses the following cytosolic enzymes are liver specific and are not inducible: sorbitol dehydrogenase (SDH; iditol dehydrogenase), arginase (ARG), ornithine carbamoyltransferase, and the mitochondrial-produced enzyme glutamate dehydrogenase (GLDH).[20,22,108] Although increases in these enzymes in the blood are highly specific for hepatocellular disease, they are not specific for the type of disease, nor are they function tests. Significant increases occur after acute hepatic necrosis. Mild increases in these enzymes may occur after hepatic hypoxemia or toxemia resulting from endotoxemia, septicemia, transient intestinal disease, hyperthermia, or administration of certain drugs (benzimidazole anthelmintics).[20,22,108]

SDH has been widely used in the evaluation of acute liver disease in horses.[78,108,109] The short half-life (less than 12 hours) of this liver cytosolic enzyme makes it ideal for the evaluation of acute ongoing disease, because values usually return to baseline within 3 to 5 days after a transient hepatic insult.[94,110] Its short half-life necessitates analysis within hours of collection. Storage of serum in the freezer (−15°C) or refrigerator results in losses of approximately 1.0% and 3.5%, respectively, of SDH activity per day.[111] Although mild variations exist among laboratories, the normal blood activity of SDH in horses is usually less than 8 U/L.[32] Foals 2 to 4 weeks of age may have SDH activity slightly greater than those of adult horses.[78,109] Increases in SDH have been reported after prolonged halothane anesthesia in horses.[112] ARG is used in the Krebs-Henseleit cycle for urea synthesis. It is found in highest activity in hepatocytes, although minute amounts also exist in renal tissue, brain, skin, testicles, and erythrocytes.[22] Increases in ARG are most indicative of acute hepatic necrosis. Like SDH, ARG has a very short half-life. GLDH is found in hepatocytes, renal tissue, brain, muscle, and intestinal cells. Like SDH and ARG, GLDH has the highest tissue activity in the liver, and increases of this enzyme in the blood can be considered specific for acute liver disease. GLDH is produced in the mitochondria, and although increases occur in almost all hepatocellular diseases, the magnitude of the increase may differ from those of SDH. The stability of the enzyme in serum may be slightly better than SDH.[108] The half-life of GLDH is 14 hours.[22]

Other cytosolic enzymes include aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and isocitrate dehydrogenase (ICD). These enzymes are also found with high activity in other tissues or are inducible. Thus increases in these enzymes are not specific for liver disease in horses. Because some of these enzymes are frequently reported in equine biochemical profiles, they may serve as a crude indicator of liver disease; however, the limitations of their usefulness must be recognized.

AST, formerly glutamic oxaloacetic transaminase (GOT), is a cytosolic- and mitochondrial-bound enzyme that catalyzes the reaction responsible for aspartate biosynthesis from carbohydrate.[20] Basically, all cells contain AST, but liver and skeletal muscle cells contain the highest activity. Cardiac muscle, erythrocytes, intestinal cells, and the kidney also are sources of AST. Hemolysis and lipemia will falsely increase the value of AST.[20] Increases are most frequently associated with muscle damage but may be seen after acute hepatic necrosis. Often the AST activity is normal in chronic hepatic disease.[100] The half-life of AST is long, and thus it may take longer than 2 weeks for the blood activity to decrease after acute hepatic disease. The AST value is most useful when analyzed with other tissue-specific enzymes. For instance, if a muscle-specific enzyme, such as creatine kinase, also is increased, an increase in AST most likely has its origin in muscle. Serial AST and SDH values can be useful for the determination of ongoing hepatic disease. If the SDH and AST values were initially increased, but subsequent evaluation reveals a normal or decreasing SDH and still elevated AST, a favorable prognosis is often indicated, because hepatic necrosis is most likely subsiding. The normal serum activity of AST is 98 to 278 U/L.[32] ICD has a distribution similar to AST.

ALT, formerly glutamic pyruvic transaminase (GPT), is responsible for alanine synthesis from carbohydrate.[20] Increases may be evident with acute hepatic disease, but myositis will also increase blood levels. Hemolysis will falsely increase the value of this enzyme, and microsomal enzyme inducers (i.e., glucocorticoids) will increase its production and release in the absence of liver disease. This enzyme is not useful for predicting liver disease in horses.[20]

ALP catalyzes the hydrolysis of monophosphate esters. This enzyme is bound to the mitochondrial membrane and thus does not leak into the blood with changes in cell membrane permeability or necrosis. Cholestasis and certain drugs, including glucocorticoids, primidone, and phenobarbital, will induce production and release of ALP. The ALP value most likely is to be increased in chronic or cholestatic liver diseases rather than acute or hepatocellular disease. Other tissues in addition to the liver contain ALP, including bone, intestine,

kidney, placenta, and leukocytes; thus increases are not necessarily indicative of cholestasis. Because of increased osteoblastic activity, foals have ALP values 2 to 3 times those of adults.[22] Pregnancy, hemolysis, and gastrointestinal disease will also cause increases in ALP.

LDH is the name for five major isoenzymes located in liver, muscle, erythrocytes, intestinal cells, and renal tissue. Increases in LDH are not liver specific unless the isoenzyme activity is determined. Isoenzyme 5 (LDH-5) is a useful indicator of acute hepatocellular disease in horses because values typically return to baseline within 4 days after a transient hepatic insult.[94,110] LDH-5 is also present in muscle, so increased serum LDH-5 activity is specific for hepatic disease if other indicators of muscle damage (i.e., creatine kinase) are normal.[110] LDH-5 is stable at room temperature for 36 hours.

γ-Glutamyltransferase (GGT) is involved in glutathione metabolism and transfer of glutamyl groups.[20] GGT primarily is associated with microsomal membranes in the biliary epithelium.[108] Cholestasis induces production and release of GGT. Renal tubule cells contain GGT, but this source is released into urine. The only other potentially significant source of GGT in the blood is pancreatic in origin. Because pancreatic disease is rare in horses and horses and foals with confirmed severe pancreatic disease have not had elevations in GGT, the blood activity of GGT is considered specific for hepatic disease in horses. Some clinicians consider GGT to be the single test of highest sensitivity when evaluating horses for evidence of liver disease.[94] The half-life of GGT is approximately 3 days; GGT is stable in serum for 2 days at room temperature or for 30 days if frozen.[22] Mild increases may be evident after acute hepatocellular necrosis and may continue to rise for 1 to 2 weeks despite improvement in clinical signs.[94] Increases are more persistent in chronic disease, especially with cholestasis.[100] Foals 2 weeks to 1 month old may have greater GGT values than adults.[78,109] The wider variation in younger horses reflects the degree and extent of hepatic maturation. Normal values for GGT in adult horses typically are less than 30 U/L[32] but may be greater in racing Thoroughbreds, healthy donkeys, burros, and asses.[113] In chronic, nonactive hepatic fibrosis and focal hepatic disease, GGT may not be significantly increased. Increased serum GGT activity has been reported in horses with cholestasis secondary to colonic displacement.[114] Racehorses occasionally have mild to moderate increases in GGT (50–140 IU/L) without any other biochemical evidence of liver disease.[115-118] This phenomenon has been observed for at least 30 years, and the elevation in GGT has been temporally linked to poor performance.[115-118] In some situations, multiple horses stabled together may have elevations in GGT. Possible causes include drug administration causing induction of GGT, toxin exposure causing disease specific for the biliary tract, viral infections causing only increases in GGT at the time of sampling, and overtraining with depletion of hepatic glycogen.[116] GGT activity between 70 and 100 U/L has previously been associated with poor health, oxidative stress, and overtraining the horse, and further, those horses with high GGT values showed a markedly reduced incidence of increased GGT following reduction in training intensity in subsequent years.[115] Furthermore, GGT levels in these horses almost always return to normal within a few weeks following rest. Recent studies demonstrated that serum GGT activity was correlated to cumulative training load and racing frequency[117] and maladaptation to training.[118]

In summary, biochemical testing for liver disease in horses should include quantitation of at least SDH or GLDH and of GGT. On rare occasions a horse may have liver disease without elevations in these enzymes. Elevation in liver enzymes are tests of disease and one should interpret these as function test.

Clearance of Pharmaceuticals

In addition to clearance of endogenous substances from the blood, liver function can be evaluated after injection of an exogenous substance. One such exogenous substance that is removed by the liver, conjugated, and excreted into bile is *bromsulfthalein (BSP)*.[22] After intravenous injection of 2.2 mg of BSP per kilogram of body weight, a clearance half-life is determined by periodically obtaining heparinized blood samples over 12 to 15 minutes. One should exercise caution when injecting BSP, because it can be thrombogenic and irritating if administered perivascularly. Blood samples for BSP quantitation should be collected from a site other than the injection site. Suggested collection times are 3, 6, 9, 12, and 15 minutes after injection. Collection of plasma at the suggested times is not crucial; however, recording the exact times at which the samples were obtained is important to ensure accurate determination of the half-life. The half-life of BSP, thus the rate of extraction by the liver, is determined by plotting the plasma concentrations against the collection times on semi-log paper. The normal half-life of BSP in horses is 2.8 to 0.5 minutes.[22]

The BSP half-life is prolonged when greater than 50% of the hepatic function is lost.[20,22] This function test is useful in horses, especially to distinguish hepatoencephalopathy from other causes of abnormal behavior or cerebral signs, and to test liver function in chronic liver disease when bilirubin, SDH, and GGT blood levels may be normal. Proper interpretation of the BSP half-life must take into account the state of hepatic blood and bile flow and the bilirubin and albumin concentrations.[20] If the hepatic blood flow is decreased significantly, as may occur with hepatic congestion or portosystemic shunts, the rate of delivery of BSP to the liver will be decreased; thus the half-life of BSP in the plasma will be prolonged. Because BSP is bound to albumin for delivery to the liver, if the blood concentration of albumin is markedly decreased, a higher proportion of unbound BSP will be delivered to the hepatocytes; thus the half-life of BSP will be shortened.[20] If the blood concentration of bilirubin is increased greatly, the bilirubin will compete with the BSP for binding sites and conjugating enzymes in the liver; thus the apparent half-life of BSP is prolonged. If significant cholestasis is present, the BSP excreted into the biliary tract will be reabsorbed into the circulation, causing an apparent prolongation of its clearance. Therefore although a BSP clearance test is very useful as a test of hepatic function, one must interpret the results in light of these limitations. Furthermore, because pharmaceutical-grade BSP is no longer commercially available, this test is basically limited to academic and research institutions. Quantitation of serum bile acid concentration has essentially replaced BSP clearance as an indicator of hepatic function.

The determination of the BSP clearance time has been suggested to be more useful for detecting liver disease than the BSP half-life.[119] It has also been suggested that the proportionality transfer constants of clearance may be more useful in predicting hepatic disease than the BSP half-life alone.[120] The clearance time is the amount of dye irreversibly removed

from the plasma per unit time. A dose of 5 mg of BSP per kilogram of body weight is given intravenously, and heparinized blood samples are obtained 2, 5, 10, 15, 25, and 30 minutes after injection. The BSP clearance time in fed normal horses is 10 mL/min/kg and 6 mL/min/kg for horses fasted 3 days.[119]

The *indocyanine green* (ICG; Beckmann & Dickinson, Baltimore, MD) clearance test has replaced the BSP clearance test in human beings.[9] Basically, the procedure and the limitations are the same as the BSP clearance test. The ICG clearance time in fed horses is 3.5 to 0.67 mL/min/kg and 1.6 to 0.57 mL/min/kg for fasted horses.[119] Although the clearance of ICG is an excellent predictor of hepatic blood flow and extraction rates, its current expense precludes its routine use in horses. Another disadvantage is that quantitation requires a spectrophotometer that reads infrared wavelengths. These limitations have hindered evaluation of ICG clearance times as a diagnostic test of liver insufficiency in horses.

The hepatic clearance of the radiopharmaceutical technetium-99m mebrofenin is used in human beings and small animals to assess hepatic function. In horses, the plasma clearance of technetium-99m mebrofenin is not affected by withholding feed.[121]

Other Nonspecific Laboratory Findings

Increased formation and release of acidic metabolic products, including ketone bodies, lactate, pyruvate, and amino acids, contribute to acidemia. Other factors that may contribute to acidemia include diarrhea and loss of renal acidification because of impaired urea synthesis. Anorexia may predispose to hypokalemia.

Although infrequently reported in horses, aldosterone and water retention may cause deranged concentrations of sodium and isosthenuria (see Polydipsia, Polyuria, and the Hepatorenal Syndrome). Azotemia may occur with hyperlipemia.[122]

Cholangiohepatitis, CAH, or a focal hepatic abscess may cause an inflammatory leukogram, anemia of chronic disease, and hyperfibrinogenemia.[89] Primary polycythemia has been reported in horses with hepatocellular carcinoma and hepatoblastoma.[123-125] Polycythemia has also been observed in horses with chronic liver inflammation and fibrosis.[116] Because fibrinogen is synthesized by the liver, widespread chronic hepatitis may mask an increase in this acute phase reactant protein. Marked elevations in total serum iron and percentage of transferrin bound iron is relatively common in adult horses with liver disease and infrequently seen in other adult horse organ system disorders.[116]

Diagnostic Imaging of the Liver

Ultrasonography is a safe, noninvasive imaging technique that uses the reflection of high-frequency sound waves from tissue interfaces to produce a visual image. Liver ultrasonography in horses is limited by the ribs, the depth and size of the liver, and its anatomic location deep to the diaphragm and lungs.[3,107] Mechanical sector or linear array scanners with 3.0-MHz crystals are most effective.[3,107] Ultrasonography can be used to assess the right and left sides of the liver and the liver shape, size, position, and texture. Hepatic vein walls are less echogenic than portal vein walls, and the biliary system is not normally visible. Ultrasonography is most useful for determining the general size of the liver; changes in the hepatic parenchyma, including abscesses, cysts, and neoplastic masses; and detecting dilated bile ducts or obstructions with choleliths. The common bile duct cannot be seen in a horse. Abnormal intrahepatic or extrahepatic blood flow or vasculature may be detectable. The liver on the left is often adjacent to the more caudally located spleen; this provides an opportunity, when visualized with ultrasound, to compare the relative echogenicity of each organ. The liver should be hypoechoic compared to the spleen, and with hepatic fibrosis the liver and spleen appear more similar in echogenicity.[3] Ultrasonography is also useful for guiding biopsy instruments into the liver (see Liver Biopsy).

Radionucleotide imaging also may be used to detect alterations in the hepatic parenchyma or blood flow.[121,126,127] Radionucleotide scanning noninvasively evaluates function and yields structural information. Two radionucleotide scanning techniques are available to evaluate the liver: liver scan and biliary scan. In the liver scan, a technetium-99m–labeled sulfur colloid is injected intravenously. Technetium-99m is a gamma-emitting radioactive compound that is detected in the body by a gamma camera. After injection, Kupffer cells phagocytose the technetium-99m sulfur colloid. Subsequent scanning with the gamma camera detects the radioactive emissions from the technetium-99m and resolves them into a two-dimensional image. Thus alterations in blood flow (portosystemic shunt) or hepatic masses such as abscesses, cysts, and neoplasia may be detectable. In the biliary scan, technetium-99m–labeled iminodiacetic acid is injected intravenously. The iminodiacetic acid is extracted by the hepatocytes, conjugated, and excreted in the bile.[128] Subsequent scanning images the biliary system and may be useful for the detection of biliary obstruction, including atresia, cholangitis, and cholelithiasis. Because radionucleotide imaging is somewhat expensive and requires a large gamma camera, this procedure is limited to research and academic institutions.

Operative mesenteric portography can be performed when a portosystemic shunt is suspected. In this procedure, a celiotomy is performed, radiopaque material is injected into a mesenteric vein, and rapid, sequential survey radiographs are obtained. Simultaneous opacification of the portal vein, azygous vein, and caudal vena cava or lack of filling of the intrahepatic portal system is indicative of portosystemic shunting.[126]

Liver Biopsy

A liver biopsy can yield important diagnostic and prognostic information. In a retrospective study of 73 horses with liver disease, a scoring system that graded the degree of fibrosis, reversible and irreversible cytopathology, inflammation, hemosiderosis, and bile duct proliferation was useful in predicting long-term survival.[129] A liver biopsy is usually performed at the right twelfth to fourteenth intercostal spaces at the intersection of a line drawn from the tuber coxae to a point midway between the elbow and the point of the shoulder. A recent study reported that using these traditional landmarks as biopsy sites may result in unsuccessful sampling of the liver in many horses and could puncture lung or intestine.[130] The study concluded that the practice of blind percutaneous liver biopsy in horses is not recommended because of the potential risk of serious complications.[130] Therefore liver biopsy in the horse should be immediately preceded by ultrasound examination and identification of an appropriate site. The procedure must be performed in a sterile manner. The area is clipped, aseptically prepared, and injected with local-acting anesthetic subcutaneously, and

a stab incision is made with a no. 15 scalpel blade. A Tru-Cut (Baxter-Travenol, St. Louis, MO) or Franklin-modified Vim-Silverman (Mueller & Co., Chicago, IL) biopsy instrument is inserted and directed such that the largest amount of liver can be penetrated. Semiautomatic biopsy instruments (EZ Core, Products Group International, Lyons, CO) and automatic biopsy guns (ProMag 2.2 Biopsy System, Manan Medical Products, Northbrook, IL) equipped with 14-gauge, 16-cm needles are very helpful for a quick and accurate liver biopsy. Ultrasound-guided biopsy will provide a more precise sampling. Samples should immediately be placed in formalin for histopathologic evaluation and, if necessary, in transport media for culture of microorganisms. Precautions to consider before performing the procedure include the risk of hemorrhage, pneumothorax, and peritonitis from bile leakage and colon or abscess puncture, as well as spread from infectious hepatitis. These complications may be reduced by performing a hemostasis profile to assess the risk of hemorrhage and by using ultrasonography to guide needle placement. Although subclinical coagulopathy has been frequently reported in horses with liver disease, clinically significant or fatal hemorrhage after performing a liver biopsy is rarely reported.[131] Biopsies may not be needed in some equine liver disorders. If historical, clinical, and laboratory information clearly indicates a diagnosis such as Theiler's disease or hepatic lipidosis, biopsy is unlikely to be of additional help in managing the case.

Summary of Diagnostic Procedures

The most useful diagnostic tests for evaluation of hepatic disease in horses are quantitation of SDH or GLDH, GGT, and serum bile acids. In the face of clinically significant liver disease, quantitation of both conjugated and unconjugated bilirubins, glucose, PT and PTT, triglycerides and ammonia is recommended. Serial testing of these indices may increase their diagnostic and prognostic value. Abnormal laboratory findings should be further investigated with ultrasonography and biopsy.

Treatment of Hepatic Insufficiency

Management techniques for hepatic insufficiency are largely supportive. (Therapies for specific liver diseases are discussed in more detail under Specific Hepatic Diseases later in this chapter.) The basic goal of treatment is to maintain the animal until enough liver regenerates to provide adequate function. Fortunately, the liver is an amazingly regenerative organ, and most acute diseases do not progress to failure. Although hepatocytes are fully differentiated cells, they are capable of active proliferation by self-duplication in response to acute external stimulation and upregulation of humoral factors, all without any contribution from hepatic stem cell progenitor cells.[27–29] Patients with severe hepatic fibrosis respond poorly because adequate regeneration often is not possible. Horses with signs of HE that are agitated, restless, or uncontrollable must be sedated to enable therapy; however, any medication should be used judiciously because most tranquilizers are metabolized by the liver or potentiate the abnormal neural function of HE. Xylazine or detomidine in small doses is safest and most effective in these instances. Use of diazepam is contraindicated because it enhances the effect of GABA on central inhibitory neurons and may exacerbate signs of HE.[49] Intravenous fluids should correct fluid deficit and acid-base or electrolyte imbalances such as hypokalemia. Because most horses with HE are anorectic and blood glucose may be decreased, particularly in foals, continuous intravenous infusion of 5% dextrose with electrolytes can be beneficial as long as pronounced hyperglycemia, hyponatremia, and hypokalemia are avoided.[116] In human beings, volume expansion with 0.9% saline solution enhances urinary ammonia excretion.[132] If bicarbonate therapy is necessary to treat acidotic patients with hyperammonemia, the rate of administration should be monitored carefully because too rapid of a rate may increase blood levels of ammonia.[49] Hypokalemia or alkalosis results in increased renal production of ammonia and increased diffusion of ammonia into the CNS; thus treatment with potassium or acidifying fluids may be beneficial.[133,134]

Therapy should also be directed at reducing the production of toxic protein metabolites by enteric bacteria or by interfering with their absorption.[135] The administration of mineral oil or magnesium sulfate per nasogastric tube is safe, assuming there is no nasal hemorrhage, and aids in reducing toxin absorption. HE in human beings with acute fulminant hepatic failure is almost always associated with cerebral edema. Thus recommendations for treatment of severe acute HE in human beings currently focus on decreasing blood ammonia levels; reducing systemic inflammation; maintaining organ perfusion, blood pH, and electrolyte balances; treatment of cerebral edema with mannitol or hypertonic saline; and treatment with antioxidants, nutritional support, and, when those fail, use of extracorporeal liver support devices.[43]

Methods to reduce the production of ammonia and other enteric toxins include the oral administration of antibiotics (neomycin, metronidazole, rifaximin, vancomycin)[132] or lactulose or lactitol (not available in the United States).[43,133,135] In horses, neomycin has been recommended at a dosage of 10 to 20 mg/kg PO every 8 hours.[116] This treatment significantly alters the gastrointestinal flora and may cause diarrhea in some horses. However, in one retrospective study in horses with liver disease, oral neomycin therapy subjectively was more helpful than lactulose in alleviating signs of HE.[34] Lactulose, a syrup containing lactose and other disaccharides, passes through the upper small intestine and is metabolized by ileal and colonic bacteria to organic acids, thereby reducing luminal pH. Proposed beneficial mechanisms of action of lactulose include increased bacterial assimilation of ammonia, decreased ammonia production, ammonia trapping in the bowel lumen by increased conversion of ammonia (NH_3) to ammonium (NH_4), enteric microflora changes, and osmotic catharsis.[136] A dose of 0.3 to 0.5 mL/kg of lactulose syrup every 8 hours per oral dose syringe or nasogastric tube has been recommended for horses.[116] If a nasogastric tube is used there should be extreme care in avoiding a nosebleed as ingested blood would increase enteric ammonia production. When given orally 3 times a day at 333 mg (0.5 mL)/kg of body weight, lactulose (Duphalac, Solvay Pharmaceuticals, Marietta, GA) significantly decreased blood ammonia values in healthy adult horses.[136] None of the horses developed diarrhea, although one horse foundered on the sixth day of treatment. Fecal pH did not decrease. As an alternative or adjunct to antimicrobials or lactulose, one may attempt modification of intestinal flora with probiotics, such as *Lactobacillus acidophilus* or *Enterococcus faecium*.[133] Many clinicians prefer not to use oral antimicrobials or lactulose but to rely on institution of a low-protein diet. Low-protein diets are recommended for hepatic diseases causing high blood ammonia or signs of

TABLE **13.3 Clinical Conditions That May Exacerbate Hepatic Encephalopathy**

Dehydration	Systemic inflammation
Constipation	Hyponatremia
Hypokalemia	Abnormally low head position
Gastrointestinal bleeding	Excessive sedation
Intestinal blood (nosebleed)	Jugular thrombosis
Infection	Hyperthermia

HE, but moderate- to normal-protein diets should be given to horses with chronic liver disease, normal mentation, and normal blood ammonia. Oral and intravenous formulations of BCAAs are commercially available for use in horses (BCAA Equine Sports, Inc., Lake Forest, IL; Baxter Healthcare Corp., Deerfield, IL), but controlled clinical trials in human beings have not shown this form of therapy to consistently ameliorate the signs of HE.[72]

As discussed previously, benzodiazepine receptor antagonists have been suggested to induce clinical and electrophysiologic remissions of HE in human beings with both acute and chronic liver failure.[36,137,138] This neuropharmacologic approach to treatment of HE holds promise, although it may not be economically feasible in horses. In human beings with HE, treatment with flumazenil, a benzodiazepine antagonist, and bromocriptine, a dopamine agonist, has provided either short-term or inconsistent results.[139,140] Zinc, levocarnitine, and ornithine aspartate enhance ureagenesis and also may reduce blood ammonia concentrations.[132,133,141] These treatments have not been evaluated in the horse. In summary, the basics for treating HE in horses include providing sedation only when needed and providing appropriate fluid and electrolyte treatments to maintain perfusion of organs and correct electrolyte and glucose abnormalities, decrease ammonia enteric production, prevent infection, control inflammation, provide adequate nutritional support, and prevent complications that may worsen HE (Table 13.3).

Antiinflammatory drugs may be beneficial in treating liver disease and include flunixin meglumine, dimethyl sulfoxide (DMSO), and pentoxifylline. DMSO (0.5–1 g/kg body weight, given intravenously diluted to 10% for 3–5 days) may help dissolve intrabiliary sludge or small calcium bilirubinate stones.[142] *Pentoxifylline* has been shown to reduce hepatic fibrosis in human beings[143] and may be given to horses at 8 mg/kg every 8 to 12 hours.[143a] Silymarin, a standardized extract of the milk thistle seeds *(Silybum marianum),* is commonly used in the treatment of chronic liver diseases in several species including the horse.[144,145] Flavonolignans are the most common class of compounds present in milk thistle extract, and of the group, silybin (silibinin) is considered to have the most hepatoprotective properties.[144] Experimental studies have demonstrated antifibrotic, antioxidant, and metabolic effects of silybin, but human studies have been insufficient in confirming the clinical efficacy in chronic liver disease.[144] In normal horses, a standard bioavailability study of orally administered silibinin found less than 1% available.[145] *S*-adenosylmethionine (SAMe) is another nutritional supplement commonly used in the treatment of chronic liver disease in small animal veterinary medicine. SAMe is a compound made primarily in the liver as a byproduct of methionine metabolism. Following oral administration, absorption, and trans-sulfuration, SAMe is converted into glutathione, a powerful antioxidant. SAMe is believed to stabilize cell membranes, promote secretion of bile, and have antiinflammatory properties. Despite these potentially favorable benefits and information to suggest that SAMe synthesis is depressed in chronic liver disease, high-quality randomized studies to prove clinical efficacy were not found.[146] There are no studies on bioavailability or efficacy in the horse.

The bile salt ursodeoxycholic acid (ursodiol) is the drug of first choice in the treatment of cholestatic liver diseases in humans[147] and has been successfully used in treating obstructive cholangiohepatitis in horses.[116] Ursodiol would not be expected to have an effect on dissolving calcium bilirubinate calculi but is an antiinflammatory and choleretic agent that increases bile production, making bile more liquid and easier to excrete.[148]

Colchicine, an antigout drug, and cyclosporine, a potent T helper cell immunosuppressant, have been used to slow hepatic fibrosis in dogs and people.[149,150] These drugs have not been fully evaluated in horses; however, colchicine has been ineffective in cases of pyrrolizidine alkaloid toxicity and may even be contraindicated due to its antimitotic effect.[149]

If any drugs are to be administered to a horse with hepatic insufficiency, it may be necessary to alter dosage. Alterations in hepatic blood flow, albumin, and biotransformation during hepatic insufficiency may prolong the half-life, as well as the dosage interval. Drugs that rely heavily on the liver for metabolism and excretion, such as chloramphenicol, erythromycin, metronidazole, and corticosteroids, should be used with caution.[24]

Once the appetite returns, a horse with HE or chronic liver disease is best managed by dietary manipulation. The ration should be high in carbohydrates and moderate to normal protein, with the protein source optimally rich in BCAAs. A mixture of two parts soaked beet pulp and one part cracked corn in molasses can be fed at a rate of 2.5 kg/100 kg body weight per day divided into six or more feedings.[113] Sorghum, bran, or milo can be substituted for the beet pulp. In horses with liver failure and neurologic signs of HE, it can be assumed that the amount of protein in the diet should initially be restricted.[116] The old concept of providing a low-protein diet to all animals with chronic liver failure is no longer believed to be standard of care in other species.[151] The beneficial effects of feeding adequate protein include maintenance of muscle mass and therefore deceased ammonia genesis associated with muscle catabolism.[72]

Multiple small feedings are optimal in horses with liver disease because of impaired gluconeogenesis. Oat or grass hay is a preferred roughage.[116] Horses should be encouraged to graze grass pastures provided they are protected from the sun. Alfalfa and legumes should be avoided if signs of HE are present because of their high protein content; however, any caloric intake is important in liver failure and alfalfa, and legumes should be allowed if the horse will not eat other feeds. Vitamins B_1, K_1, and E and selenium, zinc, and folic acid are often supplemented to horses with liver failure. Vitamin K therapy might be most beneficial when cholestasis is present.[9]

Prognosis

The prognosis for hepatic insufficiency in horses depends on the severity and type of the underlying disease. Horses with

focal or mild to moderate acute hepatic disease have the best chance for hepatic regeneration; thus they have a guarded to fair prognosis if adequate and appropriate supportive care is given. Patients with severe hepatic fibrosis and chronic liver disease have a poorer prognosis because of their inability to compensate for lost hepatic function. Regardless of the cause of liver disease, the presence of severe encephalopathy, intravascular hemolysis, profound acidosis, clinical signs of coagulopathy, diarrhea, bilateral laryngeal paralysis, markedly increased serum bile acid concentration in horses with chronic liver disease, BSP half-life greater than 10 minutes, decreased serum albumin, and histologic evidence of marked bridging fibrosis have all been associated independently with a poor to grave prognosis.[98,129,152] If a horse survives more than 5 days after an acute transient hepatic insult, the prognosis is fair. Decreasing blood levels of SBA, GLDL, and GGT are more likely to occur in horses that survive.[33] Therapy is generally unrewarding in horses with severe hepatic fibrosis.

SPECIFIC HEPATIC DISEASES

The wide variety of clinical signs with hepatic disease, coupled with the fact that the majority of the hepatic parenchyma must be affected before function is lost, makes the clinical distinction between acute and chronic liver disease a challenge. The onset of signs may be sudden, even with chronic disease. A history of progressive weight loss or "failure to thrive" may indicate chronicity. Although no single blood test is specific for distinguishing acute from chronic hepatic disease, the presence of hyperglobulinemia or hypoalbuminemia may suggest chronicity. Histopathologically, the hallmark evidence of chronicity is the presence of fibrosis. Fibrosis can be determined by ultrasound examination or preferably liver biopsy. For the purpose of discussion of specific hepatic disorders in the following sections, diseases have been classified as either *acute* or *chronic* based on the known cause of the disease (Box 13.2).

Acute Hepatic Diseases

Theiler's Disease

Sir Arnold Theiler first described this disease in South Africa in 1918 after vaccination of horses against African horse sickness with both live virus and equine-origin antiserum.[153] In 1934 and 1937, a similar disease was described in the United States after vaccination of horses against western equine encephalomyelitis using live virus and equine-origin antiserum.[86,154,155] Despite numerous accounts of this syndrome, the exact cause of Theiler's disease remains unproven. Today this disease is one of the most commonly described causes of acute diffuse hepatic necrosis in adult horses.[32,86,156-160] Theiler's disease is also referred to as *serum-associated hepatitis.* The term *serum sickness* has been used in previous literature erroneously[161]; serum sickness is an immune complex hypersensitivity to foreign protein resulting in multisystem disease within 2 weeks after foreign protein administration.[162]

Clinical Signs. Theiler's or Theiler's disease is limited to adult horses, although one report describes subclinical disease in a 2-month-old foal.[157] The onset of clinical signs of hepatic failure in Theiler's disease is acute to subacute and often rapidly progresses over 2 to 7 days. Most horses are anorectic and icteric; HE is reported in the majority of the cases.[86,156,157] Sudden death, photodermatitis, hemorrhagic diathesis, fever, dependent edema, colic, and bilirubinuria may be present.[86,156,157]

Epidemiology and Pathogenesis. The disease typically occurs sporadically, but outbreaks involving multiple horses on a single farm over several weeks have been reported.[32,86,156,157]

BOX 13.2 Liver Diseases of Horses

ACUTE DISEASE

- Theiler's disease (serum-associated hepatitis)
  - Virus-related Theiler's disease
  - Nonprimate hepacivirus (NPHV)
  - Theiler's disease–associated virus (TDAV)
  - Equine pegivirus (EPgV)
  - Equine parvovirus
- Hyperlipemia
- Tyzzer's disease
- Infectious necrotic hepatitis (black disease)
- Cholangiohepatitis
- Acute biliary obstruction
  - Cholelithiasis
  - Colon displacement
  - Hepatic torsion
- Parasitic hepatitis
  - *Parascaris equorum*
  - Large strongyles
  - *Echinococcus granulosa*
  - Schistosoma
- Toxic hepatopathy
  - Plants
  - Mycotoxins
  - Chemicals
  - Drugs
  - Iron
- Viral hepatitis
  - Equine infectious anemia (EIA)
  - Equine herpesvirus–1 (EHV-1)
  - Equine viral arteritis (EVA)
  - Giant cell hepatopathy

CHRONIC DISEASE

- Pyrrolizidine alkaloid toxicity
- Clover poisoning
- Chronic active hepatitis (CAH)
- Cholelithiasis
- Gastroduodenal obstruction
- Abscess
- Neoplasia
  - Metastatic infiltration of the liver
  - Cholangiocarcinoma
  - Hepatocellular carcinoma
  - Hepatoblastoma
  - Mixed hamartoma
- Amyloidosis
- Chronic hypoxia

CONGENITAL OR HERITABLE DISEASE

- Portosystemic shunt
- Biliary atresia
- Hyperammonemia of Morgans
- Glycogen branching enzyme deficiency

A pattern of seasonality may occur, with a larger percentage of cases presenting in summer and fall.[32,116] Frequently, horses with Theiler's disease have received an equine-origin biologic antiserum 4 to 10 weeks before the onset of hepatic failure, hence the name *serum-associated hepatitis.* Equine-origin biologic antiserums that have been associated with Theiler's disease include antisera for African horse sickness, eastern and western encephalomyelitis, *Bacillus anthracis,* tetanus antitoxin, *Clostridium perfringens, C. botulinum, Streptococcus equi* subsp. *equi,* influenza, equine herpesvirus type 1 (EHV-1), pregnant mare's serum, and plasma.[32,86,156,157,159] Some reports suggest that lactating broodmares given tetanus antitoxin after parturition are particularly prone to Theiler's disease.[156-158] Although the overall incidence of Theiler's disease in adult horses receiving tetanus antitoxin is low, tetanus antitoxin has for the past 40 years been the most common blood product associated with the disease in the United States.[116] Theiler's disease may be more commonly associated with tetanus antitoxin administration than equine plasma because of either the more frequent administration of tetanus antitoxin to horses or the fact that tetanus antitoxin is produced as a pooled product whereas commercial units of normal plasma are most often collected from a single donor. Reports of outbreaks in association with parenteral injection of homologous live virus vaccine and antiserum suggest an infectious blood-borne viral cause, and recently three newly discovered viruses (see below) have been found to be associated with the disease.[116,162] Not all cases of Theiler's disease are associated with recent administration of equine-origin plasma or serum products. Theiler's disease that is not associated with blood products appears to be most common in late summer through autumn, making the possibility of insect transmission of a viral pathogen plausible.[116] The history, onset, clinical signs, and histopathologic findings of Theiler's disease appear most similar to hepatitis B virus in human beings. Hepatitis B virus is present in all body fluids and excreta and is transmitted primarily by parenteral injection.[37]

Other previously suggested causes of Theiler's disease include exposure to hepatotoxic substances, such as mycotoxins (aflatoxin and rubratoxin), plant toxins (pyrrolizidine alkaloids, alsike clover), drugs, or chemicals.[100,158] However, these causes have not been demonstrable and usually are considered separate disease entities.

Pathology. Aside from widespread tissue icterus, pathologic findings are mostly limited to the liver. The liver appears smaller than normal in most cases. Although not pathognomonic, the histopathologic findings in Theiler disease consistently include widespread centrilobular to midzonal hepatocellular necrosis and apoptosis with hemorrhage (Fig. 13.7).[160] Moderate to severe centrilobular hepatocyte vacuolar (fatty) change and granular swelling, hemosiderosis, and bile casts may be seen.[156] A mild inflammatory infiltrate, primarily monocytes and lymphocytes, is present in portal triads. The occasional finding of mild to moderate biliary hyperplasia and fibroblastic infiltration is evidence of a slightly more chronic disease course than the clinical signs alone would suggest.[160]

Diagnosis. Recent (4–10 weeks earlier) inoculation with an equine-origin biologic antiserum or plasma, coupled with an abrupt onset of clinical signs and laboratory evidence of hepatic insufficiency, strongly suggests Theiler's disease. On ultrasound examination, the liver may subjectively appear small and hypoechoic. No single laboratory test is diagnostic. Bilirubinemia and bilirubinuria are usually present.

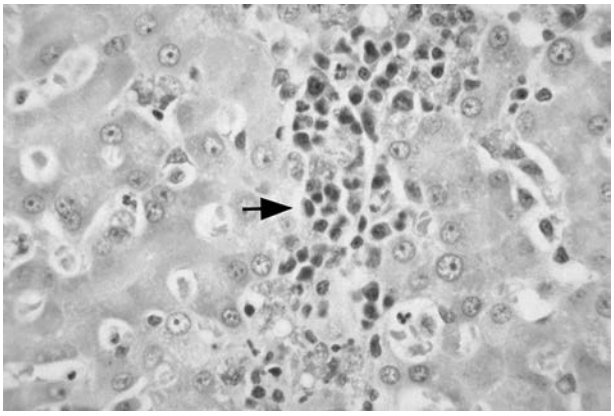


FIG. **13.7** Histopathology of the liver: Theiler's disease. Note the central area of hepatic necrosis *(arrow).*

Although both unconjugated and conjugated levels are increased, the conjugated portion generally remains less than 25% of the total.[116] Liver enzymes, including SDH, GLDH, ARG, and AST, are increased several fold. GGT is increased but not of the magnitude as are the hepatocellular enzymes.[116] The BSP half-life, PT, and APTT are often prolonged, and blood ammonia concentrations are increased. Acidosis, leukocytosis, polycythemia, and increases in serum creatine kinase activity have also been reported.[156-158] Hemolysis may occur terminally. The histopathologic findings on a liver biopsy (see previous pathogenesis section) offer the strongest diagnostic evidence of Theiler's disease.

Treatment. No specific treatment for Theiler's disease exists aside from general supportive care for hepatic insufficiency (see Treatment of Hepatic Insufficiency earlier in this chapter). Although it might be tempting to treat with interferon because of the strong possibility of viral etiology, it should be remembered that the onset of disease might be associated with attempted clearance of the virus.[163]

Prognosis and Client Education. The prognosis is poor to grave in horses with severe HE, hemorrhage, or hemolysis. Horses that survive longer than 1 week usually recover completely, although death or progressive weight loss has on rare occasions been reported during the ensuing months.[157] A favorable prognosis is warranted if serial SDH or GLDH activities are decreasing or clinical signs are waning and the horse is still eating.[116] Increased serum activity of GGT may be sustained for several weeks, despite clinical improvement and long-term survival. [116,156] No preventive agent exists, aside from the judicious use of equine-origin antiserum.

Newly Discovered Equine Hepatitis Viruses

Recently, four distinct equine hepatitis viruses have been discovered in horses: nonprimate hepacivirus (NPHV; also called hepacivirus), Theiler's disease–associated virus (TDAV), and equine pegivirus (EPgV), which are all close Flaviviridae family relatives of human hepatitis C viruses[162,164,165] and a very recently discovered DNA virus called *equine parvovirus.*[116] The horse appears to be unique among domestic animals for harboring these so-called hepatitis viruses.[166] The recent discovery of these *equine hepatitis viruses* is directly related to the use of the newer technology involving nucleic acid deep sequencing of samples. Unknown nucleotides are then reassembled using de novo computerized sequencing to identify new agents.[162]

Nonprimate Hepacivirus. Nonprimate hepacivirus was the first of the three new blood-borne viruses to be reported.[164] NPHV was found in pooled equine serum and in the serum of 8 of 103 (7.8%) normal horses, of which 35% were NPHV antibody positive; samples tested were those submitted for equine infectious anemia testing at Cornell, and it was therefore assumed that horses were healthy. Since that description in the United States, NPHV viremia has been reported during routine serum screening in 3 of 142 horses (2.1%) in Great Britain and in 7 of 210 horses (3.3%) from Germany.[167,168] Multiple NPHV infections have also been reported in Japan and Brazil.[169,170] Similar incidence of NPHV infection in clinically healthy horses is found in France (Stephane Pronost, personal communication). Currently, accumulated data from Europe, North America, and Asia indicate that nearly 40% of all adult horses are seropositive, prevalence of active infection is 4%, and approximately one fifth of those 4% are chronic carriers.[167-169] Infections from either of these three newly discovered flaviviruses (NPHV, TDAV, EPgV) are uncommon in mules or donkeys.[171] NPHV is a member of the *Hepacivirus* genus in which human hepatitis C viruses (HCV) are the prototype members, and of the three hepaciviruses found in horses, NPHV is most closely related genetically to HCV. NPHV has also been found commonly in bats, rodents, and Old World monkeys, and it is the only one of the three recently discovered horse flaviviruses known to be hepatotropic in the horse.[163,166] As regards clinical importance, the testing of a somewhat random assortment of naturally infected horses has indicated that there is little documented association between NPHV infection and clinical hepatic disease in those horses.[167,171] In NPHV experimentally infected horses there is mild disease documented by elevations in liver enzymes and lymphocytic portal inflammation and piecemeal hepatocyte necrosis.[163,172] The elevations in liver enzymes seem to occur shortly after development of measureable antibodies, suggesting that antibody-mediated activity related to the virus could cause some disease. When foals with severe combined immunodeficiency (SCID) were experimentally infected,[172] they produced no antibody and there was no increase in GGT, further supporting an immune response to the infection and association with disease.[172] The only report of clinical disease associated with NPHV infection is a case report of Theiler's disease with a horse having a high level of NPHV viremia and as the viremia diminished the horse recovered.[173] Although the data set is small, most horses that are chronically infected with NPHV do not have biochemical or clinical findings suggestive of liver disease.[116] Most horses infected with NPHV appear to have relatively slow development of NPHV-specific antibody (approximately 6–8 weeks)[163,172] followed by viral clearance in most horses in the following months. A seronegative horse inoculated with infectious clones of NPHV resulted in high RNA titers in the serum and liver of the horse establishing the molecular components of a functional NPHV genome. Delayed seroconversion, with elevated circulating liver enzymes, and mild hepatitis were observed, followed by viral clearance. Hepatotropism was confirmed by level of viremia in the liver and by finding negative-strand RNA in the liver, a hallmark of viral replication.[163]

Theiler's Disease–Associated Virus. The second equine hepatitis virus was discovered in 2012 in association with an outbreak of acute hepatitis in horses that had previously received botulinum antitoxin.[162] Seven of 17 horses treated with one particular lot of botulinum antitoxin developed clinical and/or biochemical evidence of hepatitis 6 to 8 weeks after the antitoxin administration. Hepatitis was not observed in five horses that were treated with a separate lot of botulinum antitoxin produced from different horses or in 53 farm horses that had not received antitoxin.[162]

This flavivirus virus was named *Theiler's disease–associated virus* (TDAV).[162] A reverse transcriptase–polymerase chain reaction (RT-PCR) then was used to test the serum of horses for the newly discovered equine hepatitis-associated virus (TDAV). All 17 of the horses that received the incriminated lot of botulinum antitoxin were positive for the new virus, including the 7 horses with acute hepatitis.[162] The original 17 TDAV-positive horses were retested after 1 year by qRT-PCR, and TDAV was undetectable in 13 of the previously infected horses. However, in 4 horses, persistent TDAV viremia was demonstrated.[162] A large portion of the horses from the index farm have been retested since the hepatitis outbreak, and no additional horses have become PCR positive for the virus, suggesting that direct horse-to-horse transmission or transmission by insect vectors may not occur or is not common. Follow-up studies since 2012 find that TDAV appears to be a relatively rare virus in the horse population, and it has not been found in horses in the United Kingdom and France or in any other species so far.[167] Two divergent strains of the virus were found in a European commercial equine serum product.[174] TDAV has not been found in tetanus antitoxin–associated cases of Theiler's disease. It is therefore likely that TDAV might not be a cause of Theiler's disease and that the liver disease reported in the previous outbreak may have been caused by a coinfecting virus .[116]

Equine Pegivirus. The third equine blood-borne equine flavivirus was reported by Kapoor et al.[165,167] and has been named the *equine pegivirus*. EPgV and TDAV are both members of the *Pegivirus* genus but are genetically distinct.[162,165] Pegiviruses, although a common infection in humans and other species, are not known to cause clinical disease in other species.[165,166] Three of the original 12 EPgV-infected horses from Alabama were reported to have had elevated liver enzymes but were not clinically ill.[165] qRT-PCR testing of both lymph node biopsy and liver biopsy tissue from infected horses failed to demonstrate hepatotropism of the virus.[165] Two of the horses were shown to be carriers of EPgV for at least 3.5 years. Although there are few data to support an association between EPgV infections and clinical equine liver disease at this time, this possibility cannot be ruled out.[167] Although all three equine flaviviruses (NPHV, TDAV, and EPgV) are believed to be transmitted by blood, the high seroprevalence of both NPHV and EPgV suggest that there must be modes of transmission other than plasma and antitoxin inoculations.[164,167] The role and importance of coinfection in disease are unknown.

Equine DNA Virus. The most recently discovered virus is an equine DNA virus called *equine parvovirus*, and it has been found in consecutive horses with Theiler's disease that received tetanus antitoxin or other equine-origin blood products and in a farm outbreak of Theiler's disease not associated with administration of equine-origin blood products. Experimental inoculation of a virus-negative and seronegative aged horse with a commercial product containing the virus caused a late-onset viremia (7 weeks), seroconversion, and severe hepatic disease. Further studies are under way, but this newly discovered DNA virus now appears to be the virus most closely associated with Theiler's disease in the horse and is proven on experimental inoculation to cause liver disease in horses. Some healthy horses are persistently viremic and antibody positive.

Bacterial Hepatitis

Tyzzer's Disease. *Clostridium piliforme* (formerly *Bacillus piliforme*) is a motile, spore-forming, obligate intracytoplasmic bacterium that causes acute necrotizing hepatitis. The disease was first described by E. E. Tyzzer in 1917 in a colony of waltzing mice.[174] The first documented outbreak in foals occurred in Kentucky on a single farm between 1964 and 1973, fatally infecting 23 foals.[175]

Clinical Signs. In horses, Tyzzer's disease is limited to foals between 7 and 42 days of age (average age 20 days).[176-178] The clinical signs are usually nonspecific and include loss of a suckle reflex, depression progressing to recumbency, fever, tachypnea, tachycardia, icterus, petechiation, diarrhea, dehydration, shock, seizures, and coma.[176-182] Often the foals, especially younger ones, are found dead without premonitory signs.

Epidemiology and Pathogenesis. *C. piliforme* is thought to be common in the environment, but because it is difficult to culture little is known about the epidemiology and pathogenesis of the disease. Outbreaks of Tyzzer's disease are sporadic; thus the disease is not believed to be contagious.[178] The overall incidence is likely less than 0.1% of foals per year.[178] Multiple cases have been reported on some farms, and increased environmental contamination and bacterial colonization of the mares leading to increased fecal-oral transmission are possible.[178] This was not believed to be a breed or gender predisposition. Relative risk of disease appears to be greater in the later months of the foaling season.[178] The disease has been reported in the United States, Canada, South Africa, England, and Australia.[176-182] Mice with Tyzzer's disease are infected by an oral route perinatally, with clinical signs and death occurring between 6 and 44 days of age.[178] *C. piliforme* is excreted in the feces of clinically healthy horses and can survive in the soil for at least 1 year.[177,178] Foals are infected by eating contaminated feces of their dams or by ingesting contaminated soil.[178] The bacteria replicate in the intestinal epithelium and reach the liver and heart by way of the lymphatics and blood supply.[178] In rodents and rabbits, administration of corticosteroids or sulfonamides may induce active disease.[177] The presence of antibody to the flagella of *C. piliforme* in the serum of healthy horses suggests that exposure to *C. piliforme* is not uncommon.[183]

C. piliforme causes acute multifocal hepatitis, myocarditis, and enteritis.[178] Grossly, the liver is swollen, with 1- to 5-mm white foci scattered throughout the parenchyma. Tissues are icteric, and petechial hemorrhages are present in many tissues. Microscopically, the foci are areas of coagulative necrosis with an associated inflammatory infiltrate consisting of neutrophils, macrophages, and lymphocytes. The organisms, best demonstrated at the periphery of the lesions by Warthin-Starry or Dietrerle's silver stains, appear as long bacilli organized in bundles. In addition to hepatitis, enterocolitis, myositis, pleural effusion, pulmonary congestion and edema, and lymphoid necrosis or depletion may be present.[177,180]

Diagnosis. The peracute onset and nonspecific signs confound antemortem diagnosis of Tyzzer's disease. Hematologic study may reveal hemoconcentration, leukopenia or leukocytosis, a left shift, and toxic neutrophils.[176-182] Other nonspecific laboratory findings include hyperfibrinogenemia, acidosis, hyperkalemia or hypokalemia, and profound hypoglycemia.[179,181,182] Liver enzymes are increased, including SDH, GGT, AST, ALP, and LDH-5.[176-182]

The definitive diagnosis is determined at necropsy by demonstration of the organism intracytoplasmically using silver stains or by polymerase chain reaction (PCR) analysis.[178,182] *C. piliforme* is fastidious and can only be grown in living tissue, such as embryonated chick eggs.[182] Positive serology may be supportive evidence for exposure.[183] Differential diagnosis for hepatic disease in young foals includes iron hepatotoxicity, perinatal EHV-1, leptospirosis, *Listeria monocytogenes* or *Bartonella* spp. infection, other causes of bacteremia, atresia of the bile duct, and portosystemic shunt.[184-186]

Treatment. Tyzzer's disease is highly fatal in foals. In rodents and rabbits, *C. piliforme* is sensitive to penicillin, tetracycline, erythromycin, and streptomycin. Rare reports exist of successful treatment of foals with Tyzzer's disease using penicillin, TMS, and partial parenteral nutrition.[179,182] The only confirmed case of Tyzzer's disease (PCR positive on liver biopsy) successfully treated occurred in a foal treated with ampicillin, gentamicin, and fluid and nutritional support.[182]

Prognosis and Client Education. Tyzzer's disease in foals is considered highly fatal. No preventive measures are known.

Infectious Necrotic Hepatitis

Clostridium novyi type B is the cause of *infectious necrotic hepatitis*, or *black disease.* The disease is most common in sheep and cattle, but cases have been documented in horses.[84,187-190]

Clinical Signs. The onset of disease may be peracute with sudden death. Acute signs are progressive over 24 to 72 hours and include depression, reluctance to move, fever, icterus, ataxia, colic, petechiae, periods of recumbency, tachycardia, and tachypnea.[84,187-190] Some affected horses remain standing until a short time before death.

Epidemiology and Pathogenesis. Reported cases of infectious necrotic hepatitis in horses have occurred in or adjacent to areas with a large population of sheep.[84,187-190] In sheep and cattle, infectious necrotic hepatitis is the result of multiplication of *C. novyi* in areas of liver compromised by the migration of *Fasciola hepatica.* This fluke normally does not infest horses, but migration of any parasite through the liver may predispose the animal to infectious necrotic hepatitis. Some horses with infectious necrotic hepatitis had parasitic infestations, although migrating parasites in the liver at the time of necropsy was not evident. The onset of disease in one case occurred 48 hours after administration of mebendazole.[84]

The carcass blackens rapidly after death from engorgement of subcutaneous blood vessels; hence the name *black disease.* Often, necropsy findings include serosanguineous effusion in the pericardial sac and the thoracic and abdominal cavities, widespread hemorrhages and icterus, and multifocal areas (1 to 2 mm) of coagulative hepatic necrosis. Smears or histologic sections of the hepatic lesions reveal large numbers of gram-positive rods.

Diagnosis. The acute and nonspecific nature of this disease makes antemortem diagnosis improbable. Liver-specific enzymes and bilirubin are mildly to moderately increased.[84,187-190] An abdominocentesis was performed on one horse and revealed serosanguineous peritonitis.[84] Differential diagnosis should include hepatic lobe torsion, portal vein thrombosis, and other diseases that may cause focal infection and infarction. Definitive diagnosis is based on positive staining with fluorescein-conjugated antiserum specific for *C. novyi* or isolation of the organism at necropsy.[187-190] The organism is difficult to isolate and requires rapid tissue sampling and anaerobic conditions.

Treatment. Treatment with high doses of penicillin or ampicillin is indicated, in addition to general supportive care for hepatic insufficiency.

Prognosis and Client Education. Most reported cases of infectious necrotic hepatitis in horses were fatal.

Miscellaneous Causes of Bacterial Hepatitis

Primary bacterial hepatitis is rare in adult horses. Bacterial septicemia in neonates may result in multifocal hepatitis, although clinical signs of hepatic failure rarely accompany the disease. Bacterial endotoxin, released during acute fulminant gram-negative infection or absorbed from the gastrointestinal tract after mural damage, may cause hepatic ischemia indirectly through hemodynamic alternations. Because Kupffer cells normally phagocytose endotoxin, bombardment of the liver with endotoxin may temporarily impede the function of Kupffer cells. *Cholangiohepatitis* in horses is most often a sequela to biliary stasis, cholelithiasis, CAH, hepatic neoplasia, pancreatitis, intestinal parasitism, intestinal obstruction, and proximal enteritis.[81,191-196] Clinical signs include anorexia, colic, fever, and icterus; HE may be present if there is extensive fibrosis. Laboratory abnormalities may include leukocytosis; toxemia; hyperfibrinogenemia; increased activity of SDH, ARG, GGT; increased direct-reacting bilirubin; and evidence of septic or nonseptic peritonitis.[191] Definitive diagnosis is based on histopathologic findings on liver biopsy and bacterial isolation. Isolates are most commonly enteric organisms such as *Salmonella* spp., *Escherichia coli, Citrobacter, Klebsiella, Aeromonas, Clostridium,* and *Acinetobacter* spp.[81,191,193] Treatment includes general supportive care for hepatic failure (see Treatment of Hepatic Insufficiency earlier in this chapter) and at least 4 to 6 weeks of antimicrobial therapy. The antimicrobial therapy should be based on culture and sensitivity results. Recommended antimicrobials for bacterial cholangiohepatitis in horses include trimethoprim-sulfonamide, ceftiofur, ampicillin or enrofloxacin in combination with metronidazole, penicillin and gentamicin, and chloramphenicol.[81,191]

Multisystemic Viral and Bacterial Causes of Hepatitis

Equine Herpesvirus Infection in Foals. Pregnant mares infected with EHV-1 may abort in the third trimester or deliver stillborn or weak foals. Some foals may appear normal at birth but develop clinical signs of respiratory distress, icterus, fever, and severe depression.[196,197] Secondary bacterial septicemia is common. Despite profound hepatic necrosis in herpesvirus-positive foals, serum enzyme activities were not significantly different from premature foals or foals with septicemia.[197] Pathologic lesions of aborted fetuses or neonatal foals are similar and include severe pulmonary congestion, pneumonitis, bronchiolitis, hyaline membrane disease, and intralobular coagulative hepatocellular necrosis.[196,197] Intranuclear, acidophilic inclusions are present in hepatocytes and biliary epithelium. General supportive care and prophylactic administration of broad-spectrum antimicrobials are indicated. The prognosis is poor to grave. Most affected foals die within a few days of birth. Vaccination of pregnant mares is only partially efficacious for prevention.

Equine Infectious Anemia. The causative organism of equine infectious anemia is a retrovirus that has a tropism for mononuclear phagocytes.[198] Although cyclic fever, anemia, icterus, edema, and weight loss are the systemic manifestations of the disease, the Kupffer cells in the liver are a major site of infection. Icterus is due to increased erythrocyte destruction, as well as acute hepatic necrosis.

Equine Viral Arteritis. This disease is caused by a member of the Arteriviridae family and typically is manifest clinically by depression, fever, acute upper respiratory tract infection, abortion, petechiation, and edema. After ingestion or inhalation, viral septicemia leads to vascular damage. Overt clinical signs of hepatic disease are rare, but vascular damage in the liver may cause icterus.[199]

Giant Cell Hepatopathy. A disease similar to human neonatal hepatitis has been reported in aborted midterm fetuses.[200] Histologically, disorganization of hepatic cords, multifocal necrosis, a mild mononuclear cell infiltrate, and a large hepatocyte syncytium with 8 to 10 nuclei exist. The cause in horses and human beings is unknown but may be the result of viral or bacterial infections including leptospirosis.[200]

Parasitic Hepatitis

Parasitic infestation may cause focal hepatic disease but rarely overt hepatic insufficiency. After their ingestion, as embryonated eggs, larvae of *Parascaris equorum* hatch in the small intestine and then migrate through the liver and lungs. Focal or diffuse hepatic fibrosis may result.[113] Migration of *Strongylus edentatus* or *S. equinus* through the hepatic portal veins may cause focal hepatitis, subcapsular hemorrhage, and edema, followed by focal parenchymal fibrosis and capsular fibrin deposits.[113] Focal hepatic infarcts may occur secondary to thrombotic emboli because of migrating *S. vulgaris* in mesenteric arteries. *Heterobilharzia americana* causes a remarkable starry sky image of the liver on ultrasound examination, but horses have no signs of liver disease.[201] Protozoal schizonts consistent with a *Sarcocystis* spp. other than *S. neurona* were reported at necropsy in the liver of a horse with concurrent bacterial osteomyelitis, a plasma cell tumor of the maxilla, and hepatic salmonellosis.[202] The canine cestode, *Echinococcus granulosa,* may form hydatid (larval) cysts in the liver, which is usually an incidental finding.[203,204]

Fibrosing granulomas were found in the liver, intestinal and diaphragmatic serosae, and lung of several horses submitted for necropsy.[205] The granulomas consisted of dense fibrous tissue surrounding a necrotic, laminated, mineralized center. The periphery of the granuloma contained a small rim of inflammatory cells. The typical architecture of the granulomas, combined with the finding of a residual eggshell typical of *Schistosoma* (a trematode) in one horse, led to the conclusion that the granulomas were caused by chronic schistosomiasis. Although the granulomas were considered to be incidental findings of undetermined origin in most of the horses, they caused liver failure in one horse.

Toxic Hepatopathy

Numerous chemicals, drugs, mycotoxins, and plant toxins are hepatotoxic, but only occasionally do these cause hepatic failure in horses. Clinical signs and routine laboratory diagnostic tests will generally not distinguish among these toxins; thus diagnosis largely relies on exclusion of other causes, history of exposure, and, in some cases, documentation of the toxin in the blood or liver. Some substances cause fatty change, cloudy swelling, necrosis, mild inflammatory infiltration, and fibrosis, primarily in the centrilobular location where the oxygen tension is lowest. Other substances cause the same lesions

periportally, in the area of initial exposure. Some substances are directly hepatotoxic, whereas others require biotransformation by the liver to toxic metabolites.

Hepatotoxic Plants. Plants best known for causing hepatic disease in horses are those containing pyrrolizidine alkaloids. Although pyrrolizidine alkaloid toxicity can cause acute hepatic necrosis, it more often results in hepatic fibrosis (and is discussed as a separate entity under Chronic Hepatic Diseases later in this chapter). Other plants that have been reported to cause hepatic necrosis in horses in North America include kleingrass *(Panicum coloratum, P. dichotomiflorum, P. virgatum),* lantana, lechuguilla *(Agave lechuguilla),* alsike clover *(Trifolium hybridum),* whitebrush (*Lippia* spp.), sneezeweed (*Helenium* spp.), blue-green algae (*Microcystis* and *Nodularia* spp.), lupine (*Lupine* spp.), ryegrass, and some poisonous mushrooms (*Amanita* and *Galerina* spp.).[149]

Chemical Hepatotoxins. Horses are rarely exposed to hepatotoxic chemicals in sufficient amounts to induce hepatic failure. Potential hepatotoxic chemicals include arsenic (pesticide), carbon tetrachloride (fumigant), chlorinated hydrocarbons (insecticide), carbon disulfide (botacide), pentachlorophenols (wood preservative, herbicide, fungicide), phenol (disinfectant, wood preservative), phosphorus (fertilizer), polybrominated biphenyl (fire retardant), and paraquat (herbicide).[113] Phosphine gas, associated with feeding a pelleted ration treated with aluminum phosphide, has also caused hepatic failure in a group of horses.[206] All of the above cause centrilobular necrosis, except phosphorus, which causes primarily periportal changes.

Drugs. Pharmaceutical agents can have a wide range of effects on the liver depending on the drug type, dose, frequency, duration, and route of administration; age of the animal; diet; and concurrent treatment.[207] The hepatic damage may be acute or chronic, cholestatic, zonal, vascular, or hypersensitivity mediated. Certain drugs alter hepatocellular permeability without visible injury or loss of function.

Drugs that are intrinsically hepatotoxic reproducibly cause hepatocellular necrosis.[37,113] Examples of intrinsic hepatotoxic drugs that cause zonal centrilobular necrosis include carbon disulfide and carbon tetrachloride.[113] These drugs cause hepatocellular damage in a dose-related manner, and toxicity often results in hepatic failure. Anabolic steroids cause cholestasis with little or no evidence of hepatic damage or inflammation, resulting in mild icterus, which is completely and rapidly reversible once the drug is discontinued.[37,207] Phenothiazines and macrolide antibiotics on rare occasions cause cholestatic injury, which is accompanied by significant hepatocellular necrosis and periportal inflammation.[37] Imidocarb can cause severe hepatic disease in donkeys but does not appear to do so in horses.[208] Recovery is usually expected after discontinuation of the drug. Some drugs, such as tetracycline, cause fatty infiltration of the liver but rarely cause liver dysfunction.

Idiosyncratic hepatotoxicity has been reported after administration of erythromycin, rifampin, tetracycline, isoniazid, halothane, fluothane, phenothiazines, dantrolene, diazepam, sulfonamides, phenobarbital, phenytoin, and aspirin.[37,113,149] Injury varies from mild focal hepatitis to massive necrosis. A severe toxic hepatopathy has been described in nursing foals being treated for *Rhodococcus equi* with both rifampin and doxycycline.[209] Idiosyncratic hepatopathy has a low incidence, is not dose related, and often resolves on discontinuation of the drug. Rarely, progressive liver failure ensues. Some idiosyncratic drug hepatopathies may have a pathogenesis similar to drug allergy; others may be related to biotransformation properties of the liver. For example, if an individual has enhanced biotransformation of a certain drug and the metabolite of the drug is more cytotoxic than the original unaltered drug, hepatic damage may ensue.

Finally, some drugs induce increased hepatic enzyme activities or alter hepatocellular permeability but do not cause significant hepatic damage or clinical signs. Examples include benzimidazole anthelmintics, phenobarbital, phenylbutazone, and corticosteroids. Compared with humans and dogs, corticosteroids are apparently less likely to cause hepatopathy in horses; however, one report describes steroid hepatopathy in a horse that received an overdose of triamcinolone for treatment of pruritus.[210] Three weeks after the overdose, the horse developed muscle wasting, depression, polydipsia, and laminitis. Mature neutrophilia, lymphopenia, hyperglycemia, increased serum activities of AST and GGT, and multizonal hepatocellular vacuolation were reported.

Iron Toxicity. Acute, fatal toxic hepatopathy was reported in numerous newborn foals given an oral microbial inoculum.[211] The syndrome is reproducible if *ferrous fumarate* is given orally prior to administering colostrum.[211] The incidence of hepatic failure was highest when the foals were given the inoculum before nursing. Colostrum contains abundant vitamin E and essential cofactors for glutathione. Glutathione protects against free radical damage, a proposed mechanism for iron toxicity. Thus foals that are given iron supplements before ingesting colostrum presumably are most susceptible to iron toxicity.[211] This is exacerbated by high serum iron concentrations and high percent saturation of transferrin in foals during the first 24 hours of life. Supplementation of iron during this time period results in excessive free iron and increased risk of fatal hepatotoxicity.

Clinical signs include HE, icterus, and peracute death. Laboratory abnormalities include increased blood levels of bilirubin, SDH, GGT, and ammonia; reduced BCAA-to-AAA ratio; hypoglycemia; and prolonged PT.[211] At necropsy the liver was abnormally small; histologic study revealed massive hepatic necrosis with blood-filled reticula. Some livers had mild biliary hyperplasia and periportal fibrosis. Two interesting findings from the unfortunate outbreak were that periportal fibrosis and presence of Alzheimer type 2 cells in the brain could both occur within 48 hours of toxin administration.[212] Supportive therapy may prolong life, but most foals with iron hepatotoxicosis and clinical signs die. Another less well-understood hepatic failure possibly associated with iron overload has occurred in foals with neonatal isoerythrolysis that required one or more blood transfusions.[213] Affected foals may have progressive liver disease (rising liver enzymes) within days after they receive one or more transfusions, and these foals may exhibit liver failure as soon as 5 days following transfusion or up to 3 months later. Histologic lesions in the liver consist of hepatocellular necrosis with extensive biliary proliferation. An experiment to reproduce the disease by giving young healthy foals multiple blood transfusions was unsuccessful, suggesting that a combination of hypoxia from neonatal isoerythrolysis and iron from the transfused red blood cells may be responsible for the hepatic disease.[214]

Adult horses appear to be less susceptible to iron toxicity than many other species. In one study ponies fed 50 mg/kg of ferrous sulfate for 8 weeks (National Research Council requirement is 1–2 mg/kg body weight) had no clinical signs or

histopathologic lesions in the liver although hepatic iron, serum iron, and percent saturation of transferrin increased.[215] However, rare cases of hemochromatosis have been reported.[216,217] Hemochromatosis is characterized by tissue damage and dysfunction caused by deposition of hemosiderin in parenchymal cells.[216] Hemochromatosis was diagnosed in three adult horses by histopathology and use of Prussian blue stain, which detects iron deposition (hemosiderin pigment).[216] Excessive dietary iron was not apparent. All three of the horses had signs and laboratory evidence of liver disease. Serum iron concentration was normal, and there was no evidence of saturation of the iron transport system in the serum. Hemochromatosis was accompanied by bile duct hyperplasia and hepatic fibrosis. All three horses died or were euthanized because of deterioration. Hemochromatosis was diagnosed in a pony receiving approximately 4 times the daily recommended amount of iron.[217] The diagnosis was confirmed by histopathology (severe periportal fibrosis, hemosiderosis, biliary hyperplasia) and increased serum and liver concentrations of iron. *Hemosiderosis* is the term used to describe iron deposits in Kupffer cells and hepatocytes without destruction of liver parenchyma and is a common finding during histologic examination of the normal equine liver, much of which is due to phagocytosis of heme from broken-down red blood cells. Both processes are more commonly seen around the periportal region of the liver.[26]

Mycotoxins. Feeding horses grains that are sufficiently contaminated with *Fusarium* causes the classic disease leukoencephalomalacia. The mycotoxin can also cause a concurrent hepatopathy with marked elevations in AST, GGT, and bilirubin. Lesions in the liver include hepatocyte vacuolation with centrilobular fatty change, hepatocyte necrosis, mild mononuclear infiltrate, mild bile duct proliferation, and periportal fibrosis.[218,219] Unlike in swine and some other species, liver failure caused by aflatoxins in horses is rare. It has been reported that the difference in aflatoxin toxicity in the horse could be related to differences in some of the hepatic P450 activity detoxifying aflatoxin.[220] This finding suggested that horses have microsomal-associated glutathione *S*-transferase with the capacity to collapse GSH-conjugation of reactive and toxic aflatoxin B_1, B_8, B_9 epoxide.

Experimental administration of aflatoxin B_1 to ponies has produced variable responses, from no or minimal disease (elevated liver enzymes but no clinical signs), to severe illness and death.[221-223] Prior exposure to the mycotoxin AFB_1, which affects hepatic metabolism on subsequent exposure, and duration of exposure before studies may explain the different results.[224] The target organ in horses, as in other animal species, is the liver, and horses suffering from aflatoxicosis show signs of inappetence, depression, fever, tremor, ataxia, and cough. Necropsy findings include a yellow-brown liver with centrilobular necrosis, icterus, hemorrhage, tracheal exudates, and brown urine.[222]

Acute Biliary Obstruction

Acute obstruction of the common bile duct causes icterus and signs of abdominal pain. Acute biliary occlusion may occur secondary to cholelithiasis (see Cholelithiasis) or colon displacement. Intense icterus, colic, and increased levels of direct bilirubin (greater than 85.5 µmol/L or greater than 5 mg/dL), GGT (greater than 400 U/L), and bile acids (greater than 150 µmol/L) were reported in two horses with acute colon displacements.[225] These abnormalities quickly abated after surgical correction of the displaced colon. In a comparative study of cases of colon displacement, 49% of horses with a right dorsal colon displacement had increased GGT, compared with 2% of horses with a left dorsal displacement.[114] Torsion of the left lobe of the liver was the cause of acute colic, hepatic congestion, necrosis, and focal hepatitis in a 14-year-old Arabian gelding.[226] Resection of the affected lobe resulted in complete recovery. Additional mechanical causes of biliary obstruction include hepatic neoplasia, pancreatitis, and duodenal ulcerations in foals that occur either at or just caudal to the opening of the bile duct.[116]

Hyperlipemia and Hepatic Lipidosis

Hyperlipemia, a syndrome defined as a serum triglyceride concentration in excess of 500 mg/dL with grossly discolored plasma or serum (lipemia), occurs primarily in ponies and Miniature Horses and donkeys and may lead to fatty infiltration of the liver, often accompanied by clinical signs of liver disease and a guarded prognosis.[227] *Hyperlipidemia* refers to an increase in serum triglyceride concentration (generally less than 500 mg/dL) without grossly lactescent blood or fatty infiltration of the liver. Obesity, stress, inability to satisfy metabolic energy demands, and hormonal imbalance are the major precipitating factors for hyperlipemia.[227-233]

Epidemiology and Clinical Signs. Ponies (especially Shetland Ponies and mare ponies), Miniature Horses, and donkeys (especially miniature or aged) are most susceptible to hyperlipemia. Rarely, other breeds, such as the Quarter Horse, Paso Fino, and Tennessee Walking Horse, may be affected as are a low percentage of horses with Cushing's disease. Affected ponies are usually obese, have a recent history of stress or weight loss, and are in late gestation or early lactation during the winter months.[88] Females are most commonly affected because late pregnancy and lactation appear to be significant risk factors for the disease. The disorder appears to be common in older donkeys, which could be a reflection of concurrent Cushing's disease or dental problems in those animals.[234] In Miniature Horses and donkeys, hyperlipemia can occur at any age and frequently develops as a sequela to an underlying primary illness lasting several days. The most commonly reported primary diseases predisposing Miniature Horses to hyperlipemia are enterocolitis, endotoxemia, parasitism, pituitary dysfunction, azotemia, and neonatal septicemia.[232,233] The onset of clinical signs of hyperlipemia is often acute and includes icterus, anorexia, weakness, severe depression, ataxia, muscular weakness, recumbency, diarrhea, mild colic, fever, and dependent edema.[88,228-230] In severe cases, clinical signs of hepatic failure may prevail (see Clinical Signs of Hepatic Insufficiency earlier in this chapter). Sudden death because of hepatic rupture may occur.[229] Signs of predisposing primary disease in Miniature Horses may overshadow signs of hyperlipemia.

Pathogenesis. The liver serves a unique role in energy homeostasis (see Physiology), especially in large animals in which volatile fatty acids, and not glucose, are a major energy source. Glucose is primarily manufactured in the liver from fatty acids and amino acids and is stored as glycogen for future use. If intake is decreased or energy demands are increased, glycogen stores become depleted and the major source of energy is provided by fatty acid oxidation. Obesity sets the stage for hyperlipemia by providing excessive adipose tissue stores of fatty acids, available for immediate and rapid

mobilization. Fatty acid mobilization is usually first triggered by a stress or inability to maintain energy homeostasis. Concurrent or underlying primary diseases such as enterocolitis, azotemia, infection, parasitism, or neoplasia, as well as any stressful event, such as transport or weaning, may precipitate fatty acid mobilization. Stress increases the release of catecholamines and glucocorticoids, which stimulate fatty acid release from the adipose tissue. A negative energy balance, as may occur during late gestation, early lactation, starvation, or secondary to anorexia induced by some other primary disease, exacerbates hyperlipemia by further promoting fatty acid mobilization.

After lipolysis of adipose tissue triglyceride occurs, free fatty acids, nonesterified fatty acids, and glycerol are released into the blood. Glyceride, fatty acids bound to albumin, and nonesterified fatty acids are carried to the liver, where the glyceride is converted to glucose. In the liver, free fatty acids may be oxidized to acetyl CoA and used in the tricarboxylic acid cycle for ATP production, resynthesized to triglycerides and stored in the liver, or used to make triglycerides that are released into the sinusoidal blood as VLDLs (see Fig. 13.3). Ponies with hyperlipemia have larger-diameter VLDLs containing greater concentrations of triglycerides than normal ponies.[235,236] Thus hyperlipemia in ponies and Miniature Horses is the result of efficient and excessive hepatic synthesis of triglycerides from mobilized free fatty acids, with subsequent secretion of triglyceride-laden VLDL into the blood. Endothelial lipoprotein lipase is the rate-limiting enzyme responsible for the removal of VLDL from the blood back into adipose tissue. In hyperlipemic ponies, the activity of lipoprotein lipase is not impaired and in fact is increased several fold.[235] Thus it is the overproduction of VLDL by the liver, not impairment of their removal, which is primarily responsible for hyperlipidemia in ponies.[235,236]

If the oxaloacetate supply is limited when free fatty acids are mobilized to the liver, acetyl CoA is shuttled away from the tricarboxylic acid cycle and used to make ketone bodies. Because the equine liver is quite efficient in synthesizing triglycerides, ketosis is less common than in other species.[231] However, if fatty acid mobilization and synthesis of triglycerides exceed oxidation and VLDL secretion, hepatic lipidosis ensues. Fat infiltration disrupts hepatic function, and excessive amounts of fat in the liver can result in hepatic failure and even hepatic rupture.[229]

Hormonal factors may contribute to the development of hyperlipemia and hepatic lipidosis. Insulin normally impedes the development of hyperlipemia by inhibiting tissue hormone-sensitive lipase, the enzyme responsible for lipolysis of adipose tissue. Insulin also curtails the development of hyperlipemia by stimulating gluconeogenesis in the liver and by activating lipoprotein lipase, the enzyme responsible for uptake of VLDL by adipose tissue. Despite often normal insulin levels, ponies appear to have tissue insulin insensitivity compared with horses.[229] Fasting in donkeys has been shown to cause a reduction in tissue sensitivity to insulin.[237] Glucocorticoids, catecholamines, adrenocorticotropic hormone, thyroid-stimulating hormone, growth hormone, antidiuretic hormone, and progesterone may contribute to the development of hyperlipemia by opposing the biologic actions of insulin (see Fig. 13.3). This may account for the high incidence of hyperlipemia during periods of increased cortisol levels (stress, pituitary adenoma, late pregnancy) and increased progesterone (pregnancy). In ponies, norepinephrine linearly stimulates the release of free fatty acids from adipose tissue, an effect that does not occur in horses.[238] Considering the unique effects of insulin and catecholamines on fat metabolism in ponies, it is not surprising that ponies are more susceptible than horses to the development of hyperlipemia.

Vascular thrombosis can occur secondary to hyperlipemia and fat embolism and may be seen in the lung, kidney, brain, and subcutaneous vessels. Subcutaneous thrombosis causes dependent edema. Renal nephrosis and necrotizing pancreatitis are sometimes present.[88,229] Renal disease most likely is a sequela of occlusive thrombosis. The exact cause of pancreatitis is not known; however, hyperlipemia precedes pancreatitis in the equine hyperlipemia–hepatic lipidosis syndrome. Researchers speculate that excessive lipid is deposited in and around the pancreas, which is subsequently hydrolyzed by pancreatic lipase and released as free fatty acids. Free (unbound to albumin) fatty acids are cytotoxic; when the albumin-binding capacity is exceeded, pancreatic vascular injury occurs.[239]

Azotemia inhibits lipid removal from the blood by inhibiting lipoprotein lipase. The degree of hyperlipidemia is directly correlated with the degree of azotemia.[122] Almost half of hyperlipemic Miniature Horses are azotemic.[232,233] Compared with ponies and Miniature Horses, hyperlipemia rarely develops in horses except in horses with Cushing's disease, and in those horses, hyperlipemia does not cause the fulminant hepatic lipidosis seen in ponies, miniature equines, and donkeys.[240] Marked increases in serum triglycerides without hyperlipemia may occur in horses that are anorexic, especially horses with critical illnesses.[227]

Mild increases in triglycerides (less than 500 mg/dL) may develop during azotemia.[122,230] Mild hepatic lipidosis may ensue in these cases, but hyperlipemia does not occur, and the hyperlipidemia rarely results in clinically recognizable disease.

Diagnosis. Hyperlipemia should be considered in the differential diagnosis for any obese pony, donkey, or Miniature Horse with clinical signs of severe depression, anorexia, ataxia, and icterus. The normal triglyceride level in horses and ponies should be less than 85 mg/dL but may be up to 290 mg/dL in some clinically healthy donkeys and up to 250 mg/dL in some clinically healthy pregnant pony mares.[236] In hyperlipemia, the blood grossly appears opalescent (Fig. 13.8) and the concentrations of all lipids are increased, especially triglycerides (greater than 500 mg/dL), nonesterified fatty acids, and VLDLs. Laboratory evidence of hepatic disease may include increased serum activity of SDH and GGT; increased concentrations of serum bile acids, bilirubin, and ammonia; and decreased glucose, BUN, and albumin.[228] In hyperlipemic Miniature Horses, only about half of affected patients have significant hepatic impairment.[232] The BSP half-life is prolonged. Oral and intravenous glucose challenge tests may reveal glucose intolerance because of insulin insensitivity. Metabolic acidosis is frequent, and ketoacidosis should be suspected if the anion gap is increased. Lipemia may increase serum creatinine values falsely and interfere with accurate determination of other serum chemistries. A definitive diagnosis of hepatic lipidosis is confirmed by the concurrent demonstration of increased blood concentrations of lipid, laboratory evidence of hepatic dysfunction, and ultrasonographic or histopathologic findings of fatty infiltration in the liver (Fig. 13.9). The serum concentrations of creatinine and electrolytes should be determined as an adjunct

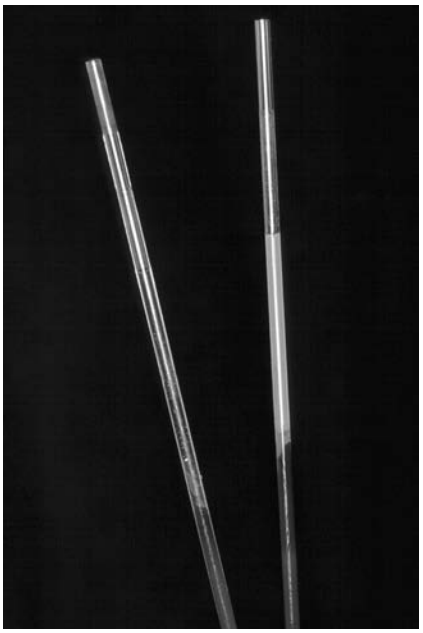


FIG. **13.8** Hyperlipemia. The spin hematocrit tube on the right is from a Miniature Horse with hyperlipemia. The hematocrit tube on the left is from an unaffected horse.

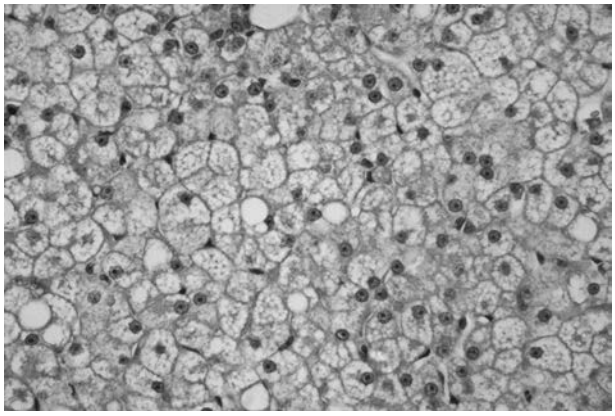


FIG. **13.9** Histopathology of the liver: fatty liver.

to therapy. Because of the incidence of hyperlipemia in ill Miniature Horses and donkeys, monitoring blood triglyceride levels would facilitate early recognition of hyperlipidemia in these patients.

Necropsy findings in affected ponies include widespread fatty change in the liver, skeletal muscle, kidney, adrenal cortex, and myocardium.[88,229] The liver and kidney are enlarged, yellow, friable, and greasy. One report has suggested that one of every five ponies with hepatic lipidosis has a ruptured liver.[229] Microscopically, hepatocytes are engorged with lipid. The nucleus is often displaced, and hepatocellular necrosis may ensue.

Treatment. The major therapeutic objectives for hyperlipemia and hepatic lipidosis include the following:

1. Treatment of hepatic failure, including hepatic encephalopathy
2. Improvement of energy intake and balance
3. Elimination of stress or treatment of concurrent disease
4. Inhibition of fat mobilization from adipose tissue
5. Increased triglyceride uptake by peripheral tissues

Treatment of hepatic failure is addressed elsewhere (see Treatment of Hepatic Insufficiency earlier in this chapter). Concentrated carbohydrate feed, such as molasses-coated grain and high-quality pasture or hay, should be encouraged. Anorectic ponies should receive 5% dextrose as a continuous infusion at a rate of 2 mL/kg per hour, and enteral nutrition should be attempted. NutriFoal, NutriPrime (KenVet, Ashland, OH), and Osmolyte HN (Ross Laboratory, Columbus, OH) have successfully been used as sources of enteral nutrition in hyperlipemic Miniature Horses.[232] Fat-free diet formulas have been proven successful when given via nasogastric tube in treating ponies with hyperlipemia.[241] These generally include glucose as the carbohydrate supply and are mixed with casein and alfalfa meal as a protein source; the combination of carbohydrate and protein intake is likely ideal. When commercially available enteral nutrition products are used, a low-fat or medium-chain triglyceride fat supplement is preferred for forced enteral feeding.[232]

If enteral nutrition is not possible, partial parenteral nutrition should be provided with equal volumes of 50% dextrose and 8.5% amino acids.[232,242] Monitoring blood glucose concentrations closely is mandatory, because some ponies have glucose intolerance (and excessive glucose will worsen acidosis and induce hypokalemia). The weaning of suckling foals may help reduce stress and the energy demand imposed on lactating mares. Concurrent diseases amenable to therapy, such as parasitism, chronic infection, and musculoskeletal disease, should be appropriately treated with anthelmintics, antimicrobials and antiinflammatory agents, or analgesics, respectively.

In addition to supplying immediate energy, glucose therapy stimulates the release of insulin. Concurrent carbohydrate and insulin administration has been used successfully to manage hyperlipemia in ponies.[228,243] The following regimen has been suggested for a 200-kg pony.[243] On day 1 the patient is administered 30 IU of protamine zinc insulin intramuscularly and 100 g of glucose orally (both twice daily). On day 2 the patient is administered 15 IU of insulin intramuscularly, twice daily, and 100 g of galactose once. Galactose is slowly converted to glucose; thus lactic acid production is minimized. This regimen may be continued for 3 days. Other insulin dose regimens are also reported.[227] Blood glucose and insulin concentrations should be closely monitored. Insulin treatment may not lower triglyceride levels, but it may help control hyperglycemia if partial parenteral nutrition is provided.[232] Nicotinic acid, thought to act by inhibiting hormone-sensitive lipase, has been used in cattle with fatty liver; however, nicotinic acid has not been evaluated in ponies with hepatic lipidosis.

Heparin potentiates the activity of lipoprotein lipase and may increase triglyceride removal from the blood. However, because lipoprotein lipase activity is already at maximum rate in affected ponies, whether or not heparin provides additional benefit is questionable.[236] Recommended dosages range between 40 and 250 IU/kg twice daily.[228,243] Because of the potential for hemorrhage, heparin therapy should be used with discretion and monitored by daily hemostatic testing. If azotemia, acidosis, and electrolyte abnormalities are present, appropriate fluid therapy is suggested.

Lipotropic agents such as choline and methionine are unlikely to be useful in horses with hyperlipemia, because triglyceride synthesis and release in the liver is usually sufficient.

Prognosis and Client Education. The prognosis for hyperlipemia in equids is reported to be poor but the prognosis is

TABLE **13.4 Pyrrolizidine Alkaloid–Containing Plants**

Species Name	Common Name	Alkaloids
Senecio jacobea	Tansy or common ragwort, stinking Willie	Jacobine, jacodine, senecionine
Senecio riddellii	Reddell's groundsel	Ridelline
Senecio longilobus	Threadleaf groundsel	Longilobine
Senecio vulgaris	Common groundsel	Senecionine, seneciphylline, retrorsine
Senecio spartioides	Broom groundsel	Seneciphylline
Senecio integerrimus	Lamb's tongue groundsel	Intergerrimine
Amsinckia intermedia	Fiddleneck, fireweed, tarweed	
Crotalaria species	Rattlebox	Monocrotaline, fulvine, crispatine
Echium plantagineum	Viper's bugloss	
Heliotropium europaeum	Common heliotrope or potato weed	Heliotrine, lasiocarpine

variable, depending on the successful and prompt correction of the predisposing cause(s) and on the ability to supply adequate nutritional support (enteral or parenteral or both). Mortality has been estimated in some studies to occur in 60% to 100% of affected ponies.[228,229] Survival rate is reportedly better in Miniature Horses, and death often results from the underlying disease and not fatty liver failure.[232,233] Most Miniature Horses with blood triglyceride levels less than 1200 mg/dL survive.[233] Prognosis should not be based upon the level of triglyceride in the serum or on liver enzyme elevations.[116]

The prevention of hepatic lipidosis revolves around identifying "at risk" equines, especially pregnant or lactating pony mares, obese or geriatric ponies and donkeys, and Miniature Horses with diarrhea and other predisposing disorders. Donkeys that have any change in their social status, such as loss of a companion, should be considered at high risk for disease. Providing adequate caloric intake is important in late pregnant and early lactation mares.[242] As a preventive, fat could be fed to increase caloric density, and feeding fat to healthy ponies increases lipoprotein lipase and decreases circulating triglycerides.[244] This would not be recommended in "at risk" equines that already have hyperlipidemia. Performing routine body condition scoring and periodic sampling of "at risk" equines for triglycerides and free fatty acids could be helpful in identifying metabolic abnormalities before the onset of clinical signs. Proper maintenance health care and husbandry are of utmost importance in "at risk" equines. Testing for Cushing's disease and dental examinations should be instituted in all older "at risk" animals.[116]

Chronic Hepatic Diseases

The distinction between acute and chronic hepatic disease is difficult. Often horses with chronic liver disease display abrupt onset of signs. With the possible exception of hyperglobulinemia, no serum biochemical parameters reliably distinguish acute from chronic hepatocellular disease. Regardless of the cause or site of injury, histopathologic evidence of fibrosis develops after chronic injury.[245] The following diseases are classified as chronic based on the presence of fibrosis in the liver or because the cause is known to result in gradual hepatic dysfunction.

Chronic Megalocytic Hepatopathy

Megalocytic hepatopathy occurs worldwide and is the most common cause of chronic liver failure in horses in certain parts of the United States.[32] It is caused by the ingestion of *pyrrolizidine alkaloid*–containing plants. The intoxication typically results in the delayed onset of chronic, progressive liver failure.



FIG. **13.10** Picture of *Crotalaria* spp. plant containing toxic pyrrolizidine alkaloids. (Courtesy Susan White.)

Clinical Signs. The development of clinical signs of liver disease is usually delayed 4 weeks to 12 months after the consumption of pyrrolizidine alkaloid–containing plants. However, individual differences in susceptibility occur (not all horses consuming the plants develop clinical signs).[246,247] The onset of obvious hepatic failure, characterized by HE and photosensitization, usually is abrupt and occurs late in the disease despite the time interval since ingestion. Premonitory signs of insidious onset may include anorexia, weight loss, exercise intolerance, and mild to moderate icterus. Diarrhea, edema, polydipsia, pruritus, laryngeal hemiparalysis, and hemolysis also have been reported to occur late in the disease.[93,113,246] Sometimes oral ulcers and halitosis develop.[248] Although uncommon, if sufficient consumption occurs during a single exposure, abortion or clinical signs of liver disease may develop acutely.

Epidemiology and Pathogenesis. Numerous species of pyrrolizidine alkaloid–containing plants exist (Table 13.4). In the United States, *Senecio jacobea* and *S. vulgaris* are found primarily along the Pacific Coast and in the western states, *Amsinckia intermedia* in the West, and *Crotalaria sagittalis* (Fig. 13.10), *C. spectabilis,* and *Heliotropium europaeum* primarily in the Southeast.

Pyrrolizidine alkaloid–containing plants are not palatable, and horses will not typically consume them unless pastures are heavily contaminated or no alternative feed source exits.[247] Some herbicides may increase palatability.[113] Pyrrolizidine

alkaloids are stable, and most intoxication occurs after ingestion of contaminated hay, pellets, or grain. *S. vulgaris* is a common contaminant in alfalfa hay, especially at the first cutting. Not all parts of the plant contain pyrrolizidine alkaloids, and the concentration may vary with the season.[249] *Amsinckia* and *Crotalaria* spp. concentrate pyrrolizidine alkaloids in their seeds; thus intoxication may occur after ingestion of contaminated oat hay or grain screenings.[249]

Horses and cattle are relatively sensitive to pyrrolizidine alkaloid intoxication compared with goats and sheep. Consumption of the plants at a dose of 2% to 5% of the body weight, fed at one time or over a few days, can result in acute toxicity.[113] The effects of pyrrolizidine alkaloids are cumulative; thus it is more common for toxicity to occur after chronic low-level exposure. Hundreds of different pyrrolizidine alkaloids exist, but only a few have proved to be toxic (see Table 13.4). The toxic pyrrolizidine alkaloids vary among plants, but they all cause the same basic lesion. Ingested pyrrolizidine alkaloids are carried to the liver via the portal circulation and are metabolized by the microsomal enzymes in zone 3 to toxic pyrrole derivatives.[248] Drugs that induce microsomal enzymes, such as mixed function oxidases, will increase the toxicity of pyrrolizidine alkaloids. The pyrroles are chemically highly reactive and capable of alkylating nucleic acids and protein; consequently the pyrroles inhibit cellular replication and protein synthesis. Because the cells cannot divide, hepatocytes enlarge, forming *megalocytes.* When the megalocytes die, fibrosis ensues. When fibrosis becomes extensive, the liver shrinks, develops a firm texture, and failure is inevitable.

Histopathologic lesions include hepatic hemorrhage and necrosis, periportal megalocytosis, portal fibrosis, bile duct hyperplasia, and multinucleate hepatocytes. When acute massive consumption exists, extensive centrilobular hepatocellular necrosis occurs, and lesions may look similar to those found in Theiler's disease. Megalocytes may not be seen in the liver on microscopic examination until 30 days or more after exposure to the pyrrolizidine alkaloid.[250]

Fibrosis around the portal vessels can cause portal hypertension, ascites, and diarrhea. Endothelial cell swelling in the portal vein is especially common with *Crotalaria* spp., further promoting veno-occlusion. Despite periportal fibrosis and veno-occlusion, portal hypertension rarely develops in horses, although it is a common manifestation of pyrrolizidine alkaloid toxicity in cattle.[113] Additional pathologic findings include myocardial necrosis, colitis, widespread hemorrhages, and adrenal cortical hypertrophy.[93] The toxic pyrrolizidine alkaloid in *Crotalaria* spp., monocrotaline, also is pneumonotoxic and may cause hydrothorax, pulmonary edema, epithelialization, and pulmonary arteritis.[248]

Pyrrolizidine alkaloids are rapidly excreted in body fluids such as milk and urine, and they can pass the placenta.[248] A report describes pyrrolizidine alkaloid toxicosis in a 2-month-old foal with a dam that consumed pyrrolizidine alkaloid–containing plants during pregnancy.[251]

Diagnosis. A presumptive diagnosis of megalocytic hepatopathy can be made from history of exposure to pyrrolizidine alkaloids, clinical signs, and laboratory evidence of hepatic disease. Early in the disease, SDH and AST may be increased, but by the time clinical signs develop, these enzymes are often normal or only mildly increased. Because fibrosis occurs periportally, GGT, ALP, and serum bile acid concentration are persistently increased. Bilirubin may be increased and the BCAA-to-AAA ratio may be decreased.[252] The BSP half-life is prolonged, and serum bile acids are usually increased late in the disease.[93]

A definitive diagnosis of pyrrolizidine alkaloid intoxication requires a liver biopsy. The histopathologic findings of megalocytosis, biliary hyperplasia, and fibrosis are highly suggestive of the disease; megalocytosis may rarely be seen with other causes of liver disease. Pyrrolizidine alkalosis must be distinguished from aflatoxicosis and alsike clover toxicity. The latter two toxicities are less common worldwide compared with pyrrolizidine alkaloids and cause periportal fibrosis and biliary epithelial hyperplasia, but generally without megalocytosis. Pyrrolizidine alkaloids can be detected in feed by high-performance liquid chromatography.[250] Some pyrrolizidine alkaloids can be identified in liver tissue. Unfortunately, because of the prolonged delay in development of clinical signs, often the original source of contamination remains unidentified.

Treatment and Prognosis. No specific antidote for pyrrolizidine alkaloid intoxication exists. Despite general supportive care, death often occurs within 10 days of the onset of obvious clinical signs of hepatic failure. A serum bile acid concentration greater than 50 μmol/L is suggestive of a poor prognosis.[93] A small number of horses with signs of liver failure and elevated bile acids may survive for many months or even years.[253] Because regeneration is not possible, if extensive megalocytosis and fibrosis are present, treatment is not warranted. Treatment with BCAA may decrease the severity of neurologic signs but does not prevent death.[93] If clinical signs or histologic changes are mild, a moderate-protein, high-energy (molasses concentrate grain) ration may be beneficial.

Attention should be directed toward asymptomatic horses that also may have consumed the plants. The source of contamination should be identified by locating the plant in hayfields or pasture or by analysis of feed. Progress can be monitored by serial liver enzyme quantitation and biopsies. Serum GGT activity may be helpful in detecting subclinical cases of pyrrolizidine alkaloid toxicity.[254]

Clover Poisoning

Alsike clover *(Trifolium hybridum)* and red clover *(Trifolium pratense)* poisoning rarely occurs in horses pastured or fed hay containing these types of clovers.[255-257] The toxic principle in alsike clover has yet to be identified. It is not known whether the agent is a toxic metabolite from the plant itself or a mycotoxin produced by a fungus *(Cymodothea trifolii)* living on the plant.[258] Both photodermatitis and liver disease have been reported. Signs of liver disease may become apparent after 2 weeks of consumption and when the diet consists of at least 20% of clover.[255] The likelihood of poisoning reportedly is greater when the pasture is heavily covered with the clover in full bloom during wet seasons. The disease is characterized histopathologically by the presence of biliary hyperplasia and periportal fibrosis without severe bile obstruction. The liver of horses that die from the disease may be noticeably enlarged with rounded edges, sometimes weighing 5% of body weight or 25 kg in a 500-kg horse. Other cases may have normal or even smaller than normal livers if there is extensive fibrosis.[256] Minimal parenchymal lesions distinguish alsike clover poisoning from pyrrolizidine alkaloid toxicity.

Chronic Active Hepatitis

Chronic active hepatitis in horses is an idiopathic, chronic, progressive hepatopathy characterized histopathologically by biliary hyperplasia, with concomitant periportal or biliary inflammation (or both) and associated hepatocellular damage.

Clinical Signs. The onset of clinical signs of CAH is insidious. Signs are compatible with progressive liver failure and include depression, exercise intolerance, weight loss, anorexia, colic, icterus, and in many cases fever. The signs may be intermittent. Although uncommon, some horses with CAH have moist exfoliative coronary dermatitis.[89]

Cause and Pathogenesis. The exact cause of CAH in horses is not known. A similar syndrome occurs in human beings and has been linked to autoimmune disease, chronic hepatitis B virus infection, non-A and non-B (viral) hepatitis, Wilson's disease, α_1-antitrypsin deficiency, and drug allergy.[259] Human beings with autoimmune-associated CAH often have marked polyclonal gammopathy and hepatic actin, smooth muscle, and antinuclear antibodies.[259] Histologic study of the liver reveals mononuclear and plasma cell infiltrates, large areas of necrosis, and fibrosis. Extrahepatic signs of autoimmune disease, including dermatitis, arthritis, and glomerulonephritis, may be present. Some horses with CAH have polyclonal gammopathy, but this is a nonspecific finding in many types of chronic hepatic diseases and is not necessarily indicative of autoimmunity.[101] Occasionally, horses with CAH have predominantly plasma and mononuclear cell infiltrates in the liver, suggesting heightened humoral activity. The presence of coronary dermatitis in horses with CAH may be a manifestation of autoimmune disease, although this has not been confirmed by immunohistologic staining. No reports have surfaced of antinuclear antibodies in horses with CAH. In addition, Wilson's disease and α_1-antitrypsin deficiency have not been documented in these animals. Idiosyncratic drug hypersensitivity has been reported in horses although not as a consistent feature of CAH.

In addition to the possibility of an autoimmune, chronic viral or hypersensitivity reaction, CAH in horses may be a manifestation of chronic cholangitis. Many horses with CAH have a suppurative inflammatory response involving the biliary system, in addition to periportal inflammation and hepatocellular necrosis.[89] Often the cholangiohepatitis is accompanied by biliary hyperplasia, fibrosis, and cholestasis. In some cases, coliform organisms have been isolated from the liver, suggestive of ascending infection from the gastrointestinal tract. In a large number of horses, histopathologic evidence of fibrosis, acute inflammation, and hepatocellular necrosis is seen, but no specific cause can be determined.

Diagnosis. The diagnostic criteria for CAH in human beings include abnormally increased serum transaminase activity for longer than 6 months, abnormal immunologic findings, and characteristic histopathologic findings.[259] In horses, serum SDH and AST activity may be mildly increased, but GGT and ALP are often markedly increased in CAH. The serum bile acid, total protein, and bilirubin (especially the direct-reacting fraction) concentrations may be increased. Bilirubinuria may be present, and the BSP half-life is usually prolonged. Hematology may reveal an inflammatory leukogram, with or without a left shift.[89] Immunodiagnostics, including antinuclear antibody titer and anti-immunoglobulin immunofluorescent staining of skin lesions, may help confirm an autoimmune phenomenon.

In the face of clinical signs and the demonstration of significant laboratory findings indicative of liver disease, a liver biopsy should be obtained (see Liver Biopsy). A sample should be submitted for histopathologic examination and for bacterial isolation and antimicrobial sensitivity testing. The definitive diagnosis of CAH depends on the presence of characteristic histopathologic findings. Progressive periportal hepatocellular necrosis obscures and distorts the limiting plate, the cord of hepatocytes that surrounds the portal triad. As this destructive process continues, bands of necrotic hepatocytes and inflammatory cells connect one liver lobule to another or extend from the portal tract to the central vein. This feature is called *bridging necrosis.* As bridging necrosis progresses, fibrosis and cirrhosis prevail. Mononuclear cells may be the predominant inflammatory infiltrate; however, neutrophils predominate if cholangiohepatitis is a feature. Biliary hyperplasia may be evidence of concurrent cholangiohepatitis.

Treatment. General supportive care for hepatic failure (see Treatment of Hepatic Insufficiency earlier in this chapter) is indicated whenever clinical signs are apparent. Specific therapy for CAH depends on the histopathologic findings. If the liver biopsy reveals abundant plasma cells, or other diagnostic tests suggest autoimmune or hypersensitivity disease, corticosteroid therapy may be beneficial. In human beings with autoimmune-associated CAH, corticosteroids improve appetite and attitude, reduce inflammation and serum transaminase activity, and hinder fibrosis. Despite short-term improvement, corticosteroids do not alter survival time, and the long-term prognosis remains poor. In horses, initial treatment with dexamethasone at 0.05 to 0.1 mg/kg/day for 4 to 7 days, followed by a gradual reduction in dose over 2 to 3 weeks, has been suggested.[116] Additional treatment with prednisolone (1 mg/kg/day) may be necessary for several weeks. In human beings, azathioprine (2 mg/kg every 24 hours) has also been used to control cases refractory to steroids,[259] although recent studies suggest that oral bioavailability of this drug in the horse is quite low, ranging from 1% to 7%.[260] If the liver biopsy reveals cholangitis, long-term (4–6 weeks) antimicrobial therapy is indicated. The choice of antimicrobial is best determined by culture and sensitivity results. Antimicrobials that are excreted in bile, such as chloramphenicol, ceftiofur, ampicillin, or broad-spectrum antimicrobials such as penicillin and gentamicin, are recommended. The decision for corticosteroid therapy should be carefully scrutinized in these cases of CAH.

Prognosis. The prognosis for CAH is variable, depending on the cause and duration of disease. The presence of cirrhosis warrants a grave prognosis.

Cholelithiasis

Bacterial cholangiohepatitis (CH) and cholelithiasis (CL) are two of the most frequent causes of obstructive hepatic disease in horses. Inflammation of the biliary tract is called *cholangitis* but in adult horses with clinical disease there is usually a concurrent inflammation of hepatocytes, hence the term *cholangiohepatitis.* CH may be an acute or chronic disease; when choleliths are present it is most likely a chronic disease, but acute onset of clinical signs is common. Studies have identified CH and CL as diseases of middle-aged to older horses with no obvious sex or breed predilection.[81,191,261,262] It should be noted that in human medicine, the term *cholelithiasis* refers to gallstones and *choledocholithiasis* refers to stones in the common bile duct. In the horse cholelithiasis has been commonly used to describe stones in any portion of the biliary system. Some publications may classify biliary stones by their location within the biliary tree: hepatoliths (within the liver) or choleliths (outside the liver).

Etiology and Pathophysiology. Although the etiopathogenesis of CH and CL in horses is uncertain, retrograde bacterial infection from the small intestine is considered probable,

and this hypothesis is supported by several reports.[81,191,263] The isolation of enteric, predominantly gram-negative organisms from clinical cases of CH and CL is consistent with this hypothesis.[81,191,261,263,264] Plant material has occasionally been found inside the stones, further supporting retrograde invasion of bacteria and even ingesta from the duodenum as a predisposing cause of the disease.[263] As bacteria gain entrance to the biliary system from the duodenum (bile is normally sterile), it would be expected that an inflammatory response would occur with tissue swelling, thus potentially increasing the pressure within the common bile duct and forcing infection into the biliary canaliculi. The ascending infection is thought to initiate a cholangitis, which may then extend into the periportal region of the liver causing CH.[81] The occurrence of enteritis or inflammatory bowel disease before the onset of CH in some cases may also support the ascending enteric bacterial infection hypothesis,[195,265] although most cases of CH do not have a clear history of those diseases.[191] The most common organisms cultured from the affected horses are *E. coli, Actinobacillus equuli, Streptococcus* spp., *Klebsiella* spp., *Enterococcus* spp., *Clostridium* spp., and *Bacteroides* spp.[191,195,266] CH may predispose to intrahepatic (hepatolith) or extrahepatic (cholelith) formation. Equine choleliths are predominantly composed of calcium bilirubinate and to a lesser extent calcium phosphate.[191,263,267,268] Ascending enteric bacterial infection is thought to be critical in the development of brown pigment stones (calcium bilirubinate) in humans.[269,270] Deconjugation of bilirubin diglucuronide by bacterial β-glucuronidase such as *Clostridium* spp., *Bacteroides* spp., *E. coli,* and *Peptococcus* spp. occurs with subsequent precipitation of calcium bilirubinate, and cementation by anionic glycoproteins is thought to result in the formation of these calculi.[269] It is clinically important to realize that calcium bilirubinate crystals may form initially, followed by sludge formation and then stone formation. If that is true, each stage of the process may signal further decreases in the efficacy of medical treatment. In the majority of clinical cases of equine CL, stones are found in more than one area of the biliary tree and can include intrahepatic and extrahepatic choleliths including choledocholiths (stones of the common bile duct).[263] Multiple stones are common, with one extreme case reported as having 1120 stones.[271] Stones in the common bile duct would almost always be expected to produce signs characteristic of obstructive failure whereas stones in other areas of the biliary tree may be silent.

Clinical Findings. Clinical signs associated with these conditions include pyrexia, colic, icterus, and, in chronic cases, significant weight loss.[81,191,261,263,272-275] In a small percentage of cases, both neurologic signs caused by hepatic encephalopathy (HE) and photosensitization may be seen.[81,276] Unless the disease is chronic and extensive fibrosis has occurred, hepatocellular function is not severely compromised, thus accounting for the absence of HE in many cases. Although nonspecific, colic and marked icterus are the most common clinical findings in horses with obstructive CH and CL.[81,191,261,267] Fever would be expected but is not always evident on a single examination. Some degree of depression may be present. Affected horses have markedly elevated serum concentrations of the hepatobiliary enzymes GGT and ALP, as well as bilirubin. A proportionate increase in the conjugated bilirubin fraction to greater than 25% of the total bilirubin is common,[81,261,267,268] but in approximately one third of cases the percentage of conjugated/total bilirubin is lower than 25%.[191] Affected horses generally have an increase in hepatocellular enzymes SDH, GLDH, and AST (commonly 2–4 times normal), although the proportionate increase above normal values is usually less than that seen with serum GGT elevations (commonly 7–20 times normal). Hyperfibrinogenemia may or may not be present.[81,191] Other notable serum biochemical abnormalities with CH include increased serum bile acids and hyperglobulinemia.[81,191] Clotting function tests (PT and PTT) are often normal but can be abnormal.[81,191,261]

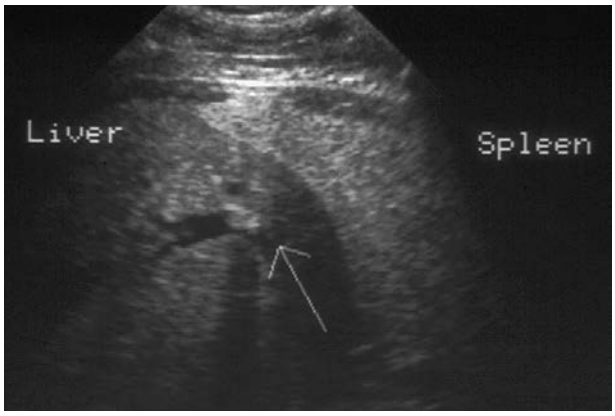


FIG. **13.11** Ultrasound image of the liver showing a hepatolith *(arrow)* casting an acoustic shadow in a dilated bile duct in a horse with obstructive cholelithiasis.

The ultrasonographic findings in horses with CH include subjective evidence of liver enlargement and distended, thickened bile ducts with variable liver echogenicity. In horses with CL, increased hepatic echogenicity, hepatomegaly, distended bile ducts, and occasional calculi are the salient features of the condition.[81,263,267,277] Stones can often best be visualized in the seventh to tenth intercostal spaces on the right side, just ventral to the lung border, and are seen in approximately 50% to 75% of horses with CL[263]; stones are uncommonly seen on the left. The biliary tree is usually not detectable ultrasonographically in normal horses, but dilated bile ducts can be seen as enlarged channels running parallel to the portal veins in approximately 40% of horses with obstructive CL, and this finding should be considered suspicious for either intrahepatic or extrahepatic biliary obstruction even if no stones are seen.[3,263] Hyperechoic choleliths with acoustic shadowing can occur, whereas other less mineralized calculi or sludge may have only faint or no acoustic shadowing (Fig. 13.11). If several bile ducts are severely distended that indicates a likely obstruction (choledocholith) in the common bile duct. If stones can be seen in the image field with classic findings of hepatic failure caused by obstruction, one can assume there is at least one other stone obstructing the common bile duct and the presence of multiple stones may lower the prognosis. Stones obstructing the common bile duct cannot be imaged on transcutaneous abdominal ultrasound, but when endoscopic examination of the duodenum is performed, the stone or a "bulge" may be noted at the bile duct opening.

Histopathology of biopsy material can provide valuable information as to the severity of inflammatory CH and the extent of accompanying hepatic fibrosis. Historically, marked periportal and bridging fibrosis have been interpreted as poor prognostic indicators in horses with suspected or known CH or CL.[191,267] Case reviews suggest that extended (months or years) survival times may be possible in some

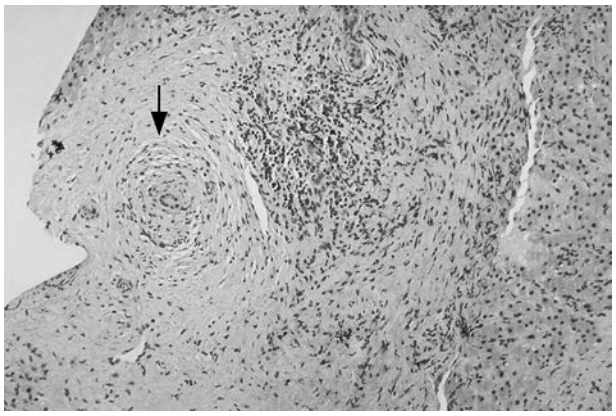


FIG. **13.12** Histopathology of the liver: concentric fibrosis *(arrow)* around a bile duct characteristic of obstruction of the common bile duct. (Photo courtesy of Dr. Kelly Butterworth.)

horses despite histologic evidence of chronic hepatobiliary damage (severe periportal and bridging fibrosis).[191] An estimate of the degree of fibrosis, based on hepatic echogenicity, can also be determined by ultrasound examination. Horses with biliary obstruction may have circumferential biliary hyperplasia and fibrosis on microscopic examination of a liver biopsy (Fig. 13.12), and this can be an extremely helpful finding in the diagnosis and prognosis when an obstructing stone cannot be seen on ultrasound or duodenoscopy and surgery is being contemplated (authors' observations). This finding does not have 100% sensitivity, so if all other clinical and laboratory findings are characteristic of CL, surgery (if an option and appropriate) should not be ruled out based on the absence of this microscopic finding. Another indication for biopsy is to provide a sample for bacterial culture, in hopes of determining the etiologic agents such that specific antimicrobial therapy can be chosen. Unfortunately, cultures obtained from biopsy samples are negative in approximately 50% of cases.[191]

Treatments and Prognosis. Based on previous studies, it would appear that long-term administration of systemic antibiotics is critical for the successful treatment of CH and in some cases of CL in horses.[191] Median treatment period in one study, where seven of nine horses had liver failure due to CH but survived, was 51 days (range 17–124 days).[191] Antibiotic selection should ideally be based on culture and sensitivity results from hepatic biopsy material, but clinicians are frequently required to select an initial antimicrobial agent empirically or when the culture results are negative. The isolation of predominantly gram-negative enteric and mixed anaerobic organisms from cases of CL[81,191,263] suggests that antibiotics with good gram-negative activity (enrofloxacin, third-generation cephalosporins, aminoglycosides, and possibly trimethoprim-sulfonamide) in addition to antimicrobials effective against enteric anaerobes (penicillin, metronidazole, chloramphenicol) would be reasonable choices. Although the liver metabolizes nearly 50% of metronidazole, its use in CH is unlikely to be a problem or require a dose adjustment unless there is marked liver fibrosis. In the absence of positive culture results, clinicians may not only have to select an agent empirically but should also be prepared to change antibiotics according to clinical response. In one study, in all horses that improved, clinical improvement was seen days to several weeks before normalization of serum biochemical indices of hepatobiliary disease (GGT, ALP, bilirubin, and bile acids).[191] Furthermore in that study, serum GGT actually increased during the initial period of clinical improvement in all horses. There is no reported level of GGT that accurately predicts treatment failure in CH, as long as the horse is clinically stable, the ultrasound examination does not reveal numerous obstructing stones or diffuse fibrosis, and both clinical and laboratory findings improve within 7 to 10 days after medical therapy commences. Continuing antimicrobial therapy until both clinical and biochemical recovery has occurred may be important in achieving a successful outcome. Supportive medical care using intravenous fluids and antiinflammatory drugs was believed to be a critical part of the treatment in one case series report.[191]

Therapeutic agents, such as the bile salts chenodeoxycholic acid and ursodeoxycholic acid (ursodiol), which are specifically used to encourage dissolution of cholesterol-rich stones in humans, have no effect on calcium bilirubinate calculi but, as antiinflammatory and choleretic agents that increase bile production, may make bile more liquid and easier to excrete.[148] There has been no recognizable evidence of toxic effects of ursodeoxycholic acid treatment in any case, but further studies on safety and efficacy need to be done. Administration of ursodiol may increase serum bile acid concentrations. There is direct experimental support for the use of DMSO in the treatment of brown pigment stones in humans because it is a direct solubilizer of calcium bilirubinate.[142]

Previous reports have described the surgical treatment of cholelithiasis.[267,272,273,276] Choledocholithotripsy (the crushing of choleliths in the bile ducts) and choledochotomy (surgical formation of an opening into the bile duct) have been performed successfully in the horse.[272,273,278] In one report on horses, external massage of the common bile duct, without choledochotomy, successfully dislodged choledocholiths into the duodenum in all of the cases in which it was attempted.[273] Unfortunately, intrahepatic calculi are largely inaccessible to the surgeon, and the fact that many cases of equine cholelithiasis possess multiple intrahepatic and extrahepatic choleliths means that recurrent biliary obstruction would be likely even if an obstructing stone is removed.

Prognosis. The prognosis of cholelithiasis depends on the extent of hepatic fibrosis, severity of clinical signs, and number and location of choleliths. Extensive or bridging fibrosis, multiple choleliths accompanied by clinical signs of hepatic failure, and severe HE warrant a poor to grave prognosis.[40,191,262] Reports of resolution of extensive fibrosis exist after reestablishment of bile flow; thus one should not consider the presence of extensive fibrosis alone to be lethal.[262,279] In contrast to reports of 30% survival for equine cholelithiasis,[81] other reviews indicate 77% to 85% survival with medical or medical and surgical treatment.[191,262] If obstruction of the bile duct requires a choledochotomy, the prognosis is guarded because of limited access to the bile duct in a horse and the risk of peritonitis. The best surgical outcome has been when there is a single stone obstructing the bile duct and when hepatic fibrosis is not severe. Surgery should be considered for horses that have intermittent or persistent abdominal pain, ultrasonographic evidence of obstruction to biliary outflow, concentric biliary fibrosis histopathologically, moderate to no hepatic fibrosis, few or no stones visible on ultrasound examination, and an unsuccessful response to medical therapy.

Neonatal Acquired Biliary Obstruction

Healing duodenal ulcers located adjacent to the hepatopancreatic ampulla in neonatal foals may result in extrahepatic biliary stenosis.[127,280] Clinical signs are compatible with gastroduodenal obstruction and include reduced suckling reflex, depression, prolonged recumbency, colic, bruxism, and firm feces. Nasogastric intubation often produces voluminous reflux. Duodenal stenosis may be confirmed radiographically by delayed gastric emptying of barium sulfate. One should consider a diagnosis of biliary stenosis if the serum levels of conjugated bilirubin and GGT are increased or if nuclear scintigraphy indicates delayed biliary excretion. Cholangiohepatitis may develop because of chronic cholestasis and may be confirmed by histopathologic examination of a liver biopsy. One must differentiate acquired biliary stenosis from congenital biliary atresia (discussed later in this chapter). Successful surgical correction of acquired biliary stenosis secondary to duodenal stricture has been described.[280]

Chronic cholangiohepatitis without biliary stenosis, another potential sequela to duodenitis or duodenal ulceration, should be considered in icteric foals with increased serum conjugated bilirubin and GGT.[32] The diagnosis is confirmed by histopathologic examination. Treatment should consist of long-term antimicrobial therapy directed by bacterial isolation and sensitivity results (see Bacterial Hepatitis).

Hepatic Abscessation

Liver abscessation is uncommon in horses. Clinical signs of hepatic abscessation depend on the extent and location of the abscess or abscesses; however, abscesses are unlikely to result in hepatic failure. Small solitary abscesses or multifocal microabscesses usually remain subclinical. Larger abscesses may be accompanied by weight loss, intermittent fever, and anorexia.[281]

Hepatic abscessation in foals may be hematogenous in origin as a sequela to bacteremia or ascending through the umbilical vein as a sequela to omphalophlebitis.[282] In the adult, bacteremia is less common than in foals, and hepatic infection or abscessation more likely originates in the intestinal lumen and ascends the bile duct or originates in the intestinal wall or mesenteric lymph nodes and is carried to the liver via the portal blood.[283] The latter is a likely sequela to primary abdominal or mesenteric abscessation because of *S. equi* subsp. *equi.* In one retrospective study of abdominal abscesses in horses, only 2 of 25 horses had solely hepatic abscesses, although some horses had abscesses involving several abdominal structures.[284] In a description of three adult horses with hepatic abscesses, disease was postulated to result from primary inflammatory bowel disease, as a sequelae to abdominal surgery, and secondary to penetration of a foreign body from the colon.[285] Laboratory diagnosis of hepatic abscessation is difficult. Smaller abscesses may not result in sufficient hepatocellular damage to impede liver function. Laboratory abnormalities characteristic of chronic inflammation and infection, including anemia of chronic disease, hyperfibrinogenemia, hypergammaglobulinemia, and mature neutrophilia, may occur with larger abscesses. Increased serum activity of liver-specific cytosolic enzymes or serum bile acid concentration rarely occurs unless liver abscessation is extensive. Ultrasonography of the liver may reveal focal changes in echogenicity. A liver biopsy should be guided by ultrasound to avoid penetration of the abscess. One should direct long-term antimicrobial therapy by culture and sensitivity results. Common isolates from abdominal abscesses in horses include *S. equi* subsp. *equi, S. equi* subsp. *zooepidemicus,* and *Corynebacterium pseudotuberculosis.*[284]

Hepatic Neoplasia

Primary hepatic neoplasia is rare in horses. A retrospective survey in 1952 indicated that hepatic tumors accounted for only 1% of all equine neoplasms.[286] Of the documented primary hepatic neoplasms in horses (cholangiocarcinoma, hepatocellular carcinoma, hepatoblastoma, and mixed hamartoma), cholangiocarcinoma is the most common.[125,287-289] Hepatic neoplasia in horses is more likely to occur after metastasis of some other primary tumor, especially lymphosarcoma.[289] Because clinical signs of hepatic failure usually are not apparent and laboratory findings are nonspecifically indicative of chronic inflammatory disease, antemortem diagnosis of hepatic neoplasia is difficult.

In one report, *cholangiocarcinoma* accounted for 9 of 10 primary liver neoplasms in horses.[286] Cholangiocarcinoma originates from the bile duct epithelium and is distinguished from hepatocellular carcinoma by its tendency to form multiple foci, its firm texture, and a whitish color produced by abundant fibrous stroma. The primary mass is typically solitary, with multiple intrahepatic secondaries. Extrahepatic metastasis is common, with transperitoneal lymphatic spread to the peritoneum and diaphragm and hematogenous spread to the lungs.[287,288] In human beings, dogs, and cats, a causative relationship appears to exist between cholangiocarcinoma and liver fluke infestation or a history of biliary tract disease.[288] A report describes cholangiocarcinoma in a horse after treatment for prolonged septic cholangiohepatitis.[290] Aberrant expression of the protein products of the tumor suppressor gene, p53, was reported at necropsy in one horse with cholangiocarcinoma.[291] Loss of function of p53 has been described in other types of carcinomas and may have contributed to malignancy. Microscopically, cholangiocarcinoma is adenocarcinomatous, producing cuboidal or columnar-lined ductules and acini. The neoplastic bile ducts do not contain bile but may contain mucus.

The clinical presentation and progression of cholangiocarcinoma in horses is not well documented, but this disorder appears to be more common in older horses.[286,287] One case report describes a 10-year-old mixed-breed horse in which the presenting signs included anorexia, weight loss, pyrexia, mild icterus, tachypnea, severe dependent edema, and abdominal distention.[292] Abnormal clinicopathologic findings included mature neutrophilia; hyperfibrinogenemia; anemia; moderately increased serum bilirubin, GGT, and SDH; and nonseptic peritonitis and pleuritis. Neoplastic cells were not detected in the body cavity effusions. Definitive antemortem diagnosis of cholangiocarcinoma was made by histopathologic examination of a liver biopsy. Necropsy revealed multiple extrahepatic metastases on the serosal surfaces of the intestine and spleen, diaphragm, omentum, pleural surfaces, and lung.

Hepatocellular carcinoma, or hepatoma, has been reported in several horses, the majority of which were less than 3 years of age.[287] Hepatocellular carcinomas are usually solitary and multilobulated. Extrahepatic metastasis occurs transperitoneally and hematogenously to lungs. Microscopically, the neoplastic cells resemble hepatocytes and retain cord arrangement. However, the normal liver architecture is lost, and sometimes the cells are difficult to differentiate. Mitotic figures are uncommon. Possible causative factors in other species include heredity, parasitism, chemical and plant carcinogens, and viral hepatitis.[288]

Reported clinical signs of hepatocellular carcinoma in horses include depression, anorexia, weight loss, intermittent diarrhea, and abdominal distention.[287] Abnormal laboratory findings included absolute erythrocytosis; persistent hypoglycemia; sanguineous peritoneal effusion; and increased serum SDH, GGT, LAP, and indirect-reacting bilirubin. In one case, absolute erythrocytosis was attributed to erythropoietin secretion by the carcinoma (inappropriate secondary erythrocytosis).[124] The same horse had a high serum concentration of α-fetoprotein, a globulin normally synthesized only by embryonic fetal liver cells that is commonly detectable in human patients with hepatocellular carcinoma.[124]

Several reports describe malignant *hepatoblastoma* in young horses.[125,287,293] Hepatoblastoma is an embryonic tumor of the liver with a wide range of histologic patterns that include both epithelial and mesenchymal elements. Clinical signs included emaciation, pyrexia, and pleural effusion. Laboratory abnormalities included mature neutrophilia, erythrocytosis with increased erythropoietin level in one case, hypergammaglobulinemia, hyperfibrinogenemia, and increased serum activity of GGT and ASP.[125,287] In one of the cases, transabdominal ultrasonography identified abnormal hepatic architecture. Increased serum α-fetoprotein may serve as a tumor marker but cannot be used alone to confirm a diagnosis.[293]

A *mixed hamartoma* was described in the liver of a late-term aborted equine fetus.[294] Histologically, the lesion appeared to be a proliferation of large hepatocyte-like cells with eccentric nuclei and voluminous cytoplasm, abnormal bile ducts, and fibroblastic fibrocystic interstitial tissue, with complete lack of structural organization.

Hepatic Amyloidosis

Amyloidosis refers to a group of diseases that are characterized by the extracellular deposition of a proteinaceous fibril substance, *amyloid,* in the tissues. Amyloid deposits are composed of nonbranching fibrils in β-pleated sheet conformation formed by the proteolytic cleavage of precursor proteins by the mononuclear phagocyte system. Depending on the organ of deposition, amyloid distorts normal tissue architecture and may lead to functional impairment. In horses, the liver and spleen are the most common organs involved in systemic amyloidosis.[295] Two forms of systemic amyloidosis exist and are distinguished by the type of precursor protein and subsequent protein fibril. The precursor in *reactive* or *secondary* systemic amyloidosis is serum amyloid protein AA, an acute-phase protein produced mostly by hepatocytes in response to chronic infection or inflammation. The AA fibrils are identified in tissues by green birefringence in polarized light, after staining with Congo red, which is lost after treatment with potassium permanganate. Most often in horses, hepatic amyloid deposits are AA fibrils and have been associated with hyperimmunization, severe parasitism, or chronic infection and inflammation.[295] Hepatic AA-amyloidosis is most commonly reported in serum-producing horses.[296] One horse in which the liver was the principal organ of amyloid deposition presented for evaluation of chronic weight loss.[295] An antemortem diagnosis of hepatic amyloidosis was not made; however, laboratory abnormalities included severe hypoalbuminemia, polyclonal gammopathy, and large numbers of ascarid and strongylid eggs in the feces. Histopathologically, the liver revealed extracellular deposits of AA amyloid periportally and adjacent to the sinusoids in the space of Disse. The amyloid deposits were accompanied by hepatocytic atrophy and a mild mononuclear cell infiltrate. Horses with hepatic amyloidosis may be asymptomatic or have clinical signs of chronic liver disease and dysfunction. GGT and bile acids may be increased in horses with moderate to marked infiltration of the liver with amyloid.[296,297] Acute abdominal hemorrhage associated with rupture of the liver may occur in some cases.

Systemic primary, immunocytic, or *idiopathic amyloidosis* is caused by the deposition of amyloid light-chain fibrils.[297] The precursor proteins in primary amyloidosis are the variable region of immunoglobulin light chains. The amyloid fibrils also stain with Congo red, but staining is retained after potassium permanganate treatment. *Local immunocytic amyloidosis,* with deposition in the upper respiratory mucosa or skin, is more common in horses than systemic primary amyloidosis. However, at least one reported case of systemic primary amyloidosis in a 14-year-old Thoroughbred mare with chronic weight loss and cutaneous nodules exists.[298] Amyloid deposits were identified in the liver, myocardium, spleen, gastrointestinal mucosa, pulmonary interstitium, pancreas, and arterial walls.

Chronic Hypoxemia

Right-sided heart failure causes the pressure in the caudal vena cava to rise. Retrograde pressure increases in the hepatic central veins, causing hypoxemia and pressure necrosis of the adjacent hepatocytes. Chronic passive congestion may cause fatty change, atrophy, and fibrosis.

Miscellaneous Vascular or Congenital Abnormalities

Portosystemic Shunt

Portosystemic shunts (PSSs) may be intrahepatic or extrahepatic, congenital or acquired. Few reports have documented congenital extrahepatic PSSs in foals.[87,194] The vascular shunts allow blood within the portal system to bypass the liver and drain into the systemic circulation, directly or indirectly, via the caudal vena cava or the azygous vein. In the reported cases, age at presentation varied between 2 and 6 months. This relatively late clinical detection could be because hindgut development in young foals is necessary to produce enough enteric ammonia to cause sufficient elevations in blood ammonia. Vague intermittent neurologic signs of blindness, ataxia, and severe depression were consistent with HE. Growth appeared to be stunted. These clinical signs are caused by the altered hepatic blood flow and hepatic insufficiency secondary to hepatocellular atrophy.

Laboratory abnormalities in the reported cases varied, but the most consistent findings included a decreased BUN, prolonged BSP half-life, and increased blood ammonia concentration. Liver biopsies revealed hepatocellular atrophy and necrosis, fibrosis, and biliary hyperplasia.[126] Antemortem diagnosis can be confirmed by liver ultrasonography (see Diagnostic Imaging of the Liver), computed tomography, ultrasound-guided percutaneous transsplenic injection of 10 mL of agitated saline into the spleen with simultaneous echocardiography of the right side of the heart ("bubble gram"), or by transrectal hepatic scintigraphy.[127,299,300] The bubble gram may have higher sensitivity because air bubbles will appear in the right side of the heart almost immediately after splenic injection if a PSS is present.[300] Theoretically, if the portal circulation is normal the liver will filter the bubbles and the bubbles will not reach the heart. Transrectal scintigraphy for the diagnosis of PSS is performed by placing a soft rubber catheter as

far into the rectum as safely possible and administering 30 mC of technetium pertechnetate, followed immediately by radioisotope scanning of both the heart and liver.[127] If the majority of technetium is detected first in the liver, portosystemic shunt can be ruled out. Once the diagnosis of PSS is made an operative mesenteric portography or CT with contrast will be needed to confirm location of the shunts(s).[299] If a liver biopsy is performed (usually unnecessary), lobular atrophy characterized by decreased distance between the central veins and portal areas and arteriolar proliferation in the portal tract with mild proliferation of interlobular arteries are usually seen. The number and size of portal veins may be subjectively less than normal.[300] The location of the shunt (or shunts) in reported cases varied, but the majority were extrahepatic, often involving a direct connection between the portal vein and the caudal vena cava. Shunts may be singular or multiple, with shunting to the caudal vena cava, azygous vein, and virtually any gastric, splenic, or mesenteric vessel.[299] Alzheimer type II astrocytes are visible in the brain, consistent with HE.[299,301] Successful treatments have included routine surgical ligation or cellophane banding.[299,300] Prior to surgery the foal should be managed to prevent HE as discussed previously. Foals with PSS may have remarkably high blood ammonia, sometimes > 200 umol/L, without obvious signs of HE.

Portal Vein Thrombosis

Acquired PSSs are rare in horses. One report describes an 11-year-old Thoroughbred with HE that subsequently was determined to have a functional PSS secondary to vascular thrombosis of the portal vein.[302] In older animals, hepatic neoplasia may predispose to thrombosis.[303] Although rare, horses with cirrhosis may occasionally develop portal vein thrombosis.

Portal vein thrombosis occurs rarely in foals following enteritis or in association with *Rhodococcus equi* infection.[304] Portal vein thrombosis has been reported in other species as a sequela to sepsis, hypercoagulability, and inflammation.

Clinical signs in foals attributed to complete portal vein thrombosis include diarrhea and HE signs.[127,304] Diarrhea may occur from increased mesenteric venous pressure and intestinal wall edema if the thrombosis is acute and completely obstructs the portal vein. Diarrhea may be a predisposing cause of or a result of the portal vein thrombosis. The neurologic signs are secondary to hyperammonemia, caused by the collateral flow of the portal blood into the vena cava and elsewhere, thus bypassing the liver. If the obstruction is not complete or portoportal collateral compensatory circulation develops, clinical signs may be absent. One foal with abdominal lymphadenitis, septic tarsitis, and hepatitis caused by *R. equi* had a large but incomplete obstruction of the portal vein, and with long-term antimicrobial therapy the patient recovered.[127] Treatment to remove portal vein thrombosis in horses has not, to the authors' knowledge, been attempted.

Biliary Atresia

Extrahepatic biliary atresia in foals has been documented.[305] An antemortem diagnosis was not possible; however, veterinary attention was sought at 4 weeks of age in both foals for anorexia, depression, lethargy, poor weight gain, colic, polydipsia, polyuria, pyrexia, and icterus. Laboratory evaluation of one of the foals was consistent with biliary obstruction, as indicated by a mildly increased serum activity of SDH and markedly increased activity of GGT and conjugated bilirubin.[305] Necropsy of both foals revealed a large, firm liver. Although not specifically documented in one case, both the entrance to the bile duct and the main bile duct were absent in the other foal.[305] Histologically, both livers appeared similar and consistent with extrahepatic biliary atresia. Bile canaliculi were present and distended, with associated degenerative hepatocytes. Biliary proliferation was extensive and surrounded by fibrous tissue with interpersed islands of hepatocytes. No bile was found in the proliferative bile ducts, and the portal triad ducts were absent.

In horses, extrahepatic biliary atresia is believed to be congenital. In human beings, other possible causes include neonatal sclerosing cholangiohepatitis, excretion of a biliary toxin, deficit of bile flow in utero, or lumen destruction because of ductal vascular insufficiency.[306] Despite the cause, extrahepatic biliary atresia induces intrahepatic biliary hypertrophy, an abortive attempt to establish continuity. The biliary hypertrophy displaces hepatocytes, causing periportal and perilobular hepatocellular degeneration, fibrous replacement, and ultimately loss of normal hepatic architecture. Although an antemortem diagnosis was not possible in either equine case, hepatobiliary scintigraphy (see Diagnostic Imaging of the Liver) successfully detected total biliary obstruction in a neonatal lamb with biliary atresia.[128]

Serous Cysts

Serous hepatic cysts have been reported and usually are an incidental finding at necropsy.[23]

Glycogen Branching Enzyme Deficiency

Autosomal recessive inheritance of glycogen branching enzyme deficiency has been reported in Quarter Horse foals (see Chapter 10 for a detailed discussion). The nonsense mutation of the affected gene, glycogen branching enzyme 1, prevents synthesis of functional enzyme. Affected foals died before 2 months of age. Clinical signs vary from stillbirth, seizures, and persistent recumbency to cardiac or respiratory failure. Leukopenia, intermittent hypoglycemia, and increased creatine kinase, GGT, and AST are common laboratory findings. Histopathology of the liver demonstrates abnormal periodic acid–Schiff (PAS)-positive globular or crystalline intracellular inclusions and reduced glycogen branching enzyme activity.[307]

Liver Lobe Torsion

Liver lobe torsion is an uncommon disorder in horses. Based on the small number of cases reported, it would appear that the left medial or accessory lobe is most commonly affected, although the entire left lobe was believed to be involved in two horses.[308-310] Predisposing causes were rarely, if ever, identified. Of the eight cases reported, only three were presented for colic. Clinical signs are most commonly anorexia of 1 or more days' duration, tachycardia, and clinical and laboratory signs associated with systemic inflammation resulting from necrosis of the twisted liver lobe and peritonitis. Peritonitis appears to be present in all cases, and peritoneal fluid is often serosanguineous. Serum levels of hepatic-derived enzymes are usually unremarkable although SDH and GLDH may be increased in association with the systemic inflammatory response. Ultrasound examination is generally unremarkable as the affected lobe may be obscured from imaging by the gastrointestinal gas. If the entire left lobe is "torsed," the affected portion may appear as a mixed echogenic mass. Surgical repair is via stapled resection of the affected lobe, and this has been successful in five cases.[308,310]

REFERENCES

1. Dyce KKM, Sack WO, Wensing CJG, et al., eds. *Textbook of veterinary anatomy*. 4th ed. Philadelphia: Saunders; 2010:551.
2. Getty R, ed. *Sisson's and Grossman's the anatomy of domestic animals*. Philadelphia: WB Saunders; 1975.
3. le Jeune S, Whitcomb MB. Ultrasound of the equine acute abdomen. *Vet Clin N Am Equine Pract*. 2014;30:353.
4. Jakowski RM. Right hepatic lobe atrophy in horses: 17 cases (1983-1993). *J Am Vet Med Assoc*. 1994; 204:1057.
5. Hall JE. The liver as an organ. In: Hall JE, ed. *Guyton and Hall textbook of medical physiology*. 12th ed. Philadelphia; 2011:837.
6. Herdt TA, Sayegh AI. Physiology of the gastrointestinal tract. In: Klein BG, ed. *Cunningham's textbook of veterinary physiology*. St. Louis: Elsevier-Saunders; 2013: 342.
7. Stalker MJ, Hayes MA. Liver pathology. In: Maxi G, ed. *Jubb, Kennedy and Palmers pathology of domestic animals*. 5th ed. Philadelphia: Saunders; 2015. p 825.3.
8. Ham AW. Pancreas, liver and galbladder. In: *Histology*. 7th ed. Philadelphia: W.B. Saunders; 1974.
9. Sodeman WA, Sodeman TM, eds. *Sodeman's pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 1985.
10. Cullen JM. Hepatobiliary system. In: Zackary JF, McGavin MD, eds. *Pathologic basis of veterinary disease*. 5th ed. Maryland Heights, MO: Mosby Elsevier; 2011. p 405.
11. Ossedryver SM, Baldwin GI, Stone BM, et al. *Indigofera spicata* (creeping indigo) poisoning of three ponies. *Aust Vet J*. 2013;91(4):143.
12. Lehninger AL. *Biochemistry*. New York: Worth Publishers Inc; 1975.
13. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem*. 1999;265(2):501.
14. Oliveira-Filho JP, Badial PR, Cunha PH, et al. Lipopolysaccharide infusion up-regulates hepcidin mRNA expression in equine liver. *Innate Immun*. 2012;18(3):438.
15. Guyton AC, Hall JE. *Textbook of medical physiology*. 10th ed. Philadelphia: WB Saunders; 2006.
16. McDermott WV. Metabolism and toxicity of ammonia. *N Engl J Med*. 1957;257:1076.
17. Zielińska M, Popek M, Albrecht J: Roles of changes in active glutamine transport in brain edema development during hepatic encephalopathy: an emerging concept, *Neurochem Res* 2014; 39(3):599.
18. Pethick DW, Rose RJ, Bryden WL, et al. Nutrient utilisation by the hindlimb of thoroughbred horses at rest. *Equine Vet J*. 1993;25:41.
19. Argenzio RA, Hintz HF. Glucose tolerance and effect of volatile fatty acid on plasma glucose concentration in ponies. *J Anim Sci*. 1970;30:514.
20. Duncan JR, Prasse KW. *Veterinary laboratory medicine*. Ames, IA: Iowa University Press; 1986.
21. Engelking LR. Evaluation of equine bilirubin and bile acid metabolism. *Compend Contin Educ Pract Vet*. 1989;11:328.
22. Coles EG. *Veterinary clinical pathology*. Philadelphia: WB Saunders; 1988.
23. Thompson RG, ed. *Special veterinary pathology*. Philadelphia: BC Decker Inc; 1988.
24. Papich MG, Davis LE. Drugs and the liver. *Veterinary clinics of North American, small animal practice*. Vol. 15. Philadelphia: WB Saunders; 1985.
25. Lakritz J, Winder BS, Noorouz-Zadeh J, et al. Hepatic and pulmonary enzyme activities in horses. *Am J Vet Res*. 2000;61(2):152.
26. Brown PJ, Whitbread TJ, Bell NJ, et al. Haemosiderin deposition in donkey *(Equusasinus)* liver: comparison of liver histopathology with liver iron contents. *Res Vet Sci*. 2011;90(2):275.
27. Theisse ND. Liver diseases. In: Kumar V, Aster JC, Abbas AK, eds. *Robbins and Cotran pathologic basis of disease*. St. Louis: Elsevier-Saunders; 2015. p 825.
28. Suzuki A. MBSJ MCC Young Scientist Award 2012 Liver regeneration: a unique and flexible reaction depending on the type of injury. *Genes Cells*. 2015;20(2):77.
29. Hall JE. Genetic control of protein synthesis, cell function and cell reproduction. In: Hall JE, ed. *Guyton and Hall textbook of medical physiology*. 12th ed. Philadelphia: Elsevier-Saunders; 2010. p 37.
30. Tennant BC. Hepatic function. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical biochemistry of domestic animals*. 6th ed. Maryland Heights, MO: Elsevier Academic Press; 1997. p 379.
31. Kumar V, Fausto N, Abbas A, eds. *Robbins pathologic basis of disease*. 7th ed. Philadelphia: WB Saunders; 2005.
32. Divers TJ. Liver disease and liver failure in horses. In: *Proceedings of the twenty-ninth American Association of Equine Practitioners*. Lexington, KY: AAEP; 1983:213.
33. West H. Clinical and pathological studies in horses with hepatic disease. *Equine Vet J*. 1996;28:146.
34. McGorum B, Murphy D, Love S, et al. Clinicopathological features of equine primary hepatic disease: a review of 50 cases. *Vet Rec*. 1999;145:134.
35. Fraser CL, Arieff AI. Hepatic encephalopathy. *N Engl J Med*. 1985;313:865.
36. Gammal SH, Jones EA. Hepatic encephalopathy. *Med Clin North Am*. 1989;73:793.
37. Goldman L, Ausiello DA, Arend W, et al. *Cecil textbook of medicine*. 23rd ed. Philadelphia: WB Saunders; 2008.
38. Coltart I, Tranah TH, Shawcross DL. Inflammation and hepatic encephalopathy. *Arch Biochem Biophys*. 2013;536:189.
39. Müller J-MV, Schulze M, Herder V, et al. Ataxia and weakness as uncommon primary manifestations of hepatic encephalopathy in a 15-year-old trotter gelding. *Equine Vet Educ*. 2011;23:5.
40. Hughes KJ, McGorum BC, Love S, et al. Bilateral laryngeal paralysis associated with hepatic dysfunction and hepatic encephalopathy in six ponies and four horses. *Vet Rec*. 2009;164:142.
41. Schiff L, Schiff ER, eds. *Disease of the liver*. Philadelphia: JB Lippincott; 1987.
42. Desjardins P, Du T, Jiang W, et al. Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure: role of glutamine redefined. *Neurochem Int*. 2012;60:690.
43. Sturgeon JP, Shawcross DL. Recent insights into the pathogenesis of hepatic encephalopathy and treatments. *Expert Rev Gastroenterol Hepatol*. 2014;8:83.
44. Sørensen M. Update on cerebral uptake of blood ammonia. *Metab Brain Dis*. 2013;28:155.
45. Lockwood AH, MacDonald JM, Reiman RE, et al. The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. *J Clin Invest*. 1979;63:449.
46. Shawcross D, Jalan R. The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation. *Cell Mol Life Sci*. 2005;62:2295.
47. Bode J, Schafer K. Pathophysiology of chronic hepatic encephalopathy. *Hepatogastroenterology*. 1985;32:259.
48. McCandless DW, ed. *Cerebral energy metabolism and metabolic energy*. New York: Plenum; 1985.
49. Albrecht J, Jones E. Hepatic encephalopathy: molecular mechanisms underlying the clinical syndrome. *J Neurol Sci*. 1999;170:138.
50. Bessman SP, Bessman AN. The cerebral and peripheral uptake of ammonia in liver disease with a hypothesis for the mechanism of hepatic coma. *J Clin Invest*. 1975;34:622.
51. Pappas SC, Ferenci P, Scafer DF, et al. Visual evoked potentials in rabbit model of hepatic encephalopathy. II. Comparison of hyperammonemic encephalopathy, postictal coma and coma induced by synergic neurotoxins. *Gastroenterology*. 1984;86:546.
52. Flannery DB, Hsia YE, Wolf B. Current status of hyperammonemia syndromes. *Hepatology*. 1982;2:495.
53. Mair TS. Ammonia and encephalopathy in the horse. *Equine Vet J*. 1997;29(1):1.

54. Peek SF, Divers TJ, Jackson CJ. Hyperammonaemia associated with encephalopathy and abdominal pain without evidence of liver disease in four mature horses. *Equine Vet J*. 1997;29:70.
55. Cohn R, Castell DO. The effect of acute hyperammonemia on the encephalogram. *J Lab Clin Med*. 1966;68:195.
56. Record CO, Mardini H, Bartlett K. Blood and brain mercaptan concentrations in hepatic encephalopathy. *Hepatology*. 1982;2:144.
57. Jones EA, Schafer DF, Ferenci P, et al. The neurobiology of hepatic encephalopathy. *Hepatology*. 1984;4:1235.
58. Palomero-Gallagher N, Zilles K. Neurotransmitter receptor alterations in hepatic encephalopathy: a review. *Arch Biochem Biophys*. 2013;536:109.
59. Tallman JF, Gallager DW. The GABA-ergic system: a locus of benzodiazepine action. *Annu Rev Neurosci*. 1985;8:21.
60. Study R, Barker J. Diazepam and (–) pentobarbitol: fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. *Proc Natl Acad Sci U S A*. 1981;A77:7486.
61. Basile AS, Gammal SH, Mullen KD. Differential responsiveness of cerebellar Purkinje neurons to GABA and benzodiazepine ligands in an animal model of hepatic encephalopathy in man. *J Neurosci*. 1988;8:2414.
62. Bassett ML, Mullen K, Skolnick P, et al. Amelioration of hepatic encephalopathy by pharmacologic antagonism of the GABA-benzodiazepine receptor complex in a rabbit model of fulminant hepatic failure. *Gastroenterology*. 1987;93:1069.
63. Ferenci P, Jones EA, Hanbauer I. Lack of evidence for impaired dopamine receptor function in experimental hepatic coma in the rabbit. *J Neurosci*. 1986;65:60.
64. Lock B, Pandit K. Is flumazenil an effective treatment for hepatic encephalopathy. *Ann Emerg Med*. 2006;47:286.
65. Ahboucha S, Butterworth R. The neurosteroid system: an emerging therapeutic target for hepatic encephalopathy. *Metab Brain Dis*. 2007;22:291.
66. Fischer JE, Baldessarine RJ. False neurotransmitters in hepatic failure. *Lancet*. 1971;2:75.
67. Gulick BA, Rogers QR, Knight HD. Plasma amino acid patterns in horses with hepatic disease. In: *Proceedings of the twenty-fourth annual convention of the American Association of Equine Practitioners*. St. Louis: AAEP; 1978:517.
68. Alexander WF, Spinder E, Harty RF, et al. The usefulness of branched chain fatty acids with acute or chronic hepatic encephalopathy. *Am J Gastroenterol*. 1989;84:91.
69. Morgan MY, Milson PJ, Sherlock S. Plasma ratio of valine, leucine, and isoleucine to phenylalanine, tyrosine in liver disease. *Gut*. 1978;19:1068.
70. Bianchi G, Marocchi R, Agostini F, et al. Update on nutritional supplementation with branched chained amino acids. *Curr Opin Clin Nutr Metab Care*. 2005;8:83.
71. Gludd LL, Dan G, Borre M, et al. Oral branched-chain amino acids have a beneficial effect on manifestations of hepatic encephalopathy in a systematic review with meta-analyses of randomized controlled trials. *J Nutr*. 2013;143:1263.
72. Holecek M. Branched-chain amino acids and ammonia metabolism in liver disease: therapeutic implications. *Nutrition*. 2013;29(10):1186.
73. Kobtan AA, El-Kalla FS, Soliman HH, et al. Higher grades and repeated recurrence of hepatic encephalopathy may be related to high serum manganese levels. *Biol Trace Elem Res*. 2016;169(2):153.
74. Lachmann V, Görg B, Bidmon HJ, et al. Precipitants of hepatic encephalopathy induce rapid astrocyte swelling in an oxidative stress dependent manner. *Arch Biochem Biophys*. 2013;536(2):143.
75. Javakumar AR, Ruiz-Cordero R, Tong XY, et al. Brain edema in acute liver failure: role of neurosteroids. *Arch Biochem Biophys*. 2013;536(2):171.
76. Odeh M. Pathogenesis of hepatic encephalopathy: the tumour necrosis factor alpha theory. *Eur J Clin Invest*. 2007;37:291.
77. Schafer DF, Fowler JM, Jones EA. Colonic bacteria: a source of gamma-aminobutyric acid in blood. *Proc Soc Exp Biol Med*. 1981;167:301.
78. Bauer JE, Asquith RL, Kivipelto J. Serum biochemical indicators of liver function in neonatal foals. *Am J Vet Res*. 1989;50:2037.
79. Divers T, Schappel K, Sweeney R, et al. Persistent hyperbilirubinemia in a healthy thoroughbred horse. *Cornell Vet*. 1993;83:237.
80. Scott DW. *Large animal dermatology*. Philadelphia: WB Saunders; 1988.
81. Johnston JK, Divers TJ, Reef VB, et al. Cholelithiasis in horses: ten cases (1982-1986). *J Am Vet Med Assoc*. 1989;194:405.
82. Jubb KVF, Kennedy PJ, Palmer N, eds. *Pathology of domestic animals*. Orlando, FL: Academic Press; 1985.
83. Johnstone IB. Antithrombin III activity in normal and diseased horses. *Vet Clin Pathol*. 1988;17:20.
84. Gay CC, Lording PM, McNeil P, et al. Infectious necrotic hepatitis (Black disease) in a horse. *Equine Vet J*. 1980;12:27.
85. Tennant BC, Hornbuckle WE. Diseases of the liver. In: Anderson NV, ed. *Veterinary gastroenterology*. Philadelphia: Lea & Febiger; 1980.
86. Tennant B. Acute hepatitis in horses: problems of differentiating toxic and infectious causes in the adult. In: *Proceedings of the twenty-fourth annual convention of the American Association of Equine Practitioners*. St. Louis: AAEP; 1978:465.
87. Prater PE, Patton CS, Held JP. Pleural effusion resulting from malignant hepatoblastoma in a horse. *J Am Vet Med Assoc*. 1989;194:383.
88. Gay CC, Sullivan ND, Wilkinson JS, et al. Hyperlipaemia in ponies. *Aust Vet J*. 1978;54:459.
89. Carlson GP. Chronic active hepatitis in horses. In *Proceedings from the seventh annual veterinary forum of the American College of Veterinary Internal Medicine*. Madison, WI: ACVIM; 1989:595.
90. Erica Twitchell L, Richard A, et al. 386 veterinary medicine today: pathology in practice. *J Am Vet Med Assoc*. 2012;240(4):385.
91. Pearson EG. Hypoalbuminemia in horses. *Compend Contin Educ Pract Vet*. 1990;12:555.
92. Robinson NE, ed. *Current therapy in equine medicine*. 9th ed. St. Louis: WB Saunders; 2009.
93. Mendle VE. Pyrrolizidine alkaloid-induced liver disease in horses: an early diagnosis. *Am J Vet Res*. 1988;49:572.
94. Divers T. Biochemical diagnosis of hepatic disease and dysfunction in the horse. *Vet Clin North Am Equine Pract*. 1993;15:15.
95. Hoffman WE, Baker G, Rieser S, et al. Alterations in selected serum biochemical constituents in equids after induced hepatic disease. *Am J Vet Res*. 1987;48:1343.
96. Kaneko J, Rudolph W, Wilson D, et al. Bile acid fractionations by high performance liquid chromatography in equine liver disease. *Vet Res Commun*. 1992;16:161.
97. West HJ. Evaluation of total plasma bile acid concentration for the diagnosis of hepatobiliary disease in horses. *Res Vet Sci*. 1989;46:264.
98. Dunkel B, Jones SA, Pinilla MJ, Foote AK. Serum bile acid concentrations, histopathological features, and short-, and long-term survival in horses with hepatic disease. *J Vet Intern Med*. 2015;29(2):644.
99. Barton M, LeRoy B. Serum bile acids concentrations in healthy and clinical ill neonatal foals. *J Vet Intern Med*. 2007;21:508.
100. Reed S, Andrews FM. The biochemical evaluation of liver function in the horse. In: *Proceedings of the thirty-second annual convention of the American Association of Equine Practitioners*. Nashville, TN: AAEP; 1986:81.
101. Parraga ME, Carlson GP, Thurmond M. Serum protein concentrations in horses with severe liver disease: a retrospective study and review of the literature. *J Vet Intern Med*. 1995;9:154.

102. Lindner A, Bauer S. Effect of temperature, duration of storage and sampling procedure on ammonia concentration in equine blood plasma. *Eur J Clin Chem Clin Biochem*. 1993;31:473.
103. Hasel K, Summers B, deLahunta A. Encephalopathy with idiopathic hyperammonaemia and Alzheimer type II astrocytes in Equidae. *Equine Vet J*. 1999;31:478.
104. Peek S, Divers T, Jackson C. Hyperammonaemia associated with encephalopathy and abdominal pain without evidence of liver disease in four mature horses. *Equine Vet J*. 1997;29:70.
105. McConnico R, Duckett W, Wood P. Persistent hyperammonemia in two related Morgan weanlings. *J Vet Intern Med*. 1997;11:264.
106. Feldman BF. Acquired disorders of hemostasis. In: *Proceedings of the Seventh Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine*. Madison, WI: ACVIM; 1989:33.
107. Nyland TG, Matton JS. *Small animal diagnostic ultrasound*. Philadelphia: WB Saunders; 1986.
108. Meyer DJ, Raquel M. The liver. In: Walton RM, ed. *Equine clinical pathology*. John Wiley & Sons; 2013:71.
109. Gossett KA, French DD. Effect of age on liver enzyme activities in serum of healthy quarter horses. *Am J Vet Res*. 1984;45:354.
110. Bernard WV, Divers TJ. Variations in serum sorbitol dehydrogenase, aspartate transaminase, and isoenzyme 5 of lactate dehydrogenase activities in horses given carbon tetrachloride. *Am J Vet Res*. 1989;50:622.
111. Sherman K, Wells R, Mattiacci M. Lability of sorbitol dehydrogenase in refrigerated and frozen horse serum. *J Equine Vet Sci*. 1991;11:176.
112. Steffey E, Giri S, Dunlop C, et al. Biochemical and haematological changes following prolonged halothane anaesthesia in horses. *Equine Vet J*. 1993;25:338.
113. Smith B, ed. *Large animal internal medicine*. 3rd ed. St. Louis: Mosby; 2003.
114. Gardner R, Nydam D, Mohammed H, et al. Serum gamma glutamyl transferase activity in horses with right or left dorsal displacements of the large colon. *J Vet Intern Med*. 2005;19:761.
115. Snow DH, Harris P. Enzymes as markers of physical fitness and training of racing horses. *Adv Clin Enzymol*. 1988;6:251.
116. Divers TJ. The equine liver in health and disease, Frank J Milne Lecture. In: *Proceedings 61st*. Las Vegas: Annual American Association of Equine Practitioners; 2015:66.
117. Mack SJ, Kirkby K, Malalana F, et al. Elevations in serum muscle enzyme activities in racehorses due to unaccustomed exercise and training. *Vet Rec*. 2014;174:145.
118. Leleu C, Haentjens F. Morphological, haemato-biochemical and endocrine changes in young Standardbreds with "maladaptation" to early training. *Equine Vet J*. 2010;38:171.
119. Engelking LR, Answer MS, Lofstedt J. Hepatobiliary transport of indocyanine green and bromosulphthalein in fed and fasted horses. *Am J Vet Res*. 1985;46:2278.
120. West HJ. Clearance of bromosulphthalein from plasma as a measure of hepatic function in normal horses and in horses with liver disease. *Res Vet Sci*. 1988;44:343.
121. Morandi F, Frank N, Avenell J, et al. Quantitative assessment of hepatic function by means of 99mTc mebrofenin in healthy horses. *J Vet Intern Med*. 2005;19:751.
122. Naylor JM, Kronfield DS, Acland H. Hyperlipemia in horses: effects of undernutrition and disease. *Am J Vet Res*. 1980;41(6):899.
123. Jeffcott LB. Primary liver-cell carcinoma in a young thoroughbred horse. *J Pathol*. 1968;97:394.
124. Roby AA, Beech J, Bloom JC, et al. Hepatocellular carcinoma associated with erythrocytosis and hypoglycemia in a yearling. *J Am Vet Med Assoc*. 1990;196:465.
125. Gold JR, Warren AL, French TW, et al. What is your diagnosis? Biopsy impression smear of a hepatic mass in a yearling Thoroughbred filly. *Vet Clin Pathol*. 2008;37:339.
126. Buonanno AM, Carlson GP, Kantrowitz B. Clinical and diagnostic features of portosytemic shunt in a foal. *J Am Vet Med Assoc*. 1988;192:387.
127. Divers TJ, Perkins G. Urinary and hepatic disorders in neonatal foals. *Clin Tech in Equine Pract*. 2003;2:67.
128. Lofstedt J, Koblik PD, Jakowski RM, et al. Use of hepatobiliary scintigraphy to diagnose bile duct atresia in a lamb. *J Am Vet Med Assoc*. 1988;193:95.
129. Durhman A, Smith K, Newton J, et al. Development and application of a scoring system for prognostic evaluation of equine liver biopsies. *Equine Vet J*. 2003;35:534.
130. Sammons SC, Norman TE, Chaffin MK, et al. Ultrasonographic visualization of the liver in sites recommended for blind percutaneous liver biopsy in horses. *J Am Vet Med Assoc*. 2014;245(8):939.
131. Johns IC, Sweeney RW. Coagulation abnormalities and complications after percutaneous liver biopsy in horses. *J Vet Intern Med*. 2008;22:185.
132. Rose CF. Ammonia-lowering strategies for the treatment of hepatic encephalopathy. *Clin Pharmacol Ther*. 2012;92:321.
133. Gerber T, Schomerus H. Hepatic encephalopathy in liver cirrhosis. *Drugs*. 2000;60:1353.
134. Weiner ID, Wino CS. Hypokalemia—consequences, causes, and corrections. *J Am Soc Nephrol*. 1987;8:1179.
135. Rothenberg M, Keeffe E. Antibiotics in the management of hepatic encephalopathy: an evidence based review. *Rev Gastroentrol Disord*. 2005;5:26.
136. Scarratt W, Warnick L. Effects of oral administration of lactulose in healthy horses. *J Equine Vet Sci*. 1998;18:405.
137. Ferenci P, Grimm G, Meryn S, et al. Successful long-term treatment of portal-systemic encephalopathy by the benzodiazepam antagonist flumazenil. *Gastroenterology*. 1989;96:240.
138. Als-Nielsen B, Gluud LL, Gluud C. Benzodiazepine receptor antagonists for hepatic encephalopathy. *Cochrane Database Syst Rev*. 2004;2:CD002798.
139. Riordan S, Williams R. Treatment of hepatic encephalopathy. *N Engl J Med*. 1997;337:473.
140. King Han M, Hyzy R. Advances in critical care management of hepatic failure and insufficiency. *Crit Care Med*. 2006;34:s225.
141. Bai M, Yang Z, Qi X, et al. L-ornithine-l-aspartate for hepatic encephalopathy in patients with cirrhosis: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol*. 2013;28(5):783.
142. Igimi H, Asakawa S, Tamura R, et al. DMSO as a direct solubilizer of calcium bilirubinate stones. *Hepatogastroenterol*. 1994;41:65.
143. Windmeier C, Gressner A. Pharmacological aspects of pentoxifylline with emphasis on its inhibitory actions on hepatic fibrogenesis. *Gen Pharmacol*. 1997;29:181.

143a. Liska DA, Akucewich LH, Marsella R, et al. Pharmacokinetics of pentoxifylline and its 5-hydroxyhexyl metabolite after oral and intravenous administration of pentoxifylline to healthy adult horses. *Am J Vet Res*. 2006;67:1621.

144. Predhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Indian J Med Res*. 2006;124:491.
145. Hackett ES, Mama KR, Twedt DC, et al. Pharmacokinetics and safety of silibinin in horses. *Am J Vet Res*. 2013;74:1327.
146. Anstee QM, Day CP. S-adenosylmethionine (SAMe) therapy in liver disease: a review of current evidence and clinical utility. *J Hepatol*. 2012;57(5):1097.
147. Camilleri M, Gores GJ. Therapeutic targeting of bile acids. *Am J Physiol Gastrointest Liver Physiol*. 2015;309(4):G209.
148. Poupon R. Ursodeoxycholic acid and bile-acid mimetics as therapeutic agents for cholestatic liver diseases: an overview of their mechanisms of action. *Clin Res Hepatol Gastroenterol*. 2012;36:S3.
149. Pearson E. Liver disease in the mature horse. *Equine Vet J*. 1999;11:87.
150. Nikolaidis N, Kountouras J, Giouleme O, et al. Colchicine treatment of liver fibrosis. *Hepatogastroenterol*. 2016;53:281.
151. Ambühl PM. Protein intake in renal and hepatic disease. *Int J Vitam Nutr Res*. 2011;81:162.

152. Durham AE, Newton JR, Smith KC, et al. Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. *Equine Vet J*. 2003;35:542.
153. Theiler A: Acute liver atrophy and parenchymatous hepatitis in horses. In Proceedings of the 5th *and* 6th *Res Dir Vet Res Dept Agr Union, S Africa* 9, 1917.
154. Madsen DE. Equine encephalomyelitis. *Utah Acad Sci Arts Letter*. 1934;11:95.
155. Marsh H. Supplementary note to article on equine encephalomyelitis. *J Am Vet Med Assoc*. 1937;91:330.
156. Guglick M, MacAllister C, Ely R, et al. Hepatic disease associated with administration of tetanus antitoxin in eight horses. *J Am Vet Med Assoc*. 1995;206:1737.
157. Messer N, Johnson P, et al. Idiopathic acute hepatic disease in horses: 12 cases (1982–1992). *J Am Vet Med Assoc*. 1994;204:1934.
158. Messer N, Johnson P. Serum hepatitis in two brood mares. *J Am Vet Med Assoc*. 1994;204:1790.
159. Aleman M, Nieto J, Carr E, et al. Serum hepatitis associated with commercial plasma transfusion in horses. *J Vet Intern Med 1*. 2005;19:120-122. 4.
160. Robinson M, Gopinth C, Hughes DL. Histopathology of acute hepatitis in the horse. *J Comp Pathol*. 1975;85:111.
161. Ryan NM, Downes MA, Isbister GK. Clinical features of serum sickness after Australian snake antivenom. *Toxicon*. 2015;108:181.
162. Chandriani S, Skewes-Cox P, Zhong W, et al. Identification of a previously undescribed divergent virus from the flaviviridae family in an outbreak of equine serum hepatitis. *Proc Natl Acad Sci U S A*. 2013;110:E1047.
163. Scheel TK, Kapoor A, Nishiuchi E, et al. Characterization of nonprimate hepacivirus and construction of a functional molecular clone. *Proc Natl Acad Sci U S A*. 2015;112:2192.
164. Burbelo PD, Dubovi EJ, Simmonds P, et al. Serology-enabled discovery of genetically diverse hepaciviruses in a new host. *J Virol*. 2012;86:6171.
165. Kapoor A, Simmonds P, Cullen JM, et al. Identification of pegivirus (GB virus-like virus) that infects horses. *J Virol*. 2013;87:7185.
166. Scheel TK, Simmonds P, Kapoor A. Surveying the global virome: identification and characterization of HCV-related animal hepaciviruses. *Antiviral Res*. 2015;115C:83.
167. Lyons S, Kapoor A, Schneider BS, et al. Viraemic frequencies and seroprevalence of nonprimate hepacivirus and equine pegiviruses in horses and other mammalian species. *J Gen Virol*. 2014;95:1701.
168. Drexler JF, Corman VM, Müller MA, et al. Evidence for novel hepaciviruses in rodents. *PloS Path*. 2013;9:e1003438.
169. Tanaka T, Kasai H, Yamashita A, et al. Hallmarks of hepatitis C virus in equine hepacivirus. *J Virol*. 2014;88(22):13352.
170. Gemaque BS, Junior Souza de Souza A, do Carmo Pereira Soares M, et al. Hepacivirus infection in domestic horses, Brazil, 2011-2013. *Emerg Infect Dis*. 2014;20(12):2180.
171. Lyons S, Kapoor A, Sharp C, et al. Nonprimate hepaciviruses in domestic horses, United Kingdom. *Emerg Infect Dis*. 2012;18:1976.
172. Ramsay JD, Evanoff R, Wilkinson Jr TE, et al. Experimental transmission of equine hepacivirus in horses as a model for hepatitis C virus. *Hepatology*. 2015;61:1533.
173. Reuter G, Maza N, Pankovics P, et al. Nonprimate hepacivirus infection with apparent hepatitis in a horse—short communication. *Acta Vet Hung*. 2014;19:1.
174. Postel A, Cavalleri JM, Pfaender S, et al. Frequent presence of hepaci and pegiviruses in commercial equine serum pools. *Vet Microbiol*. 2016;182:8.
175. Tyzzer EE. A fatal disease of Japanese Waltzing Mice caused by a spore-bearing bacillus. *J Med Res*. 1917;37:307.
176. Swerczek TW, Crowe MW, Prickett ME, et al. Focal bacterial hepatitis in foals. *Mod Vet Pract*. 1973;54:66.
177. Turk MA, Gallina AM, Perryman LE. *Bacillus piliformis* infection (Tyzzer's disease) in foals in northwest United States: a retrospective study of 21 cases. *J Am Vet Med Assoc*. 1981;178:279.
178. Swerczek TW. Tyzzer's disease in foals: retrospective studies from 1969 to 2010. *Can Vet J*. 2013;54(9):876.
179. Peek S, Byars T, Rueve E. Neonatal hepatic failure in a Thoroughbred foal: successful treatment of a case of presumptive Tyzzer's disease. *Equine Vet J*. 1994;6:307.
180. Humber KA, Sweeney RW, Saik JE, et al. Clinical and clinicopathologic findings in two foals infected with *Bacillus piliformis*. *J Am Vet Med Assoc*. 1988;193:1425.
181. Carrigan MJ, Pedrana RG, McKibbin AW. Tyzzer's disease in foals. *Aust Vet J*. 1984;61:199.
182. Borchers A, Madgesian K, Halland S, et al. Successful treatment and polymerase chain reaction (PCR) confirmation of Tyzzer's disease in a foal and clinical and pathologic characteristics in 6 additional foals (1986–2005). *J Vet Intern Med*. 2006;20:1212.
183. Hook R, Riley L, Franklin C, et al. Seroanalysis of Tyzzer's disease in horses: implications that multiple strains can infect Equidae. *Equine Vet J*. 1995;27:8.
184. Warner SL, Boggs J, Lee JK, et al. Clinical, pathological, and genetic characterization of *Listeria monocytogenes* causing sepsis and necrotizing typhlocolitis and hepatitis in a foal. *J Vet Diagn Invest*. 2012;24:581.
185. Setlakwe EL, Sweeney R, Engiles JB, et al. Identification of *Bartonella henselae* in the liver of a thoroughbred foal with severe suppurative cholangiohepatitis. *J Vet Intern Med*. 2014;28:1341.
186. Divers TJ, Byars TD. Hepatic disease of foals. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. Vol. 1. Hoboken: Wiley-Blackwell; 2011. p 409.
187. Sweeney HJ, Greg A. Infectious necrotic hepatitis in a horse. *Equine Vet J*. 1986;18:150.
188. Hollingsworth TC, Green VJ. Focal necrotizing hepatitis caused by *Clostridium novyi* in a horse. *Aust Vet J*. 1978;54:48.
189. Dumaresq JA. A case of black disease in the horse. *Aust Vet J*. 1939;15:53.
190. Oaks J, Kanaly T, Fiser T, et al. Apparent *Clostridium haemolyticum*/*Clostridium novyi* infection and exotoxemia in two horses. *J Vet Diagn Invest*. 1997;9:324.
191. Peek S, Divers T. Medical treatment of cholangiohepatitis and cholelithiasis in mature horses: 9 cases (1991-1998). *Equine Vet J*. 2000;32:301.
192. Thornberg LP, Kintner LD. Cholangiohepatitis in a horse. *Vet Med Small Anim Clin*. 1895;75:1980.
193. Clabough D, Duckett W. Septic cholangitis and peritonitis in a gelding. *J Am Vet Med Assoc*. 1992;200:1521.
194. Davis J, Blikslager A, Catto K, et al. A retrospective analysis of hepatic injury in horses with proximal enteritis (1984-2002). *J Vet Intern Med*. 2003;17:896
195. Davis JL, Jones SL. Suppurative cholangiohepatitis and enteritis in adult horses. *J Vet Intern Med*. 2003;17:583.
196. Hartley WJ, Dixon RJ. An outbreak of foal perinatal mortality due to equid herpesvirus type 1: pathologic observations. *Equine Vet J*. 1979;11:214.
197. Perkins G, Ainsworth D, Erb H, et al. Clinical, haematological and biochemical findings in foals with neonatal equine herpesvirus-I infection compared with septic and premature foals. *Equine Vet J*. 1999;31:422.
198. Clabough D. Equine infectious anemia: the clinical signs, transmission, and diagnostic procedures. *Vet Med*. 1990;85:1007.
199. Blood DC, Radostits OM. *Veterinary medicine a textbook of the diseases of cattle, sheep, pigs, goats, and horses*. 7th ed. Philadelphia: Bailliere Tindall; 1989.
200. Car BD, Anderson WI. Giant cell hepatopathy in 3 aborted midterm equine fetuses. *Vet Pathol*. 1988;25:389.
201. Carlson KL, Chaffin MK, Corapi WV, et al. Starry sky hepatic ultrasonographic pattern in horses. *Vet Radiol Ultrasound*. 2011;52:568.
202. Davis C, Barr B, Pascoe J, et al. Hepatic sarcocystosis in a horse. *J Parasitol*. 1999;85:965.

203. Hoberg E, Miller S, Brown M. *Echinococcus granulosus (Taeniidae)* and autochothonous echinococcosis in a North American horse. *J Parasitol*. 1994;80:141.
204. Benhazim A, Harmon B, Roberson E, et al. Hydatid disease in a horse. *J Am Vet Med Assoc*. 1992;200:958.
205. Buergelt C, Greiner E. Fibrosing granulomas in the equine liver and peritoneum: a retrospective morphologic study. *J Vet Diagn Invest*. 1995;7:102.
206. Easterwood L, Chaffin MK, Marsh PS, et al. Phosphine intoxication following oral exposure to aluminum-phosphide treated feed. *J Am Vet Med Assoc*. 2010;236:446.
207. Adam SE. A review of drug hepatopathy in animals. *Vet Bull*. 1972;42:683.
208. Kumar S, Gupta AK, Pal Y, Dwivedi SK. In-vivo therapeutic efficacy trial with artemisinin derivative, buparvaquone and imidocarb dipropionate against *Babesia equi* infection in donkeys. *J Vet Med Sci*. 2003;65(11):1171.
209. Venner M, Astheimer K, Lämmer M, et al. Efficacy of mass antimicrobial treatment of foals with subclinical pulmonary abscesses with. *Rhodococcus equi, J Vet Intern Med*. 2013;27:171.
210. Cohen N, Carter G. Steroid hepatopathy in a horse with glucocorticoid-induced hyperadrenocorticism. *J Am Vet Med Assoc*. 1992;200:1682.
211. Divers TJ, Warner A, Vaala WE, et al. Toxic hepatic failure in newborn foals. *J Am Vet Med Assoc*. 1983;183:1407.
212. Acland HM, Mann PC, Robertson JL, et al. Toxic hepatopathy in neonatal foals. *Vet Pathol*. 1984;21(1):3.
213. Polkes AC, Giguère S, Lester GD, et al. Factors associated with outcome in foals with neonatal isoerythrolysis (72 cases, 1988–2003). *J Vet Intern Med*. 2008;22:1216.
214. Elfenbein JR, Giguère S, Meyer SK, et al. The effects of deferoxamine mesylate on iron elimination after blood transfusion in neonatal foals. *J Vet Intern Med*. 2010;24:1475.
215. Pearson EG, Andreasen CB. Effect of oral administration of excessive iron in adult ponies. *J Am Vet Med Assoc*. 2001;218(3):400.
216. Pearson E, Hedstrom O, Poppenga R. Hepatic cirrhosis and hemochromatosis in three horses. *J Am Vet Med Assoc*. 1994;204:1053.
217. Lavoie J, Teuscher E. Massive iron overload and liver fibrosis resembling haemochromatosis in a racing pony. *Equine Vet J*. 1993;25:552.
218. Caloni F, Cortinovis C. Effects of fusariotoxins in the equine species. *Vet J*. 2010;186(2):157.
219. Voss KA, Smith GW, Haschek WM. Fumonisins: toxicokinetics, mechanism of action and toxicity. *An Feed Sci Tech*. 2007;137:299.
220. Fink-Gremmels J. Mycotoxins: their implications for human and animal health. *Vet Quart*. 1999;21:115.
221. Asquith RL, Edds GT, Aller WW, et al. Plasma concentration of iditol dehydrogenase (sorbitol dehydrogenase) in ponies treated with aflatoxin B1. *Am J Vet Res*. 1980;41(6):925.
222. Bortell R, Asquith RL, Edds GT, et al. Acute experimentally induced aflatoxicosis in the weanling pony. *Am J Vet Res*. 1983;44(11):2110.
223. Cysewski SJ, Pier AC, Baetz AL, et al. Experimental equine aflatoxicosis. *Toxicol Appl Pharmacol*. 1982;65(3):354.
224. Caloni F, Cortinovis C. Toxicological effects of aflatoxins in horses. *Vet J*. 2011;188(3):270.
225. Divers T. Diagnosis of hepatic disease and dysfunction in the horse. In: *Proceedings of the tenth annual veterinary medical forum of the American College of Veterinary Internal Medicine*. San Diego, CA: ACVIM; 1992:430.
226. Turner T, Brown C, Wilson J, et al. Hepatic lobe torsion as a cause of colic in a horse. *Vet Surg*. 1993;22:301.
227. McKenzie III HC. Equine hyperlipidemia. *Vet Clin N Am Equine Pract*. 2011;27:59.
228. Naylor NJ. Treatment and diagnosis of hyperlipemia and hyperlipidemia. In *Proceedings from the American College of Veterinary Internal Medicine Medical Forum*; 1982:47. Salt Lake City.
229. Jeffcott LB, Field JR. Current concepts of hyperlipemia in horses and ponies. *Vet Rec*. 1985;116:461.
230. Field JR. Hyperlipemia in a Quarter horse. *Compend Contin Educ Pract Vet*. 1988;10:218.
231. Bauer JE. Plasma lipids and lipoproteins of fasted ponies. *Am J Vet Res*. 1983;44:379.
232. Moore B, Abood S, Hinchcliff K. Hyperlipemia in 9 Miniature Horses and Miniature Donkeys. *J Vet Intern Med*. 1994;8:376.
233. Mogg T, Palmer J. Hyperlipidemia, hyperlipemia, and hepatic lipidosis in American Miniature Horses: 23 cases (1990-1994). *J Am Vet Med Assoc*. 1995;207:604
234. Burden FA, Du Toit N, Hazell-Smith E, et al. Hyperlipemia in a population of aged donkeys: description, prevalence, and potential risk factors. *J Vet Intern Med*. 2011;25:1420.
235. Watson T, Burns L, Love S, et al. Plasma lipids, lipoproteins, and post heparin lipases in ponies with hyperlipidemia. *Equine Vet J*. 1992;24:341.
236. Watson T, Love S. Equine hyperlipidemia. *Compend Contin Educ Pract Vet*. 1994;16:89.
237. Forhead A, Dobson H. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. *Res Vet Sci*. 1997;62:265.
238. Breidenbach A, Fuhrmann H, Deegen E, et al. Studies on equine lipid metabolism 2, lipolytic activities of plasma and tissue lipases in large horses and ponies. *Zentralbl Veterinarmed A*. 1999;46:39.
239. Valdivielso P, Ramírez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. *Eur J Intern Med*. 2014;25(8):689.
240. Dunkel B, Wilford SA, Parkinson NJ, et al. Severe hypertriglyceridaemia in horses and ponies with endocrine disorders. *Equine Vet J*. 2014;46:118.
241. Hellebeck JM, Beynen AC. A preliminary report on a fat-free diet formula for nasogastric enteral administration as treatment for hyperlipaemia in ponies. *Vet Quart*. 2001;23:201.
242. Durham AE. Hyperlipemia. In: Geor RJ, Harris PA, et al., eds. *Equine applied and clinical nutrition*. Great Britain: Saunders Elsevier; 2013. p 512.
243. Wensing TH, Schotman AJ, Kroneman J. Effect of treatment with glucose, galactose, and insulin in hyperlipemia in ponies. *Tijdschr Diergeneeskd*. 1974;99:919.
244. Geelen SN, Jansen WL, Geelen MJ, et al. Lipid metabolism in equines fed a fat-rich diet. *Int J Vitam Nutr Res*. 2013;70:148.
245. Friedman S. The cellular basis of hepatic fibrosis. *N Engl J Med*. 1993;328:1828.
246. Giles CJ. Outbreak of ragwort *(S. jacobaea)* poisoning in horses. *Equine Vet J*. 1983;15:248–250.
247. Robinson B, Gummow B. A field investigation into a suspected outbreak of pyrrolizidine alkaloid toxicosis in horses in western Queensland. *Prev Vet Med*. 2015;4:376.
248. McLean EK. The toxic actions of pyrrolizidine (Senecio) alkaloids. *Pharm Res*. 1970;22:429.
249. Johnson AE, Molyneux RJ, Merrill JR. Chemistry of toxic range plants: variation in pyrrolizidine alkaloid content of *Senecio, Amsinckia*, and *Crotalaria* species. *J Agric Food Chem*. 1985;33:50.
250. Stegelmeier BL, Gardner DR, James LF, et al. Pyrrole detection and the pathologic progression of *Cynoglossum officinale* (houndstongue) poisoning in horses. *J Vet Diagn Invest*. 1996;8:81.
251. Small A, Kelly W, Seawright A, et al. Pyrrolizidine alkaloidosis in a 2 month old foal. *J Vet Med*. 1993;40:213.
252. Lessard P, Wilson WD, Alander HJ, et al. Clinicopathologic study of horses surviving pyrrolizidine alkaloid toxicosis. *Am J Vet Res*. 1986;47:1776.
253. Lessard P, Wilson WD, Olander HJ, et al. Clinicopathologic study of horses surviving pyrrolizidine alkaloid *(Senecio vulgaris)* toxicosis. *Am J Vet Res*. 1986;47:1776.
254. Curran J, Sutherland R, Peet R. A screening test for subclinical liver diseases in horses affected by pyrrolizidine alkaloid toxicosis. *Aust Vet J*. 1996;74:236.

255. Talcott P. Alsike clover and red clover poisonings in horses. In: *Proceedings from the eighteenth annual veterinary medical forum of the American College of Veterinary Internal Medicine*. Seattle, WA; 2000:161.
256. Nation P. Hepatic disease in Alberta horses: a retrospective study of "alsike clover poisoning." *Can Vet J*. 1991;32:602.
257. Colon J, Jackson C. Hepatic dysfunction and photodermatitis secondary to alsike clover poisoning. *Compend Contin Educ Pract Vet*. 1996;189:1022.
258. Murphy MJ. Secondary photosensitization in horses ingesting *Cymodothea trifolii* infested clover. In *Proceedings fifth international symposium of poisonous plants*; May 1997.
259. Krawitt E. Autoimmune hepatitis. *N Engl J Med*. 1996;334: 897.
260. White SD, Maxwell LK, Hawkins JL, et al. Pharmacokinetics of azathioprine following single-dose intravenous and oral administration and effects of azathioprine following chronic oral administration in horses. *Am J Vet Res*. 2005;66: 1578–1583.
261. Traub JL, Rantanen N, Reed S, et al. Cholelithiasis in four horses. *J Am Vet Med Assoc*. 1982;181:59.
262. Barton M. Cholelithiasis in horses. In *Proceedings from the seventeenth annual veterinary medical forum of the American College of Veterinary Internal Medicine*. ; 1999.
263. Reef V, Johnston J, Divers T, et al. Ultrasonographic findings in horses with cholestasis: eight cases (1985–1987). *J Am Vet Med Assoc*. 1990;196:1836.
264. Grabner A. Case report. Cholangiopathy and cholelithiasis and acute hepatic syndrome. *Tierarztl Prax*. 1997;25:370.
265. Davis JL, Blikslager AT, Catto K, et al. A retrospective analysis of hepatic injury in horses with proximal enteritis (1984–2002). *J Vet Intern Med*. 2003;17:896.
266. Durham AE. Hepatitis in horses. In: Weber O, Protzer U, eds. *Comparative hepatitis*. Basel, Switzerland: Berkhauser Verlag; 2008. p 245.
267. Schneider DA. Cholestasis and biliary calculi in horses. *Comp Cont Educ Pract Vet*. 1997;19:744.
268. van der Luer RJT, Kroneman J. Three cases of cholelithiasis and biliary fibrosis in the horse. *Equine Vet J*. 1982;14:251.
269. Tabata M, Nakayama F. Bacteria and gallstones. Etiological significance. *Dig Dis Sci*. 1981;26:218.
270. Vítek L, Carey MC. New pathophysiological concepts underlying pathogenesis of pigment gallstones. *Clin Res Hepatol Gastroenterol*. 2012;36(2):122.
271. de Lima Santos R, de Oliveira T, Galvão J, et al. Cholelithiasis with atrophy of the right lateral hepatic lobe in a horse. *Ciência Rural*. 2007;37:586.
272. Roussel AJ, Becht JL, Adams SB. Choledocholithiasis in a horse. *Cornell Vet*. 1984;74:166.
273. Tulleners EP, Becht JL, Richardson DW, et al. Choledocholithotripsy in a mare. *J Am Vet Med Assoc*. 1985;186:1317.
274. Ryu SH, Bak UB, Lee CW, et al. Cholelithiasis associated with recurrent colic in a Thoroughbred mare. *J Vet Sci*. 2004;5:79.
275. Graves EA. Cholelithiasis and hepatic fibrosis in a standardbred mare. *Vet Clin North Am Equine Pract*. 2006;22(1):107.
276. Traub JL, Grant BD, Rantanen N, et al. Surgical removal of choleliths in a horse. *J Am Vet Med Assoc*. 1983;182:714.
277. Rantanen NW. Diseases of the liver. *Vet Clin North Am Equine Pract*. 1986;2(1):105.
278. Green DS, Davies JV. Successful choledocholithotomy in a horse. *Equine Vet J*. 1989;21:464.
279. Scarratt WK, Fessler RL. Cholelithiasis and biliary obstruction in a horse. *Compend Contin Educ Pract Vet*. 1985;7:s428.
280. Orsini JA, Donawick WJ. Hepaticojejunostomy for treatment of common hepatic duct obstructions associated with duodenal stenosis in two foals. *Vet Surg*. 1989;18:34.
281. Cypher EE, Kendall AT, Panizzi L, et al. Medical and surgical management of an intra-abdominal abscess of hepatic origin in a horse. *J Am Vet Med Assoc*. 2015;247(1):98.
282. Reef VB, Collatos C, Spencer PA, et al. Clinical, ultrasonographic, and surgical findings in foals with umbilical remnant infections. *J Am Vet Med Assoc*. 1989;69.
283. Ruby R, Buckles E, Pinn T, et al. Transdiaphragmatic hepatic and pulmonary abscess attributed to ileal diverticulitis in a horse. *J Vet Intern Med*. 2013;27(6):1633.
284. Rumbaugh GE, Smith BP, Carlson GP. Internal abdominal abscesses in horses: a study of 25 cases. *J Am Vet Med Assoc*. 1978;172:304.
285. Sellon DC, Spaulding K, Breuhaus BA, et al. Hepatic abscesses in three horses. *J Am Vet Med Assoc*. 2000;216:882–887.
286. Tamaschke C. Beitrage zur vergleichenden onlologie der haussaugtiere. *Wiss Z Humboldt Univ [Math Naturwiss]*. 1952;1:37.
287. Beeler-Marfisi J, Arroyo L, Caswell JL, et al. Equine primary liver tumors: a case series and review of the literature. *J Vet Diagn Invest*. 2010;22:174.
288. Moulton JE. *Tumors in domestic animals*. 2nd ed. Los Angeles: University of California Press; 1978.
289. Chaffin MK, Schmitz DG, Brumbaugh GW, et al. Ultrasonographic characteristics of splenic and hepatic lymphosarcoma in three horses. *J Am Vet Med Assoc*. 1992;201:743.
290. Durando M, McKay RJ, Staller G, et al. Septic cholangiohepatitis and cholangiocarcinoma in a horse. *J Am Vet Med Assoc*. 1995;206:1018.
291. Sironi G, Riccaboni P. A case of equine cholangiocarcinoma displaying aberrant expression of p53 protein. *Vet Rec*. 1997;141:77.
292. Mueller PO, Morris DD, Carmichael KP, et al. Cholangiocarcinoma in a horse. *J Am Vet Med Assoc*. 1992;201:899.
293. Lennox T, Wilson J, Hayden D, et al. Hepatoblastoma with erythrocytosis in a young female horse. *J Am Vet Med Assoc*. 2000;216:718.
294. Roperto F, Galati P. Mixed hamartoma of the liver in an equine fetus. *Equine Vet J*. 1984;16:218.
295. Andel AC, Gruys E, Kroneman J. Amyloid in the horse: a report of nine cases. *Equine Vet J*. 1988;20:277.
296. Abdelkader SV, Gudding R, Nordstoga K. Clinical chemical constituents in relation to liver amyloidosis in serum-producing horses. *J Comp Pathol*. 1991;105(2):203.
297. Woldemeskel M. A concise review of amyloidosis in animals. *Vet Med Int*. 2012;15:427296.
298. Hawthorne TB, Bolon B, Meyer DJ. Systemic amyloidosis in a mare. *J Am Vet Med Assoc*. 1990;196:323.
299. Fortier LA, Fubini SL, Flanders JA, et al. The diagnosis and surgical correction of congenital portosystemic vascular anomalies in two calves and two foals. *Vet Surg*. 1996;24:154.
300. Hug SA, Guerrero TG, Makara M, et al. Diagnosis and surgical cellophane banding of an intrahepatic congenital portosystemic shunt in a foal. *J Vet Intern Med*. 2012;26:171.
301. Lindsay WA, Ryder JK, Beck KA, et al. Hepatic encephalopathy caused by a portacaval shunt in a foal. *Vet Med*. 1988;83:798.
302. Beech J. Portal vein anomaly and hepatic encephalopathy in a horse. *J Am Vet Med Assoc*. 1977;170:164.
303. Patton KM, Peek SF, Valentine BA. Gastric adenocarcinoma in a horse with portal vein metastasis and thrombosis: a novel cause of hepatic encephalopathy. *Vet Pathol*. 2006;43:565.
304. Ness SL, Kennedy LA, Slovis NM. Hyperammonemic encephalopathy associated with portal vein thrombosis in a thoroughbred foal. *J Vet Intern Med*. 2013;27:382.
305. van der Luer RJ, Kroneman J. Biliary atresia in a foal. *Equine Vet J*. 1982;14:91.
306. Bates MD, Bucuvalas JC, Alonso MH, et al. Biliary atresia: pathogenesis and treatment. *Semin Liver Dis*. 1998;18:281.
307. Valberg SJ, Ward TL, Rush B, et al. Glycogen branching enzyme deficiency in quarter horse foals. *J Vet Intern Med*. 2001;15:572.
308. Turner TA, Brown CA, Wilson JH, et al. Hepatic lobe torsion as a cause of colic in a horse. *Vet Surg*. 1993;22:301.
309. Bentz KJ, Burgess BA, Lohmann KL, et al. Hepatic lobe torsion in a horse. *Can Vet J*. 2009;50:283.
310. Tennent-Brown BS, Mudge MC, Hardy J, et al. Liver lobe torsion in six horses. *J Am Vet Med Assoc*. 2012;241:615.

CHAPTER 14

Disorders of the Urinary System

Harold C. Schott II, Bryan Waldridge, and Warwick M. Bayly*

Anatomy and Development

Harold C. Schott II

ANATOMY

The urinary system of the horse, like that of most mammals, consists of paired kidneys and ureters, the bladder, and the urethra. With the exception of the abdominal portion of the urinary bladder, the entire urinary tract is located in the retroperitoneal space. In a newborn foal, each kidney weighs about 175 g. In an adult horse, the left kidney weighs between 600 and 700 g, and the right kidney is usually 25 to 50 g heavier, although this is not a consistent finding and one may observe the reverse relationship.[1,2] Thus the kidneys account for approximately 0.65% to 0.75% and 0.27% to 0.37% of the total body mass of the foal and adult horse, respectively.[1,3] The right kidney is located immediately below the dorsal extent of the last two or three ribs and the first lumbar transverse process, is shaped like a horseshoe, and measures about 15 cm long, 15 cm wide, and 5 to 6 cm high (dorsal to ventral). Craniolaterally, the right kidney is embedded into the liver, and its more craniad position compared with the left kidney prevents it from being accessible on rectal palpation. Although not the classically bean-shaped organ found in human beings and small animals, the left kidney in the adult horse is more elongated than the right kidney, with the cranial pole at the level of the hilus of the right kidney. In equids, the left kidney is about 18 cm long, 10 to 12 cm wide, and 5 to 6 cm high. Because of its more caudal location, one routinely can palpate the caudoventral aspect of the left kidney during rectal examination. The blood supply to the kidneys comes from one or more renal arteries branching from the aorta. Accessory renal arteries (which generally enter caudally) may arise from the caudal mesenteric, testicular, ovarian, or deep circumflex iliac arteries.[1,2]

The ureters are 6 to 8 mm in diameter and travel about 70 cm to their insertions in the dorsal bladder neck or trigone, close to the urethra. The distal 5 to 7 cm of each ureter courses within the bladder wall. This intramural segment of the ureter functions as a one-way valve to prevent vesicoureteral reflux with progressive bladder distention. The urinary bladder lies on the pelvic floor when empty but can increase in size and drop forward over the pelvic brim when filled with urine. The bladder can accommodate up to 3 to 4 L of urine before stimulation of micturition. In the foal, the bladder is attached to the ventral abdominal wall by the urachus and remnants of the paired umbilical arteries. Consequently, when empty, the bladder is commonly a band-shaped structure in a neonatal foal. During the first few months of life, this ventral attachment loosens as the urachal remnant becomes the middle ligament and the umbilical arterial remnants become the round ligaments of the free border of the paired lateral ligaments of the bladder.[1]

The urethra is about 2 to 3 cm long in a mare and 75 to 90 cm long in a male. In the intact male, the pelvic urethra, which is 10 to 12 cm long, widens in an elliptic pattern to a diameter of 5 cm across and 2 to 3 cm from dorsal to ventral. A rounded dorsal prominence, the colliculus seminalis, is located immediately caudal to the urethral orifice and is the site of the common openings of the ductus deferens and ducts of the seminal vesicles. The openings of the prostatic ducts are two groups of small papillae lateral to the colliculus seminalis. Between 2 and 3 cm farther caudad, the ducts of the bulbourethral glands open in paired dorsal lines. The smaller openings of the ducts of the lateral urethral glands open at the same level on the lateral aspect of the urethra.[1]

A fibrous capsule that peels easily from the normal kidney covers the surface of each kidney. The equine kidney consists of an outer cortex slightly wider than the inner medulla. The cortex is dotted with dark spots—renal corpuscles or glomeruli within Bowman's capsules. In horses, the corticomedullary junction is less distinct than in other species and is typically a deep red that contrasts well against the paler medulla and red-brown cortex. This region undulates along renal pyramids (cortex) and renal columns (medulla). The pyramids are subdivisions of the renal parenchyma, which are separated by arcuate arteries at the level of the corticomedullary junction. The equine kidney contains a total of 40 to 60 pyramids arranged in four parallel rows. The renal pelvis is the dilated proximal portion of the ureter. Microscopic examination reveals numerous small openings of the collecting ducts (ducts of Bellini). Additionally, the renal pelvis and proximal ureter are lined with compound tubular mucous glands and goblet cells that secrete the thick, viscid mucus normally found in the renal pelvis and urine of normal horses.[1,4]

*The editors and authors acknowledge and appreciate the contributions of Dr. Jennifer Taintor who assisted with updating of references for some sections.

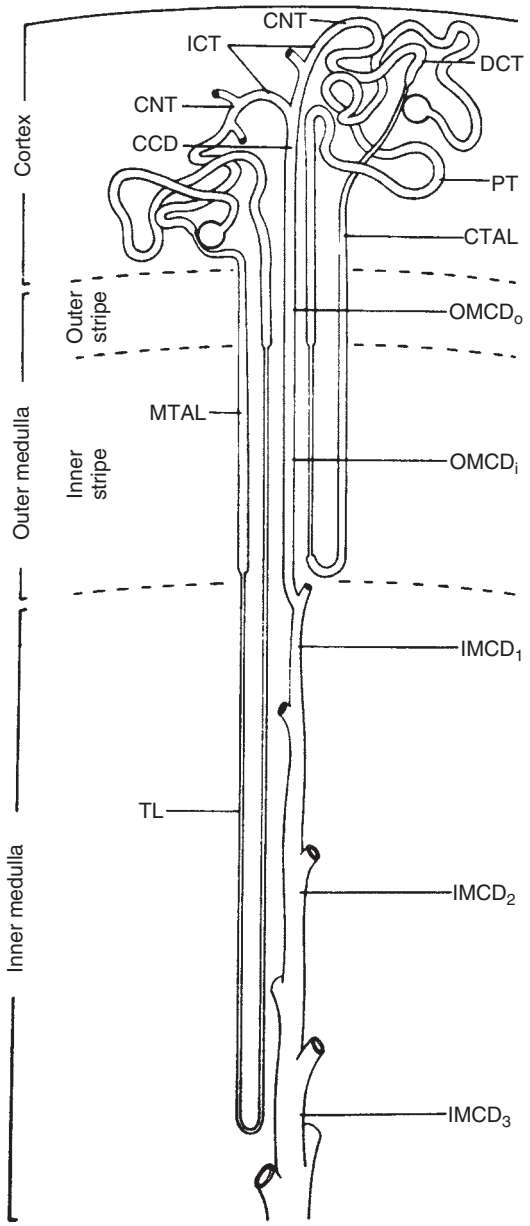


FIG. **14.1** Diagram of a superficial and juxtamedullary nephron. *TL,* Thin limb of the loop of Henle; *MTAL,* medullary thick ascending limb (mTAL) of the loop of Henle; *CCD,* cortical collecting duct; *CNT,* connecting segment; *ICT,* initial collecting tubule; *DCT,* distal convoluted tubule; *PT,* proximal tubule; *CTAL,* cortical thick ascending limb of the loop of Henle; *$OMCD_o$,* collecting duct in outer stripe of outer medulla; *$OMCD_i$,* collecting duct in inner stripe of outer medulla; *$IMCD_1$,* outer third of inner medullary collecting duct; *$IMCD_2$,* middle third of inner medullary collecting duct; *$IMCD_3$,* inner third of inner medullary collecting duct. (From Brenner BM, Rector FC, editors: *The kidney,* ed 8, Philadelphia, 2008, WB Saunders.)

The functional unit of the kidney is the nephron. Each nephron is composed of a renal corpuscle (glomerulus within Bowman's capsule), a proximal tubule (convoluted and straight components), an intermediate tubule (loop of Henle), a distal convoluted tubule, a connecting tubule, and cortical, outer medullary, and inner medullary collecting ducts (Fig. 14.1). The two populations of nephrons are (1) the superficial (or cortical) nephrons possessing short loops of Henle and (2) the juxtamedullary nephrons with long loops of Henle. Gradations exist between these two general categories of nephrons, as well as species variation in the ratio of short- to long-looped nephrons. For example, human beings have 7 times more short- than long-looped nephrons, whereas essentially 100% of nephrons in dogs and cats have long loops.[5] An early anatomic study found approximately 4 million glomeruli (nephrons) in the adult bovine kidney[6]; however, a study of kidney organogenesis using unbiased stereologic techniques to examine 45 equine left kidneys indicated that the left kidney of the horse may contain closer to 10 million glomeruli (for a total of 20 million in both kidneys).[7] The latter study also confirmed that the total number of glomeruli does not increase after birth despite continued growth of the kidney until about 1 year of age. At present, little information is available on the ratio of short- to long-looped nephrons in horses. Histologically, equine nephrons are similar to those of other mammalian species; however, the diameter and epithelial height of the tubule and collecting duct segments are comparatively larger. In addition, the equine macula densa (segment of the ascending loop of Henle that lies in close association with the juxtaglomerular apparatus of the afferent arteriole) appears more prominent than that of other mammals.[8] Whether or not these subtle histologic differences are accompanied by functional differences has not been investigated.

Relative to its size, the mammalian kidney has a richer innervation than almost any other organ.[9] Although the neuroanatomy of the equine kidney has not been well studied, autonomic nerves course from the aorticorenal and renal ganglia along the major renal vessels into the kidneys.[1] These nerves are predominantly sympathetic, because the kidneys appear to be poorly supplied by cholinergic nerves. Although the best-recognized effect of renal nerves is control of renal vascular resistance (for regulation of renal blood flow over a wide range of perfusion pressures), the nerves also act directly on renal tubules and juxtaglomerular cells. For example, low-frequency stimulation of renal nerves (below the threshold for vasoconstriction) increases proximal tubular sodium reabsorption and renin release by activation of α_1-adrenoceptors.[10] In addition to α- and β-adrenoceptors, renal vasculature is rich in dopaminergic adrenoceptors, and activation of the latter, specifically dopamine type 1 receptors, leads to increased perfusion of the outer renal medulla. Presence of these receptors is the basis for use of dopamine and the DA-1 receptor agonist fenoldopam, to improve renal blood flow in acute renal failure or decrease the risk of radiocontrast nephropathy.[11-13] Drugs also may activate renal adrenoceptors unintentionally. A common clinical example is the diuresis induced by administration of the α_2-agonists xylazine and detomidine. Although the diuresis has been attributed to a transient hyperglycemia and glucosuria, the latter is often absent.[14,15] An alternative explanation may be drug binding to α_2-adrenoceptors located on collecting duct epithelium. Activation of these receptors can lead to antagonism of the effects of antidiuretic hormone on cortical collecting ducts, which results in diuresis.[16] Renal afferent nerves appear to play a role in the pathogenesis of hypertension in species affected by this disorder.[9]

Autonomic innervation of the ureters, bladder, and urethra is important for ureteral peristalsis and micturition. The equine ureteral smooth muscle contains α_1- and β_2-adrenoceptors, which induce contraction and relaxation, respectively, when activated by norepinephrine.[17] Studies of the innervation of the equine ureter demonstrated greater densities of adrenergic neurons in the proximal (renal pelvis) and intravesicular (bladder wall) portions of the ureter.[18] Increased densities in these regions are consistent with the suspected pacemaker activity of the renal pelvis, which

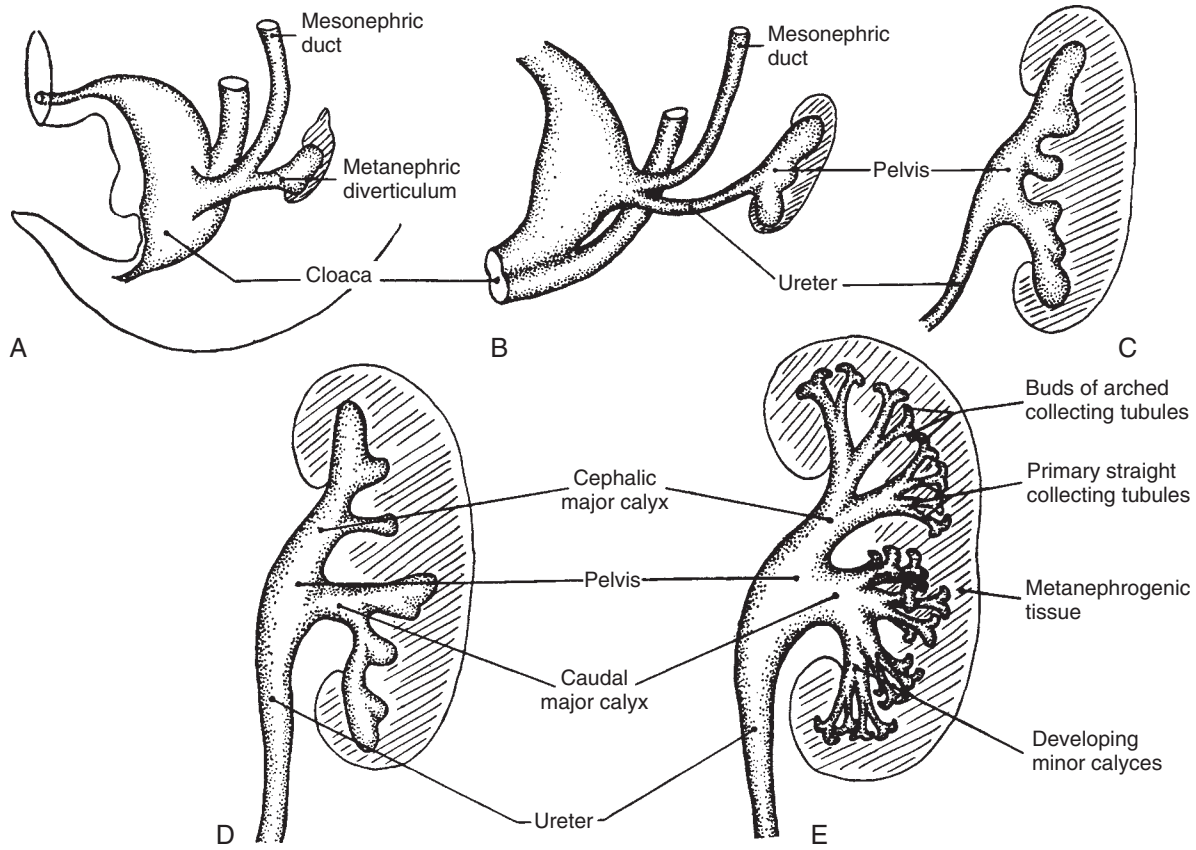


FIG. **14.2** Progression of the differentiation and development of the metanephric diverticulum into the collecting system of the mature kidney. **A,** Metanephric diverticulum originates from the caudal end of the mesonephric duct. **B,** Metanephric diverticulum develops craniad, and intermediate mesoderm (metanephrogenic tissue, hatched lines) collects about its cranial end. **C** to **E,** The metanephric diverticulum becomes the ureter and renal pelvis, and the metanephrogenic tissue becomes the collecting system and parenchyma of the mature kidney. (From Carlson BM: *Patten's foundations of embryology,* ed 6, New York, 2003, McGraw-Hill.)

initiates ureteral peristalsis and the sphincter-like function of the distal segment of the ureter. The sympathetic nerve supply to the urinary bladder is provided via the hypogastric nerve, with preganglionic fibers arriving from spinal segments L1–L4 to synapse in the caudal mesenteric ganglion. Postganglionic fibers supply the bladder (β_2-adrenergic receptors) and proximal urethra (primarily α_1- and some α_2-adrenergic receptors).[19,20] In addition to adrenergic innervation, cholinergic and peptidergic nerve fibers also innervate the equine bladder.[21] Parasympathetic innervation originates in the sacral segments of the spinal cord, with neurons joining to form the pelvic nerve.[19,20] Many complex interneuronal connections exist between sympathetic and parasympathetic nerves in the wall of the bladder, along with small adrenergic cells that facilitate interaction between sympathetic and parasympathetic pathways.[22] As a result, complete denervation of the bladder is virtually impossible. Somatic innervation of the lower urinary tract is primarily to the striated muscle of the external urethral sphincter via a branch of the pudendal nerve, which originates from the sacral cord segments (S1–S2).[1]

DEVELOPMENT

The embryonic upper urinary tract arises from bilateral primordial mesonephric ducts and intermediate mesoderm. The metanephric diverticulum originates from the caudal end of each mesonephric duct and develops craniad to become the ureter and renal pelvis. The advancing metanephric diverticuli collect about their ends intermediate mesoderm (metanephrogenic tissue), which becomes the collecting system and parenchyma of the mature kidney (Fig. 14.2). The vascular supply is derived from a branch of the aorta (renal artery) that invades the metanephrogenic tissue. The urinary bladder develops as a dilated proximal portion of the allantois. The bladder is separated from the hindgut by the craniocaudad growth of the urorectal fold, which divides the rectum from the urogenital sinus. The latter structure gives rise to the urethra (Fig. 14.3). The mesonephric and metanephric ducts initially open into the urogenital sinus, but as development continues, the distal segments of the mesonephric ducts are absorbed into the bladder wall, and the openings of the metanephric ducts are pulled craniad to their final site in the dorsal bladder neck.[23]

The fate of the mesonephric tubules (mesonephros) and mesonephric ducts varies with gender. Paired paramesonephric ducts (Müllerian ducts) arise parallel to the mesonephric ducts in both sexes. In the female, the ducts fuse distally to become the vagina and uterine body, whereas proximally they remain separate to give rise to uterine horns and oviducts. The mesonephric ducts regress into vestigial remnants termed the *epoöphoron* proximally (near the ovaries) and *Gartner's canals* distally (near the vagina and uterus; Fig. 14.4). In the

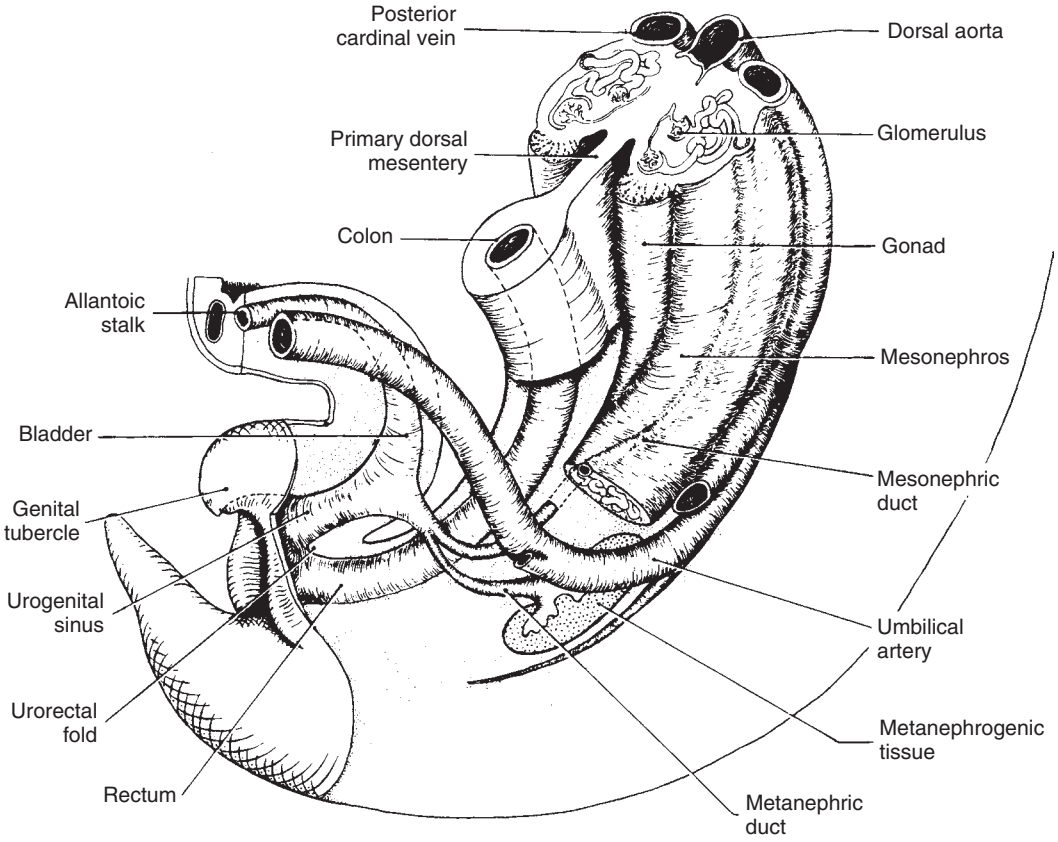


FIG. **14.3** Developing urogenital tract of the young mammalian embryo. (From Carlson BM: *Patten's foundations of embryology,* ed 6, New York, 2003, McGraw-Hill.)

male, sexual differentiation of the gonads and production of androgenic steroid hormones lead to regression of the müllerian ducts. The duct system of the male reproductive tract is appropriated from the mesonephros and mesonephric ducts (also termed *wolffian ducts*). Androgenic steroid hormones also stimulate these structures to develop into the seminiferous tubules, epididymis, and ductus deferens. The distal portion of the mesonephric duct becomes the ejaculatory duct, the terminal portion of the ductus deferens.[23]

DEVELOPMENTAL MALFORMATIONS OF THE URINARY TRACT

Anomalies of the urinary tract are uncommon in horses. A survey by Höflinger[24] revealed a similar frequency of unilateral renal agenesis (0.07%) in horses and human beings (0.10%).[5] In contrast, horseshoe kidneys (attached at the cranial or caudal poles) are the most common anomaly in human beings (0.25%) but rarely have been described in horses.[5,25]

Renal Agenesis, Hypoplasia, and Dysplasia

Renal agenesis, which may be unilateral or bilateral, results from failure of the metanephric duct to fuse with the metanephrogenic mesodermal tissue. Although unilateral anomalies have been described more frequently, this simply may reflect the incompatibility of bilateral agenesis with postnatal life.[24,26-28] Brown and colleagues[28] described a foal with bilateral renal agenesis in which severe azotemia was detected shortly after birth. Bilateral ureteral dysgenesis and cryptorchidism, agenesis of the right adrenal gland, and atresia ani accompanied renal agenesis in this foal. Unilateral defects may be incidental findings in otherwise healthy horses[29] or may be detectable during reproductive examination because many affected horses also have associated anomalies of the reproductive system. Occasionally, unilateral agenesis may result in clinical renal disease if a problem arises in the contralateral kidney. Johnson and colleagues[27] described a 4-year-old Quarter Horse with unilateral renal agenesis and a ureterolith causing contralateral hydronephrosis. The gelding was presented for weight loss, pollakiuria, and stranguria. In addition to the renal anomaly, unilateral agenesis of the ipsilateral testicle also was found on necropsy. A 9-month-old Tennessee Walking Horse colt with chronic kidney disease, unilateral renal agenesis, severe contralateral ureterolithiasis with hydronephrosis, bilateral cryptorchidism, and segmental aplasia of the ductus deferens was reported by Waldridge and colleagues.[30] Renal agenesis may be a familial disorder in several species.[25,31] Although no information is available to suggest a hereditary basis in horses, one probably should discourage repeat matings after detecting such an anomaly because congenital lesions of the urinary system are often accompanied by segmental abnormalities of the reproductive tract.

One diagnoses renal hypoplasia when one kidney is at least 50% smaller than normal or when the total renal mass is decreased by more than one third.[25] Renal hypoplasia is a quantitative defect caused by a reduced mass of metanephrogenic tissue or by incomplete induction of nephron formation by the metanephric duct. This condition may be confused with renal dysplasia. Unilateral renal hypoplasia usually is associated

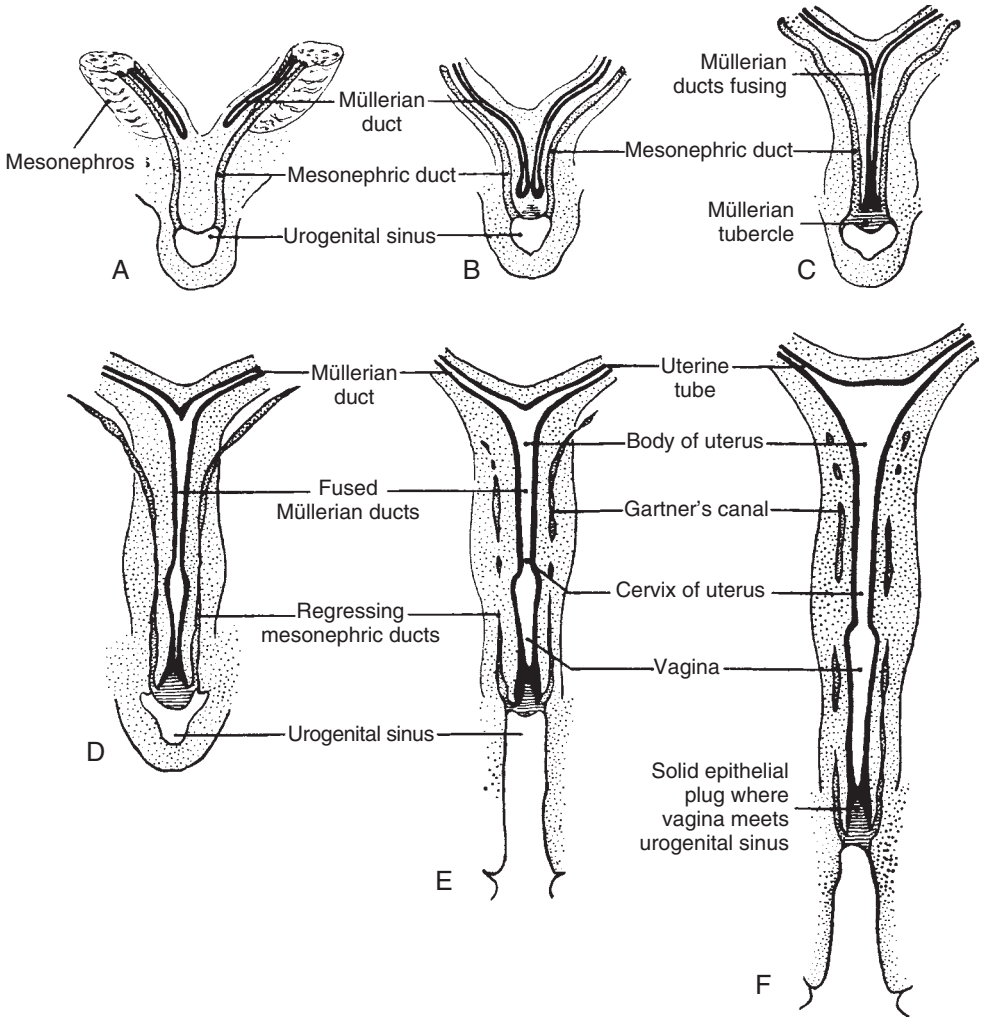


FIG. **14.4** Development of the mesonephric tubules (mesonephros) and mesonephric ducts into the female reproductive tract. **A** and **B,** Paired paramesonephric ducts (müllerian ducts) arise parallel to the mesonephric ducts in both sexes. **C** to **E,** In females, the paramesonephric ducts fuse distally to become the vagina and uterine body but remain separate proximally to give rise to uterine horns. **D** and **E,** The mesonephric ducts regress into vestigial remnants termed *Gartner's canals.* (From Carlson BM: *Patten's foundations of embryology,* ed 6, New York, 2003, McGraw-Hill.)

with contralateral hypertrophy and normal renal function, whereas bilateral hypoplasia generally leads to chronic kidney disease.[25,31] Andrews and colleagues[32] described bilateral renal hypoplasia in a foal presented after death and in three young horses with chronic kidney disease that had poor growth from birth. Anomalies in these four horses were limited to the upper urinary tract.

Renal dysplasia is disorganized development of renal tissue caused by anomalous differentiation, intrauterine ureteral obstruction, in utero viral infection, or teratogens.[25,33] Bilateral dysplasia usually leads to renal failure. In general, dysplastic kidneys are normal in size unless concurrent hypoplasia exists or the animal lives for months to years before developing renal failure. Roberts and Kelly[34] reported a case of bilateral renal dysplasia in a 19-month-old pony gelding. The pony was presented for weight loss over a 3-month period, and clinicopathologic assessment revealed chronic kidney disease. A small, firm, and nodular left kidney was palpable per rectum. At necropsy, the kidneys weighed 280 g each (33% smaller than normal for body weight) and were nodular. Renal dysplasia was suspected because glomeruli in the collapsed areas of the kidneys were small, tubules were immature, and inflammatory cells were scant. Six similar cases of bilateral renal dysplasia resulted in chronic kidney disease in horses from 2 months to 7 years of age.[35-39] Small kidneys with increased echogenicity and an indistinct corticomedullary junction were typical ultrasonographic findings,[37-39] and these findings were corroborated by computed tomography in one Miniature Horse foal.[39] At necropsy, kidneys were typically small and irregular, the cortex and medulla were not well delineated, and immature glomeruli and primitive tubules were found on histologic examination (Fig. 14.5). Renal dysplasia also may cause renal failure in neonates. For example, Zicker and colleagues[40] reported a case of renal dysplasia in a 2-day-old Quarter Horse foal presented for diarrhea and depression. Clinicopathologic assessment revealed azotemia, hyponatremia, hypochloremia, and urinary sodium wastage. At necropsy, the kidneys were normal in size (380 g), but histologic examination revealed immature glomeruli, hypoplastic tubules and vasa recta, and extensive myxomatous connective tissue occupying 90% of the

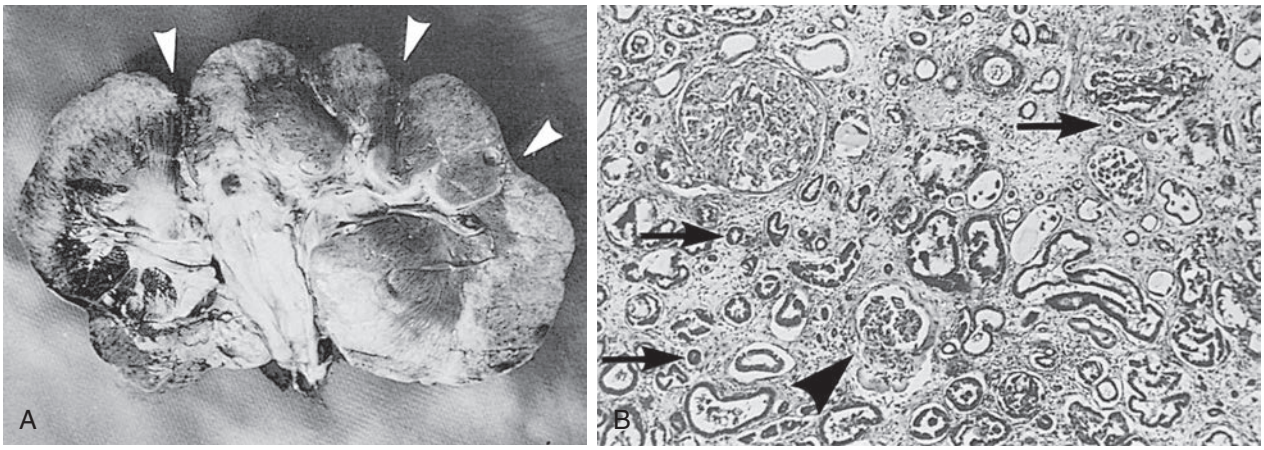


FIG. **14.5** **A,** Longitudinal section of the right kidney from a 7-year-old Arabian gelding with renal dysplasia shows focal, irregular thinning of the cortex *(arrowheads)* resulting in a nodular surface and poor delineation of the corticomedullary junction. **B,** Histologic section of same kidney (Masson trichrome stain; original magnification ×35) reveals an immature glomerulus *(large arrowhead)* and primitive tubules *(arrows)* surrounded by persistent mesenchyme. (From Ronen N, van Amstel SR, Nesbit JW, et al.: Renal dysplasia in two adult horses: clinical and pathologic aspects, *Vet Rec.* 132:269, 1993.)

total medullary volume. Finally, renal dysplasia also may be a unilateral problem that does not result in renal failure. Jones and colleagues[41] found ureteropelvic polyps to be the cause of unilateral hydronephrosis and renal dysplasia in a Trakehner colt. Poor growth and hematuria for several weeks were the presenting complaints. Renal function remained normal for 8 months after nephrectomy until the colt developed a severe bout of colic, prompting euthanasia. Ureteral obstruction by the polyps was the suggested cause of renal dysplasia, because urinary tract obstruction has been found in a large percentage of cases of human renal dysplasia.[33]

Renal Cysts

One or more renal cysts occasionally are discovered as incidental findings on necropsy examination. The cysts may arise from any portion of the nephron but more often occur in the renal cortex than in the medulla. The pathogenesis is not known, but a defect in the basement membrane that allows tubular dilation is suspected. Renal cysts vary in size from microscopic to as large as the organ itself and routinely have a clear to slightly opaque wall and contain a thin, clear fluid. Congenital cysts are differentiated easily from acquired cysts (after obstruction) by the extensive scarring that accompanies the latter. Renal cysts also may develop as a consequence of drug therapy (i.e., long-acting corticosteroids) or exposure to certain chemicals.[25,31]

Polycystic Kidney Disease, Glomerulocystic Disease, and Other Hereditary Nephropathies

Polycystic kidney disease (PKD) is a disorder in which numerous, variably sized cysts are found throughout the cortex and medulla. With glomerulocystic disease, cysts are microscopic and limited to Bowman's spaces. Cysts of the bile duct and pancreas also may occur with PKD, and both conditions have been described in stillborn offspring in many species, including foals.[25] The two major types of human PKDs are (1) a rare congenital or infantile form inherited as an autosomal recessive trait (which may be found in stillbirths) and (2) a more common adult form inherited as an autosomal dominant trait that leads to renal insufficiency in later life in association with dramatically enlarged, cystic kidneys.[42,43] The latter form of PKD develops because of mutations in genes encoding for polycystins, integral membrane proteins responsible for cell-to-cell interaction.[44] Autosomal dominant PKD also has been documented in Persian cats and related breeds and in bull terriers.[45-47] The genetic defect in Persian cats is thought to be similar to the most common defect in human beings (PKD1 gene) and leads to end-stage renal disease by 3 to 10 years of age.[46] As in human beings, the disorder is detectable by renal ultrasonographic screening of juvenile cats and preventable by avoiding subsequent mating of affected animals. Nevertheless, because of heavy inbreeding, the prevalence of PKD in Persian cats and related breeds is between 40% and 50%.[45,46]

Ramsey and colleagues[48] described polycystic kidneys in a 9-year-old Thoroughbred mare that exhibited anorexia and weight loss. Clinicopathologic assessment revealed chronic kidney disease, and euthanasia was performed. At necropsy, the kidneys were grossly enlarged, and each weighed 12 kg (Fig. 14.6). A similar case of bilateral PKD was described in a 15-year-old pony with a 4-week history of hematuria and moderate weight loss. Evaluation revealed azotemia and presence of large masses near both kidneys on rectal examination and dramatically enlarged polycystic kidneys weighing 11.4 and 9.1 kg, respectively, were found at necropsy.[49] Bertone and colleagues[50] reported a third case of adult PKD in a 10-year-old Paint gelding with weight loss. The horse was mildly azotemic, and several 2- to 15-cm-diameter cysts were imaged in both kidneys during ultrasonographic examination. In human beings, polycystic kidneys are believed to result in renal failure as cysts expand (sometimes under pressure) and compress adjacent normal renal tissue. Altered compliance of tubular basement membranes and proliferation of renal tubular epithelium result in outflow obstruction and proximal ballooning, leading to renal cyst formation.[43] In some human cases, pressure within cysts may be 5 to 10 times higher than surrounding interstitial tissue pressures. Bertone and colleagues[50] did not find increased pressure in several cysts catheterized

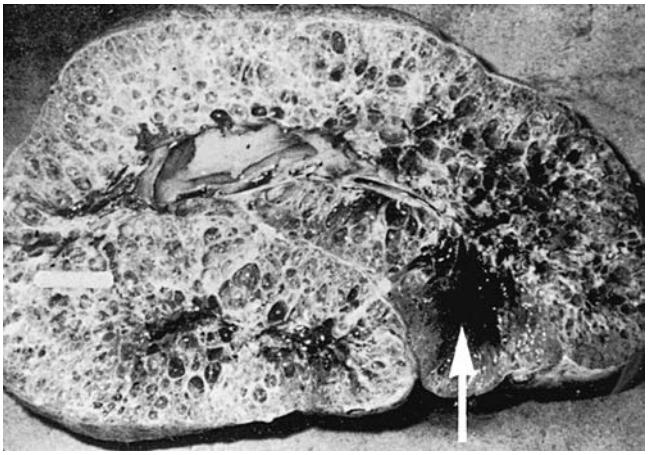


FIG. **14.6** Longitudinal section of the left kidney (35 cm long, 25 cm wide, 20 cm deep, and weighing 12 kg) from a 9-year-old Thoroughbred mare with polycystic kidneys. A calculus is located in the renal pelvis, and the arrow demonstrates the only grossly normal-looking renal parenchyma. (From Ramsey G, Rothwell TLW, Gibson KT, et al.: Polycystic kidneys in an adult horse, *Equine Vet J.* 19:243, 1987.)

percutaneously in a gelding with PKD, but differences in sodium concentrations suggested that the sampled cysts had arisen from different segments of the renal tubule. Euthanasia was performed after a prolonged hospitalization (235 days), and the kidneys were not grossly enlarged except where distorted by large cysts. Although not well documented, PKD has been described anecdotally in two additional Paint Horses, suggesting that an inherited form of PKD may occur in that breed. Another report also documented PKD in an 11-year-old Andalusian gelding.[51]

In addition to PKD, a variety of other hereditary nephropathies have been described in humans.[49] Similar disorders are starting to be recognized in domestic animals, including hereditary nephritis in bull terriers, Samoyeds, and English cocker spaniels. Analogous to Alport syndrome in human beings, a defective molecular structure of type IV collagen, an important component of the glomerular basement membrane, appears to be the cause of hereditary nephritis in these dog breeds.[47] Similarly, a syndrome of renal tubular dysplasia with autosomal recessive pattern of inheritance has been described in a population of highly inbred Japanese black cattle,[52-54] as has a syndrome of suspected hereditary renal oxalosis in Beefmaster calves.[55] Similar hereditary nephropathies are likely to occur in horses; however, to date the only one documented is a syndrome of nephrogenic diabetes insipidus in Thoroughbreds.[56]

Vascular Anomalies

Anomalies of the vascular supply to the equine urinary tract are rare but may result in hematuria, hemoglobinuria, partial ureteral obstruction, or hydronephrosis.[31,57] Latimer and colleagues[58] described a distal aortic aneurysm and associated extrarenal arteriioureteral fistula in a 5-month-old colt presented for intermittent hematuria, colic, and lameness. Partial ureteral obstruction and hydronephrosis were observed on the affected side. Intrarenal vascular anomalies, termed *renal arteriovenous malformations,* are similarly rare (reported frequency of 0.04% in humans).[59] Interestingly, vascular malformations may be silent until later in life, when varying degrees of hematuria and flank pain may ensue. The anomalous vessels are often tortuous and may be enlarged focally and devoid of elastic tissue. Hematuria and hemoglobinuria are thought to arise from areas where the anomalous vessels lie close to the collecting system.[59,60] With vascular anomalies, one should attempt to determine the extent of the defect (unilateral or bilateral) via ultrasonographic examination, contrast radiographic studies, or cystoscopy (visualization that hematuria is coming from one or both ureteral orifices). When a unilateral defect is documented in the absence of azotemia, unilateral nephrectomy or selective renal embolization has been recommended to prevent possible fatal exsanguination through the urinary tract[57,58]; however, one may consider conservative treatment if the urinary tract bleeding is minor and has not resulted in anemia.

A large vascular anomaly resulting in transient hemoglobinuria has been reported in a Quarter Horse colt.[61] Over several weeks, the large anomalous vascular structure (Fig. 14.7) spontaneously filled with a thrombus so that specific treatment (a nephrectomy) was not pursued. Severe adult-onset, idiopathic renal hemorrhage also has been described in horses.[62] Whether this latter syndrome may have been a consequence of congenital renal vascular malformations has not been determined (see Hematuria later in this chapter). Occasionally, gross hematuria with passage of blood clots can accompany omphalitis or bladder rupture.[63] One usually can detect these problems during ultrasonographic examination of the umbilical structures and sometimes can image tissue echogenicity within the bladder that is attributable to a blood clot.

Pendulant Kidney

A pendulant kidney is a rare anomaly in the horse.[64] Rectal examination reveals an extremely mobile kidney attached to the dorsal body wall by a thin band of tissue. Although a pendulous kidney could result from extreme weight loss, hydronephrosis, or perirenal trauma, the condition is usually thought to be congenital. The abnormality is an incidental finding unless displacement or rotation leads to partial or complete ureteral obstruction. As an example, the author has palpated the entire right kidney of one mare immediately craniad of the pelvic canal, and ultrasonographic imaging revealed normal size and structure of the anomalously located kidney.

Ectopic Ureter

Although ureteral ectopia occurs rarely in the horse,[65] it is the most commonly reported developmental anomaly of the equine urinary tract.[65-83] Ectopic ureters may develop when (1) the ureteric bud (metanephric duct) fails to be incorporated into the urogenital sinus or fails to migrate craniad to the bladder neck or (2) the mesonephric duct fails to regress. In the former case, the ectopic ureter opens near the urethral papilla in females or into the pelvic urethra near the colliculus seminalis in males, whereas in the latter, the ureter may open anywhere along the vagina, cervix, or uterus (but only in females because this portion of the mesonephric duct becomes the Wolffian duct system in males). In 118 reported cases of ectopic ureter in horses, 105 (89%) were females[66-69,73-78,80-83]; however, this sex distribution may reflect easier recognition of the presenting complaint of urinary incontinence in females rather than a true sex predilection. Incontinence is recognized more often in females because urine entering the pelvic urethra in males may pass retrograde into the bladder. Although a genetic predisposition for ectopic ureter exists for

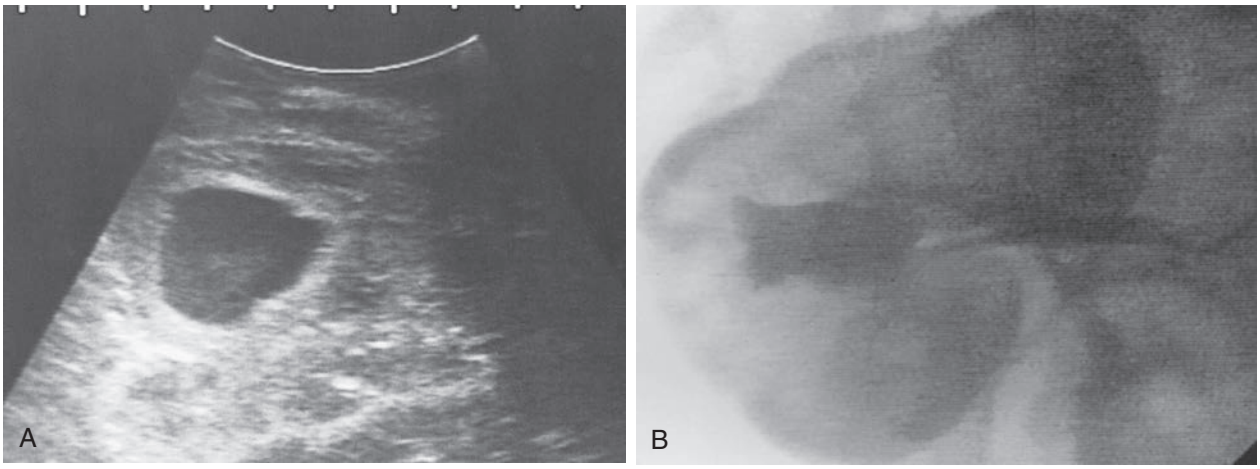


FIG. **14.7** Ultrasonographic image of the right kidney of a 9-day-old Quarter Horse colt shows a 2 × 3–cm hypoechoic cavity on a dorsal oblique view. **A,** A swirling pattern, similar in appearance to blood in the ventricles of the heart, was consistent with an arteriovenous malformation. **B,** In a selective nephrogram of the right kidney of the same colt at 20 days of age, immediately after injection of contrast, a dilated vascular space was demonstrated (contrast appears dark because of reverse gray scale) and the renal cortical tissue abaxial to this structure appeared to have reduced capillary phase contrast. (From Schott HC, Barbee DD, Hines MT, et al.: Renal arteriovenous malformation in a Quarter horse foal, *J Vet Intern Med.* 10:204, 1996.)

several dog breeds,[84] no breed predilection has been established in horses. However, Quarter Horses may be at greater risk because the condition has been reported in five Quarter Horses, three Standardbreds, two Thoroughbreds, two Appaloosas, an Arabian, a Clydesdale, a Shire, a Friesian, a Foxtrotter, and a Warmblood. The author also has seen the condition in two Quarter Horse fillies (one unilateral and one bilateral), yielding a total of 20 cases.

In horses with ureteral ectopia, urinary incontinence is generally apparent from birth, and affected animals are presented for extensive scalding of the hindlimbs. With unilateral ectopia, horses also void normally, because the other ureter enters the bladder in the appropriate location. Renal function is usually normal, but the affected ureter may be greatly dilated. Urine pooling in the vagina and uterus was a complicating factor in one case.[74] To determine the site of the ectopic ureteral orifice (or orifices), one initially visually examines the vestibule and vagina (using a blade speculum) to look for intermittent urine flow from the area of the urethral papilla. Ectopic ureteral openings usually are not apparent unless urine flow is visible. Endoscopy may be helpful in females (while inflating the vestibule and vagina with air and using a hand to form a seal at the vulva) and is required in males to visualize the ectopic ureteral opening. Intravesical placement of methylene blue dye was performed in one filly to provide evidence for ureteral ectopia. Continued dribbling of clear urine (from the ectopic ureter) followed by passage of blue, discolored urine indicated that only one ureter emptied into the bladder but provided no information on the location of the opening of the ectopic ureter.[67] Intravenous administration of dyes—including sodium fluorescein (10 mg/kg intravenously [IV]; yellow-green), indigotindisulfonate (indigo carmine, 0.25 mg/kg IV; blue-purple), azosulfamide (2.0 mg/kg IV; red), or phenolsulfonphthalein (1.0 mg/kg IV; red)—to discolor the urine may help locate ectopic ureteral openings.[85] Contrast radiography (excretory urography or retrograde contrast studies via catheterization of the bladder and ureters) has been used to detail renal architecture and the course of the ureters in some affected animals; however, results of intravenous urograms are frequently inconclusive and difficult to interpret in foals weighing more than 50 kg (contrast agent is poorly imaged). Ultrasound-guided pyelography, in which contrast agent was injected directly into the renal pelvis using a spinal needle, proved to be a more effective technique than intravenous administration of contrast agent to detail the course of an ectopic ureter, and one should consider this technique in future cases.[83]

Treatment has included ureterocystostomy (surgical reimplantation of the ectopic ureter or ureters into the bladder) or unilateral nephrectomy. Before surgery, one must determine whether the condition is unilateral or bilateral, which side is affected if unilateral, and whether urinary tract infection is present. Further, one should attempt to rule out other anomalies, especially of the reproductive tract. If the problem is bilateral (8 of 20 cases), one should establish the presence of a normal micturition response by measuring the intravesicular pressure response to progressive distention until the fluid infused is voided spontaneously. This procedure provides an estimate of bladder volume and ensures competency of the urethral sphincter before reimplantation. Among 14 cases in which surgical correction was pursued, ureterocystostomy was successful in establishing a functional urinary system in nine published cases and one foal seen by the author, but four died of postoperative complications.[69,76,83] In contrast, all four cases treated by unilateral nephrectomy had a favorable outcome.[73,74,80] Because affected ureters often are dilated and tortuous, surgical reimplantation can be difficult and may not result in a functional ureteral orifice. Consequently, when the problem is unilateral, nephrectomy of the affected side may be the preferred treatment option.[86,87]

Ureteral Defects or Tears (Ureterorrhexis)

Retroperitoneal accumulation of urine and uroperitoneum has been described in seven foals with unilateral or bilateral ureteral defects[88-94] and has been observed in three additional foals by the author. These included seven male and three female foals

of several breeds (five Standardbreds, two Thoroughbreds, one Belgian, one Oldenburg, and one Appaloosa). Clinical signs (decreased nursing, depression, abdominal distention, diarrhea, and muscle twitching or other signs of neuromuscular irritability) and clinicopathologic abnormalities (hyponatremia, hyperkalemia, hypochloremia, and azotemia) are similar to those in horses with bladder rupture but may have a slightly later onset (4–16 days of age). Mild protrusion of the vagina may occur in fillies if the peritoneum has remained intact.[95] In affected foals, ultrasonographic examination may reveal dilation of the renal pelvis and affected ureter, as well as fluid accumulation around the kidneys or farther caudad within the retroperitoneal space. As with ectopic ureters, excretory urography generally has been an unrewarding diagnostic procedure, but contrast pyelography was used successfully to image leakage of contrast agent from a proximal ureteral defect.[94] Contrast radiography has not been pursued routinely because exploratory celiotomy generally was performed shortly after a diagnosis of uroperitoneum. Catheterization of the ureters via a cystotomy and retrograde injection of methylene blue allowed localization of the defect (or defects), and surgical correction was performed successfully in four cases by suturing the defect around an indwelling catheter.[90,91,94] Although ascending urinary tract infection should be an expected complication with a stent, repair of a defect in one foal without use of an indwelling catheter resulted in further urine leakage from the ureter, prompting a nephrectomy 4 days after the initial surgery.[92] Of the remaining five foals, one died after three unsuccessful attempts at surgical repair,[89] and euthanasia was performed in four cases without attempting repair.[88,93]

At surgery or necropsy, a single defect was found in six foals, whereas bilateral defects were found in four foals and multiple defects were apparent in one ureter. In most cases the defects have been located in the proximal third of the ureter near the kidney. Interestingly, distended, tortuous ureters, occasionally accompanied by hydronephrosis, also were described in three affected foals,[89,92,94] and distal obstruction of the ureters at the bladder was suspected in two of these cases, prompting ureteroneocystostomy. Although several reports suggest that ureteral defects may be anomalies of development, the actual cause of these ureteral defects is not known. Traumatic disruption was suggested in the initial report in which histologic examination of the margins of the defect revealed hemorrhage and proliferation of immature connective tissue.[88] A traumatic cause was further supported by a subsequent report in which histologic examination of the defects revealed absence of transitional epithelium and inflammation in a foal that had been attacked by dogs.[93] Inflammation and granulation tissue also were visible in the apparently obstructed distal ureter in one of the foals with ureteral distention, again suggesting an acquired lesion. Blunt abdominal trauma, often sustained during automobile accidents, can cause retroperitoneal accumulation of urine and uroperitoneum in humans.[96] Disruption of the ureter is usually near the kidney, and this complication of trauma may not be recognized for several days after injury. In one foal evaluated by the author, multiple rib fractures found at necropsy suggested that these ureteral tears actually could be a complication of foaling trauma.

Rectourethral and Rectovaginal Fistulae

If the urorectal fold fails to separate completely the primitive hindgut from the urogenital sinus, a rectourethral fistula may be found in a colt or a rectovaginal fistula or persistent cloaca may be found in a filly.[97] These anomalies are rare in horses and when present are usually associated with atresia ani and other anomalies, including agenesis of the coccygeal vertebrae and tail, scoliosis, adherence of the tail to the anal sphincter area, angular limb deformities, and microphthalmia.[71,72,98-103] Affected foals are usually examined for atresia ani, although one also may observe signs of colic and straining. Evidence for a fistula is passage of fecal material from the vulva or urethra. In fillies, one may detect rectovaginal fistulae by digital palpation of the dorsal vestibule and vagina, but in colts a definitive diagnosis usually requires contrast radiographic procedures such as a barium enema or a retrograde urethrogram (Fig. 14.8). Surgical correction of atresia ani and fistulae has been performed successfully in several foals, but multiple surgical procedures may be required. Because ascending urinary tract infection may be a complication, one should submit a sample of urine collected via bladder catheterization (preferably during surgery) for bacterial culture.[97] In humans, evidence suggests that these anomalies are hereditary, and in one report, several foals born with atresia ani were sired by the same stallion.[98] Consequently, affected horses should not be used for breeding after surgical correction of the anomalies.



FIG. **14.8** A positive contrast urethrogram in a 3-day-old burro that had atresia ani and intermittent passage of fecal material from the urethra. A catheter passed via the urethra and contrast agent injected into the catheter resulted in accumulation of a large amount of contrast agent in the rectum and a lesser amount in the intrapelvic portion of the urethra. A small amount of contrast agent is visible in the urethrorectal fistula *(arrow)*.

A urethrorectal fistula resulting in passage of urine from the anus also has been described in a 3-year-old Thoroughbred gelding.[104] The fistula in this gelding was thought to be acquired after trauma or straining because no other developmental problems were detected, and the edges of the defect were irregular and inflamed when examined with a speculum inserted into the rectum.

Bladder Defects

Uroperitoneum may result from bladder rupture during parturition in foals (most commonly males)[105] or as a consequence of urachal leakage after infection of the umbilical structures.[106,107] In addition, Wellington described uroperitoneum in two foals that were full brothers.[108] Urine entered the abdomen from a dorsal defect in both colts, and smooth margins to the defects combined with a lack of appreciable inflammation

provided evidence in favor of anomalous development rather than trauma. Other authors have suggested that some cases of uroperitoneum likely are associated with anomalous bladder defects because of the size, location, or lack of apparent inflammation of the margins of the defects.[71,109-112] For example, Bain[109] described uroperitoneum in a foal in which the ventral portion of the bladder was absent between the lateral ligaments (umbilical artery remnants) from the umbilicus to the urethra.

Anomalous fusion of the bladder to the inner umbilical ring (absence of the urachus) has been described in one foal.[113] The malformation precluded normal contraction and evacuation of the bladder, and a megavesica (a greatly enlarged bladder) developed. The clinical appearance was similar to uroperitoneum and surgical separation of the bladder from the umbilical ring restored normal anatomic and functional integrity of the bladder. A similar case with a greatly distended bladder was reported in a foal evaluated for abdominal distention[71] that was attributed to an adhesion of the bladder to the urachus or umbilical remnant. An enlarged, flaccid bladder also was described in a foal undergoing exploratory celiotomy for suspected urinary tract disruption.[106] Adhesions to the abdominal wall were not reported, and the foal survived after the surgery, during which 50% of the distended bladder was resected. In addition to bladder distention, persistent attachment of the bladder to the area of the umbilicus via a urachal remnant was reported to cause pollakiuria and dysuria in a 15-month-old Thoroughbred filly.[114] The author also has seen postpartum bladder rupture in a mare in which a persistent urachal attachment was suspected to be a contributing factor.

Excessive bladder distention or megavesica has been described further in four stillborn foals[115] and one neonatal foal.[116] In the latter foal and in another report,[117] chronic bladder distention appeared to lead to loss of smooth muscle in the dorsal bladder wall and its replacement with collagen. The result was bladder rupture during parturition. Although these reports are similar to an early report by Rooney[105] describing the dorsal bladder wall as the anatomic weak link and likely area for rupture, they differ in that chronic distention of the bladder in utero with smooth muscle loss is not recognized in more typical bladder ruptures in neonatal foals. Why bladder distention should occur in utero without obstruction of the lower tract (not found in these cases) is not clear. Although an excessively long umbilical cord (longer than 85 cm) may lead to urachal obstruction,[115,118] urine produced in utero alternatively could drain into the amniotic cavity via the urethra. Thus megavesica remains poorly characterized and poorly understood.

Bladder distention also is recognized in some foals with hypoxic-ischemic encephalopathy. Affected foals may posture to urinate frequently, and ultrasonographic examination may reveal an enlarged bladder or incomplete bladder emptying. In recumbent foals, one may note abdominal distention, and temporary use of an indwelling bladder catheter to keep the bladder empty is helpful; however, ascending urinary tract infection may be a complication. Cystometrography would be useful to assess detrusor function in affected foals, but no reports describe this diagnostic test in equine neonates. Although administration of cholinergic drugs (e.g., bethanechol) to improve detrusor function or α-adrenergic blockers (e.g., phenoxybenzamine, acepromazine) to decrease urethral sphincter tone has been described anecdotally to be of benefit, no reports describe the efficacy of these medications in foals with this problem.

Some neonatal foals examined for abdominal pain are apparently unable to urinate normally. Ultrasound examination will reveal a normal gastrointestinal tract and a greatly enlarged but intact urinary bladder. These foals can be treated with indwelling urinary catheters for 2 to 3 days along with phenazopyridine (4 mg/kg orally [PO] every 8–12 hours) for 5 to 7 days.[119] Phenazopyridine acts as a local analgesic on the lower urinary tract and may relieve spasm or allow better relaxation of the bladder sphincters and promote normal micturition.

Patent Urachus

The urachus is the conduit through which fetal urine passes from the bladder into the allantoic cavity. Normally, the urachus closes at the time of parturition, but incomplete closure is the most common malformation of the equine urinary tract. Patent urachus occurs more commonly in foals than in other domestic species.[31] Greater than average length or partial torsion of the umbilical cord has been suggested to cause tension on the attachment of the umbilical cord to the body wall. The result is dilation of the urachus and subsequent failure to close at birth.[71,72,115,118,120] Patent urachus results in a persistently moist umbilicus after birth, from which urine may leak as drips or as a stream during micturition. One must distinguish this malformation from septic omphalitis, which also can result in urine leakage from the umbilicus within a few hours to days after birth. Ultrasound examination of the umbilical remnants is indicated to rule out or monitor for concurrent omphalophlebitis. Patent urachus has been referred to as a *congenital problem* and the latter as an *acquired problem,* but both may result in urine leakage from the urachus from birth. Neither is life threatening, but local sepsis often is accompanied by more severe illness, including septicemia or localized infection, particularly in joints.

The congenital patent urachus traditionally has been treated with frequent (two to four times daily) chemical cauterization of the urachus with swabs dipped in a concentrated phenol or 7% iodine solution or with silver nitrate applicators.[70] Because the urachus may close spontaneously in a number of cases and because these agents desiccate and irritate tissue (and may predispose to infection), the rationale for this approach is questionable.[120] In a study comparing the effects of disinfectant solutions on the bacterial flora of the umbilicus of normal foals, use of a 7% iodine solution was observed to cause rapid desiccation of the umbilical tissue and subsequent development of a patent urachus when the stump fell off a few days later.[121] Chlorhexidine diacetate (0.5%) appears to be more effective than 1% to 2% iodine solutions in reducing umbilical bacterial counts. Chlorhexidine is less irritating to tissue, binds to the stratum corneum, and has prolonged antiseptic effects.[121] The use of caustic or irritating solutions for routine umbilical dipping or treatment of patent urachus should probably be avoided because it may lead to localized necrosis and infection that may actually result in umbilical infections.

Consequently, in the absence of apparent infection, no local treatment may be indicated specifically, but affected foals frequently are given antibiotics prophylactically. For acquired patency (which may be associated with local infection, cachexia, or septicemia), broad-spectrum antibiotic therapy is indicated, and resolution of the systemic disease may be accompanied by elimination of the umbilical infection and closure of the urachus. Most cases of patent urachus without septic omphalophlebitis will resolve with supportive care, routine umbilical disinfection,

and antibiotic therapy. Empiric antibiotic therapy with antibiotics that are eliminated (and concentrated) in urine, such as potentiated sulfonamides, cephalosporins, aminoglycosides, or penicillins, usually provides adequate antimicrobial protection with uncomplicated patent urachus in otherwise healthy foals. Chemical cauterization is contraindicated with local sepsis because it may increase the risk of urachal rupture and development of uroperitoneum.[122] If one observes no decrease in urine leakage after 5 to 7 days of medical therapy, or if ultrasonography reveals abnormalities of multiple structures in the umbilicus,[123,124] surgical exploration and resection of the urachus and umbilical vessels may be indicated. In a retrospective study of 16 foals treated for sepsis of umbilical cord remnants, six of nine (67%) survived after surgical resection and antibiotic treatment, whereas only three of seven (43%) survived after antibiotic treatment alone.[125] Although this series of 16 foals often is cited in support of surgical intervention, one should note that the series studied a small number of foals and the cases were evaluated over 10 years (1975–1985), during which time many aspects of neonatal care improved. In a subsequent retrospective report of 33 foals with umbilical remnant infections, no difference in survival was observed between foals treated with antibiotics combined with surgical resection or with antibiotic therapy alone.[124] Further, emphasis was placed on the insensitivity of palpation of the umbilicus in detection of umbilical remnant infection (compared with ultrasonographic examination) and the poor outcome of cases in which the umbilical vein was involved. In addition to the possibility of omphalitis leading to urachal rupture and development of uroperitoneum, urachal leakage also may occur into the abdominal musculature and subcutaneous tissues and lead to progressive swelling and cellulitis of the ventral abdominal wall and/or prepuce.[126] Both instances require surgical intervention. Finally, trauma or tearing of the urachus also can lead to umbilical evagination of the urinary bladder,[127] which can result in partial or complete obstruction of urine flow, and surgical correction is indicated.

Renal Physiology

Harold C. Schott II

The kidneys perform two essential functions in the maintenance of homeostasis: (1) elimination of nitrogenous and organic waste products and (2) control of body water content and ion composition. In addition, the kidneys are important endocrine organs that produce renin, erythropoietin, and the active form of vitamin D; they also play an important role in the degradation and excretion of a number of other hormones, including gastrin and parathormone. To gain an understanding of the pathophysiologic alterations associated with renal disorders in horses, one must first review some aspects of normal renal physiology in this species.

PRODUCTION AND ELIMINATION OF NITROGENOUS AND ORGANIC WASTES

The two most commonly recognized waste products excreted in urine are urea and creatinine (Cr), but many other nitrogenous or organic wastes are produced each day and subsequently are eliminated by the kidneys (Box 14.1).[128]

BOX 14.1 Compounds Excreted by the Kidneys

- Urea
- Phenols
- Indoles
- Skatoles
- Hormones
- Polyamines
- Trace elements
- Serum proteases
- Creatinine (Cr)
- Pyridine derivatives
- Guanidino compounds
- β_2-Microglobulin
- Hippurate esters
- Aliphatic amines
- Aromatic amines
- Middle molecules

Urea Metabolism

A molecule of urea is produced in the liver from two ammonium ions that are liberated during catabolism of amino acids. For each urea molecule, the carbon atom is derived from bicarbonate. One ammonium ion is cleaved from an amino acid via an α-ketoglutarate–dependent transamination coupled to oxidative deamination of glutamate. The second ammonium ion is derived from aspartate in the urea cycle.[129] Urea synthesized in the liver is released into the blood, and clearance by the kidneys represents the major pathway (75%–100%) of excretion. Extrarenal urea excretion includes losses in sweat and through the gastrointestinal tract. With normal intestinal function, enteric excretion is minimal because of enterohepatic recirculation (reabsorption of ammonia from the degradation of urea by bacterial ureases and subsequent reformation of urea in the liver).[130]

In human beings, inborn errors of metabolism leading to deficiency of a specific transaminase or of one of the five enzymes of the urea cycle can result in accumulation of ammonia and other intermediates of amino acid catabolism. These disorders typically are inherited as autosomal recessive traits, and their consequence is moderate to severe mental dysfunction because the accumulated intermediates can be toxic to the central nervous system (CNS; i.e., ammonia) or can act as false neurotransmitters (i.e., aromatic amines).[128] Because urea production is limited in these disorders, blood urea nitrogen (BUN) concentration is often low.[129] Although such defects in metabolism appear to be rare in domestic animals,[131] development of encephalopathy in association with hyperammonemia has been recognized in horses.[132,133] Furthermore, in one report of two related Morgan weanling fillies, persistent hyperammonemia was suspected to be caused by a defect in a mitochondrial ornithine transporter similar to an autosomal recessive syndrome of hyperornithinemia, hyperammonemia, and homocitrullinuria in human beings.[134]

BUN concentration depends on age, diet, rate of urea production, and renal function. For example, a low BUN typically is found in neonatal foals because of an anabolic demand for amino acids.[135] Investigations of nitrogen use in ponies have demonstrated that urea production is proportional to dietary protein content. Similarly, urinary urea excretion increases in proportion with urea production.[136,137] As a result, with

increased levels of dietary protein or when urea is supplemented in the diet, BUN may increase twofold or greater.[138-140]

In human beings and small animals, BUN is routinely higher in samples collected postprandially because diets are typically high in protein.[130] Postprandial elevation of BUN has not been described in horses or other herbivores. However, fasting leads to enhanced protein catabolism to meet energy demands and increased BUN in horses.[141,142] In ponies, however, BUN decreases with fasting.[143] This opposite response suggests differences in the metabolic responses of horses and ponies to anorexia, consistent with a greater capacity of ponies to mobilize and use fat during starvation. Other causes of protein catabolism, including fever, infection, trauma, myositis, burns, and corticosteroid therapy, also can produce an increase in BUN.[130] Finally, a decrease in renal blood flow or function increases BUN. The former may occur with dehydration or during periods of anesthesia or exercise; the latter is a reflection of renal disease.[130] With short bouts of moderate to intensive exercise, BUN often does not change[140,144]; however, during prolonged exercise BUN can increase by 50% or more because of the combined effects of decreased renal blood flow and protein catabolism.[145,146]

Most renal nitrogen excretion occurs in the form of urea in urine. One must recognize that urea excretion is completely passive and that the high concentrations achieved in urine are merely a consequence of medullary tonicity produced by the countercurrent-multiplier function of the loop of Henle. Thus although variations in dietary protein intake lead to parallel changes in urea excretion, the idea that low-protein diets decrease the workload on the kidney is a fallacy.[130] Urinary urea nitrogen concentrations can vary from as low as 50 mg/dL in neonatal foals or horses with primary polydipsia to greater than 2500 mg/dL in normal horses on high-protein diets. Total daily urea excretion usually ranges between 100 and 300 g/day in horses with normal renal function.

Creatinine Metabolism

Cr is produced by the nonenzymatic, irreversible cyclization and dehydration of creatine. Three amino acids in the kidney, liver, and pancreas are indirectly responsible for its production; subsequently Cr is transported to other organs such as muscle and brain, where it is phosphorylated to store energy in the form of phosphocreatine.[130,147] In human beings, 1.5% to 2.0% of the creatine pool is converted to Cr daily and results in a fairly constant excretion of Cr within a given individual.[130] With normal renal function, a direct relationship exists between daily Cr production, serum Cr concentration, and Cr excretion, all three being proportional to total muscle mass. The fact that Cr is 30% higher in male humans than in females and urinary Cr excretion is correlated to body size across a wide range of animal species supports this relationship.[130,148] Cr is excreted principally in urine, but sweat and the gastrointestinal tract are secondary routes of excretion.[130] In contrast to urea, enterohepatic recycling of Cr does not occur, and the gastrointestinal tract may represent a major route of excretion when renal function is compromised. For example, in a group of azotemic human patients, between 15% and 65% of radiolabeled Cr was found to be excreted through the intestine.[149] Cr excreted by this route is degraded rapidly by bacteria so that little is found in feces.

Like BUN, Cr can vary with age, activity level, and renal function. In contrast, dietary protein intake has little influence on Cr in horses.[138] Newborn foals routinely have Cr values 30% to 50% higher than those measured in the mare, and values as high as 20 to 30 mg/dL have been measured in some premature or asphyxiated foals.[135] These high values may result from limited diffusion of Cr across the placenta. For example, the Cr concentration in equine amniotic fluid at term is proportionately much greater than urea nitrogen concentration (Cr, 10.1 mg/dL; urea nitrogen, 38.8 mg/dL).[150] If the foal appears healthy and all other laboratory values are within reference ranges, a Cr value in the range of 5 to 15 mg/dL should not cause alarm. In most healthy foals with normal renal function, Cr decreases to values below 3.0 mg/dL within the first 3 to 5 days of life.[149] After the first few days of life, Cr is usually lower in foals than in adults[139] because of the combined effect of rapid growth and the fact that skeletal muscle comprises a smaller percentage of body weight in foals than in adult horses. Other nonrenal factors that may influence Cr include fasting, rhabdomyolysis or muscle wasting caused by disease, and exercise. Although fasting can increase the measured value for Cr, a substantial portion of this increase actually is due to other compounds (possibly ketones) that increase during fasting and are measured as non-Cr chromagens in the commonly used Jaffe's colorimetric assay for Cr determination (see Examination of the Urinary System).[139,141,151] In contrast, the increase in Cr (up to 80% in some reports) associated with various types of exercise is likely the combined result of increased release of creatine from muscle and decreased urinary Cr excretion during exercise.[139,141,144-146]

Cr is filtered freely at the glomerulus and is concentrated to values of 100 to 300 mg/dL in equine urine, which results in a total daily urine excretion of 15 to 25 g of Cr.[152,153] In comparison to urea, Cr excretion is responsible for only one tenth as much urinary nitrogen excretion. Minor species and sex differences have been reported for renal tubular handling of Cr, with a weak proximal tubular secretory mechanism in humans and male dogs (accounting for 7%–10% of total urinary Cr excretion).[130,147] To determine whether tubular secretion of Cr occurs in equine kidneys, Finco and Groves[154] fitted anesthetized ponies with ureteral catheters and performed simultaneous inulin and exogenous Cr clearance studies. Because inulin is filtered freely at the glomerulus and is neither secreted nor reabsorbed by renal tubules, inulin clearance (Cl_{In}) provides a standard of comparison for Cr clearance (Cl_{Cr}). Tubular secretion of Cr should result in a greater value for Cl_{Cr} than for Cl_{In}, whereas the opposite should occur with tubular reabsorption of Cr. To magnify any minor tubular secretion of Cr, stop-flow studies were performed by temporarily occluding the ureteral catheters. During obstruction, tubular lumen pressure increased and tubular flow decreased. As a consequence, fluid remained in contact with tubular epithelium for a prolonged period, enhancing local tubular secretory or resorptive processes. Analysis of a series of urine samples collected after release of ureteral occlusion revealed no differences in tubular handling of inulin or Cr, leading to the conclusion that Cr neither was reabsorbed nor secreted by equine kidneys. In contrast, simultaneous measurement of endogenous Cl_{Cr} and Cl_{In} in several horses with chronic kidney disease (author's unpublished observations) has revealed higher values for Cl_{Cr}, indicating that tubular secretion of Cr may develop in horses as renal function declines (see Chronic Renal Failure). Whether significant excretion of Cr occurs in sweat or through the gastrointestinal tract has not been investigated in horses.

Metabolism of Other Nitrogenous and Organic Compounds

Although the kidneys excrete a number of nitrogenous and organic wastes in addition to urea and Cr (see Box 14.1), these compounds are quantitatively unimportant in terms of nitrogen balance.[128] Two of the more commonly recognized molecules are ammonia and uric acid. In proximal tubular epithelial cells, ammonium ions and α-ketoglutarate are produced from glutamine. Subsequent metabolism of α-ketoglutarate results in generation of two bicarbonate molecules that are returned to the systemic circulation. Ammonium ions are secreted in exchange for sodium into the tubule lumen, where they remain trapped, because tubules are relatively impermeable to ammonium ions. Furthermore, because the pK_a for ammonia is greater than 9.0, most of the tubular ammonia remains in the form of ammonium ions, even in alkaline equine urine. Although ammonium ion excretion is of little significance in overall nitrogen excretion, it plays an important role in acid (hydrogen ion) excretion. In fact, glutamine metabolism and ammonium ion excretion can increase several-fold in response to metabolic acidosis.[155] Although urinary ammonium concentration is not measured routinely, one can estimate it because it is directly related to the urinary anion gap ($[Na^+ + K^+] - Cl^-$) in human patients with normal anion gap metabolic acidosis.[156] More important, impairment of this proximal tubular acid secretion pathway contributes to the development of metabolic acidosis in patients with renal insufficiency.

Uric acid is a product of purine nucleotide degradation and is the major nitrogenous waste product formed in amphibians and reptiles. In mammals, however, uric acid excretion (mostly in the ionic form of urate) is unimportant in terms of overall nitrogen excretion.[128] Uric acid metabolism has received little attention in veterinary species with the exception of Dalmatian dogs. This breed exhibits high urate excretion rates and is predisposed to uric acid stone formation; however, this problem results from decreased hepatic uricase activity rather than any abnormality in renal urate handling.[157] Finally, hyperuricemia (leading to gout in humans) also can be attributed to a lack of uricase activity in human tissues and greater renal reabsorption of urate compared with other mammalian species. Thus crystallization of urate in tissues appears to be limited to humans.[128] Urate metabolism has been studied little in horses, although Keenan[144] observed that plasma concentrations increased dramatically in response to exercise (from less than 1 μmol/L at rest to 150–200 μmol/L 1 hour after racing) and that these increases were accompanied by a transient increase in urinary urate excretion (from less than 40 μmol/L at rest to 250–1270 μmol/L after racing).

The proximal tubule is also the major site of excretion (by tubular secretion) of a number of endogenous organic anions and cations.[128] The anions share the common pathway measured by p-aminohippurate clearance, the substance traditionally used to measure effective renal plasma flow (because more than 90% is excreted via this pathway). A number of exogenous compounds also are excreted via these pathways—acetazolamide, furosemide, probenecid, penicillin G, sulfadiazine, salicylate, atropine, cimetidine, and neostigmine. Thus administration of these compounds can interfere with tubular secretion of endogenous organic wastes or other exogenous products by healthy kidneys.[158] More important, the pharmacokinetic effects of these products vary widely in patients with renal insufficiency. Combined with the fact that anion binding to plasma proteins is decreased with azotemia, dosing protocols of many medications may need to be readjusted for patients with renal failure.

BODY WATER AND ELECTROLYTE BALANCE

Body Fluids: Volume and Composition

Water accounts for at least 60% of total body mass, equivalent to 300 L in a 500-kg horse.[128,156-161] About 200 L of total body water is intracellular fluid, and the remaining 100 L is extracellular fluid. Extracellular fluid is divided between plasma (4%–6% of body mass, approximately 25 L), interstitial fluid and lymph (10%–12% of body mass, approximately 45 L), and transcellular fluid (6%–10% of body mass, approximately 30 L, most of which is in the lumen of the gastrointestinal tract). Despite significant differences in ion composition (Table 14.1), the extracellular fluid and intracellular fluid compartments exchange water freely to maintain osmotic equilibrium.[155]

From the values in Table 14.1, one can estimate the total amount of exchangeable sodium, potassium, and chloride in the body fluids of a 500-kg horse: approximately 16,000 mEq, 28,800 mEq, and 10,800 mEq, respectively (including gastrointestinal fluid ion contents). These values are accurate except for that of sodium, which may be twice as great; however, 40% to 50% is sequestered in bone and is not readily available to buffer sodium alterations in body fluids.[159-161] Thus the 16,000-mEq estimate is accurate for the exchangeable sodium content in body fluids. Similarly, one can estimate body fluid contents of calcium, magnesium, and phosphorus at approximately 1000 mEq (20 g), 6875 mEq (84 g), and 8150 mEq (140 g), respectively (excluding gastrointestinal fluid ion contents, because these vary with the amount and solubility of the dietary source). As for sodium, the values underestimate the total body content of calcium, magnesium, and phosphorus, because more than 99%, 70%, and 85% of these elements, respectively, are contained in the skeleton.[130]

TABLE **14.1 Approximate Ionic Compositions (mEq/L) of Plasma, Interstitial Fluid, and Intracellular Fluid (Skeletal Muscle)**

Electrolyte Cations	Plasma	Interstitial Fluid	Skeletal Muscle Cell
Na^+	140	143	10
K^+	4.0	4.1	142
Ca^{2+}	2.5	2.4	4.0
Mg^{2+}	1.1	1.1	34
Total	**147.6**	**150.6**	**190**
ANIONS			
Cl^-	100	113	4
HCO_3^-	25	28.2	12
$H_2PO_4^-$, HPO_4^{-2}	2.0	2.3	40
Protein	14	0.0	50
Other	6.6	7.1	84[a]
Total	**147.6**	**150.6**	**190**

Modified from Rose BD: *Clinical physiology of acid-base and electrolyte disorders,* ed 3, New York, 1989, McGraw-Hill.
[a]This largely represents organic phosphates such as adenosine triphosphate.

Water Balance

Appropriate water balance maintains plasma osmolality in a narrow range (270–300 mOsm/kg) and is achieved by matching daily water intake with water loss.[162-164] Water is provided from three sources: (1) free water intake (drinking), (2) water in feed, and (3) metabolic water (Table 14.2). Horses consume most of their water by drinking (about 85%), but feed and metabolic water provide about 5% and 10% of daily water, respectively. Water can be lost by three routes: (1) in urine, (2) in feces, and (3) as insensible losses (evaporation) across the skin and respiratory tract (Table 14.3). Investigations of water balance have revealed a maintenance water requirement of 60 to 65 mL/kg/day or 27 to 30 L/day for a 500-kg horse.[162,165] These values are consistent with traditional recommendations that 5 to 10 gallons/day of fresh water be provided to a stabled horse under mild environmental conditions.[166] Urinary and fecal water losses range between 20% to 55% and 30% to 55%, respectively, of the total daily water loss.[162,165,167,168] The remaining (insensible) loss accounted for up to 15% to 40% of daily water loss, despite mild ambient conditions and the lack of observed sweating in most studies of water balance.

Water drinking and urine production are the mechanisms by which water balance is finely tuned; however, they can vary widely among individual horses and also are affected by age, environmental conditions, level of exercise, and diet. Often, for example, neonatal foals consume milk in excess of 20% of their body mass daily,[169] which equates to fluid intake approaching 250 mL/kg/day. Water intake by horses increased 15% to 20% when ambient temperature increased from 13°C to 25°C.[170] Under conditions of high ambient temperature and humidity, urine concentration also may increase to conserve water, whereas fecal water content tends to remain fairly stable, at about 75% of fecal weight. Exercising horses, especially endurance horses and racing horses treated with furosemide, can increase water consumption by 100% to 200% to replace body water lost in sweat (and urine). Horses and ponies on all-roughage diets also drink more and have greater daily fecal water loss (because of greater daily fecal volume) than animals fed a large amount of concentrate or complete pelleted diets.[167,168] Diets high in nitrogen (protein) and calcium, such as legume hays, typically increase urine volume by 50% or more and are associated with a similar increase in urinary nitrogen excretion. These diets are also more digestible so that fecal water excretion generally decreases because of a decrease in total fecal material.[136,141,167,168] Although high dietary levels of salt have been suggested to increase drinking and promote diuresis, no increase in water consumption or urine volume was observed in ponies fed 5 to 10 times the daily salt requirement (equivalent to about 350 g of sodium chloride [NaCl] for a 500-kg horse).[171] The effects of water access, continuous versus intermittent, have received less attention, although a study reported in 1999 showed no difference in water balance in horses provided water three times daily compared with horses that had continuous access to water.[172] Furthermore, horses drink the most water within the hour after feeding,[173] and feral horses and ponies often drink only once or twice daily.[174] Thus horses are unlikely to require continuous access to water. An obvious exception is a patient with renal insufficiency that should have access to fresh water at all times.

Two main stimuli for thirst are (1) increased plasma osmolality and (2) hypovolemia or hypotension.[175] The former is mediated through osmoreceptors in the hypothalamus that have a high threshold for activation (about 295 mOsm/kg) in humans. Hemodynamic stimuli are mediated by low- and high-pressure baroreceptors. Osmotic and hemodynamic stimuli can produce their dipsogenic effect in part by activating a local renin-angiotensin-aldosterone system in the central nervous system.[176,177]

Renal water reabsorption is controlled principally by the action of arginine vasopressin (antidiuretic hormone) on the collecting ducts.[178] Vasopressin is produced in the neurosecretory neurons of the supraoptic nuclei, packaged in granules, and transported down axons for storage in the neurohypophysis (pars nervosa or posterior pituitary). As for thirst, increases in plasma osmolality and hypovolemia or hypotension are the stimuli for vasopressin release. Osmoreceptors for vasopressin release also are located in the hypothalamus, adjacent to the osmoreceptors mediating thirst. Activation of these receptors is the signal for vasopressin release from the neurohypophysis. Furthermore, these osmoreceptors are not equally sensitive to all plasma solutes. For example, increases in plasma sodium concentration and infusion of mannitol are potent stimuli, whereas increases in plasma glucose and urea concentrations are weak stimuli. These differences have led to the suggestion that osmoreceptor activation is caused by an osmotic water shift that produces cell shrinkage (which would be greater for sodium and mannitol than for glucose or urea). Activation of osmoreceptors signaling vasopressin release also appears to have a threshold value; however, this threshold appears to vary

TABLE **14.2 Water Balance in Hay-Fed Horses in a Cool Climate**

Water Intake (L)	Water Loss (L)
Consumption 23.6	Feces 14.0
Hay 1.1	Urine 4.9
Metabolic 2.7	Insensible 8.5
Total 27.4	Total 27.4

Data from Tasker JB: Fluid and electrolyte studies in the horse. 3. Intake and output of water, sodium, and potassium in normal horses, *Cornell Vet.* 57:649, 1967.

TABLE **14.3 Water and Electrolyte Balance in Horses Receiving a Low-Sodium Diet (Alfalfa and Timothy Hay)**

	Intake	Urinary Loss	Fecal Loss	Unmeasured[a]
TASKER[b]				
Water (L)	27.4	4.9	14	8.5 (31%)
Sodium (mEq)	329	7	116	206 (63%)
Potassium (mEq)	3930	2196	993	741 (19%)
GROENENDYK, ENGLISH, ABETZ[c]				
Water (L)	27.6[d]	9.9	7.2	10.5 (38%)
Sodium (mEq)	986	527	253	206 (21%)
Potassium (mEq)	3320	2661	504	155 (5%)
Chloride (mEq)	3008	2347	174	487 (16%)

[a]Unmeasured losses include insensible water losses and electrolyte losses thought to occur in sweat; value in parentheses is the percentage represented by these unmeasured losses.
[b]Tasker JB: Fluid and electrolyte studies in the horse. 3. Intake and output of water, sodium, and potassium in normal horses, *Cornell Vet.* 57:649, 1967.
[c]Groenendyk S, English PB, Abetz I: External balance of water and electrolytes in the horse, *Equine Vet J.* 20:189, 1988.
[d]Water intake includes imbibed water (23.6 L), water in feed (1.1 L), and metabolic water (2.9 L).

highly among individuals. In addition, the threshold for vasopressin release in human beings is significantly lower (270–285 mOsm/kg) than that for thirst. Thus vasopressin release can be thought of as the initial line of defense against a mild increase in plasma osmolality, whereas thirst and drinking are secondary responses to even greater increases.

Studies in horses, ponies, and donkeys have demonstrated that increased plasma osmolality (induced by water deprivation or infusion of hypertonic saline) and hypovolemia (induced by furosemide administration) are stimuli for thirst.[172,178-182] Furthermore, after a period of water deprivation, dehydrated ponies, horses, and donkeys appear to be able to replace water deficits within 15 to 30 minutes of gaining access to water. The increases in plasma osmolality and vasopressin concentration associated with water deprivation also are corrected in this same period of time, indicating that imbibed water is absorbed rapidly from the gastrointestinal tract.[179] Although increases in plasma vasopressin concentration have been measured in horses and ponies during water deprivation,[179,183] vasopressin also appears to be a "stress hormone" in equids, because substantially greater concentrations (tenfold greater than those induced by water deprivation) have been measured after application of a nose twitch, nasogastric intubation, or exercise.[184,185] Thus increases in plasma vasopressin concentration after water deprivation would be expected to vary in horses, and separating osmotic effects from stress effects may be difficult sometimes.

Once released, vasopressin acts on V_2-receptors on the basolateral membrane of collecting duct epithelial cells, leading to insertion of water channels (transmembrane proteins) in the apical membrane.[175] These channels increase the water permeability of the apical membranes and lead to increased water reabsorption. Action of V_2-receptors is mediated by activation of adenylyl cyclase and a stimulatory transmembrane G protein. Interestingly, V_2-receptor activation can be antagonized by activation of adjacent α_2-adrenoceptors and by a prostaglandin E_2–mediated effect on an inhibitory G protein.[16,186] Although effects of these antagonists vary with species and have not yet been studied in horses, the diuresis associated with administration of α_2-agonists to horses[14,15] likely may be attributable to vasopressin antagonism at the collecting duct.

As mentioned previously, most water drinking in equids occurs periprandially; thus feeding practices affect timing of water intake.[173] If a horse eats a large meal once or twice daily, both increased plasma sodium concentration and decreased plasma volume (because of a shift of fluid into the bowel) stimulate thirst and vasopressin release. The result is a simultaneous increase in water intake and a decrease in urine output.[187] In addition, hypovolemia further stimulates activation of the renin-angiotensin-aldosterone system, which leads to enhanced renal sodium conservation as an additional means of restoring plasma volume. Although the increase in plasma sodium concentration with meal feeding is rather small (1%–3%), the decrease in plasma volume is much greater (5%–25%). The magnitude of this fluid shift (and the degree of activation of the renin-angiotensin-aldosterone system) can be attenuated largely by feeding small meals four to six times throughout the day.[188,189] Thus more frequent feeding causes less perturbation of body fluids and likely has a protective effect against development of some forms of colic.

Although balance of daily water intake and output is critical for maintenance of homeostasis, it warrants mention that equids tolerate water deprivation well.[190-196] For example, after horses were deprived of water for 72 hours (which resulted in body weight loss in excess of 10%), most of the weight lost (90% of which was assumed to be water) was recovered within the first hour after being provided access to water.[193] Similarly, even greater body weight losses (approaching 20%) induced by water deprivation and desert walking in donkeys and burros were replaced largely within the first few minutes after water was provided.[191,192] Thus in terms of water balance, equids (especially donkeys and burros) truly can be considered desert-adapted animals.[196,197] An important reason for their ability to tolerate water deprivation appears to be a substantial intestinal reserve of water and electrolytes that they call on during periods of dehydration for the maintenance of plasma volume.[3,198] Despite rapid fluid replacement by equids that have been dehydrated by water deprivation, horses that become dehydrated because of prolonged exercise or diarrheal disease (colitis) often do not drink. This behavior can be attributed to the fact that these conditions produce loss of body water and osmoles in the form of sweat or diarrhea. As a result, plasma osmolality does not increase, and osmotic thirst stimulus is not produced. In human endurance athletes, this state of mild to moderate dehydration that does not induce thirst has been called *voluntary* and *involuntary* dehydration,[199,200] and although less well documented, a similar response appears to occur in endurance horses.[201] Another form of involuntary dehydration, which may be accompanied by increases in plasma osmolality and protein concentration, also has been described anecdotally in mares after foaling.

Electrolyte Balance

Intake and loss of electrolytes also must be matched appropriately to maintain body content of electrolytes within narrow ranges. This balance is most important for the exchangeable ions (Na^+, K^+, and Cl^-) because these have minimal tissue (skeletal) reserves that can be called on during times of need. An exception is the fluid and electrolyte reserve in the lumen of the gastrointestinal tract, which may be able to provide replacement of 10% or more of the body content of these electrolytes.[198] Three sources provide electrolytes: (1) feed, (2) water (usually minimal amounts), and (3) a number of dietary supplements. Electrolytes also can be lost by three routes: (1) in urine, (2) in feces, and (3) in sweat (insensible losses; see Table 14.3). Investigations of electrolyte balance have revealed that most horses eating predominantly hay or pasture grass ingest excess potassium and chloride. In contrast, sodium intake varies and with some diets may be marginal.[162,164,165] A maintenance requirement for sodium of 0.4 to 0.8 mEq/kg/day or 200 to 400 mEq/day (6–12 g/day) for a 500-kg horse has been suggested.[165,181] However, exercising horses that may lose 500 to 1000 mEq of sodium per hour in sweat or are treated with furosemide have greater dietary requirements to replace such losses.[164] Thus addition of 50 to 75 g of common table salt (which provides 850–1275 mEq, because 1 g of NaCl provides approximately 17 mEq sodium) is a safe and economical method of providing daily supplemental sodium and chloride to athletic horses.

Data from the water and electrolyte balance studies performed by Tasker[162] and by Groenendyk, English, and Abetz[165] (see Table 14.3) provide a good illustration of the capacity of the equine kidneys to conserve sodium when dietary intake is low (see Tasker's data[162] at top) compared with when intake is unlimited (see Groenendyk and colleagues' data[165] at bottom).

Furthermore, these studies demonstrate that urinary excretion is the major route for loss of potassium and chloride. Although dietary intake of potassium is usually excessive, equine kidneys do not appear to have a great capacity to conserve potassium during periods of food and water deprivation or with anorexia associated with disease.[162,164,200] Thus urinary potassium concentration and total excretion can remain substantial in the face of decreased intake. Consequently, with decreased feed intake horses can develop significant total body potassium depletion and often benefit from supplemental dietary potassium (25–50 g/day of potassium chloride [KCl] provides 375–750 mEq, because 1 g KCl provides approximately 15 mEq of potassium).

In horses the stimuli for electrolyte intake (salt appetite) have received much less attention than stimuli for drinking. Houpt and colleagues[181] found that horses that had marginal sodium intake (250 mEq/day) and that were treated with furosemide ate more salt in the hours after treatment than did placebo-treated horses on the same diet; however, salt intake (which was comparable for eating salt from a block or drinking a 0.9% NaCl solution) was excessive in both treatment groups (in excess of 100 g). Thus salt appetite, unlike water intake, is less closely regulated to balance intake with losses. In fact, when salt is available ad libitum, horses appear to consume more than their maintenance needs. The excess is eliminated by increased urinary sodium excretion. Although this apparently excessive salt appetite may seem inappropriate, one could consider it advantageous for exercising horses, which have a much greater daily salt requirement.[202]

RENAL REGULATION OF BODY WATER CONTENT AND ION COMPOSITION

The kidneys are the organs responsible for fine-tuning body water content and ion composition within narrow ranges. The important components of renal regulation of water and ion content include renal blood flow, glomerular filtration, and tubular modification of glomerular filtrate to produce the final urine.

Renal Blood Flow

At rest, the kidneys receive about 15% to 20% of the cardiac output, or about 7.5 to 10.0 L/min for an average-size horse.[203,204] This high tissue perfusion, 500 to 600 mL/min per 100 g of kidney compared with 50 to 100 mL/min per 100 g of brain tissue, is necessary for the kidney to function as an effective filter and regulator of extracellular fluid composition. Furthermore, tubular reabsorption of glomerular filtrate requires energy. Because more than 99% of the filtrate is reabsorbed, the metabolic rate of the kidney is high (second only to that of the heart), and despite the fact that the kidneys account for less than 1% of body weight, they are responsible for about 10% of whole body oxygen use.[128] Next, renal blood flow is distributed preferentially to the renal cortex. In fact, renal medullary blood flow, which is derived largely via the vasa recta that arise from the efferent arterioles of juxtamedullary glomeruli, accounts for less than 20% of total renal blood flow.[204] Consequently, the renal medullary tissue normally functions in a hypoxic environment. Medullary hypoxia has been described as "an inevitable accompaniment of efficient urinary concentration" as a consequence of countercurrent exchange.[205] Although the latter mechanism would suggest that oxygen tension should decrease progressively toward the inner medulla, the lowest values, often no greater than 10 mm Hg, are found in the inner portion of the outer medulla, termed the *inner stripe* (Fig. 14.9).[206] This finding can be explained by the substantial metabolic activity of epithelial cells lining the medullary thick ascending limb (mTAL) of the loop of Henle in the inner stripe. The sodium-potassium-adenosine triphosphatase pumps (Na^+,K^+-ATPase) in the basal membrane of these cells are responsible for the greatest ATP use (and thereby oxygen consumption) in the medulla.[207] The combined effects of low oxygen delivery and a high rate of use produce the lowest oxygen tension in the inner stripe. Fortunately, several "protective" mechanisms exist to preserve medullary blood flow and tissue oxygenation during periods of renal hypoperfusion and include a preferential reduction in cortical blood flow along with redistribution of renal blood flow to the corticomedullary region, accumulation of adenosine with depletion of ATP, and production of prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) and nitric oxide.[205-207] Of clinical importance is that the use of nonsteroidal antiinflammatory drugs (NSAIDs) in patients with poor renal perfusion can exacerbate tissue hypoxia because PGE_2 acts as a vasodilator and an inhibitor of Na^+,K^+-ATPase. In fact, the earliest lesions of analgesic nephropathy include degeneration and necrosis of mTAL cells before development of overt papillary necrosis.[207]

Renal blood flow of horses has been measured by a variety of techniques including p-aminohippurate clearance (Cl_{PAH}) by classic clearance techniques involving timed urine

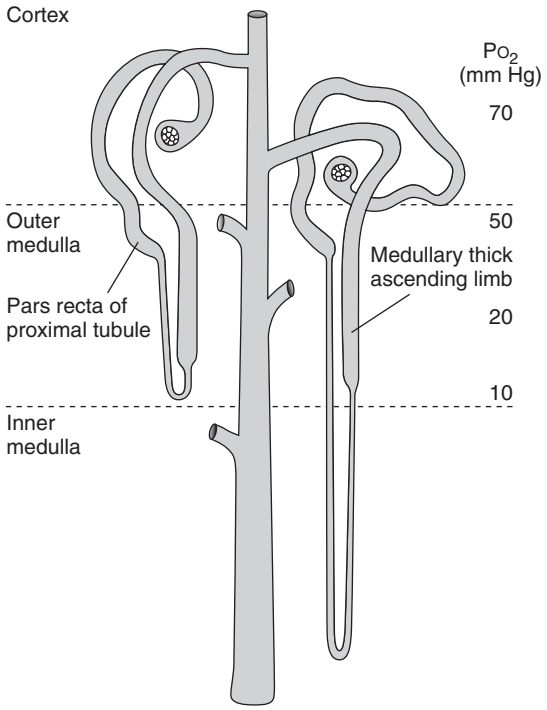


FIG. **14.9** Schematic drawing of a nephron. In the outer medulla is the site of lowest intrarenal oxygen tension (Po_2), in the final (straight) portion of the proximal tubule and the medullary thick ascending limb (mTAL) of the loop of Henle. Limited medullary blood flow and high metabolic rates make these outer medullary nephron segments the most susceptible to damage during periods of renal hypoperfusion.

collections and by plasma disappearance curves, as well as clearance of radionuclides, microsphere injection, and use of ultrasonic Doppler flow probes placed around the renal artery (Table 14.4).[11,203,204,208-223] The latter technique does not provide absolute blood flow values but rather measures changes in renal blood flow from a baseline value.[11] Plasma disappearance curves for ^{131}I-orthoiodohippuric acid and 99mTc-mercaptoacetyltriglycine have been validated in normal horses in an attempt to establish radionuclide techniques for rapid and noninvasive measurement of renal blood flow in hospitalized horses.[219,223] Although values for renal blood flow determined by these radionuclide techniques compared well with previous data (Table 14.5),[224-234] their future use in a clinical setting likely will remain limited because of moderate expense and the need to perform serial measurements to provide clinically relevant information in patients with reduced renal function.[235]

Intrinsic and extrinsic factors play a role in the control of renal blood flow. The former include autoregulation and action of renal nerves; the latter include vasoconstrictors (catecholamines, renin-angiotensin system, arginine vasopressin) and vasodilators (prostaglandins, dopamine, atrial peptides, bradykinin, adenosine, and nitric oxide).[128]

TABLE **14.4 Reported Values for Effective Renal Plasma Flow and Renal Blood Flow in Horses and Ponies**

Number of Animals	Method[a]	Effective Renal Plasma Flow (Mean ± SD/SE mL/min/kg [Range])	Renal Blood Flow (RBF) (mL/min/kg)[b]	Reference
One female horse	$Cl_{Diodrast}$	6.91 ± 0.81	13.2 ± 1.6	208
One female pony	$Cl131_{I\text{-}o\text{-}HA}$	Bolus injection: 12.85 ± 1.81	21.7 ± 3.1	209[c]
		Constant infusion: 11.45 ± 1.25	19.4 ± 2.1	
Three female horses	$Cl131_{I\text{-}o\text{-}HA}$	Bolus injection: 11.97 ± 2.63	20.2 ± 4.4	
		Constant infusion: 9.56 ± 1.84	16.2 ± 3.1	
Six female ponies	Cl_{PAH}	12.09 ± 0.34 (7.86–21.62)	20.4 ± 0.6	210
Two female horses		9.59 ± 0.86 (4.75–19.78)	16.2 ± 1.4	
Five female/gelding horses	$Cl131_{I\text{-}o\text{-}HA}$	8.24 ± 2.88 (5.66–12.89)	13.9 ± 4.9	211
Eight female horses	Cl_{PAH}	Bolus injection: 12.0 ± 1.7	20.3 ± 2.9	214
Four-day-old foals		Bolus injection: 15.2 ± 1.5	25.7 ± 2.7	
Five horses (three male, two female)		Infusion (Cl-plasma): 18.2 ± 2.0	30.8 ± 3.4	
Three ponies (one male, two female)		Infusion (Cl-urine): 11.9 ± 1.9	20.1 ± 3.2	
Six female horses	Cl_{PAH}	8.5–10.8[d]	14.4–18.3	215
Six female horses	Cl_{PAH}	11.9 ± 1.0	20.0 ± 1.7	216
Six horses[e]	$Cl131_{I\text{-}o\text{-}HA}$	6.26 (4.33–6.80)	10.58 (7.32–11.50)	217
Eight female horses	Cl_{PAH}	9.65 ± 0.84 (5.60–12.54)	16.31 ± 1.42	219
	$Cl131_{I\text{-}o\text{-}HA}$	11.32 ± 1.03 (7.82–15.71)	19.14 ± 1.74	
Six pony foals (three female, three male)	Cl_{PAH}	16.63 (15.61–17.26)	28.11	221
Ten horses (four female, six gelding)	$Cl99_{mTc\text{-}MAG\text{-}3}$	7.92 ± 1.51 (5.58–10.62)	13.39 ± 2.55	222
Four ponies[e]	Microspheres	—	208 ± 58[f,g]	204
Eleven ponies[e]	Microspheres	—	548 ± 87[f]	203
Eight ponies[e]	Microspheres	—	483 ± 79[f]	212
Eleven ponies[e]	Microspheres	—	670 ± 50[d,f]	213
Three horses (female, male, and gelding)	Microspheres	—	535 ± 93[f]	218
Nine horses[e]	Microspheres	—	589 ± 50[f]	220
Four gelding ponies	Microspheres	—	428 ± 49[f]	223

[a]$Cl_{Diodrast}$, Clearance of 3,5-diiodo-4-pyridine-*N*-acetic acid; $Cl131_{I\text{-}o\text{-}HA}$, clearance of ^{131}I-*o*-iodohippurate; Cl_{PAH}, clearance of *p*-aminohippurate; $Cl99_{Tc\text{-}MAG3}$, clearance of 99mTc-mercaptoacetyltriglycine.
[b]RBF values presented have been calculated from effective renal plasma flow (ERPF) data using extraction ratios (ERs) of 0.80 for diodrast and 0.91 for ^{131}I-*o*-iodohippurate and *p*-aminohippurate: RBF = (ERPF/ER)/(1 – hematocrit); hematocrit was assumed to be 0.35.
[c]Other horses and ponies also were studied after bolus injection of ^{131}I-*o*-iodohippurate and yielded ERPF values of 16.93 ± 6.05 and 10.65 ± 2.73 mL/min/kg for ponies and horses, respectively; these values corresponded to a RBF of 8.6 ± 10.2 mL/min/kg in ponies and 18.0 ± 4.6 mL/min/kg in horses.
[d]Values estimated from figure.
[e]Sex not reported.
[f]RBF values for microsphere studies are expressed in units of milliliters per minute per 100 g of kidney tissue; a value of 500 mL/min/100 g would correlate to an RBF value of 18 mL/min/kg (or 3.6 and 9 L/min for a 200-kg pony and a 500-kg horse, respectively).
[g]Values reported are for ponies under general anesthesia (halothane in oxygen); these authors also reported that renal medullary blood flow was 2.6% to 18.8% of total RBF in two ponies.

Although not unique to the kidney, autoregulation of blood flow is a physiologic response that maintains cortical renal blood flow in the normal range through a rather wide range of perfusion pressures (75–180 mm Hg in humans). This response is thought to be independent of neural or hormonal mechanisms and is attributed to a myogenic response to changes in arterial wall tension. The local action of renal nerves or release of vasoconstrictor substances leads to an increase in renal vascular resistance that may occur in response to disease states (hypovolemic or endotoxic shock), drugs (particularly anesthetic agents), or physical stress (exercise). Renal blood flow may or may not decrease, depending on the degree of vasoconstriction. For example, renal vascular resistance increases during low-intensity exercise to divert a greater portion of the cardiac output to the working muscles. Thus the fraction of cardiac output delivered to the kidneys decreases; however, because cardiac output also increases in response to exercise, total renal blood flow remains unchanged.[216] In contrast, during halothane anesthesia in ponies, redistribution of cardiac output occurs without an increase in cardiac output. Under these circumstances, renal vasoconstriction is accompanied by a decrease in renal blood flow to about 60% of the awake value at 1.0 to 1.5 minimal alveolar concentration of halothane. As the plane of anesthesia deepens (to 2.0 minimal alveolar concentration), a greater increase in renal vascular resistance (or degree of vasoconstriction) further decreases renal blood flow to about 25% of the awake value, likely because of further vasodilatation of other vascular beds and a mild decrease in cardiac output.[212] Although renal blood flow was not measured, results of a study of prolonged (18 hours) anesthesia with sevoflurane are of interest in terms of probable renal hypoperfusion and damage.[236] After 10 hours of anesthesia, an increase in urine production was accompanied by evidence of tubular dysfunction (e.g., glucosuria, enzymuria). Furthermore, microscopic lesions after anesthesia were limited to the more distal nephron (mTAL and distal tubule), providing support that these normally hypoxic nephron segments may be the first to succumb to prolonged hypoperfusion.

When renal blood flow decreases, counteracting vasodilatory mediators usually are released in an attempt to ameliorate the decrease in renal blood flow. The best studied of these vasodilatory mediators include renal prostaglandins (PGE_2 and PGI_2) and dopamine. Although the role of renal prostaglandins in the control of basal or resting renal blood flow is thought to be insignificant, renal prostaglandins are important mediators of vasodilation in response to a number of vasoconstrictive stimuli.[237] Furthermore, production of renal prostaglandins is several times greater in medullary tissue so that action of these mediators leads to a greater increase in inner cortical (region of juxtamedullary glomeruli) and medullary blood flow. As mentioned previously, one should not be surprised that the lesion associated with antagonism of prostanoid production by use of NSAIDs is medullary or papillary necrosis.[238,239] With or without renal vasoconstriction, activation of dopamine receptors (DA_1 type) leads to renal vasodilation. Because the receptors are located on most renal arterioles, blood flow increases in the renal cortex and the medulla. For this reason, dopamine infusions are touted to be of benefit in treating acute renal failure because this catecholamine has been shown to increase renal blood flow and urine output by 30% to 190% in normal horses.[11]

Glomerular Filtration

Approximately 20% of the blood entering the glomeruli passes through small pores in the filtration barrier into Bowman's capsule. The primary force driving filtration is glomerular capillary transmural hydraulic pressure. A relatively constant pressure across the glomerular capillary wall is maintained by greater resistance in the arteriole leaving the glomerulus (efferent arteriole) than in the arteriole entering the glomerulus (afferent arteriole). This difference in vascular resistance generates the hydraulic pressure that forces plasma water out of the glomerular capillaries.[128] The filtration barrier is made up of three layers: (1) endothelium of the glomerular capillaries, (2) basement membrane, and (3) foot processes of the epithelial cells (podocytes) lining Bowman's capsule. The pore size of the filtration barrier, about 8 to 10 nm in diameter, prevents filtration of cells and larger proteins. As a result, the fluid that enters Bowman's capsule is an ultrafiltrate that is essentially identical to plasma except that it has less than 0.05% of the protein content of plasma. Interestingly, the diameter of albumin is about 6 nm, so its size should not prevent filtration. Glycosaminoglycans containing heparan sulfate and sialic acid residues impart a significant negative charge to the filtration barrier. Thus charge repulsion of albumin (which is similarly negatively charged) may be more important than molecular size in preventing significant loss of albumin into the filtrate; however, metabolic disturbances (metabolic acidosis) can neutralize the glomerular charge barrier, and one can observe transient proteinuria in the absence of structural damage to the glomerular barrier.[240]

By definition, glomerular filtration rate (GFR) is the volume of plasma filtered per unit of time and commonly is described in milliliters per minute per kilogram of body mass. The GFR of horses and ponies ranges between 1.6 and 2.0 mL/kg/min, with some authors reporting slightly higher values for ponies. This range is similar to those of other animals and humans. For a 500-kg horse, this value equals 800 to 1000 mL/min or about 1200 to 1400 L/day. This value represents filtration of the total plasma volume 60 to 70 times per day. Because urine production is about 10 L/day, more than 99% of the glomerular filtrate is reabsorbed.

Like renal blood flow, GFR has been measured in horses by a variety of techniques, including Cl_{In} by classic clearance techniques, Cl_{Cr}, and clearance or plasma disappearance of radionuclides (see Table 14.5). Plasma disappearance curves for 99mTc-diethylenetriamine pentaacetic acid (99mTc-DTPA) compare well in normal horses with Cl_{In} (the gold standard).[219] Although this technique is less expensive than ^{131}I-orthoiodohippuric acid clearance for estimating renal blood flow,[235] clinical use is limited by availability of nuclear medicine capabilities and expense (because one must take multiple measurements to assess disease progression or response to treatment). Gleadhill and colleagues[241] described use of a three blood sample technique to estimate GFR by plasma disappearance of 99mTc-DTPA. Interestingly, rather than expressing GFR on the basis of per kilogram of body mass or a body surface area, they suggested that GFR should be compared with extracellular fluid volume. Because plasma activity of 99mTc-DTPA also can be used to estimate extracellular fluid volume, one can make this estimate of GFR using 99mTc-DTPA alone. Standardization of GFR based on extracellular fluid volume is attractive and warrants further consideration because it eliminates the effect of variable body composition (e.g., specifically differences in body fat) when expressing GFR based on body mass. The authors subsequently used this method to estimate the decrease in GFR accompanying exercise.[233]

TABLE **14.5** **Reported Values for Glomerular Filtration Rate in Horses and Ponies**

Number of Animals	Method[a]	Glomerular Filtration Rate (GFR) (Mean ± SD/SE mL/min/kg [Range])	Cl_{CRend}/Cl_{In} Ratio[a]	Reference
Not reported[b]	Cl_{In}	0.83 ± 0.13[c,d]	1.02	208[b]
	Cl_{CRend}	0.85 ± 0.22[c,d]		
One horse[e]	Cl_{In}	1.4	—	208[b]
Twelve female horses	Cl_{In}	1.66 ± 0.33[c] (1.17–2.28)	0.88 ± 0.11	208
	Cl_{CRend}	1.46 ± 0.24[c] (1.10–1.65)		
One female pony	$Cl125_{\text{I-iothalamate}}$	Bolus injection: 5.43 (one study)	—	209[f]
		Constant infusion: 6.10 ± 1.27	—	
Three female horses	$Cl125_{\text{I-iothalamate}}$	Bolus injection: 4.20 ± 1.13		
		Constant infusion: 3.14 ± 0.53		
Thirteen ponies[e]	Cl_{CRend}	1.93 ± 0.37[c] (1.36–2.70)	—	224
Nine (no NaCl supplement)		2.06 ± 0.34[c] (1.64–2.70)		
Four (NaCl supplement)		1.63 ± 0.27[c] (1.36–1.99)		
Seven female horses	Cl_{CRend}	3.68 ± 1.18[c] (2.07–4.99)	—	225
Four female horses	Cl_{In}	1.65 ± 0.07[c] (1.34–2.04)	0.96 ± 0.02	226
	Cl_{CRend}	1.62 ± 0.03[c] (1.29–2.15)		
Six female ponies	Cl_{In}	1.92 ± 0.06[c] (0.64–3.37)	0.86[c]	210[g]
	Cl_{CRend}	2.24 ± 0.06[c] (1.04–4.15)		
Two female horses	Cl_{In}	1.86 ± 0.14[c] (0.71–3.68)	1.11[c]	
	Cl_{CRend}	1.67 ± 0.13[c] (0.68–3.09)		
Twelve female/gelding horses	$Cl99m_{\text{Tc-DTPA}}$	1.93 ± 0.27 (1.39–2.53)	—	211
Four gelding horses	Cl_{CRend}	1.34 ± 0.51[c] (1.01–2.10)	—	145
One female pony	Cl_{CRend}	1.15 ± 0.08	—	227
Four horses (one gelding, three female)	Cl_{CRend}	1.45 ± 0.21[c]		
Ten gelding horses	Cl_{CRend}	1.88 ± 0.46	—	152
Twelve female horses	Cl_{CRend}	1.48 ± 0.04	—	228[h]
One male pony	$Cl14_{\text{C-In}}$	1.74 ± 0.15	0.61 ± 0.11	154[i]
	Cl_{CRend}	1.06 ± 0.10		
Four ponies (two male, two female)	$Cl14_{\text{C-In}}$	1.66 ± 0.38[c] (1.34–2.22)	1.02 ± 0.07[c]	
	Cl_{CRex}	1.70 ± 0.39[c] (1.43–2.27)		
Six female horses	Cl_{CRend}	1.92 ± 0.51 (1.49–2.74)	—	153
Two female horses	Cl_{CRend}	Awake horses: 2.65[c]	—	229[j]
		During anesthesia: 1.32[c]		
		After anesthesia: 2.50[c]		
Eight female horses	Cl_{In}	Bolus injection: 1.63 ± 0.33		214
Four-day-old foals	Cl_{CRend}	2.81 ± 0.55	1.00[c,k]	
Five horses (three male, two female)	Cl_{In}	Bolus injection: 2.30 ± 0.34		
Three ponies (one male, two female)		Infusion (Cl-plasma): 2.56 ± 0.30		
		Infusion (Cl-urine): 2.82 ± 0.32		
Six female horses	Cl_{CRex}	2.56 ± 0.60[c]	—	230
Six female horses	Cl_{In}	1.88 ± 0.67	—	216
Eight female horses	Cl_{In}	1.83 ± 0.21 (0.89–2.95)	—	219
	$Cl99m_{\text{Tc-DTPA}}$	1.79 ± 0.18 (1.08–2.51)		
Twelve female horses	Cl_{In}	1.55 ± 0.04[c] (0.98–2.22)	—	231
	$Cl99m_{\text{Tc-DTPA}}$	1.47 ± 0.27[c] (0.91–1.82)		
	$Cl99m_{\text{Tc-DTPA(cam)}}$	1.55 ± 0.22[c,l]		

TABLE **14.5 Reported Values for Glomerular Filtration Rate in Horses and Ponies—cont'd**

Number of Animals	Method[a]	Glomerular Filtration Rate (GFR) (Mean ± SD/SE mL/min/kg [Range])	Cl_{CRend}/Cl_{In} Ratio[a]	Reference
Thirty horses (7 male, 23 female)	Cl_{In}	1.73	1.03[c]	232
	Cl_{CRend}	1.79		
Six pony foals (three female, three male)	Cl_{In}	3.21 ± 0.36 (2.73–3.64)	0.60[c]	221
	Cl_{CRend}	1.92 ± 0.14 (1.60–2.14)		
Five horses	$Cl99m_{Tc\text{-}DTPA}$	3.3 ± 0.4	—	233
Six female horses	Cl_{CRend}	1.20–1.87	—	234[a]

[a]Cl_{In}, Inulin clearance; Cl_{CRend}, endogenous creatinine clearance; $Cl125_{I\text{-}iothalamate}$, clearance of ^{125}I-iothalamate; $Cl99m_{Tc\text{-}DTPA}$, clearance of 99mTc-diethylenetriamine pentaacetic acid (99mTc-DTPA); $Cl14_{C\text{-}In}$, clearance of ^{14}C-inulin; Cl_{CRex}, exogenous creatinine clearance; $Cl99m_{Tc\text{-}DTPA(cam)}$, clearance of 99mTc-DTPA determined by serial imaging at the body surface with a gamma camera.
[b]Values taken from Knudsen.[208]
[c]Values presented have been calculated from original data.
[d]Low GFR values were attributed to rapidly declining plasma inulin concentrations (non–steady-state conditions) during the urine collection periods.
[e]Sex not reported.
[f]Other horses and ponies also were studied after bolus injection of ^{125}I-iothalamate and yielded GFR values of 5.39 ± 1.79 and 3.44 ± 1.11 mL/min/kg for ponies and horses, respectively.
[g]Attempts at measuring GFR by plasma disappearance after bolus injection of inulin were unsuccessful.
[h]Value presented is for control group; GFR was not different after phenylbutazone administration (1.36 ± 0.04 mL/min/kg) or phenylbutazone and furosemide administration (1.44 ± 0.12 mL/min/kg) but was reported to increase to 1.75 ± 0.16 mL/min/kg after water loading (25 L) and to 1.77 ± 0.18 mL/min/kg after water loading and phenylbutazone administration.
[i]Ponies were anesthetized during the studies.
[j]Mares studied before, during, and after 1.2 minimum alveolar concentration halothane anesthesia.
[k]Value calculated from urinary clearance values for inulin and creatinine (Cr).
[l]Despite correction for differences in depth (right kidney closer to lateral body surface than left kidney), the clearance of 99mTc-DTPA determined by serial imaging at the body surface with a gamma camera showed a greater (–60% of total) GFR by the right kidney compared with the left kidney (–40% of total). Because similar differences have not been demonstrated in microsphere studies of renal blood flow (RBF) (in which both kidneys receive equal blood flow), this technique requires further refinement before it can be used to provide accurate measures of GFR in horses.

The mechanisms responsible for control of renal blood flow (autoregulation, neural input, hormonal factors) also play a role in control of GFR. In addition, GFR is affected further by factors such as plasma protein concentration (oncotic pressure) and alterations in the filtration barrier. As discussed previously, a balance exists between that action of vasoconstrictor and vasodilator substances during periods of decreased renal blood flow. Interestingly, GFR decreases less than renal blood flow with moderate to severe renal vasoconstriction. This sparing effect on GFR has long been attributed to greater vasoconstrictive effects of angiotensin II on efferent arterioles compared with afferent arterioles.[242] Such a response could increase the glomerular capillary transmural hydraulic pressure driving filtration and would be manifested by an increase in filtration fraction. In fact, the latter response has been documented in exercising horses.[216] However, other vasoconstrictors (endothelins) and vasodilators (endothelium-derived relaxing factors, nitric oxide) have been shown to play a role in the control of glomerular capillary hemodynamics and filtration so that a singular role for angiotensin II is likely an oversimplified explanation for the sparing effect on GFR.[243]

Renal Tubular Function

Once the glomerular filtrate enters the renal tubule, it is modified extensively in the process of becoming the final product excreted into the renal pelvis. A complete review of renal tubular function is beyond the scope of this text; however, a few general concepts warrant mention, and a number of specific aspects are addressed elsewhere in this chapter. First, most glucose, amino acid, electrolyte, and water reabsorption occurs across epithelial cells lining the proximal tubule; however, these substances are not all reabsorbed to the same extent. For example, this tubule segment is responsible for reabsorption of essentially all filtered glucose and amino acids, about 90% of bicarbonate, 70% of sodium, and 60% of chloride.[155] Furthermore, at the end of the proximal tubule, fluid is no more concentrated than it was in Bowman's space. Tubular sodium concentration is unchanged, whereas tubular chloride concentration actually has increased (because of preferential bicarbonate reabsorption). Despite limited modification of these tubular fluid components, net reabsorption of between 60% and 80% of the total filtered load of sodium, chloride, and water occurs within the proximal tubule. Proximal tubular epithelial cells are also responsible for secretion of ammonium ions and a number of organic anions and cations, as described previously.

Tubular fluid passing into the loop of Henle becomes progressively more concentrated (hypertonic) as it travels to the inner medulla because the descending limb is permeable to water, urea, and electrolytes (the latter to a lesser degree).[155] In contrast, the ascending limb is relatively impermeable to water but actively reabsorbs sodium, chloride, and potassium via the apical $Na^+/K^+/2Cl^-$ cotransporter (blocked by furosemide), which is coupled to Na^+,K^+-ATPase on the basolateral membrane. As a result, fluid leaving this nephron segment is actually less concentrated (hypotonic) than the original filtrate. The loop of Henle is responsible for reabsorption of an additional 15% to 20% of filtered sodium and chloride, along with addition of urea to the tubular fluid. More important, the loop of Henle is responsible for generation of the medullary osmotic gradient via countercurrent multiplication. This function results from the combined effects of different permeability

characteristics of the descending and ascending limbs of the loop of Henle and active removal of sodium and chloride in the ascending limb.

The distal tubule is quantitatively less important in reabsorption of electrolytes and water; however, the distal tubule is the nephron segment in which the final qualitative changes in urine occur.[155] For example, the distal tubule is an important site of calcium, potassium, and acid excretion. The latter two typically are exchanged for sodium under the influence of aldosterone. Tubule fluid passes from the distal tubule into the outer or cortical collecting ducts, which are impermeable to urea. In addition to further modification of fluid in the cortical collecting ducts, tubular urea concentration increases steadily as water is removed (under the influence of vasopressin) as fluid travels to the inner medulla. In contrast, in the absence of vasopressin (as with diabetes insipidus), the collecting ducts are impermeable to water and produce hypotonic urine. The collecting ducts remain impermeable to urea (which accounts for up to 50% of the osmoles in urine) except for the innermost medullary segments, which allow urea to be recycled into the interstitium for maintenance of the medullary osmotic gradient.

Reabsorption of glomerular filtrate by renal tubules requires a close association with the vascular system that carries reabsorbed solute and water to the circulation. Proximal tubules are adjacent to peritubular capillaries, which have a tremendous capacity to accommodate the massive flux of solute and water across proximal tubule epithelial cells. Equally important in maintenance of the medullary osmotic gradient are the vasa recta, hairpin capillaries that travel deep into the renal medulla in association with loops of Henle derived from the population of juxtamedullary nephrons. Blood flow through these capillaries is typically slow, allowing for countercurrent exchange of solute in the medullary interstitium, which is necessary for generation and maintenance of medullary hypertonicity. Urea leaving the descending limb of Henle and being recycled across the innermost portion of the medullary collecting duct is responsible for about half of this medullary hypertonicity.

These basic aspects of tubular function have a number of important clinical implications. First, proximal tubule epithelial cells have a high metabolic rate. Although most of the proximal tubule is in the more highly perfused renal cortex, renal hypoperfusion leads to a relative hypoxia surrounding these cells because of ongoing metabolic activity. Consequently, the proximal tubule is highly susceptible to injury when cortical blood flow is reduced (e.g., with hypovolemia or other states accompanied by a decrease in renal blood flow). Second, as discussed previously, the renal medulla receives only a small fraction of the total renal blood flow, leading to a normally hypoxic local environment. Thus any degree of renal hypoperfusion also is accompanied by exacerbation of medullary hypoxia, especially in the inner stripe because of the metabolic activity of epithelial cells lining the mTAL. In fact, in cases of acute renal failure in humans, histologic examination of renal tissue actually may show more severe lesions in the more distal nephron (mTAL) rather than in the proximal tubule.[244] Recognition of this more distal tubular damage has also led to consideration of therapeutic interventions to reduce damage to this nephron segment during periods of poor renal perfusion (e.g., continuous infusion of furosemide to decrease the metabolic activity of the mTAL). Third, despite the fact that the distal tubule and collecting ducts are responsible for reabsorption of less than 5% of the total glomerular filtrate, a decrease in reabsorption of only 1% to 2% can be quantitatively significant and can lead to dramatic polyuria (see Polyuria and Polydipsia later in this chapter). Next, generation of a maximal medullary concentration gradient requires slow flow of tubular fluid for countercurrent multiplication and slow flow of blood through the vasa recta to maximize countercurrent exchange. Thus conditions that increase tubular flow rates (high-volume intravenous fluids) or increase vasa recta blood flow (endogenous PGE_2 and PGI_2 production consequent to renal hypoperfusion) compromise the medullary concentration gradient (partial medullary washout) and lead to production of more dilute urine with increased urinary sodium concentration (and excretion).

A final aspect of tubular function that appears to be unique to horses among the domestic species is excretion of calcium. Equine urine is well recognized as being cloudy and viscid. These qualities can be attributed to the large amount of calcium excreted in normal equine urine, largely in the form of calcium carbonate ($CaCO_3$) crystals, and mucus secreted by glands in the renal pelvis and proximal ureter that acts to "lubricate" the lower urinary tract to minimize adherence of crystal to the epithelium lining the ureters, bladder, and urethra. Although the nature of this unique tubular calcium excretion has been studied little in horses, one report of the role of vitamin D in calcium and phosphorus homeostasis in horses suggested that this vitamin-hormone was less important in horses than in other species.[245] This fascinating difference between horses and other species evaluated by large animal internists clearly warrants further investigation.

Excretion of Solute and Water

Renal function traditionally is thought of in terms of glomerular filtration, tubular modification of the filtered fluid, and excretion of the final urine. This concept accommodates excretion of nitrogenous and organic wastes and the major aspects of regulation of body water content and ionic balance. Urine concentration and volume also are affected by solute excretion, and another way to think about renal function is in terms of total solute and water excretion. For example, a horse could produce 6 L of urine daily with an osmolality of 900 mOsm/kg to excrete 5400 mOsm of solute or, if the solute load were doubled to 10,800 mOsm, the horse could produce 12 L of urine with an osmolality of 900 mOsm/kg to eliminate the additional solute. Thus urine osmolality reflects the ability of the kidney to dilute or concentrate the final urine but does not necessarily provide an accurate estimate of the "quantitative ability" to excrete solute or retain water. One assesses these functions by calculating osmolal clearance (C_{osm}) and free water clearance (C_{H_2O}).[155] Like other clearances, these calculations require measurement of urine flow (via timed urine collection) and measurement of plasma and urine osmolality.

These measures of renal solute and water handling are conceptualized by considering urine to have two components: (1) that which contains all the urinary solute in a solution that is isosmotic to plasma (C_{osm}, usually expressed in milliliters per minute or liters per day), and (2) that which contains free water without any solute (C_{H_2O}, also expressed in milliliters per minute or liters per day). The sum of these two components is the actual urine flow rate in milliliters per minute or liters per day. Because urine is typically more concentrated than plasma, C_{H_2O} typically has a negative value, indicating water conservation. In fact, the inverse of free water clearance

is termed *renal water reabsorption*. Returning to the foregoing example, excretion of the 5400 mOsm would require production of 18 L of urine that is isosmotic with plasma (using a value of 300 mOsm/kg for plasma). However, because 6 L of concentrated urine actually was produced during the period measured, the kidneys quantitatively have reabsorbed 12 L of free water per day. In contrast, despite production of urine with an identical urine osmolality (900 mOsm/kg), excretion of 10,800 mOsm would require production of 36 L of urine isosmotic with plasma. Free water clearance would be 30 L/day (i.e., 30 L/day of free water would be reabsorbed by the kidneys). Thus although concentrated urine always will have a negative C_{H_2O} value, indicating renal water reabsorption, and dilute urine always will have a positive value for C_{H_2O}, indicating renal water excretion, quantitative assessment of renal solute and water handling requires measurement of osmolal and free water clearances.

Excretion of free water by the kidney occurs by generation of hypotonic tubule fluid in the ascending limb of the loop of Henle, and the amount or volume of free water produced depends on the amount of tubule fluid presented to that segment. Free water consequently is excreted by keeping the collecting ducts relatively impermeable to water (lack of vasopressin). Assessment of C_{H_2O} is most helpful in patients with hyponatremia and hypo-osmolality that cannot be attributed to another primary disease process (diarrhea or bladder rupture). For hyponatremia to develop, water excretion must be defective. For example, hyponatremia can develop with prerenal failure (hypovolemia) or with oliguric renal failure after a reduction in GFR and the amount of filtrate presented to the loop of Henle. Hyponatremia and hypo-osmolality may also develop with use of loop diuretics, because less free water is generated in the ascending limb of the loop of Henle because of blockade of the apical $Na^+/K^+/2Cl^-$ cotransporter (smaller amounts of solute are removed). A final cause of true hyponatremia may be the syndrome of inappropriate vasopressin secretion or syndrome of inappropriate antidiuretic hormone secretion. Although the latter condition has not been documented in horses, occasionally it may play a role in the development of hyponatremia in a foal.[246]

Examination of the Urinary System

Harold C. Schott II

HISTORY AND PHYSICAL EXAMINATION

To begin the evaluation of a horse with urinary tract disease, one should collect a complete history and perform a thorough physical examination. Important historical information includes duration and clinical signs, number of horses affected, diet, medications administered, and response to treatment. One also should assess water intake and urine output. For example, owners may mistake pollakiuria (frequent urination) for polyuria (increased urine production), and distinguishing between the two is helpful for forming a diagnostic plan. Pollakiuria frequently occurs in females during estrus or in either sex of horse with cystic calculi or cystitis. In contrast, polyuria more often accompanies renal disease, pituitary pars intermedia dysfunction (PPID), behavior problems (primary polydipsia), diabetes insipidus, or diabetes mellitus. Astute owners may note increased thirst after exercise or a change in urine character, such as a clearer stream, to support polydipsia and polyuria.

One can determine water intake over 24 hours by turning off any automatic-watering devices and providing a known volume of water to the horse.[247] Water intake can vary widely with environmental conditions, level of activity, and diet (see Renal Physiology) so that repeated measurements over several 24-hour periods may be more rewarding in documenting average daily water consumption. Urine output, which should range between 5 and 15 L in a horse with normal renal function, is more difficult to determine. One can apply a urine collection harness for 24-hour urine collections[248-251]; alternatively, one can use an indwelling Foley catheter attached to a collection apparatus to quantify urine output in mares. Although horses used for research tolerate these devices fairly well, they have limited application to clinical patients. One can construct a practical collection device for geldings and stallions by cutting off the bottom of a large plastic bottle, padding it, and fitting it over the prepuce. One covers the opening of the bottle with a rubber tube and clip, and urine can be removed every few hours.[252] During the collection period, horses usually are tied or restrained in stocks to minimize interference with the collection device.

The most common presenting complaints for horses with urinary tract disease are weight loss and abnormal urination. Other clinical signs vary with the cause and site of the problem and may include fever, anorexia, depression, ventral edema, oral ulceration, excessive dental tartar, colic, or scalding or blood staining of the perineum or hindlimbs. Although lumbar pain and hindlimb lameness have been attributed to urinary tract disease, a musculoskeletal problem is the usual cause of these clinical signs. Decreased performance may be an early presenting complaint for renal disease, but poor performance likely results from changes associated with uremia (mild anemia and lethargy) rather than from renal pain. Occasionally a horse with urolithiasis or renal neoplasia may have a history of recurrent colic or "kidney colic," but renal disease is an uncommon cause of abdominal pain. Prolonged or repeated posturing to urinate and dysuria or hematuria would be important findings to implicate the urinary tract as the probable source of abdominal pain in such patients.

In addition to a thorough physical examination, one should include rectal palpation in the evaluation of all horses with suspected urinary tract disease. One should palpate the bladder to determine size, wall thickness, and presence of cystic calculi or mural masses. If the bladder is full, one should palpate the bladder again after bladder catheterization or urination. One can palpate the caudal pole of the left kidney for size and texture. The ureters generally are not palpable unless enlarged or obstructed by disease, but one should palpate the dorsal abdomen (retroperitoneal course of ureters) and trigone to determine whether they are detectable. Dilation of a ureter may occur with pyelonephritis or ureterolithiasis; in mares, palpation of the distal ureters through the vaginal wall may be more rewarding. One also should palpate the reproductive tract to assess whether a reproductive problem could be causing the clinical signs.

HEMATOLOGY AND SERUM BIOCHEMISTRY

A complete blood count (CBC) that reveals an elevated white blood cell count and total protein or fibrinogen concentration

would support an inflammatory or infectious disease process. One may observe mild anemia (packed cell volume [PCV], 20%–30%) consequent to decreased renal erythropoietin production and a shortened red blood cell life span in horses with chronic renal disease.

BUN and Cr concentrations are the most commonly used indices of renal function, specifically the GFR.[5,130,253] One must remember that BUN and Cr do not increase until the majority of nephrons (generally considered about 75%) become nonfunctional.[254] Although this commonly used percentage is based on studies of partially nephrectomized laboratory animals, several clinical reports in which unilateral nephrectomy was used successfully to manage disorders of the upper urinary tract support a similar renal reserve capacity in horses.[41,74,255-257] In addition, renal function remained within normal ranges, and animals maintained body weight after experimental unilateral nephrectomy in ponies[258] and in horses.[259] Thus measurement of BUN and Cr is of little use in evaluating early or minor changes in GFR. Once elevated, however, small increases in BUN and Cr are more sensitive indicators of further deterioration in GFR, because one can interpret doubling of BUN or Cr as a 50% decline in remaining renal function (Fig. 14.10).

Urea can be measured by a variety of methods, categorized broadly as direct or indirect analyses.[5,130] The direct method is the diacetyl monoxime reaction, in which urea reacts with diacetyl after hydrolysis of diacetyl monoxime to diacetyl and hydroxylamine. One determines urea concentration spectrophotometrically by measuring the yield of the yellow diazine reaction product. Indirect analysis is based on enzymatic conversion of urea to ammonia and carbonic acid by urease. Several methods exist for subsequently determining ammonia concentration, and the one used most often is the enzyme-coupled reaction with glutamate dehydrogenase. Although the term *blood urea nitrogen* is widely accepted, one must remember that the actual measurement reported is the urea concentration in serum or plasma.

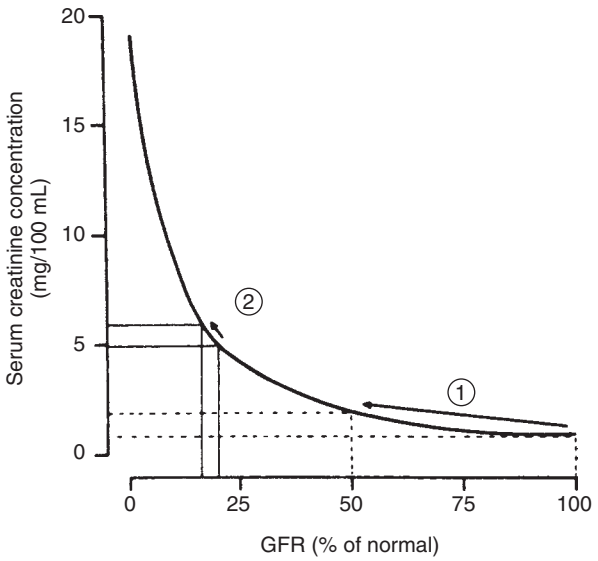


FIG. **14.10** Relationship between glomerular filtration rate (GFR) and serum creatinine (Cr). When renal function is normal, a large decrease in GFR (as with acute renal failure) results in a minor increase in Cr *(arrow 1).* In contrast, when renal function is decreased (as with chronic renal failure [CRF]), a much smaller decrease in GFR results in a similar increase in Cr *(arrow 2).* (From Brenner BM, editor: *Brenner and Rector's the kidney,* ed 8, Philadelphia, 2008, WB Saunders.)

Cr can also be assayed by several methods, but the one used most often is the Jaffe's reaction, which is a colorimetric assay based on the formation of a complex between Cr and alkaline picrate.[5,130,253] Unfortunately, a number of other substances in plasma or serum contribute to the yellow color, which leads to a 20% overestimation of actual Cr in human beings and in horses.[139] These non-Cr chromagens include glucose, pyruvate, acetoacetate, fructose, uric acid, ascorbic acid, and some drugs. Interference by non-Cr chromagens is greatest when Cr is in the normal range, which leads to a high coefficient of variation in repeated measurements of the same sample. With azotemia, Cr measurement by the Jaffe's reaction becomes more accurate because the contribution from non-Cr chromagens does not increase significantly (non-Cr chromagens are responsible for less than 5% of the color development when Cr is greater than 5.0 mg/dL). Non-Cr chromagens do not interfere significantly with urine Cr measurement.

In addition to the factors discussed in the preceding section that influence urea and Cr metabolism (see Renal Physiology), spurious increases in Cr may be reported in various metabolic disorders or after administration of certain cephalosporin antibiotics.[5] When such increases in Cr are thought to be spurious, one can measure true Cr by several methods, which include use of an automated analyzer that distinguishes Cr and non-Cr chromagens by their different rates of color development or performance of the Cr imidohydrolase enzyme assay. The latter yields ammonia, which can be quantified by colorimetric methods. As an example, Cr measured by the Jaffe's reaction increased 16% after horses were fasted for 3 days; however, when serum was analyzed by the enzymatic method, no increase in Cr was detected.[139] In addition to spurious increases in Cr, other substances can cause spurious decreases in serum Cr. The most common one is bilirubin, which, when greater than 5.0 mg/dL, may decrease measured Cr by 0.1 to 0.5 mg/dL.[5]

The reporting of serum or plasma urea and Cr concentrations also varies among different countries. In the United States BUN and Cr are reported in milligrams per deciliter, whereas in other parts of the world they are reported in standard international units of millimoles per liter and micromoles per liter, respectively. One can convert BUN from milligrams per deciliter to millimoles per liter and Cr from milligrams per deciliter to micromoles per liter by multiplying by 0.357 and 88, respectively.[5]

Azotemia is the term used to describe an elevation in BUN and Cr concentrations; thus it is strictly a laboratory abnormality. Azotemia can be prerenal in origin because of decreased renal perfusion. It may be attributable to primary renal disease or may accompany obstructive diseases or disruption of the urinary tract (postrenal azotemia).[254,260] Thus one should interpret BUN and Cr considering hydration status of the patient, presenting complaint, and physical findings. In general, animals with prerenal azotemia tend to have smaller increases in BUN and Cr than animals with intrinsic renal failure, whereas animals with postrenal failure may have the greatest degree of azotemia.[261] Unfortunately, BUN and Cr can cover a wide range of values for all three categories of azotemia; thus specific ranges do not identify the type of azotemia.[261-264] In an attempt to characterize azotemia better, use of the BUN-to-Cr ratio also has been examined. In theory,

the ratio should be higher for prerenal azotemia (because of increased reabsorption of urea with low tubule flow rates) and postrenal azotemia (because of preferential diffusion of urea across peritoneal membranes in cases of uroperitoneum) than for azotemia associated with intrinsic renal failure. As for categoric values for BUN and Cr, BUN-to-Cr ratios measured in azotemic dogs with naturally occurring diseases were distributed over wide, nondiscriminatory ranges for all three types of azotemia.[261,262] In horses, the BUN-to-Cr ratio more often has been used to separate acute renal failure from chronic kidney disease. In the acute renal failure, Cr tends to increase proportionately more than BUN, leading to a BUN-to-Cr ratio of less than 10:1.[265] In contrast, with chronic kidney disease, the BUN-to-Cr ratio often exceeds 10:1. Although a clear explanation for this difference has not been established, the difference may be related to different volumes of distribution for urea and Cr. Urea, a nonpolar molecule, diffuses freely into all body fluids, whereas Cr, a charged molecule, likely requires longer to diffuse out of the extracellular fluid space. Thus a sudden decrease in renal perfusion leads to a greater increase in Cr than in BUN. Muscle breakdown or damage, as with rhabdomyolysis, may be an additional factor contributing to the rapid increase in serum Cr concentration. Furthermore, the BUN-to-Cr ratio value provides only a suggestion of the duration of azotemia in horses, because one can find exceptions for acute and chronic renal failure. Finally, the BUN-to-Cr ratio also may be useful in assessing adequacy of dietary protein intake in cases of chronic kidney disease (see Chronic Kidney Disease).[266]

The terms *prerenal azotemia* and *prerenal failure* describe the reversible increase in BUN and Cr associated with renal hypoperfusion.[254,260,264,267] Although these terms are entrenched firmly in human and veterinary medical literature, they likely contribute to the failure to recognize the renal damage that accompanies several medical and surgical conditions. Lack of recognition is attributable to the large renal functional reserve. In fact, in many cases of prerenal failure, one can demonstrate altered glomerular and tubule function by proteinuria and cast formation, impaired concentrating ability, and changes in electrolyte excretion.[268,269] Although such functional alterations are usually reversible, a degree of permanent nephron loss can occur and could explain microscopic evidence of renal disease in as many as one third of equine kidneys examined.[270] Thus considering prerenal failure as a transient and reversible period of compromised renal function that can lead to permanent but clinically silent decreased renal functional mass may be more appropriate. Furthermore, periods of decreased renal blood flow or prerenal failure are accompanied by a number of compensatory renal responses that are mobilized to preserve renal blood flow (autoregulatory response of the afferent arterioles) and GFR (increase in filtration fraction because of angiotensin II–mediated efferent arteriolar constriction). In addition, increased intrarenal production of vasodilatory prostaglandins (PGE_2 and PGI_2) is an important response to renal ischemia that maintains or even increases medullary blood flow (see Renal Physiology). Thus one also can consider prerenal failure as a period of decompensation from the numerous renal compensatory responses to hypoperfusion.[271]

Prerenal azotemia traditionally is differentiated from intrinsic renal failure by assessing urinary concentrating ability. With prerenal azotemia, maintenance of urinary concentrating ability is demonstrated by a urine specific gravity greater than 1.020 and urine osmolality exceeding 500 mOsm/kg. In contrast, urinary concentrating ability typically is lost with intrinsic renal failure, and urine specific gravity and osmolality are less than 1.020 and 500 mOsm/kg, respectively, in the face of dehydration.[272] Such assessment is challenging, however, because it is valid only when performed on urine collected before initiation of fluid therapy or administration of any medications (α_2-receptor agonists, furosemide) that can affect urine flow and concentration.[14-16,273] In addition to these measures of urinary concentrating ability, urine-to-serum ratios of osmolality, urea nitrogen and Cr concentrations, and fractional sodium clearance may provide useful information to differentiate prerenal azotemia from intrinsic renal failure (Table 14.6).[272,273] For example, urine-to-serum Cr ratios exceeding 50:1 (reflecting concentrated urine) and fractional sodium clearance values less than 1% (indicating adequate tubule function) would be expected in horses with prerenal azotemia, whereas ratios less than 37:1 and clearance values greater than 0.8% were reported in a group of horses determined to have primary renal disease.[272] Although these values can be helpful, the data in Table 14.6 illustrate that renal hypoperfusion is accompanied by a progressive loss of concentrating ability because the ranges of these ratios tend to be lower for horses with prerenal azotemia than for clinically normal horses. Thus these data also support the concept that the progression from prerenal failure to intrinsic renal failure is associated with decompensation of the intrarenal responses to hypoperfusion.[271] Clinically, this decompensation is recognized as persistence of azotemia, whereas prerenal azotemia rapidly improves (by 30%–50% within 24 hours and completely by 72 hours) in response to fluid therapy and other supportive treatments.

In patients at risk for developing acute renal failure, including horses with serious gastrointestinal disorders or rhabdomyolysis and those receiving nephrotoxic medications, serial assessment of urine specific gravity or osmolality, sodium concentration, and fractional sodium clearance may be useful in identifying significant changes in renal function before the onset of azotemia. Similarly, if one determines urine flow rate during a timed urine collection period, assessment of renal water reabsorption (C_{H_2O}; see Renal Physiology) can be a sensitive predictor of impending renal failure.[274-276] Unfortunately, monitoring of these parameters often is complicated by use of intravenous fluid support in such patients. Although

TABLE **14.6 Diagnostic Indices That May Be Useful for Separating Prerenal from Renal Azotemia in Horses**

Diagnostic Index	Normal Horses	Prerenal Azotemia	Renal Azotemia
Urine osmolality (mOsm/kg)	727–145	458–961	226–495
Uosm/Posm	2.5–5.2	1.7–3.4	0.8–1.7
UUN/PUN	34.2–100.8	15.2–43.7	2.1–14.3
UCr/PCr	2.0–344.4	51.2–241.5	2.6–37.0
FCl_{Na}	0.01–0.70	0.02–0.50	0.80–10.10

Modified from Grossman BS, Brobst DF, Kramer JW, et al.: Urinary indices for differentiation of prerenal azotemia and renal azotemia in horses, *J Am Vet Med Assoc.* 180:284, 1982.
Uosm, Urine osmolality; *Posm,* plasma osmolality; *UUN,* urine urea nitrogen; *PUN,* plasma urea nitrogen; *UCr,* urine creatinine; *PCr,* plasma creatinine; *FCl_{Na},* fractional sodium clearance.

intravenous fluids can complicate interpretation of many of these indexes of renal function, Roussel and colleagues[273] found that the urine-to-plasma osmolality ratio remained greater than 1.7:1 in healthy horses receiving 20 L of an intravenous polyionic solution over 4 hours. Thus serial measurement of urine specific gravity or osmolality may provide useful information for patients at high risk for acute renal failure.

One usually considers a diagnosis of postrenal azotemia resulting from obstruction or disruption of the urinary tract based on clinical signs, including dysuria and renal colic. With bladder rupture, however, some affected foals and adult horses continue to void urine, although progressive abdominal distention usually accompanies development of uroperitoneum. One most often confirms urinary tract disruption by measuring a twofold or greater value for peritoneal fluid Cr concentration compared with serum Cr concentration. Occasionally in a foal with a urachal problem or a gelding with a disrupted urethra, postrenal azotemia may be accompanied by considerable swelling in the abdominal wall or in the prepuce, respectively.

In addition to screening for azotemia and concentrating ability, the laboratory database should include serum electrolyte, protein (albumin and globulin), and glucose concentrations, as well as muscle enzyme activity.[5,260,263-267] Hyponatremia and hypochloremia are common in horses with renal disease. Serum potassium concentration may be normal or may be elevated in cases of acute or chronic kidney disease. Hyperkalemia is often most extreme and most serious with urinary tract disruption and uroperitoneum. Calcium and phosphorus concentrations vary in horses with renal disease. Hypercalcemia and hypophosphatemia often occur in horses with chronic kidney disease, especially when they are fed alfalfa hay (see Chronic Kidney Disease), whereas hypocalcemia and hyperphosphatemia are more common with acute renal failure. With protein-losing glomerulopathies, albumin tends to be lost to a greater extent than the higher molecular weight globulins. One can find low total protein and albumin concentrations in severe cases of chronic renal disease, whereas other horses may have an increased globulin concentration consistent with a chronic inflammatory response. Hyperglycemia (values greater than 150–175 mg/dL) after stress, exercise, sepsis, PPID, α_2-agonist administration, or diabetes mellitus can result in glucosuria.[277,278] Finally, when pigmenturia is a complaint, muscle enzyme activity measurements and urine sediment examination are helpful in differentiating myoglobinuria from hematuria or hemoglobinuria.

URINALYSIS

One should perform urinalysis whenever one suspects urinary tract disease. One can collect urine as a midstream catch during voiding, via urethral catheterization, or via cystocentesis in foals. Unlike cows, horses cannot be stimulated easily to urinate, but they often urinate within a few minutes after being placed in a freshly bedded stall. Manual compression of the bladder during rectal palpation may stimulate urination after the rectal examination is completed. One should evaluate color, clarity, odor, viscosity, and specific gravity at the time of collection.[279,280] Normal equine urine is pale yellow to deep tan and often is turbid because of the large amounts of $CaCO_3$ crystals and mucus. Urine appearance commonly changes during urination, especially toward the end of micturition, when more crystals tend to be voided. If pigmenturia or hematuria is present, then noting the timing and duration of passage of discolored urine may help localize the source. Pigmenturia throughout urination is most consistent with myonecrosis or a bladder or kidney lesion, whereas passage of discolored urine at the start or end of urination more often occurs with lesions of the urethra or accessory sex glands (see Hematuria).

Assessment of Urine Concentration

Urine specific gravity is a measure of the number of particles in urine and is a useful estimate of urine concentration. Although determination of specific gravity with a refractometer is quick and easy (reagent strips should not be used to measure specific gravity in horses),[279] one must recognize that urine concentration is determined most accurately by measurement of urine osmolality, because the presence of larger molecules in urine, such as glucose or proteins, leads to overestimation of urine concentration by assessment of specific gravity. Clinically, overestimation is a problem in patients with diabetes or heavy proteinuria.[155] Urine specific gravity is used to separate urine concentration into three categories: (1) urine that is more dilute than serum (hyposthenuria or specific gravity less than 1.008 and osmolality less than 260 mOsm/kg); (2) urine and serum of similar osmolality (isosthenuria or specific gravity of 1.008–1.014 and osmolality of 260–300 mOsm/kg); and (3) urine that is more concentrated than serum (specific gravity greater than 1.014 and osmolality greater than 300 mOsm/kg). Although urine of most normal horses is concentrated (3 to 4 times more concentrated than serum with specific gravity of 1.025–1.050 and an osmolality of 900–1200 mOsm/kg), occasionally a normal horse produces dilute or highly concentrated urine. For example, in response to water deprivation for 24 to 72 hours, horses with normal renal function often produce urine with a specific gravity greater than 1.045 and an osmolality greater than 1500 mOsm/kg.[194,195,200] In contrast, foals typically have hyposthenuric urine consequent to their mostly milk diet.[169] Although constant polyuria decreases the ability of the neonate to generate a large osmotic gradient in the medullary interstitium, dehydrated foals still can produce urine with a specific gravity greater than 1.030. With chronic renal insufficiency, the ability to produce concentrated (specific gravity greater than 1.025) or dilute (specific gravity less than 1.008) urine is lost. Thus horses with chronic kidney disease typically manifest isosthenuria. As discussed previously, urine specific gravity is helpful in differentiating prerenal from renal azotemia in horses that exhibit dehydration or shock with many disorders.

Reagent Strip Analysis

The pH of equine urine is usually alkaline (7.0–9.0 pH).[279,280,281] Vigorous exercise or bacteriuria can result in acidic pH. Bacteriuria can impart an ammonia odor to the urine secondary to breakdown of urea by bacteria with urease activity. Concentrate feeding generally decreases urine pH toward the neutral value.[281] Similarly, the more dilute the urine sample is, the closer the pH is to 7.0. The dilute urine produced by foals typically is neutral to mildly acidic and is relatively free of crystalline material. Interestingly, calcium oxalate crystals are more prevalent in urine of foals than in that of adults.[282] Occasionally, one detects aciduria in a dehydrated or anorectic horse. Although aciduria typically has been attributed to metabolic acidosis, many patients actually may have hypochloremic metabolic alkalosis accompanied

by paradoxic aciduria. The mechanism for paradoxic aciduria is likely similar to that described in ruminants with abomasal outflow obstruction.[283] Briefly, after all chloride has been reabsorbed from the glomerular filtrate, further sodium reabsorption occurs by exchange with (excretion of) potassium or hydrogen ions. Thus paradoxic aciduria is most likely to occur with concomitant hypokalemia or whole-body potassium depletion.

Commercially available urine reagent strips can yield false-positive results for protein with alkaline samples. Thus proteinuria can be assessed better by performing the semiquantitative sulfosalicylic acid precipitation test or by specific quantification with a colorimetric assay (e.g., the Coomassie brilliant blue dye method[284] or other assays that are used routinely on cerebrospinal fluid [CSF]). In normal mares, a mean value of 3.2 mg/kg (1.6 g) per day and a range of 3.6 to 22.3 mg/kg (1.8–11.2 g) per day for urinary protein excretion have been reported by Schott and colleagues[285] and Kohn and Strasser,[153] respectively. These values translate into urinary protein concentrations of less than 100 mg/dL in most normal horses. Comparison of the quantitative protein result (milligrams per deciliter) to urine Cr concentration (milligrams per deciliter) in the form of a urine protein-to-Cr ratio also is recommended. This technique is more practical because it obviates timed urine collection. Although a normal range has not yet been reported for horses, values exceeding 1.0:1.0 and 3.5:1.0, respectively, are considered above normal for dogs[286] and indicate nephrotic range proteinuria in human beings.[5] Thus a urine protein-to-Cr ratio greater than 2:1 likely supports significant proteinuria in an equine patient. Proteinuria may occur with glomerular disease, bacteriuria, or pyuria or may transiently follow exercise.[285]

Normal equine urine should not contain glucose. Although the renal threshold for glucose has not been evaluated thoroughly in horses, an early study by Link[287] suggested that the threshold may be lower (about 150 mg/dL) than that of small animals and human beings. Thus glucosuria can accompany hyperglycemia associated with the causes described previously or with administration of dextrose-containing fluids or parenteral nutrition.[277,278] In addition, glucosuria may accompany sedation with α_2-agonists or exogenous corticosteroid administration.[14,15] When one detects glucosuria in the absence of hyperglycemia, one should suspect primary tubule dysfunction (Fanconi syndrome). Glucosuria more often has been detected in horses with acute renal failure (mostly in experimental models of nephrotoxicity) than in those with chronic renal disease. Unlike ruminants, ketones rarely are detected in equine urine, even in advanced catabolic states or with diabetes mellitus. A positive result for blood on a urine reagent strip can reflect the presence of hemoglobin, myoglobin, or intact erythrocytes in the urine sample. Evaluation of serum for hemolysis and of urine sediment for red blood cells, combined with an ammonium sulfate precipitation test to detect myoglobin,[288] can be rewarding in differentiating among these pigments (see Hematuria). Finally, occasionally one detects bilirubinuria on reagent strip analysis of equine urine. Bilirubinuria is associated with intravascular hemolysis, hepatic necrosis, and obstructive hepatopathies. In most instances, one more commonly detects hemolysis and hepatic disease by abnormal biochemical data such as elevated serum bilirubin concentration and increased hepatic enzyme activity.

Sediment Examination

Sediment examination is probably the most underused diagnostic technique available for evaluation of urinary tract disorders in horses. In humans, sediment examination has been demonstrated to be a useful predictor for occurrence and severity of acute renal failure.[289] Unfortunately, a major limitation is that sediment should be examined within 30 to 60 minutes after collection. To perform sediment examination, 10 mL of fresh urine is centrifuged (usually in a conical plastic tube) at 1000 rpm for 3 to 5 minutes. The supernatant urine is discarded (or used for quantitative protein determination), and the pellet is resuspended in the few drops of urine remaining in the tube. A drop of sediment is transferred to a glass slide, and a coverslip is applied. One first examines the sediment at low power to evaluate for casts and subsequently at high power to quantify erythrocytes, leukocytes, and epithelial cells, as well as to determine whether bacteria are present. Casts are molds of Tamm-Horsfall glycoprotein and cells that form in tubules and subsequently pass into the bladder. Casts are rare in normal equine urine but may be associated with inflammatory or infectious processes. Casts are unstable in alkaline urine; thus, one should evaluate sediment as soon as possible after collection to ensure accurate assessment. Fewer than five erythrocytes per high-power field should be seen in an atraumatically collected urine sample. Increased numbers of urinary red blood cells can result from inflammation, infection, toxemia, neoplasia, or exercise (see Hematuria). Pyuria (more than 10 white blood cells per high-power field) most often is associated with infectious or inflammatory disorders. Normal equine urine should have few bacteria, if any. The absence of bacteria on sediment examination does not rule out their presence, however, and bacterial culture of urine collected by catheterization or cystocentesis (foals) should be performed when one suspects cystitis or pyelonephritis. Finally, equine urine is rich in crystals. Most of these are $CaCO_3$ crystals of variable size, but calcium phosphate crystals and occasionally calcium oxalate crystals also are visible in normal equine urine (Fig. 14.11).[279,280,290] Addition of a few drops of 10% acetic acid solution may be necessary to dissolve crystals for accurate assessment of urine sediment.[279]

Enzymuria

Renal tubules are metabolically active, being responsible for absorption or excretion of a wide range of substances. Transport of these compounds is facilitated by several enzymes, which are found in large amounts in lysosomes within or in the brush borders of tubular epithelial cells. Regular turnover of these cells and release of endocytotic vesicles and lysosomes into the tubular lumen results in activity of enzymes in urine (enzymuria).[291] A number of substances that are filtered at the glomerulus (including bile acids, aminoglycoside and cephalosporin antibiotics, mannitol, dextrans, radiographic contrast media, and heavy metals) are taken up via endocytosis into proximal tubular epithelial cells. Endocytotic vesicles combine with lysosomes and substances that are not broken down by lysosomal enzymes subsequently are extruded into the tubule lumen through evacuation of residual bodies.

Inflammation or necrosis of tubular epithelial cells results in elevated urinary activity of lysosomal and brush border enzymes. Because proximal tubular epithelial cells are the most metabolically active of all kidney cells, they are particularly susceptible to ischemic injury. Furthermore, they can be injured similarly by exposure to large quantities of

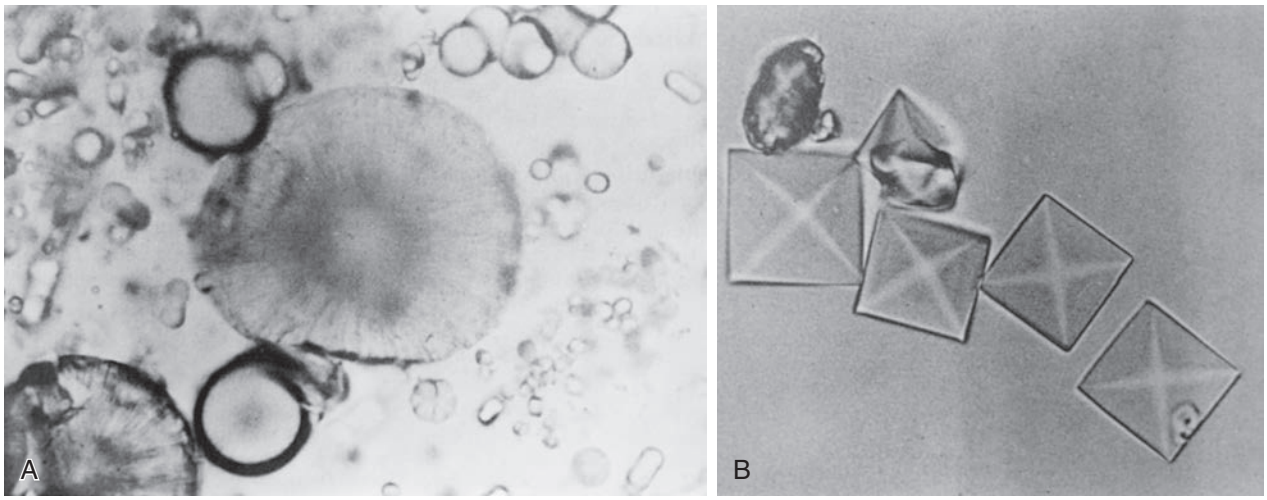


FIG. **14.11** Crystals commonly observed in equine urine sediment (original magnification, ×160). **A,** Large, round calcium carbonate ($CaCO_3$) crystals (center and lower left) and smaller calcium phosphate crystals (oblong). **B,** Calcium oxalate dihydrate crystals. (Reproduced from Osborne CA, O'Brien TD, Ghobrial HK, et al.: Crystalluria: observations, interpretations, and misinterpretations, *Vet Clin North Am Small Anim Pract.* 16:45, 1986.)

nephrotoxins in glomerular filtrate. As a result, determination of the activities of certain urinary enzymes can provide evidence of tubular damage several days before azotemia may develop.[292-295] Additional contributors to increased urinary enzyme activity include (1) low-molecular-weight enzymes such as amylase that normally are filtered by glomeruli and reabsorbed in the proximal tubules, (2) postrenal genitourinary tract epithelia that usually contribute a negligible amount to the overall urinary enzyme activity (unless they become neoplastic), and (3) secretions from accessory sex glands. Contributions from the latter explain why intact males tend to have higher urinary activities of lactate dehydrogenase (LDH) and *N*-acetyl-β-D-glucosaminidase (NAG).

Although more than 40 enzymes have been detected in urine of different species, only a few appear to be of diagnostic relevance. To be of clinical use, a urinary enzyme must have measurable activity in the kidney; its activity must lie within a narrow range in urine of healthy animals; it must be sufficiently large (molecular weight greater than 60,000) so as not to be filtered freely across the glomerulus; and its activity must increase early enough during renal injury to permit institution of corrective treatment. Finally, the activity of the urinary enzyme should remain fairly stable in urine for several days without the need for special processing. In human beings and dogs, a number of enzymes, including NAG, LDH, β-glucuronidase, alanine aminopeptidase, alkaline phosphatase (ALP), leucine aminopeptidase, γ-glutamyltransferase (GGT), and kallikrein have been demonstrated to be sensitive indicators of renal damage.[292-294,296,297] With respect to horses, normal values have been established for activities of GGT, ALP, NAG, LDH, and kallikrein.[298-302] Attempts to assay aspartate aminotransferase and alanine aminotransferase activities were unsuccessful in normal horse urine.[299]

ALP and GGT are membrane-associated enzymes found primarily in the brush border of the proximal tubular epithelium.[293-297,303] Their activity in distal tubular epithelium is negligible.[257] These enzymes have activity in other tissues, but because they are not filtered by the glomerulus, elevated activity in urine in the absence of significant proteinuria is presumed to originate from the kidneys. Measurable activity in normal urine is attributed to cell turnover.[293] LDH is a more ubiquitous tubular epithelial enzyme, being as active in the distal tubules and medullary papillae as in proximal tubular epithelium.[296-303] NAG is a proximal tubular lysosomal enzyme.[293-303] Only GGT, ALP, LDH, and NAG have been assayed in the urine of horses known to have, or thought to have, some form of renal dysfunction. Determination of NAG activity can be difficult in normal equine urine because of its alkalinity, and normal values may be less than detectable assay limits when one uses a spectrophotometric, rather than a fluorometric, method.[304-307] Published normal activities (expressed per gram of Cr) for these enzymes in equine urine are GGT: 0 to 25 IU/g Cr; ALP: 0 to 28 IU/g Cr; LDH: 0 to 12 IU/g Cr; and NAG: less than 1 IU/g Cr (less than 2 IU/L urine). Comparison with Cr concentration, which is relatively constant, results in less volume-related variation and allows interpretation of a randomly collected specimen (compared with a urine sample obtained during a timed collection period).[298,308,309]

Factors one must consider when measuring urinary enzyme activities include conditions under which the urine sample has been stored, urinary pH, diurnal variation, gender- and age-related variations, and other naturally occurring inhibitors or promoters of enzyme activity in urine (albumin, mucoproteins, proteolytic agents, amino acids, and ammonia). Although these factors have received limited study respecting equine urine, it has been documented that freezing can decrease activity of all enzymes, especially GGT. Furthermore, the colder the temperature, the more rapid the loss of enzyme activity.[298] To obtain the most accurate results, one should refrigerate samples (4°C) and assay them within 72 hours of collection. In species that have a slightly acidic or neutral urinary pH, assay of NAG is considered one of the most valuable diagnostic tests available. However, its activity appears to be susceptible to pH changes. In human beings receiving nephrotoxic medications, NAG activity in urine became undetectable at pH values greater than 8.[310] Similarly, NAG activities were undetectable (less than 2 IU/L) in urine

of normal horses.[311] In a study of monensin toxicosis in horses, NAG activity increased as urinary pH decreased.[300] Certain amino acids and ammonia further act as inhibitors of lysosomal enzymes such as ALP and NAG in the urine of humans, dogs, and rats,[312,313] and techniques have been developed to remove these agents from the urine by gel filtration before assay of their activity.[314]

Theoretically, assessment of changes in the urinary activity of selected enzymes may assist the clinician in identifying the segment of the nephron suffering the greatest dysfunction or damage. Although NAG, GGT, and ALP are associated primarily with proximal tubular epithelium, LDH usually is associated with distal tubular epithelial cells. Increases in urinary GGT and ALP activity have been induced experimentally in horses receiving gentamicin and neomycin for 5 to 10 days.[315,316] Increases also have been measured in horses with diarrhea, acute abdominal crises, and endotoxic shock. In the latter instances, enzymuria was assumed to indicate tubular damage after ischemia. Five consecutive days of furosemide administration also produced moderate increases in GGT and ALP activity, with ALP increasing more rapidly.[311] However, 48 hours of water deprivation failed to induce any changes in GGT, ALP, or LDH activity.[302] Similarly, no change in urinary LDH activity was observed in horses administered phenylbutazone (8.8 mg/kg PO) daily for 6 days.[302]

Although increases in urinary enzyme activities generally indicate acute tubular damage, one must interpret urine enzyme-to-Cr ratios carefully. Threshold values above which elevations are significant have not been well documented, although one study reported that a GGT/Cr value greater than 25 IU/g indicated tubular damage.[298] In contrast, in a study of gentamicin-induced nephrotoxicity in pony mares, Hinchcliff and colleagues[315] measured GGT/Cr values exceeding 100 IU/g several days before measuring an increase in Cr concentration. Furthermore, one frequently may measure GGT/Cr values between 25 and 100 IU/g in horses receiving gentamicin at recommended doses. Similar to gentamicin pharmacokinetics, enzymuria in these horses has been highly variable; these horses have not been recognized to be at risk of developing acute renal failure. Although enzymuria likely reflects a degree of tubular damage in these patients, one should interpret increases in GGT/Cr values between 25 and 100 IU/g with caution, whereas increases greater than 100 IU/g are more likely to be clinically significant.

Horses with chronic renal disease may have normal or reduced enzyme activities that reflect cellular changes that occur in the nephron in response to chronic inflammation. Just as BUN and Cr concentrations may be normal during the early stages of renal disease; urinary enzyme activities may not accurately reflect renal dysfunction later in the disease course when results of blood tests and urinalysis are more likely to be abnormal. A possible reason for this phenomenon is that substantial destruction of tubular epithelium occurring early in the disease leaves fewer epithelial cells to be an ongoing source of elevated enzyme activities. Alternatively, regenerating tubular epithelial cells may be more refractory to the effects of the toxin.

All in all, determination of urinary enzyme activities has failed to gain acceptance as a routine measure of renal tubular damage in most equine hospitals. This lack of acceptance can be attributed to the high sensitivity for detection of subclinical renal tubular damage. For example, although a urine GGT/Cr ratio elevated to a value of 75 supports tubular damage, it does not provide the clinician a quantifiable risk for development of acute renal failure. Thus, as a single measurement, the ratio is of limited use in deciding whether one should discontinue use of a nephrotoxic medication (e.g., gentamicin). In contrast, more dramatic elevations of the GGT/Cr ratio may precede development of azotemia and could be a useful warning that one may need to discontinue a medication or, at a minimum, prolong the dosing interval. Until values for urinary enzyme activities are reported for a larger number of equine patients with various diseases, the true value of assessing urinary enzyme activity will remain unclear.

Fractional Clearance of Electrolytes

Urinary electrolyte losses, which reflect tubular function, can be expressed as excretion rates (total amount of electrolyte excreted during a given time period, expressed as milliequivalents per minute) or as clearance rates. Determination of clearance rates uses the same clearance concept to measure GFR. In brief, a clearance rate (Cl_A) is a measure of the volume of plasma that is cleared completely of the substance in question (A) during a given time period. One calculates the Cl_A by performing a timed urine collection (to determine urine flow in milliliters per minute) and measuring the concentration of the desired substance in plasma and urine (Cr or inulin for determination of GFR)[5]:

$$Cl_A = (\text{urine [A]/plasma [A]}) \times \text{urine flow}$$

As with protein, urinary clearance of many substances, including electrolytes, often is compared with that of Cr.[5] Basically, a substance that is filtered mostly across the glomerulus but is neither reabsorbed nor secreted by renal tubules (inulin) will have a clearance rate similar to that of Cr. In contrast, a substance that is poorly filtered (larger molecule) or reabsorbed to a great extent by renal tubules (sodium or chloride) will have a lower clearance value than that of Cr. Similarly, clearance values for substances that are eliminated by filtration and tubular secretion (potassium) may exceed that measured for Cr. An advantage of comparing the clearance of a substance (A) to Cl_{Cr} (expressed as a fraction of Cl_{Cr}) is that it obviates the need to perform a timed urine collection, because the urine flow factor is cancelled out in the calculation:

$$\frac{Cl_A}{Cl_{Cr}} = \frac{(\text{Urine [A]/Plasma [A]}) \times \text{Urine flow}}{(\text{Urine [Cr]/Plasma [Cr]}) \times \text{Urine flow}}$$

that, by rearrangement and expression as a percentage, becomes

$$\frac{Cl_A}{Cl_{Cr}} = \frac{\text{Urine [A]}}{\text{Plasma [A]}} \times \frac{\text{Plasma [Cr]}}{\text{Urine [Cr]}} \times 100$$

This calculation is called the *fractional Cr clearance value*.[5,317] More often, however, the term *fractional excretion* has been used to describe this value. Although most sources recommend that blood and urine samples be collected at the same time for determination of fractional clearance values, serum electrolyte and Cr concentrations are usually stable (except in patients with prerenal azotemia or acute renal failure), so one can use blood values measured within a few days of the urine sample in the clearance calculations. Consequently, leaving a specimen cup for the client to use to collect a voided sample is acceptable and obviates bladder catheterization in many cases.

As previously discussed (see Renal Physiology), the equine kidneys function to conserve more than 99% of filtered sodium and chloride ions. In contrast, potassium ions are conserved poorly except during periods of whole body potassium depletion (anorexia, prolonged exercise). Thus normal fractional clearance values are less than 1% for sodium but are considerably higher for potassium (Table 14.7).[318-325] Increases in fractional sodium and chloride clearance values may reflect an appropriate renal regulatory response to dietary excess, as with psychogenic salt consumption.[265] Alternatively, increases in fractional clearance values specifically for sodium and phosphorus also can be early indicators of renal tubule damage[272,295,315,324,326]; however, one must interpret results of these calculations in light of fluid therapy because fractional clearance values can be increased artifactually in horses receiving intravenous polyionic solutions.[272] Similarly, medication (furosemide) or light exercise also can result in increased urine flow and fractional sodium and chloride clearance values.[327]

The kidneys play an important role in equine calcium and phosphorus homeostasis, and renal loss varies with dietary intake. Thus fractional clearances of calcium and phosphorus also have been used to assess adequacy of dietary intake. Although diet is evaluated more appropriately on a herd basis by analyses of hay and concentrates, fractional clearances may be useful in individual animals or when feed analysis is impractical (e.g., forage consists of pasture). Determination of fractional calcium and phosphorus clearances has received limited study with a focus on young racing horses.[227,320,321,328-330] For example, excessive dietary phosphorus intake (which can lead to nutritional secondary hyperparathyroidism) leads to increased fractional clearance of phosphorus. Evaluation of fractional calcium clearance is hampered by the fact that most of the calcium in equine urine is in the form of $CaCO_3$ crystals. To measure the urinary calcium concentration reliably, one must collect the entire contents of the bladder during voiding or via catheterization to ensure collection of the initial crystal-poor and final crystal-rich fractions. Subsequently, one treats a well-mixed aliquot of urine with acetic acid or nitric acid to solubilize the crystals.[324] In one report, fractional calcium and phosphorus clearance values greater than 2.5% and less than 4% were considered consistent with adequate dietary intake (adequate calcium intake with phosphorus intake that was not excessive).[320] Unfortunately, because the ranges for fractional calcium and phosphorus clearances can be wide in normal horses (see Table 14.7), measurement of these clearances may not be sensitive enough to detect minor dietary imbalances. Like potassium, the fractional clearance of magnesium is high (29%± 8%) in horses fed a diet adequate in magnesium. Horses fed a low-magnesium diet reduced fractional clearance to 6%± 3%.[331]

Determination of fractional electrolyte clearance values also has been advocated in the evaluation of horses with recurrent rhabdomyolysis.[319,332,333] Low sodium and potassium clearances have been reported in some affected horses. Whether these low fractional clearance values reflected total body electrolyte depletion (as a consequence of repeated bouts of exercise in hot weather or repeated furosemide administration) or a true physiologic predisposition to rhabdomyolysis was not determined. Nevertheless, low fractional clearance values document the need for electrolyte supplementation in equine athletes. Harris and Snow[332] also described another population of horses that exhibited recurrent rhabdomyolysis that had increased fractional phosphorus clearance and was reported to respond to dietary supplementation with ground limestone. Thus although

TABLE **14.7 Fractional Electrolyte Clearance Values for Horses and Ponies**

Sodium	Potassium	Chloride	Phosphorus	Calcium	Reference
ADULTS					
0.16 ± 0.24	27.0 ± 14.6	0.17 ± 0.11	NR	NR	224[a]
0.02–1.00	15–65	0.04–1.60	0.00–0.50	NR	318
0.11–0.87	10.8–28.5	NR	0.07–0.74	NR	225
0.01–0.70	NR	NR	NR	NR	272
0.27 ± 0.02	38.52 ± 7.26	1.01 ± 0.24	NR	1.49 ± 1.58	152
0.0–0.46	23.9–75.1	0.48–1.64	0.04–0.16	NR	153
0.032–0.52	23.3–48.1[b]	0.59–1.86	0–20[b]	0.0–6.72[b]	70
0.034 ± 0.095	42.4 ± 9.8	0.352 ± 0.190	0.710 ± 0.250	NR	195
0.04–0.52	35–80	0.70–2.10	0.00–0.20	NR	319
0.0002–2.43	1.0–42.7[c]	0.012–3.47	0.023–2.77	NR	280
NR	NR	NR	0.115–0.302	NR	227
NR	NR	NR	0.08–5.53[b]	2.10–4.60[b]	320
NR	NR	NR	0.61–0.75	11–33	321[d]
FOALS					
0.31 ± 0.18	13.26 ± 4.49	0.42 ± 0.32	3.11 ± 3.81	2.85 ± 3.26	322
AFTER FUROSEMIDE ADMINISTRATION					
12.0	207	9.5			318

NR, Not reported.
[a]Values calculated from data provided.
[b]Fractional clearance of potassium may exceed upper limit on high potassium diets; a fractional clearance of phosphorus exceeding 4% suggests excessive dietary intake; a fractional clearance for calcium should exceed 2.5% with adequate intake.
[c]Low range attributed to low urine potassium concentrations determined by ion-specific electrodes.
[d]Fractional clearance value for magnesium reported at 7 to 30.

determination of fractional electrolyte clearances may be helpful in the evaluation of horses with recurrent rhabdomyolysis, only a small portion of affected horses are likely to show significant clinical improvement in response to dietary electrolyte supplementation alone.

Finally, the methodology used to determine fractional electrolyte clearances should be standardized because several factors may lead to erroneous results. For example, a note of caution is warranted when ion-specific electrodes (instead of a flame photometer) are used to measure urinary potassium concentration, because components of animal urine can interfere with the ion-specific electrode and lead to spurious low values. One usually can avoid this problem by performing the analysis on urine diluted with water.[334] As mentioned previously, light exercise may increase urine flow rate and sodium excretion.[327] Thus "spot" urine samples are best collected in the morning before feeding and exercise. McKenzie and colleagues[234] compared fractional electrolyte clearance values between single-sample spot urine collections to 24-hour volumetric urine collections over 3 days in horses receiving diets varying in cation-anion balance. They found substantial variation in fractional clearance values within individual horses over the 3-day study, despite feeding of a consistent diet. When they assessed the effect of the differing diets, fractional clearance values for sodium, potassium, and chloride were generally similar when calculated using urine electrolyte concentrations determined in spot urine samples or well-mixed 24-hour collection samples. However, they found greater variability with fractional clearance values for calcium and magnesium. The fact that they detected considerable variability should not be surprising when one remembers that fractional clearance values are a calculation using four measurements (Cr and electrolyte concentrations in plasma serum and urine). Thus small variations in each measured value have the potential to magnify error in the final calculation. All in all, this study should serve as a reminder that determination of fractional electrolyte clearances is only one of the diagnostic tools one should use to evaluate patients with renal disease or recurrent rhabdomyolysis or to assess diet in a group of horses.

IMAGING TECHNIQUES

Ultrasonography

One can perform ultrasonographic examination of the urinary tract transrectally or transabdominally.[335-342] Bladder imaging is performed best transrectally using a 5-MHz probe. One must remember the character of equine urine while imaging the bladder; the urine will be an inhomogeneous, swirling, echogenic fluid because of the presence of mucus and crystals. The latter can appear as echogenic sediment in the ventral aspect of the bladder, and balloting the bladder may cause this echogenic material to swirl. One also can confirm the presence of a cystic calculus because calculi have a highly echogenic surface and produce an acoustic shadow (Fig. 14.12). Similarly, one may image and palpate masses in the bladder wall during the examination.

The right kidney is triangular or horseshoe shaped and is imaged best transabdominally via the dorsolateral aspect of the last two or three intercostal spaces (Fig. 14.13A). The left kidney is somewhat a more typical bean shape, lies deep to the spleen, and can be imaged via the last two intercostal spaces or via the paralumbar fossa. Because the left kidney is deeper than the right kidney, it can be difficult to image completely and is

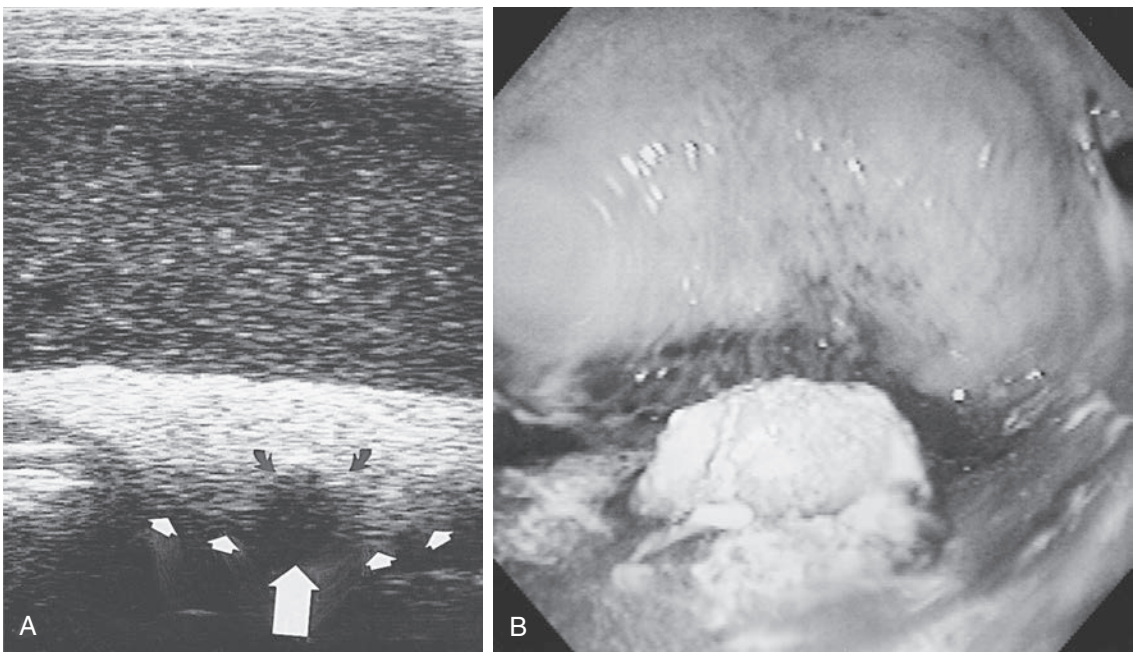


FIG. **14.12** Transrectal ultrasonographic and cystoscopic images of the bladder of a Miniature Horse mare with recurrent cystitis and urolithiasis. **A,** The ultrasonographic image shows a layer of echogenic crystalline material in the ventral aspect of the bladder (*small, white arrows* outline ventral bladder wall) and presence of a small cystolith (outlined by *small black arrows* and *large white arrow*). **B,** After lavage of the urine sediment, cystoscopy confirmed the presence of a small urolith, which was amenable to removal by digital manipulation.

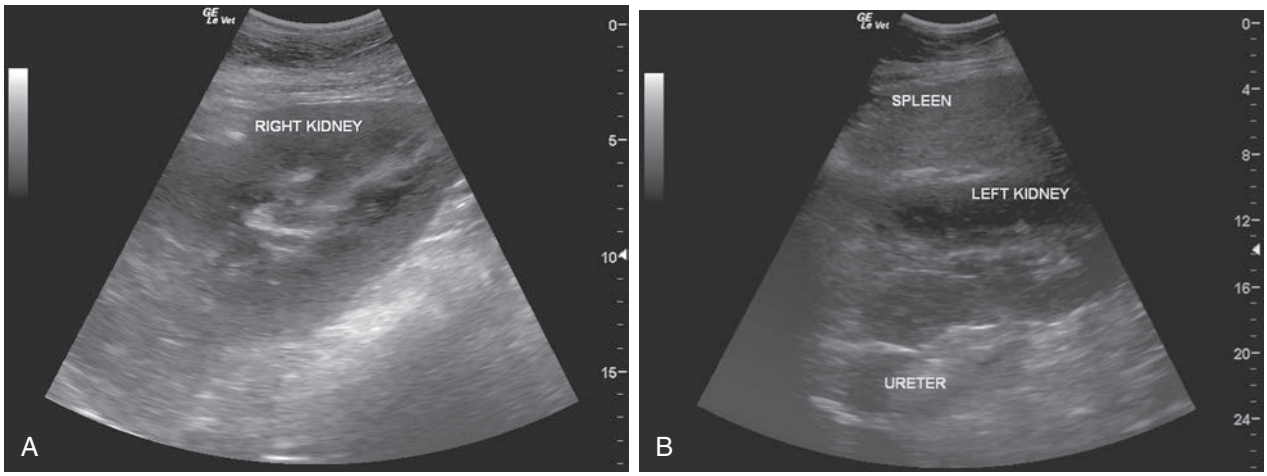


FIG. **14.13** Transabdominal ultrasonographic image of a normal **(A)** right kidney **(B)** and left kidney. The renal medulla is more hypoechoic than the renal cortex, except for the renal pelvis, which varies in echogenicity. The left kidney is imaged deep to the spleen.

examined best with a 2.5- or 3-MHz probe (Fig. 14.13B). One should assess systematically the size and shape of both kidneys, architecture, and echogenicity of the parenchyma, including imaging the kidneys in dorsal, sagittal, transverse, and transverse oblique anatomic planes.[340] In a full-size horse, the right kidney should not measure more than 15 cm in the longest axis, whereas the left kidney may measure up to 18 cm if imaged longitudinally.

In acute renal failure the kidneys may be normal or enlarged, and abnormalities of parenchymal detail are not often detected. When present, abnormal findings may include perirenal edema, widening of the renal cortex, and loss of a distinct corticomedullary junction.[336,337,339,341-343] Chronic kidney disease can result in decreased kidney size, irregular surfaces, and increased echogenicity because of renal fibrosis. Cystic or mineralized areas in renal parenchyma can be associated with chronic renal disease or congenital anomalies. Although uncommonly recognized, distinct curvilinear hyperechoic bands in the outer renal medulla parallel to the corticomedullary junction have been imaged in foals and adults horses with renal disease attributable to acute or chronic phenylbutazone toxicity.[342,344,345] This finding, termed the *medullary rim sign,* is thought to result from damage (and possible secondary mineralization) to the inner stripe of the outer medulla, the location of highly metabolically active nephron segments including the distal extent of the proximal tubule and the thick ascending limb of the loop of Henle. Calculi in the renal pelvis generally cast an acoustic shadow and can result in hydronephrosis of the affected kidney (Fig. 14.14).[336,337,339] Occasionally, one cannot image one or both kidneys because of a gas-filled bowel between the kidney and abdominal wall. Reexamination at a later time generally is required for successful imaging in such cases. In addition, administration of high rates of fluid therapy, especially in foals, can lead to iatrogenic pyelectasia (fluid distention of the renal pelvis).[342,346] One should use caution so as not to interpret this mild distention of the renal pelvis falsely as evidence of ureteral or lower urinary tract obstruction.

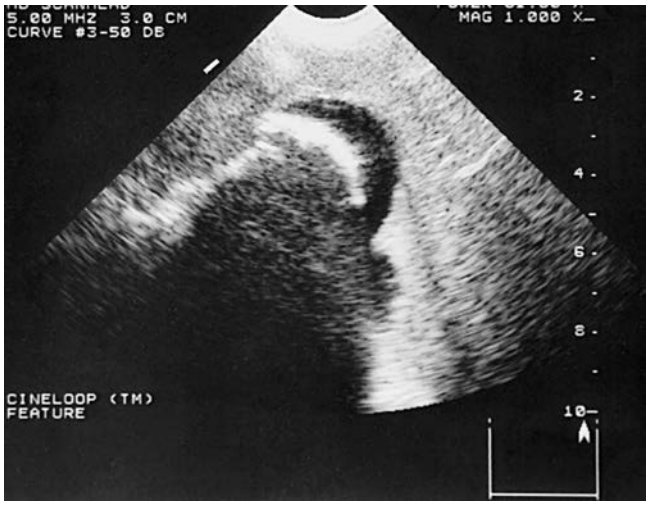


FIG. **14.14** Transrectal ultrasonographic image of the left kidney of a mare with nephrolithiasis and hydronephrosis. The nephrolith has an echogenic surface and produces an acoustic shadow. The echolucent crescent moon-shaped structure is a fluid-filled remnant of the renal parenchyma, consistent with hydronephrosis.

Radiography

Radiography rarely is used to evaluate urinary tract disease in adult horses. One usually can obtain diagnostic radiographs of the urinary tract only in foals or Miniature Horses. Excretory urography using intravenous contrast material or pyelography using contrast agent delivered into the renal pelvis under ultrasonographic guidance may be useful to identify a nonfunctional or hypoplastic kidney or an ectopic or torn ureter.[78,83] The latter procedure may be more rewarding, but both require general anesthesia to be performed safely, and these radiographs can be very difficult to interpret due to extensive superimposition and poor image quality. One also can use retrograde contrast studies to evaluate the ureters in mares[347]; however, they have been used most often in foals suspected of having an ectopic ureter or a ruptured bladder. Contrast radiographic studies also can help to identify strictures or masses in the urethra or bladder, but endoscopy is generally more useful for these problems. In small animals, abdominal survey radiographs are most useful for assessing

kidney size and shape, whereas ultrasonography provides more information about parenchymal changes associated with renal disease.[348] Thus use of a standardized protocol for ultrasonographic evaluation of the equine kidneys should provide essentially the same amount of information as the combined use of survey radiography and ultrasonography in small animal patients.[340]

Nuclear Scintigraphy

Nuclear scintigraphy is an additional imaging modality often used to assess renal anatomy and function in human beings and small animals. One can use various radionuclides and pharmaceuticals, depending on the type of scintigraphic examination being pursued.[349-351] In fact, scintigraphy is used routinely for quantitative measurement of GFR in these species. Walsh and Royal[231] compared use of renal scintigraphy (using 99mTc-DTPA, which is similar to inulin in that it is neither secreted nor reabsorbed after filtration) for measurement of GFR in horses but found greater variability compared with GFR values measured by plasma disappearance of inulin or the same radionuclide (99mTc-DTPA). Nevertheless, renal scintigraphy using 99mTc-DTPA can provide qualitative information about renal function and is the only method currently available for assessing split renal function (assessing individual kidney function) in horses. Renal scintigraphy also has been performed with 99mTc tagged to glucoheptonate (taken up by the proximal tubule epithelial cells to provide anatomic detail) or mercaptoacetyltriglycine (MAG_3, which is similar to p-aminohippurate because it is eliminated by proximal tubular secretion) to provide qualitative information about renal anatomy and function (Fig. 14.15).[352,353] Thus one may use renal scintigraphy to document the presence of a functional kidney in horses when multiple ultrasonographic examinations have been complicated by interfering bowel or when one desires information about individual kidney function.

Endoscopy

Endoscopy of the urinary tract is a useful diagnostic aid when the complaint is abnormal urination.[279,354-356] A flexible endoscope with an outside diameter of 12 mm or less and a minimum length of 1 m is adequate for examination of the urethra and bladder of an adult horse of either sex. One should sterilize the endoscope before endoscopy of the lower urinary tract. Tranquilization of the patient is recommended, and one should clean the distal end of the penis or the vulva thoroughly. The endoscope is passed as one passes a urinary catheter, using the air control intermittently to inflate the urethra or bladder. It is important to not insufflate with air excessively while the endoscope is underneath urine in the bladder because the mucus will bubble and prevent complete visualization. Normal urethral mucosa is pale pink with longitudinal folds. When dilated with air the mucosa flattens and may appear redder than normal, and a prominent submucosal vascular pattern may be apparent. Passage of a catheter before endoscopy (for sample collection or to empty the bladder) can produce mild irritation and erythema of the urethral mucosa; therefore, one should not mistake this for abnormal findings. The regions of the ischial arch (where the urethra begins to widen into the ampullar portion) and of the colliculus seminalis (in the roof of the pelvic urethra just distal to the urethral sphincter) should be examined closely, because these are common sites of posturination or postbreeding hemorrhage in geldings or stallions (see Hematuria). Subsequent passage of the endoscope through the urethral sphincter and air distention allows evaluation of the bladder for calculi, inflammation, and masses (see Fig. 14.12). Viewing the ureteral openings in the dorsal aspect of the trigone can help determine the source of hematuria or pyuria (see Hematuria). A small volume of urine should pass from each ureteral opening approximately once each minute or more frequently if the horse is well hydrated or has been sedated with an α_2-agonist. One can perform individual ureteral catheterization to obtain urine samples from each kidney by passing sterile polyethylene tubing via the biopsy channel of the endoscope. Additionally, one can take biopsies of masses in the bladder or urethra.

SPECIALIZED DIAGNOSTIC TECHNIQUES

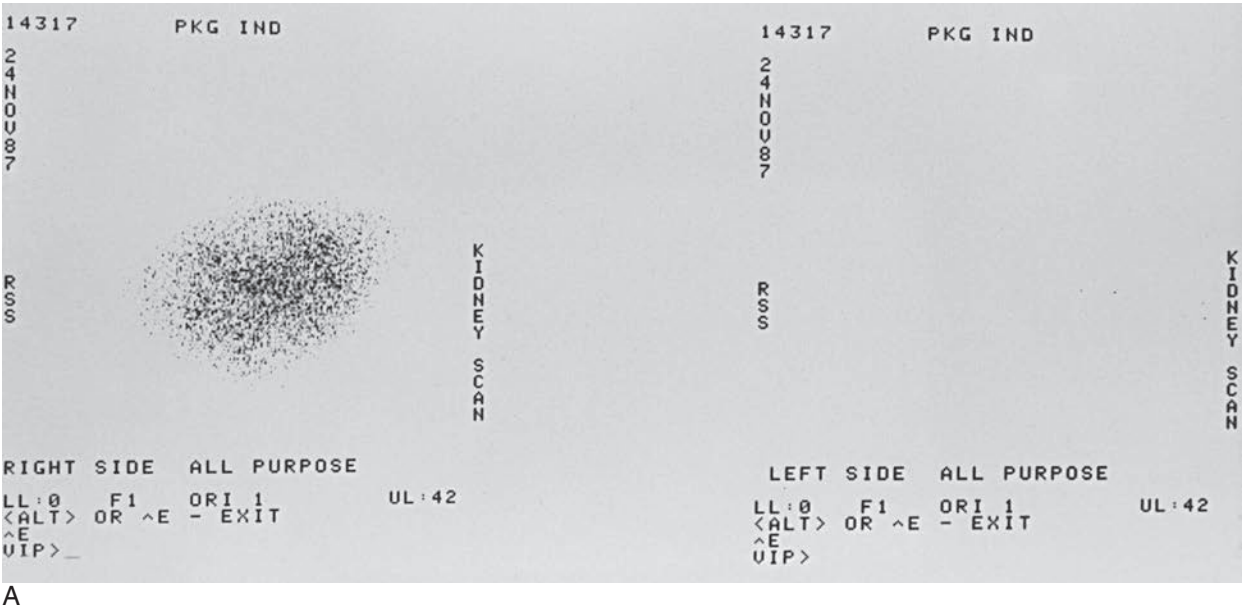
Ureteral Catheterization

With the development of high-resolution videoendoscopic equipment, retrograde instrumentation of the bladder, ureter, and renal pelvis rapidly is replacing surgical exploration for diagnostic evaluation and therapeutic management of urinary tract disorders in human beings and dogs.[349,357-359] Similarly, retrograde ureteral catheterization and instrumentation can be a valuable technique for evaluation and treatment of horses with unilateral disorders of the upper urinary tract.[29,360,361] In addition to localization of unilateral renal hemorrhage, pyelonephritis, and neoplasia, ureteral catheterization further enables retrograde pyelography.[347] As mentioned previously, one may perform this technique successfully in male and female horses during cystoscopic examination.[279,354-356]

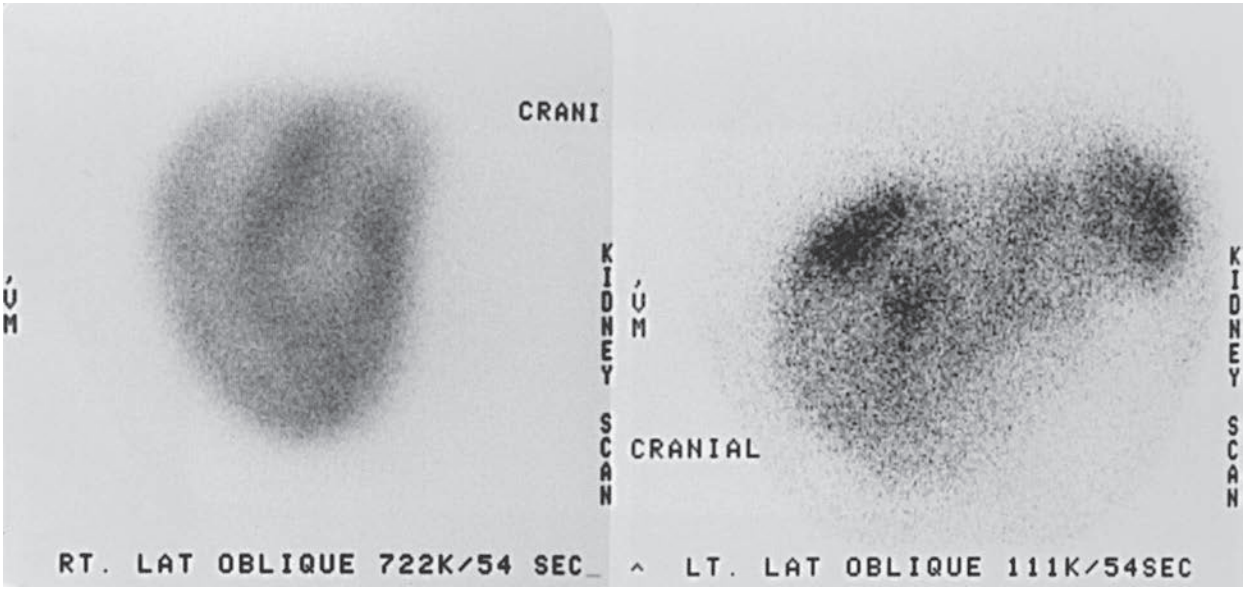
In mares, one also can catheterize the ureters manually without endoscopic guidance.[362] After preparation of the vulva, one catheterizes the bladder and drains the urine. One removes the bladder catheter and dilates the urethra manually until two fingers can be passed into the bladder. One can palpate the ureteral orifices dorsally as small, soft projections. When this is done, a catheter is placed between the fingertips, passed through the urethra, and directed into the ureter (Fig. 14.16). A relatively rigid catheter with a rounded end (no. 8–10 Fr polypropylene catheter) facilitates passage into the ureter. After advancing the catheter 5 to 10 cm into the ureter, one attaches a syringe to the other end of the catheter and collects a urine sample. During sampling, the catheter is held in place, and the ureteral opening is occluded with the fingertips to minimize loss of urine around the catheter.

Water Deprivation and the Antidiuretic Hormone Challenge Test

Water deprivation is a simple test to determine whether hyposthenuric polyuria is caused by a behavior problem such as primary (psychogenic) polydipsia or results from central or nephrogenic diabetes insipidus.[252] One should not perform a water deprivation test in an animal that is clinically dehydrated or azotemic. A baseline urinalysis should be performed (sample collected by catheterization to empty the bladder at the start of the test), and serum concentrations BUN and Cr and body weight should be measured before removal of water (food does not necessarily need to be removed, but this may help prevent gastrointestinal complications of water deprivation). One measures urine specific gravity and weight loss after 12 (usually overnight) and 24 hours. Horses with normal renal function



A



B

FIG. **14.15** Renal scintigraphic images of horses with renal disease. **A,** Renal scintigraphic image using 99mTc-diethylenetriamine pentaacetic acid (99mTc-DTPA) revealed absence of functional left renal tissue, compared with an image of the right kidney, in a stallion with chronic renal failure (CRF). A nonfunctional hypoplastic left kidney was found on necropsy examination. **B,** Renal scintigraphic image using technetium-99m-glucoheptonate (99mTc-GH) in a gelding with unilateral left-sided pyelonephritis revealed inhomogeneous uptake of the radionuclide and lesser count emission over the same time compared with the normal right kidney. The scintigraphic study, which provided anatomic and functional information, supported pursuit of unilateral nephrectomy, rather than prolonged antibiotic administration, as the treatment of choice for this horse.

typically produce urine with a specific gravity greater than 1.045 and an osmolality greater than 1500 mOsm/kg in response to water deprivation within 24 to 72 hours.[194,195,200] Practically, one can stop the test when urine specific gravity reaches 1.025 or greater. Furthermore, the test should be stopped if more than 5% of body weight is lost or clinical evidence of dehydration becomes apparent. With long-standing primary polydipsia, affected horses may not be able to concentrate urine fully (to a specific gravity greater than 1.025) because of partial washout of the medullary interstitial osmotic gradient. Extending the test period beyond 24 hours for such patients offers little benefit; however, affected horses should respond more favorably to water deprivation (producing urine with a higher specific gravity) after a period of partial water deprivation (termed a *modified water deprivation test*) during which daily water intake is restricted to 40 mL/kg for several days, which should allow time for restoration of the medullary interstitial osmotic gradient.[252] Horses with central or nephrogenic diabetes insipidus

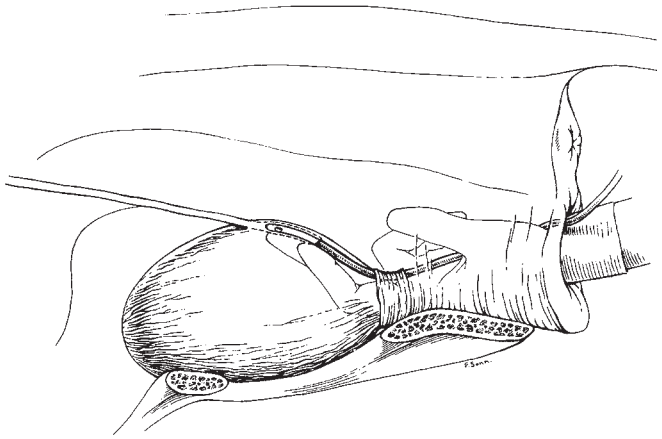


FIG. **14.16** Manual placement of a polypropylene catheter into the ureter of a mare. (From Schott HC, Hodgson DR, Bayly WM: Ureteral catheterization in the horse, *Equine Vet Educ.* 2:140, 1990.)

cannot concentrate urine in response to a water deprivation test.[56,252,363,364] When one suspects these problems, patients should be monitored every few hours because significant dehydration may ensue within 6 hours of water deprivation.

In the absence of azotemia or clinical signs of early renal failure, inability to concentrate urine in response to water deprivation supports a diagnosis of diabetes insipidus; however, the test does not distinguish between the neurogenic and nephrogenic forms of the disorder. One can differentiate these by exogenous administration of vasopressin (antidiuretic hormone).[56,363,364] Currently, two approaches exist for performing an antidiuretic hormone challenge test. First, one can administer aqueous synthetic vasopressin (20 U/mL for intramuscular or subcutaneous injection) as an intravenous infusion (5 U [0.25 mL] added to 1 L of a 5% dextrose solution and administered IV at a rate of 2.5 mU/kg over 60 minutes [250 mL to a 500-kg horse]) or an intramuscular injection (0.5 U/kg). An increase in urine specific gravity to 1.020 or greater after 60 to 90 minutes would be the expected response, whereas failure to increase urine concentration would support nephrogenic diabetes insipidus. Second, one can use desmopressin acetate (DDAVP), a synthetic analog of arginine vasopressin. Administration of DDAVP is considered the safer diagnostic technique in small animals because the change in structure of this vasopressin analog has decreased pressor actions and less effect on visceral smooth muscle compared with an enhanced antidiuretic effect. One microgram of DDAVP has an antidiuretic activity of 4 U of arginine vasopressin. The author and coworkers have validated the use of DDAVP as a replacement for the antidiuretic hormone challenge test in normal horses. In this study, the nasal spray form of DDAVP (0.1 mg/mL DDAVP) was diluted in sterile water, and 0.05 μg/kg was administered intravenously (25 μg, equal to 100 U of antidiuretic activity, to a 500-kg horse) to horses that had polyuria induced by repeated nasogastric intubation with water for 3 days preceding DDAVP challenge. Urine was collected for 8 hours after DDAVP administration, and an increase in urine specific gravity to values greater than 1.020 was observed from 2 to 7 hours after DDAVP administration (Fig. 14.17).[365] Furthermore, DDAVP administration had no effect on heart rate or systemic blood pressures. These data demonstrate that intravenous administration of DDAVP is a safe and useful diagnostic tool for evaluation of horses with diabetes insipidus.

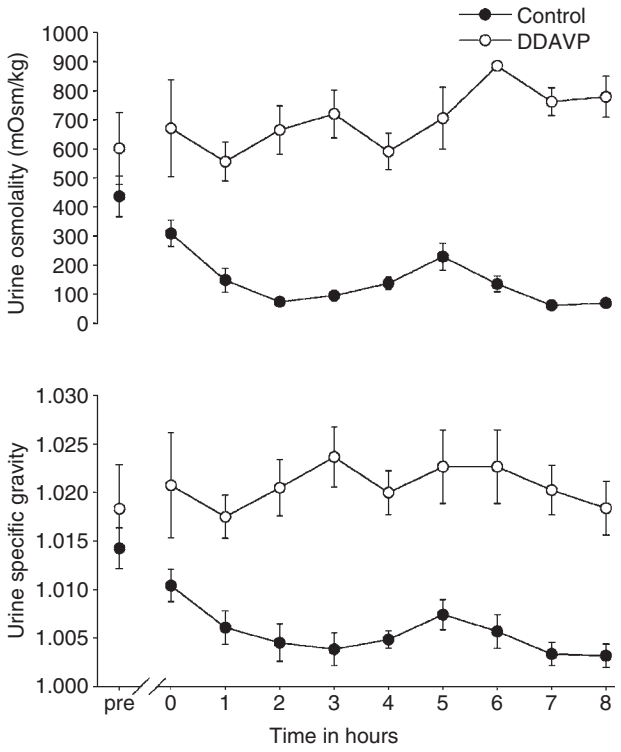


FIG. **14.17** Urine osmolality and specific gravity in six horses administered 0.05 μg/kg desmopressin acetate (DDAVP) *(open circles)* or placebo *(filled circles)* intravenously at time 0. The horses had polyuria induced by repeated nasogastric intubation with water (40 mL/kg) twice daily for 3 days preceding DDAVP challenge and again 4 hours after administration of DDAVP. Urine was collected for 8 hours after treatment, and an increase in urine specific gravity to values greater than 1.020 was observed from 2 to 7 hours after DDAVP administration.[365]

A final test for evaluating polyuria and polydipsia is an intravenous challenge with hypertonic saline (Hickey-Hare test).[182] The goal is to produce an increase in plasma osmolality, which should trigger release of endogenous vasopressin. One protocol for the test would be to measure plasma osmolality and endogenous vasopressin concentrations before and within 30 minutes after administration of 1 to 2 mL/kg of a 7.5% NaCl solution. A normal response, expected in horses with primary polydipsia, would be concurrent increases in plasma vasopressin concentration and urine specific gravity. Horses with nephrogenic diabetes insipidus also would respond with an increased plasma vasopressin concentration; those with neurogenic diabetes insipidus would not. Urine specific gravity would not be expected to increase in response to hypertonic saline administration with either form of diabetes insipidus. One could evaluate plasma vasopressin concentrations similarly before and at the end of a water deprivation test.[179,183] Unfortunately, however, assays for plasma vasopressin concentration are not currently available at commercial laboratories. Thus pursuit of this diagnostic test requires cooperation of a research laboratory.

Quantitative Measures of Renal Function

As mentioned previously, azotemia does not develop until more than 75% of nephrons cease to function; therefore measurement of BUN and Cr, although readily available, provides a poor reflection of smaller declines in renal function.

A number of methods are available to quantitate renal function in horses.[279,235] Basically, these tests can be separated into plasma disappearance curves or clearance studies involving use of timed urine collections. Generation of plasma disappearance curves requires collection of an initial blood sample, intravenous administration of one of a number of compounds (inulin, Cr, sodium sulfanilate, phenolsulfonphthalein [phenol red], or radionuclides), and collection of another series of blood samples over the subsequent 60 to 90 minutes.* One can express results in terms of an elimination half-life in minutes or as clearance values in milliliters per kilogram per minute. Mean elimination half-lives of 39.5 ± 4.4, 32.8 ± 4.1, and 16.4 ± 2.3 minutes have been reported for sodium sulfanilate, 99mTc-MAG_3, and phenolsulfonphthalein, respectively, in healthy horses.[222,366,367] The difference in these values can be explained in part by the fact that 99mTc-MAG_3 and phenolsulfonphthalein are eliminated mostly by tubular secretion and consequently are cleared more rapidly from plasma than is sodium sulfanilate (eliminated mostly by glomerular filtration). Plasma elimination half-times are most useful when measured sequentially throughout the course of renal disease; for example, Bertone and colleagues[50] reported a progressive increase in the sodium sulfanilate elimination half-time from 90 to 150 minutes during a 240-day course of chronic kidney disease in a horse with PKD.

When one uses plasma disappearance curves of a substance to estimate GFR, the compound being used must meet all the requirements of a filtration agent[211,214,219]: (1) no significant binding to plasma proteins, (2) ability to be filtered freely across the glomerulus, and (3) absence of tubular reabsorption or secretion. The gold standard compound is inulin. More traditionally, GFR has been measured by timed urine collection periods (to measure urine flow rate) and measurement of plasma and urine concentrations of a test compound that meets the listed requirements.[5,235] Several protocols exist for performing urine clearance studies. Ideally, the urine collection period should span 24 hours, although for practicality, one may use shorter collection periods.[252,235] For all protocols, one documents the bladder as being empty at the start of the collection period by catheterization or observation of voiding. All urine produced during the study period is collected and pooled into one sample. At the end of the collection period, the bladder should be emptied again via catheterization, the total volume of urine produced should be recorded, and an aliquot of the pooled urine sample should be assayed for the test substance. Similarly, one measures the concentration of the test substance in a blood sample collected near the midpoint of the urine collection period. For urine collections of 12 to 24 hours, endogenous Cr is the test substance used because it is the only one that does not have to be given by steady intravenous infusion during the collection period. GFR is calculated as the Cl_{Cr},

$$\text{GFR} = (\text{Urine [Cr]}/\text{Plasma [Cr]}) \times \text{Urine flow}$$

with the modification that the result usually is divided by body mass (in kilograms) to express GFR in term of milliliters per minute per kilogram. Although endogenous Cr is a convenient test substance, its use typically underestimates GFR because non-Cr chromagens in serum artifactually increase the value in the denominator.[235] Similarly, significant tubular secretion of Cr can be one of the compensatory responses to renal failure. This can lead to overestimation of GFR calculated using endogenous Cl_{Cr}. Despite these limitations, the endogenous Cl_{Cr} technique for measurement of GFR can provide useful information, especially when it is performed on several occasions during the course of renal failure (see Chronic Kidney Disease).

To avoid the limitations of endogenous Cl_{Cr} as a measure of GFR, one can administer a number of filtration markers (inulin, Cr, radionuclides) as an intravenous infusion throughout the urine collection period.* One starts the infusion as a bolus to increase the plasma concentration of the test substance to the desired level (e.g., the desired plasma concentration for exogenous Cr is 5–10 mg/dL, to minimize the influence of non-Cr chromagens) and subsequently continues a steady infusion throughout the remainder of the collection period. One usually performs this type of study for a shorter period with the horse restrained in stocks. One should empty the bladder of the horse at the start of the study and collect urine after two or three 30-minute intervals. One should pass a catheter and empty the bladder completely at the end of each 30-minute period. Alternatively, an indwelling bladder catheter can be used for the entire collection. After urine volumes are measured, an aliquot of each urine sample is assayed, and a blood sample is collected at the midpoint of each urine collection period for the test substance; then the GFR is calculated as the mean value for the two or three collection periods. For all practical purposes, these types of GFR measurements (with the exception of exogenous Cl_{Cr}) usually are limited to research studies, because commercial laboratories do not offer inulin assays. One also can use this shorter protocol for GFR determination using endogenous Cr as the test substance, without the need for an intravenous infusion. (The results of several studies measuring GFR in normal horses are presented in a previous section of this chapter; see Renal Physiology.)

Renal Biopsy

Renal biopsy is a useful diagnostic technique for identifying the affected region of the nephron, the type of lesion, and the chronicity and severity of disease.[5,368-371] Although biopsy is a safe procedure when performed with ultrasonographic guidance, it has risks, including perirenal hemorrhage or hematuria and, less commonly, penetration of bowel. In human beings, perinephric hematomas are common and have been detected in 57% to 85% of patients the day after biopsy. Microscopic hematuria occurs in virtually all patients for the first couple of days after biopsy, and gross hematuria occurs in 5% to 10% of patients. Most of these complications are inconsequential, but in 1% to 3% of patients the complications have resulted in the need for postbiopsy transfusions.[5] Similarly, in a group of seven normal horses subjected to renal biopsy, postbiopsy macroscopic and microscopic hematuria occurred in five animals. Furthermore, perirenal hemorrhage was a prominent finding during necropsy examination of five of these animals (including one examined 27 days after biopsy tissue was collected).[371] Thus renal biopsies remain controversial in human and equine renal failure patients.[372-374] Renal biopsies should be approached with caution and are indicated only when the results would alter the therapeutic plan or prognosis. Information about the effect of renal biopsy results on therapy and outcome of renal disease in human beings is limited; however, in one prospective study, biopsy results were found to influence

* References 211, 214, 219, 222, 231, 366, 367.

* References 154, 208, 210, 214, 216, 219, 226, 230.

physicians' decisions on about half of cases when the technique was performed.[375] In general, renal biopsy is pursued more aggressively in human beings with acute renal insufficiency than in those with chronic renal insufficiency, especially when it is difficult to determine the type of renal disease based on results of urinalysis and sediment examination.[5] In the equine patient, one performs a renal biopsy with the horse sedated and restrained in a stocks. Penetration of the needle (a Tru-Cut™–type biopsy needle or, preferably, a triggered biopsy device) into the renal parenchyma is imaged sonographically by triangulating the ultrasound beam with the biopsy instrument and the kidney or by determination of the site and depth of biopsy needle placement via ultrasonographic imaging immediately before biopsy. One should place the tissue collected in formalin for histopathologic and possibly electron microscopic evaluation. Additional samples can be collected for bacterial culture and for immunofluorescence testing (placed in Michel's transport medium or quick-frozen after coating with a preservative such as Tissue-Tek). One should determine appropriate sample processing beforehand by contacting the pathologist who will examine the biopsy samples.

Although renal biopsy results could provide useful diagnostic and prognostic information about the type of renal disease in horses with acute renal failure (e.g., glomerulonephritis, tubular necrosis, interstitial nephritis), they have been used more often to document pathology in horses with chronic kidney disease. In most cases of chronic kidney disease, one cannot detect the inciting cause unless it can be associated with a historical event or immunofluorescence testing is pursued. This limitation can be attributed to the fact that significant renal disease develops before onset of azotemia. Pathologic lesions are widespread at this point, and involvement of all nephron segments and the interstitium often leads to the interpretation of end-stage kidney disease. In occasional cases, the results may help separate infectious (pyelonephritis) or congenital (renal dysplasia) causes from nonspecific causes of renal failure. Although such results could be useful in the therapeutic approach to these patients, one should consider the limitations and risks of renal biopsy before performing this technique in horses with chronic kidney disease.

Urodynamic Procedures

Cystometrography and urethral pressure profiles are used to evaluate detrusor and urethral muscle function, respectively. Both techniques involve measurement of intraluminal pressure during inflation of the bladder or urethra. These techniques have been useful for diagnosis of myogenic and neurogenic disorders of the bladder and urethra in dogs and human beings.[376] The procedures have been performed experimentally in normal horses and ponies,[377-379] but little information is available about the use of these techniques in clinical cases (see Urinary Incontinence and Bladder Dysfunction).

Acute Renal Failure

Warwick M. Bayly

Acute renal failure (ARF) is a clinical syndrome associated with abrupt reduction in GFR. Sustained reduction in GFR is associated with failure of the kidneys to excrete nitrogenous wastes causing azotemia and with disturbances in fluid, electrolyte, and acid-base homeostasis. The human medical literature is full of various terms and definitions for different forms of ARF. Veterinary medical definitions are simpler. Basically, ARF can result from decreased renal perfusion without associated cell injury, as with dehydration (prerenal failure); obstruction or disruption of the urinary outflow tract (postrenal failure); or ischemic or toxic damage to the tubules, tubular obstruction, acute glomerulonephritis leading to a primary reduction in the filtering capacity of the glomeruli, or tubulointerstitial inflammation and edema. Any of these intrarenal causes can be associated with intrinsic renal failure. Prerenal azotemia, dehydration, and ischemic tubular insults or necrosis represent a continuum, the former resulting in the latter when perfusion is compromised sufficiently to result in death of tubule cells.[380] Classically, ARF has been associated with oliguria and occasionally with anuria, and these are certainly the most commonly noted clinical signs associated with this disease in horses. However, nonoliguric forms of ARF, particularly intrinsic renal failure, exist and are characterized by slower development of azotemia, lower peaks in creatinine concentrations, subtler increases in urinary sodium clearance, and a more rapid recovery of renal function in response to treatment. Nonoliguric renal failure seems to be diagnosed less commonly in horses, although it is not uncommon to recognize mildly azotemic horses that have apparently normal urine output. In some cases, localized proximal tubule damage and reduced solute reabsorption actually may lead to enhanced distal delivery of filtrate, which may result in polyuric ARF.

In horses, ARF is usually prerenal or renal in origin and most often is caused by dehydration, hemodynamic, or nephrotoxic insults.[70] With the exception of bladder rupture or ureteral or urachal rents in neonates, postrenal failure is uncommon in horses. Identification and correction of the cause of ARF are important because, in the early stages, renal dysfunction is frequently reversible, whereas established ARF often requires extensive supportive care and carries a guarded prognosis. By identifying patients at increased risk and attempting to interrupt the cycle of events leading to ARF, one possibly may reduce the incidence of this condition.

CAUSES

Prerenal failure is associated with conditions that result in decreased cardiac output or increased renal vascular resistance (or in both) and is the most common cause of reversible azotemia. In horses, the most common causes of dehydration, reduced cardiac output, and therefore reduced renal perfusion are associated with diarrhea, endotoxemia, acute blood loss, septic shock, and prolonged exercise. The resultant reductions in renal blood flow, GFR, and urine production usually cause azotemia and retention of water and electrolytes. Anesthesia and decreased mean arterial pressure also may decrease cardiac output and urine production enough to result in a degree of prerenal azotemia. NSAIDs also can precipitate prerenal azotemia in patients with decreased renal blood flow.[381] Although prostaglandins play only a minor role in maintenance of renal blood flow in the normal state, PGE_2 and PGI_2 are important vasodilatory mediators of renal blood flow under conditions of reduced renal perfusion. Thus administration of NSAIDs to dehydrated or toxemic patients may contribute further to renal hypoperfusion by exacerbating a decrease in renal blood flow. In some cases, this may be sufficient to produce ischemic renal parenchymal damage, thus causing intrinsic renal

failure. Generally, the parenchymal lesion associated with NSAID toxicity is medullary crest or papillary necrosis. Such lesions develop because the renal medulla normally receives much less blood flow than the renal cortex and consequently is much more susceptible to NSAID-induced changes in renal blood flow.

In human beings, intrinsic renal diseases that lead to ARF generally are categorized according to the primary site of injury: tubules, interstitium, glomeruli, or vessels.[380] Acute tubular necrosis (ATN) is the intrinsic renal failure recognized most often in horses (interstitial and primary glomerular disease being recognized occasionally and vascular disease almost never). Ischemia, especially when associated with microvascular coagulation (which often leads to irreversible cortical necrosis[265]), and nephrotoxins probably are the most common causes of equine ATN. Important nephrotoxins include aminoglycoside antibiotics and NSAIDs. Less commonly, ATN develops after exposure to endogenous pigments (myoglobin or hemoglobin), heavy metals such as mercury (contained in some counterirritants), or vitamins D or K_3 (menadione).[382-385] Use of aminoglycosides, particularly gentamicin, is a common cause of equine ATN.[386] Aminoglycoside toxicity is a result of damage to proximal tubular epithelial cells that is mediated by impaired cell organelle function. Administration of potentially nephrotoxic agents such as NSAIDs, polymyxin B, or furosemide (which can exacerbate hypovolemia) can increase the risk of aminoglycoside nephrotoxicity.

Myoglobinuria and hemoglobinuria have been associated with the development of ARF in horses (pigmentary nephropathy).[387] Myoglobinuric nephrosis can follow exertional rhabdomyolysis, heat stroke, or extensive crush injuries. Causes of intravascular hemolysis and hemoglobinuria include incompatible blood transfusions, immune-mediated hemolytic anemia, fulminant hepatic failure, and toxicosis from ingesting onions (*Allium* spp.) or withered red maple leaves *(Acer rubrum)*. Although the mechanism of pigment-induced renal injury is still not well understood, increased hydroxyl radical formation associated with reduction of ferrous iron compounds and tubular obstruction by casts of heme proteins are likely contributing factors. The fact that pigmentary nephropathy is uncommon in well-hydrated horses suggests a possible link to renal perfusion. Myoglobin and hemoglobin have also been suggested to induce renal vasoconstriction.

Acute interstitial nephritis often is not recognized but is believed usually to result from an allergic reaction to drugs such as β-lactam and sulfonamide antibiotics. Immune-mediated and embolic or ascending bacterial infections also may be associated with the condition, which is characterized by edema and inflammatory cell infiltration of the interstitium. Tubules frequently contain leukocytes and erythrocytes, which pass into the lumen through the disrupted tubular basement membrane.

Glomerulonephritis often is identified postmortem in aged horses[70,270] and apparently is most often immune mediated. Glomerulonephritis often results in subacute or nonoliguric renal failure. Although the condition is theoretically reversible with immunosuppressive agents, in horses such undertakings are usually impractical and rarely are tried for long.

Postrenal obstructive failure can develop with disease of the renal pelves, ureters, bladder, or urethra. The severity of renal failure depends on the extent of the obstruction. Frequently, problems are not recognized in horses until urine output is obviously reduced or renal function is impaired to the point that systemic problems are manifested. Although a neurogenic bladder can cause a functional obstruction, postrenal failure in horses most often results from intraluminal blockage by uroliths, which can cause obstruction anywhere in the urinary outflow tract.[388,389] Other possible intraluminal causes include neoplasia or stricture formation. Extraluminal obstructive lesions such as retroperitoneal tumors, adhesions, or bladder displacements also occasionally are associated with the development of postrenal failure.

PATHOPHYSIOLOGY

The pathophysiology of equine ARF has received little study, and the mechanisms at work are assumed essentially to be the same as those identified in experimental studies of other animal species. Several mechanisms have been demonstrated to be involved in the development of ARF, the actual pathogenesis being complex and depending somewhat on the cause of the disease, hydration status, and exposure to potential nephrotoxins. Multiple factors probably operate in different combinations at different times and in different nephrons. These different mechanisms are discussed next in relation to the type of failure with which they are associated (i.e., prerenal, intrinsic, and postrenal failure).

The pathophysiology of prerenal failure and ischemic ARF tends to involve the same processes. Toxins that cause ATN also share many pathophysiologic features with ischemic ARF.[205] The heterogeneity of intrarenal blood flow is an important factor in the development of this condition. The kidneys are particularly susceptible to ischemic and toxic injury because of their unique anatomic and physiologic features. Although they receive approximately 20% of the cardiac output, only about 10% to 20% of total renal blood flow reaches the medulla via the vasa recta. This low medullary blood flow is necessary to ensure a functional countercurrent mechanism in this region of the kidney; however, low blood flow also creates a large corticomedullary oxygen gradient and renders the renal medulla hypoxic and highly susceptible to ischemic injury. Conversely, the renal cortex receives 80% to 90% of total renal blood flow and is particularly susceptible to toxins.

Hypovolemia and dehydration trigger compensatory systemic and renal responses. The systemic responses include activation of the autonomic nervous system and renin-angiotensin system and release of antidiuretic hormone. Peripheral vasoconstriction is one of the effects of these responses. Renal responses to decreases in circulating blood volume have several phases. Initially, tubular reabsorption of sodium and water increases and is mediated by nerves and hormones. Reabsorption usually is associated with reduced clearance of urea and an associated increase in serum urea nitrogen concentration in the face of preservation of GFR, which in turn maintains plasma creatinine concentration in the normal range. More severe hypovolemia overcomes renal autoregulation responses, with renal blood flow being redistributed from the cortex to the medulla and GFR thus declining. The renal circulatory changes further enhance tubule solute reabsorption in the face of the decreased GFR. The net effect is production of small amounts of concentrated urine, a high urine-to-plasma creatinine ratio, and low fractional sodium clearance.[272]

A further reduction in renal blood flow results in a syndrome considered intermediate between the prerenal and intrinsic ischemic forms of ARF. Urine concentrating ability apparently is disrupted earlier than is sodium reabsorptive

capability, with the result that urine osmolality decreases and output increases, but fractional sodium clearance stays low. Patients may appear to be mildly polyuric.[390] With more severe or prolonged renal hypoperfusion and ischemic insult, urinary sodium and fractional sodium clearance start to increase, and the animal develops nonoliguric, and then oliguric, ARF. These changes are associated with progressively more severe tubular necrosis as the ARF progresses from prerenal to renal. The more severe the insult, the poorer is the prognosis.

Intrarenal vasoconstriction is caused by an imbalance between vasoconstrictive and vasodilating factors (systemic or local) that act on small renal vessels in particular. These potent vascular mediators include the vasodilator nitric oxide and the vasoconstrictor endothelin. Hypercalcemia is associated with increases in free calcium in the vascular smooth muscle and leads to enhanced vascular tone. Vasopressin and angiotensin II also have been shown to induce significant vasoconstriction under certain experimental conditions, as well as endotoxin and myoglobin. Some nephrotoxins (e.g., gentamicin, heavy metals, and radiographic contrast agents) can cause renal vasoconstriction in addition to direct toxic effects on the proximal tubules.[391-393]

Prostaglandins E_2 and I_2 are potent mediators that are responsible for a critical vasodilatory response in the face of reduced renal blood flow when activation of the renin-angiotensin system alone would result in further vasoconstriction. Increases in circulatory concentrations of angiotensin II stimulate synthesis of renal prostaglandins. Consequently, the vasoconstrictive effects of stimulating the renin-angiotensin system usually are blunted somewhat by concomitant increases in PGE_2 and PGI_2; however, in the face of increased angiotensin II and simultaneous inhibition of prostaglandin synthesis (e.g., because of NSAID administration), a significant increase in renal vascular resistance results.[394]

The reduction in GFR associated with reduced renal perfusion is associated with a number of mechanisms involving the glomeruli, vasculature, and tubules. In the initial phases, a reduction in glomerular capillary hydrostatic pressure is associated with a net drop in renal blood flow and a rise in renal vascular resistance. The latter phenomenon usually is associated with afferent arteriolar vasoconstriction and efferent arteriolar vasodilation and initially is reversible with volume expansion. Later (2 days or more), restoration of renal blood flow does not necessarily improve GFR, and a drop in renal blood flow is associated with a disproportionately greater drop in GFR.[395] Even when vasoconstriction is reversed, GFR may not improve; this reflects loss of renal blood flow autoregulation. This disproportionate reduction in GFR suggests a fall in the ultrafiltration coefficient of the glomeruli after a reduction in total filtering area. The mechanism for this is unclear but may be associated with increases in angiotensin II concentration because it is known to induce mesangial contraction and a drop in the ultrafiltration coefficient.[396]

In addition to the aforementioned vascular changes that can affect GFR, the possible effects on GFR in the juxtamedullary region of even mild changes in renal blood flow warrant specific mention. The glomeruli and adjacent straight portion of the proximal tubule and thick ascending limbs of the loops of Henle in this region are apparently particularly susceptible to hypoxia (i.e., ischemia) because of their high oxygen requirements. Swelling of endothelial and tubule cells after prolonged ischemia in this region results in increased vascular resistance and continued compromise of the medullary circulation, even when cortical renal blood flow has been restored.[205,397]

GFR also can be compromised significantly by obstruction of tubular lumina by casts of cellular debris, inflammatory cells, and endogenous pigments. Increased intratubular pressure decreases the net driving pressure for glomerular filtration in the same way that obstruction of the urinary outflow tract ultimately can lead to a lower GFR. One of the reasons that treatments to accelerate the solute excretion rate (e.g., furosemide, mannitol) may be therapeutic adjuncts is that they are believed to help disperse these luminal blockages.

Tubuloglomerular feedback is a regulatory mechanism that lowers GFR whenever solute (most notably NaCl) concentrations at the macula densa are increased. In ARF, impaired transport in the thick ascending limb of the loop of Henle in the context of preserved glomerular response to signals from the macula densa results in decreased GFR. This feedback is a normal protective mechanism mediated principally by the renin-angiotensin system, although prostaglandin, intracellular calcium, and adenosine may play a role in signal transmission or regulation. In essence, the mechanism serves to prevent massive fluid losses associated with a reduction of tubular reabsorptive capacity; however, in cases of renal hypoperfusion and ischemia, the effect is the opposite, because basically the feedback mechanism exacerbates the effects of already reduced renal blood flow.

Proximal tubule cells undergo morphologic changes early in cases of ischemia. They lose their brush borders and polarity and the integrity of their tight junctions is disrupted, probably after alterations in the actin and microtubular cytoskeletons.[398,399] Under these conditions, the glomerular filtrate is able to leap back to the peritubular circulation, thus reducing the net (or effective) GFR. This mechanism is thought to be an important source of GFR reduction only in more severe cases of ischemia or nephrotoxin exposure.

Tubule cells involved in solute reabsorption have a high metabolic rate and a high demand for oxygen. The corticomedullary oxygen gradient makes these cells vulnerable to the effects of hypoxia and ischemia, the thick ascending limb of the loop of Henle in the outer medulla being most susceptible.[400] Early in ischemic and toxic ARF, decreases in ATP and adenosine diphosphate tissue levels occur with associated elevations in adenosine monophosphate and inorganic phosphate concentrations. Much of the adenosine monophosphate is broken down further to adenosine and then to xanthine. Adenosine is a potent constrictor of cortical blood flow and probably enhances the effect of the tubuloglomerular feedback system. Depletion of ATP in tubular cells inhibits cell volume regulation, and the resultant swelling probably contributes to luminal obstruction and increased vascular resistance. Redistribution of Na^+-K^+-ATPase from the basolateral to the apical membrane of the tubule cells reduces the ability of the cells to extrude sodium into the peritubular fluid and circulation.[401] Redistribution of integrins to the apical surface contributes to the breakdown of tight junctions.[402] Depletion of ATP in tubule cells also leads to an increase in cytosolic calcium concentration. In addition to being a vasoconstrictor, calcium activates proteases and phospholipases, interferes with mitochondrial energy metabolism, and can break down the cytoskeleton.[380,403] Administration of calcium channel blockers has helped ameliorate ARF in some experimental situations.[404]

Reperfusion of renal tissue after a period of ischemia is associated with rapid production of oxygen free radicals and with significant tissue damage. Xanthines, neutrophils, phospholipase A_2, mixed-function oxidases, and mitochondrial

electron transport are associated with the production of these oxidants.[405] Phospholipase A_2 hydrolyzes phospholipids in cell and mitochondrial membranes to free fatty acids and lysophospholipids and produces arachidonic acid. Arachidonate in turn is converted to eicosanoids that are vasoconstricting and chemotactic for neutrophils.[406] Cell membranes are particularly susceptible to phospholipase activity after reperfusion. Reperfusion injury is a major consideration in transplant surgery; however, the role it plays in the pathogenesis of prerenal and ischemic ARF is not clear.

Many of the cellular biochemical and structural changes that occur with ischemic ARF are also important parts of the pathogenesis of ATN associated with exposure to nephrotoxins. Toxin-associated dysfunction and necrosis of cells result in increased tubular pressure and a decreased glomerular capillary hydrostatic pressure gradient. Loss of reabsorptive capability triggers tubuloglomerular feedback and further reduces GFR by lowering renal blood flow after vasoconstriction and a decrease in the glomerular ultrafiltration coefficient. Transepithelial back leak of solutes into the circulation further interferes with the excretory function of the kidneys.

Acute Tubular Necrosis

Many agents are recognized to have potential nephrotoxic effects. Aminoglycoside-induced renal toxicity results from accumulation of these antibiotics within the renal cortex. Streptomycin is the least nephrotoxic of the aminoglycosides, whereas gentamicin and kanamycin are intermediate nephrotoxins. Neomycin is the most nephrotoxic. Most cases of aminoglycoside toxicity are associated with conditions that cause reduced renal perfusion because the healthy kidney usually can tolerate some degree of aminoglycoside overdosing, especially with every-24-hour administration, which allows tubule cells time to recover as concentrations decrease. After being freely filtered at the glomerulus, aminoglycosides bind to polyphosphoinositides on the brush border of proximal tubules and subsequently are reabsorbed by pinocytosis. Accumulation of these antibiotics interferes with lysosomal, mitochondrial, and Na^+-K^+-ATPase function by inhibiting phospholipase A activity. Binding to the brush border is saturable, with the result that sustained exposure of the proximal cells to the drug over time (as with more frequent dosing) results in greater accumulation of the drug and increased nephrotoxicity. High trough concentrations are more nephrotoxic than high peak concentrations because tubule cells are exposed to higher aminoglycoside concentrations over a longer period of time. As a result, once-daily dosing may attenuate the risk of nephrotoxicosis while maintaining or improving therapeutic efficacy (because it results in higher peak concentrations and postantibiotic effect).[407,408] Calcium in the glomerular filtrate competitively inhibits aminoglycoside binding in the proximal convoluted tubule. Experimentally, it has been demonstrated that both intravenous calcium supplementation (calcium gluconate 20 mg/kg q 12 h)[409] and increased dietary calcium intake through alfalfa hay[410] had a protective effect against experimental gentamicin toxicity. Many mild cases of aminoglycoside toxicity notably are associated with nonoliguric ARF and therefore may go unrecognized in horses.

Some cephalosporins, such as cephaloridine, have considerable nephrotoxic potential. These agents cause necrosis because of mitochondrial toxicity after intracellular accumulation of the antibiotic.[411]

Polymyxin B is a polypeptide antibiotic that can bind endotoxin. It is often administered to horses at risk for ARF with colitis, endotoxemia, NSAID administration, and dehydration. In rats, renal histopathologic lesions of experimental polymyxin B toxicity include proximal tubular epithelial cell cytoplasmic vacuolization and focal necrosis, tubular dilation, and interstitial edema.[412] More frequent administration resulted in a quicker onset of renal toxicity and higher renal concentrations than once-daily dosing, which suggested an active uptake mechanism for polymyxin B in proximal tubule cells. Polymyxin B (6000 IU/kg IV q 8 h) did not significantly affect the urinary GGT/Cr ratio and appeared safe in healthy horses.[413]

Bisphosphonates may be administered to horses with osteopenic disease or other orthopedic conditions, and these drugs may sometimes cause acute tubular necrosis. Renal function should be evaluated in horses treated with bisphosphonates that develop clinical signs of ARF after treatment, and perhaps renal function should be routinely assessed before and after treatment. Higher doses and rapid intravenous administration or concurrent treatment with NSAIDs have been associated with a higher risk for ARF.[414]

Not all cases of ATN are caused by direct toxic changes in tubule cells. For instance, whether myoglobin and hemoglobin are truly nephrotoxic is still debatable. The principal characteristics of pigmentary nephropathy caused by these agents are tubule obstruction and reduced renal blood flow (caused by direct vasoconstrictor effects). Whether obstruction is physical, caused by pigment accumulation, or the aggregation of sloughed cells is not clear. Myoglobin tends to be associated with nephropathy more frequently than hemoglobin. Patients often quickly become oliguric, presumably because of the widespread tubule obstruction.

Acute Interstitial Nephritis

Distinguishing between drug-induced ATN and acute interstitial nephritis can be difficult, and the latter rarely is diagnosed in horses. Making a distinction may be a particular problem when continued use of antibiotics is indicated. With ATN, one may alter the dosing regimen by reducing the dose or prolonging the treatment interval, whereas with interstitial nephritis, short-term corticosteroid therapy is often of benefit in human beings. Interstitial nephritis often is marked by eosinophiluria and eosinophilia and is more likely to be associated with the presence of red blood cells than is ATN. The exact immune mechanism by which interstitial nephritis develops is unclear, although it is thought most likely to be caused by delayed cell-mediated hypersensitivity or the presence of antitubule basement membrane antibodies.[415] The prognosis is usually grave in horses with acute interstitial nephritis.

Acute Glomerulonephritis

Acute glomerular nephropathy is rare in horses; however, when it occurs, it usually is manifested by nephrotic syndrome, although hematuria and oliguria sometimes are apparent. Deposits of γ-globulin and complement are found along the basement membrane (global form) or in the mesangial area (mesangioproliferative form).[416] Group C streptococcal antigens have been identified along with equine glomerulonephritis, and equine infectious anemia (EIA) viral antigen-antibody complexes have been recognized in the glomeruli of horses that were not in renal failure.[270,417] Deposition of immune complexes activates the complement cascade. Formation of

C_3b and C_5a causes platelet aggregation and attracts neutrophils. Tissue damage results from deposition of complement per se and from inflammation associated with neutrophil activation and release of reactive oxygen radicals, proteases, elastases, and other lysozymes. These enzymes, plus platelet-activating factor and leukotriene B_4, increase vascular permeability and upregulate expression of adhesion molecules, thus promoting further inflammation.[406] Severe reduction in GFR results from large drops in the glomerular permeability coefficient, which is associated with widening of Bowman's spaces after inflammation and deposition of immune complexes.

Basically, increases in ureteral pressure for any reason result in GFR reduction because of a drop in the glomerular capillary hydrostatic pressure gradient, some tubular backleaking, a decrease in the glomerular permeability coefficient, and ultimately, a reduction in renal blood flow. (See previous discussion of the pathophysiology of postrenal ARF.)

CLINICAL SIGNS

In most horses with hemodynamically mediated (i.e., prerenal, ischemic, dehydration) ARF, clinical signs are usually more pronounced from the primary problem, such as acute colic or enterocolitis, sepsis, coagulopathies, rhabdomyolysis, or heavy metal poisoning, rather than renal dysfunction. Therefore the predominant clinical signs are often dehydration (with or without diarrhea), depression, and anorexia. Other signs can include tachycardia, hyperemic mucous membranes, pyrexia, mild abdominal pain, and laminitis. Because clinical signs usually relate to the inciting problem, ARF may not be suspected or detected unless the veterinarian specifically evaluates renal function as part of the workup for a more obvious disease. In general, the clinical manifestations of ARF reflect the systemic effects of toxic substances usually excreted in the urine (i.e., azotemia generally is reflected by anorexia and depression), urinary tract dysfunction, and derangements of fluid, electrolyte, and acid-base balance. One may observe signs of encephalopathy or hyperlipemia in horses with severe azotemia.

Although oliguria is considered the hallmark of ARF, urine production in horses varies. Oliguria frequently occurs in the early stages of hemodynamically mediated ARF and is the most frequently reported clinical sign that is related directly to urinary tract dysfunction. As outlined in the section on pathophysiology, however, nonoliguric and polyuric stages of prerenal and intrinsic ARF also can be associated with renal hypoperfusion. Anuria is rare. Nonoliguric or polyuric ARF also can be associated with exposure to nephrotoxins (ATN), and polyuria is common during the recovery phase of ARF, regardless of its cause. The magnitude of azotemia tends to be lower in nonoliguric than in oliguric ARF, possibly indicating less severe damage in the former condition. Similarly, nonoliguric ARF is associated with a more favorable prognosis.

Patients with ARF often are treated initially with large volumes of intravenous or oral fluids for the primary disease to restore plasma volume. In these cases, oliguria may progress to polyuria. When significant renal damage has been sustained, persistence of oliguria in the face of fluid administration usually is manifested as failure to produce a significant volume of urine in response to fluid therapy. The degree of change of azotemia also is minimal in the initial 24 to 36 hours of treatment. If one does not monitor these patients carefully, then fluid retention may lead to development of subcutaneous and pulmonary edema. Hematocrit, total protein concentration, and renal indices should be frequently measured to objectively assess response to fluid therapy and renal function. Soft feces caused by fluid retention also may be apparent in patients with oliguric ARF.

Postrenal or obstructive uropathy usually is characterized by mild to severe abdominal pain and pollakiuria and stranguria (see Obstructive Disease of the Urinary Tract).

DIAGNOSIS

Increases in plasma urea nitrogen and creatinine concentrations (i.e., azotemia) are frequently the initial findings that suggest compromised renal function. Azotemia simply reflects a reduction in GFR; it has almost no differential diagnostic value. After establishing recent development of azotemia, the equine internist must proceed systematically to differentiate among six possible syndromes associated with ARF: (1) prerenal ARF, (2) ischemic ARF, (3) ATN, (4) acute interstitial nephritis, (5) acute glomerulonephritis, and (6) obstructive (postrenal) ARF. A useful way to go about this is first to try to rule out prerenal and postrenal ARF. If this is possible, then the patient must have a type of intrinsic ARF; therefore one can direct further diagnostic efforts to identifying the disease subtypes.

As described in greater detail later (see Urolithiasis), the diagnosis of postrenal obstructive disease usually is based on a combination of clinical signs, history, and the results of rectal palpation, ultrasonography, and urinary tract endoscopy. The frequency and volume of urination can vary with these cases, and total obstruction causes anuria. Salt and water reabsorption often are impaired as the problem persists, which results in hyponatremia (i.e., plasma sodium concentration in the low-normal range). Although the mechanisms for this sodium wasting are not completely clear, it apparently is related to impaired function of the ascending limb of the loop of Henle and an increased medullary blood flow after prostaglandin release. Both of these events greatly diminish the magnitude and effect of the medullary countercurrent concentrating mechanisms. Prostaglandin also directly inhibits the effect of antidiuretic hormone.[418]

Measurement of urine specific gravity in the azotemic horse is a commonly practiced means of detecting prerenal ARF. In these cases, urine specific gravity is usually greater than 1.025 and often as high as 1.055. In human beings, one can use a number of reliable indexes to differentiate renal azotemia from prerenal azotemia[419-421] that are based on urine osmolality and the ratio of urine-to-plasma osmolality (U/P_{osm}), urine sodium concentration, and fractional sodium clearance and ratios of urine-to-plasma urea concentrations (U/P_{UN}) and urine-to-plasma Cr concentrations (U/P_{Cr}). Fractional sodium clearance in particular is a good indicator of solute reabsorption and proximal tubule function, whereas U/P_{Cr}, and to a lesser extent U/P_{UN}, are useful indexes of the ability of the tubules to reabsorb water. The utility of these indexes in horses also has been investigated and has been shown to have considerable differential diagnostic value.[272] Although these tests are discriminatory, some degree of overlap occurs between them respecting prerenal and parenchymal or intrinsic problems. One also must bear in mind that these indexes do not allow differentiation between intrinsic and postrenal disease. A number of horses with prerenal (nonoliguric) ARF have urine osmolality less than 360 mOsm. A small percentage

of horses in prerenal ARF have U/P_{Cr} less than 30 (usually greater than 50) and fractional sodium clearance greater than 0.80% (usually less than 0.50%), most likely because of the unrecognized existence of tubule damage before volume depletion or because of a natriuretic effect of some treatments such as diuretics or intravenous fluids. Natriuresis is particularly likely to be induced with bicarbonate administration because sodium cations are lost with unreabsorbed bicarbonate anions. In these cases, the determination of the fractional chloride clearance may be a better indicator of the response of the kidney to hypoperfusion.

No parameter reliably differentiates between prerenal and ischemic ARF because of the pathophysiologic continuum between these diseases. At one end is reduced GFR with preserved tubule function and concentrating mechanisms. This form is readily reversible with appropriate therapy. Additional or more prolonged decreases in GFR lead to disturbances in tubule function and slower reversal of damage until the other end of the spectrum is reached, in which complete and irreversible loss of renal function occurs. Assessment of urine specific gravity before initiation of fluid therapy is helpful in differentiating prerenal failure from renal failure. As normally functioning kidneys would preserve salt and water maximally in response to a transient decrease in renal blood flow with prerenal failure, so urine specific gravity and osmolality are greater than the values associated with serum, whereas the urine produced by horses with intrinsic ARF is often isosthenuric (specific gravity less than 1.020). In a clinical situation, assessment of the response to fluid therapy is the most practical way to differentiate prerenal failure from intrinsic forms of ARF. Azotemia caused by prerenal problems should resolve quickly with replacement of fluid deficits and restoration of renal perfusion. In prerenal failure, volume repletion also should restore renal function, with the result that the magnitude of azotemia should decrease by 50% or more during the first day of therapy. In contrast, fluid therapy usually does not lead to prompt resolution of azotemia associated with intrinsic problems. Application of the measurement of U/P_{Cr} and U/P_{UN} ratios is limited to use on urine samples collected before initiation of fluid therapy or the first urine sample voided after fluid therapy has been started.

In prerenal ARF, electrolyte and acid-base abnormalities generally reflect problems caused by a primary disease (e.g., enterocolitis, colic, blood loss). Most frequently, horses are mildly acidotic, hyponatremic, and hypochloremic. Plasma concentrations of potassium and calcium vary according to what disease is causing renal hypoperfusion. Potassium concentration also is affected to some extent by urine output and feed intake, hyperkalemia being most common in association with oliguria and anuria.

The technique for biopsy of the left kidney has been well described.[369] The main indication of kidney biopsy is to help differentiate between types of intrinsic renal diseases when one thinks that this distinction will have therapeutic and prognostic relevance. One often can diagnose ischemic failure and ATN without biopsy. The primary complication associated with the biopsy procedure is renal hemorrhage, which can be severe. Ultrasonographic guidance and use of a spring-loaded biopsy instrument may reduce the risk of complications. Ultrasonography also allows biopsy of the right kidney. As the knowledge of equine renal physiology and pathology improves and advances in molecular genetics lead to techniques that supplant or supplement standard histopathologic methods, the diagnostic usefulness of renal biopsy may increase. For example, biopsy results may dictate the use of immunosuppressive agents in the treatment of some forms of renal parenchymal diseases (e.g., interstitial nephritis).

Identification of subtypes of intrinsic ARF often depends on analysis of urine and urine sediment. The history of exposure to ischemic insults or potential nephrotoxins such as aminoglycosides certainly helps in this regard, but determination of the severity of the condition and its prognosis still generally relies on the analysis of urine. Ischemic tubule disease is similar to ATN. In both cases a slight to moderate proteinuria with specific gravities usually less than 1.020 and urine osmolality less than 350 mOsm occur. Fractional sodium clearance is nearly always greater than 1.0, regardless of urine output. Granular casts frequently are visible, particularly with ATN. Enzymuria and phosphaturia are frequently prominent early in the course of ATN.[295,422] Plasma sodium and chloride concentrations are usually low. Plasma concentrations of calcium and inorganic phosphate vary greatly: increases, decreases, and normal values are possible, depending on the diet of the horse, the nephrotoxin, and the location and severity of damage to the nephron.[295,417,422]

One must remember that GFR is reduced in cases of ATN and is most likely caused by the increase in presentation of sodium and chloride at the macula densa after dysfunction of proximal sodium reabsorption. Stimulation of the macula densa results in release of renin and local production of angiotensin II, thus increasing renal vascular resistance and lowering renal blood flow. The low GFR may mask the absolute magnitude of the damage in tubule function; however, if one studies renal sodium reabsorption carefully over time in cases of ATN, this defect appears to improve more rapidly than that of GFR.[423]

Acute interstitial nephritis often is not recognized in horses; however, in a study conducted about 40 years ago, the condition was diagnosed in approximately one eighth of all human patients who needed renal biopsy for diagnosis of unexplained ARF.[424]

Edema and diffuse or focal patches of interstitial inflammation characterize the disease. In human medicine, the numbers of drugs, toxins, and infectious agents known to induce this disease are growing. No reason exists to believe that similar agents are not capable of causing the same disease in equids. Inflammatory cells surround the tubules and can move between epithelial cells into the tubule lumina. As a result, white blood cell casts are common. The leukocytes are also capable of disrupting the tubule basement membrane, making cell repair much less likely; this is an important distinguishing feature between ATN and interstitial nephritis. With the former disease the basement membrane usually stays intact. Reduction of GFR and azotemia probably result from the interstitial edema, intratubular obstruction, and release of vasoactive agents.

Acute interstitial nephritis and ATN have similar fractional sodium clearance, U/P_{Cr}, and U/P_{osm} values; however, the urine sediment may be different for each disease. Sterile pyuria and microscopic hematuria commonly occur with interstitial nephritis, although red blood cell casts are rare. Mild proteinuria and eosinophiluria are also common. Eosinophiluria generally seems to be limited to renal interstitial disease, mainly drug-induced interstitial nephritis but also chronic pyelonephritis and systemic lupus erythematosus.[425,426] Eosinophiluria may or may not be accompanied by

eosinophilia. Eosinophils in equine urine should be easy to observe using Wright's stain, given the alkaline nature of the fluid. Occasionally, fevers are believed to be associated with the development of interstitial nephritis. This clinical sign is relatively nonspecific and might be misleading if the animal is being treated with antibiotics for an infection. What one might see with close monitoring of the patient is an initial reduction or resolution of the fever after the onset of antimicrobial therapy followed by recurrence of the fever and development of azotemia. If this happens, then acute interstitial nephritis should be on the rule-out list.

The nephrotic syndrome usually characterizes acute glomerulonephritis. Proteinuria is moderate to severe, and the urine usually is concentrated. Urine osmolality and U/P_{osm} are comparable with the values with prerenal ARF and are higher than those values normally seen in ATN. Urinary sodium concentration and fractional sodium clearance tend to be much lower than with other intrinsic renal and postrenal causes of ARF. The ratios of U/P_{UN} and U/P_{Cr} are often similar to those associated with prerenal failure and higher than those in ischemic, tubular, interstitial, or postrenal disease. Renal tubular secretion of creatinine is increased in glomerulonephritis, with the result that creatinine concentrations may not rise quickly and urea nitrogen-to-creatinine ratios stay high, as they frequently do with prerenal problems. Therefore although urinary indexes should make possible differentiation of acute glomerulonephritis from other parenchymal and postrenal diseases, they overlap much with those associated with prerenal ARF. One is going to differentiate ARF caused by acute glomerulonephritis from that caused by hypoperfusion based on the significant proteinuria and red blood cell numbers usually associated with the former disease. Erythrocyte casts are more common with glomerulonephritis than with other intrinsic causes of ARF. Some causes of postrenal ARF also may manifest red blood cell casts, but the proteinuria is not normally as great in those situations.

TREATMENT

Initially, treatment of horses with ARF should focus on reversing the inciting or underlying cause and correcting fluid and electrolyte imbalances. Early identification of patients at risk and prevention of problems by rapid restoration and accurate maintenance of intravascular fluid volume, glomerular filtration, and urine production (with fluids and possibly diuretics) is obviously preferable to treatment. The importance of prevention cannot be overstated. One should correct initial fluid deficits over the first 6 to 12 hours of treatment. Physiologic saline or a balanced electrolyte solution is the fluid of choice unless the patient is hypernatremic, as may be the case in prerenal ARF or acute glomerulonephritis. In the event of hypernatremia, solutions of 0.45% NaCl and 2.5% dextrose are recommended. The addition of 50 to 100 g of dextrose per liter of fluid to saline or polyionic fluids helps address the caloric needs of anorectic horses. If fluid administration is begun early in the course of the disease or if the problem is not severe, then diuresis should result. When this occurs, one should maintain intravenous fluid therapy at least at twice maintenance level (up to 100 mL/kg/day or more) until serum creatinine concentration decreases dramatically. One then reduces the rate of fluid administration to 40 to 50 mL/kg/day until the creatinine concentration is normal or the horse is eating and drinking adequately. The horse must be carefully observed to ensure that it urinates appropriately in response to fluid therapy and does not develop edema and renal indices decrease as desired.

In the event that the horse remains oliguric 10 to 12 hours after starting fluid therapy, administration of dopamine in 5% dextrose slowly (3–5 μg/kg/min) may improve renal blood flow and urine output. One should monitor blood pressure during dopamine infusion because the drug can induce significant hypertension. Administration of dopamine should be discontinued if blood pressure starts to rise. The infusion can be restarted at a lower rate when blood pressure has returned to normal. Blood pressure also may increase because of overhydration of oliguric patients. Regular monitoring of body weight, hematocrit, and serum total protein concentration, central venous pressure, and lung sounds is important to prevent problems caused by overhydration.

The use of diuretic agents such as mannitol and loop diuretics to treat ARF is controversial. Furosemide and ethacrynic acid are the loop diuretics most commonly used. Furosemide is a particularly potent short-acting agent that acts by blocking the $Na/K/2Cl^-$ cotransporter in the ascending limb of the loop of Henle. In addition to promoting diuresis, cotransporter inhibition also may protect these tubule cells by reducing their metabolic rate and thus oxygen demand in the face of limited oxygen availability after hypoperfusion. To be effective, loop diuretics must gain access to the tubule lumina. Therefore loop diuretics may be of limited value in prerenal or ischemic problems, although the potential protective effect of furosemide on the especially vulnerable cells of the thick ascending limb may warrant its administration. Loop diuretic administration also may exacerbate or induce volume depletion in cases of ARF that are characterized by isosthenuria or polyuria (e.g., early ATN), thus making the condition worse and rendering the patient more susceptible to the effects of nephrotoxins such as gentamicin. Loop diuretics appear to be most beneficial when used in cases characterized by tubule obstruction (e.g., pigmenturia) because the diuretic-induced increase in solute retention apparently helps flush these blockages and casts from the tubules. In any case, urine production should first be stimulated with volume replacement rather than diuretic administration.

Although loop diuretics have no direct effect on GFR, 20% mannitol (0.25–1.0 g/kg) given over 15 to 20 minutes may help combat oliguria by increasing renal blood flow and GFR. The increase occurs after reductions in plasma protein concentration and oncotic pressure. These changes in turn result from the systemic effects of the increase in intravascular osmolality induced by mannitol. Mannitol also may induce synthesis of the vasodilator PGE_2 and release of atrial natriuretic peptide, which also would increase renal blood flow and GFR. Once filtered, it also acts as an osmotic diuretic, thus decreasing urine solute concentration and increasing urine volume. Consequently, mannitol also can be effective in treating conditions characterized by tubular obstruction and swelling of tubule cells.

Hyperkalemia is uncommon in equine ARF, except in some postrenal cases. When present, hyperkalemia is usually mild and responds to administration of potassium-free intravenous fluids. When hyperkalemia (greater than 6.5 mEq/L) persists, correction of any associated acidosis with sodium bicarbonate or administration of glucose (up to 10% solution), or both, usually helps. In the worst or most refractory cases, insulin may be necessary. Potassium supplementation (KCl 20–40

mEq/L IV) may be necessary during the polyuric phase of recovery from ARF.

Calcium metabolism often is disrupted in cases of equine ARF, with hypocalcemia and hyperphosphatemia or hypercalcemia and hypophosphatemia having been reported variously. Hypercalcemia usually resolves with a switch to a grass or grass-hay die (avoid legumes) and time. Hypocalcemia probably occurs for a number of reasons, including skeletal resistance to parathyroid hormone during the early stages of ARF and deficiency of 1,25-dihydroxycholecalciferol, which results from downregulation of renal 1,25-hydroxylase with hyperphosphatemia, dysfunction of this enzyme after renal parenchymal damage, or both.[422,427] Hypoalbuminemia and enhanced deposition of calcium in injured tissues, as can occur in cases of rhabdomyolysis, are other factors to consider. Administration of a calcium salt with intravenous fluid therapy protocol and feeding legumes such as alfalfa are usually sufficient to correct hypocalcemia.

Severe uremia often decreases erythrocyte life span and induces platelet dysfunction. Consequently, anemia (possibly also caused by decreased production of erythropoietin) and bleeding tendencies may be associated with ARF. These conditions may require symptomatic treatment with whole blood transfusions.

Because aminoglycoside toxicity is one of the more commonly recognized causes of equine ARF,[265,386,428] continued aminoglycoside use in horses with ARF warrants specific attention. Gentamicin and amikacin are the aminoglycoside antibiotics most often used in equine practice, and their pharmacokinetics have been well studied in healthy animals. Large interindividual and age variations occur in the volume of distribution and clearance of these drugs in normal horses, and this variability is even greater in diseased horses. Therefore when a patient with ARF requires aminoglycoside antibiotics, monitoring serum trough concentrations of the drug to adjust the dosing interval or dose is strongly indicated, because it provides the best protection against exacerbating renal damage. Therapeutic drug monitoring of aminoglycoside concentration can be helpful to maintain desired concentration, prevent toxicity, and assess GFR. Increased trough concentration of aminoglycosides can indicate reduced GFR because their excretion is dependent on glomerular filtration. Renal dysfunction associated with aminoglycoside toxicity usually is indicated by an increase of at least 0.3 mg/dL in creatinine concentration. When this occurs, discontinuing therapy until renal indices have normalized, prolonging the dosing interval, or reducing the dose should be considered. One must remember that changes in urine sediment (casts) and development of enzymuria, mild proteinuria, glucosuria, and decreased urine-concentrating ability develop a number of days before creatinine concentration increases and are good indicators of the development of aminoglycoside nephrotoxicity. One usually can address the problem successfully by maintaining or increasing intravenous fluid therapy to guard against renal hypoperfusion and by appropriately adjusting the dosing interval with the antibiotic.

Intermittent hemodialysis has been used to successfully treat oxytetracycline toxicosis and ARF in a 4-day-old foal.[429] Oxytetracycline can be directly nephrotoxic at high doses (as for contracted tendons in neonates), and oxidation of oxytetracycline can result in the formation of nephrotoxic compounds. Hemodialysis is an excellent ancillary therapy in cases of toxin-induced ARF, if feasible. In 2013 Wong and colleagues[430] reported a technique of renal replacement therapy in healthy adult horses using a commercially available machine for humans. Another dialysis technique that may be more practical is continuous-flow peritoneal dialysis.[431] Continuous-flow peritoneal dialysis involves the placement of two indwelling catheters in the flank (ingress) and ventral abdomen (egress) and continuous infusion of dialysate solution. The use of two catheters and continuous flow of the dialysate solution, rather than a single ventral abdominal catheter and intermittent infusion, may both increase the surface area of the peritoneum for dialysis and have fewer complications.

PROGNOSIS

The prognosis for equine ARF depends on the underlying cause, the duration of renal failure, the response to initial treatment, and the development of secondary complications such as diarrhea, thrombophlebitis, and laminitis. Generally speaking, the duration of ARF before beginning therapy is the most important determinant of prognosis. Of the primary causes of ARF, severe ischemic failure and acute interstitial nephritis probably carry the worst prognosis in horses. ARF of any cause, however, should be associated with a reduced or poor prognosis in the event that early interruption of the pathophysiologic events leading to ARF is not achieved or that the animal displays prolonged oliguria or anuria (longer than 12 hours) after institution of vigorous therapy. Most cases of postrenal ARF carry a favorable prognosis and are reversible, provided the initiating disease is treated successfully. When discussing prognosis with horse owners, practitioners always must bear in mind that a successful outcome is not always associated with complete return of normal function. Many horses live long after a bout of ARF but never fully regain the ability to concentrate urine as well as they did before the disease; in other cases, they remain constantly polyuric. ATN, after nephrotoxicity in particular, carries a favorable prognosis when tubule basement membranes remain intact.

Chronic Kidney Disease

Harold C. Schott II

PREDISPOSING CONDITIONS

Chronic kidney disease (CKD or chronic renal failure) is recognized infrequently in horses. In dogs and cats, the prevalence of CKD has been reported to be 0.9% and 1.6%, respectively,[432] whereas the Veterinary Medical Database at Purdue University reported that only 515 of 442,535 horses admitted to participating veterinary teaching hospitals during the years 1964 to 1996 had CKD (prevalence of 0.12%). In actuality, this may be an underestimate, because when a diagnosis of CKD is established for a horse, it likely may be destroyed without presentation to a veterinary teaching hospital. As in dogs and cats, CKD appears to be a greater problem in older horses (the prevalence increased to 0.23% in horses older than 15 years). A 0.51% prevalence in intact males older than 15 years of age also suggests that stallions may be at greater risk.

Although the clinical syndrome of CKD is uncommon, one widely cited abattoir study revealed that 16% of 45 horses examined had glomerular lesions on light microscopic

examination and 42% (22 of 53 horses examined) exhibited deposits of immunoglobulin or complement with immunofluorescence staining of tissue samples.[270] Although these findings suggest that as many as one third of horses may have microscopic evidence of renal disease, only one of the horses in this survey exhibited signs of CKD. This disparity can be attributed to the large renal reserve capacity, because clinical signs of renal failure do not become apparent until two thirds to three fourths of functional nephrons have been lost.[254] Although this rule of thumb is based on studies of partially nephrectomized laboratory animals, support for a similar renal reserve capacity in horses is found in several clinical reports of unilateral nephrectomy used successfully to manage disorders of the upper urinary tract.[41,74,255-257] In addition, after experimental unilateral nephrectomy in ponies[258] and horses,[259] renal function remained within normal ranges, and the animals maintained body weight.

Renal disorders leading to CKD may be congenital or acquired. In horses younger than 5 years of age with a history that does not include an event that might have been complicated by ARF, one should suspect a congenital renal disorder: renal agenesis, hypoplasia, dysplasia, or polycystic kidney disease.[27,28,32,34-40,48-51] Although each of these congenital abnormalities is occasionally recognized, acquired disease consequent to glomerular or tubular injury is more often the cause of CKD in horses.[70,95,260,266,433,434] Acquired disease is usually insidious in onset, and renal injury may have started years earlier. Thus identifying the cause of CKD is challenging because many horses have evidence of advanced glomerular and tubular disease, termed *end-stage kidney disease (ESKD),* by the time clinical signs of CKD develop. Nevertheless, knowing the various causes of CKD affords a better overall understanding of CKD in horses.

Glomerulonephritis

Glomerular injury is a common precipitant of renal insufficiency and CKD in horses. Although immune-mediated glomerular injury is implicated most often in glomerulonephritis, glomerular integrity can be disrupted by a number of unrelated disease processes, including ischemia, toxic insults, and infection.[435] These mechanisms usually lead to significant vascular and tubulointerstitial changes in addition to glomerular injury. Thus the designation glomerulonephritis typically is reserved to denote renal disease of which immune-mediated glomerular damage is suspected to be the initiating factor in the development of renal failure. Until the past decade, glomerulonephritis was considered a rare cause of CKD in domestic animals, and interstitial nephritis was implicated more often.[436-438] Refinement of histologic examination techniques with immunofluorescence staining and electron microscopy examination of renal tissues has led to increased recognition of subclinical and clinically significant glomerulonephritis.[438]

A brief review of the subgross anatomy of the glomerulus sheds light on the pathophysiology of glomerulonephritis. The renal corpuscle, or glomerulus, is comprised of a tuft of glomerular capillaries surrounded by epithelial cells that line Bowman's capsule. The root that supports the pedicle of the glomerular capillary network is similar to the mesenteric root that supports the intestinal tract, and Bowman's capsule is analogous to the peritoneum (Fig. 14.18A). On a microscopic level, components of the glomerulus include capillary endothelial cells, mesangium (cells and matrix), glomerular basement membrane (GBM), and visceral epithelial cells. At the vascular

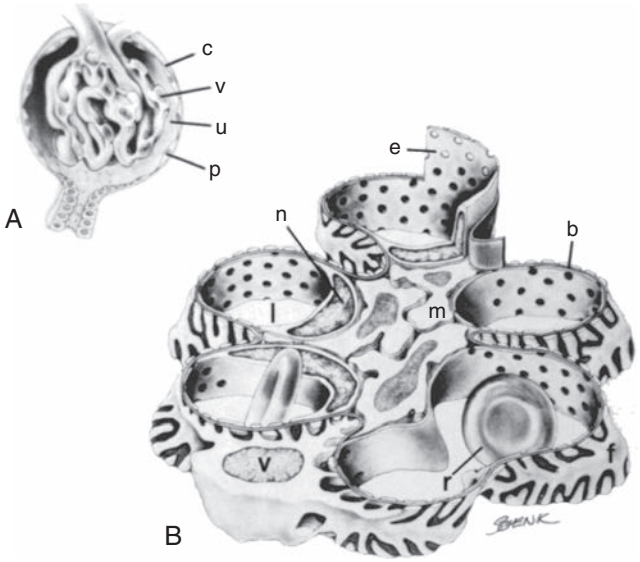


FIG. **14.18** Subgross anatomy of a renal corpuscle. **A,** A renal corpuscle with a tuft of glomerular capillaries surrounded by Bowman's capsule *(**c**)* shows visceral *(**v**)* and parietal *(**p**)* epithelial cells separated by the urinary space *(**u**)*. **B,** Cross-section of a portion of a glomerulus shows the nucleus *(**n**)* and fenestrations *(**e**)* of capillary endothelial cells, the glomerular basement membrane (GBM) *(**b**)*, mesangial cells *(**m**)* separated by mesangial matrix, and the nucleus *(**v**)* of a visceral epithelial cell. A red blood cell (RBC) *(**r**)* is in the lumen *(**l**)* of one of the glomerular capillaries. (From Osborne CA, Hammer RF, Stevens JB, et al.: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med.* 21:207, 1977.)

pedicle, the latter become contiguous with parietal epithelial cells that line Bowman's space, much as mesothelial cells covering bowel serosa and mesentery become contiguous with the peritoneum at the mesenteric root.[435] Glomerular capillary endothelial cells are unique in that they are fenestrated with pores that are the initial barrier for passage of blood components into the urinary space (Fig. 14.18B). The mesangium, which lies between endothelial and epithelial cells, is the support structure of the glomerular capillaries and is analogous to the mesenteric tissue supporting bowel. Mesangial cells are a component of the reticuloendothelial system and phagocytize macromolecular substances, including fragments of old GBM or larger molecules that pass through endothelial cell pores but cannot pass subsequently through the GBM. In addition, mesangial cells have contractile elements that allow them to participate in regulation of glomerular hemodynamics. Furthermore, these cells proliferate in response to glomerular injury and can release a number of cytokines that modulate the glomerular inflammatory response.[5] The GBM lies between endothelial and epithelial cells and surrounds glomerular capillaries, except where mesangium is present (Fig. 14.19A). Returning to the abdominal cavity analogy, the GBM would lie beneath bowel serosa except in the area of mesenteric attachment, which would contain mesangial cells and matrix. The GBM consists of a central electron-dense layer, the lamina densa, and two thinner, more electron-lucent layers, the lamina rara externa and the lamina rara interna (Fig. 14.19B).[5,435] The main components of the GBM are collagen-like molecules and matrix glycoproteins that are produced principally by visceral epithelial cells. Normal GBM undergoes steady turnover

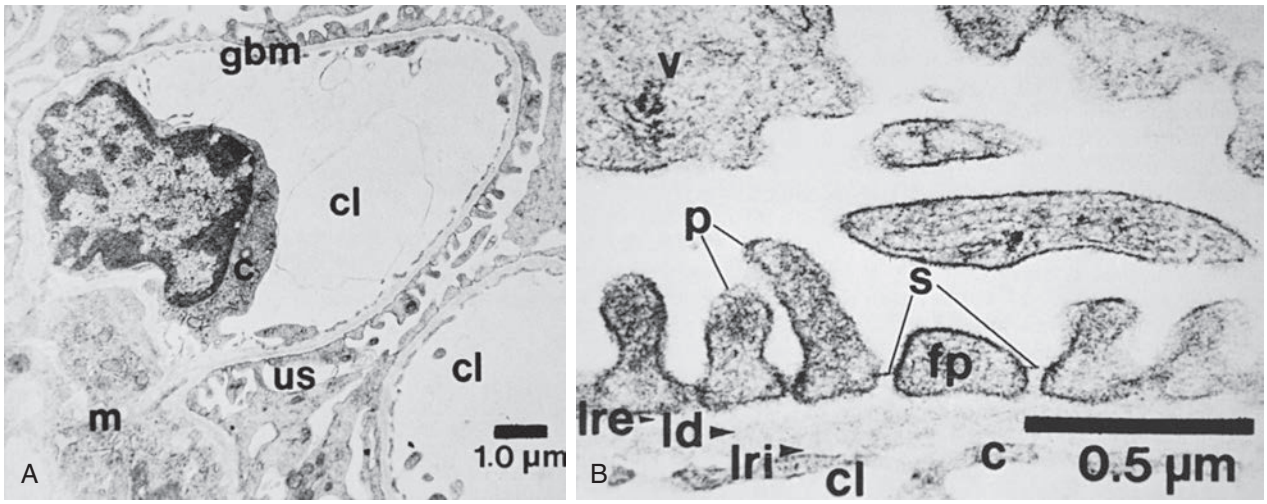


FIG. **14.19** Electron micrograph of a normal glomerulus. **A,** Low-power magnification illustrates patent capillary lumens *(**cl**)* separated from the urinary space *(**us**)* by fenestrated endothelial cell cytoplasm *(**c**)*, the glomerular basement membrane (GBM) *(**gbm**)*, and foot processes of visceral epithelial cells. Mesangial cells and matrix *(**m**)* are also apparent (bar = 1.0 μm). **B,** Higher power magnification reveals the ultrastructural features of the filtration barrier including, from bottom to top, the capillary lumen *(**cl**)* and endothelial cell cytoplasm *(**c**)*; the lamina rara interna *(**lri**)*, lamina densa *(**ld**)*, and lamina rara externa *(**lre**)* of the GBM; and cytoplasm *(**v**)*, foot processes *(**fp**)*, and slit diaphragms *(**s**)* of the visceral epithelial cells. Deposits of glomerular polyanion or glycosaminoglycans *(**p**)* are visible on the foot processes (bar = 0.5 μm). (From Osborne CA, Hammer RF, Stevens JB, et al.: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med.* 21:207, 1977.)

with removal of debris by mesangial cells. The visceral epithelial cells, also called *podocytes,* cover the uriniferous duct side of the GBM and have many cytoplasmic extensions called *foot processes* that form extensive interdigitations with foot processes of adjacent epithelial cells. The narrow gap between foot processes—the filtration slit or slit pore—is bridged by a thin membrane, the slit pore diaphragm.[5,435]

The filtration barrier of the glomerulus consists of fenestrated endothelial cells, GBM, and slit pores between epithelial cell foot processes. These structures constitute a size and charge selective filtration barrier. Although all components of this barrier are anionic (i.e., they repel anionic macromolecules), the GBM is thought to be the principal agent for the permeability characteristics of the filtration barrier. The GBM is rich in glycosaminoglycans, containing heparin sulfate and sialic acid residues. These strongly anionic molecules are responsible for its negative charge barrier, which limits filtration of anionic macromolecules, predominantly albumin.[5,432]

Glomerulonephritis is initiated by deposition of circulating immune complexes along the GBM and in the mesangium, leading to complement activation and leukocyte infiltration and adherence. Release of oxidants and proteinases by neutrophils and macrophages; production of eicosanoids, cytokines, and growth factors by macrophages and mesangial cells; and platelet aggregation and activation of coagulation factors lead to endothelial and epithelial cell swelling (with fusion of foot processes), formation of microthrombi in glomerular capillaries, and mesangial cell proliferation.[432,435,439] Immune complexes can be deposited in a subendothelial, intra-GBM, or subepithelial site, depending on their size and charge properties, but most tend to be found in the subendothelial space. On electron microscopic examination, these immune complexes appear as electron-dense granular deposits (Fig. 14.20A), and staining with antiimmunoglobulin G and anticomplement (C3) antibodies reveals an irregular (granular or "lumpy bumpy") immunofluorescence staining pattern (Fig. 14.20B).[270,432,435] The GBM proliferates to surround the immune deposits, leading to irregular thickening of the filtration barrier. Despite widening of the filtration barrier, the size and charge selective filtration properties are compromised, and microscopic hematuria and proteinuria result. In rare instances, glomerulonephritis may be attributed to a true autoimmune disorder in which autoantibodies directed against GBM components (e.g., type IV collagen) are produced. Electron microscopic examination in these cases also reveals GBM thickening with predominantly subepithelial electron-dense deposits (Fig. 14.21A), and immunofluorescence staining shows a more regular or smooth linear pattern of immunofluorescence (Fig. 14.21B).[435,440,441] Immune-mediated glomerulonephritis, accompanied by proteinuria, also has been described as one of the manifestations of systemic lupus erythematosus in horses.[442] Another immune mechanism of glomerulonephritis in horses is production of mixed or monoclonal cryoglobulins and deposition of antibody-antibody immune complexes along the glomerular GBM.[443,444] Cryoglobulinemia is associated with a number of diseases in human beings[5,443] but has been described in only a few horses.[444,445] Deposition of antibody-antibody immune complexes along the GBM may be a more important precipitant of glomerulonephritis than was recognized previously because electron microscopic examination is required to demonstrate characteristic fibrillar or crystalline intracapillary and subendothelial deposits associated with the condition.[443] Regardless of what immune mechanism leads to glomerular injury, the end result is thickening of the filtration barrier, retarded GFR, and development of CKD in severe cases.

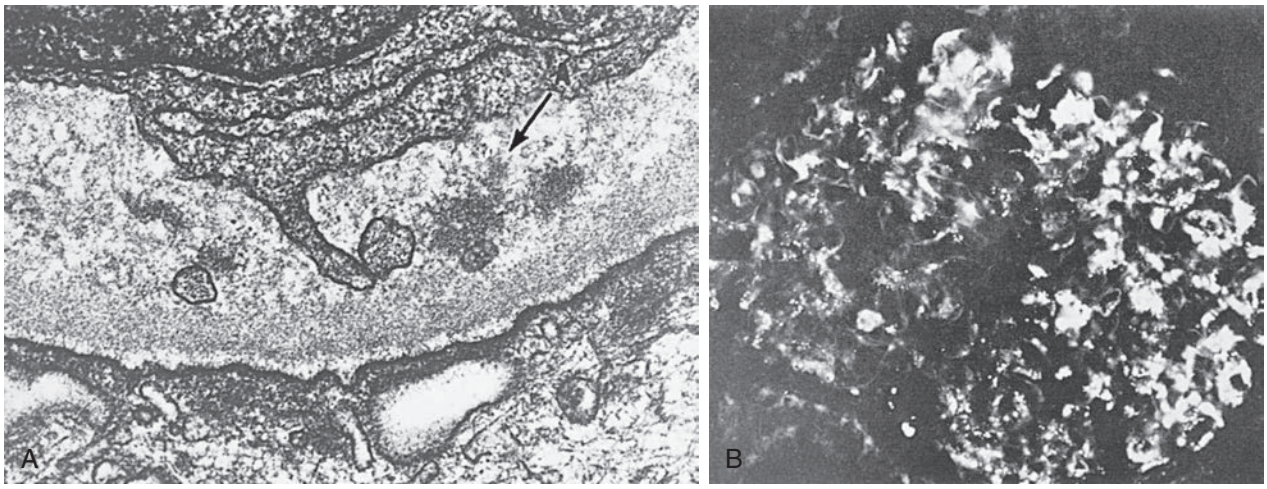


FIG. **14.20** Immune-mediated glomerulonephritis in a horse 165 days after experimental infection with equine infectious anemia virus (EIAV). **A,** An electron micrograph (×32,500) shows endothelial cell swelling *(top),* thickening of the glomerular basement membrane (GBM) with electron-dense immune deposits in a predominantly subendothelial location *(arrow),* and fusion of foot processes of the visceral epithelial cells. **B,** Immunofluorescent staining with fluorescein-tagged antiequine immunoglobulin G antibody (×100) demonstrates granular or "lumpy bumpy" deposits of immunoglobulin G along the GBM and in the mesangium. (From Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 1. Pathogenesis in persistent infection by equine infectious anemia virus, *Lab Invest.* 26:701, 1972.)

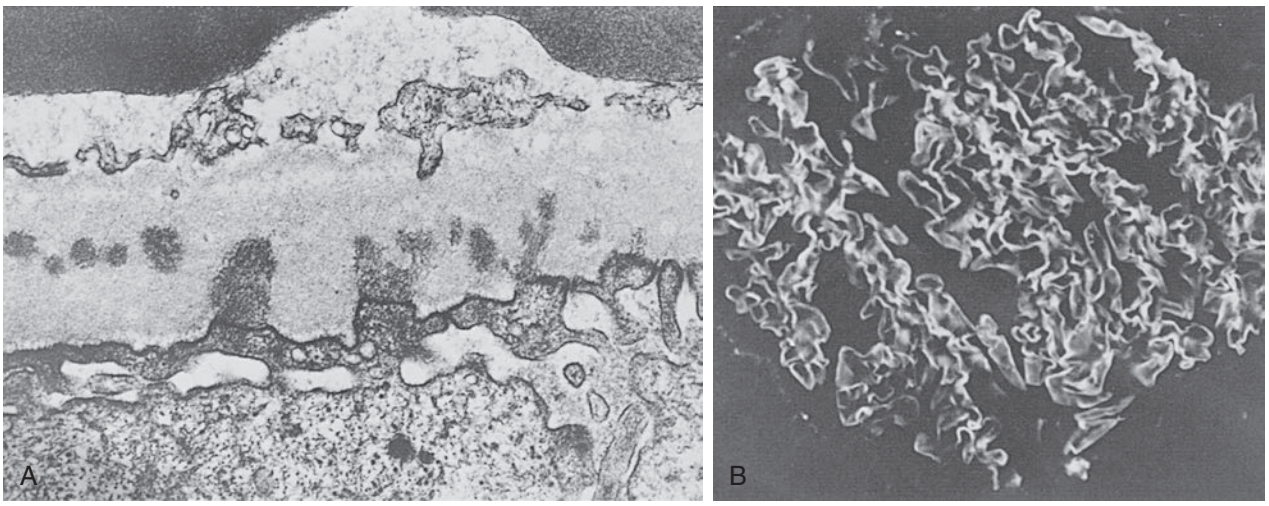


FIG. **14.21** Spontaneous immune-mediated glomerulonephritis in a horse. **A,** An electron micrograph (×22,750) shows a red blood cell (RBC) in the capillary lumen *(top),* relatively normal fenestrated endothelial cytoplasm, thickening of the glomerular basement membrane (GBM) with electron-dense immune deposits in a predominantly subepithelial location, and fusion of foot processes of the visceral epithelial cells. **B,** Immunofluorescent staining with fluorescein-tagged antiequine immunoglobulin G antibody (×160) demonstrates smooth, linear deposits of immunoglobulin G. (From Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 2. Antiglomerular basement membrane antibody and other mechanisms of spontaneous disease, *Lab Invest.* 26:708, 1972.)

Although a number of terms are used to describe the specific morphologic changes associated with glomerular injury, glomerulonephritis is categorized most broadly histologically as *proliferative* or *membranous.*[432,435,438] Proliferative (or mesangioproliferative) glomerulonephritis describes glomerular injury associated with influx of inflammatory cells and proliferation of mesangial cells. The predominant histologic finding is increased cellularity in glomeruli (Fig. 14.22A–B). This lesion tends to be associated with the more acute stages of glomerulonephritis during which immune complexes are being deposited in a predominantly subendothelial site. Membranous glomerulonephritis describes glomerular injury accompanied by significant thickening of the capillary wall and GBM; with periodic acid–Schiff (PAS) staining, the predominant histologic finding is increased in the mesangial area and on the GBM (Fig. 14.22C). Methenamine silver stain further

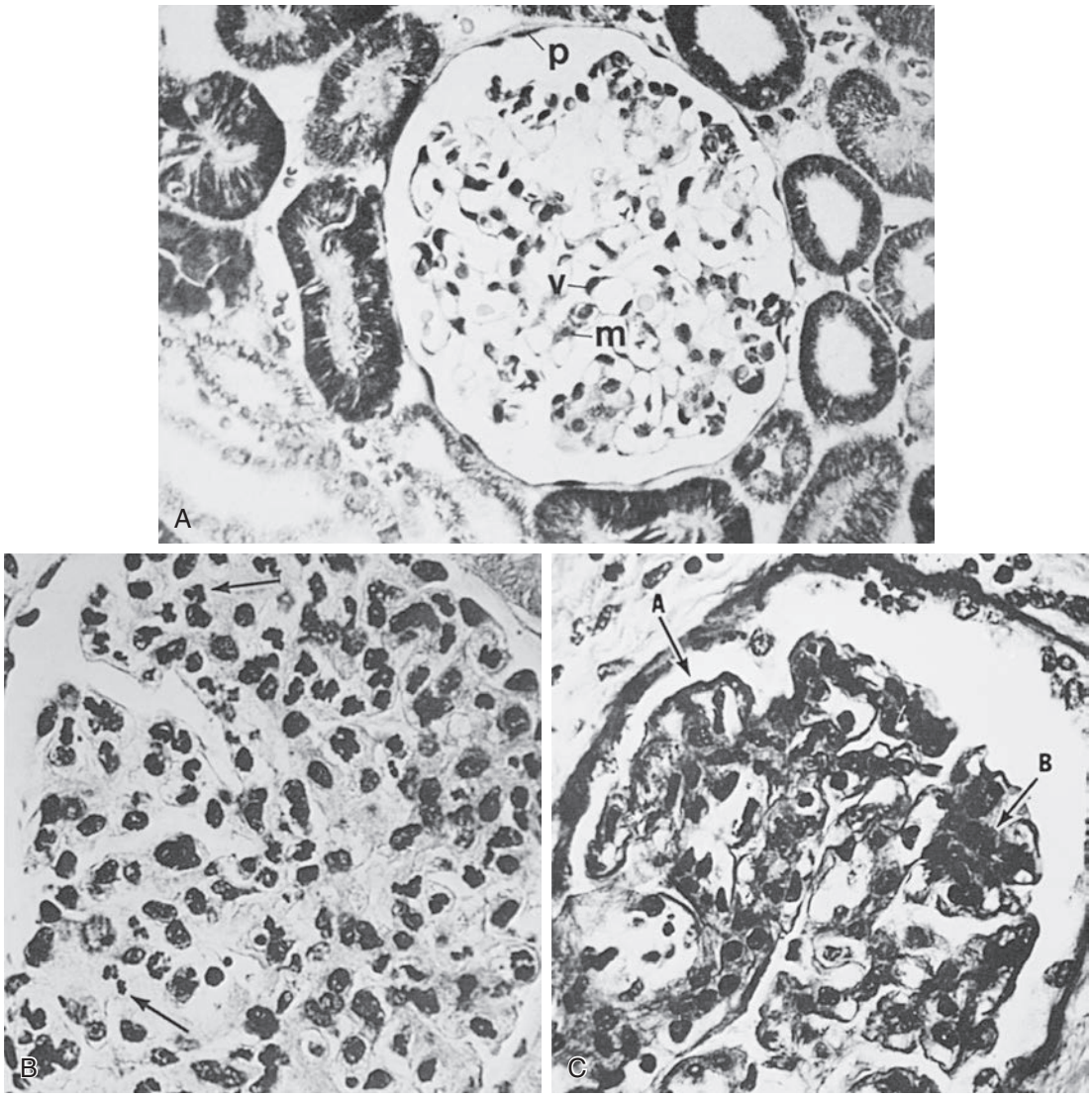


FIG. **14.22** Histologic changes in equine glomerulonephritis. ***A,*** Photomicrograph of a normal glomerulus. Bowman's capsule is lined by flattened parietal epithelial cells *(**p**)*. Visceral epithelial cells *(**v**)* are adjacent to the glomerular basement membrane (GBM), which is of uniform thickness. Mesangial cells *(**m**)* are surrounded entirely by glomerular capillaries (periodic acid–Schiff [PAS] stain, ×100). ***B,*** Photomicrograph demonstrates proliferative glomerulonephritis in a horse after experimental infection with equine infectious anemia virus (EIAV). A combination of neutrophil infiltration *(arrows)* and mesangial cell proliferation is apparent (hematoxylin–eosin stain, ×160). ***C,*** Photomicrograph demonstrates membranous glomerulonephritis in a horse after experimental infection with EIAV. The GBMs are thickened **(A),** and mesangial areas contain PAS-positive material **(B)** (PAS stain, ×160). (**A** from Osborne CA, Hammer RF, Stevens JB, et al.: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med.* 21:207, 1977; **B** and **C** from Banks KL, Henson JB, McGuire TC: Immunologically mediated glomerulitis of horses. 1. Pathogenesis in persistent infection by equine infectious anemia virus, *Lab Invest.* 26:701, 1972.)

enhances visibility of the thickening of the GBM.[435,438] Membranous glomerulonephritis tends to be associated with more soluble immune complexes or autoantibodies that can pass through the GBM and localize in a predominantly subepithelial site, resulting in less infiltration of inflammatory cells. As would be expected, a spectrum of lesions is visible in naturally occurring glomerulonephritis, leading to varying histologic descriptions of the disease (membranoproliferative glomerulonephritis). As glomerular injury progresses, proliferation of the parietal epithelium also occurs, likely in response to filtration of macromolecules and cellular debris. Lesions associated with parietal cell proliferation can include layering of epithelial cells (termed *crescents*) on the inner aspect of Bowman's capsule (Fig. 14.23A), adhesion formation between the glomerular tuft and Bowman's capsule (Fig. 14.23B), and tuft collapse. Glomerulosclerosis describes the end stage of progressive, irreversible glomerular injury in which replacement of glomerular components with hyaline material is visible on histologic examination (Fig. 14.23C).[435]

Specific histologic categorization of glomerulonephritis provides causative and prognostic information for human beings with renal failure attributable to glomerular disease.[5] Subcategorization

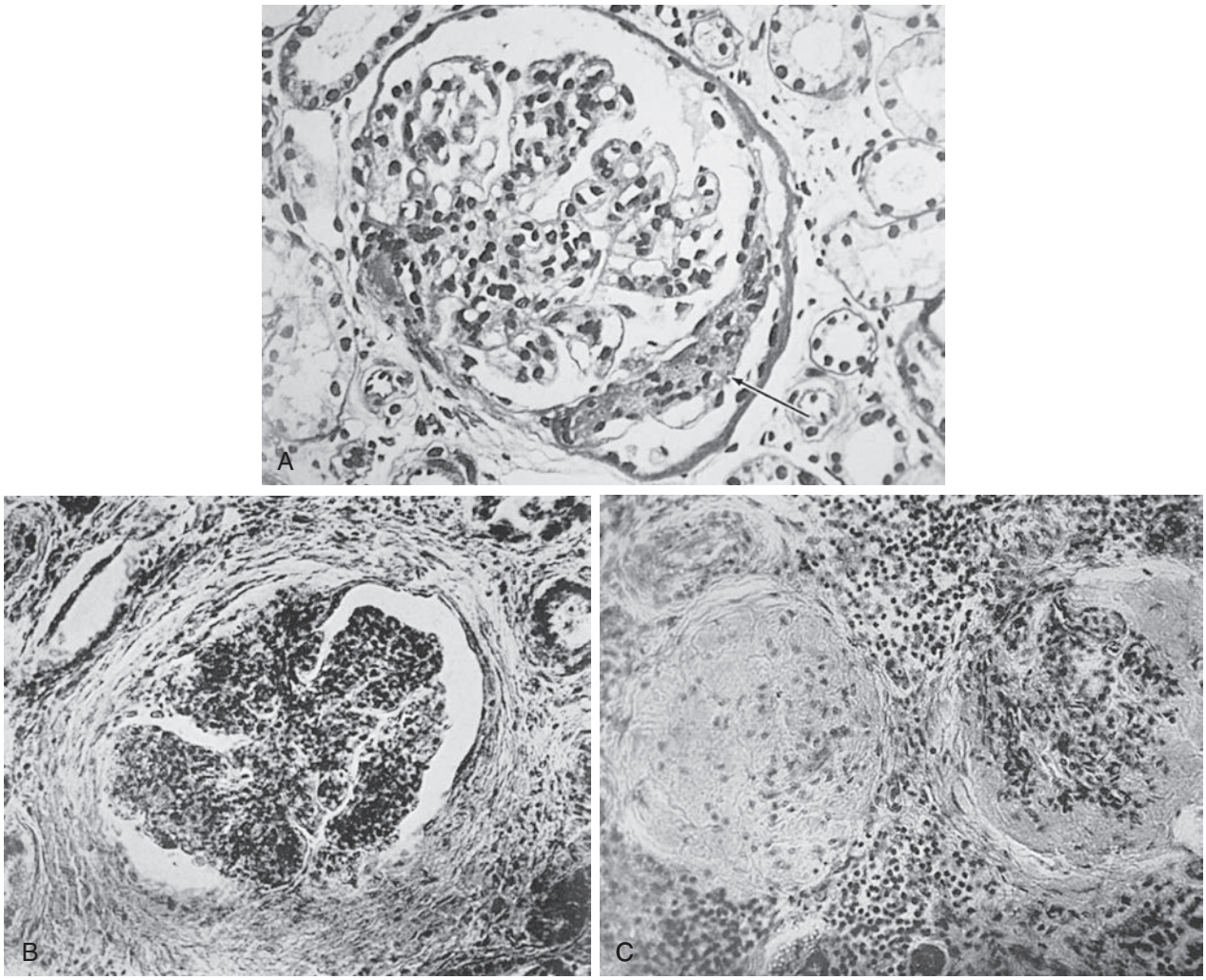


FIG. **14.23** Progressive histologic changes in equine glomerulonephritis. **A,** Photomicrograph of a renal biopsy specimen illustrates membranoproliferative glomerulonephritis (an increase in cell numbers and thickening of the glomerular basement membrane [GBM]) and parietal epithelial cell proliferation resulting in crescent formation (*arrow;* periodic acid–Schiff stain [PAS], ×100). **B,** Photomicrograph demonstrates progressive glomerulonephritis resulting in adhesion formation between the capillary tuft and proliferating parietal epithelial cells. **C,** Photomicrograph of end-stage glomerulosclerosis shows replacement of glomerular components with hyaline material (more complete in glomerulus on the left). (**A** from Osborne CA, Hammer RF, Stevens JB, et al.: The glomerulus in health and disease: a comparative review of domestic animals and man, *Adv Vet Sci Comp Med.* 21:207, 1977; **B** and **C** from Fincher MG, Olafson P: Chronic diffuse glomerulonephritis in a horse, *Cornell Vet.* 24:356, 1934.)

of glomerulopathies also has been done in a prospective study of naturally occurring chronic renal disease in canines. Although classifications—including focal glomerulonephritis, mesangioproliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, crescentic glomerulonephritis, and sclerosing glomerulonephritis—could be made, histologic findings were not prognostically useful.[446] This study also revealed that glomerular disease was responsible for 52% of canine CKD; however, an inciting disease could be identified in only one of 31 dogs with glomerulonephritis (vegetative endocarditis).

Glomerulonephritis also appears to be an important cause of CKD in horses.[266] Of 60 reported cases of CKD attributable to acquired disease, glomerulonephritis was identified as the inciting cause of CKD in 32 (53%) (Table 14.8).*

Glomerulonephritis also may be the initiating disease process in cases of ESKD for which gross and histopathologic changes are so extensive that a primary mechanism of renal injury cannot be identified. A number of systemic inflammatory and infectious disease processes in horses may be accompanied by glomerulonephritis, but progression to CKD appears to be a rare sequela. For example, experimental *Leptospira pomona* infection produced subacute glomerulonephritis characterized by hypercellularity and edema of capillary tufts,[472] but leptospirosis appears to be a rare cause of clinical renal disease in horses.[473-475] Similarly, experimental infection with equine infectious anemia virus (EIAV) produced histologic and immunofluorescent evidence of glomerulonephritis in 75% and 87% of infected horses, respectively.[440] Immunoglobulins with anti-EIA activity were eluted from glomeruli collected from experimentally infected horses, but none of the

* References 238, 260, 263, 266, 388, 416, 417, 433, 439, 443, 444, 447-471.

TABLE **14.8 Causes of Chronic Renal Failure in 75 Horses (Excluding Reports of Congenital Renal Failure and Experimental Induction of Chronic Renal Failure)**

Disorders	Number of Cases	References (Number of Cases)
CONGENITAL DISORDERS (15 OF 75 [20%])		
Renal agenesis/contralateral obstruction and hydronephrosis	1	27 (1)
Renal hypoplasia	4	32 (3), 39 (1)
Renal dysplasia	6	34 (1), 35 (1), 36 (2), 37 (1), 38 (1)
Polycystic kidney disease (PKD)	4	48 (1), 49 (1), 50 (1), 51 (1)
ACQUIRED DISORDERS (60 OF 75 [80%])[a]		
Glomerulonephritis	32	433 (1), 260 (2), 266 (1), 439 (1), 443 (7), 444 (1), 447 (1), 448 (1), 449 (1), 416 (1), 263 (1), 450 (1), 451 (1), 452 (1), 453 (5), 454 (2), 455 (1), 456 (2), 417 (1)
Chronic interstitial nephritis (CIN)	2	457 (1), 458 (1)
With obstructive nephrolithiasis and/or ureterolithiasis	11	459 (1), 460 (1), 388 (7), 461 (1), 462 (1)
With pyelonephritis	8	433 (1), 266 (1), 453 (1), 463 (1), 464 (1), 465 (1), 466 (1), 467 (1)
With papillary necrosis	2	266 (1), 238 (1)
End-stage kidney disease (ESKD)[b]	5	458 (1), 468 (1), 469 (1), 470 (1), 471 (1)

[a]In many reports of acquired renal disease leading to chronic renal failure, histopathologic changes involve glomeruli and interstitium. Categorization in this table is based on the authors' conclusions in these reports. When severe lesions involved glomeruli and interstitium, a categorization of ESKD was made.
[b]ESKD and reports of oxalate nephropathy.

horses showed clinical signs of renal disease. Poststreptococcal glomerulonephritis is a well-recognized cause of renal disease in human beings,[5,476] and Roberts and Kelly[477] speculated that glomerulonephritis in a horse with chronic pleuritis and purpura hemorrhagica likely resulted from circulating immune complexes involving streptococcal antigens. Divers and colleagues[417] provided support for this hypothesis by eluting group C streptococcal antigens from immune complex deposits in glomeruli collected from a horse with CKD. Finally, an occasional case of equine glomerulonephritis may result from true autoimmune disease, and in one instance, anti-GBM antibody was eluted from glomeruli with linear GBM immunofluorescent staining pattern isolated from a horse.[270] Little information is available about histopathologic subcategories of glomerulonephritis in horses, although several reports have attempted to make comparisons with glomerular lesions that have been better characterized in other species.[443,455,478] No reports have attempted to correlate histologic changes with the degree of renal failure in horses. Thus assessment of the severity of renal disease associated with glomerulonephritis in horses currently is based more on clinical findings (e.g., body condition, magnitude of azotemia) than on histologic changes in a renal biopsy sample.

Chronic Interstitial Nephritis

Tubulointerstitial disease usually results from ATN after ischemia, endotoxemia, sepsis, or exposure to nephrotoxic compounds. Hypovolemia associated with acute blood loss, colic, diarrhea, endotoxemia, or sepsis can lead to renal hypoperfusion and ischemic damage.[265] Severe localized infection (e.g., pleuritis, peritonitis) or septicemia also may be accompanied by tubule damage. Aminoglycoside antibiotics, NSAIDs, vitamin D, vitamin $K_{3\,(menadione)}$, acorns, and heavy metals such as mercury are potentially nephrotoxic.[382] Intravascular hemolysis or rhabdomyolysis also can lead to acute tubular damage after the nephrotoxic effects of hemoglobin and myoglobin (see Acute Renal Failure). In horses, tubulointerstitial disease culminating in CKD also can be caused by ascending urinary tract infections resulting in pyelonephritis[266,433,453,463-467,479] or bilateral obstructive disease caused by ureteroliths or nephroliths.[38,266,388,459-462,480] In other cases, a cause of the tubular disease may not be identified.[457,458] Finally, although this has not yet been described in horses, immune mechanisms, including antitubular basement membrane disease, can lead to chronic interstitial nephritis (CIN) in human beings.[5]

CIN is defined most strictly by clinical signs of renal disease associated with histologic changes of tubular damage and an interstitial inflammatory cell infiltrate (Fig. 14.24). Inflammatory cells include lymphocytes, monocytes, and occasionally plasma cells. Neutrophils are uncommon; however, eosinophilic infiltrates suggest drug reactions in human patients.[5] Major glomerular and vascular lesions are not apparent. The hallmark that distinguishes CIN from acute tubular and interstitial disease is interstitial fibrosis.

Although CIN has a fairly strict histologic definition, a number of disease processes can lead to tubular damage. For all practical purposes, *CIN* is a catchall term for extraglomerular causes of CKD in horses. As a consequence, gross findings in horses with CIN can vary dramatically. For example, NSAID toxicity, of which phenylbutazone has the greatest nephrotoxic potential,[481] can produce papillary necrosis[238,239] manifested by hematuria[482] in the early stages of disease, whereas chronic disease may be associated with nephrolithiasis and hydronephrosis. An area of papillary necrosis serves as a nidus for stone formation, and subsequent obstructive disease leads to hydronephrosis.[388,480] Similarly, upper urinary tract infection can lead to minor or major changes in the architecture of the kidneys and to variable histologic changes.

Using CIN as a broad category for nonglomerulonephritic causes of CKD in horses, this group of tubulointerstitial diseases was responsible for 38% (23 of 60) of the previously reported cases of CKD in horses (see Table 14.8). Although in

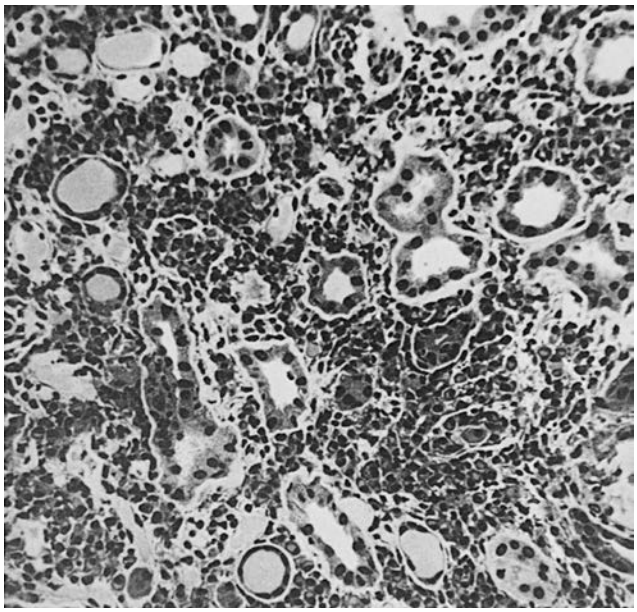


FIG. **14.24** Histologic changes in interstitial nephritis. The interstitium contains dense infiltrates of lymphocytes and plasma cells superimposed on moderate interstitial fibrosis and tubular atrophy (hematoxylin–eosin stain, ×275). (From Brenner BM, editor: *Brenner and Rector's the kidney,* ed 8, 2008, Philadelphia, WB Saunders.)

theory one would expect CIN to be accompanied by greater evidence of tubular dysfunction (e.g., enzymuria, glucosuria, increased fractional clearance of sodium), abnormal measures of tubular function have not been detected regularly. Similarly, microscopic hematuria and proteinuria, the hallmarks of glomerular disease, would not be expected.

End-Stage Kidney Disease

End-stage kidney disease describes the severe gross and histologic changes in kidneys collected from animals in the final stages of CKD. Grossly, the kidneys typically are pale, shrunken, and firm, and they may have an irregular surface and an adherent capsule. Histologically, severe glomerulosclerosis occurs along with hyalinization and extensive interstitial fibrosis. The end-stage lesions make determining the initiating cause of renal disease virtually impossible. In several cases with a pathologic description consistent with ESKD, the underlying cause of renal injury could not be established.[458,468-471]

Chronic Kidney Disease of Other Causes

Several early cases of CKD in horses were attributed to oxalate poisoning because oxalate crystals were observed in renal tubules.[457,468] Horses appear to be more resistant than other domestic species to oxalate-induced renal damage[25]; however, experimental administration of various forms of oxalate (in large doses) produced hypocalcemia and gastrointestinal signs rather than renal failure.[483] In fact, the early reports of oxalate nephropathy in horses failed to demonstrate that the affected horses had been exposed to oxalates.[457,468] Furthermore, long-term ingestion of plants containing oxalate produces fibrous osteodystrophy (oxalates bind calcium in the intestinal tract, decreasing intestinal calcium absorption), but renal damage in affected horses is minimal.[484] Formation of oxalate crystals in diseased equine kidneys now is recognized as a secondary change likely related to stasis of urine in damaged renal tubules.[451]

Amyloidosis is an unusual cause of CKD in horses.[25,485] Amyloid deposits associated with systemic disease typically are composed of aggregates of the amino-terminal fragment of serum amyloid A protein, an acute phase protein.[486-489] Concentrations of serum amyloid A increase with many inflammatory disease processes, and chronic elevations can result in amyloid deposition (AA type) in a variety of tissues, a condition termed *systemic reactive amyloidosis.*[490,491] Incidence and tissue localization of amyloid exhibit considerable species variation. Dogs appears to be affected most often, but the fact that the disease is familial in Abyssinian cats implies a genetic basis for the disease.[491] Amyloid deposits accumulate as stacks of protein in a β-pleated sheet conformation and are identified in tissue samples by their extracellular location and homogeneous, eosinophilic appearance when stained with hematoxylin and eosin. They are birefringent when viewed under polarized light, and staining with alkaline Congo red solution imparts a characteristic green color.[490,491] Amyloid deposition in the kidney is most common in dogs and cattle, and renal amyloidosis is a significant cause of CKD in dogs.[491] In horses, localized amyloidosis of the upper airway (around the nares or nasal septum) or skin is more common than systemic amyloidosis.[490] In the localized form, amyloid deposits are composed of immunoglobulin light chains (AL type). Although reports describe systemic reactive amyloidosis consequent to heavy parasite infestation in horses, renal involvement was minimal or not apparent.[492,493] Systemic amyloidosis has been recognized most often in horses hyperimmunized for antiserum production, and hepatic and splenic involvement may be more common than renal involvement in these horses.[25,485,487]

A final acquired cause of CKD, renal neoplasia, is discussed in greater detail elsewhere in this chapter. Although horses with renal neoplasia may have weight loss, tumors are usually unilateral and development of CKD is uncommon.

UREMIC TOXINS AND THE UREMIC SYNDROME

Regardless of the underlying cause of nephron loss, the ensuing renal insufficiency leads to development of azotemia and its clinical manifestation, the uremic syndrome.[5,486-488,494] The uremic syndrome is a multisystemic disorder that develops as a result of the effects of uremic toxins on cell metabolism and function. Although the uremic syndrome originally was attributed to the effects of increased BUN concentration, a number of nitrogenous compounds now are known to accumulate in CKD and contribute to alterations of cell metabolism and function. In fact, the correlation between the severity of the uremic syndrome and the magnitude of azotemia is poor. For example, urea administered to human beings with normal renal function results simply in fluid shifts, osmotic diuresis, and increased thirst.[495] Urea toxicity also has been studied in nephrectomized dogs and in human patients with CKD sustained by dialysis. Increasing the urea concentrations of the dialysate to maintain BUN at artificially higher values has produced variable results. For example, BUN values up to about 150 mg/dL were associated with few clinical signs, whereas lethargy, weakness, anorexia, vomiting, and bleeding diathesis (caused by platelet dysfunction) were produced when BUN was increased to values exceeding 175 to 200 mg/dL. Excess circulating urea also can degrade spontaneously to ammonia, carbonate, or cyanate. Cyanate can react with

N-terminal amino groups on a number of proteins and, by altering tertiary structure, can interfere with enzyme activity and structural integrity of cell membranes.[5] Thus accumulation of urea is likely responsible for some of the signs of uremic syndrome.

Creatinine and other guanidino compounds also accumulate in renal failure. These compounds are strong organic bases that contain an amidino group (N–C = NH).[5] With CKD, urinary excretion of creatinine and other guanidino compounds actually may increase (because of tubular secretion) but not enough to prevent increases in blood concentrations. For example, the author has found that in naturally occurring cases of equine CKD (author's unpublished data), endogenous Cl_{Cr}, when measured simultaneously with Cl_{In}, overestimates GFR by 50% to 100%. These data indicate that tubular secretion of creatinine, which Finco and Groves were unable to document in healthy ponies,[154] is initiated by or is quantitatively much greater with CKD. Although metabolic pathways have not been elucidated fully, guanidino compounds appear to be produced predominantly in the liver, and their concentration in blood and tissues increases with decreased renal function or increased dietary protein intake.[495] The relationship between guanidino compounds and the syndrome of uremia is also unclear. For example, administration of methylguanidine to healthy dogs resulted in weight loss, neurologic signs, and anemia but only when blood concentrations were an order of magnitude greater than those in spontaneous cases of CKD.[5] In contrast, administration of another guanidino compound, guanidinopropionic acid, led to hemolysis by depleting erythrocyte glutathione concentrations (supporting a role for this uremic toxin in the anemia of CKD).

Products of intestinal bacterial metabolism—including secondary methylamines (from metabolism of choline and lecithin), aromatic amines (from metabolism of tyrosine and phenylalanine), polyamines (from metabolism of lysine and ornithine), and tryptophan breakdown products (indole, skatole, indoleacetic acid, and others)—can contribute to the clinical signs associated with the uremic syndrome and neurologic disease. Some of these compounds have been studied thoroughly (e.g., the inhibitory effect of the polyamine, spermine, on erythropoiesis), whereas others are ill understood but likely contribute to altered neuromuscular and neurologic function.[5,494,495]

Another group of larger uremic toxins have been termed *middle molecules*.[5,495] These compounds are higher molecular weight compounds (500–3000 D) that are removed more readily from uremic patients by peritoneal dialysis than by hemodialysis. These compounds are not well characterized, and their existence is supported more by the difference in clinical response to peritoneal dialysis and hemodialysis in uremic human patients awaiting renal transplantation.

In addition to nitrogenous compounds, abnormal metabolism of hormones and trace minerals also accompanies the decline in renal function associated with acute and CKD. Secondary hyperparathyroidism (leading to osteodystrophy) and insulin insensitivity are well-recognized endocrine contributions to the uremic syndrome in human and small animal patients.[5,495-497] Endocrine dysfunction can be attributed to several factors: (1) decreased production of renal hormones (erythropoietin, vitamin D_3), (2) decreased hormone clearance prolonging plasma half-life (parathormone, gastrin), (3) decreased hormone production (testosterone), (4) tissue insensitivity (insulin, parathormone), and (5) hypersecretion to reestablish homeostasis (parathormone).[5] Regarding trace minerals, uremia may be accompanied by aluminum toxicity and zinc deficiency. Aluminum may contribute to some of the neurologic signs associated with azotemia (especially with ARF), whereas zinc has been implicated in testicular atrophy and abnormal taste in uremic human patients.[5,495] Presumed uremic encephalopathy has been reported in horses with CKD when neurologic dysfunction could not be attributed to other causes.[498] All five reported cases died or were euthanized. Swollen astrocytes were observed histologically in all horses that were necropsied.

Clearly, because so many potential uremic toxins exert their effects as renal function declines, it is unlikely that a single compound or even a few compounds will ever be identified as the primary cause of the uremic syndrome in patients with CKD. Furthermore, these compounds together are more likely to impair basic cell function in a number of tissues, and the signs of uremia are more likely to reflect multisystemic organ dysfunction. Methylation of membrane proteins has been identified as one of the common mechanisms of cell dysfunction.[499] What is truly remarkable, however, is the tremendous adaptive capacity of the failing kidneys to maintain sodium and water balance within narrow ranges until GFR declines to 20% of the normal value or less.[500]

CLINICAL SIGNS

Horses with CKD present relatively late in the disease course, when their owners note lethargy, anorexia, and weight loss. A history of months to years of polydipsia and polyuria in some cases supports renal disease of long duration. In other animals, preexisting disease (e.g., colic, colitis, pleuropneumonia) or prolonged medication (e.g., aminoglycoside antibiotics, NSAIDs) may provide important information about the initiation and duration of renal failure. In most cases, however, the onset is insidious, and identifying a precipitating event or gauging the duration of renal disease is not possible.

Chronic weight loss is the most common presenting complaint for horses with CKD.[70,95,260,266,433,434] Partial anorexia, ventral edema, polydipsia and polyuria, rough hair coat, lethargy, and poor athletic performance are other owner concerns. In addition, horses with advanced CKD may have a characteristic odor that likely reflects the combined effects of uremic halitosis and increased urea excretion in sweat. For the horses presented in Table 14.8, weight loss, ventral and peripheral edema, and polydipsia and polyuria were reported in 53 of 63 (84%), 24 of 56 (43%), and 21 of 48 (44%), respectively, of the cases. Lethargy and weight loss can be attributed to several factors. An increase in the concentration of nitrogenous wastes in blood can have a direct central appetite suppressant effect that can lead to partial or complete anorexia.[5,495] Next, as azotemia develops, excess urea diffuses across gastrointestinal epithelium and is metabolized to ammonia and carbon dioxide by bacterial urease. Also, when blood urea concentration is high, urea is excreted in saliva. In the oral cavity, excess ammonia can lead to excessive dental tartar formation (Fig. 14.25), gingivitis, and oral ulcers. In the gastrointestinal tract, excess urea and ammonia can lead to ulceration and mild to moderate protein-losing enteropathy, and severely uremic animals may produce soft feces.[5,432,495] The prolonged half-life of gastrin (eliminated

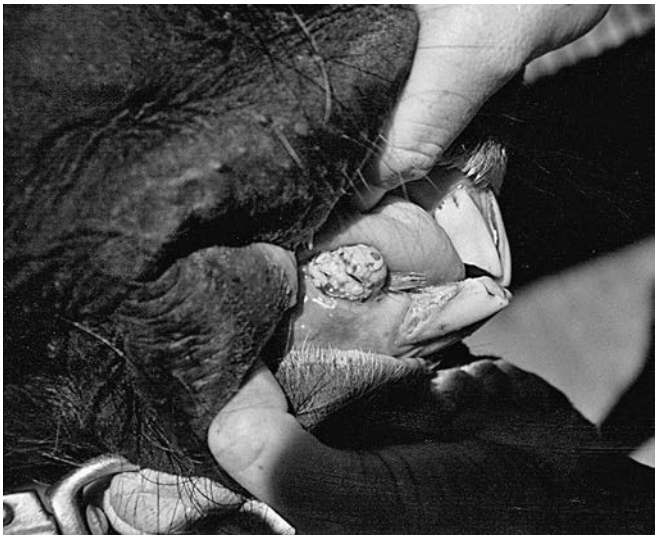


FIG. **14.25** Excessive dental tartar on the canine tooth and lower corner incisor of a horse with chronic renal failure (CRF).

through the kidneys) may contribute further to gastric ulcer formation because of increased acid secretion.[35] Finally, as the combined effects of uremic toxins render the affected patient catabolic, body mass declines as body reserves are tapped to meet basal energy requirements.[5,495]

Mild ventral edema with CKD may be attributable to three factors: (1) decreased oncotic pressure, (2) increased vascular permeability, and (3) increased hydrostatic pressure. Because albumin accounts for approximately 75% of plasma colloid oncotic pressure, decreases in albumin concentration (less than 2 g/dL) can decrease plasma oncotic pressure despite a normal total plasma protein concentration.[155,501] The effects of uremic toxins on endothelial cell membranes can alter vascular permeability, which contributes to edema.[5,495] Chronic renal insufficiency can lead to renal hypoxia and hypoperfusion, which stimulate renal juxtaglomerular cells to release renin. Activation of the renin-angiotensin system tends to elevate blood (and capillary hydrostatic) pressure and contributes to edema. Activation of the renin-angiotensin system also leads to increased sodium reabsorption in the proximal (direct effect of angiotensin II) and distal (effect of aldosterone) tubules.[155] Sodium retention leads to expansion of circulating volume, which is another factor in edema formation. Alterations in blood pressure in horses with CKD have not been evaluated routinely (as they have in small animal and human patients), and increased circulating concentrations of angiotensin II or aldosterone in horses have not been documented extensively. The nephrotic syndrome (characterized by edema, hypoalbuminemia, and heavy proteinuria) is not as well documented in horses as in small animals and human beings with CKD. However, horses with CKD appear less at risk for the significant pleural effusion or ascites that can accompany the nephrotic syndrome in small animals.[432]

Polydipsia and polyuria are variable findings in horses with CKD. The magnitude of polydipsia and polyuria theoretically is related to the degree of tubulointerstitial damage; however, the degree of polyuria does not appear to correlate with the magnitude of azotemia in clinical cases. Typically, polyuria is not as severe with CKD as with diabetes insipidus or psychogenic water drinking and may not be noticed by an owner (see the Polyuria and Polydipsia section of this chapter). The wide variation in water intake in normal horses and common use of automatic waterers and large stock tanks also make observation of polydipsia more difficult. The mechanisms of polyuria with CKD can include (1) increased tubular flow rate in surviving nephrons, (2) decreased medullary hypertonicity, and (3) impaired response of collecting ducts to vasopressin (acquired nephrogenic diabetes insipidus). Although these mechanisms may contribute to the polyuria of CKD, which of them may be most important is not known.[432,502]

An early complaint for horses with CKD is poor performance, which may be related to mild anemia (PCV 25%–30%) and lethargy. Although the anemia of CKD has been attributed to several factors—including blood loss, decreased erythrocyte survival time, nutritional deficiencies, and decreased erythropoietin production—the latter clearly has emerged as the principal cause of anemia in human beings and small animals with CKD.[5,432,503] In fact, administration of recombinant human erythropoietin (rhEPO) to human patients awaiting kidney transplant has been one of the most significant advances in management of CKD because it has eliminated the need for blood transfusions, improved exercise capacity, and decreased morbidity associated with the uremic syndrome.[5,503] Administration of rhEPO has also benefited anemic dogs and cats with CRF, although both species may need iron supplementation to support erythropoiesis.[5,432] The response often has been only temporary because many small animal patients develop refractory anemia after production of antirhEPO antibodies within a few weeks to months after initiation of treatment.[432] Plasma concentrations of erythropoietin have been determined in normal horses,[504] and rhEPO administration (15 IU/kg IV, three doses per week for 3 weeks) has been reported to increase the hematocrit in splenectomized horses[505]; however, no reports describe rhEPO administration to horses with CKD. Furthermore, moderate to severe anemia that may be fatal has been reported to develop after repeated rhEPO administration to racehorses.[506-508] The risks of adverse reactions far outweigh the potential benefits of rhEPO, and its use in horses should be avoided.

DIAGNOSIS AND LABORATORY EVALUATION

One establishes a diagnosis of CKD when persistent isosthenuria (specific gravity of 1.008–1.014) accompanies azotemia and typical clinical signs.[70,95,260,264,266] Rectal palpation of the left kidney may be normal or may reveal a kidney that is small or firm with an irregular surface. Less often, the kidneys and ureters may be enlarged if obstructed by uroliths or if infection or neoplasia is present. The ratio of BUN-to-creatinine concentration (BUN-to-Cr ratio) in horses with CKD is typically greater than 10:1 (Table 14.9); that for horses with prerenal azotemia or ARF is often less than 10:1.[266] This difference can be attributed in part to different volumes of distribution for urea (all body fluids) and creatinine (primarily the extracellular fluid space). As a consequence, with acute reductions in renal blood flow, the increase in creatinine concentration is usually greater, on a relative or percentage basis, than the increase in BUN. The point deserves emphasis, however, that BUN is influenced by dietary protein intake so that BUN-to-Cr values do not always distinguish ARF from CKD. In fact, one also can use the BUN-to-Cr ratio to assess adequacy of

TABLE **14.9 Abnormal Laboratory Values Reported for Horses with Chronic Renal Failure**

Parameter	Number[a]	Percentage
Blood urea nitrogen/serum creatinine concentrations (BUN/Cr) >10	29/34	85%
BUN/Cr >15	17/34	50%
Anemia (packed cell volume [PCV] <30%)	12/30	40%
Hypoalbuminemia (<2.5 g/dL)	12/14	86%
Hypoalbuminemia (<2.0 g/dL)	7/14	50%
Hyponatremia (Na^+ <135 mEq/L)	26/40	65%
Hyperkalemia (K^+ >5.0 mEq/L)	23/41	56%
Hypochloremia (Cl^- <95 mEq/L)	19/41	46%
Hypercalcemia (Ca^{2+} >13.5 mg/dL)	26/39	67%
Hypophosphatemia (P <1.5 mg/dL)	17/36	47%
Acidosis (pH <7.35)	3/5	60%

[a]Number of reports with this laboratory finding/total number of reports in which this laboratory parameter was reported.

dietary protein intake in the management of horses with CKD because a value greater than 15:1 can reflect excessive protein intake.[95]

In addition to azotemia, further abnormal laboratory data accompanying CKD can include mild anemia, hypoalbuminemia, hyponatremia, hyperkalemia, hypochloremia, hypercalcemia, hypophosphatemia, and metabolic acidosis (see Table 14.9). A nonregenerative anemia is related largely to a deficient supply of the renally secreted glycoprotein, erythropoietin; however, reduced erythrocyte life span may be another significant contributor to anemia. Normally, equine erythrocytes have a life span of 140 to 155 days.[507] With uremia, erythrocyte life span is shortened because excessive nitrogenous waste products alter the integrity of erythrocyte membranes and the function of ion channels, which regulate erythrocyte volume.[154] These less resilient erythrocytes are more likely to be removed from the circulation by the reticuloendothelial system.

The electrolyte alterations associated with CKD reflect loss of tubule function. Because sodium, chloride, bicarbonate, and phosphate are conserved by renal tubules, excessive urinary loss of these electrolytes accompanies CKD. Although fractional electrolyte clearance values (see Examination of the Urinary System) may remain within normal ranges or increase only slightly in horses with CKD, significant daily urinary loss of electrolytes still may occur. As an example, if a horse with CKD and creatinine and sodium concentrations of 5 mg/dL and 130 mEq/L, respectively, is producing 20 L of urine daily with respective creatinine and sodium concentrations of 50 mg/dL and 12.5 mEq/L, the fractional clearance of sodium is 1.0% and daily urinary sodium loss is 250 mEq. An increase in urinary sodium concentration to 25 mEq/L after another decrease in tubular reabsorption would result in an increase in fractional sodium clearance to 2% but would represent an additional 250 mEq of daily sodium loss in the urine. The latter value would approach 1% to 2% of the exchangeable sodium in the body and would require an additional 15 g of salt intake daily to accommodate this loss. Hypercalcemia and hypophosphatemia (Williams-Smith syndrome) are fairly common findings in horses with CKD (see Table 14.9), and the degree of hypercalcemia appears to vary with the amount of calcium in the diet.[433,450,453,509] In human beings with ESKD, hypercalcemia is an occasional finding attributed to hyperparathyroidism, vitamin D supplementation, or use of calcium-containing dialysate solutions. Furthermore, the osteodystrophy of CKD is associated with aluminum deposition in bone, which has been speculated to reduce skeletal buffering capacity for increases in serum calcium concentration and thereby contributes to hypercalcemia.[5] Because the equine kidney is an important route of calcium excretion (via $CaCO_3$ crystals), impaired tubular function in the face of continued intestinal absorption is the most common explanation for calcium accumulation in blood. This explanation is supported by the rapid development of hypercalcemia after experimental bilateral nephrectomy in ponies fed alfalfa hay (Fig. 14.26).[258] In addition, parathormone concentrations have been found to be below the lower limit of detection in horses with CKD, indicating that hyperparathyroidism does not play a role in development of hypercalcemia.[51,458] However, parathormone clearance by the kidney also is reduced with CKD and may be associated with a change in the regulatory set point for serum calcium concentration in uremic human beings.[5] Whether hypercalcemia in horses with CKD leads to exacerbation of renal disease or tissue mineralization remains unknown. Nevertheless, one can demonstrate the effect of dietary calcium by changing the type of hay fed to horses with CKD. Horses with serum calcium concentrations exceeding 20 mg/dL on a diet of mostly alfalfa can have their serum calcium concentrations returned to the normal range within a few of days after changing the diet to grass hay.[50,266] Similarly, nephrectomized ponies fed grass hay did not become hypercalcemic.[266] The cause of hypophosphatemia in horses with CKD also remains undocumented, although it has been explained by the law of mass action, which leads to a decrease in serum phosphate concentration in association with hypercalcemia.[5,509] A similar response is not observable in horses that eat *Cestrum diurnum* (leading to a syndrome of hypervitaminosis D)—they develop hypercalcemia without hypophosphatemia.[510] Another possibility that has been suggested is that hypophosphatemia may result from long-standing anorexia associated with CKD.[458] Regardless of the cause, clinical problems associated with hypophosphatemia have not yet been recognized in horses with CKD.

A degree of metabolic acidosis accompanies CKD in human beings and small animals and is attributed to decreased ability of failing kidneys to excrete hydrogen ions and regenerate bicarbonate.[5,432] Normally, acid-base balance is maintained by reabsorption of filtered bicarbonate and excretion of hydrogen ions along with ammonia and phosphate. As renal function declines in the early stages of renal failure, hydrogen ion excretion via renal ammoniagenesis and ammonium excretion increases. As renal failure progresses, compromised renal ammoniagenesis and decreased medullary recycling of ammonia caused by structural renal damage likely contribute to impaired ammonium excretion. Because hepatic glutamine synthesis is required for renal ammoniagenesis, the earlier increase in ammonium excretion may contribute to protein malnutrition in patients with CKD.[432] Metabolic acidosis also contributes to a number of the clinical signs of the uremic syndrome and may exacerbate some of the electrolyte alterations (e.g., hyperkalemia) of CKD. Metabolic acidosis has been reported in a limited number of horses with CKD (see Table 14.9); however, in the author's experience and that of others,[260,458] most horses with CKD have normal acid-base status

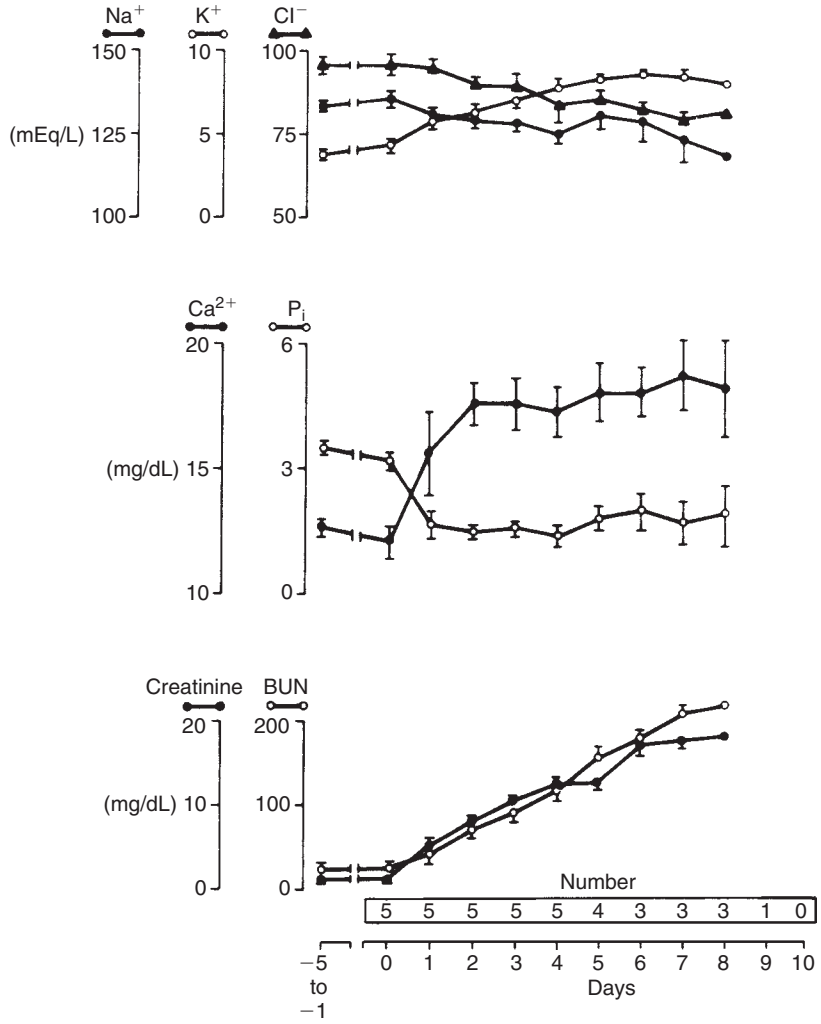


FIG. **14.26** Changes in serum electrolyte concentrations, blood urea nitrogen (BUN) concentration, and serum creatinine (Cr) concentration after bilateral nephrectomy in Shetland ponies. (From Tennant B, Lowe JE, Tasker JB: Hypercalcemia and hypophosphatemia in ponies following bilateral nephrectomy, *Proc Soc Exp Biol Med.* 167:365, 1981.)

or are alkalotic until the terminal stages of the disease, when metabolic acidosis typically develops. Metabolic alkalosis has been attributed to enhanced bicarbonate reabsorption and production, in association with hypochloremia and increased renal ammoniagenesis, respectively. In some instances, hypochloremic metabolic alkalosis may be accompanied by paradoxic aciduria.[458] The mechanism of paradoxic aciduria is likely similar to that of hypochloremic metabolic alkalosis in ruminants with abomasal outflow obstruction.[283] Briefly, after all chloride has been reabsorbed from the glomerular filtrate, further sodium reabsorption occurs by exchange with (excretion of) potassium or hydrogen ions. Thus paradoxic aciduria is most likely to occur with concomitant hypokalemia or whole body potassium depletion.

Horses with CKD also can develop hypercholesterolemia and hyperlipidemia (hypertriglyceridemia), and occasionally an animal has grossly lipemic plasma (hyperlipemia).[95,264,266] In fact, Naylor, Kronfeld, and Acland[511] reported a positive correlation between serum triglyceride and creatinine concentrations in a group of azotemic horses. Twelve of 13 cases of hypertriglyceridemia in horses reviewed by Dunkel and McKenzie[512] were azotemic. With azotemia, hyperlipidemia can develop as a result of increased synthesis, decreased degradation, or increased mobilization of triglycerides from fat stores.[5] Lipoprotein lipase, which stimulates triglyceride uptake into cells, is inhibited by azotemia.[513] Heparin treatment (40 IU/kg subcutaneously every 8 hours) has been recommended to stimulate lipoprotein lipase in an attempt to clear the serum.[95,266] Hypercholesterolemia and hyperlipidemia increase the risk of atherosclerotic cardiovascular disease in human beings with CKD.[5] Furthermore, these conditions stimulate mesangial cell proliferation and matrix production in diseased glomeruli and thus accelerate progression to glomerulosclerosis.[514]

The failing kidneys have a tremendous adaptive capacity to maintain tubular function until GFR is low.[500] In the author's experience, tubular dysfunction resulting in significant sodium or phosphorous wasting (manifested by increased fractional clearance values), glucosuria, or enzymuria is more common with ARF than with CKD. When present, however, abnormal tubule function rarely leads to values for fractional sodium clearance in excess of 5%.[272,461,471] In contrast, loss of

concentrating ability resulting in isosthenuria is a consistent feature of CKD that usually develops before azotemia. The associated polyuria may or may not be observed by the client, but the horse usually maintains water balance well through polydipsia. Polyuria typically results in urine that is clear and essentially devoid of crystals and mucus. Sediment examination is generally unremarkable, but increased numbers of red or white blood cells may occur with nephrolithiasis or ureterolithiasis or with pyelonephritis, respectively. Gross hematuria further supports urolithiasis or neoplasia. One should include a quantitative urine culture in the diagnostic plan for all horses with CKD because bacteriuria may not always be accompanied by pyuria.

Alterations in the integrity of the highly anionic glomerular filtration barrier also can lead to loss of protein, predominantly albumin, in the urine. Few quantitative data are available on urinary protein loss in horses with CKD because in most previous reports proteinuria was assessed using urine reagent strips. Reagent strip results of +++ or ++++ correlate with protein concentrations of 100 to 300 and 1000 to 2000 mg/dL, respectively, depending on which reagent strip one uses. In a proteinuric horse that is producing 20 L of urine daily, these values would yield a wide range of urinary protein loss (20–400 g daily). The latter value would approach 25% of the total protein content of plasma and so is not realistic. In human beings with CKD, urinary protein loss exceeding 3.5 g/day (50 mg/kg/day for a 70-kg person) is classified as *nephrotic-range proteinuria,* and some patients with heavy proteinuria may lose in excess of 15 g per day (more than 200 mg/kg/day).[5] The upper limit of acceptable urinary protein excretion in dogs is 20 mg/kg/day.[286] Using these values, estimates for the upper acceptable limits for urinary protein loss and nephrotic-range proteinuria in a 500-kg horse would be 10 g and in excess of 25 g/day, respectively. These values agree well with a mean value of 3.2 mg/kg (1.6 g/day) and a range of 3.6 to 22.3 mg/kg (1.8–11.2 g) per day in normal mares reported by Schott, Hodgson, and Bayly[285] and by Kohn and Strasser,[153] respectively.

Another method to document proteinuria is to determine the ratio of urinary protein to urinary creatinine (in milligrams per deciliter). This technique is more practical because it avoids a timed urine collection period. Although a normal range has not yet been reported for horses, values in excess of 1.0:1 and 3.5:1 are considered above normal and indicate nephrotic-range proteinuria in dogs[286] and human beings,[5] respectively. Thus a urine protein-to-Cr ratio greater than 2.0:1 likely supports significant proteinuria in a horse with CKD. Finally, a horse with CKD and heavy proteinuria (more than 200 mg/kg/day) could excrete as much as 100 g of protein daily (5%–7% of total plasma protein). Proteinuria of this magnitude can increase urine specific gravity to 1.020 or greater and certainly would be great enough to lead to a decline in serum albumin (and total protein) concentration, despite increased hepatic albumin production. In some horses with CKD and a normal total plasma protein concentration, increased globulin concentration offsets mild hypoalbuminemia, whereas in other cases hyperglobulinemia actually can result in an increase in total plasma protein concentration.

One can use ultrasonography to determine kidney size in horses with CKD and to evaluate for the presence of cysts or nephroliths. Horses with ESKD typically have small kidneys that are more echogenic than normal (because of sclerosis and possible tissue mineralization).[335,336,339,341,342] Cystoscopy also can be a useful diagnostic aid to assess urine production qualitatively in each kidney and is particularly useful when ultrasonographic imaging fails to identify a kidney.[335] Renal scintigraphy is another imaging option to detect functional renal tissue that may not be apparent by ultrasonography.[352] One can perform renal biopsy using ultrasonographic guidance to document renal disease. Unfortunately, because most horses are presented for evaluation in the later stages of disease, biopsy results typically reveal glomerular, tubular, and interstitial lesions consistent with ESKD. Rarely do the lesions provide information about the inciting cause of the renal disease unless immunofluorescence testing and electron microscopy are pursued. These require placing samples in special fixatives, in addition to the sample placed in formalin for routine histopathologic examination. In some cases, renal biopsy results supporting pyelonephritis or a congenital anomaly (dysplasia) as the cause of CKD are useful in developing a therapeutic plan or providing a prognosis.

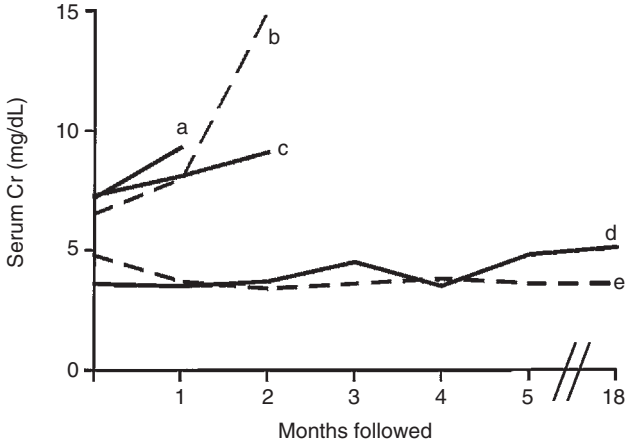


FIG. **14.27** Serum creatinine *(**Cr**)* concentrations in five horses with chronic renal failure (CRF). The three horses with an initial Cr concentration greater than 5 mg/dL *(**a** to **c**)* had rapid progression of renal disease over a 1- to 2-month period, necessitating euthanasia, whereas the two horses with an initial Cr concentration less than 5 mg/dL *(**d** and **e**)* were maintained with supportive care for longer than 18 months.

One can assess the severity of CKD in affected horses using available sophisticated techniques. The magnitude of azotemia is the most readily available and practical parameter, but it is an insensitive one.[5] Azotemia becomes apparent only after 75% or more of renal function is lost. Furthermore, the degree of azotemia can vary with nonrenal factors such as diet, body mass, and hydration. In general, creatinine concentration is a more reliable measure than BUN, and doubling of creatinine roughly correlates to a 50% decline in GFR (see Fig. 14.6). Creatinine concentrations in the range of 5 to 10 mg/dL indicate a significant decline in renal function, and values exceeding 15 mg/dL are consistent with a grave prognosis. In contrast, horses with a creatinine concentration below 5 mg/dL may exhibit few clinical signs and can be managed for months to years (Fig. 14.27). Plotting the inverse of creatinine concentration (1/Cr) over time also has been used to monitor progression of CKD in human beings[515,516] and in one horse[50] in an attempt to predict the end point of the disease process (Fig. 14.28). Unfortunately, these plots are subject to considerable variation (because of changes in tubular secretion of creatinine) and have not proved to be of any more value than monitoring creatinine over time.[515,516]

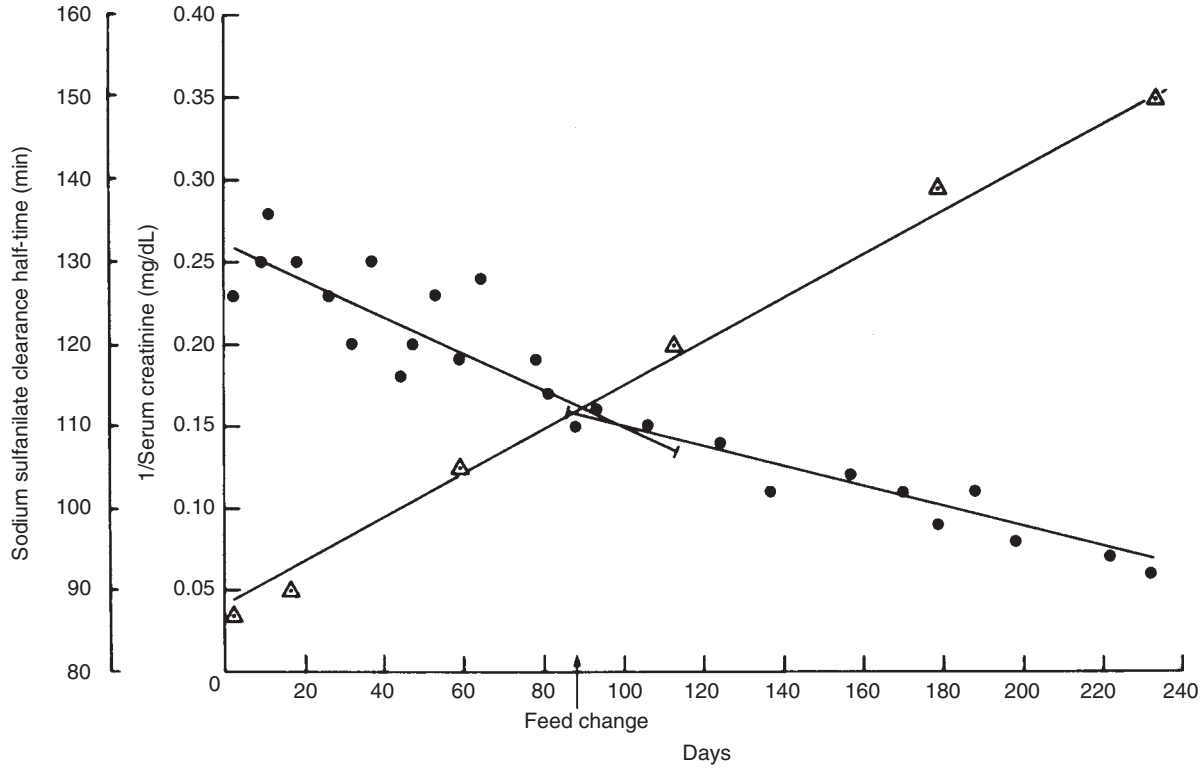


FIG. **14.28** The inverse of the serum creatinine (Cr) concentration *(filled circles)* and the whole-blood sodium sulfanilate clearance half-time in a horse with chronic renal failure (CRF) associated with polycystic kidney disease (PKD). The slope of the line describing the reciprocals of Cr concentration from day 1 to day 98 was significantly different ($P < 0.05$) from that from day 99 to 235, and the slope change was associated with a change from alfalfa to grass hay. (From Bertone JJ, Traub-Dargatz JL, Fettman MJ, et al.: Monitoring the progression of renal failure in a horse with polycystic kidney disease: use of the reciprocal of serum creatinine concentration and sodium sulfanilate clearance half-time, *J Am Vet Med Assoc.* 191:565, 1987.)

Measurement of GFR provides the most accurate quantitative assessment of renal function but is pursued rarely because it is more time consuming and technically demanding than measurement of creatinine concentration. Although a number of methods are available to measure GFR,[235] measurement of endogenous Cl_{Cr} or the plasma disappearance of exogenous creatinine, sulfanilate,[270,366] or 99mTc-DTPA[219] are the most practical methods in a clinical setting. The former requires timed urine collections, whereas the latter may require special assays or nuclear medicine capabilities (see Examination of the Urinary System). Horses in the earlier stages of CKD can develop tubular creatinine secretion, which could lead to overestimation of GFR measured by the Cl_{Cr} technique. As renal disease progresses, however, creatinine excretion decreases faster than GFR declines because of loss of compensatory secretion. Despite these limitations, repeated measurement of Cl_{Cr} in a single animal can be useful for monitoring progression of CKD over time (Fig. 14.29).

DISEASE PROGRESSION

One of the hallmarks of CKD is the progressive nature of the disease,[517-519] and progression appears to be largely independent of the inciting cause because of the final common pathways of renal injury. The response to a decrease in renal functional mass is a compensatory increase in filtration (termed *single-nephron GFR*) and tubular function (e.g., creatinine secretion, ammoniagenesis) by remaining nephrons. The increase in single-nephron GFR results from increased glomerular capillary blood flow and hydrostatic pressure leading to glomerular hyperfiltration. Hyperfiltration is associated with increased permeability of the GBM and proteinuria. Furthermore, increased filtration of macromolecules leads to activation and proliferation of mesangial and epithelial cells and eventually progression to glomerulosclerosis.[5,501,517-519] In addition to causing proteinuria, increased filtration of protein is accompanied by increased proximal tubular protein reabsorption. The latter leads to upregulation of genes encoding vasoactive and inflammatory mediators by tubular and interstitial cells and contributes substantially to injury of the renal interstitium.[519]

A number of studies investigating the mechanisms of glomerular hypertension have demonstrated that activation of the renin-angiotensin system and angiotensin II production are of considerable importance, because angiotensin II is a potent constrictor of glomerular efferent arterioles.[520-522] Activation of the intrarenal renin-angiotensin system can produce significant glomerular hypertension without producing increases in systemic angiotensin II concentration or blood pressure. In fact, administration of specific angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors has been demonstrated to decrease glomerular capillary hydrostatic pressure and the magnitude of proteinuria in experimental studies of renal disease.[520-523] Angiotensin-converting enzyme inhibitors have helped to control hypertension and proteinuria

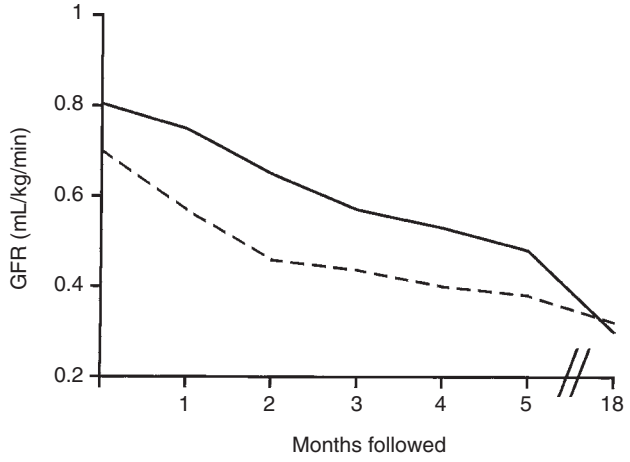


FIG. **14.29** Glomerular filtration rate (GFR) as measured by endogenous creatinine clearance (Cl_{Cr}) *(solid line)* and inulin clearance (Cl_{In}) *(dashed line)* in a mare (see horse in Fig. 14.28) with chronic renal failure (CRF). GFR declined steadily over the 18-month period, despite minimal change in serum creatinine (Cr) concentration. Compared with Cl_{In}, endogenous Cl_{Cr} overestimated GFR until the terminal stage of the disease process. This difference likely reflects tubule secretion of Cr in the earlier stages of CRF, which subsequently diminished during the final weeks of the disease course.

in small animals,[432,524] but no reports describe use of angiotensin-converting enzyme inhibitors in horses with CKD.

The role of dietary protein in the progression of CKD has been the subject of a number of investigations in human beings and small animals with a variety of renal diseases.[525-530] A well-established fact is that one can ameliorate clinical signs of uremia by decreasing dietary protein content; however, whether decreasing dietary protein slows progression of renal disease remains controversial. Increased dietary protein has been demonstrated to increase activation of the renin-angiotensin system; therefore decreasing dietary protein could be protective for the reasons discussed in the preceding paragraph. Next, a decrease in protein intake leads to production of smaller amounts of urea and other nitrogenous wastes. In theory, the workload on the kidneys is similarly decreased. Because all work (aerobic ATP production and use) is associated with a degree of free radical production, greater protein intake would generate more free radicals. Free radicals appear to be especially injurious to diseased kidneys because their scavenging mechanisms are compromised. Increased dietary protein also requires increased ammoniagenesis by renal tubules to excrete the associated proton load; however, ammoniagenesis can lead to noninflammatory, nonimmune complement activation in proximal tubule epithelial cells. One can ameliorate this proinflammatory consequence of ammoniagenesis by supplementation with sodium bicarbonate. In summary, excessive dietary protein leads to a greater degree of uremia and has the potential to exacerbate renal damage in CKD by several mechanisms.

In contrast, protein-calorie malnutrition is associated strongly with increased morbidity and mortality in human CKD patients and can be attributed to increased protein catabolism when dietary protein intake is marginal to low. In human beings a number of serum components, including prealbumin, cholesterol, and insulin-like growth factor; plasma and muscle amino acid profiles; and body composition measurements have been studied as potential indexes of nutritional status. However, serum albumin concentration is the most practical and most extensively studied nutritional index. In addition to heavy proteinuria, hypoalbuminemia also can be attributed to protein-calorie malnutrition. Furthermore, a BUN-to-Cr ratio less than 10:1 may be another indication of inadequate protein intake. Thus current dietary recommendations call for dietary protein and caloric intake at levels that meet or slightly exceed predicted requirements, which should render nitrogen balance neutral.[531] Finally, a critically important but often overlooked aspect of nutritional management of CKD patients is provision of a palatable diet.[432,531] Feeding smaller meals more frequently and varying the diet help to increase food intake in CKD patients of all species.

Experimental studies of CKD in horses are limited to reports that describe the effects of prolonged daily dosing of horses with mercurial compounds.[532-535] In these four reports, each involving a single horse, the subjects were destroyed between 85 and 191 days after mercury administration began. Anorexia, weight loss, and nonpruritic urticaria were the major clinical signs. The renal lesion was CIN, which was accompanied by a decrease in concentrating ability and an increase in water consumption. Additional tubular dysfunction included glucosuria in all four horses and a variable degree of proteinuria. Interestingly, clinical signs and tubular dysfunction preceded the development of azotemia in all horses, and excretion of sodium or chloride did not increase. The final report focused principally on the effects on renal function, and azotemia did not develop until the final week of life; GFR (assessed by endogenous Cl_{Cr}) decreased to 35% of the highest measured value on the day before euthanasia.[535] Aciduria and increased phosphate excretion, in addition to glucosuria and proteinuria, also were detected during the final week of life.

TREATMENT

At the time of presentation most horses with CKD exhibit obvious weight loss and other clinical signs. Because of the progressive and irreversible nature of the renal disease, the long-term prognosis is grave. Specific corrective treatment for CKD (renal transplantation) is not available for horses, and maintenance by peritoneal dialysis or hemodialysis would only be practical for valuable breeding animals. Pyelonephritis could be considered an exception because antibiotic treatment in theory could lead to resolution of infection and improved renal function. Unfortunately, significant renal damage usually has occurred by the time the diagnosis is established in most cases of bilateral pyelonephritis, so the prognosis for a return to normal renal function is guarded to poor. In contrast, the short-term prognosis may be more favorable. Some horses with CKD may maintain creatinine concentration of less than 5 mg/dL for months with minimal deterioration. To predict which patients will deteriorate more rapidly is difficult, but a recent history and initial ability to counteract weight loss with improved management are useful indicators. Laboratory analysis of blood samples at 2- to 4-week intervals to follow the degree of azotemia and serum electrolyte alterations may be useful in monitoring disease progression. In general, animals that are eating well and maintaining reasonable body condition carry the best short-term prognosis and may still be able to perform some limited work. Their use as breeding animals may be reduced because azotemia and eventual weight

loss would reduce the chance for normal conception and gestation. Horses with less severe to moderate CKD may do well clinically until they face a hypovolemic crisis, such as colic or colitis, and severe azotemia occurs. The goal in each case is to provide appropriate supportive care and to monitor the horse closely so as to provide humane euthanasia before the patient develops uremic decompensation.

As previously discussed, once significant renal disease is established, irreversible decline in GFR and progression of renal failure generally ensue.[5,517-519] Thus management of the equine patient afflicted with CKD involves palliative efforts to minimize further loss of renal function. The goals are to prevent complicating conditions (e.g., by providing plenty of water), to discontinue administration of nephrotoxic agents, and to provide a palatable diet to encourage appetite and minimize further weight loss.[70,95,266,434,536] Intravenous fluid therapy to promote diuresis, usually with 0.9% NaCl, is of much greater benefit in cases of acute, reversible renal failure but also may benefit patients that suffer a sudden exacerbation of CKD. One must administer intravenous fluid therapy cautiously because horses with CKD are at a higher risk to develop significant peripheral or pulmonary edema.

Supportive care also can include supplementation of sodium bicarbonate (50–150 g/day) when serum bicarbonate concentration is consistently less than 20 mEq/L.[536] One may need to add supplemental bicarbonate to a bran mash. If sodium bicarbonate supplementation aggravates ventral edema, one should limit or discontinue supplementation. Edema is not usually a significant problem, and the horse should tolerate mild edema (rather than being treated with diuretics that could be ineffective or lead to further electrolyte wastage) unless the edema interferes with ambulation. In a previous edition of this text, the author also recommended supplementation with NaCl to replace potential loss of these electrolytes in urine in horses with CKD. However, one study[537] found that supplementation with NaCl (used in an attempt to increase water intake) in cats with preexisting renal disease led to progression of renal disease that remained after salt supplementation was discontinued. Thus one should approach salt supplementation in horses with CKD with caution and should consider it only for patients with hyponatremia and hypochloremia.

Substituting high-calcium and high-protein feed sources such as alfalfa hay with good-quality grass hay and carbohydrates (corn and oats) may help control hypercalcemia and the magnitude of azotemia. Ideally, the hay and grain should contain an adequate but not excessive amount of protein (less than 10% crude protein), which should maintain the BUN-to-Cr ratio in a target range between 10:1 and 15:1.[266] Providing unlimited access to fresh water and encouraging adequate energy intake by feeding a variety of palatable feeds is important. In fact, if appetite for grass hay deteriorates, then offering less ideal feeds (e.g., alfalfa hay, increased amounts of concentrate) to meet energy requirements and reduce the rate of weight loss is preferable. Often horses continue to graze at pasture when their appetite for hay is diminished. Administration of B vitamins or anabolic steroids for their touted appetite-stimulating effects may benefit some animals. Although dietary fat is calorie dense, one must approach supplementation judiciously in patients with hyperlipidemia and hypercholesterolemia, and excessive fat supplementation can result in feed refusal.

Administration of corticosteroids or NSAIDs can limit the intrarenal inflammatory response associated with renal failure and also may attenuate renal injury. For example, administration of meclofenamate limited proteinuria in a group of human patients with severe manifestations of the nephrotic syndrome[538]; however, nonspecific blockade of prostaglandin production by corticosteroids and most available NSAIDs has the adverse effect of decreasing production of important renal vasodilating agents (prostaglandin E_2 and prostacyclin). Production of these prostanoids increases during periods of renal vasoconstriction or ischemia to maintain intrarenal blood flow, particularly to the renal medulla. With excessive or long-term NSAID administration, renal papillary necrosis develops after medullary ischemia.[238,239,388,461] Thus the negative effects of corticosteroids and NSAIDs on renal blood flow outweigh possible benefits, and they are not recommended routinely for the management of CKD in horses.

The progressive renal injury that occurs in CKD is associated with continued damage to glomerular and tubular membranes mediated by ongoing activation of the inflammatory cascade. In theory, treatment with antioxidant medications and free radical scavengers could be of benefit, but experimental data in horses do not bear this out. Similarly, interest in the role of dietary fatty acids as precursors of eicosanoids has been considerable. Specifically, dietary supplementation with sources rich in ω-3 fatty acids (linolenic acid; fish and flax oils) compared with ω-6 fatty acids (linoleic acid), appear to decrease generation of more damaging fatty acid metabolites during activation of the inflammatory cascade. In horses, dietary supplementation with ω-3 fatty acids (in the form of linseed oil) has been effective at ameliorating the effects of endotoxin in studies in vitro,[539-541] and supplementation with fish oil (another rich source of ω-3 fatty acids) slowed the progression of renal failure in laboratory animals.[542,543] Unfortunately, the effects of endotoxin in vivo were not ameliorated by feeding linseed oil in preliminary equine studies,[544] and the possible benefits of feeding ω-3 fatty acids to horses with CKD are not known at this time.

Control of hypertension and reduction of proteinuria have been recognized as the most successful interventions to limit progression of renal disease in human beings with CKD.[519] Thus monitoring the blood pressure and the level of proteinuria (urine protein-to-urine Cr ratio) in horses with CKD seems to be prudent. Treatment with angiotensin-converting enzyme inhibitors could be beneficial in horses with either of these problems but has not yet been pursued because of the expense of the available medications. Attention also has been directed to use of more specific antiinflammatory or immunosuppressant medications to limit renal injury in immune-mediated glomerulonephritis. For example, inhibition of thromboxane synthetase activity (thromboxane A_2 is a potent vasoconstricting agent and platelet activator) was demonstrated to limit renal histologic and functional changes in a canine model of immune-mediated glomerulonephritis.[545] Similarly, cyclosporine was used as an adjunct treatment in a prospective study of naturally occurring canine glomerulonephritis. Unfortunately, renal function declined in cyclosporine-treated dogs, as it did in control dogs. The lack of any beneficial effect, along with the adverse reactions to cyclosporine, led to the conclusion that cyclosporine was of no use for treating CKD.[546] As these studies demonstrate, specific manipulation of the inflammatory or immune response can limit renal injury when one can administer medications before or early in the course of renal

disease; however, with long-standing, naturally occurring disease, such treatments are much less likely to retard progression of renal failure significantly. Finally, other investigators are focusing on developing therapeutic strategies that may modulate or limit renal fibrosis. Their studies of the effects of cytokines, lymphokines, and proteoglycans on matrix synthesis and degradation by mesangial cells and on fibroblast activation in damaged glomeruli may lead to novel treatment options in the future.[547,548]

Urinary Tract Infections

Harold C. Schott II

In human beings, bacterial urinary tract infections (UTIs) are among the most common infections.[5] In contrast, bacterial UTIs are uncommon in horses.[70,95,434,549] As in other species, ascending UTIs are more common, although septic nephritis may be an occasional consequence of septicemia, especially in neonatal foals.[550] Mares are at higher risk for UTIs than geldings or stallions because of their shorter urethra.

Development of a UTI requires initial urethral colonization with pathogenic bacteria, entry of pathogens into the bladder, and subsequent multiplication in the bladder.[5,551] Urethral colonization involves adherence to uroepithelial cells, typically by enteric bacteria that possess fimbrial adhesins (pili) that bind to specific glycolipid receptors on uroepithelial cells. Not surprisingly, pathogenic *Escherichia coli* are rich in these specific surface adhesins, whereas nonpathogenic *E. coli* have few specific surface adhesins. Further characterization of human pathogenic *E. coli* strains by their somatic (O), flagellar (H), and capsular (K) antigens has revealed that a small number of *E. coli* strains are responsible for a large percentage of UTIs.[5,551,552] Normal vulvar and preputial flora protect against urethral colonization by pathogenic bacteria, but any anatomic defect leading to turbulent urine flow compromises maintenance of normal flora and may increase the likelihood of colonization by pathogens.[553,554] Although broodmares have not been proven to be at greater risk, intercourse is a well-established risk factor for the development of UTIs in women. In addition, human prostatic secretions contain a heat-stable cationic protein that has potent antibacterial activity.[5] Thus stallions may be at lower risk than geldings for UTIs.

Once a pathogen has colonized the distal urethra, rapid multiplication between micturitions allows invasion of the proximal urethra and bladder, which do not have a protective flora. Host defenses in the bladder include immunoglobulins in urine and a mucopolysaccharide layer rich in glycosaminoglycans covering the uroepithelial surface.[551,552] Production of protective glycosaminoglycans is under hormonal control by estrogen and progesterone in rabbits.[555] Thus the lack of these beneficial hormonal effects has been suggested as an explanation for the increased risk for UTIs in prepubertal and postmenopausal women and spayed dogs.[556] Furthermore, women with recurrent UTIs have been speculated to have decreased concentrations of secretory immunoglobulin A in their urine.[557] Although continued urine production dilutes proliferating bacteria, once pathogens have gained access to the bladder, the rate of replication far outweighs any dilution effect and allows the UTI to become established.[5] Although antibiotic therapy is highly effective in eliminating most UTIs, recurrent UTIs can be a challenge to manage. In addition to thorough evaluation to eliminate predisposing factors in these patients, one may consider additional approaches to prevention. For example, fimbrial vaccines are effective against experimental UTIs in monkeys.[552]

URETHRITIS

Bacterial urethritis has been described as a cause of hemospermia in stallions[558,559]; however, with the exception of traumatic, parasitic (habronemiasis), or neoplastic conditions of the penis or urethra that interfere with urine flow, the author is unaware of documented cases of primary bacterial urethritis resulting in dysuria.[87,560] Furthermore, hemospermia likely attributable to urethritis in a number of previous cases probably was caused by urethral rents at the ischial arch that involve the corpus spongiosum penis, which have become easier to identify with urethral videoendoscopy (see Hematuria).[561] Bacterial infections of accessory sex glands or the prepuce also may cause dysuria. Accessory sex gland infections generally are limited to intact males and are more likely to cause infertility or hemospermia than dysuria.[87,562] Preputial infections can occur after trauma, presence of a foreign body, habronemiasis, or neoplasia, and affected horses typically have a malodorous, swollen sheath. Examination of the sheath and penis, along with biopsy of abnormal tissue, allows diagnosis of the primary problem. Occasionally, an older gelding may develop recurrent sheath swelling or infection that cannot be attributed to a primary disease process. The pathogenesis of this problem is not known, although fat accumulation, poor hygiene, and inactivity may be contributing factors. Treatment involves repeated sheath cleaning, application of topical anti-inflammatory and antibacterial ointments, and when involvement is more severe, systemic antibiotic administration.

CYSTITIS

Primary cystitis is uncommon in horses, and predisposing causes should be ruled out whenever cystitis is suspected. Bacterial cystitis is usually a secondary problem that may accompany alterations in urine flow caused by urolithiasis, bladder neoplasia, bladder paralysis, an anatomic defect of the bladder or urethra, or entry into the urinary tract (e.g., catheterization, endoscopy).* Dysuria may be manifested by pollakiuria, stranguria, hematuria, or pyuria. One may observe scalding and accumulation of urine crystals on the perineum of affected mares or on the front of the hindlimbs of affected male horses. These findings should not be confused with normal estrous activity in the mare. Although nosocomial UTIs are a well-documented problem in hospitalized human[5] and small animal patients,[567] this complication has not been well recognized in equine patients except for ill neonatal foals.

Diagnostic evaluation includes physical and rectal examinations and collection of a urine sample for urinalysis and quantitative bacterial culture. In the absence of uroliths or other bladder masses, transrectal palpation of the bladder is usually within normal limits; however, endoscopic and ultrasonographic examination of the bladder may be helpful in assessing mucosal damage and wall thickening in horses with cystitis.[335,354] Because normal equine urine is rich in mucus and crystals, gross examination may be unrewarding, but sediment examination may reveal increased numbers of white

* References 70, 95, 389, 434, 553, 554, 563-566.

blood cells (more than 10 leukocytes per high-power field) and presence of bacteria in some cases of cystitis. Normal sediment examination results do not rule out UTI. A definitive diagnosis requires quantitative culture results exceeding 10,000 colony-forming units (CFUs) per milliliter in a urine sample collected by midstream catch or urethral catheterization.[95,279,549] For best results, one should evaluate urine sediment within 30 to 60 minutes of collection and should cool samples for culture during transport because bacterial numbers may increase in samples left at room temperature. Organisms that may be isolated on culture include *E. coli, Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* and rarely *Corynebacterium renale.*[70,95,434,549,564] Isolation of more than one organism is common. *Salmonella* spp. occasionally have been isolated from the urine of apparently healthy horses, and *Candida* spp. infections of the lower urinary tract also have been documented in sick neonatal foals receiving broad-spectrum antibiotics.[70,95]

Successful treatment of bacterial cystitis requires correction of predisposing problems such as urolithiasis and administration of systemic antibiotics. Ideally, selection of an antibiotic is based on the results of susceptibility testing of isolated organisms, and the initial recommended course of treatment traditionally is at least 1 week.[70,95,434,549] A trimethoprim-sulfonamide combination, ampicillin, penicillin, and an aminoglycoside or ceftiofur can be initial choices. In human beings with uncomplicated cystitis, single-dose antimicrobial therapy, which is less costly and is associated with fewer adverse effects, has a success rate comparable with that of longer term conventional therapy (75% or greater). Furthermore, relapse after single-dose therapy is not accompanied by more severe clinical signs or more extensive urinary tract involvement[5]; however, if clinical signs recur after treatment is discontinued, one should repeat urine culture along with additional diagnostic evaluation to determine a cause for altered urine flow or bacterial persistence.

Treatment of recurrent UTI usually requires long-term medication (4–6 weeks), and ease of administration and cost become additional considerations in antibiotic selection. Trimethoprim-sulfonamide combinations and β-lactams are excreted via the kidneys and concentrated in urine. Although results of in vitro susceptibility testing of isolated pathogens may reveal resistance, these drugs may have effective antimicrobial activity against the causative organisms because of their high concentrations achieved in urine.[549] Metabolism of the antibiotic is another consideration. For example, sulfamethoxazole is metabolized mostly to inactive products before urinary excretion, whereas sulfadiazine is excreted mostly unchanged in urine.[568]

Additional treatments for recurrent UTI can include supplementation with 50 to 75 g of loose salt in the diet[556] or provision of warm water during cold weather in an attempt to increase water intake and urine production. Administration of a urine-acidifying agent like ammonium chloride (20–40 mg/kg PO daily) also has been recommended in cases of cystitis and urolithiasis.[563] Use of ammonium chloride at this dose, however, has not produced a consistent decrease in urine pH. Larger oral doses of ammonium chloride (60–520 mg/kg/day),[569-571] methionine (1 g/kg every 24 hours), vitamin C (1–2 g/kg/day),[281] or ammonium sulfate (175 mg/kg/day)[572] were more successful in reducing urine pH to less than 6.0 in a limited number of horses; however, at these doses, medications were usually unpalatable and had to be administered by dose syringe or stomach tube. Adding grain to the diet is another simple way to decrease urine pH, although the decline is modest, and urine pH typically remains greater than 7.0.[281] A final treatment aid is bladder lavage. This procedure most benefits horses with accumulations of large amounts of crystalloid material in the bladder, a condition that has been termed *sabulous urolithiasis.*[572] Whenever sabulous urolithiasis is present, neurologic control of bladder function should be assessed. Incomplete emptying of the bladder can result in excessive accumulation of urinary sediment. Although one can add a number of antiseptics to the sterile polyionic lavage fluid, the most important consideration is adequate volume to flush the crystalline debris completely from the bladder. Concurrent cystoscopy is a useful tool to assess the efficacy of bladder lavage.

One report of experimental induction of cystitis in equids exists.[573] After chemical irritation of the bladder mucosa, 2.5×10^{13} CFUs of *Proteus mirabilis* were instilled into the bladders of nine female ponies. Three days later all ponies demonstrated stranguria, and culture results yielded 20,000 to 100,000 CFUs/mL of *P. mirabilis.* Sediment examination revealed increased numbers of white blood cells (more than 10 per high-power field) in seven of nine ponies, and bacteria were observed in all samples (although in low numbers of one to three per high-power field). In two untreated ponies, the cystitis resolved spontaneously between 2 and 4 weeks after inoculation; however, resolution was complete within 3 to 6 days in ponies treated with trimethoprim-sulfadiazine.

Epizootics of cystitis also have been reported in the southwestern United States[564,574] and Australia.[575] In the former reports, a syndrome of ataxia and urinary incontinence was associated with ingestion of Sudan grass and Johnson grass (hybrids of *Sorghum* spp.). Both problems were attributed to sublethal intoxication with hydrocyanic acid in the plants, which resulted in demyelination of the lower spinal cord and bladder paralysis. Pyelonephritis was often the cause of death in affected horses.[574] Another outbreak of cystitis, manifested by hematuria more than incontinence, occurred in the Northern Territories and Western Australia in 1963.[575] The kidneys and ureters were not affected, and ataxia was not observed in these horses. The problem, which resulted in loss of more than 200 horses, began shortly after the end of the wet season. Affected horses were on range pasture, and, although this was not proved, a fungal toxin was suspected because sporidesmin (a toxin produced by *Pithomyces chartarum*) was known to cause bladder lesions in sheep and cattle. An environmental cause was substantiated further when no additional cases developed in 1964, after a "dry" wet season.

PYELONEPHRITIS

Upper UTIs involving the kidneys and ureters are rare in horses.[70,95,549] The course of the distal segment of the ureters into the dorsal bladder wall creates a physical barrier or valve to prevent vesicoureteral reflux, a prerequisite for ascending pyelonephritis. Vesicoureteral reflux is more common in children because the intramural portion of the distal ureter lengthens with growth. Furthermore, congenital vesicoureteral reflux often occurs in families, which suggests a genetic tendency for the problem, which has been associated with developmental anomalies of the intramural insertion of one or both ureters.[5] More obvious problems that interfere with this barrier and increase the risk for vesicoureteral reflux

and associated upper UTIs include ectopic ureter or bladder distention, which may occur with bladder paralysis or urethral obstruction. Over time, vesicoureteral reflux leads to progressive ureteral dilation and renal scarring (Fig. 14.30). This effect explains the common finding of ureteral dilation in young horses with ectopic ureters and provides further support for unilateral nephrectomy, rather than reimplantation of the ureter as the treatment of choice for ectopic ureter.[69,76] In addition to vesicoureteral reflux, intrarenal urinary reflux also is required to initiate renal damage, predisposing the parenchyma to ascending infection. The renal papillae contain collections of papillary duct openings. These are typically conical structures that protrude into the renal pelvis. Similar to the protective nature of the intramural ureteral segment against vesicoureteral reflux, this morphology protects against intrarenal reflux; however, in human infants and young pigs, a second type of large, concave, "refluxing" papillae also have been described in the areas of the renal pelvis most often affected with renal scarring.[5] Thus vesicoureteral and intrarenal reflux are important in development of pyelonephritis.

The role of recurrent lower UTI in the development of pyelonephritis is less clear. For example, pyelonephritic scarring (without infection) can develop after high-pressure urinary tract obstruction (urethrolithiasis) and can predispose to future upper UTI. In contrast, many cases of recurrent cystitis never proceed to involve the upper urinary tract.[5] Because the kidneys are densely vascular organs, septic nephritis may develop in association with septicemia in neonatal or adult horses.[550] Unless renal involvement is extensive, upper UTI may go undetected but could lead to development of nephrolithiasis or chronic kidney disease months to years later. As in the bladder, defense mechanisms within the normal kidney act to minimize bacterial colonization and proliferation. Efficacy of renal clearance varies with the species of bacteria entering the kidney. In addition, obstruction of urine flow (unilateral ureterolithiasis or nephrolithiasis) increases, rather than decreases, the risk of bacterial proliferation in the obstructed kidney.

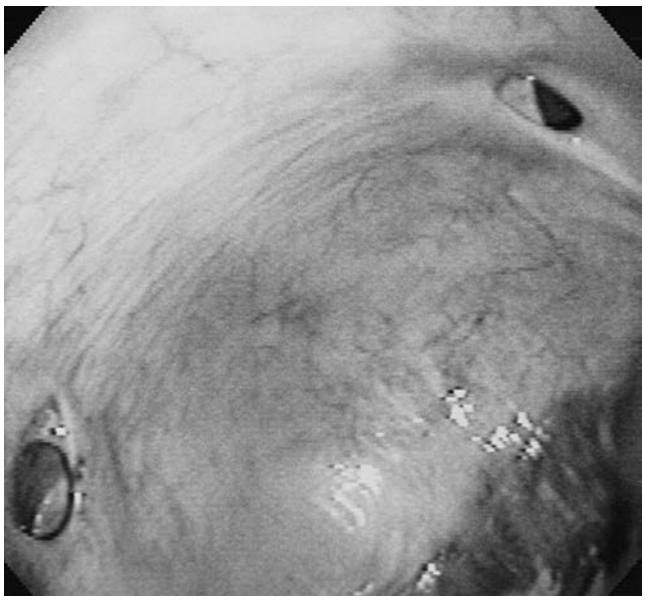


FIG. **14.30** Endoscopic image of the bladder of a pony mare with recurrent cystitis and pyelonephritis. The ureteral openings were distended easily by insufflation of the bladder with air. Ureteral dilation resulted from long-term vesicoureteral reflux.

Pyelonephritis in horses has been described in association with urolithiasis, recurrent cystitis, and bladder paralysis.[70,95,389,549,574] Other causes have included accidental amputation of the penis during castration,[576] foreign bodies in the bladder,[467] and lower urinary tract neoplasia.[465,565] With pyelonephritis, dysuria is manifested by hematuria or pyuria rather than stranguria and pollakiuria (as for cystitis). Some cases of pyelonephritis may have severe hematuria. In addition, horses with upper UTIs generally have other clinical signs, including fever, weight loss, anorexia, or depression.* Upper UTIs also can be accompanied by nephrolithiasis, ureterolithiasis, or both.[266] In such cases, whether urolithiasis or infection develops first or whether both are consequences of vesicoureteral reflux, intrarenal reflux, and renal parenchymal damage is unclear. In an occasional case, small uroliths may travel down the ureter and lead to recurrent urethral obstruction with renal colic as the presenting complaint.

As for cystitis, diagnostic evaluation includes physical and rectal examinations, urinalysis, and a quantitative urine culture. Careful palpation may allow detection of an enlarged ureter or kidney, although the kidney also may become shrunken in long-standing cases. In addition to the organisms causing cystitis, one can isolate *Actinobacillus equuli, Streptococcus equi* subsp. *equi, Rhodococcus equi,* or *Salmonella* spp. from horses with hematogenous septic nephritis.[95,550] In horses with upper UTIs, one should perform a CBC and serum biochemistry profile to assess the systemic inflammatory response and renal function. Finally, cystoscopy (including watching for urine flow from each ureteral opening) and ultrasonographic imaging of the bladder, ureters, and kidneys are helpful adjunct diagnostic procedures.[335,354] Ureteral catheterization (by passing polyethylene tubing via the biopsy channel of the endoscope or by using a no. 8–10 Fr polypropylene catheter, which can be passed blind in mares) may allow collection of urine samples from each ureter to distinguish unilateral from bilateral disease.[362]

Treatment for upper UTIs includes a prolonged course of appropriate systemic antibiotics (selected based on susceptibility testing results on isolated pathogens). In select cases with unilateral disease, one may consider surgical removal of the affected kidney and ureter.[87,255,256] Prerequisites for a nephrectomy include documentation of unilateral disease by normal laboratory results for renal function (absence of azotemia), recovery of insignificant numbers of bacteria (fewer than 10,000 CFUs/mL) from urine collected from the ureter leading to the unaffected kidney, and ultrasonographic evidence of abnormal structures (e.g., fluid-filled structures, nephrolithiasis) in the affected kidney. Alternatively, poor response to several weeks of appropriate antimicrobial therapy and recurrence of clinical signs of pyelonephritis are additional indications for nephrectomy. Unfortunately, successful treatment of bilateral pyelonephritis is rare, but the poor prognosis likely is related to failure to establish the diagnosis until late in the disease course.

PARASITIC INFECTIONS

One occasionally finds parasitic lesions associated with the nematodes *Strongylus vulgaris, Halicephalobus gingivalis*

* References 70, 95, 255, 256, 453, 463, 464, 466, 479, 549.

(previously *Micronema deletrix*), and *Dioctophyma renale* in equine kidneys.[64] Although larval migration of *S. vulgaris* in the renal artery and parenchyma is considered aberrant,[577] larval migration was found in more than 20% of horses in one abattoir survey. Passage through renal tissue may result in infarction or subcapsular or pelvic hemorrhage when parasites localize in these sites.[578] Although rare, *H. gingivalis* infection is often life threatening because of concurrent CNS involvement leading to a variety of neurologic deficits that generally require euthanasia.[579-583] *Halicephalobus gingivalis* has been suggested to be the most important cause of verminous meningoencephalomyelitis in horses.[584] Only the female parasite has been identified in equine tissues, typically in highly vascular organs. One usually finds large, granulomatous lesions that are full of the rhabditiform nematodes in the kidneys. The life cycle of *H. gingivalis* is unknown, but the apparent saprophyte appears to have worldwide distribution. The finding of gingival lesions and oral granulomata in some horses suggests that ingestion is the likely route of infection. Attempts to find nematode larvae or eggs in urine have been unsuccessful, and whether the horse is an accidental host or is important for the life cycle of the parasite is unclear.[579] The free-living form is found in decaying organic debris (e.g., tree stumps) and also has been reported to affect human beings.[583] Antemortem diagnosis has not been made, and cytologic changes in CSF from affected horses cannot differentiate between nematodiasis and protozoal encephalomyelitis.[582] Renal involvement is typically inapparent, although one affected horse demonstrated a 2-week course of stranguria and polyuria before onset of neurologic deficits.[580]

Dioctophyma renale is a large, bright-red nematode, and the female may reach 100 cm in length. The typical hosts are carnivorous species, but the parasite occasionally affects horses that ingest the intermediate host (annelid worm) while grazing or drinking from natural water sources.[585] Once localized in the kidney, the parasite may live 1 to 3 years; eggs are shed in the urine. The parasite completely destroys the renal parenchyma, and death of the parasite leads to shrinking of the host kidney into a fibrous mass. Occasionally, hydronephrosis or renal hemorrhage may be a serious complication of parasitic infection.[586,587]

In contrast to the nematodes, infection with the coccidian parasite *Klossiella equi* appears common, yet no reports describe clinical disease associated with this coccidial infection.[588-593] Although disorders accompanied by immunosuppression have been suggested to increase its likelihood, *K. equi* infection is still considered an incidental finding in affected horses. The life cycle has not been elucidated fully, but one proposal is that ingested sporocysts (or sporozoites) enter the bloodstream and undergo schizogony in endothelial cells of the glomeruli. Merozoites are released into the urinary space and undergo one or more additional rounds of schizogony in tubular epithelial cells. Eventually, a population of merozoites develop into microgametes and macrogametes. Little evidence of an inflammatory response to parasite replication in renal tissues is apparent. Sporogony follows with the subsequent release of sporocysts into the urine.[589,593] Although *K. equi* has not been associated with clinical disease, it warrants mention that the organism has been found worldwide in horses, ponies, donkeys, burros, and zebras, and a postmortem survey of 47 horses in Australia revealed that six (12.8%) were infected.[593]

Obstructive Disease of the Urinary Tract

Harold C. Schott II

Most cases of obstructive urinary tract disease in horses are caused by urolithiasis. Urinary tract displacement, trauma, and neoplasia are other causes.[70,95,434] Urinary tract obstruction can result in a variety of clinical signs depending on the site and degree of obstruction. Incomplete obstruction can result in dysuria, incontinence, and mild abdominal pain, whereas complete obstruction usually results in moderate to severe pain termed *renal* or *kidney colic*. True "kidney colic" is an uncommon cause of abdominal pain, although horses may show signs similar to those of dysuria with gastrointestinal pain. Another complication of complete obstruction is rupture of the bladder or urethra. Signs of pain subside after rupture but are replaced by depression and inappetance, which accompany postrenal acute renal failure. In some cases of disruption of the urinary tract, one also may observe progressive abdominal distention and enlargement of the ventral midline, penis, and prepuce.

EPIDEMIOLOGY OF UROLITHIASIS

The epidemiology of urolithiasis varies with species.[389,563,594,595] Lower urinary tract stones predominate in veterinary species, whereas upper urinary tract stones are more common in human beings. Historically, lower urinary tract stones were a more substantial problem in human beings as well, and they remain the more common form of urolithiasis in underdeveloped countries. The shift in prevalence from lower to upper urinary tract stones appears to have accompanied industrialization, but the reasons for this are not entirely clear.[595]

From 1970 to 1989, urolithiasis was responsible for 0.11% of equine admissions to 22 veterinary teaching hospitals and accounted for 7.8% of the diagnoses of urinary tract disease.[389] Male horses, especially geldings, are predisposed to urolithiasis (75% of all cases), and a breed predisposition has not been described. This sex predilection has been attributed to the shorter, distensible urethra of the mare, which likely permits voiding of small calculi.[563] Urolithiasis is typically an adult disease, and the mean age of affected horses is about 10 years.[389] Nevertheless, young horses can be affected, and the author has seen bilateral nephrolithiasis in a weaning foal (likely a consequence of neonatal septicemia) and dysuria in a 3-month-old colt caused by multiple cystoliths that formed on sutures used to repair a ruptured bladder as a neonate. Uroliths are most common in the urinary bladder (60%), although they also may develop in the kidneys (12%), ureters (4%), and urethra (24%).[389] Interestingly, as many as 10% of affected horses have uroliths in multiple locations.[389] Uroliths can vary greatly in size. In one mare, a cystolith weighing more than 6 kg was present as an apparently incidental finding in a horse destroyed for a limb fracture.[596]

PATHOPHYSIOLOGY OF UROLITHIASIS

In general, two steps are required for calculus formation: (1) nucleation and (2) crystal growth.[5,597-599] Factors that contribute to precipitation of urinary crystals and nucleation include

supersaturation of urine; prolonged urine retention; genetic tendencies to excrete larger amounts of calcium (hypercalciuria), uric acid (hyperuricosuria), or oxalates (hyperoxaluria); and inhibitors and promoters of crystal growth. For two or more ions in a solution to precipitate into a crystal, the product of their individual ion activities must exceed the equilibrium solubility product (K_{sp}). A supersaturated solution is one in which the ion activity product exceeds the K_{sp}. A mildly supersaturated solution is termed *metastable,* because crystals tend to precipitate and dissolve at similar rates, so that crystal growth does not occur and the solution remains clear. However, once the ion activity product exceeds a critical value (formation product ratio), precipitation outpaces dissolution, rapid crystal growth occurs, and the solution becomes cloudy.[5,597-599] Normal human urine typically is supersaturated with one or more ion activity products; however, the formation product ratios are considerably higher in urine (10 times greater than K_{sp}) than they are in an aqueous solution because of the presence of inhibitors of crystal growth.[5] This activity explains why observation of crystals in urine sediment examination is common, yet calculus formation is uncommon. Furthermore, although K_{sp} values are constant for each type of crystal, they vary with temperature and pH. Typically, cooling promotes crystal formation (as when samples are refrigerated), whereas effects of pH vary with the type of calculus (acidification leads to dissolution of calcium crystals but promotes crystallization of urate crystals).[597] Next, any problem resulting in urine retention or incomplete bladder emptying increases the chance of crystal growth. Although not described for horses, genetic variations in ion excretion rates are well-documented risk factors for human and canine urolithiasis. For example, hypercalciuria is inherited as an autosomal dominant trait in human beings and is responsible for 30% to 40% of nephroliths.[5] Similarly, dogs with cystine urolithiasis have an inherited defect in renal tubular transport of cystine, whereas Dalmatians are afflicted with urate stones because of a defect in uric acid metabolism in the breed.[594]

Normal urine is rich in a number of inhibitors of crystal growth, including pyrophosphate, citrate, magnesium ions, glycosaminoglycans, and several glycoproteins, including nephrocalcin.[5,597,599] The degree of inhibitory activity varies with crystal type; for example, pyrophosphate is responsible for 50% of the inhibitory activity against calcium phosphate stone formation in human urine but has a much less inhibiting effect on calcium oxalate stone formation.[5] Although poorly documented, inhibitors of crystal growth in equine urine, including its high mucus content, likely play an important protective role against calculus formation, which would seem especially true in light of the substantial urinary excretion of $CaCO_3$ crystals by normal horses. Similar to the risk associated with increased ion excretion, one should not be surprised that defects in inhibitor activity also have been documented in syndromes of human urolithiasis.[5,597] Other urine components may act as promoters of crystal growth. These components principally include organic matrix components of calculi: matrix substance A, uromucoid, and a number of serum proteins.[597,598] Finally, some urine components may have inhibitor and promoter activity. For example, Tamm-Horsfall glycoprotein, a protein secreted in the ascending limb of the loop of Henle that forms the backbone of urine casts, has been shown to promote struvite crystal formation in feline urine.[600] In contrast, this glycoprotein also inhibits calcium oxalate crystal aggregation, and a group of human patients with calcium oxalate urolithiasis were demonstrated to have an abnormality in Tamm-Horsfall mucoprotein.[601]

Because normal urine of most species typically is supersaturated and is in balance between crystal precipitation and dissolution, spontaneous nucleation rarely initiates calculus growth. Rather nucleation generally requires stasis of urine flow, increasing the chance of contact between crystalloid material and uroepithelium (as occurs in areas of the renal pelvis) or a damaged uroepithelial surface.[597-600] The latter results in local activation of inflammatory and clotting pathways, producing a nidus for local crystal adherence.[602] In addition, desquamated epithelial cells, leukocytes, or necrotic debris may provide a nidus for crystal growth at more distal sites in the urinary tract. Tissue damage from a variety of causes is likely the most important factor for the development of uroliths in horses. For example, after urinary tract catheterization or endoscopy, areas of traumatized uroepithelium are covered rapidly with a fine layer of crystalline material. This material usually resolves spontaneously unless infection develops. Similarly, nephroliths may form in the renal medulla or pelvis after renal papillary necrosis secondary to NSAID toxicity. Once crystal growth begins around a nidus, equine urine has the disadvantage of being highly alkaline, favoring crystallization of most urolith components, especially $CaCO_3$.

Horses develop two basic forms of uroliths, and both are composed primarily of $CaCO_3$.[563,603] More than 90% are yellow-green spiculated stones that easily fragment (type I urolith) (Fig. 14.31A). Less commonly, uroliths are gray-white smooth stones that are more resistant to fragmentation (type II urolith; Fig. 14.31B). The latter stones often contain phosphate in addition to $CaCO_3$. The crystalline composition of normal equine urine sediment ($CaCO_3$ crystals predominate, although calcium oxalate and phosphate crystals also occur) and uroliths is similar: $CaCO_3$ in the form of calcite (a hexagonal crystal form of $CaCO_3$) is most common, followed by vaterite (a metastable, hexagonal crystal form in which $CaCO_3$ is replaced partially by magnesium or to a lesser extent by manganese, strontium, and sulfur). Other less common components include aragonite (an orthorhombic crystal form of $CaCO_3$), weddellite (calcium oxalate dihydrate), struvite (magnesium ammonium phosphate hexahydrate), hydroxyapatite, and uric acid (Table 14.10).[290,603-607] Neumann and colleagues[603] examined the cut surface of a number of equine uroliths by scanning electron microscopy and described a pattern of irregular, concentric bands around the core (Fig. 14.32A) that were separated by small spherules of crystalline material (Fig. 14.32B). This pattern suggested that calculus growth occurs by accretion of preexisting microscopic spherules (the crystals already present in normal equine urine) on the surface of the growing urolith rather than de novo crystal formation at the surface of the urolith. Furthermore, banding was speculated to represent growth through incorporation of organic matrix on the surface of the urolith at times when fewer spherules were present in urine. The gaps between adjacent spherules result in porosity to the urolith. Because precipitation and dissolution occur simultaneously during growth of urinary calculi, porosity allows exposure of inner aspects of the urolith to urine, which can lead to dissolution as urine composition changes. Neumann and colleagues[603] described two types of porosities observed by electron microscopy: (1) primary porosity, consisting of the original pores or gaps between spherules, and (2) secondary porosity, which developed after dissolution of inner areas of the uroliths. Greater dissolution or secondary porosity developed preferentially in areas of the urolith with

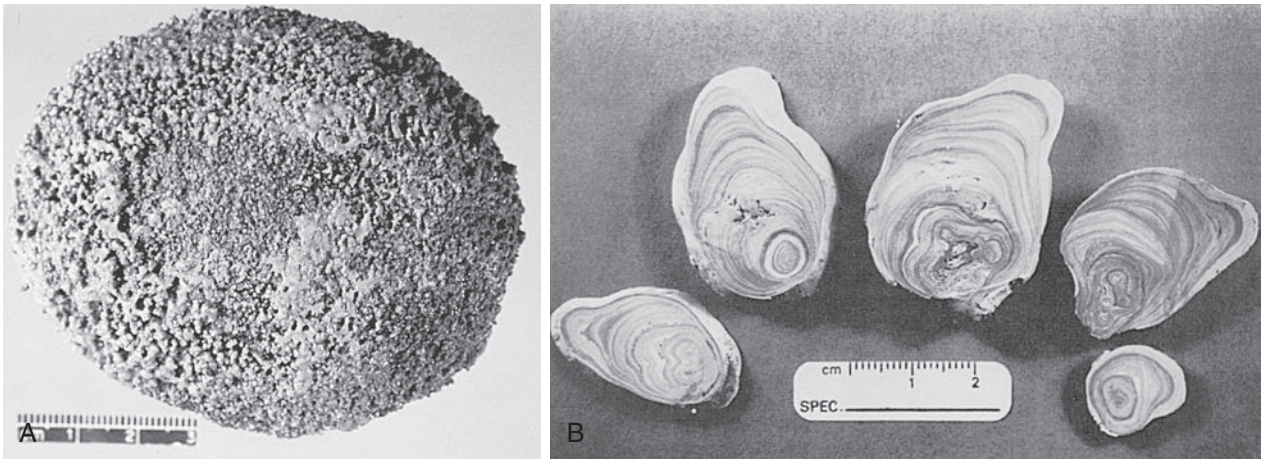


FIG. **14.31** Equine cystic calculi. **A,** The more common flattened, spherical type of bladder calculus usually is spiculated. **B,** The less common form of gray, smooth-surfaced calculus may be more irregular in shape. (**B** from DeBowes RM: Surgical management of urolithiasis, *Vet Clin North Am Equine Pract.* 4:461, 1988.)

TABLE **14.10 Published Results of Radiographic Crystallographic Analysis of Equine Urinary Calculi**

Type of Calculus	Reference			
	604	605	290	603
Total number of calculi	4	157	18	17
Calcite	2	58	2	11
Calcite/vaterite	1	11	9	5
Calcite/aragonite	1	—	—	1
Calcite/weddellite	—	63	4	—
Calcite/whewellite	—	2	—	—
Calcite/hydroxyapatite	—	8	—	—
Calcite/octacalcium phosphate	—	5	—	—
Calcite/struvite	—	3	—	—
Calcite/gypsum	—	2	—	—
Calcite/vaterite/weddellite	—	2	2	—
Calcite/whewellite/weddellite	—	3	—	—
Calcite/vaterite/sodium acid urate	—	—	1	—

higher magnesium content (vaterite). More extensive development of secondary porosity theoretically leads to increased urolith fragility, which has the therapeutic benefit of increasing the chance that the urolith can be crushed or fragmented before removal.

The role of UTI in the development of urolithiasis appears to vary with species.[5,594,598] Struvite urolithiasis in human beings and dogs appears to be almost exclusively a consequence of UTI, whereas most struvite uroliths in cats and sheep are not associated with infection.[598] In addition to contributing to uroepithelial injury and nidus formation, UTI with urease-positive bacteria (*Proteus* spp. and coagulase-positive staphylococci are most common) allows splitting of urea into two ammonia molecules, which are hydrolyzed rapidly to ammonium ions (a component of struvite crystals).[5,598,599] In a review of 68 horses with urolithiasis, Laverty and colleagues[389] reported positive urine culture results in only 2 of 19 horses in which urine culture was performed; however, culture of material from the centers of 30 calculi yielded positive results from 19 (63%), and a variety of different bacterial species were isolated. Only 1 of 28 calculi examined in this study contained struvite. The significance of finding bacteria in the center of equine $CaCO_3$ uroliths is not known, especially for isolates other than coagulase-positive staphylococci and *Proteus* spp.; culture of an appropriately collected sample of urine always is preferred over culture of a calculus.[389,608] Nephroliths and ureteroliths also are found in some cases of pyelonephritis.[461-463,479] Laing and colleagues[462] described a 2-year-old colt with bilateral nephrolithiasis and chronic kidney disease. *Proteus* spp. were isolated from urine, and the nephroliths were composed principally of $CaCO_3$ but also contained lesser amounts of struvite. In contrast, Ehnen and colleagues[388] found evidence of infection in only one of eight horses with nephrolithiasis or ureterolithiasis (or both) and chronic kidney disease. In the author's experience, the presence of stones in the upper urinary tract or presence of multiple uroliths warrants concurrent evaluation for UTI, because the author has seen two horses with recurrent urinary tract obstruction with ureteroliths that were determined ultimately to be sequelae of unilateral pyelonephritis. Holt and Pearson[609] described a similar case in which a renal calculus and abscess were found 5 months after removal of a cystic calculus.

NEPHROLITHIASIS AND URETEROLITHIASIS

It used to be that renal or ureteral calculi rarely were described as forms of equine urolithiasis[610]; however, since the advent of diagnostic ultrasound, nephrolithiasis and ureterolithiasis are now more commonly recognized.[257,360,361,388,389,459-462] In a review of 68 horses with urolithiasis by Laverty and colleagues,[389] 16% had uroliths in the kidneys and ureters, and a few horses with cystic calculi also had calculi in the upper urinary tract. Interestingly, 9 of 15 horses with nephroliths in this review were stallions, 3 of 15 were geldings, and 3 of 15 were mares. Whether a true increase in prevalence of nephrolithiasis and ureterolithiasis has occurred, or whether

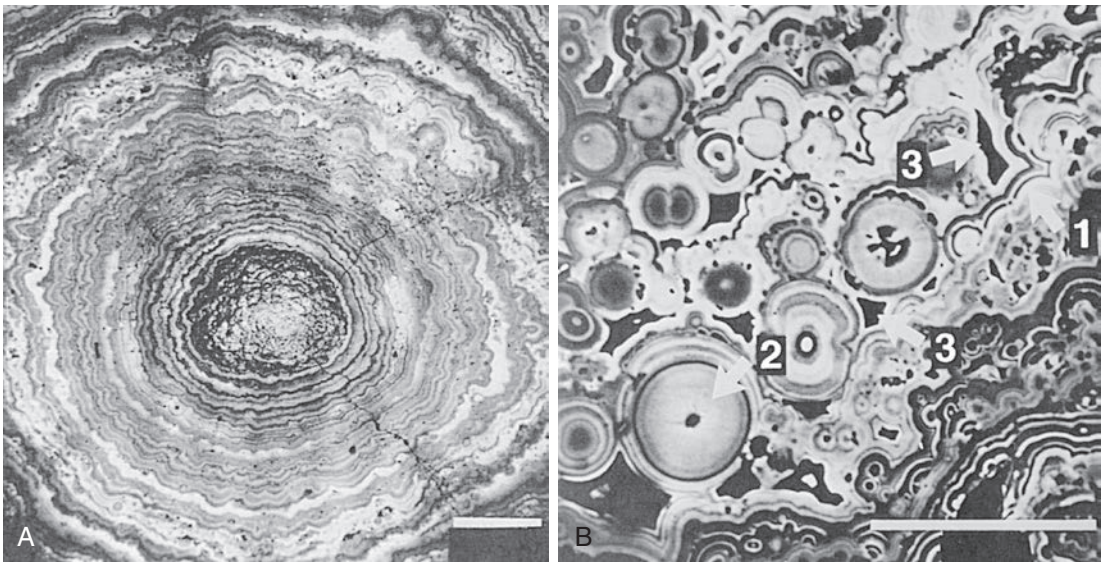


FIG. **14.32** Scanning electron microscopic appearance of the cut surface of equine cystic calculi. **A,** Lower power micrograph reveals the intricate pattern of concentric banding around the core (bar = 500 μm). **B,** Higher power micrograph reveals the ultrastructural features, including bands (1), spherules (2), and primary porosity in black (3) (bar = 50 μm). (From Neumann RD, Ruby AL, Ling GV, et al.: Ultrastructure and mineral composition of urinary calculi from horses, *Am J Vet Res.* 55:1357, 1994.)

these conditions have become easier to document with the simultaneous development of ultrasonographic imaging as a diagnostic tool for equine medicine, is not clear. An undocumented speculation is that young racehorses may be at greater risk of developing renal calculi because of the common use of NSAIDs (and increased risk for development of papillary necrosis) in these athletes.[388] The important point is that one should not overlook upper urinary tract lithiasis in horses.

Nephroliths may develop around a nidus associated with a variety of renal diseases, including polycystic kidney disease (see Fig. 14.6), pyelonephritis (Fig. 14.33A), papillary necrosis (Fig. 14.33B), or neoplasia. At present, data on upper urinary tract stones in horses are insufficient to know if they develop spontaneously (in the absence of tissue damage) as in human beings or if they differ significantly in mineral composition from cystic calculi. Although nephrolithiasis and ureterolithiasis are painful conditions in human beings, horses with nephroliths or ureteroliths often remain asymptomatic until bilateral obstructive disease leads to development of acute or chronic renal failure.

Upper urinary tract stones also may be an incidental finding at necropsy.[610] When clinical signs are present, nonspecific presenting complaints consistent with uremia (poor performance, lethargy, inappetance, and weight loss) are more common than signs of obstructive disease (colic, stranguria, hematuria). Occasionally in the horse, a stone or nidus may pass down the ureter and lead to urethral obstruction and signs of acute obstructive disease. Rectal palpation may reveal an enlarged kidney or ureter (or bladder with urethral obstruction), and ureteral calculi may be palpable in an enlarged ureter. Because normal ureters are not palpable on rectal examination, one should palpate the entire course of the ureters (retroperitoneally along the dorsal abdominal wall to the dorsolateral aspects of the pelvic canal to their insertion at the dorsal bladder neck) because an enlarged ureter is easy to overlook.

One usually diagnoses renal and ureteral calculi during rectal or ultrasonographic examination (Fig. 14.34). Although ultrasonographic imaging may provide information on the presence, number, and location of calculi, one can miss stones smaller than 1 cm in diameter despite complete examination. Other ultrasonographic findings to support upper tract lithiasis include dilation of the renal pelvis or proximal ureter and, in long-standing cases, hydronephrosis.[336,339] Although azotemia generally accompanies bilateral disease, horses with unilateral disease often maintain normal renal function. For reasons detailed previously, one should perform a quantitative urine culture in all horses with nephrolithiasis or ureterolithiasis to assess possible concurrent UTI.

Because most horses with nephrolithiasis or ureterolithiasis have chronic kidney disease by the time the diagnosis is established,[266,388,480] few cases are amenable to treatment. Thus reports of successful management of horses with renal and ureteral calculi are few. Removal of the calculus, limited to horses with unilateral disease or mild azotemia, has been the only effective means of treatment.[257,360,361] In the absence of azotemia, nephrectomy is the preferred technique for management of unilateral renal calculi.[257] Furthermore, removal of the affected kidney and ureter should eliminate any associated upper UTI or chance of recurrence. The approach involves a dorsal flank incision, rib resection, and blunt retroperitoneal dissection to expose the kidney.[87,434,611] In one horse with mild azotemia, nephrotomy (via an approach similar to nephrectomy) was performed successfully to remove obstructing calculi in the renal pelvis and proximal ureter. Unfortunately, little improvement in azotemia occurred, and the horse was destroyed a few weeks later.[388] Ureteral calculi also have been removed by ureterolithectomy via ventral celiotomy and paralumbar approaches.[360,459] A basket stone dislodger (Dormia Stone Dislodger, V Mueller Co., McGow Park, IL), introduced through a vestibulourethral approach and guided by rectal

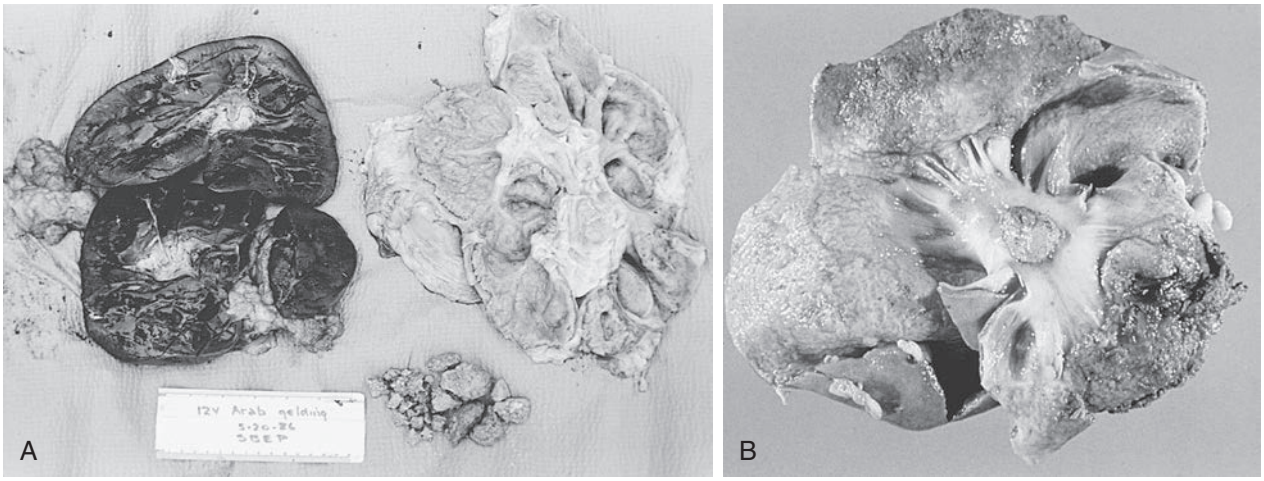


FIG. **14.33** **A,** Multiple nephroliths developed in association with unilateral pyelonephritis in a gelding that was presented for repeated urethral obstruction. **B,** A small nephrolith lodged in the renal pelvis resulted in ureteral obstruction and development of hydronephrosis in a Standardbred gelding that had a 4-year history of phenylbutazone therapy.

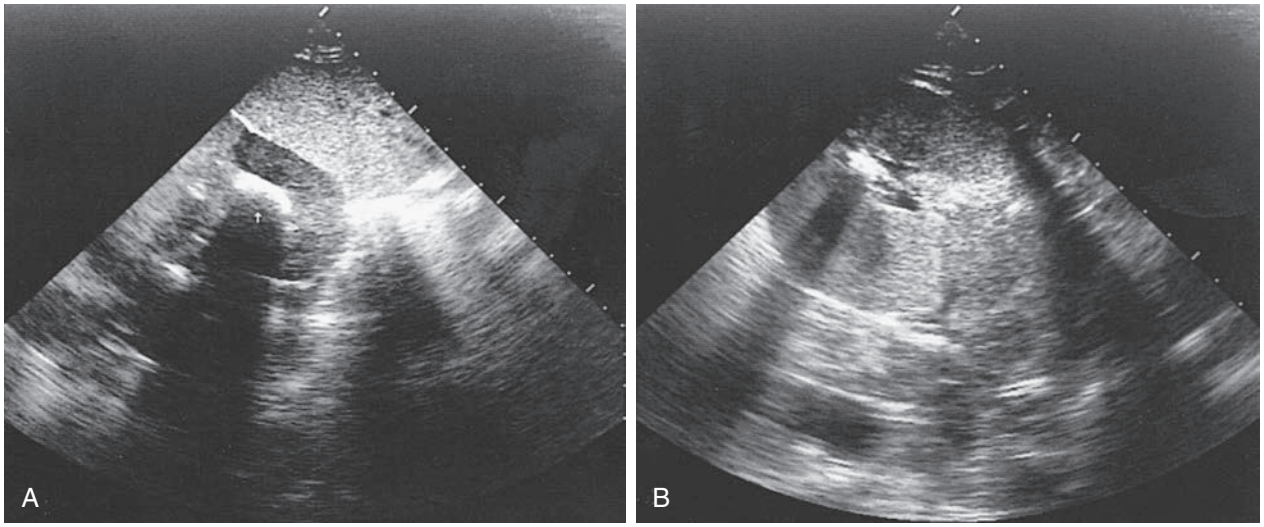


FIG. **14.34** Ultrasonographic images of the left, **A,** and right, **B,** kidneys of a 10-month-old Arabian filly that developed bilateral nephrolithiasis and chronic renal failure (CRF) as sequelae of neonatal septicemia. The nephroliths are highly echogenic and cast acoustic shadows in both kidneys.

palpation, also has been used for removal of distal ureteral calculi in the mare.[360] Medical management (antibiotics, grass hay, and salt to promote diuresis) of bilateral ureterolithiasis was attempted in a 3-year-old Thoroughbred filly with incomplete ureteral obstruction and mild azotemia.[459] After 4 weeks, deterioration of clinical signs and more severe azotemia prompted ureterolithectomy to remove stones from the left ureter. Percutaneous nephrostomy was used successfully for placement of a catheter in the right renal pelvis (to establish percutaneous urine flow) for short-term management of azotemia in the postoperative period. Unfortunately, the filly was destroyed after developing cecal impaction 6 days later, and necropsy revealed a shrunken, nonfunctional left kidney and a previously undetected nephrolith in the right kidney. This case demonstrated the feasibility and potential benefits of accessing the renal pelvis of the horse via percutaneous nephrostomy.[612,613]

Rodger and colleagues[361] described the successful use of electrohydraulic lithotripsy through a ureteroscope to disintegrate a single, unilateral ureterolith in a horse with evidence of bilateral renal disease. Electrohydraulic lithotripsy is a means of converting electric energy into mechanical energy that can be directed to fragment the urolith.[361,614,615] Basically, the device produces an electric discharge arc (a spark) between two electrodes at the tip of the instrument. The heat associated with the discharge causes a small amount of the liquid medium (urine) to burst into gas bubbles, and the associated shock wave fractures the urolith. One must keep the end of the instrument adjacent to the urolith yet away from the mucosa, which could be disrupted by the same shock waves that destroy the calculus. Although the technique has not been highly successful for treatment of canine uroliths,[616] equine uroliths may be more amenable to its use because they are commonly porous (and fragile). Although electrohydraulic lithotripsy has

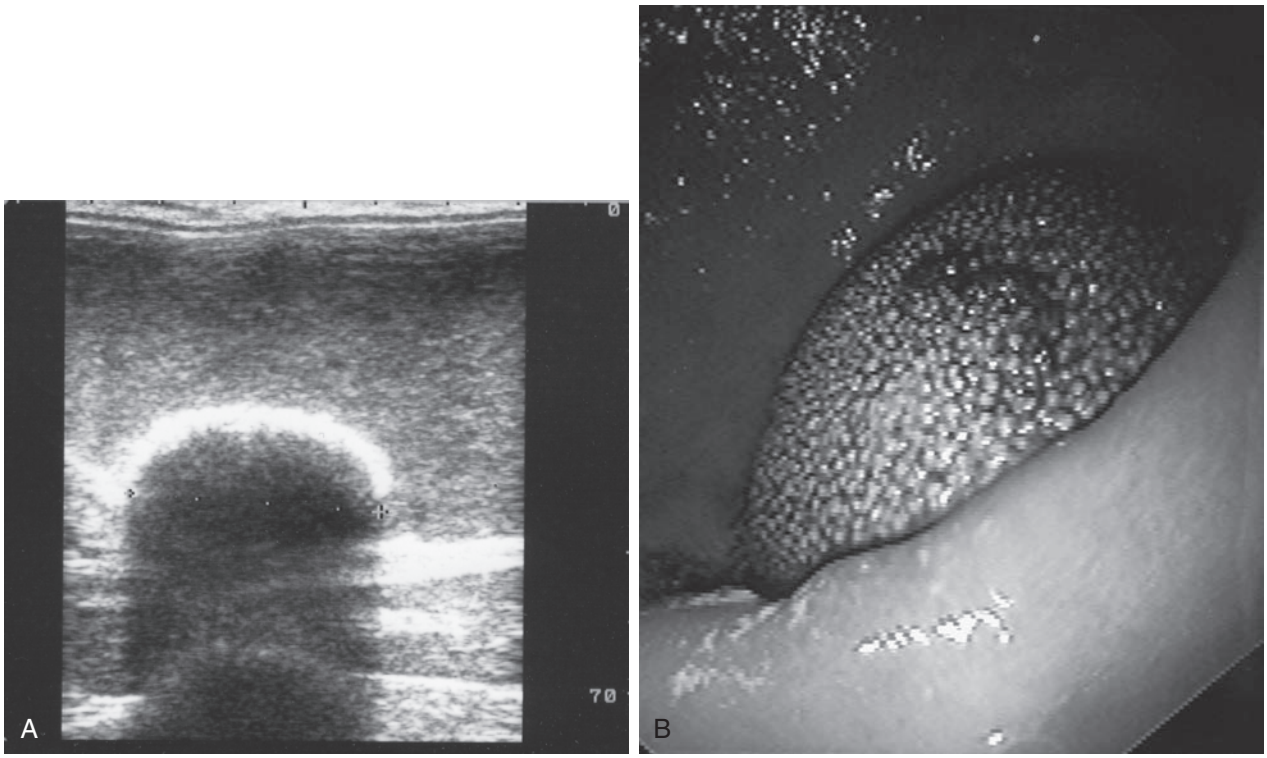


FIG. **14.35** **A,** Transrectal ultrasound examination of the bladder using a 5-mHz linear array probe. A single large calculus is present with acoustic shadowing ventral to the urolith. **B,** A type I urolith within the bladder as seen during cystoscopy.

been effective in treating selected equine cystoliths[614,615] and one ureterolith,[361] the expense of the equipment and availability of other surgical options likely will limit its use to selected cases that are not amenable to routine surgical treatments. Extracorporeal shock wave lithotripsy has been utilized for upper tract stone removal in human beings and dogs[613] and may have application in equids. This noninvasive technology uses a reflector to focus the energy from a shock wave generated outside the body on a nephrolith in situ and has proven to be an effective treatment for human nephrolithiasis.

CYSTIC CALCULI

Cystic calculi are the most commonly recognized form of equine uroliths.[70,95,389,434,563,609] Cystoliths typically are flattened, spherical stones with a spiculated or smooth surface. Dysuria resulting from cystoliths may include hematuria, stranguria, pollakiuria, pyuria, or incontinence. Hematuria is usually more apparent after exercise. An affected male horse may demonstrate stranguria by repeatedly dropping its penis and posturing to urinate but voiding little or no urine. An affected mare also may posture repeatedly to urinate and demonstrate winking, and these signs could be confused with estrous activity. Less common signs include an irritable attitude, recurrent colic, and loss of condition; one burro was presented for recurrent rectal prolapse.[617]

One usually diagnoses cystic calculi by palpation and ultrasound examination of the bladder per rectum (Fig. 14.35). Bladder uroliths are usually large enough to be detected easily; however, if the bladder is distended, one may need to empty it by passing a catheter to facilitate palpation of the stone. Bladder catheterization also allows assessment of urethral patency and collection of samples for urinalysis and quantitative culture. One should perform a CBC and serum biochemical profile to document whether anemia, inflammation, or azotemia has developed. Cystoscopic examination is helpful in assessing the size and number of cystic calculi and the severity of damage to the bladder mucosa, as well as any asymmetry in appearance or function of the ureteral openings (Fig. 14.36).[335,354] Because one may find calculi in multiple sites in the urinary tract, thorough evaluation of the upper urinary tract is warranted for all cases of cystic urolithiasis.

In contrast to upper urinary tract lithiasis, many reports[569,614,615,617-632] and several reviews detail the clinical signs and surgical options for management of cystic calculi.[570,571]

The size of the calculus, gender of the horse, and surgeon's preference play roles in treatment selection. The preferred technique in males, especially for larger stones, is laparocystotomy through a ventral midline or paramedian incision with the horse in dorsal recumbency under general anesthesia. Laparoscopic or laparoscopic-assisted cystotomy may also be used for urolith removal in geldings.[633,634] For removal of smaller cystoliths, one can perform a perineal urethrotomy in the standing male horse with the use of local or epidural anesthesia. One catheterizes the urethra to facilitate identification of the urethra and makes an incision at the level of the ischial arch. After incising the urethra, forceps are used to grasp and remove the calculus, and the bladder is lavaged to remove remaining debris. One may attempt removal of larger calculi via this approach by using a lithotrite to crush the urolith into smaller fragments. The urethral incision can be closed, but it usually is allowed to heal by second intention. Although this approach can be performed less expensively and the risks associated with general anesthesia

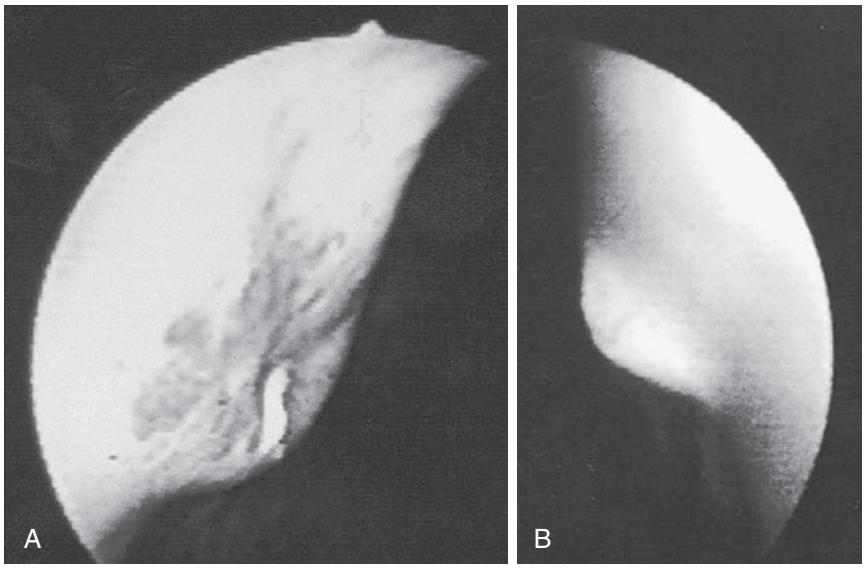


FIG. **14.36** View of the abnormal left **(A)** and normal right **(B)** ureteral openings of a horse with unilateral pyelonephritis and recurrent cystic calculi. The diagnosis was confirmed by culture of urine samples collected from each ureter.

can be avoided, doing so increases the risk of complications, including urethral trauma and stricture formation,[559,609,635] urethrolith formation at the surgical site,[389,636] development of a urethral diverticulum,[636] and persistent urine passage through a fistula at the surgical site.[635,637] The distensible urethra of the mare allows retrieval of cystic calculi via this route in many cases. Using sedation and epidural anesthesia, one can remove the stone intact with forceps or by direct grasping if the surgeon has small hands. One may crush a spiculated urolith with a lithotrite to ease removal. Spiculated stones or urolith fragments can be manipulated further into a sterile plastic bag or palpation sleeve to minimize trauma to the urethral mucosa during removal. If necessary, the urethral lumen can be enlarged by performing sphincterotomy at the dorsal aspect of the urethra.[638] A pararectal (Gökels) approach for dorsal cystotomy[611,632] and electrohydraulic lithotripsy,[614,615] as described for ureterolithiasis, also have been used in male and female horses to treat cystic calculi. Ballistic shockwave lithotripsy using an operating cystoscope via a perineal urethrostomy approach has also been used in a gelding with a cystolith.[639] Successful laser lithotripsy in horses depends on the composition of the urolith and type of laser.[640] Pulsed-dye lasers may be more efficacious for fragmentation of equine uroliths. However, reports of laser lithotripsy in horses are limited, and it appears that certain lasers cannot be assumed to be uniformly successful against all types of equine uroliths.

After surgical removal of cystoliths, one administers systemic antibiotics and an antiinflammatory drug for a minimum of 1 week. As for cystitis, antibiotic selection should be based on susceptibility testing of recovered isolates. If culture results are negative, then a sulfonamide-trimethoprim combination is an appropriate selection. An early report by Lowe[636] described excellent long-term results—and no recurrence—after removal of cystic calculi by laparocystotomy in four horses. Similar low rates of recurrence have been echoed in several reviews of equine urolithiasis.[563,571,609,611] In contrast to these favorable reports (most of which do not provide supporting data), Laverty et al.[389] reported that clinical signs of urolithiasis recurred in 12 of 29 horses (41%) for which follow-up data were available. The interval between episodes of recurrence was 1 to 32 months (mean: 13 months). As Lowe initially described in 1965,[636] Laverty and colleagues[389] also found greater recurrence of cystic calculi after treatment by perineal urethrotomy (7 of 15 horses) compared with laparocystotomy. Other complications of cystic calculi unrelated to the surgical approach have included vesiculoureteral reflux and renal failure[641] and concurrent urolithiasis at other sites.[389] A more common complication of urethral obstruction,[559] bladder rupture, also may occur after cystic urolithiasis. The author also has seen a case of squamous cell carcinoma of the bladder that was associated with a large cystic calculus and development of uroperitoneum after bladder rupture (Fig. 14.37).

Postoperative recommendations that may help prevent recurrence include control of UTI and use of urinary acidifiers, although the benefits of the latter are not well documented (see Cystitis in the Urinary Tract Infections section of this chapter).[281,563,569-571] Other considerations for decreasing recurrence include dietary modifications to decrease calcium excretion and to promote diuresis. Changing the diet from a high-calcium hay (e.g., alfalfa or clover) to grass or oat hay decreases dietary calcium and thus should decrease urinary calcium excretion because fecal calcium excretion is relatively constant in horses.[642] This dietary change should decrease total calcium excretion and may decrease urinary nitrogen excretion and daily urine volume.[168] The latter changes could enhance supersaturation of urine. In theory, the addition of 50 to 75 g of loose salt daily to the concentrate portion of the diet should promote diuresis; however, in a study of ponies fed NaCl as 1%, 3%, or 5% of the total dry matter of the diet (1% approximates 75 g of NaCl for a 500-kg horse), no differences in water intake, urine production, or calcium excretion were observed.[171] Another factor that affects urine pH and urine calcium excretion is dietary cation-anion balance (DCAB = [Na + K] − [Cl + S]). A lower DCAB has been associated with

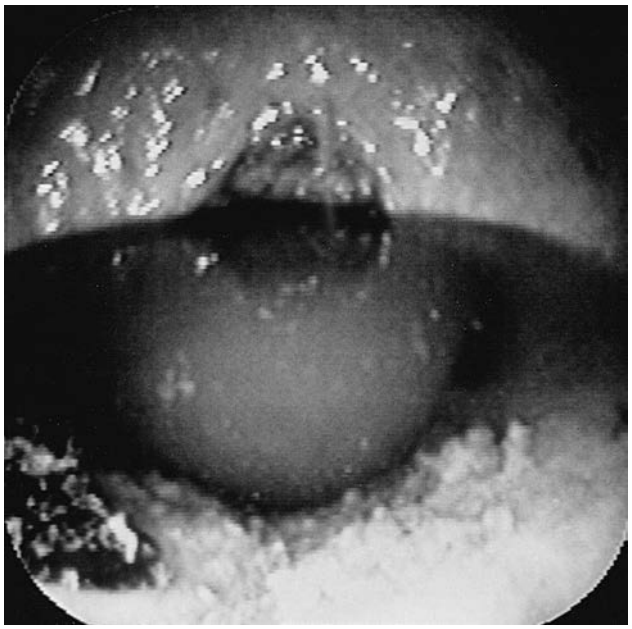


FIG. **14.37** A large urolith is present in the bladder below the surface of the pool of urine. The cystolith was accompanied by bacterial cystitis and squamous cell carcinoma of the bladder.

a decrease in urine pH and an increase in urinary calcium excretion.[643-645] Increasing the amount of grain in the diet, changing to lower quality hay, or adding one or more minerals to the diet (e.g., ammonium chloride, calcium chloride, ammonium sulfate) usually lowers DCAB. Not surprisingly, supplements that decrease DCAB are familiar as urinary acidifying agents. Because a diet low in calcium and DCAB could result in a negative calcium balance, a possible long-term effect could be decreased skeletal calcium content.

Despite the success of dietary management (low protein, phosphorous, and magnesium) for medical dissolution of canine[646] and feline[647] uroliths, dietary management is unlikely to replace surgical treatment of cystic urolithiasis in horses. This problem can be attributed to the fact that dietary management for small animals has been directed at struvite urolithiasis and these stones are not common in horses. Nevertheless, one should not overlook dietary management as one of the postoperative recommendations for urolithiasis, because it could decrease the risk of recurrence. At the least, legume hays and dietary supplements containing calcium should be avoided, and the diet could be supplemented with 50 to 75 g of loose salt daily. Remillard and colleagues[572] used these dietary manipulations effectively, along with administration of ammonium sulfate as a urine-acidifying agent, to manage one recurrent case of equine urolithiasis.

URETHRAL CALCULI

Urethral calculi are primarily a problem of male horses,* although they have been detected in a few mares.[389] In the absence of predisposing urethral damage or stricture formation, urethroliths are usually small cystoliths passed into the urethra. Thus most urethroliths initially lodge where the urethra narrows as it passes over the ischial arch. They may pass

* References 70, 95, 389, 434, 559, 563, 636, 648, 649.

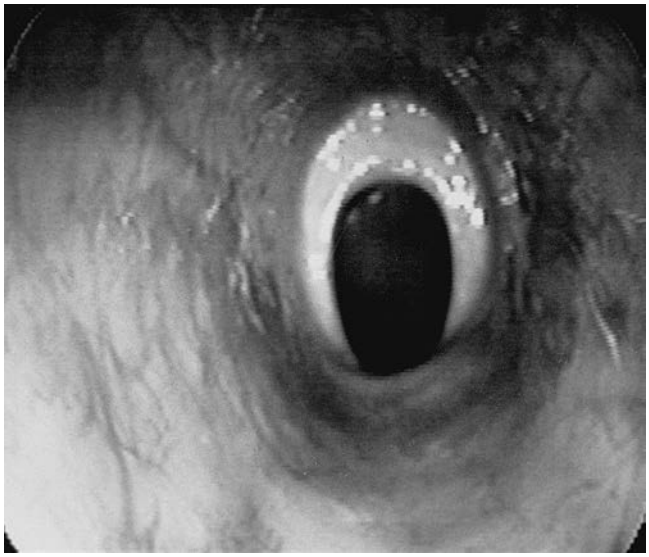


FIG. **14.38** Stricture formation in the urethra at the level of the ischial arch formed as a complication of a perineal urethrotomy.

slowly farther down the urethra, until complete obstruction produces signs of renal colic. One should consider an obstructing urethral calculus when male horses show colic signs and frequently posture to urinate. Occasionally, one may see blood on the end of the urethra. Palpation of the penis may reveal repeated urethral contractions or a firm mass in the urethra. Rectal palpation reveals a distended bladder that is turgid, unlike the flaccid bladder distention of bladder paralysis. If the bladder ruptures, colic signs are supplanted by progressive depression and anorexia after the development of postrenal acute renal failure.[389,559,648,649] The diagnosis is confirmed by passage of a urinary catheter that is then obstructed by the urolith or by endoscopic examination of the urethra. One can confirm suspected bladder rupture by measuring a twofold or greater increase in peritoneal fluid creatinine concentration compared with serum creatinine concentration.

One can remove calculi lodged at the ischial arch through a perineal urethrotomy. Passage of a catheter into the bladder, if not achieved before surgery, is necessary to ensure a patent urinary tract after stone removal. The urethrotomy is allowed to heal by second intention, and temporary use of an indwelling bladder catheter usually is not necessary. One often can remove calculi lodged in the distal urethra from a sedated horse by gentle transurethral crushing of the urolith with a hand or forceps. For a calculus lodged distal to the ischial arch and unpalpable in the distal portion of the penis, general anesthesia and positioning the horse in dorsal recumbency are generally necessary for surgical removal of the stone. One may close the urethra or allow it to heal by second intention. A commercial extracorporeal shock wave machine designed for orthopedic conditions in horses has been used to successfully break up a distally lodged urethrolith in a standing sedated horse.[650]

Follow-up endoscopic examination of the urethra allows assessment of urethral healing and possible stricture formation (Fig. 14.38). Further treatment includes administration of antibiotics and antiinflammatory agents until dysuria resolves. Although initial treatment of urinary tract obstruction caused by a urethrolith is straightforward, the prognosis for affected horses should remain guarded because a number of potential

complications of perineal urethrotomy (described previously) may occur. Furthermore, a substantial number of horses have had poor outcomes because of associated bladder rupture and peritonitis[389,559,648] or upper urinary tract lithiasis or pyelonephritis.[389,609]

SABULOUS UROLITHIASIS

Another form of equine urolithiasis termed *sabulous urolithiasis* also has been described in a limited number of horses.[609,566] Sabulous (Greek for *sand*) urolithiasis refers to the accumulation of large amounts of crystalloid urine sediment in the ventral aspect of the bladder. This condition is a secondary problem resulting from bladder paralysis or other physical or neurologic disorders interfering with complete bladder emptying.[564,574,575] Affected horses usually are presented for evaluation of urinary incontinence or hindlimb weakness and ataxia, and one may detect accumulation of urine sediment in a distended bladder during rectal palpation. A complete neurologic examination should be performed on horses with sabulous urolithiasis or bladder dysfunction. Common differential diagnoses for bladder paresis or paralysis include equine herpesvirus–1, polyneuritis equi, illicit tail block, equine protozoal myeloencephalitis, and sacral fracture or osteomyelitis. Symptomatic treatment includes repeated bladder lavage, medications that promote bladder emptying, and broad-spectrum antibiotics, but the condition carries a poor prognosis unless the primary problem resulting in bladder paralysis can be resolved (see Urinary Incontinence and Bladder Dysfunction).

BLADDER DISPLACEMENT

Displacement of the urinary bladder is a rare cause of obstruction and dysuria.[70,651-654] In mares, two types of bladder displacements can occur: (1) extrusion through a tear in the floor of the vagina or (2) true prolapse with eversion of the bladder.[655] Urethral obstruction also may occur with vaginal or uterine prolapse. In male horses scrotal herniation of the bladder has been described, but this type of bladder displacement is rare.[656] Bladder displacements typically result from repeated abdominal contractions or straining. Thus they most often are associated with parturition and to a lesser extent with colic. Perineal lacerations after trauma or foaling may lead to extrusion, whereas excessive straining without laceration leads to prolapse and eversion. Because the bladder turns inside out with the latter problem, one establishes the diagnosis by recognizing the appearance of the bladder mucosa and ureteral openings. Eversion does not always result in obstruction.

With urethral obstruction, one should pass a catheter into the bladder before correction of the displacement. In the absence of obstruction, one corrects extrusions during repair of the perineal or vaginal laceration. One should institute a course of broad-spectrum antibiotics and an antiinflammatory agent because pelvic abscess and peritonitis are potential complications. Manual reduction of bladder eversions may be successful in some cases, but more often than not, one may need to perform urethral sphincterotomy to replace the bladder.[654] In some cases, reduction via laparotomy may be necessary because the everted bladder may be filled with the pelvic flexure, complicating manual reduction.[653] A purse-string suture placed in the area of the external urethral sphincter may be of benefit to prevent recurrence of the prolapse, and medical treatment should include broad-spectrum antibiotics and an antiinflammatory agent because UTI is a potential complication.

PENILE TRAUMA

Urinary tract obstruction is an occasional complication of penile trauma or paraphimosis, and one should consider the patency of the urethra in all cases of penile injury. Causes may include blunt trauma, breeding injuries, use of stallion rings, sedation with phenothiazine tranquilizers, or laceration during castration.[70,657-659] In addition to preputial swelling, injury may result in a penile hematoma or possible paraphimosis.[660,661] In one report, hematoma formation in the corpus spongiosum penis of a Quarter Horse stallion resulted in complete obstruction and bladder rupture.[662] In addition to ensuring patency of the urinary tract, treatment includes administration of antibiotics and antiinflammatory agents until most of the swelling resolves. One may close lacerations of the urethra or leave them to heal by second intention depending on location and condition of the wound. Because stricture formation is a possible complication, some wounds may be treated better by phallectomy than by urethral repair.[658]

Hematuria

Harold C. Schott II

Hematuria can be a presenting complaint for a variety of disorders of the urinary tract, including vascular malformation, urinary tract infection, urolithiasis, and neoplasia.[663] In addition to these problems, which are discussed elsewhere in this chapter, several other specific causes of hematuria exist. These causes range from microscopic hematuria accompanying exercise to more severe conditions that can result in life-threatening urinary tract hemorrhage. In addition, normal equine urine may contain pyrocatechin, an oxidizing agent that can cause urine to turn red to brown after exposure to air, snow, or bedding (especially wood shavings).

Although values have not been determined in horses, normal human urine contains about 5000 (range: 2000–10,000) red blood cells (RBCs) per milliliter.[5] This range of RBC excretion should yield negative results on reagent strip analysis and a report of not more than five RBCs per high-power field on sediment examination. Increases in RBC excretion may lead to microscopic or macroscopic hematuria. Microscopic hematuria, which implies an increase in RBC excretion that is not visible grossly, usually is associated with increases in the range of 10,000 to 2.5 million RBCs per milliliter of urine. On sediment examination, at least 10 RBCs per high-power field should be apparent. Reagent strip analysis results can range from trace to +++. One must realize that reagent strip results, which use the peroxidase-like activity of hemoglobin and myoglobin to oxidize a chromogen in the test pad, do not differentiate between hemoglobin and myoglobin.[664] Thus positive results are not specific for hematuria and may be more appropriately termed *pigmenturia.* Despite this limitation, one can use reagent strips to differentiate hematuria from hemoglobinuria or myoglobinuria when the color change is limited to scattered spots on the test pad. This pattern implies that intact RBCs were adsorbed onto the pad, underwent lysis, and produced a localized color change caused by hemoglobin activity on the chromogenic

substrates. Ability to differentiate hematuria from excretion of the heme pigments is limited to a threshold of 250,000 to 300,000 RBCs per milliliter of urine, unless urine samples are diluted with normal saline. Other limitations of reagent strip analysis include false-positive reactions when urine samples are contaminated with oxidizing agents (e.g., disinfectants) or false-negative reactions when urine samples contain vitamin C or have been preserved with formalin.[664]

Macroscopic or gross hematuria indicates RBC excretion in excess of 2.5 to 5 million RBCs per milliliter of urine (or about 0.5 mL of blood per liter of urine).[5,285,664,665] One can differentiate macroscopic hematuria from other causes of pigmenturia by centrifuging a sample of urine to produce a red cell pellet and yellow supernatant urine. Quantification of erythrocyte numbers in macroscopic hematuria is of little clinical value. In contrast, urinary RBC numbers may provide diagnostic and prognostic information in cases of microscopic hematuria in human beings.[5] However, variations in urine concentration complicate accurate counts. In concentrated urine (specific gravity greater than 1.020), RBCs tend to become crenated because of the osmotic shift of water out of the cells. In urine with a specific gravity less than 1.010, osmotic swelling and dilution of hemoglobin lead to "ghost cell" formation.[5,666] Furthermore, many RBCs lyse in dilute urine (especially alkaline urine) so that RBC excretion is vastly underestimated. Reagent strip analysis can be useful in dilute urine samples to detect hemoglobin released from lysed erythrocytes.[665]

Microscopic examination of urine sediment in cases of hematuria is helpful in distinguishing glomerular from nonglomerular bleeding. The hallmark of glomerular bleeding is a substantial variation in RBC size, shape, and hemoglobin content (termed *dysmorphism*), whereas bleeding from other sites produces a more uniform population of urinary erythrocytes.[5,665,667] Dysmorphism is attributed to membrane deformation, which occurs as erythrocytes traverse the glomerular filtration barrier.[667] Urinary RBCs in normal persons are typically dysmorphic, indicating glomerular origin, but the excretion rate is low.[5] Thus one must interpret urinary RBC morphologic characteristics along with urinary RBC numbers to determine significance.[667,668] The volume of dysmorphic cells tends to be lower than that of erythrocytes of nonglomerular origin, so that measurement of mean corpuscular volume also has been used to separate glomerular from nonglomerular bleeding.[669] The presence of RBC or hemoglobin casts is also pathognomonic for glomerular bleeding.[5,665,667] These casts form when urinary RBCs and hemoglobin from the proximal portion of the nephron (glomerulus) combine with Tamm-Horsfall mucoprotein secreted in the ascending limb of the loop of Henle. Because urinary RBCs and casts deteriorate rapidly in urine samples, other methods of detecting glomerular hematuria, such as immunocytochemical staining for Tamm-Horsfall glycoprotein, have been developed but have not gained widespread use.[670]

Noting the timing of hematuria is usually a more practical means of initially localizing the site of urinary tract hemorrhage.[666] Hematuria throughout urination is consistent with hemorrhage from the kidneys, ureters, or bladder, whereas hematuria at the beginning of urination often is associated with lesions in the distal urethra. Hematuria at the end of urination usually results from hemorrhage from the proximal urethra or bladder neck. A thorough diagnostic evaluation, including physical examination, rectal palpation, analyses of blood and urine, endoscopy of the lower tract, and ultrasonography, usually is rewarding in establishing the source and cause of urinary tract hemorrhage.[663]

Urinary tract infection, although uncommon in horses, may result in hematuria. With infection of the upper urinary tract, partial anorexia, weight loss, and fever may be additional presenting complaints, whereas horses with cystitis generally manifest stranguria and pollakiuria; however, hematuria has been the presenting complaint in several reports of cystitis and pyelonephritis.[479,575,671-673] The presence of uroliths at any level of the urinary tract may cause mucosal irritation and hemorrhage, resulting in hematuria.[257,389,563] Typically, affected horses also show signs of renal colic or painful urination (stranguria or pollakiuria), especially with uroliths in the bladder or urethra. Finally, neoplasia of the kidneys, ureters, bladder, or urethra may result in hematuria as the presenting complaint.[565,674-676] These conditions are discussed in detail in other sections in this chapter.

TOXICITIES

Nephrotoxicity, particularly after administration of NSAIDs (especially phenylbutazone), may result in microscopic or gross hematuria.[238,239,482,677,678] Historical or current use of nephrotoxic medications supports this diagnosis, and discontinuation of the nephrotoxic agent and supportive care are the appropriate treatments. Horses that consume alfalfa hay containing blister beetles (equine cantharidiasis) may void red urine. Cantharidin is excreted in urine and is very irritating to the lower urinary tract, resulting in cystitis and hemorrhage from the urinary bladder mucosa.[679]

URETHRAL DEFECTS

Although a widely recognized cause of hemospermia in stallions, rents or tears of the proximal urethra at the level of the ischial arch can also cause hematuria in geldings.[561,663,680,681] Because the defects are difficult to detect without videoendoscopic equipment, the lesions likely may have been missed in previous reports of urethral bleeding.[558,559] Consequently, hematuria has been attributed to urethritis or hemorrhage from "varicosities" of the urethral vasculature.[559,680] Because the vasculature underlying the urethral mucosa becomes prominent when the urethra is distended with air during endoscopic examination, especially in the proximal urethra (to the point that blood can be seen flowing in the submucosal vasculature), one easily would suspect that hemorrhage could arise from apparent urethritis or urethral varicosity.

Urethral rents or tears in geldings typically occur at the ischial arch and involve the corpus spongiosum penis (CSP). The urethral rent into the vascular space of the penis results in hematuria at the end of urination in association with urethral contraction.[561,663,680] Affected geldings generally void a normal volume of urine that is not discolored. At the end of urination, a series of urethral contractions results in squirts of bright-red blood. Occasionally, the horse may pass a smaller amount of darker blood at the start of urination. In most instances the condition does not appear painful or result in pollakiuria. Interestingly, most affected stallions with hemospermia and geldings with hematuria have been Quarter Horses or Quarter Horse crosses that have been free of other complaints.[559,561] Treatment with antibiotics for suspected cystitis or urethritis routinely has been unsuccessful, although hematuria has resolved spontaneously in approximately half of the cases seen by the author.

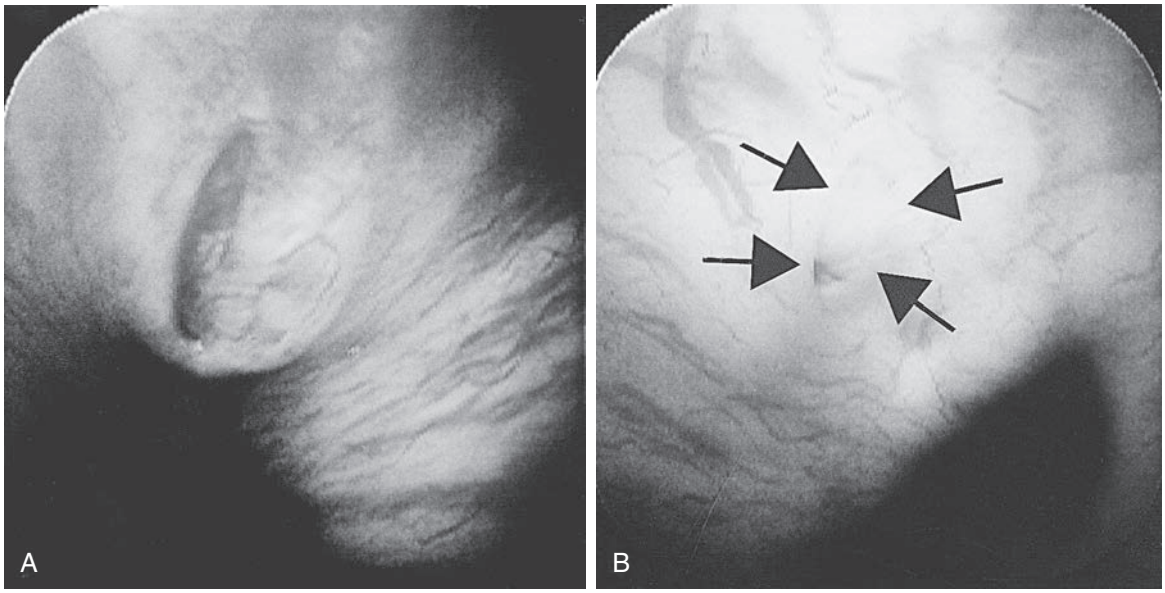


FIG. **14.39** Endoscopic images of urethral defects or tears at the level of the ischial arch. **A,** A more acute lesion (hematuria for 2 weeks) is surrounded by a raised rim of tissue. **B,** A chronic lesion (hematuria for 6 months) is flat to recessed *(between arrows).* Evidence of inflammation around both lesions is minimal.

Examination of affected horses is often unremarkable. In comparison, horses with hematuria caused by neoplasms involving the distal urethra or penis usually have additional complaints such as pollakiuria, a foul odor to the sheath, or presence of a mass in the sheath or on the penis.[682] With urethral rents, laboratory analysis of blood reveals normal renal function, although mild anemia can be an occasional finding. Urine samples collected midstream or by bladder catheterization appear grossly normal. Urinalysis may have normal results, or the number of RBCs may be increased on sediment examination (a finding that also would result in a positive reagent strip result for blood). Bacterial culture of urine yields negative results.

One makes the diagnosis via endoscopic examination of the urethra, during which one typically sees a lesion along the dorsocaudal aspect of the urethra at the level of the ischial arch (Fig. 14.39A). With hematuria lasting several weeks, the lesion appears as a fistula communicating with the vasculature of the CSP (Fig. 14.39B). External palpation of the urethra in this area is usually unremarkable but can help localize the lesion, because external digital palpation is visible via the endoscope as movements of the urethra.

Although the complete pathophysiology of this condition remains unclear, the defect has been speculated to result from a "blowout" of the CSP into the urethral lumen (Fig. 14.40).[561] Hemorrhage from urethral rents occurs with higher pressure within the CSP during urination in geldings and ejaculation in stallions.[681] Pressure in the CSP during urination is higher in geldings because of their smaller CSP diameter compared with stallions. Hematuria at the end of urination in geldings with urethral rents is speculated to occur when a sudden decrease in intraluminal urethral pressure occurs while pressure in the CSP is still high. Once created, the lesion is maintained by bleeding at the end of each urination, and the surrounding mucosa heals by formation of a fistula into the overlying vascular tissue.[561] An explanation for the consistent location along the dorsocaudal aspect of the urethra at the level of the

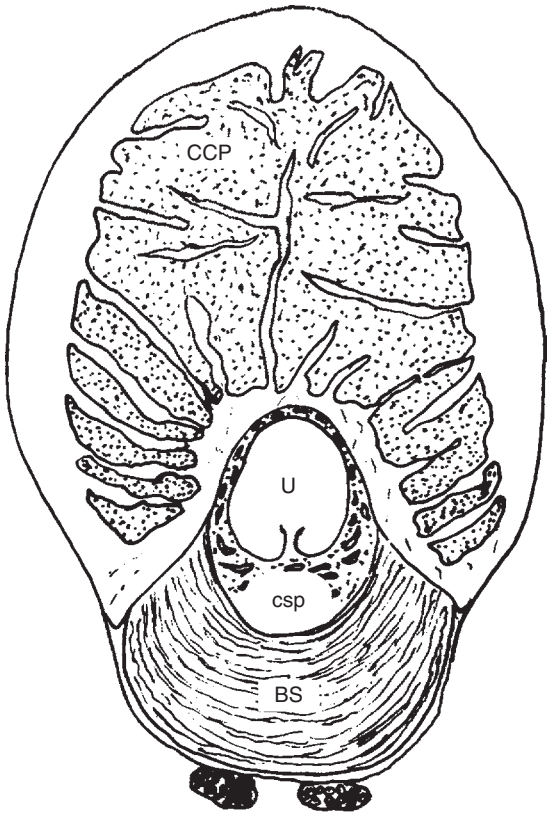


FIG. **14.40** Cross-sectional diagram of the equine penis at the level of the ischial arch shows a defect between the corpus spongiosum penis (CSP) ***(csp)*** and the urethral lumen ***(U)***. The CSP is a cavernous tissue surrounding the urethra that is distinct from the corpus cavernosum penis ***(CCP)***; the CSP is also adjacent to the bulbospongiosus muscle ***(BS)*** caudally. (From Schumacher J, Varner DD, Schmitz DG, et al.: Urethral defects in geldings with hematuria and stallions with hemospermia, *Vet Surg.* 24:250, 1995.)

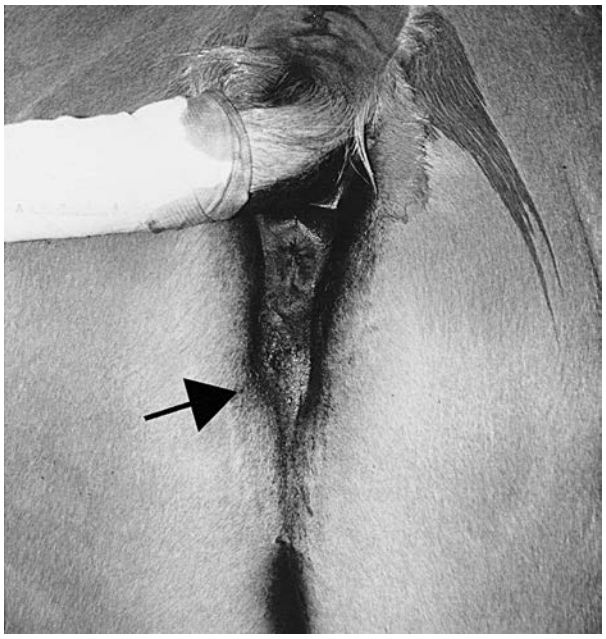


FIG. **14.41** Perineum of Paint gelding with hematuria associated with a proximal urethral defect. The asymmetry of the perineal musculature is visible at the level of the ischial arch. (*Arrow* shows where asymmetry is more prominent on left side.)

ischial arch has not been documented but may be related to the anatomy of the musculature supporting the base of the penis and an enlargement of the CSP in this area. Furthermore, a narrowing of the lumen of the urethra at the distal extent of the ampullar portion of the urethra occurs that also may contribute to the location of the defects. However, histologic studies in both geldings and stallions have shown that no differences exist in the urethral mucosa, lamina propria, or CSP between the ischial arch and other sites in the penis.[681] An anatomic predisposition in Quarter Horses has not been documented but could be speculated to be based on an apparently increased risk in this breed. In addition, some of the affected horses have asymmetry to the musculature under the tail in this area, supporting a possible developmental defect (Fig. 14.41).

Because hematuria may resolve spontaneously, no treatment may be required initially. If hematuria persists longer than 1 month or if significant anemia develops, then a temporary subischial urethrotomy has been successful in a number of affected geldings. With sedation and epidural or local anesthesia, one makes a vertical incision down to a catheter placed in the urethra. The surgical wound requires several weeks to heal, and moderate hemorrhage from the CSP is apparent for the first few days after surgery. Additional treatment consists of local wound care and prophylactic antibiotic treatment (typically a trimethoprim-sulfonamide combination) for 7 to 10 days. Hematuria should resolve within 1 week after this procedure. Limiting the incision into the CSP but not extended into the urethral lumen also has been a successful treatment and is now the preferred surgical treatment.[561] This treatment option provides support for the vascular blowout theory and lessens the risk of urethral stricture formation. Furthermore, the treatment decreases the chance of a permanent fistula forming at the site, a surgical complication that has occurred in geldings.

IDIOPATHIC RENAL HEMATURIA

Idiopathic renal hematuria is a syndrome characterized by sudden onset of gross, often life-threatening hematuria.[62,683] Hemorrhage arises from one or both kidneys and is manifested by passage of blood clots in urine. Endoscopic examination of the urethra and bladder usually reveals no abnormalities of these structures, but one may see blood clots exiting one or both ureteral orifices. Although a definitive cause of renal hemorrhage may be established in some horses (e.g., renal adenocarcinoma, arteriovenous or arterioureteral fistula),[58,61] the disorder is termed *idiopathic* when a primary disease process cannot be determined. Both sexes, a wide age range, and several breeds of horses (including a mammoth donkey and a mule) have been affected. However, more than 50% of animals with idiopathic renal hematuria have been Arabians.

Use of the term *idiopathic renal hematuria* to describe this syndrome of horses was adopted from its use in human patients and dogs with severe renal hemorrhage.[5,61,684-690] *Benign essential hematuria* and *benign primary hematuria* are other terms that describe less severe hematuria that is not associated with trauma or other obvious causes of hematuria. In the latter species, hematuria is more commonly a unilateral than a bilateral problem, similar to what has been observed in the few affected horses. The pathophysiology remains poorly understood, but macroscopic hematuria has been associated with immune-mediated glomerular damage (e.g., acute postinfectious glomerulonephritis, membranoproliferative glomerulonephritis, immunoglobulin A nephropathy, Berger's disease), thin basement membrane nephropathy, and the loin pain–hematuria syndrome in human patients.

Although hematuria and pigmenturia can accompany a number of systemic diseases in horses,[477,691,692] patients affected with idiopathic renal hematuria have spontaneous, severe hematuria in the absence of other signs of disease. Although one report suggested that severe renal hemorrhage resulted from pyelonephritis,[673] supportive data were lacking. In cases managed by the author, neither urinary tract infection nor urolithiasis has been detected; the magnitude of hematuria often resulted in the need for repeated blood transfusions. As with guttural pouch mycosis, the syndrome may produce episodic hemorrhage. Initially, one notes hemorrhage by finding a large amount of clotted blood in stall bedding or in the pasture. However, other clinical signs (e.g., depression, anorexia, weight loss) are typically absent. Examination may reveal dried blood at the end of the penis or in the sheath of males or on the vulvar lips and between the hindlimbs of mares. In both sexes, neoplasia of the external genitalia or urinary tract is an important differential diagnosis, whereas in mares, one also must consider varicosities in the area of the vestibulovaginal sphincter, especially in multiparous mares. When blood is not detected in the sheath or vulvar areas, further evaluation may be unrewarding because the renal bleeding may cease spontaneously. Bleeding has been attributed anecdotally to cystitis and pyelonephritis in the absence of positive urine culture results, because hemorrhage stops during a course of antimicrobial therapy. More likely, spontaneous resolution has occurred. Furthermore, the magnitude of hematuria is considerably greater with idiopathic renal hematuria than with most urinary tract infections, pyuria is absent, and urine culture results are negative. In the author's experience, one or two initial episodes of hemorrhage are followed by a more severe hemorrhagic crisis within months to several years after

observation of the initial bleeding episode. Interestingly, renal colic has been notably absent in the history of affected horses.

One diagnoses idiopathic renal hematuria by exclusion of systemic disease, other causes of hematuria, and alterations in hemostasis. Physical examination may reveal tachycardia, tachypnea, and pale mucous membranes consistent with acute blood loss. Rectal palpation may reveal an enlarged, irregular bladder because of the presence of blood clots. Azotemia is inconsistent. Endoscopic examination is important to document that hematuria is originating from the upper urinary tract and to determine whether hemorrhage is unilateral or bilateral (Fig. 14.42). Repeated examinations may be required to answer the latter question. Ultrasonographic imaging is necessary to rule out nephrolithiasis or ureterolithiasis and occasionally may reveal a distended vascular space or renal vascular anomaly as the cause of hematuria. Renal scintigraphy can be a useful technique in affected horses because it may provide semiquantitative information about renal function when a nephrectomy is being considered. Renal biopsy and immunofluorescent staining may assist in documenting immune-mediated glomerular injury, but the significance of such results is not well understood at this time.

Treatment for idiopathic renal hematuria consists of supportive care for acute blood loss, including blood transfusions. Medications intended to promote hemostasis (e.g., aminocaproic acid, formalin) also have been administered, but their efficacy has not been validated. Treatment with corticosteroids may be useful in cases with potential immune-mediated mechanisms as the cause of bleeding. Because the condition may be self-limiting in some patients, supportive care is warranted. With severe and recurrent unilateral hematuria, a nephrectomy may be indicated, but one should warn owners of the risk of hematuria developing in the contralateral kidney. In the author's experience, risk of contralateral renal bleeding appears to be greater in the Arabian breed.



FIG. **14.42** Endoscopic image of a large blood clot exiting the right ureteral opening of a gelding with idiopathic renal hematuria.

EXERCISE-ASSOCIATED HEMATURIA

Exercise is accompanied by increased filtration of RBCs and protein across the glomerular barrier in a large percentage of human and equine athletes.[285,693] Typically the hematuria is microscopic, but occasionally one may observe gross discoloration of urine. Gross hematuria more often may result from bladder erosions, which may be induced traumatically by the abdominal contents pounding the bladder against the pelvis during exercise.[356] Detection of focal bladder erosions or ulcers with a contrecoup distribution and a history of emptying the bladder immediately before the exercise bout are characteristic for this problem. A diagnosis of exercise-associated hematuria should be one of exclusion after diagnostic evaluation has ruled out other causes of hematuria such as presence of a cystolith.

PIGMENTURIA ASSOCIATED WITH SYSTEMIC DISEASE

With any systemic disease that may lead to hemolysis, thrombocytopenia, coagulopathy, or alterations in vascular permeability, hematuria or hemoglobinuria may develop. Discolored urine has the potential to be accompanied by a degree of nephrotoxicity because of the interaction of iron ions of heme molecules with surface molecules on proximal tubular epithelial cells. With transient pigmenturia (as with exercise-associated hematuria), changes in renal function may not be apparent, but with more severe disease processes, acute renal failure may develop. In human beings, a syndrome of hemolytic anemia and thrombocytopenia leading to development of acute renal failure has been termed the *hemolytic-uremic syndrome*.[694] The syndrome is recognized more commonly in children than in adults and is the most common cause of acute renal failure in children. Similar syndromes have been described in a limited number of horses.[477,691,692] Furthermore, hemolysis and hemoglobinuria may be recognized with liver disease, medications (e.g., penicillin-induced immune-mediated hemolytic anemia, concentrated intravenous DMSO solutions), or intoxications (e.g., ingestion of red maple leaves). Finally, conditions accompanied by extensive rhabdomyolysis also may result in pigmenturia and acute renal failure.[695] Assessment of muscle enzyme activities in these cases usually is rewarding in establishing myoglobin as the most likely cause of pigmenturia. In addition, one can use the Blondheim test (ammonium sulfate precipitation) to differentiate myoglobinuria from hemoglobinuria.[288] Furthermore, serum is discolored with intravascular hemolysis, whereas serum appears normal if hematuria or myoglobinuria is present. Centrifugation of urine and sediment analysis will differentiate if pigmenturia or hematuria is the cause of discolored urine.

Polyuria and Polydipsia

Harold C. Schott II

For small animals, polyuria and polydipsia have been defined as urine output exceeding 50 mL/kg/day and fluid intake of more than 100 mL/kg/day.[696,697] These values would equate to 25 L of urine production and 50 L of water consumption for a 500-kg horse. Compared with normal values for daily urine production and water consumption of 5 to 15 L and 20

TABLE **14.11 Relationship of the Percentage of Water Filtered That Is Reabsorbed to Daily Urine Output and Renal Water Absorption**

Glomerular Filtration Rate (GFR) (L/day)	Percentage of Filtered Water Reabsorbed	Urine Production (L/day)	Urine Osmole Excretion (mOsm)	Urine Osmolality (mOsm/kg)	Renal Water Reabsorption[a] (L/day)
1000	99%	10	10,000	1000	23.3
1000	98%	20	10,000	500	13.3
1000	96%	40	10,000	250	6.7

[a]Renal water reabsorption (the inverse of free water clearance) is a calculated volume of water that is retained or lost by the kidney. Renal water reabsorption is calculated from actual urine volume and the calculated volume of urine required to excrete all osmoles in urine that is isosmotic with plasma. In this table a urine osmolality value of 300 mOsm/kg is assumed to be isosmotic to plasma, 1 kg of water is assumed to equal 1 L of water, and a total of 10,000 Osm is assumed to be excreted daily. Thus when 98% of filtered water is reabsorbed, the 20 L of urine produced (if isosmotic) would contain 6000 mOsm. Because an additional 13.3 L of water would be needed to excrete the remaining 4000 mOsm (as isosmotic urine), the kidneys are considered to be actively reabsorbing 13.3 L of water.

to 30 L, respectively,* these definitions of polyuria and polydipsia appear applicable to horses as well. One must remember that urine production and water consumption vary with age, diet, workload, environmental temperature, and gastrointestinal water absorption. For example, urine production increases by 50% to 100% when the diet is changed from a grass to a legume hay.[168] Although this increase in urine production has been associated with higher dietary protein intake and urinary nitrogen excretion,[136] increases in calcium intake and urinary calcium excretion may be another contributing factor. Similarly, horses that are exercised heavily, are stabled in hot climates, or have chronic diarrhea may have a water intake exceeding 100 L per day yet produce normal volumes of urine.[163]

A brief review of water turnover by the equine kidneys provides insight into how a small change in renal water reabsorption can lead to a dramatic increase in urine production (polyuria). In normal horses, glomerular filtration exceeds 1000 L/day, a volume that is 10 times greater than the total extracellular fluid volume; however, approximately 99% of this water is reabsorbed in the renal tubules and collecting ducts, resulting in production of 5 to 15 L of urine daily. The result is urine that is 3 to 4 times more concentrated than plasma (urine osmolality of 900–1200 mOsm/kg and a specific gravity of 1.025–1.050). Furthermore, urea (in urine) has replaced sodium (in plasma) as the most important solute. If only 98% of water is reabsorbed, urine volume would double, and the additional water would result in more dilute urine (urine osmolality of 450–600 mOsm/kg and a specific gravity of 1.015–1.025). If water reabsorption decreased to 96% of filtered water, the horse would produce approximately 40 L of urine with a urine osmolality of 225 to 300 mOsm/kg and a specific gravity of 1.005 to 1.010 (Table 14.11). In the latter case, urine is more dilute than plasma (hyposthenuria), and the kidneys are excreting or losing water actively. Under certain conditions, active water excretion by the kidneys is important for maintenance of plasma osmolality in the normal range. The best example is a neonatal foal that may ingest a volume of milk exceeding 20% of its body weight daily.[169] This ingestion equates to a fluid intake approaching 250 mL/kg/day, and failure to produce a large volume of hyposthenuric urine could result in water retention, decreased plasma osmolality, and clinical hyponatremia (manifested by neurologic signs).

Determining that a horse is producing more urine than normal is often difficult, especially in horses kept at pasture. Owners may report that a horse is polyuric when in fact the frequency of urination has increased (pollakiuria) rather than the volume. Pollakiuria occurs with conditions such as cystitis and urolithiasis or during estrus in the mare. Horses housed in stalls bedded with straw are difficult to evaluate because excessive urine may not be obvious to the casual observer. For those bedded on shavings or sawdust, excessively wet bedding may be easier to recognize, but this is a subjective impression. Occasionally, polyuria may be so severe that urine may flow from the stall into the barn aisle. When doubt exists as to whether a horse has polyuria and polydipsia, documentation of water consumption over one or more 24-hour periods may be necessary.[247] One can quantify urine production by collecting urine over a 12- or 24-hour period. For geldings and stallions, one can construct a collection device by cutting off the bottom of a large plastic bottle, padding it, and fitting it over the prepuce. One covers the opening of the bottle with a rubber tube and clip to allow removal of urine every few hours. In mares, one can place an indwelling Foley catheter in the bladder or apply a urine collection harness.[248-252] During the collection period, horses usually are tied to minimize interference with the collection device.

The major causes of polyuria in horses include renal failure, pituitary pars intermedia dysfunction (PPID), and primary or psychogenic polydipsia.[70,252] Less common causes include excessive salt consumption, central and nephrogenic diabetes insipidus, diabetes mellitus, sepsis and endotoxemia, and iatrogenic causes (sedation with α_2-agonists, corticosteroid therapy, or diuretic administration).

RENAL FAILURE

Horses with acute renal failure usually have a transient period of anuria or oliguria. If horses survive the acute phase of renal disease, tubule damage results in a subsequent period during which impaired concentrating ability results in polyuria.[252,260] Urine is frequently hyposthenuric during this period of tubular repair. Owners should provide horses recovering from acute renal failure with adequate water, salt, and a low-nitrogen (protein) and low-calcium diet. Such a diet consists of good-quality grass hay or nonleguminous pasture. Repair of tubules and return of concentrating ability may take several weeks. Although these animals appear to have normal renal function after this recovery period, a permanent reduction in total renal function likely persists because most animals can maintain apparently normal health with only about 30% to 50% of functioning nephrons.

Chronic kidney disease may develop after damage from nephrotoxins. In addition, immune-mediated mechanisms,

* References 152, 153, 162, 165, 167, 194, 200, 247.

chronic infection, and nephrolithiasis may give rise to chronic kidney disease.[260,266,698] Horses that do not recover from the ischemic renal damage occurring with hypovolemic or endotoxic shock also may progress to chronic kidney disease. Signs vary and include polyuria and polydipsia in some cases. When present, polyuria and polydipsia are usually moderate compared with the dramatic increases in urine production observed with primary polydipsia or diabetes insipidus. Most horses with chronic kidney disease also exhibit other signs, including poor performance, weight loss, and ventral edema. A variable degree of azotemia is present, and urinalysis reveals isosthenuria (urine is isosmotic with plasma [260–300 mOsm/kg] with a specific gravity of 1.008–1.014).

The mechanisms of polyuria after acute and chronic kidney disease are not entirely clear.[158] Increased tubular flow rate in surviving nephrons is one possible mechanism that would result in less time for water removal from tubular fluid. Next, medullary hypertonicity may decrease because of diminished transport of sodium and chloride out of tubular fluid passing through the ascending limb of the loop of Henle (diluting segment of the nephron) along with increased blood flow through the remaining medullary tissue. A third possibility is impaired response of collecting ducts to vasopressin (acquired nephrogenic diabetes insipidus). Although all these mechanisms may contribute to the polyuria of renal failure, which particular mechanism may be the most influential is not known. Furthermore, because the horse can produce hyposthenuric urine during the recovery phase of acute renal failure, the mechanisms of polyuria differ somewhat for acute and chronic kidney disease.

PITUITARY PARS INTERMEDIA DYSFUNCTION

PPID is a common disorder of older horses that is often referred to as *equine Cushing's disease* because of its similarity to Cushing's disease in dogs and humans.[70,699-706] The clinical syndrome of PPID in horses is associated with hypertrichosis, laminitis, weight loss, and polydipsia and polyuria. The disorder is discussed in detail elsewhere in this text.

In one review of 17 horses with PPID, polyuria and polydipsia were found in 13 (76%)[706]; however, in another series of 21 horses with PPID, polyuria and polydipsia were not a historical complaint in any of the affected horses.[705] Thus polyuria and polydipsia associated with PPID are generally less severe than that observed with primary polydipsia or diabetes insipidus. PPID may lead to polyuria by several mechanisms. First, polyuria may result from actions of hormones derived from proopiomelanocortin, most specifically adrenocorticotropin. Hyperadrenocorticism resulting from excessive adrenocorticotropin activity on the adrenal cortex can lead to hyperglycemia, which may exceed the renal tubular threshold for reabsorption. The renal threshold for glucose in horses appears to be lower than in small animals (about 150 mg/dL).[287] When plasma glucose concentrations exceed this threshold value, the resultant glucosuria can lead to an osmotic diuresis. Although commonly implicated as the cause of polyuria in horses with PPID, glucosuria was found in only one of five affected horses in a clinical report.[705] Furthermore, horses with hyperglycemia and glucosuria still may be able to concentrate their urine in response to water deprivation.[702] A second mechanism implicated in the development of polyuria is cortisol antagonism of the action of vasopressin on the collecting ducts. Although frequently cited as the mechanism of polyuria in canine hyperadrenocorticism, experimental evidence to support this mechanism is lacking in dogs and horses. Furthermore, considerable species heterogeneity exists in the effects of corticoids on vasopressin activity, and in some species a primary dipsogenic effect may be more important. Next, growth of the pars intermedia may lead to impingement on the posterior pituitary and hypothalamic nuclei (located immediately dorsal to the pituitary gland), which are the sites of vasopressin storage and production, respectively. Decreased vasopressin production and release would result in a partial central diabetes insipidus as a third mechanism for polyuria.[699] Central diabetes insipidus, however, is not the cause of polyuria in all cases because some affected horses can concentrate their urine when deprived of water.[702] Consequently, the polyuria and polydipsia seen in many horses with PPID is likely the combined result of several mechanisms.

PRIMARY POLYDIPSIA

Although rare, primary or psychogenic polydipsia is probably the most common cause of polyuria and polydipsia in adult horses for which clients have a primary complaint of excessive water consumption and urination.[70,252] Primary polydipsia can be attributed to the fact that horses that exhibit this problem are generally in good body condition and are not azotemic. Furthermore, the magnitude of polyuria typically is much greater than that observed with renal failure or PPID. Owners may report that horses with primary polydipsia drink two to three times more water than their stablemates and their stalls often are flooded with urine. In some instances, primary polydipsia appears to be a stable vice that reflects boredom in affected horses, whereas in other cases it may develop after a change in environmental conditions, stabling, diet, or medication administration. Anecdotally, primary polydipsia is reported to be more common in southern states during periods of high temperature and humidity. In human beings, primary polydipsia can be a compulsive behavior associated with mental illness or may be caused by a primary abnormality in the osmoregulation of thirst, in which case it is referred to as *dipsogenic diabetes insipidus*.[5] The latter may be idiopathic or may occur after neurologic disease involving the hypothalamic osmoreceptors regulating thirst. Excessive water consumption causes expansion and dilution of body fluids, leading to a decrease in plasma osmolality and suppression of vasopressin release. With low plasma vasopressin concentrations, collecting ducts become impermeable to water, and hyposthenuria is induced to allow the rate of water excretion to balance intake. In human beings the magnitude of polydipsia and resultant polyuria varies considerably among affected persons, and similar variation, although undocumented, likely occurs in affected horses as well.

One diagnoses primary polydipsia by exclusion of renal failure and hyperadrenocorticism. In addition, other factors, such as excessive salt supplementation and medication administration, must be excluded. One excludes diabetes insipidus and confirms the diagnosis of primary polydipsia by demonstrating urine-concentrating ability after water deprivation.[194,200] Specific gravity should exceed 1.025 after water deprivation of sufficient duration (12–24 hours) to produce a 5% loss in body weight. In cases of long-standing polyuria, the osmotic gradient between the lumen of the collecting tubule and the medullary

interstitium may be diminished (medullary washout). In these cases, vasopressin activity may not lead to an increase in urine specific gravity to values greater than 1.020. Consequently, horses with primary polydipsia lasting several weeks that fail to concentrate their urine after 24 hours of water deprivation may be given a modified water deprivation test. One performs the test by restricting water intake to approximately 40 mL/kg/day for 3 to 4 days. By the end of this time, urine specific gravity should exceed 1.025 in a horse that has had medullary washout. If the urine specific gravity remains in the isosthenuric range (1.008–1.014), then one should evaluate the polyuric horse further for early chronic kidney disease, in which urine-concentrating ability may be compromised before the onset of significant azotemia. In theory, chronic kidney disease could occur when between two thirds and three fourths of functional nephrons have been lost. Subtle signs of decreased performance and mild weight loss also would support early renal failure. Finally, horses with primary polydipsia typically produce hyposthenuric urine. Although such dilute urine would be an unlikely finding in the early stages of chronic kidney disease, it could be found in the polyuric recovery phase after acute renal failure. In the latter instance a thorough history should reveal whether any event that may have been complicated by acute renal failure may have affected the horse recently.

Management of horses with primary polydipsia is empiric. Because the diagnosis is one of exclusion, once it is established that the horse is not suffering from significant renal disease, one may safely consider restricting water intake to meet maintenance, work, and environmental requirements of the horse. In addition, one should take steps to improve the attitude of the horse by reducing boredom. Increasing the amount of exercise and turning the horse out to pasture are possible options, as well as providing a companion or toys in the stall. Increasing the frequency of feeding or the amount of roughage in the diet also may increase the time spent eating and thus reduce the habitual drinking.

EXCESSIVE SALT CONSUMPTION

In an occasional case of apparent primary polydipsia, polyuria and polydipsia may be attributed to excessive salt consumption and are manifested by increased fractional sodium clearance. Psychogenic salt eaters appear to be less common than psychogenic water drinkers, because the former would have to consume a substantial amount of salt to develop polyuria. In fact, the authors are aware of only one well-documented report of psychogenic salt eating in which a yearling Paint filly drank in excess of 500 mL/kg/day and had excessive urination when provided free access to salt.[326] The fractional clearances of sodium (3.4%) and chloride (2.6%) were increased and supported excessive intake. Although salt intake was not quantified for this filly, it may have exceeded 10% of the dry matter intake and appeared to be associated causally with muscle fasciculations and a stilted gait. Such a high salt intake is suggested because no increases in water consumption or urine volume were detected in one study in which ponies were fed diets containing 1%, 3%, and 5% of NaCl.[171] The 5% NaCl diet contained 5 to 10 times the daily requirement of NaCl and was similar to feeding about 350 g of NaCl daily to a 500-kg horse. Similar to cases of primary polydipsia, the filly described in this report was able to concentrate urine in response to water deprivation, and the problem was managed successfully by limiting water intake to 50 mL/kg/day and preventing access to salt.

DIABETES INSIPIDUS

Diabetes insipidus results in polyuria and polydipsia because of vasopressin deficiency or insensitivity of the renal collecting duct epithelial cells to vasopressin. In human beings, vasopressin deficiency, central or neurogenic diabetes insipidus, is the more common form, with hereditary and acquired forms being described. The hereditary form appears to result from decreased numbers of neurosecretory neurons in the supraoptic nuclei of the hypothalamus and is inherited in an autosomal dominant fashion. However, polyuria and polydipsia do not develop until after the first few years of life in affected persons, suggesting progressive loss of neurosecretory tissue. The acquired form of neurogenic diabetes insipidus results from degeneration of neurons in the supraoptic nuclei after trauma, vascular abnormalities, infection, a variety of tumors, or occasionally pressure from pituitary enlargement (PPID).[5,707,708] As with the hereditary form, polyuria and polydipsia usually are not manifested until 80% to 90% of the neurosecretory neurons are destroyed.

In equids, two well-documented cases of neurogenic diabetes insipidus have been described.[363,364] Neither animal could concentrate urine in response to water deprivation, but administration of exogenous vasopressin resulted in an increase in urine concentration and a decrease in urine volume. In a Welsh pony in which the condition was considered idiopathic, the absence of an increase in plasma vasopressin concentration after water deprivation (compared with control ponies) further supported a diagnosis of neurogenic diabetes insipidus.[364] Acquired neurogenic diabetes insipidus after encephalitis was confirmed histologically in the other horse.[363] Two other reports of diabetes insipidus in horses more likely described cases of primary polydipsia because both animals demonstrated an ability to concentrate urine during water deprivation or had random urine specific gravities greater than 1.020.[709,710]

Nephrogenic diabetes insipidus results from resistance of the cortical and medullary collecting ducts to the antidiuretic action of vasopressin.[5,707,708] In the absence of systemic disease, nephrogenic diabetes insipidus is most commonly a familial disorder in human beings with an X-linked semirecessive mode of inheritance. As such, the disorder is carried by females and expressed in male offspring.[711] Nephrogenic diabetes insipidus has been reported in three colts, two of which were sibling Thoroughbreds, suggesting that an inherited form of nephrogenic diabetes insipidus also may occur in horses.[56,712] Two of the three colts were underweight for their age. Affected colts could not increase urine concentration in response to water deprivation, although they did show appropriate increases in plasma vasopressin concentration. Furthermore, minimal response to exogenous vasopressin administration in all three colts confirmed resistance of the cortical and medullary collecting ducts to the antidiuretic action of vasopressin.

Nephrogenic diabetes insipidus also may be acquired after drug therapy or a variety of metabolic, infectious, or mechanical (postobstruction) disorders. Anomalous or neoplastic disorders resulting in structural deformation of the kidneys are another potential cause of nephrogenic diabetes insipidus.[5,707,708] Necropsy and histopathology revealed no lesions in one colt and few focal interstitial aggregates of lymphocytes, plasma cells, and rare areas of interstitial fibrosis in the kidneys with focal necrosis of the renal pelvis and ureteral epithelium

in another.[56] Histopathologic lesions found in renal biopsies from one case[712] included severe vacuolization and intracytoplasmic eosinophilic granular changes in distal collecting duct epithelium and occasional slightly increased cellularity of glomeruli. However, these lesions were apparently reversible and not observed in repeat biopsies performed later, after dietary and supportive therapy for diabetes insipidus.

Neurogenic and nephrogenic forms of diabetes insipidus share some similarities in pathogenesis. Polyuria because of a lack of vasopressin activity results in net water loss and an increase in plasma osmolality. As plasma osmolality increases, stimulation of thirst results in a compensatory increase in water consumption. In normal individuals and those with nephrogenic diabetes insipidus, the osmoreceptors in the hypothalamus sense the increase in plasma osmolality and subsequently signal for vasopressin release. As little as a 1% increase in plasma osmolality (about 3 mOsm/kg) results in a 1-pg/mL increase in plasma vasopressin concentration. In normal individuals, this small change is substantial enough to increase urine osmolality and decrease urine flow. With greater increases in plasma osmolality, more vasopressin is secreted. In human beings, urine osmolality approaches a maximum after an increase in vasopressin concentration to about 5 pg/mL (from a resting value of about 1 pg/mL).[5,708]

Limited studies in ponies and horses suggest that a similar degree of vasopressin release occurs in response to minor increases in plasma osmolality[179,182,183]; however, vasopressin also appears to be a "stress hormone" in horses because substantially greater concentrations (tenfold higher than those induced by water deprivation) have been measured after application of a nose twitch, nasogastric intubation, or exercise.[185] Thus increases in plasma vasopressin concentration after water deprivation would be expected to vary in horses, and separating osmotic effects from stress effects in an individual horse may be difficult. A word of caution also is warranted about subjecting horses suspected of having diabetes insipidus to water deprivation. Because urine-concentrating ability may show minimal improvement with either form of diabetes insipidus, affected horses may continue to excrete excess water in the face of water deprivation. As a result, they may become substantially dehydrated (10%–15%) within the first 12 hours of water deprivation. Thus one should carefully monitor horses suspected of having diabetes insipidus during the period of water deprivation to decrease the risk of inducing serious hypertonic dehydration.

The Hickey-Hare test to diagnose diabetes insipidus involves infusion of hypertonic (2.5%) saline (0.25 mL/min/kg IV for 45 minutes) to stimulate vasopressin release and water retention.[712] Normal animals will respond with concentrated urine and reduced urinary volume after sodium infusion. Horses with diabetes insipidus did not concentrate urine after hypertonic saline administration.[56,712]

In addition to assessing the effects of water deprivation on urine-concentrating ability and plasma vasopressin concentrations, the final diagnostic manipulation is often administration of exogenous vasopressin (2.5 mU/kg in 5% dextrose [5 U vasopressin/L] constant rate infusion over 60 minutes or 0.5 U/kg intramuscularly [IM]).[713] In normal horses, urine specific gravity should increase to at least 1.020 at 60 to 90 minutes after vasopressin administration. Alternatively, DDAVP, a synthetic vasopressin analog, may be used. DDAVP is available as a nasal solution (0.1 mg/mL) for humans with central diabetes insipidus. The nasal spray can be diluted in sterile water and administered (0.05 μg/kg IV) for the diagnosis of diabetes insipidus in horses. Normal horses that underwent experimentally induced diuresis by repeated dosing of water via nasogastric tube increased urine specific gravity to greater than 1.020 from 2 to 7 hours after administration of DDAVP.

The medullary interstitial osmotic gradient develops as a result of countercurrent exchange, and the magnitude of the gradient is related inversely to tubular flow. Medullary washout may occur with the high tubular flow rates that can accompany renal disease. Although partial medullary washout may contribute to the concentrating defect in diabetes insipidus, the rapid response to exogenous vasopressin administration in cases of neurogenic diabetes insipidus (increase in urine osmolality to 900 mOsm/kg or greater) indicates that the medullary concentration gradient is not severely compromised.[5,714] This response further explains why in some cases of diabetes insipidus, urine osmolality can be greater than plasma osmolality after water deprivation. The mechanism for this response is thought to be a decrease in tubule flow rate that allows more time for passive water extraction from the hypoosmotic tubular fluid. With nephrogenic diabetes insipidus, one also may observe mild improvement in urine-concentrating ability (an increase in urine osmolality up to 500 mOsm/kg) in response to exogenous vasopressin administration. This response has been attributed to partial sensitivity of the collecting ducts to vasopressin and to vasopressin activity at other portions of the renal tubule.[5,707]

Treatment of diabetes insipidus should aim to manage polydipsia and polyuria. With neurogenic diabetes insipidus, recovery of vasopressin secretion is rare once the secretory neurons have degenerated to the degree that polyuria and polydipsia become apparent. Consequently, treatment consists of hormone replacement. In the past, intramuscular injection of Pitressin tannate in oil every 2 to 3 days was successful in limiting polyuria; however, development of resistance or allergic reactions was an occasional problem, and long-acting vasopressin formulations are no longer available. Vasopressin has relatively short antidiuretic effects and a short plasma half-life, which makes replacement therapy in horses difficult.[715] With the development of potent vasopressin analogs (desmopressin), effective treatment by nasal insufflation is now available.[5] Desmopressin eye drops have also been used to increase urine specific gravity in a foal with central diabetes insipidus.[716] The use of desmopressin for diagnosis and treatment of neurogenic diabetes insipidus in small animals has been described.[714,717] Largely by chance, other oral medications, including chlorpropamide and clofibrate, have been found efficacious in treating neurogenic diabetes insipidus. The mechanism of action of these drugs is uncertain, but they are thought to potentiate the effect of vasopressin on the collecting ducts.[5,714]

With nephrogenic diabetes insipidus, replacement hormone therapy is ineffective, and the only practical form of treatment for many years has been to restrict sodium and water intake or to administer thiazide diuretics. The latter treatment may reduce polyuria by 50% in many cases.[5] Thiazide diuretics inhibit sodium reabsorption in the distal tubule (diluting segment of the nephron) and increase solute delivery to the collecting duct. The mechanism by which such therapy paradoxically benefits patients with nephrogenic diabetes insipidus is poorly understood. Explanations include enhanced proximal tubular fluid reabsorption (via glomerulotubular balance) and decreased glomerular filtration and tubular flow

(via a greater osmotic stimulus to the macula densa and subsequent tubuloglomerular feedback).[5,708] Treatment with prostaglandin inhibitors or amiloride has also decreased polyuria in patients with nephrogenic diabetes insipidus. The former agents probably work by decreasing renal blood flow and glomerular filtration, whereas the latter drug, a sodium channel blocker, is thought to act in a manner similar to the thiazide diuretics.[5] One horse clinically improved with only dietary salt restriction (no additional salt other than what was contained in feed) and limited but regular access to water.[712]

DIABETES MELLITUS

Diabetes mellitus is a state of chronic hyperglycemia usually accompanied by glucosuria.[277,718] The resultant osmotic diuresis is an occasional cause of polyuria and polydipsia in horses that has been described to result in a water intake exceeding 80 L/day.[719,720] Type 1 (insulin-dependent) diabetes mellitus results from a lack of insulin that in human beings is usually attributable to viral or autoimmune disease. Those with type 2 (non–insulin-dependent) diabetes mellitus have normal to high insulin concentrations, but their tissues are insulin insensitive. Thus the response to an oral carbohydrate load or an intravenous glucose challenge is impaired, resulting in prolonged hyperglycemia.[718] The mechanism of insulin resistance is not well documented for horses but may result from insulin antagonism, decreased numbers of insulin receptors, or lack of insulin receptor activation in response to insulin binding. The most common causes of equine non–insulin-dependent diabetes mellitus are equine metabolic syndrome and PPID, both of which are discussed in detail elsewhere in this text.[700-704]

Although very uncommon, a few reports describe insulin-dependent and non–insulin-dependent diabetes mellitus that were not caused by PPID or equine metabolic syndrome and that resulted in polyuria and polydipsia as one of the presenting complaints.[719-724] Diagnostic evaluation reveals consistent hyperglycemia but negative dexamethasone suppression test results (a normal decrease of plasma cortisol to low concentrations). Treatment in most instances is supportive, although replacement insulin therapy may be helpful in cases in which measured serum insulin concentrations are low (insulin-dependent diabetes mellitus). In one case of pancreatic β-cell failure,[720] treatment with glyburide (0.02 mg/kg) and metformin (1.9 mg/kg) lowered interstitial fluid glucose concentrations to normal ranges. Insulin therapy may be of some benefit in horses with elevated serum insulin concentrations because pharmacologic doses in part may overcome the insulin insensitivity. In such cases, synthetic insulin products may be preferable over protamine zinc insulin, because one horse with PPID and secondary diabetes mellitus developed antiinsulin antibodies and had a relapse in clinical signs after 7 weeks of insulin therapy.[704]

SEPSIS AND ENDOTOXEMIA

Polyuria and polydipsia also have been reported as clinical signs in horses with sepsis or endotoxemia, although other clinical signs such as fever, abdominal pain, and weight loss predominate.[725] The mechanism is unclear but may result from endotoxin-induced prostaglandin production. Prostaglandin E_2 is a potent renal vasodilating agent in laboratory animals, and it antagonizes the effects of antidiuretic hormone on the collecting ducts.[186] Some horses with chronic gram-negative bacterial infections (e.g., peritonitis, pleuritis) may have low-grade or intermittent endotoxemia as a mechanism for polyuria, similar to the polyuria observed with canine pyometra.[726]

IATROGENIC POLYURIA

Finally, polyuria can be iatrogenic, occurring after a number of management practices or medical treatments. The most obvious iatrogenic cause is fluid therapy, for which polyuria is a desired response. Polyuria also has been observed with exogenous corticoid administration, although as for PPID the mechanism remains unclear. Human beings and dogs appear to experience a potent thirst response to exogenous corticoids; thus polydipsia may be an important cause of the polyuria observed. In horses receiving long-term dexamethasone treatment for immune-mediated disorders, one may observe profound glucosuria (2–3 g/dL) that leads to osmotic diuresis in these patients. Finally, transient diuresis or polyuria accompanies sedation with the α_2-agonists xylazine and detomidine.[14,15] Although these agents cause hyperglycemia for a few hours after administration, and occasionally glucosuria, a more likely mechanism for the transient polyuria is the existence of α_2-adrenoreceptors on collecting duct epithelial cells. Activation of these receptors is another mechanism of vasopressin antagonism.[16]

Renal Tubular Acidosis

Warwick M. Bayly

The veterinary profession recognizes well that horses with acute or chronic kidney disease are frequently acidotic. These animals are almost invariably azotemic, hypochloremic, and normokalemic or hyperkalemic and frequently have significant abnormalities on urinalysis. A less frequent renal disease associated with acidosis is renal tubular acidosis (RTA). RTA is a clinical syndrome of impaired renal acidification characterized by a hypokalemic, hyperchloremic acidosis without azotemia. Affected horses frequently have normal urinalysis. The condition has been well described in human beings,[727-729] and several case reports documenting its existence in horses have appeared since the mid-1980s.[730-738]

The causes and pathogenic mechanisms responsible for the development of RTA are ill understood. In human beings RTA may be primary (genetic or idiopathic) or secondary, occurring after a variety of conditions, including hyperglobulinemia, various autoimmune disorders, kidney disease such as polynephritis and obstructive uropathy, cirrhosis, drug- or toxin-induced nephropathies (including amphotericin B), metabolic disorders involving nephrocalcinosis, and a number of genetic diseases. All reported equine cases apparently have been idiopathic, because no signs of primary renal, hepatic, or immune-mediated disease or disturbance of calcium metabolism have been evident, and the horses have had no history of access to toxins. In some cases, however, low-grade renal tubular disease has been suspected because of mild proteinuria.

General agreement is that three types of RTA exist.[155] Type 1, also known as *distal* or *classic RTA,* arises from the inability of the cells of the distal tubule to establish a steep hydrogen ion gradient between the blood and the urine. This inability results from failure of normal H^+ excretion from the distal tubules. In many cases, this gradient may be less than 10:1. Whether

TABLE **14.12 Features Used to Differentiate Type 1 and Type 2 Renal Tubular Acidosis**

Variable	Type 1	Type 2
Acidosis	Severe	Less severe, self-limiting
Hypokalemia	Severe	Mild to moderate
Glycosuria, proteinuria	Absent	Often present
Urine pH during mild/moderate acidosis	Inappropriately high	Inappropriately high
Severe acidosis	Inappropriately high	Normal
Effect of alkali administration	Decreases or worsens hypokalemia, depending on stage of disease	Worsens hypokalemia
Amount of HCO_3^- needed to correct acidosis	Low	High
Ammonium chloride challenge	Failure to excrete acid (urine does not acidify)	Urine pH decreases
Bicarbonate loading	Filtered bicarbonate is reabsorbed	Bicarbonate is lost in the urine

or not this low ratio is caused by an insufficient number of proton-secreting pumps in the distal nephron or by H^+ diffusing back across the lumenal membrane after being secreted is not clear. Accelerated K^+ secretion occurs because of the existing electrochemical driving forces in the distal nephron and the lack of protons to offset them. In addition to having high urinary K^+ clearance, patients may be hypercalciuric and hyperphosphaturic, although in horses this may be difficult to assess. Equine urinary concentrations of K^+ and Ca^{+2} in particular are high. In human beings, about 70% of adults with distal RTA develop some form of urolithiasis.[739] This condition has not been recognized in the few reported equine cases and may not be, given the significant differences between the two species.

Type 2, or proximal, RTA is caused by a failure of HCO_3^- resorption in the proximal tubule. This part of the nephron usually reabsorbs the bulk of the filtered HCO_3^- via Na^+ and H^+ exchange and the subsequent breakdown of carbonic acid to carbon dioxide and water under the influence of carbonic anhydrase. Disruption of normal Na^+ and H^+ exchange or carbonic anhydrase activity therefore results in excess flow of HCO_3^- to the distal tubule where the ability to resorb the anion is poor. This bicarbonaturia also results in accelerated K^+ secretion and hypokalemia. In humans with this form of the disease, the suggestion is that a reduction occurs in the threshold concentration at which HCO_3^- is reabsorbed from the proximal nephron. As a result, reabsorption resumes once serum HCO_3^- concentration drops below this threshold, and a new steady state is said to develop. More likely because of the urinary HCO_3^- loss, plasma HCO_3^- concentration decreases, as does glomerular filtration of HCO_3^-. Eventually the distal tubule reaches a point at which it can handle the amount of HCO_3^- presented. Urine pH may start to decrease because of the lower amount of HCO_3^- being excreted. A new steady plasma HCO_3^- concentration is established, albeit considerably below the normal range, and acid-base homeostasis may return gradually. Consequently, type 2 RTA often appears to be self-limiting in humans.[740] This form of the disease occurs rarely by itself in humans and almost always is associated with other proximal tubule dysfunction such as defective resorption of glucose, amino acids, and phosphate (Fanconi syndrome). Type 2 RTA has been reported in two horses.[730]

Fanconi syndrome has been rarely reported in horses, either experimentally with mercuric chloride toxicity[535] or as an idiopathic transient syndrome of weight loss, glucosuria, lactic aciduria, and aminoaciduria in two adult Quarter Horses.[741] A metabolic screening test that detects carbohydrates and organic acids in urine for the diagnosis of Fanconi syndrome in dogs has also been applied to horses. Animals with Fanconi syndrome have aminoaciduria and possibly proteinuria with other indications of tubular disease such as glycosuria, bicarbonaturia, and lactic aciduria. One of the Quarter Horses reported by Ohmes and colleagues[741] had chronic kidney disease, but both horses responded within days to months with intravenous and oral supplementation of bicarbonate and potassium.

Type 4 RTA is characterized by hyperkalemic, hyperchloremic acidosis and is common in humans, but it has not been reported in horses. Type 4 RTA apparently is associated with hypoaldosteronism or resistance of the distal nephron cells to the effects of aldosterone. As a result, the renal clearance of K^+ and H^+ is reduced. The associated natriuresis ultimately may lead to a reduced ability to concentrate urine because of "washout" of the medullary concentrating gradient, if the condition becomes chronic. Another form of RTA (type 3), which formerly had been described as having characteristics of type 1 and type 2 RTA, now is considered to be a variation of type 1.[155]

Differentiating between the distal (H^+ retention) and proximal (HCO_3^- wasting) forms of RTA is theoretically important if the approach to the human form of the disease is any guide, because in human beings the two types differ with respect to clinical severity, treatment, and prognosis.[742] The ability to differentiate between the forms of RTA in horses appears to be less critical, because treatment does not seem to differ much according to the suspected type. Table 14.12 summarizes some differential characteristics of the two types of RTA. In humans with type 2 RTA, plasma HCO_3^- concentrations tend to be higher than those associated with the distal form of the disease, but this does not appear to be the case in horses, because all the horses the author has seen, plus those that have been described in the literature, have been severely acidotic (HCO_3^- concentrations 10 mmol/L or less). In horses, differentiation between the distal and proximal types of RTA has been based on measurement of urine pH. Evaluation of urine ammonium excretion, urine net charge or anion gap, and urinary P_{CO_2} have not been reported in horses, although they are regarded as critical to the specific differentiation of RTA in humans.[156,743,744] Because of the alkalinity of normal equine urine, measurement of urine P_{CO_2} is probably of little

benefit. With type 1 RTA, urine pH tends to stay high (i.e., in the normal to alkaline range). In type 2, urine pH is generally neutral or slightly acidic. A suggested way of making this differentiation is to assess the urinary response to the administration of the urine-acidifying agent ammonium chloride (0.1 g/kg). Typically, one gives this solution orally, and it should lower the urine pH to less than 7.0, which it supposedly does in normal horses and those with type 2 RTA. In cases of distal (type 1) RTA, the urine pH remains high in the face of the increased acid load. Because the administration of such acidifying agents potentially could worsen the degree of acidosis in cases of type 2 RTA (as a result of reduced buffering capacity), performance of this test is not recommended until one has attempted at least partial replacement of the HCO_3^- deficit. In the author's experience, the ammonium chloride challenge test has proved unreliable, having failed to acidify the urine of normal healthy horses even when given intravenously. The dose and rate of administration may need to be increased before the test can be considered worthwhile. A more suitable alternative may be to infuse sodium sulfate as is done sometimes in humans.[727] The author could find no reports of the use of this test in horses.

Another diagnostic option that may be worthy of investigation in horses is calculation of the urine net charge. As in any body fluid, the sum of urinary cations must equal the sum of its anions. Therefore in urine,

$$Na^+ + K^+ + Mg^{2+} + NH_4^+ = Cl^- + HPO^- + SO^- + \text{other urine buffers} + \text{organic anions}$$

In human beings, Ca^{2+} and Mg^{2+} tend to be present in urine in small constant quantities. Excretion of HPO^-, SO^-, titratable buffers, and organic anions is also constant and exceeds the Ca^{+2} and Mg^{+2} by an amount that is referred to as the *urine anion gap* (AG_u). Thus

$$Na^+ + K^+ - Cl^- = AG_u + NH_4^+$$

The expression ($Na^+ + K^+ - Cl^-$) is referred to as the *urine net charge* and reflects the excretion of NH_4^+.[156] Given the aforementioned plasma electrolyte and acid-base abnormalities, type 2 RTA has been shown likely to exist when the urine net charge is negative (i.e., Cl^- greater than [$Na^+ + K^+$]) because NH_4^+ is greater than AG_u. In other words, H^+ continues to be secreted normally from the distal tubule and plenty of NH_4^+ is being produced. When ($Na^+ + K^+$) is greater than Cl^-, the net charge is positive, indicating that NH_4^+ excretion is low because of failure to excrete H^+ (i.e., type 1 or distal RTA exists).

No breed or sex predilection exists for RTA in horses, and the average age for 16 affected horses was 7 years.[737] The most common clinical signs in affected horses are depression, poor performance, weight loss, and anorexia. Various other signs such as chronic weight loss, ataxia, dysphagia, and periodic collapse also have been reported. The latter three signs may be manifestations of severe weakness, which probably is caused by the severe hypokalemia. The reduced K^+ concentration also may be associated with bradycardia.

On clinical examination, affected horses are usually afebrile. Mild icterus is common, presumably because of anorexia, although significant indirect hyperbilirubinemia and increases in serum activities of GGT and ALP have been observed. Although cirrhosis of the liver has been associated with the development of RTA in human beings, equine cases that the author is aware have demonstrated no clinicopathologic changes compatible with severe liver disease. Sulfobromophthalein clearance half-time was normal in the one case in which it was measured.[731]

One should suspect RTA whenever a horse has a severe hyperchloremic metabolic acidosis in the absence of any obvious cause of extrarenal hypovolemia such as diarrhea or small intestinal ileus. In such cases, the plasma anion gap usually is widened, whereas with RTA it remains normal. However, hypoalbuminemia may mask widening of the anion gap because albumin is normally responsible for much of the normal anion gap.[745] Therefore the anion gap value should be smaller in hypoalbuminemic horses. Azotemia has not been recognized in horses with RTA, although signs of tubular dysfunction, such as mild proteinuria and increases in fractional K^+ clearance and urine GGT activity, have been observed. Renal biopsy and ultrasonic examination of the kidneys are normal.

Definitive diagnosis of RTA is based on the demonstration of severe hypokalemia (2.3 mmol/L or less) and a urine pH that is generally neutral or alkaline in the face of a severe acidosis (venous blood pH less than 7.15), although theoretically one could observe a slight aciduria with type 2 RTA. Hypokalemia in the face of severe acidosis is uncommon. Classically, the reverse is expected because of intracellular buffering of H^+ and the reciprocal role of H^+ and K^+ in maintaining electroneutrality of the extracellular fluid. Occasionally, serum chloride concentrations are in the high normal range rather than being elevated. Blood gas measurement usually reveals some degree of compensatory respiratory alkalosis ($Paco_2$ = 25–35 mm Hg).

The differential diagnosis of RTA should be included for any animal that has sudden weakness, depression, and possible collapse: cardiovascular failure, neurologic disease (including rabies), hypoglycemia, and peracute toxemia or endotoxemia. One usually can rule out all of these based on a thorough physical examination. These findings, plus demonstration of the aforementioned electrolyte and acid-base abnormalities, suggest RTA. Conditions such as hypokalemic periodic paralysis, Addison's disease, pituitary pars intermedia dysfunction, and chronic corticosteroid or diuretic use could cause some of these clinicopathologic disturbances but not the combination of hyperchloremia, acidosis, hypokalemia, and a normal plasma anion gap.

Prompt recognition of the disease and quick institution of therapy are important because the untreated condition is potentially fatal. All reported equine cases of RTA have responded satisfactorily to treatment, as have the cases that the author has seen. Although the ability to differentiate between type 1 and type 2 RTA may be important in humans from a therapeutic perspective, the distinction has not seemed to be essential in horses. Regardless of the suspected type of the disease, treatment of the condition in horses is associated with HCO_3^- and K^+ replacement therapy. Although the administration of $KHCO_3$ would seem to be ideal, it is not readily available, and the need for prompt therapy means that treatment usually involves a combination of orally administered KCl and intravenously administered $NaHCO_3$ and KCl. It must be emphasized that one should not give $NaHCO_3$ without some form of potassium supplementation to ensure that hypokalemia does not get worse. Generally, a steady improvement in the patient occurs after 12 to 24 hours of treatment. Oral fluids usually are given 4 to 6 times per day for the first 48 hours, whereas intravenous fluids frequently

are given at a slow but steady rate. Inclusion of glucose or dextrose in intravenous fluids helps to promote intracellular uptake of K^+. Serum chloride concentration usually decreases as HCO_3^- concentration increases. In the author's experience, a steady improvement occurs in HCO_3^- concentration and tends to improve more rapidly than K^+ concentration. Serum concentrations of potassium appear to be slower to return to normal, possibly because intracellular stores are replaced first. Estimating the total potassium deficit before the onset of treatment is impossible because of the huge intracellular deficit these animals suffer. In the author's experience, total deficits of K^+ frequently exceed 4000 mmol.

With correction of the acid-base and electrolyte disturbances, muscle strength and appetite generally return within 48 hours of the onset of treatment. At this point, one usually can reduce potassium supplementation and subsequently discontinue it once the forage intake of the horse has returned to normal. Obviously, giving the horse a diet that includes ample amounts of good quality hay is important because of the high potassium content of this feedstuff. Bicarbonate therapy generally continues longer, although one usually discontinues intravenous fluids with return of appetite. Long-term oral supplementation with sodium bicarbonate is often required for the maintenance of normal acid-base status. Periodic rechecking is advised until it becomes clear that the condition is stable. Long-term follow-up of cases that the author has seen and those that have been reported in the literature suggest that the prognosis is favorable, but horses with evidence of renal disease may be more likely to relapse.[737]

Neoplasia of the Urinary Tract

Harold C. Schott II

Neoplasia of the urinary tract in horses is uncommon.[64,70,95,746-748] Primary renal neoplasms, which represent fewer than 1% of all equine tumors, include adenomata, renal cell carcinomata, and nephroblastomata.[749-751] Renal adenomata are small well-circumscribed lesions in the renal cortex that are usually incidental necropsy findings.[25]

The most commonly reported and best-described renal tumor in the horse is renal cell carcinoma or adenocarcinoma.[674,675,750-760] These neoplasms arise from the epithelium of the proximal convoluted tubules in most cases. In human beings, renal cell carcinomata are known as the *internist's tumor* because of their diverse and often obscure presenting signs. Although a classic triad of symptoms including flank pain, gross hematuria, and palpable renal mass has been described, these symptoms occur in fewer than 10% of affected human beings at presentation.[5] Similarly, affected horses usually have nonspecific presenting complaints, including poor performance, depression, weight loss, and recurrent colic (Table 14.13).[751] A paraneoplastic process leading to severe hypoglycemia has been reported in two cases of equine renal cell carcinoma, probably caused by production of an insulin-like factor, an abnormal variant of insulin-like growth factor II, or less likely from excessive glucose use by the tumor.[758,759] More specific clinical signs that increase suspicion of renal cell carcinoma include hematuria and detection of a palpable mass on rectal examination (see Table 14.13).[751] Renal cell carcinomata are typically unilateral, and the contralateral kidney maintains normal renal function. Therefore indices of renal function such as BUN and creatinine concentration are usually normal. A retrospective study reported leukocytosis with mature neutrophilia in 59% of cases and hyperfibrinogenemia in only 33%.[751] Abdominocentesis revealed hemoabdomen or exfoliated neoplastic cells in 71% of cases. Although nephrectomy is the treatment of choice in human beings, tumors are typically large and adherent to surrounding organs, especially local vascular structures, by the time that they are detected in horses. Thus surgical removal usually is not possible. Furthermore, frequent metastases (see Table 14.13) usually make renal adenocarcinoma an untreatable problem. The poor prognosis can be attributed to the fact that clinical signs of intraabdominal neoplasia in horses often are not apparent until the disease is advanced.[761] In one report of renal carcinoma, clinical signs of the tumor were absent until the horse was anesthetized for laryngeal surgery. After an uncomplicated surgery, the horse was repositioned for recovery but died shortly thereafter. Compression of the caudal vena cava by a large renal carcinoma was suspected to have led to a decrease in venous return as the cause of sudden death.[762] In another horse, clinical signs were not observed until bony metastasis to the olecranon caused lameness.[760]

Other neoplastic diseases that may affect the kidneys include nephroblastoma,[763-765] transitional cell carcinoma,[761,766] and squamous cell carcinoma.[767] Nephroblastoma (Wilms' tumor) is an embryonal tumor that arises in primitive nephrogenic tissue or in foci of dysplastic renal tissue, whereas the latter tumor types arise from the uroepithelium of the renal pelvis or ureter.[25] Papillary renal adenoma of distal nephron differentiation has been described as an incidental finding during necropsy of an aged mare.[768] Neoplastic involvement of the upper urinary tract also may occur with dissemination of lymphosarcoma, hemangiosarcoma, melanoma, or adenocarcinoma arising from other tissues in the abdomen.[25,95,749,761,769]

Finally, although they are not truly cancerous disease processes, mucinous hyperplasia of the renal pelvic and proximal ureteral uroepithelium or ureteropelvic polyp formation can lead to development of a tissue mass in either kidney, ureteral obstruction, and hydronephrosis.[41,770] Renal mucous gland cystadenomas have been found incidentally at necropsy.[771] Mucous gland hyperplasia of the renal pelvis or proximal urethra are differentiated from cystadenomas by their thin fibrous connective tissue capsule, compression of adjacent renal parenchyma, multiple proliferative intraluminal papillary projections, and large mucus-filled cystic lumens.

In addition to rectal palpation for a mass in the area of either kidney and examination of urine for red blood cells (hematuria), ultrasonographic evidence of a tissue mass destroying the normal architecture of the kidney would support renal neoplasia. Unfortunately, attempts to establish a definitive antemortem diagnosis were successful in only two previous case reports. In the first case, neoplastic cells were detected on analysis of peritoneal fluid,[755] whereas percutaneous biopsy of the tissue mass in a second horse demonstrated neoplastic tissue. Renal biopsy should be performed with caution as neoplasia can increase the risk of complications such as hemorrhage and colic. An ultrasound-guided renal biopsy is recommended to ensure sampling of the suspected mass. However, histopathologic examination may be inconclusive as renal neoplasms can often have poor histologic detail.[750] The majority of renal carcinomas are definitely diagnosed during postmortem examination. Thus these procedures are warranted in all horses with a mass consistent with a renal neoplasm. In addition to

TABLE **14.13 Clinical Features of 15 Horses with Renal Cell Carcinoma**

Breed	Age (Years)	Sex	Unilateral or Bilateral	Presenting Complaint	Weight Loss	Pain/Colic	Hematuria	Palpable Mass on Rectal Examination	Azotemia	Postmortem Findings	Reference
American Saddlebred	15	G	Bilateral	Recurrent colic	Yes	Yes	No	Yes	No	25-cm-diameter renal calculus, metastases to liver and other kidney	752
Albino	10	F	Unilateral, left	Abortion and ascites	No	No	No	Yes	Mild	75-cm-diameter, 31-kg mass, metastases to peritoneum	754
Standardbred	16	F	Unilateral, right	Weight loss, hematuria	Yes	No	Yes	Yes	No	30-cm-diameter, 8-kg mass, adhesions to liver and bowel, no metastases	754
Thoroughbred	16	F	Unilateral, left	Weight loss	Yes	No	Yes	Yes	Mild	35-cm-diameter, 20-kg mass, hemoperitoneum, no metastases	755
Thoroughbred	16	F	Unilateral, right	Weight loss, soft feces	Yes	No	No	No	Mild	Metastases to liver and lungs	756
Pony	15	G	Unilateral, left	Weight loss, hematuria	Yes	No	Yes	Yes	No	6.6-kg mass, metastases to liver and lungs	757
Pony	10	G	Unilateral, right	Weight loss, back pain, colic, polyuria/ polydipsia	Yes	Yes	Yes	No	No	6-cm-diameter mass hemoperitoneum, no metastases	757
Thoroughbred	4	G	Unilateral, left	Respiratory noise	No	No	NR	NR	No	30-cm-diameter mass, local invasion of sublumbar muscles	757
Grade	7	G	Unilateral, right	Hematuria, followed by weight loss and colic	Yes	Yes	Yes	No	Mild	40-cm-diameter, 23-kg mass, hemothorax and hemoperitoneum, metastases to liver and lungs	757
Thoroughbred	9	F	Unilateral, right	Recurrent colic, weight loss	Yes	Yes	No	Yes	No	30-cm-diameter mass, metastases to omentum and muscle	674
Shire	4	F	Unilateral, right	Hematuria, followed by weight loss	Yes	Yes	Yes	Yes	No	65-cm-diameter, 47.7-kg mass, adhesions to liver and bowel, no metastases	675
Grade	14	F	Unilateral, left	Pyrexia, hematuria, diarrhea	Yes	No	Yes	Yes	No	5-kg mass; metastases to liver, pancreas, and lungs	758

Mustang[a]	14	G	Unilateral, right	Collapse Hypoglycemia Colic Anorexia Diarrhea	Yes	Yes	No	No	No		760
Thoroughbred[a]	6	G	Unilateral, right	Depression Head pressing Obtunded Weight loss Weakness	Yes	Yes	No	Yes	No	35-kg mass, duodenum and jejunum loosely adhered to mass, metastases to liver	759
Arabian	21	G	Unilateral, right	Forelimb lameness Poor appetite	No	Yes	No	NR	NR	2 × 5–cm extrarenal mass at cranial pole of kidney, single metastasis to lung	5

G, Gelding; *F,* mare; *NR,* not reported.
[a]Paraneoplastic syndrome (hypoglycemia).

analyzing urine for hematuria, cytologic examination of urine for neoplastic cells is also indicated.[565]

The most common presenting complaint for bladder neoplasia is hematuria.[565,680,772] Unlike dogs, in which transitional cell carcinoma is the most commonly described bladder neoplasm, squamous cell carcinoma has been reported most frequently in horses.[565,680,773] Squamous epithelial cells are found to occur in the normal equine bladder, and it has been suggested that this may be the reason squamous cell carcinoma is more common in the horse than transitional cell carcinoma, which is a common bladder neoplasm in other species.

Other types of bladder neoplasms affecting horses include transitional cell carcinoma, lymphosarcoma, leiomyosarcoma, rhabdomyosarcoma, and fibromatous polyps. In contrast to cattle, which develop bladder neoplasia in association with chronic ingestion of bracken fern and other plants (enzootic hematuria), dietary factors have not been incriminated in the development of bladder cancer in horses.[25] Common presenting complaints often mimic those of cystic calculi: stranguria, pollakiuria, and hematuria.[773] One can establish diagnosis of bladder neoplasms by rectal palpation or ultrasonographic imaging of a bladder mass, endoscopic examination, biopsy (Fig. 14.43), and urine cytologic examination. Some horses with bladder neoplasia may develop secondary pyelonephritis. Treatment has included partial bladder resection or intravesicular instillation of 5-fluorouracil, but successful outcomes have not been reported.[565,773,774] Doxorubicin (30 mg/m^2 IV) therapy was unsuccessful for the treatment of an extensive and poorly differentiated bladder leiomyosarcoma in one case.[775] By the time that clinical signs are observed, most affected horses will have local metastatic involvement, notably of the aorta, iliac arteries, and lymph nodes, and prognosis is poor.

Tumors of the urethra and external genitalia are the most common urinary tract neoplasms of horses and account for 6% to 10% of all neoplasms, with squamous cell carcinoma being the most commonly reported.[776,777] Although reports describe a paraurethral lipoma[554] and fibrosarcomata[465] resulting in frequent urination or dribbling and urinary tract infection, respectively, tumors of the external genitalia are more common than urethral tumors.[86,87,560] Tumors affecting the external genitalia include squamous cell carcinoma, sarcoid, melanoma, mastocytoma, hemangioma, and papillomata or warts. Habronemiasis used to be a significant cause of genital lesions before the widespread use of ivermectin but should remain on the differential list because it can be distinguished from squamous cell carcinoma or sarcoid only by microscopic examination. Biopsy or simple impression smear of *Habronema* lesions will reveal extensive infiltration of eosinophils, which distinguishes habronemiasis from neoplasia, and habronemiasis is usually very pruritic.[778,779] Breeds with nonpigmented genitalia (Appaloosas and Paints) appear to be predisposed to squamous cell carcinoma. Similarly, a predilection for older geldings and stallions has been associated with a carcinogenic potential of smegma, which may cause chronic irritation that stimulates the progression of papillomas to squamous cell carcinoma.[777-780] As in men, infection with papillomavirus is a risk factor for the development of squamous cell carcinoma.[778,779] Presenting complaints include masses involving the penis, prepuce, or vulva; edema of the penis or vulva; frequent extrusion of the penis; and a malodorous sheath or hematuria if the distal urethra is involved. Urinary tract obstruction is uncommon unless the tumors are large. Diagnosis is often made by direct examination of the external genitalia, finding raised and ulcerated single or multiple lesions. Advanced lesions appear cauliflower-like with areas of ulceration or necrosis. Although metastasis is uncommon (12%–16%), rectal palpation and ultrasonographic examination of the penis, local tissues, and regional lymph nodes is recommended.[777,779] Diagnosis is by visual examination and collection of a biopsy. Collection of the latter often is by complete excision of the lesion. In addition to surgical removal, a rather high rate of recurrence has led to a number of adjunct therapies, including intralesional chemotherapy, immunotherapy, cryotherapy, hyperthermia, and radiation therapy. In male horses, surgery can range from local excision to posthioplasty, partial phallectomy, or en bloc resection with penile retroversion.[781] Some squamous cell carcinomas may respond well to adjunctive treatment with piroxicam (80 mg PO q 24 h).[782] Tumor response is likely dependent on expression of cyclooxygenase-2 (COX-2), and not all equine squamous cell carcinomas produce COX-2 equally. Piroxicam is a selective COX-2 inhibitor in other species. Inhibition of COX-2 may induce apoptosis in neoplastic cells, inhibit angiogenesis, or act as an immunostimulant. Adverse gastrointestinal effects are possible during piroxicam therapy and may be avoided by administration every 48 to 72 hours. Piroxicam administration has also been recommended in the treatment of transitional cell carcinoma in small animals.[715] Because all treatment combinations have had variable success, some authors recommend aggressive surgical treatment early in the course of the disease.[783] Local injection of cisplatin, a cytotoxic antineoplastic agent, has been used with a high success rate to treat equine sarcoid and squamous cell carcinoma.[784,785] Topical 5% 5-fluorouracil cream (every 24 hours in mares; every 14 days in males) with or without surgical debulking may be effective for treatment of noninvasive squamous cell carcinoma of the external genitalia.[786] Incidence of recurrence of squamous cell carcinoma is 11% to 30%.[777-779] Because of varying responses and success, a combination of surgical and adjunctive therapy is recommended.

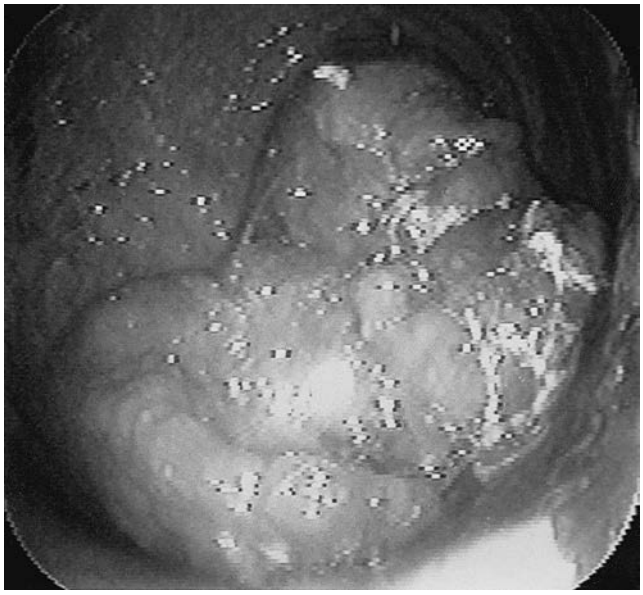


FIG. **14.43** Leiomyosarcoma of the urinary bladder resulted in hematuria. (Courtesy R. MacKay, University of Florida.)

Urinary Incontinence and Bladder Dysfunction

Warwick M. Bayly

Loss of control of bladder function is an infrequent problem in horses. When the condition is recognized, it is usually because a degree of incontinence develops, which by definition means that intravesicular pressure exceeds resting urethral pressure. Although a number of recognized abnormalities of bladder emptying can afflict human beings, dysfunctions of equine bladder control and micturition tend to fall into one of three categories, with the extent to which they are recognized depending on clinical signs. Basically, the three types of problems are (1) the reflex or upper motor neuron (UMN) bladder (also known as *spastic* or *autonomic bladder*), (2) paralytic or lower motor neuron (LMN) bladder, and (3) the myogenic or nonneurogenic bladder. Either of the last two conditions can be associated with the atonic bladder syndrome, which is probably appropriate given the similarity of clinical signs and subsequent treatment once either of the two types of conditions is recognized. In fact, although signs of UMN bladder dysfunction are initially different from those of the other two groups, this condition in horses also usually is not recognized until a degree of incontinence develops. A final form of bladder neuropathy that has been described in humans and dogs, but not horses, is reflex dyssynergia. In this condition, loss of coordination of detrusor contraction and urethral relaxation occurs, and the animal may make efforts to urinate but fail to do so. Such cases frequently appear similar to those of urethral obstruction, and one must distinguish between the two.

Although treatment options are limited and tend to be the same, regardless of what type of condition is responsible for incontinence, determining the origin of the problem is prognostically important. To accomplish this, the internist needs to have a good working knowledge of how micturition normally is controlled.

CONTROL OF MICTURITION

From a neurologic perspective, one can regard the bladder as having a body and an outlet, which can be divided further into the neck (or trigone) and the proximal urethra. Functionally, the bladder alternates between filling and storage and emptying and elimination phases.[787] Dysfunction in any of these locations or phases may result in clinical problems. Somatic innervation is primarily to the striated muscle of the urethra via a branch of the pudendal nerve, which originates from the sacral spinal cord (S1–S2). Other branches of this nerve go to the anal sphincter and perineum. The sympathetic nerve supply is provided via the hypogastric nerve, with the preganglionic fibers arriving from L1–L4 and synapsing in the caudal mesenteric ganglion. From there, postganglionic fibers supply the bladder (β_2-receptors) and proximal urethra (primarily α_1- and some α_2-adrenergic receptors).[19] Parasympathetic innervation also originates in the sacral cord, with neurons combining to form the pelvic nerve. Many complex interneuronal connections exist between sympathetic and parasympathetic nerves in the wall of the bladder, as well as small adrenergic cells that facilitate contact between sympathetic and parasympathetic pathways.[22] As a result, complete denervation of the bladder is virtually impossible. During the filling phase, the tone of smooth and striated muscles that together comprise the external and internal urethral sphincters increases. The pudendal nerve and the sympathetic nerves, respectively, innervate these muscles. Contraction of these muscles during filling maintains continence. Although the striated muscle forms a definite sphincter around the pelvic urethra, the anatomic existence of a bona fide internal sphincter is debatable.[788] However, restriction to urine outflow that follows stimulation of the α-adrenergic receptors at the neck of the bladder has a sphincter-like effect. The smooth muscle of the bladder, referred to as the *detrusor muscle,* is innervated by the parasympathetic pelvic nerve and α_2-adrenergic postganglionic fibers. Neurologic lesions anywhere in the spinal cord, not just the sacral segments, have the potential to produce bladder dysfunction.[789]

The storage or filling phase is dominated by sympathetic nerve activity and provides an excellent example of the effects of reciprocal innervation.[790] During filling, the detrusor muscle relaxes because of α-receptor–mediated inhibition of pelvic nerve afferents and stimulation of sympathetic β_2-receptors in the smooth muscle of the bladder body. The latter is a reflex response that involves sensory input from bladder stretch and pressure receptors via the afferent pelvic nerve fibers to the sacral spinal cord, interneurons in the cord, and preganglionic and postganglionic sympathetic axons in the hypogastric nerve. Relaxation of this muscle allows accumulation of large volumes of urine with little or no increase in intravesicular pressure.

Intravesicular pressure starts to rise once detrusor muscle fibers are stretched fully. Receptors in the bladder wall detect these increases, and impulses are transmitted via the pelvic nerve and ascending spinoreticular cord tracks to the pons, cerebrum, and cerebellum, where they are interpreted as the sensation of bladder fullness. Signals responsible for voluntary micturition originate in the cerebrum and exert their influence via the brainstem, from which UMNs descend in reticulospinal tracts to the sacral parasympathetic nuclei. This triggers the emptying phase. From these sacral segments, pelvic nerve impulses stimulate detrusor muscle contraction, and action potentials traveling via parasympathetic ganglia in the pelvic plexus or bladder wall to postganglionic fibers stimulate the smooth muscle. Depolarization spreads throughout the bladder via tight junctions, resulting in a strong, coordinated, contractile process. Simultaneous inhibition of the pudendal nerve and hypogastric α- and β_2-sympathetic activity further facilitates detrusor muscle activity and relaxation of the external and internal urethral sphincters, respectively. Part of this inhibitory activity represents reflex neuronal activity linking pelvic and pudendal nerve axons in the sacral spinal cord and inhibiting internuncial connections between sacral segments and sympathetic neurons in the lumbar spinal cord. Urethral sphincter relaxation also is coordinated centrally in a number of areas, including the cerebellum. Detrusor muscle contraction pulls the bladder neck open, and micturition occurs. The emptying phase ends when the bladder stretch receptors sense that the bladder is empty and afferent parasympathetic (pelvic nerve) impulses cease. Pelvic nerve efferent activity also stops and pudendal motor and sympathetic nerve activity resumes (because it is no longer inhibited), with the result that the detrusor muscle relaxes, restoring external and internal urethral sphincter tone.

CLINICAL SIGNS OF BLADDER DYSFUNCTION

Control of bladder function obviously is complex, and many sites exist where disruption of normal micturition can originate. In reality, problems are usually detectable in horses only when a degree of incontinence develops, which usually is manifested as constant or periodic dribbling of urine from the vulva or penis. In chronic cases, one frequently finds evidence of scalding and associated depilation of the perineum in mares and ventral abdomen in males, as well as the hindlimbs. Any activity that results in increased intraabdominal pressure, such as coughing or exercise, may exacerbate signs or be associated with their initial observation. Adult horses develop these problems much more frequently than foals. The duration of bladder dysfunction hugely affects prognosis and treatment because overflow incontinence may take years to months to be observed by horse owners and may not recognized until there is significant detrusor muscle atony and accumulation of large amounts of sabulous.[789]

Academically, being able to differentiate between the different neurogenic or myogenic forms of bladder dysfunction may seem important. In reality, the clinical signs and treatments tend to be the same, regardless of cause. UMN disease is characterized by increased urethral resistance, despite the presence of a full bladder, and may make catheterization or manual emptying of the bladder via rectal compression difficult. The condition usually occurs in association with broad, deep spinal cord lesions. In horses, these problems rarely are recognized because of the severe nature of associated clinical problems such as recumbency and myopathies. Frequently, such situations are deemed incompatible with life. Possibly, a focal lesion caused by a disease such as equine protozoal myeloencephalopathy or aberrant parasite migration could result in the development of spastic bladder without associated neurologic signs.

If horses suffering from this type of problem are able to stand, are kept in a sling, or have an isolated problem, in time they may develop the ability to urinate reflexively. This reflex develops because of the stimulation of pressure receptors connected to pelvic nerve afferents, which reflexively activate pelvic (parasympathetic) nerve efferents and the pudendal nerve. This activation results in detrusor muscle contraction and relaxation of the striated urethral muscle (external sphincter) leading to frequent urination, especially if the increases in abdominal pressure are regular, as may occur with any movement. These patients usually have some residual urine volume in the bladder after voiding. In such cases, urine dribbling usually is reported to occur intermittently, which is an important part of differentiating this type of dysfunction from paralytic (i.e., LMN) causes of incontinence.

As for the LMN bladder, lumbosacral trauma, equine herpesvirus–1 (EHV-1) myeloencephalitis, and cauda equina neuritis are probably the most common causes of this type of dysfunction, although in some parts of the world, Sudan grass toxicity[574] and sorghum cystitis-ataxia (enzootic ataxia cystitis)[564,574] are major problems. Tumors of the lumbosacral spinal cord, such as lymphosarcoma and melanoma, are also capable of inducing paralytic bladder. Finally, iatrogenic paralysis after epidural administration of alcohol occasionally has been reported in show horses. Because this practice is discouraged and unethical, how frequently it leads to complications is impossible to know.

With a paralytic (LMN) bladder, one usually sees other signs of LMN and lumbosacral dysfunction, including all or some of the following: loss of anal sphincter tone, tail paralysis, analgesia or hypalgesia over the perineum, atrophy of the muscles of the hip and hindlimb, and hindlimb weakness. Damage to the pudendal nerve and loss of external urethral sphincter integrity are therefore particularly important in the development of this problem. The bladder is atonic and distended with the urethral muscles relaxed, which results in urine dribbling because of overflow from the bladder. This incontinence may appear continuous, which helps to differentiate it from spastic (UMN) dysfunction. Sometimes the penis or vulva also may appear paralyzed. The prognosis is usually poor because of the development of secondary cystitis and general damage to the bladder wall and the detrusor muscle.

Idiopathic incontinence in geldings is a syndrome of bladder paresis or paralysis and sabulous urolithiasis without overt neurologic deficits.[789] Some cases may have a history of subtle neurologic disease or possible musculoskeletal conditions that prevent posturing to urinate and complete emptying of the bladder, which eventually leads to progressive detrusor muscle atony and accumulation of urinary sediment. The condition generally develops slowly, in association with the accumulation of large amounts of sabulous or mucoid urinary sediment or sludge in the bladder, which mainly is comprised of $CaCO_3$ crystals. In time, the weight and volume of this material, when coupled with that of normal urine accumulation, progressively stretch the detrusor muscle. Incontinence is not normally notable until the cranial aspect of the bladder begins to protrude over the edge of the pubis, which results in craniad and ventrad displacement of the sediment. This results in a bladder muscle that is stretched beyond its normal modulus of elasticity, and normal contraction and micturition are no longer possible. Severe distention and stretching also lead to breakdown in tight junctions that prevents depolarizing waves from passing from muscle fiber to muscle fiber. Eventually, overdistention becomes so significant that the ability to maintain sphincter function is lost and incontinence develops. These cases usually occur without any other signs of neurologic disease; however, because of the lack of any identifiable cause or specific pathophysiologic mechanism, specific focal lesions of a peripheral nerve such as the hypogastric nerve in fact could lead to a similar syndrome. Certainly, retention of urine in the bladder for any period results in the deposition of large quantities of sediment that in turn exacerbate the condition. One must recognize that part of the problem in cases of myogenic atony may stem from secondary cystitis, which develops after retention of urine for any period. The urea in retained urine breaks down to ammonia, which is irritating to the mucosal wall. Subsequent inflammation damages the bladder musculature further.

Cystitis and chronic urethritis per se can be nonneurogenic, nonmyogenic causes of apparent incontinence. Irritation of stretch receptors in the bladder wall apparently causes regular stimulation of stretch receptors in parasympathetic afferents and stimulates detrusor contractions that cannot be inhibited voluntarily. This effect results in an apparent increase in the frequency of urination (pollakiuria) and an inability to control when it occurs. The condition frequently is referred to as *urge incontinence* and also may occur in association with a unilateral ectopic ureter in which the bladder is much smaller than usual (because of disuse) and incapable of storing a normal volume of urine. The frequency of micturition again is increased.

In mares, incontinence may occur with cystolithiasis or secondary to foaling trauma to the urethral sphincter in multiparous mares, with or without a history of dystocia.[789] Hypoestrogenism also has been reported as a cause of nonneurogenic incontinence in an 18-year-old female Shetland pony.[791] A similar condition has been noted in older spayed bitches. The pathophysiologic mechanism of the condition is not known but probably is linked to a modulating effect of estrogen on norepinephrine and α-adrenergic receptor activity in the internal urethral sphincter.[792] In the documented case, the patient responded well to small doses (2 mg IM q 48 h) of estradiol cypionate.

A transient postoperative condition that results in urine retention after abdominal or perineal surgery has been noted in human beings.[793] The cause has not been explained, although the hypothesis is that a reflex depression of parasympathetic nerves may be responsible for detrusor muscle stimulation. This condition is distinct from the pain and reluctance to contract abdominal muscles after surgery. Whether such a condition exists in horses is unknown.

DIAGNOSIS

Careful physical and neurologic examinations are the basis for characterization of bladder dysfunction in equine patients and the basis for any efforts aimed at identifying a cause. Although never good, the prognosis appears to be most positive in cases in which the horse still can generate significant increases in intravesicular pressure. Therefore tests aimed at evaluating bladder and urethral pressure capacities may be diagnostically useful. Cystometry, which involves the inflation of the bladder with sterile water, isotonic saline, or carbon dioxide, has been described in horses and pony mares.[377,378] Briefly, one introduces a large-gauge (30-Fr) catheter into the bladder connected to a three-way stopcock to an infusion pump and pressure transducer. The pressure transducer is connected to a chart recorder. One fills the bladder until micturition occurs. Intravesicular pressure is recorded continuously. Usually a gradual increase in pressure is related to the infusion of fluid until the pressure suddenly rises, reflecting the onset of detrusor muscle contraction. The pressure at the point of this sharp increase is regarded as the contraction threshold. In normal horses, this threshold is about 90 ± 20 cm H_2O.[377] One determines the urethral pressure profile by using a catheter with multiple side openings and positioning the tip at the urethral sphincter. One then fills the catheter with fluid and withdraws it at a constant rate while recording the intraurethral pressure. Normal values are usually greater than 50 cm H_2O. Pressures were significantly lower in recordings of three incontinent mares.[378] Although these tests have not been used widely, they have considerable prognostic potential. Put simply, the higher the pressures are, the better the prognosis. One also can determine the distance over which high pressure is present in the urethra. Such determination also may be important because it gives some further information on the integrity of the urethral sphincters.

TREATMENT

Only 21% of cases (8 of 37) of urinary incontinence reported by Schott et al.[789] were successfully treated, and approximately 50% were eventually euthanized because of incontinence. Horses that respond to treatment for neurologic disease or with correctable predisposing conditions, such as cystoliths in mares or congenital lesions such as ectopic ureter, have a generally favorable prognosis. Prognosis is also better for horses that regain detrusor and bladder sphincter muscle function within 10 to 14 days after the onset of clinical signs. Chronically affected horses will require constant grooming and hygiene to treat and prevent urine scald, sporadic treatment for urinary tract infection, lowered dietary calcium (avoid legumes) to reduce urinary crystals, and occasional bladder lavage to remove sabulous and potentially improve bladder emptying and reduce cystitis.

Some horses with UMN disease recover gradually, especially if one can determine and treat a specific cause. A major complication with paralytic and myogenic forms of bladder dysfunction is that cases often are not recognized soon enough and atonia is irreversible. By that time, the prognosis tends to be poor, and identification of an initiating cause is difficult—and likely irrelevant. In such cases, treatment is often futile; however, one often can determine this definitively only by assessing the response to attempted treatment. Regardless of whether dealing with UMN, LMN, or myogenic disorders, treatment tends to be the same concerning the bladder.

If a definitive cause such as EHV-1 myeloencephalitis or equine protozoal myeloencephalopathy is identified, one should institute specific treatment for that disease. Concerning bladder dysfunction, the basic aim is to provide support while hoping for spontaneous recovery, which may take a long time (e.g., months in cases of pelvic nerve damage). A basic aim of therapy is to prevent retention of urine because of the secondary problems that this entails. Therefore promotion of bladder emptying is an important goal that one may attempt by regular catheterization or an indwelling catheter. (In male patients, one normally inserts such catheters via a perineal urethrostomy.) Although facilitating drainage, intermittent or indwelling catheterization appears to predispose the horse to secondary bacterial cystitis and therefore should not be used without some forethought. Some horses that are chronically incontinent seem to survive comfortably without any catheterization.[564] Antimicrobial therapy is important for treating any incontinence cases but is especially critical when one uses indwelling or regular catheterization.

One may give the α-adrenergic blocker phenoxybenzamine (0.7 mg/kg PO q 6 h) to eliminate any urethral resistance, thus facilitating emptying in the reflex UMN or in situations of great atonic overdistention. Bethanechol chloride is a parasympathomimetic agent resistant to the action of acetylcholinesterase and apparently has a selective effect on the smooth muscle of the gastrointestinal tract and bladder. The drug is used principally to stimulate detrusor muscle activity, and it acts by stimulating postganglionic parasympathetic effector cells rather than motor end plates. The recommended dosage ranges from 0.25 to 0.75 mg/kg subcutaneously every 8 hours. Starting the horse on the smallest dose is recommended. This drug reportedly has varying results.[794] Bethanechol has no effect when the bladder is completely atonic or areflexic. If the muscle is capable of generating weak contractions, bethanechol may be useful. One could determine whether contractions are possible by cystometry. Although bethanechol can increase intravesicular pressure, whether it helps evacuate the bladder also depends on the status of the urethral sphincter and striated muscle. One must remember that drugs such as phenoxybenzamine and bethanechol can have undesired side effects on other body systems. The use of general muscle relaxants

and an α-adrenergic blocker may be useful for achieving urethral muscle relaxation. Diazepam (0.2–0.5 mg/kg IV) and dantrolene are the most commonly used relaxants. Diazepam is effective in large doses, which also usually result in sedation. Dantrolene (10 mg/kg PO loading dose, then 2.5 mg/kg PO maintenance q 6 h) slows release of calcium from the sarcoplasmic reticulum and has been tried in dogs with varying effects.

Surgical removal of the sabulous sludge found in a number of these horses has been tried via perineal urethrostomy or cystotomy, with poor results.[566] Removal via cystotomy is not recommended because of difficulties in evacuating the material without contaminating the peritoneal cavity. Cystotomy to remove sabulous urolithiasis is unnecessary, and sediment is likely to return within a few weeks to months.[789] Perineal urethrostomy, combined with irrigation with large volumes of fluid while the horse is anesthetized, seems to be the most effective way of removing sabulous. Five affected horses were managed for up to 3 years by repeatedly emptying the bladder through a urinary catheter and using saline lavage with cystoscopic guidance to remove residual sabulous material.[795] Cystitis was treated with antimicrobial and antiinflammatory medications, and bethanechol chloride was also administered. Frequent catheterization resulted in a urethral stricture in one horse. Four horses returned to work, and one was retired because of persistent incontinence.[795] The prognosis for most affected horses is poor because of chronic irreversible changes in the bladder wall, which seem to prohibit any possible return of normal detrusor muscle function.

REFERENCES

1. Getty R, ed. *Sisson and Grossman's the anatomy of domestic animals*. 5th ed. Philadelphia: WB Saunders; 1975.
2. Schummer A, Nickel F, Sack WO. *The viscera of the domestic animals*. 2nd ed. New York: Springer-Verlag; 1979.
3. Webb AI, Weaver BQM. Body composition of the horse. *Equine Vet J*. 1979;11:39–47.
4. Calhoun ML. Comparative histology of the ureters of domestic animals. *Anat Rec*. 1959;133:365.
5. Brenner BM, ed. *Brenner and Rector's the kidney*. 8th ed. Philadelphia: WB Saunders; 2008.
6. Rytand DA. The number and size of mammalian glomeruli as related to kidney and to body weight, with methods for their enumeration and measurement. *Am J Anat*. 1938;62:507.
7. Beech DJ, Sibbons PD, Rossdale PD, et al. Organogenesis of lung and kidney in Thoroughbreds and ponies. *Equine Vet J*. 2001;33:438.
8. Yadava RP, Calhoun ML. Comparative histology of the kidney of domestic animals. *Am J Vet Res*. 1958;19:958.
9. DiBona GF. The function of renal nerves. *Rev Physiol Biochem Pharmacol*. 1982;94:75.
10. DiBona GF. Neural regulation of renal tubular sodium reabsorption and renin secretion. *Fed Proc*. 1985;44:2816.
11. Trim CM, Moore JN, Clark ES. Renal effects of dopamine infusion in conscious horses. *Equine Vet J Suppl*. 1989;7:124.
12. Denton MD, Chertow GM, Brady HR. Renal-dose dopamine for the treatment of acute renal failure: scientific rationale, experimental studies and clinical trials. *Kidney Int*. 1996;49:4.
13. Stone GW, Tumlin JA, Madyoon H, et al. Design and rationale of CONTRAST: a prospective, randomized, placebo-controlled trial of fenoldopam mesylate for the prevention of radiocontrast nephropathy. *Rev Cardiovasc Med*. 2001;2(suppl 1):S31.
14. Thurmon JC, Steffey EP, Zinkl JG, et al. Xylazine causes transient dose-related hyperglycemia and increased urine volume in mares. *Am J Vet Res*. 1984;45:224.
15. Trim CM, Hanson RR. Effects of xylazine on renal function and plasma glucose in ponies. *Vet Rec*. 1986;118:65.
16. Gellai M. Modulation of vasopressin antidiuretic action by renal alpha 2-adrenoceptors. *Am J Physiol*. 1990;259:F1.
17. Prieto D, Hernandez M, Rivera L, et al. Catecholaminergic innervation of the equine ureter. *Res Vet Sci*. 1994;54:312.
18. Labadiáa A, Rivera L, Costa G, et al. Alpha and beta adrenergic receptors in the horse ureter. *Rev Esp Fisiol*. 1987;43:421.
19. Labadia A, Rivera L, Costa G, et al. Influence of the autonomic nervous system in the horse urinary bladder. *Res Vet Sci*. 1988;44:282.
20. Prieto D, Benedito S, Rivera L, et al. Autonomic innervation of the equine urinary bladder. *Anat Histol Embryol*. 1990;19:276.
21. Prieto D, Benedito S, Rodrigo R, et al. Distribution and density of neuropeptide Y-immunoreactive nerve fibers and cells in the horse urinary bladder. *J Auton Nerv Syst*. 1989;27:173.
22. de Groat WC, Booth AM. Physiology of the urinary bladder and urethra. *Ann Intern Med*. 1980;92:312.
23. Carlson BM. *Patten's foundations of embryology*. 6th ed. New York: McGraw-Hill; 2003.
24. Höflinger VH. Zur Kenntnis der kongenitalen unilateralen Nierenagenesie bei Haustieren II. Ihr Vorkommen bei den einzelnen Tierarten. *Schweiz Arch Tierheilkd*. 1971;13:330.
25. Jubb KVF, Kennedy PC, Palmer N, eds. *Pathology of domestic animals*. 3rd ed. vol. 2. San Diego: Academic Press; 1985.
26. Huston R, Saperstein G, Leipold HW. Congenital defects in foals. *J Equine Med Surg*. 1977;1:146.
27. Johnson BD, Klingborg DJ, Heitman JM, et al. A horse with one kidney, partially obstructed ureter, and contralateral urogenital anomalies. *J Am Vet Med Assoc*. 1976;169:217.
28. Brown CM, Parks AH, Mullaney TP, et al. Bilateral renal dysplasia and hypoplasia in a foal with an imperforate anus. *Vet Rec*. 1988;122:91.
29. Schott HC, Papageorges M, Hodgson DR. Diagnosis of renal disease in the nonazotemic horse (abstract #15). *J Vet Intern Med*. 1989;3:116.
30. Waldridge BM, Lenz SD, Hudson J, Rodriguez-Hurtado I. Multiple congenital urogenital abnormalities in a Tennessee Walking Horse colt. *Equine Vet Educ*. 2009;21:315.
31. Jones TC, Hunt RD. *Veterinary pathology*. Philadelphia: Lea & Febiger; 1983.
32. Andrews FM, Rosol TJ, Kohn CW, et al. Bilateral renal hypoplasia in four young horses. *J Am Vet Med Assoc*. 1986;189:209.
33. Taxy JB. Renal dysplasia: a review. *Pathol Annu*. 1985;20:139.
34. Roberts MC, Kelly WR. Chronic renal failure in a young pony. *Aust Vet J*. 1980;56:599.
35. Anderson WI, Picut CA, King JM, et al. Renal dysplasia in a Standardbred colt. *Vet Pathol*. 1988;25:179.
36. Ronen N, van Amstel SR, Nesbit JW, et al. Renal dysplasia in two adult horses: clinical and pathological aspects. *Vet Rec*. 1993;132:269.
37. Ramirez S, Williams J, Seahorn TL, et al. Ultrasound-assisted diagnosis of renal dysplasia in a 3-month-old Quarter Horse colt. *Vet Radiol Ultrasound*. 1998;39:143.
38. Woolridge AA, Seahorn TL, Williams J, et al. Chronic renal failure associated with nephrolithiasis, ureterolithiasis, and renal dysplasia in a 2-year-old Quarter Horse gelding. *Vet Radiol Ultrasound*. 1999;40:361.
39. Gull T, Schmitz A, Bahr A, et al. Renal hypoplasia and dysplasia in an American Miniature foal. *Vet Rec*. 2001;149:199.
40. Zicker SC, Marty GD, Carlson GP, et al. Bilateral renal dysplasia with nephron hypoplasia in a foal. *J Am Vet Med Assoc*. 2001;196:1990.
41. Jones SL, Langer DL, Sterner-Kock A, et al. Renal dysplasia and benign ureteropelvic polyps associated with hydronephrosis in a foal. *J Am Vet Med Assoc*. 1994;204:1230.
42. Grantham JJ. Polycystic kidney disease: a predominance of giant nephrons. *Am J Physiol*. 1983;244:F3.

43. Gardner KD. Pathogenesis of human cystic renal disease. *Annu Rev Med*. 1988;39:185.
44. Zerres K, Eggermann T, Rudnik-Schoneborn S. DNA diagnosis in hereditary nephropathies. *Clin Nephrol*. 2001;56:181.
45. Cannon MJ, MacKay AD, Barr FJ, et al. Prevalence of polycystic kidney disease in Persian cats in the United Kingdom. *Vet Rec*. 2001;149:409.
46. Barrs VR, Gunew M, Beatty JA, et al. Prevalence of autosomal dominant polycystic kidney disease in Persian cats and related-breeds in Sydney and Brisbane. *Aust Vet J*. 2001;79:257.
47. O'Leary CA, Ghoddusi M, Huxtable CR. Renal pathology of polycystic kidney disease and concurrent hereditary nephritis in bull terriers. *Aust Vet J*. 2002;80:353.
48. Ramsey G, Rothwell TLW, Gibson KT, et al. Polycystic kidneys in an adult horse. *Equine Vet J*. 1987;19:243.
49. Scott PC, Vasey J. Progressive polycystic renal disease in an aged horse. *Aust Vet J*. 1986;63:92.
50. Bertone JJ, Traub-Dargatz JL, Fettman MJ, et al. Monitoring the progression of renal failure in a horse with polycystic kidney disease: use of the reciprocal of serum creatinine concentration and sodium sulfanilate clearance half-time. *J Am Vet Med Assoc*. 1987;191:565.
51. Aguilera-Tejero E, Estepa JC, Lopez I, et al. Polycystic kidneys as a cause of chronic renal failure and secondary hypoparathyroidism in a horse. *Equine Vet J*. 2000;32:167.
52. Ohba Y, Kitagawa H, Okura Y, et al. Clinical features of renal tubular dysplasia, a new hereditary disease in Japanese black cattle. *Vet Rec*. 2001;149:115.
53. Ohba Y, Kitagawa H, Kitoh K, et al. Inheritance of renal tubular dysplasia in Japanese black cattle. *Vet Rec*. 2001;149:153.
54. Sasaki Y, Kitagawa H, Kitoh K, et al. Pathological changes of renal tubular dysplasia in Japanese black cattle. *Vet Rec*. 2002;150:628.
55. Rhyan JC, Sartin EA, Powers RD, et al. Severe renal oxalosis in five young Beefmaster calves. *J Am Vet Med Assoc*. 1992;201:1907.
56. Schott HC, Bayly WM, Reed SM, et al. Nephrogenic diabetes insipidus in sibling colts. *J Vet Intern Med*. 1993;7:68.
57. Colahan PT, Mayhew IG, Merritt AM, et al. Equine medicine and surgery. 5th ed. vol. 2. St. Louis: Mosby; 1999.
58. Latimer FG, Magnus R, Duncan RB. Arterioureteral fistula in a colt. *Equine Vet J*. 1991;23:483.
59. Crotty KL, Orihuela E, Warren MM. Recent advances in the diagnosis and treatment of renal arteriovenous malformations and fistulas. *J Urol*. 1993;150:1355.
60. Takaha M, Matsumoto A, Ochi K, et al. Intrarenal arteriovenous malformation. *J Urol*. 1980;124:315.
61. Schott HC, Barbee DD, Hines MT, et al. Renal arteriovenous malformation in a Quarter Horse foal. *J Vet Intern Med*. 1996;10:204.
62. Schott HC, Hines MT. Severe urinary tract hemorrhage in two horses. *J Am Vet Med Assoc*. 1994;204:1320 (letter).
63. Spiro I. Hematuria and a complex congential heart defect in a newborn foal. *Can Vet J*. 2002;43:375.
64. Wintzer HJ, ed. *Equine diseases: a textbook for students and practitioners*. New York: Springer-Verlag; 1986.
65. Baker JR, Ellis CE. A survey of post mortem findings in 480 horses 1958 to 1980: (1) causes of death. *Equine Vet J*. 1981;13:43.
66. Ordidge RM. Urinary incontinence due to unilateral ureteral ectopia in a foal. *Vet Rec*. 1976;98:384.
67. Rossdale PD, Ricketts SW. *Equine stud farm medicine*. 2nd ed. London: Baillière Tindall; 1980.
68. Christie B, Haywood N, Hilbert B, et al. Surgical correction of bilateral ureteral ectopia in a male Appaloosa foal. *Aust Vet J*. 1981;57:336.
69. Modransky PD, Wagner PC, Robinette JD, et al. Surgical correction of bilateral ectopic ureters in two foals. *Vet Surg*. 1983;12:141.
70. Robinson NE, ed. *Current therapy in equine medicine*. 6th ed. St. Louis: WB Saunders; 2009.
71. Richardson DW. Urogenital problems in the neonatal foal. *Vet Clin North Am Equine Pract*. 1985;1:179.
72. Robertson JT, Embertson RM. Surgical management of congenital and perinatal abnormalities of the urogenital tract. *Vet Clin North Am Equine Pract*. 1988;4:359.
73. Houlton JEF, Wright IM, Matic S, et al. Urinary incontinence in a Shire foal due to ureteral ectopia. *Equine Vet J*. 1987;19:244.
74. Sullins KE, McIlwraith CW, Yovich JV, et al. Ectopic ureter managed by unilateral nephrectomy in two female horses. *Equine Vet J*. 1988;20:463.
75. MacAllister CG, Perdue BD. Endoscopic diagnosis of unilateral ectopic ureter in a yearling filly. *J Am Vet Med Assoc*. 1990;197:617.
76. Pringle JK, Ducharme NG, Baird JD. Ectopic ureter in the horse: three cases and a review of the literature. *Can Vet J*. 1990;31:26.
77. Squire KRE, Adams SB. Bilateral ureterocystostomy in a 450-kg horse with ectopic ureters. *J Am Vet Med Assoc*. 1992;201:1213.
78. Blikslager AT, Green EM, MacFadden KE, et al. Excretory urography and ultrasonography in the diagnosis of bilateral ectopic ureters in a foal. *Vet Radiol Ultrasound*. 1992;33:41.
79. Blikslager AT, Green EM. Ectopic ureter in horses. *Compend Contin Educ Pract Vet*. 1992;14:802.
80. Odenkirchen S, Huskamp B, Scheidemann W. Two anomalies of the urinary tract of horses: ectopia ureteris and diverticulum vesicae. *Tierarztl Prax*. 1994;22:462.
81. Tech C, Weiler H. Ectopia ureteris: a contribution to diagnosis, therapy, and pathology. *Pferdeheilkunde*. 1996;12:843.
82. Jansson N, Thofner M. Ureterocystotomy for treatment of unilateral ureteral ectopia in a 300 kg horse. *Equine Vet Educ*. 1999;11:132.
83. Tomlinson JE, Farnsworth K, Sage AM, et al. Percutaneous ultrasound-guided pyelography aided diagnosis of ectopic ureter and hydronephrosis in a 3-week-old filly. *Vet Radiol Ultrasound*. 2001;42:349.
84. Holt PE, Thrusfield MV. Hotston Moore A: breed predisposition to ureteral ectopia in bitches in the UK. *Vet Rec*. 2000;146:561.
85. Rossoff IS. *Handbook of veterinary drugs and chemicals*. 2nd ed. Taylorville, IL: Pharmatox Publishing; 1994.
86. Walker DF, Vaughan JT. *Bovine and equine urogenital surgery*. Philadelphia: Lea & Febiger; 1980.
87. Auer JA, Stick JA, eds. *Equine surgery*. 3rd ed. St. Louis: WB Saunders; 2006.
88. Stickle RL, Wilcock BP, Huseman JL. Multiple ureteral defects in a Belgian foal. *Vet Med Small Anim Clin*. 1975;70:819.
89. Richardson DW, Kohn CW. Uroperitoneum in the foal. *J Am Vet Med Assoc*. 1983;182:267.
90. Robertson JT, Spurlock GH, Bramlage LR, et al. Repair of ureteral defect in a foal. *J Am Vet Med Assoc*. 1983;183:799.
91. Divers TJ, Byars TD, Spirito M. Correction of bilateral ureteral defects in a foal. *J Am Vet Med Assoc*. 1988;192:384.
92. Cutler TJ, MacKay RJ, Johnson CM, et al. Bilateral ureteral tears in a foal. *Aust Vet J*. 1997;75:413.
93. Jean D, Marcoux M, Louf CF. Congenital bilateral distal defect of the ureters in a foal. *Equine Vet Educ*. 1998;10:17.
94. Morisset S, Hawkins JF, Frank N, et al. Surgical management of a ureteral defect with ureterorrhaphy and of ureteritis with ureteroneocystostomy in a foal. *J Am Vet Med Assoc*. 2002;220:354.
95. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
96. Kawashima A, Sandler CM, Corriere JN, et al. Ureteropelvic junction injuries secondary to blunt abdominal trauma. *Radiology*. 1997;205:487.
97. Chandler JC, MacPhail CM. Congenital urethrorectal fistulas. *Compend Contin Educ Pract Vet*. 2001;23:995.
98. Fuchsloser RK, Rusch K. Atresia recti bei einem Vollblutfohlen. *Dtsch Tierarztl Wochenschr*. 1971;78:519.

99. Gideon L. Anal agenesis with rectourethral fistula in a colt. *Vet Med*. 1977;72:238.
100. Chaudhry NI, Cheema NI. Atresia ani and rectovaginal fistula in an acaudate filly. *Vet Rec*. 1980;107:95.
101. Kingston RS, Park RD. Atresia ani with an associated urogenital tract anomaly in foals. *Vet Clin North Am Equine Pract*. 1982;4(1):32.
102. Furie WS. Persistent cloaca and atresia ani in a foal. *Vet Clin North Am Equine Pract*. 1983;5(1):30.
103. Jansson N. Anal atresia in a foal. *Compend Contin Educ Pract Vet*. 2002;24:888.
104. Cruz AM, Barber SM, Kaestner SBR, et al. Urethrorectal fistula in a horse. *Can Vet J*. 1999;40:122.
105. Rooney J. Rupture of the urinary bladder in the foal. *Vet Pathol*. 1971;8:445.
106. Adams RA, Koterba AM, Cudd TC, et al. Exploratory celiotomy for suspected urinary tract disruption in neonatal foals: a review of 18 cases. *Equine Vet J*. 1988;20:13.
107. Kablack KA, Embertson RM, Bernard WV, et al. Uroperitoneum in the hospitalized equine neonate: retrospective study of 31 cases, 1988–1997. *Equine Vet J*. 2000;32:505.
108. Wellington JKM. Bladder defects in newborn foals. *Aust Vet J*. 1972;48:426.
109. Bain AM. Diseases of foals. *Aust Vet J*. 1954;30:9.
110. Pascoe RR. Repair of a defect in the bladder of a foal. *Aust Vet J*. 1971;47:343.
111. Crowe MW, Swerczek TW. Equine congenital defects. *Am J Vet Res*. 1985;46:353.
112. Radostits OM, Blood DC, Gay CC. *Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats, and horses*. 9th ed. Philadelphia: Baillière Tindall; 2000.
113. Dubs VB. Megavesica zufolge Urachusmangel bei einem neugeborenen Fohlen. *Schweiz Arch Tierheilkd*. 1976;118:395.
114. Dean PW, Robertson JT. Urachal remnant as a cause of pollakiuria and dysuria in a filly. *J Am Vet Med Assoc*. 1988;192:375.
115. Whitwell KE, Jeffcott LB. Morphological studies on the fetal membranes of the normal singleton foal at term. *Res Vet Sci*. 1975;19:44.
116. Rossdale PD, Greet TRC. Mega vesica in a newborn foal. *Int Soc Vet Perinatol Newsletter*. 1989;2(2):10.
117. Oikawa M, Yoshihara T, Katayama Y, et al. Ruptured bladder associated with smooth muscle atrophy of the bladder in a neonatal foal. *Vet Clin North Am Equine Pract*. 1993;15(7):38.
118. Whitwell KE. Morphology and pathology of the equine umbilical cord. *J Reprod Fertil Suppl*. 1975;23:599.
119. Britt B, Byars TD. Hagyard-Davidson-McGee: proceedings. *Am Assoc Equine Pract*. 1997;43:170–177.
120. Turner TA, Fessler JF, Ewert KM. Patent urachus in foals. *Vet Clin North Am Equine Pract*. 1982;4(1):24.
121. Lavan RP, Madigan J, Walker R, et al. Effect of disinfectant treatments on the bacterial flora of the umbilicus of neonatal foals. In: *Proceedings of the fortieth annual meeting of the American Association of Equine Practitioners*. Vancouver. Canada; 1994:37.
122. Ford J, Lokai MD. Ruptured urachus in a foal. *Vet Med Small Anim Clin*. 1982;77:94.
123. Reef VB, Collatos C. Ultrasonographic examination of normal umbilical structures in the foal. *Am J Vet Res*. 1988;49(2143).
124. Reef VB, Collatos C, Spencer PA, et al. Clinical, ultrasonographic, and surgical findings in foals with umbilical remnant infections. *J Am Vet Med Assoc*. 1989;195:69.
125. Adams SB, Fessler JF. Umbilical cord remnant infections in foals: 16 cases (1975-1985). *J Am Vet Med Assoc*. 1987;190(316).
126. Lees MJ, Easley KJ, Sutherland JV, et al. Subcutaneous rupture of the urachus, its diagnosis and surgical management in three foals. *Equine Vet J*. 1989;21:462.
127. Textor JA, Goodrich L, Wion L. Umbilical evagination of the urinary bladder in a neonatal filly. *J Am Vet Med Assoc*. 2001;219:953.
128. Brenner BM, ed. *Brenner and Rector's the kidney*. 8th ed. vol. 2. Philadelphia: WB Saunders; 2008.
129. Dimski DS. Ammonia metabolism and the urea cycle: function and clinical implications. *J Vet Intern Med*. 1994;8:73.
130. Kaneko JJ, ed. *Clinical biochemistry of domestic animals*. 3rd ed. New York: Academic Press; 1980.
131. Strombeck DR, Meyer DJ, Freedland RA. Hyperammonemia due to a urea cycle enzyme deficiency in two dogs. *J Am Vet Med Assoc*. 1975;166:1109.
132. Peek SF, Divers TJ, Jackson CJ. Hyperammonaemia associated with encephalopathy and abdominal pain without evidence of liver disease in four mature horses. *Equine Vet J*. 1997;29:70.
133. Hasel KM, Summers BA, De Lahunta A. Encephalopathy with idiopathic hyperammonaemia and Alzheimer type II astrocytes in equidae. *Equine Vet J*. 1999;31:478.
134. McConnico RS, Duckett WM, Wood PA. Persistent hyperammonemia in two related Morgan weanlings. *J Vet Intern Med*. 1997;11:264.
135. Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Lea & Febiger; 1990.
136. Prior RL, Hintz HF, Lowe JE, et al. Urea recycling and metabolism of ponies. *J Anim Sci*. 1974;38:565.
137. Hintz HF, Schryver HF. Nitrogen utilization in ponies. *J Anim Sci*. 1972;34:592.
138. Reitnour CM, Treece JM. Relationship of nitrogen source to certain blood components and nitrogen balance in the equine. *J Anim Sci*. 1971;32:487.
139. Landwehr K. *Untersuchungen über die Beeinflussung von Kreatinin und Harnstoff im Blutplasma des Pferdes durch extrarenale Faktoren, inaugural dissertation*. Hannover Germany: Tierärztliche Hochschule Hannover; 1986.
140. Miller PA, Lawrence LM. The effect of dietary protein level on exercising horses. *J Anim Sci*. 1988;66(2185).
141. Patterson PH, Coon CN, Hughes IM. Protein requirements of mature working horses. *J Anim Sci*. 1985;61:187.
142. Sticker LS, Thompson DL, Bunting LD, et al. Feed deprivation in mares: plasma metabolite and hormonal concentrations and responses to exercise. *J Anim Sci*. 1995;73:3696.
143. Baetz AL, Pearson JE. Blood constituent changes in fasted ponies. *Am J Vet Res*. 1972;33:1941.
144. Keenan DM. Changes of blood metabolites in horses after racing, with particular reference to uric acid. *Aust Vet J*. 1979;55:54.
145. Snow DH, Kerr MG, Nimmo MA, et al. Alterations in blood, sweat, urine and muscle composition during prolonged exercise in the horse. *Vet Rec*. 1982;110:377.
146. Rose RJ, Ilkiw JE, Arnold KS, et al. Plasma biochemistry in the horse during 3-day event competition. *Equine Vet J*. 1980;12:132.
147. Narayanan S, Appleton HD. Creatinine: a review. *Clin Chem*. 1980;26:1119.
148. Gärtner VK, Reulecke W, Hackbarth H, et al. Zur Abhängigkeit von Muskelmasse und Körpergröβe im Verleich von Maus, Ratte, Kaninchen, Hund, Mensch und Pferd. *Dtsch Tierarztl Wochenschr*. 1987;94:52.
149. Jones JD, Burnett PC. Creatinine metabolism in humans with decreased renal function: creatinine deficit. *Clin Chem*. 1974;20:1204.
150. Schott HC, Mansmann RA. Biochemical profiles of normal equine amniotic fluid at parturition. *Equine Vet J Suppl*. 1988;5:52.
151. Mascioli SR, Bantle JP, Freier EF, et al. Artifactual elevation of serum creatinine level due to fasting. *Arch Intern Med*. 1984;144:1575.
152. Morris DD, Divers TJ, Whitlock RH. Renal clearance and fractional excretion of electrolytes over a 24-hour period in horses. *Am J Vet Res*. 1984;45:2431.
153. Kohn CW, Strasser SL. 24-Hour renal clearance and excretion of endogenous substances in the mare. *Am J Vet Res*. 1986;47:1332.

154. Finco DR, Groves C. Mechanism of renal excretion of creatinine by the pony. *Am J Vet Res*. 1985;46:1625.
155. Rose BD, ed. *Clinical physiology of acid-base and electrolyte disorders*. 3rd ed. New York: McGraw-Hill; 1989.
156. Goldstein MB, Bear R, Richardson RMA, et al. The urine anion gap: a clinically useful index of ammonium excretion. *Am J Med Sci*. 1986;292:198.
157. Gronwall R, Brown MP. Probenicid infusion in mares: effect on para-aminohippuric acid clearance. *Am J Vet Res*. 1988;49:250.
158. Boveáe KC, ed. *Canine nephrology*. Media, PA: Harwal; 1984.
159. Carlson GP. *Thermoregulation and fluid balance in the exercising horse, Equine exercise physiology*. Cambridge: Granta Editions; 1983.
160. Carlson GP. *Hematology and body fluids in the equine athlete: a review. Equine exercise physiology*. 2nd ed. Davis, CA: ICEEP Publications; 1987.
161. Schott HC, Hinchcliff KW. Fluids, electrolytes, and bicarbonate. *Vet Clin North Am Equine Pract*. 1993;9:577.
162. Tasker JB. Fluid and electrolyte studies in the horse. III. Intake and output of water, sodium, and potassium in normal horses. *Cornell Vet*. 1967;57:649.
163. Carlson GP. Fluid and electrolyte dynamics in the horse. *Proc Annu Vet Med Forum Am Coll Vet Intern Med*. 1986;4:7–29.
164. Rose RJ. Electrolytes: clinical applications. *Vet Clin North Am Equine Pract*. 1990;6:281.
165. Groenendyk S, English PB, Abetz I. External balance of water and electrolytes in the horse. *Equine Vet J*. 1988;20:189.
166. Hinton M. On the watering of horses: a review. *Equine Vet J*. 1978;10:27.
167. Fonnesbeck PV. Consumption and excretion of water by horses receiving all hay and hay-grain diets. *J Anim Sci*. 1968;27:1350.
168. Cymbaluk NF. Water balance of horses fed various diets. *Vet Clin North Am Equine Pract*. 1989;11(1):19.
169. Martin RG, McMeniman NP, Dowsett KF. Milk and water intakes of foals sucking grazing mares. *Equine Vet J*. 1992;24:295.
170. Caljuk EA. Water metabolism and water requirements of horses. *Nutr Abstr Rev*. 1962;32:574.
171. Schryver HF, Parker MT, Daniluk PD, et al. Salt consumption and the effect of salt on mineral metabolism in horses. *Cornell Vet*. 1987;77:122.
172. Freeman DA, Cymbaluk NF, Schott HC, et al. Clinical, biochemical, and hygiene assessment of stabled horses provided continuous or intermittent access to drinking water. *Am J Vet Res*. 1999;60:1445.
173. Sufit E, Houpt KA, Sweeting M. Physiological stimuli of thirst and drinking patterns in ponies. *Equine Vet J*. 1985;17:12.
174. Keiper RR, Keenan MA. Nocturnal activity patterns of feral ponies. *J Mammal*. 1980;61:116.
175. 8th ed. Brenner BM, ed. *Brenner and Rector's the kidney*. vol. 1. Philadelphia: WB Saunders; 2008.
176. Andersson B, Augustinsson O, Bademo E, et al. Systemic and centrally mediated angiotensin II effects in the horse. *Acta Physiol Scand*. 1987;129:143.
177. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. *Physiol Rev*. 1998;78:583.
178. Houpt KA. Drinking: the behavioral sequelae of diuretic treatment. *Vet Clin North Am Equine Pract*. 1987;9(9):15.
179. Houpt KA, Thorton SN, Allen WR. Vasopressin in dehydrated and rehydrated ponies. *Physiol Behav*. 1989;45:659.
180. Jones NL, Houpt KA, Houpt TR. Stimuli of thirst in donkeys (Equus asinus). *Physiol Behav*. 1989;46:661.
181. Houpt KA, Northrup A, Wheatley T, et al. Thirst and salt appetite in horses treated with furosemide. *J Appl Physiol*. 1991;71:2380.
182. Irvine CHG, Alexander SL, Donald RA. Effect of an osmotic stimulus on the secretion of arginine vasopressin and adrenocorticotropin in the horse. *Endocrinology*. 1989;124:3102.
183. Sneddon JC, van der Walt J, Mitchell G, et al. Effects of dehydration and rehydration on plasma vasopressin and aldosterone in horses. *Physiol Behav*. 1993;54:223.
184. McKeever KH, Hinchcliff KW, Schmall LM, et al. Plasma renin activity and aldosterone and vasopressin concentrations during incremental treadmill exercise in horses. *Am J Vet Res*. 1992;53:1290.
185. Nyman S, Hydbring E, Dahlborn K. Is vasopressin a "stress hormone" in the horse? *Pferdeheilkunde*. 1996;12:419.
186. Kinter LB, Huffman WF, Stassen FL. Antagonists of the antidiuretic activity of vasopressin. *Am J Physiol*. 1988;254:F165.
187. Clarke LL, Argenzio RA, Roberts MC. Effect of meal feeding on plasma volume and urinary electrolyte clearance in ponies. *Am J Vet Res*. 1990;51:571.
188. Youket RJ, Carnevale JM, Houpt KA, et al. Humoral, hormonal and behavioral correlates of feeding in ponies: the effects of meal frequency. *J Anim Sci*. 1985;61:1103.
189. Clarke LL, Ganjam VK, Fichtenbaum B, et al. Effect of feeding on renin-angiotensin-aldosterone system of the horse. *Am J Physiol*. 1988;254:R524.
190. Tasker JB. Fluid and electrolyte studies in the horse. IV. The effects of fasting and thirsting. *Cornell Vet*. 1967;57:658.
191. Yousef MK, Dill DB, Mayes MG. Shifts in body fluids during dehydration in the burro, Equus asinus. *J Appl Physiol*. 1970;29:345.
192. Maloiy GMO. Water economy of the Somali donkey. *Am J Physiol*. 1970;219:1522.
193. Carlson GP, Rumbaugh GE, Harrold D. Physiological alterations in the horse produced by food and water deprivation during periods of high environmental temperatures. *Am J Vet Res*. 1979;40:982.
194. Brobst DF, Bayly WM. Responses of horses to a water deprivation test. *J Equine Vet Sci*. 1982;2:51.
195. Genetzky RM, Lopanco FV, Ledet AE. Clinical pathologic alterations in horses during a water deprivation test. *Am J Vet Res*. 1987;48:1007.
196. Sneddon JC, van der Walt JG, Mitchell G. Water homeostasis in desert-dwelling horses. *J Appl Physiol*. 1991;71:112.
197. Sneddon JC. Physiological effects of hypertonic dehydration on body fluid pools in arid-adapted mammals: how do Arab-based mammals compare? *Comp Biochem Physiol*. 1993;104A:201.
198. Meyer H, Coenen M. Influence of exercise on the water and electrolyte content of the alimentary tract. *Proc Equine Nutr Physiol Symp*. 1989;11:3.
199. Hubbard RW, Sandick BL, Matthew WT, et al. Voluntary dehydration and alliesthesia for water. *J Appl Physiol*. 1984;57:868.
200. Rumbaugh GE, Carlson GP, Harrold D. Urinary production in the healthy horse and in horses deprived of feed and water. *Am J Vet Res*. 1982;43:735.
201. Greenleaf JE. Problem: thirst, drinking behavior, and involuntary dehydration. *Med Sci Sports Exerc*. 1992;24:645.
202. Butudom P, Schott HC, Davis MW, et al. Drinking salt water enhances rehydration in horses dehydrated by furosemide administration and endurance exercise. *Equine Vet J Suppl*. 2002;34:513.
203. Parks CM, Manohar M. Distribution of blood flow during moderate and strenuous exercise in horses. *Am J Vet Res*. 1983;44:1861.
204. Staddon GE, Weaver BMQ, Webb AI. Distribution of cardiac output in anaesthetized horses. *Res Vet Sci*. 1979;27:38.
205. Brezis M, Rosen S. Hypoxia of the renal medulla: its implication for disease. *N Engl J Med*. 1995;332:647.
206. Epstein FH. Oxygen and renal metabolism. *Kidney Int*. 1997;51:381.
207. Heyman SN, Rosen S, Brezis M. The renal medulla: life at the edge of anoxia. *Blood Purif*. 1997;15:232.
208. Knudsen E. Renal clearance studies on the horse. I. Inulin, endogenous creatinine and urea. *Acta Vet Scand*. 1959;1:52.
209. Paul JW. A comparative study of renal function in horses and ponies and a study of the pharmacokinetics of oxytetracycline in the horse. Master's thesis. Columbus: The Ohio State University; 1973.

210. Zatzman ML, Clarke L, Ray WJ, et al. Renal function of the pony and the horse. *Am J Vet Res*. 1982;43:608.
211. Hood DM, Amoss MS, Gremmel SM, et al. Renovascular nuclear medicine in the equine: a feasibility study. *Southwest Vet*. 1982;35:19.
212. Manohar M, Goetz TE. Cerebral, renal, adrenal, intestinal, and pancreatic circulation in conscious ponies and during 1.0, 1.5, and 2.0 minimal alveolar concentrations of halothane-O_2 anesthesia. *Am J Vet Res*. 1985;46:2492.
213. Manohar M. *Furosemide and systemic circulation during severe exercise. Equine exercise physiology*. 2nd ed. Davis, CA: ICEEP Publications; 1987.
214. Brewer BD, Clement SF, Lotz WS, et al. A comparison of inulin, para-aminohippuric acid, and endogenous creatinine clearances as measures of renal function in neonatal foals. *J Vet Intern Med*. 1990;4:301.
215. Hinchcliff KW, McKeever KH, Schmall LM, et al. Renal and systemic hemodynamic responses to sustained submaximal exertion in horses. *Am J Physiol*. 1990;258:R1177.
216. Schott HC, Hodgson DR, Bayly WM, et al. *Renal responses to high intensity exercise. Equine exercise physiology*. 3rd ed. Davis, CA: ICEEP Publications; 1991.
217. Held JP, Daniel GB. Use of nonimaging nuclear medicine techniques to assess the effect of flunixin meglumine on effective renal plasma flow and effective renal blood flow in healthy horses. *Am J Vet Res*. 1991;52:1619.
218. Armstrong RB, Esseán-Gustavsson B, Hoppeler H, et al. O_2 delivery at VO_{2max} and oxidative capacity in muscles of Standardbred horses. *J Appl Physiol*. 1992;73:2274.
219. Matthews HK, Andrews FM, Daniel GB, et al. Comparison of standard and radionuclide methods for measurement of glomerular filtration rate and effective renal blood flow in female horses. *Am J Vet Res*. 1992;53:1612.
220. Manohar M, Goetz TE, Saupe B, et al. Thyroid, renal, and splanchnic circulation in horses at rest and during short-term exercise. *Am J Vet Res*. 1995;56:1356.
221. Holdstock NB, Ousey JC, Rossdale PD. Glomerular filtration rate, effective renal plasma flow, blood pressure and pulse rate in the equine neonate during the first 10 days postpartum. *Equine Vet J*. 1998;30:335.
222. Woods PR, Drost WT, Clarke CR, et al. Use of 99mTc-mercaptoacetyltriglycine to evaluate renal function in horses. *Vet Radiol Ultrasound*. 2000;41:85.
223. McConaghy FF, Hodgson DR, Hales JRS, et al. Thermoregulatory-induced compromise of muscle blood flow in ponies during intense exercise in the heat: a contributor to the onset of fatigue? *Equine Vet J Suppl*. 2002;34:491.
224. Rawlings CA, Bisgard GE. Renal clearance and excretion of endogenous substances in the small pony. *Am J Vet Res*. 1975;36:45–48.
225. Traver DS, Salem C, Coffman JR, et al. Renal metabolism of endogenous substances in the horse: volumetric vs clearance ratio methods. *J Equine Med Surg*. 1977;1:378.
226. Gelsa H. The renal clearance of inulin, creatinine, trimethoprim and sulphadoxine in horses. *J Vet Pharmacol Ther*. 1979;2:257.
227. Lane VM, Merritt AM. Reliability of single-sample phosphorous fractional excretion determination as a measure of daily phosphorous renal clearance in equids. *Am J Vet Res*. 1983;44:500.
228. Gronwall R. Effect of diuresis on urinary excretion and creatinine clearance in the horse. *Am J Vet Res*. 1985;46:1616.
229. Smith CM, Steffey EP, Baggott JD, et al. Effects of halothane anesthesia on the clearance of gentamicin sulfate in horses. *Am J Vet Res*. 1988;49:19.
230. McKeever KH, Hinchcliff KW, Schmall LM, et al. Renal tubular function in horses during sustained submaximal exercise. *Am J Physiol*. 1991;261:R553.
231. Walsh DM, Royal HD. Evaluation of 99mTc-labeled diethylenetriaminepentaacetic acid for measuring glomerular filtration rate in horses. *Am J Vet Res*. 1992;53:776.
232. Bickhardt K, Deegen E, Espelage W. Kidney function tests in horses: methods and reference values in healthy animals. *Dtsch Tierarztl Wochenschr*. 1996;103:117.
233. Gleadhill A, Marlin D, Harris PA, et al. Reduction of renal function in exercising horses. *Equine Vet J*. 2000;32:509.
234. McKenzie EC, Valberg SJ, Godden SM, et al. Comparison of volumetric urine collection versus single-sample urine collection in horses consuming diets varying in cation-anion balance. *Am J Vet Res*. 2003;64:284–291.
235. Matthews HK, Andrews FM, Daniel GB, et al. Measuring renal function in horses. *Vet Med*. 1993;88:349.
236. Driessen B, Zarucco L, Steffey EP, et al. Serum fluoride concentrations, biochemical and histopathological changes associated with prolonged sevoflurane anesthesia in horses. *J Vet Med A Physiol Pathol Clin Med*. 2002;49:337.
237. Dunn MJ, Zambraski EJ. Renal effects of drugs that inhibit prostaglandin synthesis. *Kidney Int*. 1980;18:609.
238. Gunson DE. Renal papillary necrosis in horses. *J Am Vet Med Assoc*. 1983;182:263.
239. Gunson DE, Soma LR. Renal papillary necrosis in horses after phenylbutazone and water deprivation. *Vet Pathol*. 1983;20:603.
240. Kanwar YS. Biology of disease: biophysiology of glomerular filtration and proteinuria. *Lab Invest*. 1984;51:7.
241. Gleadhill A, Marlin D, Harris PA, et al. Use of a three-blood-sample plasma clearance technique to measure GFR in horses. *Vet J*. 1999;158:204.
242. Steinhausen M, Endlich K, Wiegman DL. Glomerular blood flow. *Kidney Int*. 1990;38:769.
243. Lüscher TF, Bock HA, Yang Z, et al. Endothelium-derived relaxing and contracting factors: perspectives in nephrology. *Kidney Int*. 1991;39:575.
244. Molitoris BA, Finn WF, eds. *Acute renal failure: a companion to Brenner & Rector's the kidney*. Philadelphia: WB Saunders; 2001.
245. Breidenbach A, Schlumbohm C, Harmeyer J. Peculiarities of vitamin D and of the calcium and phosphate homeostatic system in horses. *Vet Res*. 1998;29:173.
246. Lakritz J, Madigan J, Carlson GP. Hypovolemic hyponatremia and signs of neurologic disease associated with diarrhea in a foal. *J Am Vet Med Assoc*. 1992;200:1114.
247. Sneddon JC, Colyn P. A practical system for measuring water intake in stabled horses. *J Equine Vet Sci*. 1991;11:141.
248. Vander Noot GW, Fonnesbeck PV, Lydman RK. Equine metabolism stall and collection harness. *J Anim Sci*. 1965;24:691.
249. Warwick IS. Urine collection apparatus for male horses. *J Sci Technol*. 1966;12:181.
250. Tasker JB. Fluid and electrolyte studies in the horse. II. An apparatus for the collection of total daily urine and feces from horses. *Cornell Vet*. 1966;56:77.
251. Harris P. Collection of urine. *Equine Vet J*. 1988;20:86.
252. Brown CM, ed. *Problems in equine medicine*. Philadelphia: Lea & Febiger; 1989.
253. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem*. 1992;38:1933.
254. Osborne CA, Polzin DJ. Azotemia: a review of what's old and what's new. I. Definition of terms and concepts. *Compend Contin Educ Pract Vet*. 1983;5:497.
255. Irwin DHG, Howell DW. Equine pyelonephritis and unilateral nephrectomy. *J S Afr Vet Assoc*. 1980;51:235.
256. Trotter GW, Brown CM, Ainsworth DM. Unilateral nephrectomy for treatment of a renal abscess in a foal. *J Am Vet Med Assoc*. 1984;184:1392.
257. Juzwiak JS, Bain FT, Slone DE, et al. Unilateral nephrectomy for treatment of chronic hematuria due to nephrolithiasis in a colt. *Can Vet J*. 1988;29:931.
258. Tennant B, Lowe JE, Tasker JB. Hypercalcemia and hypophosphatemia in ponies following bilateral nephrectomy. *Proc Soc Exp Biol Med*. 1981;167:365.
259. DeBowes R. Personal communication; 1991.

260. Koterba AM, Coffman JR. Acute and chronic renal disease in the horse. *Compend Contin Educ Pract Vet*. 1981;3:S461.
261. Finco DR, Duncan JR. Evaluation of blood urea nitrogen and serum creatinine concentrations as indicators of renal dysfunction: a study of 111 cases and a review of related literature. *J Am Vet Med Assoc*. 1976;168:593.
262. Osborne CA, Polzin DJ. Azotemia: a review of what's old and what's new. II. Localization. *Compend Contin Educ Pract Vet*. 1983;5:561.
263. Brobst DF, Grant BD, Hilbert BJ, et al. Blood biochemical changes in horses with prerenal and renal disease. *J Equine Med Surg*. 1977;1:171.
264. Bayly WM. A practitioner's approach to the diagnosis and treatment of renal failure in horses. *Vet Med*. 1991;86:632.
265. Divers TJ, Whitlock RH, Byars TD, et al. Acute renal failure in six horses resulting from haemodynamic causes. *Equine Vet J*. 1987;19:178.
266. Divers TJ. Chronic renal failure in horses. *Compend Contin Educ Pract Vet*. 1983;5:S310.
267. Tennant B, Dill SG, Rebhun WC, et al. Pathophysiology of renal failure in the horse. In: *Proceedings of the thirty-first annual meeting of the American Association of Equine Practitioners*. Toronto, Canada; 1985:627.
268. Grauer GF. Clinicopathologic evaluation of early renal disease in dogs. *Compend Contin Educ Pract Vet*. 1985;7:32.
269. Allen TA, Fettman MJ. Comparative aspects of nonoliguric renal failure. *Compend Contin Educ Pract Vet*. 1987;9:293.
270. Banks KL, Henson JB. Immunologically mediated glomerulitis of horses. II. Antiglomerular basement membrane antibody and other mechanisms of spontaneous disease. *Lab Invest*. 1972;26:708.
271. Badr KF, Ichikawa I. Prerenal failure: a deleterious shift from renal compensation to decompensation. *N Engl J Med*. 1988;319:623.
272. Grossman BS, Brobst DF, Kramer JW, et al. Urinary indices for differentiation of prerenal azotemia and renal azotemia in horses. *J Am Vet Med Assoc*. 1982;180:284.
273. Roussel AJ, Cohen ND, Ruoff WW, et al. Urinary indices of horses after intravenous administration of crystalloid solutions. *J Vet Intern Med*. 1993;7:241.
274. Baek SM, Brown RS, Shoemaker WC. Early prediction of acute renal failure and recovery. I. Sequential measurements of free water clearance. *Ann Surg*. 1973;177:253.
275. Baek SM, Makabali GG, Brown RS, et al. Free-water clearance patterns as predictors and therapeutic guides in acute renal failure. *Surgery*. 1975;77:632.
276. Kosinski JP, Lucas CE, Ledgerwood AM. Meaning and value of free water clearance in injured patients. *J Surg Res*. 1982;33:184.
277. Taylor FGR, Hillyer MH. The differential diagnosis of hyperglycemia in horses. *Equine Vet Educ*. 1992;4:135.
278. Chapman DI, Haywood PE, Lloyd P. Occurrence of glycosuria in horses after strenuous exercise. *Equine Vet J*. 1981;13:259.
279. Kohn CW, Chew DJ. Laboratory diagnosis and characterization of renal disease in horses. *Vet Clin North Am Equine Pract*. 1987;3:585.
280. Edwards DJ, Brownlow MA, Hutchins DR. Indices of renal function: reference values in normal horses. *Aust Vet J*. 1989;66:60.
281. Wood T, Weckman TJ, Henry PA, et al. Equine urine pH: normal population distributions and methods of acidification. *Equine Vet J*. 1990;22:118.
282. Edwards DJ, Brownlow MA, Hutchins DR. Indices of renal function: values in eight normal foals from birth to 56 days. *Aust Vet J*. 1990;67:251.
283. Gingerich DA, Murdick PW. Paradoxic aciduria in bovine metabolic alkalosis. *J Am Vet Med Assoc*. 1975;166:227.
284. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248.
285. Schott HC, Hodgson DR, Bayly WM. Haematuria, pigmenturia and proteinuria in exercising horses. *Equine Vet J*. 1995;27:67.
286. Grauer GF, Thomas CB, Eicker SW. Estimation of quantitative proteinuria in the dog, using the urine protein-to-creatinine ratio from a random, voided sample. *Am J Vet Res*. 1985;46:2116.
287. Link RP. Glucose tolerance in horses. *J Am Vet Med Assoc*. 1940;97:261.
288. Blondheim SH, Margoliash E, Shafrir E. A simple test for myohemoglobinuria (myoglobinuria). *J Am Med Assoc*. 1958;167:453.
289. Marcussen N, Schumann J, Campbell P, et al. Cytodiagnostic urinalysis is very useful in the differential diagnosis of acute renal failure and can predict the severity. *Ren Fail*. 1995;17:721.
290. Mair TS, Osborn RS. The crystalline composition of normal equine urine deposits. *Equine Vet J*. 1990;22:364.
291. Burchardt U, Peters JE, Neef L, et al. Der diagnostiche Wert von Enzymbestimmugen im Harn. *Z Med Lab Diagn*. 1977;18:190.
292. Raab WP. Diagnostic value of urinary enzyme determinations. *Clin Chem*. 1972;18:5.
293. Price RG. Urinary enzymes, nephrotoxicity and renal disease. *Toxicology*. 1982;23:99.
294. Stroo WE, Hook JB. Enzymes of renal origin in urine as indicators of nephrotoxicity. *Toxicol Appl Pharmacol*. 1977;39:423.
295. Bayly WM, Brobst DF, Elfers RS, et al. Serum and urinary biochemistry and enzyme changes in ponies with acute renal failure. *Cornell Vet*. 1986;76:306.
296. Prescott LF. Assessment of nephrotoxicity. *Br J Clin Pharmacol*. 1982;13:303.
297. Mahrun D, Paar D, Bock KD. Lysosomal and brush-border enzymes in urine of patients with renal artery stenosis and with essential hypertension. *Clin Biochem*. 1978;12:228.
298. Adams R, McClure JJ, Gossett KA, et al. Evaluation of a technique for measurement of γ-glutamyltransferase in equine urine. *Am J Vet Res*. 1986;46:147.
299. Brobst DF, Carroll RJ, Bayly WM. Urinary enzyme concentrations in healthy horses. *Cornell Vet*. 1986;76:229.
300. Amend J, Nicholson R, King R, et al. Equine monensin toxicosis: useful ante-mortem and post-mortem clinicopathologic tests. In: *Proceedings of the thirty-first annual meeting of the American Association of Equine Practitioners*. Toronto, Canada; 1985:361.
301. Giusti EP, Sampaio AM, Michelacci YM, et al. Horse urinary kallikrein I: complete purification and characterization. *Biol Chem*. 1988;369:387.
302. Schmitz DG, Green RA. Effects of water deprivation and phenylbutazone administration on urinary enzyme concentrations in healthy horses. In: *Proceedings of the thirty-third annual meeting of the American Association of Equine Practitioners*. New Orleans; 1987:301.
303. Guder W, Ross B. Enzyme distribution along the nephron. *Kidney Int*. 1984;26:101.
304. Jung K, Pergande M, Schreiber G, et al. Stability of enzymes in urine at 37° C. *Clin Chim Acta*. 1983;131:185.
305. Goren M, Wright R, Osborne S, et al. Two automated procedures for N-acetyl-β-D-glucosaminidase determination evaluated for detection of drug-induced tubular nephrotoxicity. *Clin Chem*. 1986;32(2052).
306. Leaback D, Walker P. Studies on glucosaminidase. IV. The fluorometric assay of N-acetyl-β-d-glucosaminidase. *Biochem J*. 1961;78:151.
307. Irie A, Tabuchi A, Ura T. Influence of pH and temperature on the activities of the urinary enzymes. *Jpn J Clin Pathol*. 1985;13:441.
308. Vestergaard P, Leverett R. Constancy of urinary creatinine excretion. *J Lab Clin Med*. 1958;51:211.
309. Werner M, Heilbron DC, Mahrun D, et al. Patterns of urinary enzyme excretion in healthy subjects. *Clin Chim Acta*. 1970;29:437.
310. Mahrun D, Fuchs I, Mues G, et al. Normal limits of urinary excretion of eleven enzymes. *Clin Chem*. 1976;22:1567.

311. Akins JA. *Evaluation of equine urinary N-acetyl-β-d-glucosaminidase, gamma glutamyltransferase, and alkaline phosphatase as markers for early renal tubular damage, master's thesis*. Pullman, WA: Washington State University; 1989.
312. Mattenheimer H, Frolke W, Grotsch H, et al. Identification of inhibitors of urinary alanine aminopeptidase. *Clin Chim Acta*. 1986;160:125.
313. Reusch C, Vochezer R, Weschta E. Enzyme activities of alanine aminopeptidase (AAP) and N-acetyl-β-d-glucosaminidase (NAG) in healthy dogs. *Am J Vet Med*. 1991;38:90.
314. Werner A, Mahrun D, Atoba A. Use of gel filtration in the assay of urinary enzymes. *J Chromatogr*. 1969;40:234.
315. Hinchcliff KW, McGuirk SM, MacWilliams PS. Gentamicin nephrotoxicity. In: *Proceedings of the thirty-third annual meeting of the American Association of Equine Practitioners*. New Orleans; 1987:67.
316. Edwards DJ, Love DN, Rause J, et al. The nephrotoxic potential of neomycin in the horse. *Equine Vet J*. 1989;21:206.
317. Constable PD. Letter to the editor. *J Vet Intern Med*. 1991;5:357.
318. Traver DS, Coffman JR, Moore JN, et al. Urine clearance ratios as a diagnostic aid in equine metabolic disease. In: *Proceedings of the twenty-second annual meeting of the American Association of Equine Practitioners*. Dallas; 1976:177.
319. Harris P, Colles C. The use of creatinine clearance ratios in the prevention of equine rhabdomyolysis: a report of four cases. *Equine Vet J*. 1988;20:459.
320. Caple IW, Doake PA, Ellis PG. Assessment of the calcium and phosphorous nutrition in horses by analysis of urine. *Aust Vet J*. 1982;58:125.
321. Cuddeford D, Woodhead A, Muirhead R. Potential of alfalfa as a source of calcium for calcium deficient horses. *Vet Rec*. 1990;126:425.
322. Brewer BD, Clement SF, Lotz WS, et al. Renal clearance, urinary excretion of endogenous substances, and urinary indices in healthy neonatal foals. *J Vet Intern Med*. 1991;5:28.
323. Coffman J. Percent creatinine clearance ratios. *Vet Med Small Anim Clin*. 1980;75:671.
324. King C. Practical use of urinary fractional excretion. *J Equine Vet Sci*. 1994;14:464.
325. McKenzie EC, Valberg SJ, Godden SM, et al. Plasma and urine electrolyte and mineral concentrations in Thoroughbred horses with recurrent exertional rhabdomyolysis after consumption of diets varying in cation-anion balance. *Am J Vet Res*. 2002;63:1053.
326. Buntain BJ, Coffman JR. Polyuria and polydipsia in a horse induced by psychogenic salt consumption. *Equine Vet J*. 1981;13:266.
327. Schott HC, Bayly WM, Hodgson DR. Urinary excretory responses in exercising horses: effects on fractional excretion values. In: *Proceedings of the eleventh annual meeting of the Association for Equine Sports Medicine*. Orlando, FL; 1992:23.
328. Caple IW, Bourke JM, Ellis PG. An examination of the calcium and phosphorous nutrition of Thoroughbred racehorses. *Aust Vet J*. 1982;58:132.
329. Mason DK, Watkins KL, McNie JT. Diagnosis, treatment and prevention of nutritional secondary hyperparathyroidism in Thoroughbred race horses in Hong Kong. *Vet Clin North Am Equine Pract*. 1988;10(3):10.
330. Ronen N, van Heerden J, van Amstel SR. Clinical and biochemistry findings, and parathyroid hormone concentrations in three horses with secondary hyperparathyroidism. *J S Afr Vet Assoc*. 1992;63:134.
331. Stewart AJ, Hardy J, Kohn CW, et al. Validation of diagnostic tests for determination of magnesium status in horses with reduced magnesium intake. *Am J Vet Res*. 2004;65:422.
332. Harris PA, Snow DH. Role of electrolyte imbalances in the pathophysiology of the equine rhabdomyolysis syndrome. In: Persson SGB, Lindholm A, Jeffcott LB, eds. *Equine exercise physiology*. 3rd ed. Davis, CA: ICEEP Publications; 1991.
333. Beech J, Lindborg S. Potassium concentrations in muscle, plasma, and erythrocytes and urinary fractional excretion in normal horses and those with chronic intermittent exercise-associated rhabdomyolysis. *Res Vet Sci*. 1993;55:43.
334. Brooks CL, Garry F, Swartout MS. Effect of an interfering substance on determination of potassium by ion-specific potentiometry in animal urine. *Am J Vet Res*. 1988;49:710.
335. Traub-Dargatz JL, McKinnon AO. Adjunctive methods of examination of the urogenital tract. *Vet Clin North Am Equine Pract*. 1988;4:339.
336. Rantanen NW. Diseases of the kidneys. *Vet Clin North Am Equine Pract*. 1986;2:89.
337. Reef VB. Ultrasonic evaluation of large animal renal diseases. *Proc Annu Vet Med Forum Am Coll Vet Intern Med*. 1986;4:2–45.
338. Penninck DG, Eisenberg HM, Teuscher EE, et al. Equine renal ultrasonography: normal and abnormal. *Vet Radiol*. 1986;27:81.
339. Kiper ML, Traub-Dargatz JL, Wrigley RH. Renal ultrasonography in horses. *Compend Contin Educ Pract Vet*. 1990;12:993.
340. Hoffman KL, Wood AKW, McCarthy PH. Sonographic-anatomic correlation and imaging protocol for the kidneys of horses. *Am J Vet Res*. 1995;56:1403.
341. Divers TJ, Yeager AE. The value of ultrasonographic examination in the diagnosis and management of renal diseases in horses. *Equine Vet Educ*. 1997;7:334.
342. Reef VB. *Equine diagnostic ultrasound*. Philadelphia: WB Saunders; 1998.
343. Bayly WM, Elfers RS, Liggitt HD, et al. A reproducible means of studying acute renal failure in the horse. *Cornell Vet*. 1986;76:287.
344. Leveille R, Miyabayashi T, Weisbrode SE, et al. Ultrasonographic renal changes associated with phenylbutazone administration in three foals. *Can Vet J*. 1996;37:235.
345. Ramirez S, Seahorn TL, Williams J. Renal medullary rim sign in 2 adult Quarter horses. *Can Vet J*. 1998;39:647.
346. Jakovljeric S, Rivers WJ, Chun R, et al. Results of renal ultrasonography performed before and during administration of saline (0.9% NaCl) solution to induce diuresis in dogs without evidence of renal disease. *Am J Vet Res*. 1999;60:405.
347. Rapp HJ, Tellhelm B, Spurlock SL. Die röntgenologische Darstellung der Harnableitenden wege der Stute mit Hilfe retrograder Kontrastmittelgabe. *Pferdeheilkunde*. 1987;3:309.
348. Konde LJ, Park RD, Wrigley RH, et al. Comparison of radiography and ultrasonography in the evaluation of renal lesions in the dog. *J Am Vet Med Assoc*. 1986;188:1420.
349. Tanagho EA, McAnninch JW, eds. *Smith's general urology*. 12th ed. Norwalk: Appleton & Lange; 1988.
350. Blaufox MD. Procedures of choice in renal nuclear medicine. *J Nucl Med*. 1991;32:1301.
351. Twardock AR, Krawiec DR, Lamb CR. Kidney scintigraphy. *Semin Vet Med Surg (Small Anim)*. 1991;6:164.
352. Schott HC, Roberts GD, Hines MT, et al. Nuclear scintigraphy as a diagnostic aid in the evaluation of renal disease in horses. In: *Proceedings of the thirty-ninth annual meeting of the American Association of Equine Practitioners*. San Antonio, TX; 1993:251.
353. Schott HC. Recurrent urolithiasis associated with unilateral pyelonephritis in five equids. In: *Proceedings of the forty-eighth annual meeting of the American Association of Equine Practitioners*. Orlando, FL; 2002:136.
354. Sullins KE, Traub-Dargatz JL. Endoscopic anatomy of the equine urinary tract. *Compend Contin Educ Pract Vet*. 1984;6(11):S663.
355. Rapp HJ, Sernetz M. Urethroskopie und Ureterenkatheterisierung bei der Stute. *Pferdeheilkunde*. 1985;1:197.
356. Traub-Dargatz JL, Brown CM, eds. *Equine endoscopy*. 2nd ed. St. Louis: Mosby; 1997.
357. Huffman JL, Bagley DH, Lyon ES. Extending cystoscopic techniques into the ureter and renal pelvis. *JAMA*. 1983;250:2002.
358. Ensor RD, Boyarksy S, Glenn JF. Cystoscopy and ureteral catheterization in the dog. *J Am Vet Med Assoc*. 1966;149:1067.

359. Senior DF, Newman RC. Retrograde ureteral catheterization in female dogs. *J Am Anim Hosp Assoc*. 1986;22:831.
360. MacHarg MA, Foerner JJ, Phillips TN, et al. Two methods for the treatment of ureterolithiasis in a mare. *Vet Surg*. 1984;13:95.
361. Rodger LD, Carlson GP, Moran ME, et al. Resolution of a left ureteral stone using electrohydraulic lithotripsy in a Thoroughbred colt. *J Vet Intern Med*. 1995;9:280.
362. Schott HC, Hodgson DR, Bayly WM. Ureteral catheterization in the horse. *Equine Vet Educ*. 1990;2:140.
363. Filar J, Ziolo T, Szalecki J. Diabetes insipidus in the course of encephalitis in the horse. *Med Weter*. 1971;27:205.
364. Breukink HJ, Van Wegen P, Schotman AJH. Idiopathic diabetes insipidus in a Welsh pony. *Equine Vet J*. 1983;15:284.
365. Unpublished data.
366. Brobst DF, Bramwell K, Kramer JW. Sodium sulfanilate clearance as a method of determining renal function in the horse. *J Equine Med Surg*. 1978;2:500.
367. Hinchcliff KW, McGuirk SM, MacWilliams PS. Pharmacokinetics of phenolsulfonphthalein in horse and pony mares. *Am J Vet Res*. 1987;48:1256.
368. Osborne CA, Fahning ML, Schultz RH, et al. Percutaneous renal biopsy in the cow and the horse. *J Am Vet Med Assoc*. 1968;153:563.
369. Bayly WM, Paradis MR, Reed SM. Equine renal biopsy: indications, technique, interpretation, and complications. *Mod Vet Pract*. 1980;61:763.
370. Modransky PD. *Comparative evaluation of ultrasound-directed biopsy techniques in the horse, master's thesis*. Pullman, WA: Washington State University; 1983.
371. Barratt-Boyes S, Spensley MS, Nyland TG, et al. Ultrasound localization and guidance for renal biopsy in the horse. *Vet Radiol*. 1991;32:121.
372. Striker GE. Controversy: the role of renal biopsy in modern medicine. *Am J Kidney Dis*. 1982;1:241.
373. Morel-Maroger L. The value of renal biopsy. *Am J Kidney Dis*. 1982;1:244.
374. Donadio JV. The limitations of renal biopsy. *Am J Kidney Dis*. 1982;1:249.
375. Turner MW, Hutchinson TA, Barre PE, et al. A prospective study on the impact of renal biopsy in clinical management. *Clin Nephrol*. 1986;26:217.
376. Gleason DM, Bottaccini MR, Drach GW. Urodynamics. *J Urol*. 1976;115:356.
377. Clark SE, Semrad SD, Bichsel P, et al. Cystometrography and urethral pressure profiles in healthy horse and pony mares. *Am J Vet Res*. 1987;48:552.
378. Kay AK, Lavoie JP. Urethral pressure profilometry in mares. *J Am Vet Med Assoc*. 1987;191:212.
379. Ronen N. Measurements of urethral pressure profiles in the male horse. *Equine Vet J*. 1994;26:55.
380. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. *N Engl J Med*. 1996;334:1448.
381. Shankel SW, Johnson DC, Clark PS, et al. Acute renal failure and glomerulopathy caused by nonsteroidal anti-inflammatory drugs. *Arch Intern Med*. 1992;152:986.
382. Schmitz DG. Toxic nephropathy in horses. *Compend Contin Educ Pract Vet*. 1988;10:104.
383. Markel MD, Dyer RM, Hattel AL. Acute renal failure associated with application of a mercuric blister in a horse. *J Am Vet Med Assoc*. 1984;185:92.
384. Harrington DD, Page EH. Acute vitamin D_3 toxicosis in horses: case reports and experimental studies of the comparative toxicity of vitamins D_2 and D_3. *J Am Vet Med Assoc*. 1983;182:1358.
385. Rebhun WC, Tennant BC, Dill SG, et al. Vitamin K_3-induced renal toxicosis in the horse. *J Am Vet Med Assoc*. 1984;184:1237.
386. Riviere JE, Traver DS, Coppoc GL. Gentamicin toxic nephropathy in horses with disseminated bacterial infection. *J Am Vet Med Assoc*. 1982;180:648.
387. Brown C. Equine nephrology. *Vet Annu*. 1986;26:1.
388. Ehnen SJ, Divers TJ, Gillette D, et al. Obstructive nephrolithiasis and ureterolithiasis associated with chronic renal failure in horses: eight cases (1981-1987). *J Am Vet Med Assoc*. 1990;197:249.
389. Laverty S, Pascoe JR, Ling GV, et al. Urolithiasis in 68 horses. *Vet Surg*. 1992;21:56.
390. Miller PD, Krebs RA, Neal BJ, et al. Polyuric prerenal failure. *Arch Intern Med*. 1980;140:907.
391. Baylis C. The mechanism of the decline in glomerular filtration in gentamicin induced acute renal failure in the rat. *J Antimicrob Chemother*. 1980;6:381.
392. Flamenbaum W, McNeil JS, Kotchen TA, et al. Experimental acute renal failure induced by uranyl nitrate in the dog. *Circ Res*. 1972;31:682.
393. Katzberg RW, Morris TW, Schulman G, et al. Reactions to intravenous contrast media. II. Acute renal response in euvolemic and dehydrated dogs. *Radiology*. 1983;147:331.
394. Levenson DJ, Simmons Jr CD, Brenner BM. Arachidonic acid metabolism, prostaglandins, and the kidney. *Am J Med*. 1982;72:354.
395. Reineck HJ, O'Connor GJ, Lifschitz MD, et al. Sequential studies on the pathophysiology and glycerol-induced acute renal failure. *J Lab Clin Med*. 1980;96:356.
396. Dworkin LD, Ichikawa I, Brenner VN. Hormonal modulation of glomerular function. *Am J Physiol*. 1983;244:F95.
397. Frega NS, DiBona DR, Guertter B, et al. Ischemic renal injury. *Kidney Int*. 1976;10:517.
398. Molitoris BA. Ischemia-induced loss of epithelial polarity: potential role of the actin cytoskeleton. *Am J Physiol*. 1991;260:F769.
399. Abbate M, Bonventre JV, Brown D. The microtubule network of renal epithelial cells is disrupted by ischemia and reperfusion. *Am J Physiol*. 1994;267:F971.
400. Venkatachalam MA, Bernard DB, Donohoe DF, et al. Ischemic damage and repair in the rat proximal tubule: differences among the S1, S2, and S3 segments. *Kidney Int*. 1978;14:31.
401. Molitoris BA, Dahl R, Geerde SA. Cytoskeleton disruption and apical redistribution of proximal tubule Na^+/K^+ATPase during ischemia. *Am J Physiol*. 1992;263:F483.
402. Goligorsky MS, DiBona GF. Pathogenetic role of Arg-Gly-Asp-recognizing integrins in acute renal failure. *Proc Natl Acad Sci U S A*. 1993;90:5700.
403. Kribben A, Widder ED, Wetzels JFM, et al. Evidence for role of cytosolic free calcium in hypoxia-induced proximal tubule injury. *J Clin Invest*. 1994;93:1922.
404. Bonventre JV. Mechanisms of ischemic acute renal failure. *Kidney Int*. 1993;43:1160.
405. Johnson KJ, Weinberg JM. Postischemic renal injury due to oxygen radicals. *Curr Opin Nephrol Hypertens*. 1993;2:625.
406. Klausner JM, Paterson IS, Goldman G, et al. Postischemic renal injury is mediated by neutrophils and leukotrienes. *Am J Physiol*. 1989;256:F794.
407. Hinchcliff KW, McGuirk SM, MacWilliams TS. Gentamicin nephrotoxicity. *Proc Am Assoc Equine Pract*. 1988;33:67.
408. Hostetler K, Hall L. Aminoglycoside antibiotics inhibit lysosomal phospholipase A and C from rat liver in vitro. *Biochim Biophys Acta*. 1982;710:506.
409. Brashier MK, Geor RJ, Ames TR, et al. Effect of intravenous calcium administration on gentamicin-induced nephrotoxicosis in ponies. *Am J Vet Res*. 1998;59:1055.
410. Schumacher J, Wilson RC, Spano JS, et al. Effect of diet on gentamicin-induced nephrotoxicosis in horses. *Am J Vet Res*. 1991;52:1274.
411. Blantz RC. Intrinsic renal failure: acute. In: Seldin DW, Giebsich G, eds. *The kidney: physiology and pathophysiology*. New York: Raven Press; 1985.
412. Abdelraouf K, Braggs KH, Yin T, et al. Characterization of polymyxin B-induced nephrotoxicity: implications for dosing regimen design. *Antimicrob Agents Chemother*. 2012;56:4625.

413. Morresey PR, Mackay RJ. Endotoxin-neutralizing activity of polymyxin B in blood after IV administration in horses. *Am J Vet Res*. 2006;67:642.
414. Nieto JE, Maher O, Stanley SD, et al. Pharmacokinetics, pharmacodynamics, and safety of zoledronic acid in horses. *Am J Vet Res*. 2013;74:550.
415. Galpin J, Shinaberger J, Stanley T, et al. Acute interstitial nephritis due to methicillin. *Am J Med*. 1978;17:756.
416. McCausland IP, Milestone BA. Diffuse mesangioproliferative glomerulonephritis in a horse. *N Z Vet J*. 1976;24:239.
417. Divers TJ, Timoney JF, Lewis RM, et al. Equine glomerulonephritis and renal failure associated with complexes of group-C streptococcal antigen and IgG antibody. *Vet Immunol Immunopathol*. 1992;32:93.
418. Shimizu K, Kurosawa T, Maeda T, et al. Free water excretion and washout of renal medullary urea by prostaglandin E_1. *Jpn Heart J*. 1969;10:437.
419. Eliahou HD, Bata A. The diagnosis of acute renal failure. *Nephron*. 1965;2:287.
420. Miller TR, Anderson RG, Linas SL, et al. Urinary diagnostic indices in acute renal failure. *Ann Intern Med*. 1978;89:47.
421. Espinel CH, Gregory AW. Differential diagnosis of acute renal failure. *Clin Nephrol*. 1980;13:73.
422. Elfers RS, Bayly WM, Brobst DF, et al. Alterations in calcium, phosphorus, and C-terminal parathyroid hormone levels in equine acute renal disease. *Cornell Vet*. 1986;76:317.
423. Meroney WH, Rubini MD. Kidney function during acute tubular necrosis: clinical studies and a theory. *Metabolism*. 1959;8:1.
424. Wilson DM, Turner DR, Cameron JS, et al. Value of renal biopsy in acute intrinsic renal failure. *BMJ*. 1976;2:459.
425. Simenhoff NL, Guild WR, Gammin GJ. Acute diffuse interstitial nephritis. *Am J Med*. 1968;44:618.
426. Ruffing KA, Hoope SP, Blend D, et al. Eosinophils in urine revisited. *Clin Nephrol*. 1994;41:163.
427. Llach F, Felsenfeld AJ, Haussler MR. Pathophysiology of altered calcium metabolism in rhabdomyolysis-induced acute renal failure: interactions of parathyroid hormone, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol. *N Engl J Med*. 1981;305:117.
428. Sweeney RW, MacDonald M, Hall J, et al. Kinetics of gentamicin elimination in two horses with acute renal failure. *Equine Vet J*. 1988;20:182.
429. Vivrette S, Cowgill LD, Pascoe J, et al. Hemodialysis for treatment of oxytetracycline-induced acute renal failure in a neonatal foal. *J Am Vet Med Assoc*. 1993;203:105.
430. Wong DM, Witty D, Alcott CJ, et al. Renal replacement therapy in healthy adult horses. *J Vet Intern Med*. 2013;27:308.
431. Gallatin LL, Couëtil LL, Ash SR. Use of continuous-flow peritoneal dialysis for the treatment of acute renal failure in an adult horse. *J Am Vet Med Assoc*. 2005;226:756. Chronic Kidney Disease.
432. 6th ed. Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. vol. 2. St. Louis: WB Saunders; 2005.
433. Tennant B, Kaneko JJ, Lowe JE, et al. Chronic renal failure in the horse. In: *Proceedings of the twenty-third annual meeting of the American Association of Equine Practitioners*. St. Louis; 1978:293.
434. Kobluk CN, Ames TR, Geor RJ, eds. *The horse, diseases and clinical management*. Philadelphia: WB Saunders; 1995.
435. Osborne CA, Hammer RF, Stevens JB, et al. The glomerulus in health and disease: a comparative review of domestic animals and man. *Adv Vet Sci Comp Med*. 1977;21:207.
436. Langham RF, Hallman ET. The incidence of glomerulonephritis in domesticated animals. *J Am Vet Med Assoc*. 1949;49:471.
437. Slauson DO, Lewis RM. Comparative pathology of glomerulonephritis in animals. *Vet Pathol*. 1979;16:135.
438. Winter H, Majid NH. Glomerulonephritis: an emerging disease? *Vet Bull*. 1984;54:327.
439. Van Biervliet J, Divers TJ, Porter B, et al. Glomerulonephritis in horses. *Compend Contin Educ Pract Vet*. 2002;24:892.
440. Banks KL, Henson JB, McGuire TC. Immunologically mediated glomerulitis of horses. I. Pathogenesis in persistent infection by equine infectious anemia virus. *Lab Invest*. 1972;26:701.
441. Banks KL. Animal model of human disease: antiglomerular basement antibody in horses. *Am J Pathol*. 1979;94:443.
442. Geor RJ, Clark EG, Haines DM, et al. Systemic lupus erythematosus in a filly. *J Am Vet Med Assoc*. 1990;197:1489.
443. Sabnis SG, Gunson DE, Antonovych TT. Some unusual features of mesangioproliferative glomerulonephritis in horses. *Vet Pathol*. 1984;21:574.
444. Maede Y, Inaba M, Amano Y, et al. Cryoglobulinemia in a horse. *J Vet Med Sci*. 1991;53:379.
445. Traub-Dargatz J, Bertone A, Bennett D, et al. Monoclonal aggregating immunoglobulin cryoglobulinaemia in a horse with malignant lymphoma. *Equine Vet J*. 1985;17:470.
446. MacDougall DF, Cook T, Steward AP, et al. Canine chronic renal disease: prevalence and types of glomerulonephritis in the dog. *Kidney Int*. 1986;29:1144.
447. Fincher MG, Olafson P. Chronic diffuse glomerulonephritis in a horse. *Cornell Vet*. 1934;24:356.
448. Frank ER, Dunlap GL. Chronic diffuse glomerulo-tubular nephritis in a horse. *North Am Vet*. 1935;16:20.
449. Kadaás I, Szaázados I. Membrano-proliferative diffuse glomerulonephritis in a horse. *Dtsch Tierarztl Wochenschr*. 1974;81:618.
450. Brobst DF, Lee HA, Spencer GR. Hypercalcemia and hypophosphotemia in a mare with renal insufficiency. *J Am Vet Med Assoc*. 1978;173:1370.
451. Roberts MC, Seiler RJ. Renal failure in a horse with chronic glomerulonephritis and renal oxalosis. *J Equine Med Surg*. 1979;3:278.
452. Dobos-Kovaács M. Chronic, diffuse, membrane-proliferative glomerulonephritis and its complications in a horse. *Magy Aállatorvosok Lapja*. 1981;36:533.
453. Tennant B, Bettleheim P, Kaneko JJ. Paradoxic hypercalcemia and hypophosphotemia associated with chronic renal failure in horses. *J Am Vet Med Assoc*. 1982;180:630.
454. Morris DD, Lee JW. Renal insufficiency due to chronic glomerulonephritis in two horses. *Vet Clin North Am Equine Pract*. 1982;4(8):21.
455. Waldvogel A, Wild P, Wegmann C. Membranoproliferative glomerulonephritis in a horse. *Vet Pathol*. 1983;20:500.
456. Scarratt WK, Sponenberg DP. Chronic glomerulonephritis in two horses. *J Equine Vet Sci*. 1984;4:252.
457. Webb RF, Knight PR. Oxalate nephropathy in a horse. *Aust Vet J*. 1977;53:554.
458. Brobst DF, Bayly WM, Reed SM, et al. Parathyroid hormone evaluation in normal horses and horses with renal failure. *J Equine Vet Sci*. 1982;2:150.
459. Byars TD, Simpson JS, Divers TJ, et al. Percutaneous nephrostomy in short-term management of ureterolithiasis and renal dysfunction in a filly. *J Am Vet Med Assoc*. 1989;195:499.
460. Hope WD, Wilson JH, Hager DA, et al. Chronic renal failure associated with bilateral nephroliths and ureteroliths in a two-year-old Thoroughbred colt. *Equine Vet J*. 1989;21:228.
461. Hillyer MH, Mair TS, Lucke VM. Bilateral renal calculi in an adult horse. *Equine Vet Educ*. 1990;2:117.
462. Laing JA, Raisis AL, Rawlinson RJ, et al. Chronic renal failure and urolithiasis in a 2-year-old colt. *Aust Vet J*. 1992;69:199.
463. Held JP, Wright B, Henton JE. Pyelonephritis associated with renal failure in a horse. *J Am Vet Med Assoc*. 1986;189:688.
464. Carrick JB, Pollitt CC. Chronic pyelonephritis in a brood mare. *Aust Vet J*. 1987;64:252.
465. Sloet van Oldruitenborgh-Oosterbaan MM, Klabec HC. Ureteropyelonephritis in a Fresian mare. *Vet Rec*. 1988;122:609.
466. Mair TS, Taylor FGR, Pinsent PJN. Fever of unknown origin in the horse: a review of 63 cases. *Equine Vet J*. 1989;21:260.

467. Hamlen H. Pyelonephritis in a mature gelding with an unusual urinary bladder foreign body: a case report. *J Equine Vet Sci*. 1993;13:159.
468. Andrews EJ. Oxalate nephropathy in a horse. *J Am Vet Med Assoc*. 1971;159:49.
469. Buntain B, Greig WA, Thompson H. Chronic nephritis in a pony. *Vet Rec*. 1979;104:307.
470. Alders RG, Hutchins DR. Chronic nephritis in a horse. *Aust Vet J*. 1987;64:151.
471. Snyder JR, Batista, de Cruz J. Chronic renal failure in a stallion. *Compend Contin Educ Pract Vet*. 1984;6:S134.
472. Morter RL, Williams RD, Bolte H, et al. Equine leptospirosis. *J Am Vet Med Assoc*. 1969;155:436.
473. Divers TJ, Byars TD, Shin SJ. Renal dysfunction associated with infection of Leptospira interrogans in a horse. *J Am Vet Med Assoc*. 1992;201:1391.
474. Hogan PM, Bernard WV, Kazakevicius PA, et al. Acute renal disease due to Leptospira interrogans in a weaning. *Equine Vet J*. 1996;28:331.
475. Frazer ML. Acute renal failure from leptospirosis in a foal. *Aust Vet J*. 1999;77:499.
476. Srivastava T, Warady BA, Alon US. Pneumonia-associated acute glomerulonephritis. *Clin Nephrol*. 2002;57:175.
477. Roberts MC, Kelly WR. Renal dysfunction in a case of purpura haemorrhagica in a horse. *Vet Rec*. 1982;110:144.
478. Wimberly HC, Antonovych TT, Lewis RM. Focal glomerulosclerosis-like disease with nephrotic syndrome in a horse. *Vet Pathol*. 1981;18:692.
479. Boyd WL, Bishop LM. Pyelonephritis of horses and cattle. *J Am Vet Med Assoc*. 1937;90:154.
480. Divers TJ. Nephrolithiasis and ureterolithiasis in horses and their association with renal disease and failure. *Equine Vet J*. 1989;21:161 (editorial).
481. MacAllister CG, Morgan SJ, Borne AT, et al. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc*. 1993;202:71.
482. Behm RJ, Berg IE. Hematuria caused by renal medullary crest necrosis in a horse. *Compend Contin Educ Pract Vet*. 1987;9:698.
483. Stewart J, McCallum JW. The anhydraemia of oxalate poisoning in horses. *Vet Rec*. 1944;56:77.
484. Walthall JC, McKenzie RA. Osteodystrophia fibrosa in horses at pasture in Queensland: field and laboratory investigations. *Aust Vet J*. 1976;52:11.
485. Jakob W. Spontaneous amyloidosis of animals. *Vet Pathol*. 1971;8:292.
486. Nunokawa Y, Fujinaga T, Taira T, et al. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci*. 1993;55:1011.
487. Husebekk A, Husby G, Sletten K, et al. Characterization of amyloid protein AA and its serum precursor SAA in the horse. *Scand J Immunol*. 1986;23:703.
488. Sletten K, Husebekk A, Husby G. The amino acid sequence of an amyloid fibril protein AA isolated from the horse. *Scand J Immunol*. 1987;26:79.
489. Sletten K, Husebekk A, Husby G. The primary structure of equine serum amyloid A (SAA) protein. *Scand J Immunol*. 1989;30:117.
490. van Andel ACJ, Gruys E, Kroneman J, et al. Amyloid in the horse: a report of nine cases. *Equine Vet J*. 1988;20:277.
491. DiBartola SP, Benson MD. The pathogenesis of reactive systemic amyloidosis. *J Vet Intern Med*. 1989;3:31.
492. Hayden DW, Johnson KH, Wolf CB, et al. AA amyloid-associated gastroenteropathy in a horse. *J Comp Pathol*. 1988;98:195.
493. Vanhooser SL, Reinemeyer CR, Held JP. Hepatic AA amyloidosis associated with severe strongylosis in a horse. *Equine Vet J*. 1988;20:274.
494. Bovée KC. The uremic syndrome. *J Am Anim Hosp Assoc*. 1976;12:189.
495. Wills MR. Uremic toxins, and their effect on intermediary metabolism. *Clin Chem*. 1985;31:5.
496. Malluche H, Faugere MC. Renal bone disease 1990: an unmet challenge for the nephrologist. *Kidney Int*. 1990;38:193.
497. Nagode LA, Chew DJ. Nephrocalcinosis caused by hyperparathyroidism in progression of renal failure. *Semin Vet Med Surg (Small Anim)*. 1992;7:202.
498. Frye MA, Johnson JS, Traub-Dargatz JL, et al. Putative uremic encephalopathy in horses: five cases (1978-1998). *J Am Vet Med Assoc*. 2001;218:560–566.
499. Perna AF, Ingrosso D, Galletti P, et al. Membrane protein damage and methylation reactions in chronic renal failure. *Kidney Int*. 1996;50:358.
500. Hayslett JP. Functional adaptation to reduction in renal mass. *Physiol Rev*. 1979;59:137.
501. Pearson EG. Hypoalbuminemia in horses. *Compend Contin Educ Pract Vet*. 1990;12:555.
502. Bovée KC. Functional responses to nephron loss. In: Bovée KC, ed. *Canine nephrology*. Media, PA: Harwal; 1984.
503. Ersley AJ. Erythropoeitin. *N Engl J Med*. 1991;324:1339.
504. Jaussand P, Audran M, Gareau RL. Kinetics and haematological effects of erythropoietin in horses. *Vet Res*. 1994;25:568.
505. Effects of erythropoietin on plasma and red cell volume. VO_{2max}, and hemodynamics in exercising horses. *Med Sci Sports Exerc*. 1993;25:S25.
506. Geor RJ, Weiss DJ. Drugs affecting the hematologic system of the performance horse. *Vet Clin North Am Equine Pract*. 1993;9:649.
507. Piercy RJ, Swardson CJ, Hinchcliff KW. Erythroid hypoplasia and anemia following administration of recombinant human erythropoietin to two horses. *J Am Vet Med Assoc*. 1998;212:244.
508. Woods PR, Campbell G, Cowell RL. Nonregenerative anaemia associated with administration of recombinant human erythropoietin to a Thoroughbred racehorse. *Equine Vet J*. 1997;29:326–328.
509. Matthews HK, Kohn CW. Calcium and phosphorous homeostasis in horses with renal disease. In: *Proceedings of the eleventh annual Forum of the American College of Veterinary Internal Medicine*. San Diego; 1993:623.
510. Krook L, Wasserman RH, Shively JN, et al. Hypercalcemia and calcinosis in Florida horses: implication for the shrub Cestrum diurnum as the causative agent. *Cornell Vet*. 1975;65:26.
511. Naylor JM, Kronfeld DS, Acland H. Hyperlipemia in horses: effects of undernutrition and disease. *Am J Vet Res*. 1980;41:899.
512. Dunkel B, McKenzie III HC. Severe hypertriglyceridaemia in clinically ill horses: diagnosis, treatment and outcome. *Equine Vet J*. 2003;35:590–595.
513. Mogg TD, Palmer JE. Hyperlipidemia, hyperlipemia, and hepatic lipidosis in American Miniature Horses: 23 cases (1990–1994). *J Am Vet Med Assoc*. 1995;207:604–607.
514. Gröne HJ, Hohbach J, Gröne EF. Modulation of glomerular sclerosis and interstitial fibrosis by native and modified lipoproteins. *Kidney Int*. 1996;49(suppl 54):S18.
515. Levey AS. Measurement of renal function in chronic renal disease. *Kidney Int*. 1990;38:167.
516. Walser M. Progression of chronic renal failure in man. *Kidney Int*. 1990;37:1195.
517. Klahr S, Schreiner G, Ichikawa I. The progression of renal disease. *N Engl J Med*. 1988;318:1657.
518. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med*. 1998;339:1448.
519. Reggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. *Lancet*. 2001;357:1601.
520. Yoshioka T, Mitarai T, Kon V, et al. Role for angiotensin II in an overt functional proteinuria. *Kidney Int*. 1986;30:538.
521. Heeg JE, de Jong PE, van der Hem GK, et al. Reduction of proteinuria by angiotensin converting enzyme inhibition. *Kidney Int*. 1987;32:78.

522. Keane WF, Anderson S, Aurell M, et al. Angiotensin converting enzyme inhibitors and progressive renal insufficiency: current experience and future directions. *Ann Intern Med*. 1989;111:503.
523. Taal MW, Brenner BM. Renoprotective effects of RAS inhibition: from ACEI to angiotensin II antagonists. *Kidney Int*. 2000;57:1803.
524. Brown SA, Walton C, Crawford P, et al. Long-term effects of antihypertensive regimens on renal hemodynamics and proteinuria in diabetic dogs. *Kidney Int*. 1993;43:1210.
525. Ihle BU, Becker GJ, Whitworth JA, et al. The effect of protein restriction on the progression of renal insufficiency. *N Engl J Med*. 1989;321:1773.
526. Mitch WE. Dietary protein restriction in patients with chronic renal failure. *Kidney Int*. 1991;40:326.
527. Fouque D, Laville M, Boissel JP, et al. Controlled low protein diets in chronic renal insufficiency: meta-analysis. *BMJ*. 1992;304:216.
528. Klahr S, Level AS, Beck GJ, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. *N Engl J Med*. 1994;330:877.
529. Polzin DJ, Osborne CA, Hayden DW, et al. Influence of reduced protein diets on morbidity, mortality, and renal function in dogs with induced chronic renal failure. *Am J Vet Res*. 1984;45:506.
530. Brown SA, Finco DR, Crowell WA, et al. Dietary protein intake and the glomerular adaptations to partial nephrectomy in dogs. *J Nutr*. 1991;121:S125.
531. Ikizler TA, Hakim RM. Nutrition in end-stage renal disease. *Kidney Int*. 1996;50:343.
532. Seawright AA, Roberts MC, Costigan P. Chronic methylmercurialism in a horse. *Vet Hum Toxicol*. 1978;20:6.
533. Roberts MC, Ng JC, Seawright AA. The effects of prolonged daily low level mercuric chloride dosing in a horse. *Vet Hum Toxicol*. 1978;20:410.
534. Roberts MC, Seawright AA, Ng JC. Chronic phenylmercuric acetate toxicity in a horse. *Vet Hum Toxicol*. 1979;21:321.
535. Roberts MC, Seawright AA, Ng JC, et al. Some effects of chronic mercuric chloride intoxication on renal function in a horse. *Vet Hum Toxicol*. 1982;24:415.
536. Divers TJ. Management of chronic renal failure in the horse. In: *Proceedings of the thirty-first annual meeting of the American Association of Equine Practitioners*. Toronto, Canada; 1985:1.
537. Kirk CA. Dietary salt and FLUTD: risk or benefit? In: *Proceedings of the twentieth annual forum of the American College of Veterinary Internal Medicine*. Toronto, Canada; 2002:553.
538. Velosa JA, Torres VE, Donadio JV. Treatment of severe nephrotic syndrome with meclofenamate: an uncontrolled pilot study. *Mayo Clin Proc*. 1985;60:586.
539. Morris DD, Henry MM, Moore JN, et al. Effect of dietary linolenic acid on endotoxin-induced thromboxane and prostacyclin production by equine peritoneal macrophages. *Circ Shock*. 1989;29:311.
540. Henry MM, Moore JN, Feldman EB. The effect of dietary alpha linolenic acid on equine monocyte procoagulant activity and eicosanoid synthesis. *Circ Shock*. 1990;32:173.
541. Morris DD, Henry MM, Moore JN, et al. Dietary alpha linolenic acid reduces endotoxin- induced production of tumor necrosis factor activity by peritoneal macrophages. *Am J Vet Res*. 1991;52:528.
542. Barcelli UO, Weiss M, Pollack VE. Effects of dietary prostaglandin precursor on the progression of experimentally induced chronic renal failure. *J Lab Clin Med*. 1982;100:786.
543. Scharschmidt LA, Gibbons NB, McGarry L, et al. Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. *Kidney Int*. 1987;32:700.
544. Henry MM, Moore JN, Fischer JK. Influence of an omega-3 fatty acid-enriched ration on in vivo responses of horses to endotoxin. *Am J Vet Res*. 1991;52:523.
545. Longhofer SL, Frisbie DD, Johnson HC, et al. Effects of thromboxane synthetase inhibition on immune complex glomerulonephritis. *Am J Vet Res*. 1991;52:480.
546. Vaden SL, Breitschwerdt EB, Armstrong PJ, et al. The effects of cyclosporine versus standard care in dogs with naturally occurring glomerulonephritis. *J Vet Intern Med*. 1995;9:259.
547. Müller GA, Schettler V, Müller CA, et al. Prevention of progression of renal fibrosis: how far are we? *Kidney Int*. 1996;49(suppl 5):S75.
548. Davies M, Kastner S, Thomas GJ. Proteoglycans: their possible role in renal fibrosis. *Kidney Int*. 1996;49(suppl 54):S55.
549. Divers TJ. Diagnosis and management of urinary tract infections in the horse. In: *Proceedings of a symposium on trimethoprim/sulfadiazine, clinical application in equine medicine, Princeton Junction*. Yardley, PA: Veterinary Learning Systems; 1984.
550. Robinson JA, Allen GK, Green EM, et al. A prospective study of septicaemia in colostrum-deprived foals. *Equine Vet J*. 1993;25:214–219.
551. Reid G, Sobel JD. Bacterial adherence in the pathogenesis of urinary tract infection: a review. *Rev Infect Dis*. 1987;9:470.
552. Senior DF. Bacterial urinary tract infections: invasion, host defenses, and new approaches to prevention. *Compend Contin Educ Pract Vet*. 1985;7:334.
553. Johnson PJ, Goetz TE, Baker GJ, et al. Treatment of two mares with obstructive (vaginal) urinary outflow incontinence. *J Am Vet Med Assoc*. 1987;191:973.
554. Sertich PL, Hamir AN, Orsini P, et al. Paraurethral lipoma in a mare associated with frequent urination. *Equine Vet Educ*. 1990;2:121.
555. Mulholland SG, Qureshi SM, Fritz RW, et al. Effect of hormonal deprivation on the bladder defense mechanism. *J Urol*. 1982;127:1010.
556. Roberts JA. Bacterial adherence and urinary tract infection. *South Med J*. 1987;80:347.
557. Reidasch G, Heck P, Rauterberg E, et al. Does low urinary sIgA predispose to urinary tract infection? *Kidney Int*. 1983;23:759.
558. Voss JL, Pickett BW. Diagnosis and treatment of haemospermia in the stallion. *J Reprod Fertil Suppl*. 1975;23:151.
559. Sullins KE, Bertone JJ, Voss JL, et al. Treatment of hemospermia in stallions: a discussion of 18 cases. *Compend Contin Educ Pract Vet*. 1988;10:1396.
560. McKinnon AO, Voss JL, eds. *Equine reproduction*. Philadelphia: Lea & Febiger; 1993.
561. Schumacher J, Varner DD, Schmitz DG, et al. Urethral defects in geldings with hematuria and stallions with hemospermia. *Vet Surg*. 1995;24:250.
562. Blanchard TL, Varner DD, Hurtgen JP, et al. Bilateral seminal vesiculitis and ampullitis in a stallion. *J Am Vet Med Assoc*. 1988;192:525.
563. DeBowes RM, Nyrop KA, Boulton CH. Cystic calculi in the horse. *Compend Contin Educ Pract Vet*. 1984;6:S268.
564. Adams LG, Dollahite JW, Romane WM, et al. Cystitis and ataxia associated with sorghum ingestion. *J Am Vet Med Assoc*. 1969;155:518.
565. Fischer AT, Spier S, Carlson GP, et al. Neoplasia of the urinary bladder as a cause of hematuria. *J Am Vet Med Assoc*. 1985;186:1294–1296.
566. Holt PE, Mair TS. Ten cases of bladder paralysis associated with sabulous urolithiasis in horses. *Vet Rec*. 1990;127:108.
567. Wise LA, Jones RL, Reif JS. Nosocomial canine urinary tract infections in a veterinary teaching hospital. *J Am Anim Hosp Assoc*. 1990;26(148):1983–1988.
568. Nouws JFM, Firth EC, Vree TB, et al. Pharmacokinetics and renal clearance of sulfamethazine, sulfamerazine, and sulfadiazine and their N_4-acetyl and hydroxy metabolites in horses. *Am J Vet Res*. 1987;48:392.
569. Johnson PJ, Crenshaw KL. The treatment of cystic and urethral calculi in a gelding. *Vet Med*. 1990;85:891.

570. White NA, Moore JN, eds. *Current practice of equine surgery*. Philadelphia: JB Lippincott; 1990.
571. Mair TS, Holt PE. The aetiology and treatment of equine urolithiasis. *Equine Vet Educ*. 1994;6:189.
572. Remillard RL, Modransky PD, Welker FH, et al. Dietary management of cystic calculi in a horse. *J Equine Vet Sci*. 1992;12:359.
573. Divers TJ, Byars TD, Murch O, et al. Experimental induction of Proteus mirabilis cystitis in the pony and evaluation of therapy with trimethoprim-sulfadiazine. *Am J Vet Res*. 1981;42:1203.
574. Van Kempen KR. Sudan grass and sorghum poisoning of horses: a possible lathyrogenic disease. *J Am Vet Med Assoc*. 1970;156:629.
575. Hooper PT. Epizootic cystitis in horses. *Aust Vet J*. 1968;44:11.
576. Roberts MC. Ascending urinary tract infection in ponies. *Aust Vet J*. 1979;55:191.
577. Cranley JJ, McCullagh KG. Ischaemic myocardial fibrosis and aortic strongylosis in the horse. *Equine Vet J*. 1981;13:35.
578. Poynter D. The arterial lesions produced by Strongylus vulgaris and their relationship to the migratory route of the parasite in its host. *Res Vet Sci*. 1960;1:205.
579. Rubin HL, Woodard JC. Equine infection with Micronema deletrix. *J Am Vet Med Assoc*. 1974;165:256.
580. Alstad AD, Berg JE, Samuel C. Disseminated Micronema deletrix infection in the horse. *J Am Vet Med Assoc*. 1979;174:264.
581. Blunden AS, Khalil LF, Webbon PM. Halicephalobus deletrix infection in a horse. *Equine Vet J*. 1987;19:255.
582. Darien BJ, Belknap J, Nietfeld J. Cerebrospinal fluid changes in two horses with central nervous system nematodiasis (Micronema deletrix). *J Vet Intern Med*. 1988;2:201.
583. Angus KW, Roberts L, Archibald DRN, et al. Halicephalobus deletrix infection in a horse in Scotland. *Vet Rec*. 1992;131:495. letter.
584. Lester G. Parasitic encephalomyelitis in horses. *Compend Contin Educ Pract Vet*. 1992;14:1624.
585. Cheng TC. *General parasitology*. New York: Academic Press; 1973.
586. Smits GM, Misdorf W. Dioctophyma renale beim Hund in den Neiderlanden. *Zentralbl Veterinarmed B*. 1965;12:327.
587. Szwejkowski H. Sektionsbild der Dioctophymease der Hunde. *Arch Exp Veterinarmed*. 1960;14:1184.
588. Newberne JW, Robinson VB, Bowen NE. Histological aspects of Klossiella equi in the kidney of a zebra. *Am J Vet Res*. 1958;19:304.
589. Vetterling JM, Thompson DE. Klossiella equi Baumann, 1946 (Sporozoa: Eucoccidia: Adeleina) from equids. *J Parasitol*. 1972;58:589.
590. Todd KS, Gosser HS, Hamilton DP. Klossiella equi Baumann, 1946 (Sporozoa: Eucoccidiorida) from an Illinois horse. *Vet Med Small Anim Clin*. 1977;72:443.
591. Lee CG, Ross AD. Renal coccidiosis of the horse associated with Klossiella equi. *Aust Vet J*. 1977;53:287.
592. Austin RJ, Dies KH. Klossiella equi in the kidneys of a horse. *Can Vet J*. 1981;22:159.
593. Reppas GP, Collins GH. Klossiella equi infection in horses: sporocyst stage identified in urine. *Aust Vet J*. 1995;72:316.
594. DiBartola, Chew DJ. Canine urolithiasis. *Compend Contin Educ Pract Vet*. 1981;3:226.
595. Sutor DJ, Wooley SE, Illingsworth JJ. A geographical and historical survey of the composition of urinary stones. *Br J Urol*. 1974;46:393.
596. Wharrier J. Cystic calculus in the horse. *Vet Rec*. 1964;76:187 (letter).
597. Smith LH. The medical aspects of urolithiasis: an overview. *J Urol*. 1989;141:707.
598. Osborne CA, Polzin DJ, Abdullahi SU, et al. Struvite urolithiasis in animals and man: formation, detection, and dissolution. *Adv Vet Sci Comp Med*. 1985;29:1.
599. Senior DF, Finlayson B. Initiation and growth of uroliths. *Vet Clin North Am Small Anim Pract*. 1986;16:19.
600. Buffington CA, Blaisdell JL, Sako T. Effects of Tamm-Horsfall glycoprotein and albumin on struvite crystal growth in urine of cats. *Am J Vet Res*. 1994;55:965.
601. Hess B, Nakagawa Y, Parks JH, et al. Molecular abnormality of Tamm-Horsfall glycoprotein in calcium oxalate nephrolithiasis. *Am J Physiol*. 1991;260:F569.
602. See WA, Williams RD. Urothelial injury and clotting cascade activation: common denominators in particulate adherence to urothelial surfaces. *J Urol*. 1992;147:541.
603. Neumann RD, Ruby AL, Ling GV, et al. Ultrastructure and mineral composition of urinary calculi from horses. *Am J Vet Res*. 1994;55:1357.
604. Sutor DJ, Wooley SE. Animal calculi: an x-ray diffraction study of their crystalline composition. *Res Vet Sci*. 1970;11:299.
605. Grünberg W. Carbonate urinary calculi in herbivorous domestic animals. *Zentralbl Veterinarmed A*. 1971;18:767.
606. Mair TS. Crystalline composition of equine urinary calculi. *Res Vet Sci*. 1986;40:288.
607. Osborne CA, Sanna JJ, Unger LK, et al. Analyzing the mineral composition of uroliths from dogs, cats, horses, cattle, sheep, goats, and pigs. *Vet Med*. 1989;84:750.
608. Ruby AL, Ling GV. Bacterial culture of uroliths: techniques and interpretation of results. *Vet Clin North Am Small Anim Pract*. 1986;16:325.
609. Holt PE, Pearson H. Urolithiasis in the horse: a review of 13 cases. *Equine Vet J*. 1984;16:31.
610. Jackson OE. Renal calculi in a horse. *Vet Rec*. 1972;91:7.
611. DeBowes RM. Surgical management of urolithiasis. *Vet Clin North Am Equine Pract*. 1988;4:461.
612. Donner GS, Ellison GW, Ackerman N, et al. Percutaneous nephrolithotomy in the dog: an experimental study. *Vet Surg*. 1987;16:411.
613. Mulley AG. Management of nephrolithiasis: new approaches to "surgical" kidney stones. *Annu Rev Med*. 1988;39:347.
614. MacHarg MA, Foerner JJ, Phillips TN, et al. Electrohydraulic lithotripsy for treatment of a cystic calculus in a mare. *Vet Surg*. 1985;14:325.
615. Eustace RA, Hunt JM. Electrohydraulic lithotripsy for treatment of cystic calculus in two geldings. *Equine Vet J*. 1988;20:221.
616. Senior DF. Electrohydraulic shock-wave lithotripsy in experimental canine struvite bladder stone disease. *Vet Surg*. 1984;13:143.
617. Snyder JR, Pascoe JR, Williams JW. Rectal prolapse and cystic calculus in a burro. *J Am Vet Med Assoc*. 1985;187:421.
618. Kendrick JW. Cystic calculi in a horse. *Cornell Vet*. 1950;40:187.
619. Usenik EA, Larson LL, Sauer F. Cystotomy and removal of a urolith in a Shetland mare. *J Am Vet Med Assoc*. 1956;128:453.
620. Menon MN, Lingam UM. Laparo-cystotomy in a horse. *Indian Vet J*. 1958;35:482.
621. Lowe JE. Suprapubic cystotomy in a gelding. *Cornell Vet*. 1960;50:510.
622. Furness TR. Cystic calculus in a three-year-old gelding. *Can Vet J*. 1960;1:221.
623. Wright JG, Neal PA. Laparo-cystotomy for urinary calculus in a gelding. *Vet Rec*. 1960;72:301.
624. Lowe JE. Surgical removal of equine uroliths via the laparocystotomy approach. *J Am Vet Med Assoc*. 1961;139:345.
625. Reed DG. Suprapubic cystotomy in a stallion. *Can J Comp Med Vet Sci*. 1964;28:95.
626. Williams KR. Laparo-cystotomy in a gelding. *Vet Rec*. 1964;76:83.
627. Williams PFB. Removal of an urinary calculus from a gelding. *N Z Vet J*. 1979;27:223.
628. Mair TS, McCaig J. Cystic calculus in a horse. *Equine Vet J*. 1983;15:173.
629. Belling TH. Equine laparocystotomy. *Vet Clin North Am Equine Pract*. 1983;5(1):16.
630. Kaneps AJ, Shires GMH, Watrous BJ. Cystic calculi in two horses. *J Am Vet Med Assoc*. 1985;187:737.
631. Crabbe BG, Bohn AA, Grant BD. Equine urocystoliths. *Vet Clin North Am Equine Pract*. 1991;13(1):12.

632. van Dongen PL, Plenderleith RW. Equine urolithiasis: surgical treatment by Gökels pararectal cystotomy. *Equine Vet Educ*. 1994;6:186.
633. Röcken M, Stehle C, Mosel G, et al. Laparoscopic-assisted cystotomy for urolith removal in geldings. *Vet Surg*. 2006;35:394–397.
634. Lund CM, Ragle CA, Lutter JD. Laparoscopic removal of a bladder urolith in a standing horse. *J Am Vet Med Assoc*. 2013;243:1323.
635. Dyke TM, Maclean AA. Urethral obstruction in a stallion with possible synchronous diaphragmatic flutter. *Vet Rec*. 1987;121:425.
636. Lowe JE. Long-term results of cystotomy for removal of uroliths from horses. *J Am Vet Med Assoc*. 1965;147:147.
637. Trotter GW, Bennett DG, Behm RJ. Urethral calculi in five horses. *Vet Surg*. 1981;10:159.
638. Firth EC. Urethral sphincterotomy for delivery of vesical calculus in the mare: a case report. *Equine Vet J*. 1976;8:99.
639. Koenig J, Hurtig M, Pearce S, et al. Ballistic shockwave lithotripsy in an 18-year-old Thoroughbred gelding. *Can Vet J*. 1999;40:185.
640. May KA, Pleasant RS, Howard RD, et al. Failure of holmium: yttrium-aluminum-garnet laser lithotripsy in two horses with calculi in the urinary bladder. *J Am Vet Med Assoc*. 2001;219:957–961.
641. Crabbe BG, Grant BD. Complications secondary to a chronic urocystolith. *Vet Clin North Am Equine Pract*. 1991;13(3):8.
642. Schryver HF, Hintz HF, Lowe JE. Calcium and phosphorous in the nutrition of the horse. *Cornell Vet*. 1974;64:493.
643. Hintz HF. Dietary cation-anion balance. *Vet Clin North Am Equine Pract*. 1991;13(10):6.
644. Wall DL, Topliff DR, Freeman DW, et al. Effects of dietary cation-anion balance on urinary mineral excretion in exercised horses. *J Equine Vet Sci*. 1992;12:168.
645. Cooper SR, Kline KH, Foreman JH, et al. Effects of dietary cation-anion balance on blood pH, acid-base parameters, serum and urine mineral levels, and parathyroid hormone (PTH) in weanling horses. *J Equine Vet Sci*. 1995;15:417.
646. Osborne CA, Polzin DJ, Kruger JM, et al. Medical dissolution of canine struvite urocystoliths. *Vet Clin North Am Small Anim Pract*. 1986;16:349.
647. Osborne CA, Lulich JP, Kruger JM, et al. Medical dissolution of feline struvite urocystoliths. *J Am Vet Med Assoc*. 1990;196:1053.
648. McCue PM, Brooks PA, Wilson WD. Urinary bladder rupture as a sequela to obstructive urethral calculi. *Vet Med*. 1989;84:912.
649. Gibson KT, Trotter GW, Gustafson SB. Conservative management of uroperitoneum in a gelding. *J Am Vet Med Assoc*. 1992;200:1692.
650. Verwilghen D, Ponthier J, Van Galen G, et al. The use of radial extracorporeal shockwave therapy in the treatment of urethral urolithiasis in the horse: a preliminary study. *J Vet Intern Med*. 2008;22:1449.
651. Pascoe JR, Pascoe RRR. Displacements, malpositions, and miscellaneous injuries of the mare's urogenital tract. *Vet Clin North Am Equine Pract*. 1988;4:439.
652. Donaldson RS. Eversion of the bladder in a mare. *Vet Rec*. 1973;92:409.
653. Haynes PF, McClure JR. Eversion of the urinary bladder: a sequel to third-degree perineal laceration in the mare. *Vet Surg*. 1980;9:66.
654. Alvarenga J, Oliveira CM. Correia da Silva LCL: prolapse with eversion of the urinary bladder in a mare. *Vet Clin North Am Equine Pract*. 1995;17(8):8.
655. Jennings PB, ed. *The practice of large animal surgery*. vol. 2. Philadelphia: WB Saunders; 1984.
656. Noone JP. Scrotal herniation of the urinary bladder in the horse. *Ir Vet J*. 1966;20:11.
657. Wheat JD. Penile paralysis in stallions given propiopromazine. *J Am Vet Med Assoc*. 1966;148:405.
658. Yovich JV, Turner AS. Treatment of a postcastration urethral stricture by phallectomy in a gelding. *Compend Contin Educ Pract Vet*. 1986;8:S393.
659. Todhunter RJ, Parker JE. Surgical repair of urethral transection in a horse. *J Am Vet Med Assoc*. 1988;193:1085.
660. Gibbons WJ. Hematoma of penis. *Mod Vet Pract*. 1964;45:76.
661. Memon MA, McClure JJ, Usenik EA. Preputial hematoma in a stallion. *J Am Vet Med Assoc*. 1987;191:563.
662. Firth EC. Dissecting hematoma of corpus spongiosum and urinary bladder rupture in a stallion. *J Am Vet Med Assoc*. 1976;169:800.
663. Schumacher J, Schumacher J, Schmitz D. Macroscopic haematuria of horses. *Equine Vet Educ*. 2002;4:255.
664. Osborne CA, Stevens JB. *Handbook of canine and feline urinalysis*. St. Louis: Ralston Purina Company; 1981.
665. Fairley KF, Birch DF. Microscopic urinalysis in glomerulonephritis. *Kidney Int*. 1993;44(suppl 42):S9.
666. Hitt ME. Hematuria of renal origin. *Compend Contin Educ Pract Vet*. 1986;8:14.
667. Pollock C, Pei-Ling L, György AZ, et al. Dysmorphisms of urinary red blood cells: value in diagnosis. *Kidney Int*. 1989;36:1045.
668. Jai-Trung L, Hiroyoshi W, Hiroshi M, et al. Mechanism of hematuria in glomerular disease: an electron microscopic study in a case of diffuse membranous glomerulonephritis. *Nephron*. 1983;35:68.
669. Gibbs DD, Lynn KL. Red cell volume distribution curves in the diagnosis of glomerular and non-glomerular hematuria. *Clin Nephrol*. 1990;33:143.
670. Janssens PMW. New markers for analyzing the cause of hematuria. *Kidney Int*. 1994;46(suppl 47):S115.
671. Johnson JK, Neely DP, Latterman SA. Hematuria caused by abdominal abscessation in a foal. *J Am Vet Med Assoc*. 1987;191:971.
672. Bernard WV, Williams D, Tuttle PA, et al. Hematuria and leptospiruria in a foal. *J Am Vet Med Assoc*. 1993;203:276.
673. Kisthardt KK, Schumacher J, Finn-Bodner ST, et al. Severe renal hemorrhage caused by pyelonephritis in 7 horses: clinical and ultrasonographic evaluation. *Can Vet J*. 1999;40:571.
674. Owen RR, Haywood S, Kelly DF. Clinical course of renal adenocarcinoma associated with hypercupraemia in a horse. *Vet Rec*. 1986;119:291.
675. West HJ, Kelly DF, Ritchie HE. Renal carcinomatosis in a horse. *Equine Vet J*. 1987;19:548.
676. Patterson-Kane JC, Tramontin RR, Giles Jr RC, et al. Transitional cell carcinoma of the urinary bladder in a Thoroughbred, with intra-abdominal dissemination. *Vet Pathol*. 2000;37:692.
677. DiPiro JT, Talbert RL, Hayes PE, et al. *Pharmacotherapy: a pathophysiologic approach*. New York: Elsevier; 1989.
678. Edwards JF, Carter GK. Severe renal pelvic necrosis and hematuria of Arabian horses associated with possible analgesic nephrosis. In: *Proceedings of the forty-second annual meeting of the American College of Veterinary Pathology*. ; 1991:45.
679. Helman RG, Edwards WC. Clinical features of blister beetle poisoning in equids: 70 cases (1983–1996). *J Am Vet Med Assoc*. 1997;211:1018–1021.
680. Lloyd KCK, Wheat JD, Ryan AM, et al. Ulceration in the proximal portion of the urethra as a cause of hematuria in horses: four cases (1978–1985). *J Am Vet Med Assoc*. 1989;194:1324.
681. Taintor J, Schumacher J, Schumacher J, et al. Comparison of pressure within the corpus spongiosum penis during urination between geldings and stallions. *Equine Vet J*. 2004;36:362–364.
682. Mair TS, Walmsley JP, Phillips TJ. Surgical treatment of 45 horses affected by squamous cell carcinoma of the penis and prepuce. *Equine Vet J*. 2000;32:406.
683. Schott HC. Idiopathic renal hematuria. In: *Proceedings of the eighteenth annual forum of the American College of Veterinary Internal Medicine*. Seattle; 2000:190.
684. Pardo V, Berian MG, Levi DF, et al. Benign primary hematuria: clinicopathologic study of 65 patients. *Am J Med*. 1979;67:817.

685. Lano MD, Wagoner RD, Leary FJ. Unilateral essential hematuria. *Mayo Clin Proc*. 1979;54:88.
686. Aber GM, Higgins PM. The natural history and management of the loin pain/hematuria syndrome. *Br J Urol*. 1982;54:613.
687. Hughes JH, Stanisic TH, Buster D, et al. Massive nontraumatic hematuria: a challenging demand demanding immediate action. *Postgrad Med J*. 1990;67:97.
688. Stone EA, DeNovo RC, Rawlings CA. Massive hematuria of nontraumatic renal origin in dogs. *J Am Vet Med Assoc*. 1983;183:868.
689. Holt PE, Lucke VM, Pearson H. Idiopathic renal hemorrhage in the dog. *J Small Anim Pract*. 1987;28:253.
690. Kaufman AC, Barsanti JA, Selcer BA. Benign essential hematuria in dogs. *Compend Contin Educ Pract Vet*. 1994;16:1317.
691. Morris CF, Robertson JL, Mann PC, et al. Hemolytic uremic-like syndrome in two horses. *J Am Vet Med Assoc*. 1987;191:1453.
692. Dolente BA, Seco OM, Lewis ML. Streptococcal toxic shock in a horse. *J Am Vet Med Assoc*. 2000;217:64.
693. Abarbanel J, Benet AE, Lask D, et al. Sports hematuria. *J Urol*. 1990;143:887.
694. Corrigan Jr JJ, Boineau FG. Hemolytic-uremic syndrome. *Pediatr Rev*. 2001;22:365.
695. Sprayberry K, Madigan J, LeCouteur RA, et al. Renal failure, laminitis, and colitis following severe rhabdomyolysis in a draft horse-cross with polysaccharide storage myopathy. *Can Vet J*. 1998;39:500.
696. Grauer GF. The differential diagnosis of polyuric-polydipsic diseases. *Compend Contin Educ Pract Vet*. 1981;3:1079.
697. Hughes D. Polyuria and polydipsia. *Compend Contin Educ Pract Vet*. 1992;14:1161.
698. Tennant B, Kaneko JJ, Lowe JE, et al. Chronic renal failure in the horse. *Proc Am Assoc Equine Pract*. 1978;23:293.
699. Love S. Equine Cushing's disease. *Br Vet J*. 1993;149:139.
700. King JM, Kavanaugh JF, Bentinck-Smith J. Diabetes mellitus with pituitary neoplasms in a horse and a dog. *Cornell Vet*. 1962;52:133.
701. Loeb WF, Capen CC, Johnson LE. Adenomas of the pars intermedia associated with hyperglycemia and glycosuria in two horses. *Cornell Vet*. 1966;56:623.
702. Green EM, Hunt EL. Hypophyseal neoplasia in a pony. *Compend Contin Educ Pract Vet*. 1985;7:S249.
703. Horvath CJ, Ames TR, Metz AL, et al. Adrenocorticotropin-containing neoplastic cells in a pars intermedia adenoma in a horse. *J Am Vet Med Assoc*. 1988;192:367.
704. Staempfli HR, Eigenmann EJ, Clarke LM. Insulin treatment and development of anti-insulin antibodies in a horse with diabetes mellitus associated with a functional pituitary adenoma. *Can Vet J*. 1988;29:934.
705. van der Kolk JH, Kalsbeek HC, van Garderen E, et al. Equine pituitary neoplasia: a clinical report of 21 cases (1990–1992). *Vet Rec*. 1993;133(594).
706. Hillyer MH, Taylor FGR, Mair TS. Diagnosis of hyperadrenocorticism in the horse. *Equine Vet Educ*. 1992;4:131.
707. Coggins CH, Leaf A. Diabetes insipidus. *Am J Med*. 1967;42:806.
708. Robertson GL. Differential diagnosis of polyuria. *Annu Rev Med*. 1988;39:425.
709. Chenault L. Diabetes insipidus in the equine. *Southwest Vet*. 1969;22:321.
710. Satish C, Sastry KNV. Equine diabetes insipidus: a case report. *Indian Vet J*. 1978;55:584.
711. Forssman H. Two different mutations of the X-chromosome causing diabetes insipidus. *Am J Hum Genet*. 1955;7:21.
712. Brashier M. Polydipsia and polyuria in a weanling colt caused by nephrogenic diabetes insipidus. *Vet Clin North Am Equine Pract*. 2006;22:219–227.
713. Bertone JJ, Horspool LJ, eds. *Equine clinical pharmacology*. Philadelphia: WB Saunders; 2004.
714. Greene CE, Wong P, Finco DR. Diagnosis and treatment of diabetes insipidus in two dogs using two synthetic analogs of antidiuretic hormone. *J Am Anim Hosp Assoc*. 1979;15:371.
715. Plumb DC. *Plumb's veterinary drug handbook*. 6th ed. Ames, IA: Blackwell; 2008.
716. Kranenburg LC, Thelen MHM, Westermann CM, et al. Use of desmopressin eye drops in the treatment of equine congenital central diabetes insipidus. *Vet Rec*. 2010;167:790.
717. Kraus KH. The use of desmopressin in diagnosis and treatment of diabetes insipidus in cats. *Compend Contin Educ Pract Vet*. 1987;9:752.
718. Corke MJ. Diabetes mellitus: the tip of the iceberg. *Equine Vet J*. 1986;18:87 (editorial).
719. Muylle E, van den Hende C, DePrez P, et al. Non-insulin dependent diabetes mellitus in a horse. *Equine Vet J*. 1986;18:143.
720. Johnson PJ, Scotty NC, Weidmeyer C, et al. Diabetes mellitus in a domesticated Spanish Mustang. *J Am Vet Med Assoc*. 2005;226:584–588.
721. Siegel ET. Diabetes mellitus in a horse. *J Am Vet Med Assoc*. 1966;149:1016.
722. Jeffrey JR. Diabetes mellitus secondary to chronic pancreatitis in a pony. *J Am Vet Med Assoc*. 1968;153:1168.
723. Baker JR, Richie HE. Diabetes mellitus in the horse: a case report and review of the literature. *Equine Vet J*. 1974;6:7.
724. Ruoff WW, Baker DC, Morgan SJ, et al. Type II diabetes mellitus in a horse. *Equine Vet J*. 1986;18:143.
725. Traver DS, Moore JN, Coffman JR, et al. Peritonitis in a horse: a cause of acute abdominal distress and polyuria-polydipsia. *J Equine Med Surg*. 1977;1:36.
726. Hardy RM, Osborne CA. Canine pyometra: pathophysiology, diagnosis and treatment of uterine and extra-uterine lesions. *J Am Anim Hosp Assoc*. 1974;10:245.
727. Gennari FJ, Cohen JJ. Renal tubular acidosis. *Annu Rev Med*. 1978;29:521.
728. Fauci AS, Braunwald E, Kasper DL, et al. *Harrison's principles of internal medicine*. 17th ed. New York: McGraw-Hill; 2008.
729. Maxwell MM, Kleeman DR, Narins RG, eds. *Clinical disorders of fluid and electrolyte metabolism*. 4th ed. New York: McGraw-Hill; 1987.
730. Trotter GW, Miller D, Parks A, et al. Type II renal tubular acidosis in a mare. *J Am Vet Med Assoc*. 1986;188:1050.
731. Hansen TO. Renal tubular acidosis in a mare. *Compend Contin Educ Pract Vet*. 1986;8:864.
732. Ziemer EL, Parker HR, Carlson GP, et al. Renal tubular acidosis in two horses: diagnostic studies. *J Am Vet Med Assoc*. 1987;190:289.
733. Van der Kolk JH, Kalsbeek HC. Renal tubular acidosis in a mare. *Vet Rec*. 1993;133:44.
734. Van der Kolk JH, de Graaf-Roelfsema E, Joles JA, et al. Mixed proximal and distal renal tubular acidosis without aminoaciduria in a mare. *J Vet Intern Med*. 2007;21:1121–1125.
735. Gull T. Type: 1 renal tubular acidosis in a broodmare. *Vet Clin North Am Equine Pract*. 2006;22:29–237.
736. Stewart AJ. Secondary renal tubular acidosis in a quarter horse gelding. *Vet Clin North Am Equine Pract*. 2006;22:e47–e61.
737. Aleman MR, Kuesis B, Schott HC. Renal tubular acidosis in horses (1980–1999). *J Vet Intern Med*. 2001;15:136–143.
738. MacLeay JM, Wilson JH. Type-II renal tubular acidosis and ventricular tachycardia in a horse. *J Am Vet Med Assoc*. 1998;212:1597–1599.
739. Van den Berg CJ, Harrington TM, Bunch TW, et al. Treatment of renal lithiasis associated with renal tubular acidosis. *Proc Eur Dial Transplant Assoc*. 1983;20:473.
740. Battle D. Renal tubular acidosis: symposium on acid-base disorders. *Med Clin North Am*. 1983;67:859.
741. Ohmes CM, Davis EG, Beard LA, et al. Transient Fanconi syndrome in Quarter horses. *Can Vet J*. 2014;55:147.
742. Frolich ED, ed. *Pathophysiology: altered regulatory mechanisms in disease*. Philadelphia: JB Lippincott; 1984.
743. Halperin ML, Richardson RMA, Bear R, et al. Urine ammonium: the key to the diagnosis of distal renal tubular acidosis. *Nephron*. 1988;50:1.

744. Rodriguez-Soriano J, Vallo A. Renal tubular acidosis. *Pediatr Nephrol*. 1990;4:268.
745. van Leeuwen AM. Net cation equivalency (base-binding power) of the plasma proteins. *Acta Med Scand*. 1964;176(suppl 422):36.
746. Brown CM. Equine nephrology. *Vet Annu*. 1986;26:1.
747. Sundberg JP, Burnstein T, Page EH, et al. Neoplasms of equidae. *J Am Vet Med Assoc*. 1977;170:150.
748. Cotchin E. A general survey of tumors in the horse. *Equine Vet J*. 1977;9:16.
749. Theilen GH, Madewell BR, eds. *Veterinary cancer medicine*. 2nd ed. Philadelphia: Lea & Febiger; 1987.
750. Tyner GA, Nolen-Walston RD, Hall T, et al. A multicenter retrospective study of 151 renal biopsies in horses. *J Vet Intern Med*. 2011;25:532.
751. Wise LN, Bryan JN, Sellon DC, et al. A retrospective analysis of renal carcinoma in the horse. *J Vet Intern Med*. 2009;23:913.
752. Berggren PC. Renal adenocarcinoma in a horse. *J Am Vet Med Assoc*. 1980;176:1252.
753. Haschek WM, King JM, Tennant BC. Primary renal cell carcinoma in two horses. *J Am Vet Med Assoc*. 1981;179:992.
754. Pomroy W. Renal adenocarcinoma in a horse. *Equine Vet J*. 1981;13:198.
755. Van Amstel SR, Huchzermeyer D, Reyers F. Primary renal cell carcinoma in a horse. *J S Afr Vet Assoc*. 1984;55:35.
756. Brown PJ, Holt PE. Primary renal cell carcinoma in four horses. *Equine Vet J*. 1985;17:473.
757. Van Mol KAC, Fransen JLA. Renal carcinoma in a horse. *Vet Rec*. 1986;119:238.
758. Swain JM, Pirie RS, Hudson NPH, et al. Insulin-like growth factors and recurrent hypoglycemia associated with renal cell carcinoma in a horse. *J Vet Intern Med*. 2005;19:613–616.
759. Baker JL, Aleman M, Madigan J. Intermittent hypoglycemia in a horse with anaplastic carcinoma of the kidney. *J Am Vet Med Assoc*. 2001;218:235–237.
760. Rumbaugh ML, Latimer FG, Porthouse KP, et al. Renal carcinoma with osseous and pulmonary metastases in an Arabian gelding. *Equine Vet J*. 2003;35:107–109.
761. Traub JL, Bayly WM, Reed SM, et al. Intraabdominal neoplasia as a cause of chronic weight loss in the horse. *Compend Contin Educ Pract Vet*. 1983;5:S526.
762. Robertson SA, Waterman AE, Lane JG, et al. An unusual cause of anaesthetic death in a horse. *Equine Vet J*. 1985;17:403.
763. Nyka W. Sur une tumeur renal du cheval issue du blastème meátaneáphrique. *Bull Cancer*. 1928;17:241.
764. Köhler H. Nephroblastom in der Niere eines Pferds. *Dtsch Tierarztl Wochenschr*. 1977;84:400.
765. Jardine JE, Nesbit JW. Triphasic nephroblastoma in a horse. *J Comp Pathol*. 1996;114:193.
766. Servantie J, Magnol JP, Regnier A, et al. Carcinoma of the renal pelvis with bony metaplasia in a horse. *Equine Vet J*. 1986;18:236.
767. Vivotec J. Carcinomas of the renal pelvis in slaughter animals. *J Comp Pathol*. 1977;87:129.
768. Matsuda K, Kousaka Y, Nagamine N, et al. Papillary renal adenoma of distal nephron differentiation in a horse. *J Vet Med Sci*. 2007;69:763–765.
769. Carrick JB, Morris DD, Harmon BG, et al. Hematuria and weight loss in a mare with pancreatic adenocarcinoma. *Cornell Vet*. 1992;82:91.
770. Kim DY, Cho DY, Snider III TG. Mucinous hyperplasia in the kidney and ureter of horse. *J Comp Pathol*. 1994;110:309.
771. Loynachan AT, Bryant UK, Williams NM. Renal mucus gland cystadenomas in a horse. *J Vet Diagn Invest*. 2008;20:520–522.
772. Sweeney RW, Hamir AN, Fisher RR. Lymphosarcoma with urinary bladder infiltration in a horse. *J Am Vet Med Assoc*. 1991;199:1177.
773. Traub-Dargatz JL. Urinary tract neoplasia. *Vet Clin North Am Equine Pract*. 1998;14:495.
774. Zantingh AJ, Gaughan EM, Bain FT. Case report: squamous cell carcinoma of the urinary bladder in a horse. *Compend Contin Educ Vet*. 2012;34(10):E1.
775. Hurcombe SDA, Slovis NM, Kohn CW, et al. Poorly differentiated leiomyosarcoma of the urogenital tract in a horse. *J Am Vet Med Assoc*. 2008;233:1908–1912.
776. van den Top JG, de Heer N, Klein WR, et al. Penile and preputial tumours in the horse: a retrospective study of 114 affected horses. *Equine Vet J*. 2008;40:528.
777. McCue PM. Neoplasia of the female reproductive tract. *Vet Clin North Am Equine Pract*. 1998;14:505.
778. Strafuss AC. Squamous cell carcinoma in horses. *J Am Vet Med Assoc*. 1976;168:61.
779. van den Top JG, de Heer N, Klein WR, et al. Penile and preputial squamous cell carcinoma in the horse: a retrospective study of treatment of 77 affected horses. *Equine Vet J*. 2008; 40:533.
780. Plaut A, Kohn-Speyer AC. The carcinogenic action of smegma. *Science*. 1947;105:656.
781. Rizk A, Mosbah E, Karrouf G, et al. Surgical management of penile and preputial neoplasms in equine with special reference to partial phallectomy. *J Vet Med*. 2013;891413:2013.
782. Moore AS, Beam SL, Rassnick KM, et al. Long-term control of mucocutaneous squamous cell carcinoma and metastasis in a horse using piroxicam. *Equine Vet J*. 2003;35:715–718.
783. Markel MD, Wheat JD, Jones K. Genital neoplasms treated by en bloc resection and penile retroversion in horses: 10 cases (1977-1986). *J Am Vet Med Assoc*. 1988;192(396).
784. Theáon AP, Pascoe JR, Carlson GP, et al. Intratumoral chemotherapy with cisplatin in oily emulsion in horses. *J Am Vet Med Assoc*. 1993;202:261.
785. Theáon AP, Pascoe JR, Meagher DM. Perioperative intratumoral administration of cisplatin for treatment of cutaneous tumors in Equidae. *J Am Vet Med Assoc*. 1994;205:1170.
786. Fortier LA, MacHarg MA. Topical use of 5-fluorouracil for treatment of squamous cell carcinoma of the external genitalia of horses: 11 cases (1988-1992). *J Am Vet Med Assoc*. 1994;205:1183–1185.
787. Khanna OMP. Disorders of micturition. *Urology*. 1976;8:316.
788. Brorasmussen F, Sorensen AH, Bredahl E, et al. The structure and function of the urinary bladder. *Urol Int*. 1965;19:280.
789. Schott HC, Carr EA, Patterson JS, et al. Urinary incontinence in 37 horses, in Proceedings. *Am Assoc Eq Pract*. 2004;50:345.
790. Learmonth JR. Contribution to neurophysiology of urinary bladder in man. *Brain*. 1930;54:147.
791. Madison JB. Estrogen-responsive urinary incontinence in an aged pony mare. *Compend Contin Educ Pract Vet*. 1984;6:S390.
792. Hodgson DJ, Dumas S, Bolling DR, et al. Effect of estrogen on sensitivity of the rabbit bladder and urethra to phenylephrine. *Invest Urol*. 1978;16:67.
793. Starr I, Ferguson LK. Beta-methylcholine-urethane: its action in various normal and abnormal conditions, especially postoperative urinary retention. *Am J Med Sci*. 1940;200:372.
794. Finkbeiner AE. Is bethanechol chloride clinically effective in promoting bladder emptying? A literature review. *J Urol*. 1985;134:443.
795. Rendle DI, Durham AE, Hughes KJ, et al. Long-term management of sabulous cystitis in five horses. *Vet Rec*. 2008;162:783–788.

CHAPTER 15

Disorders of the Hematopoietic System

Bettina Dunkel

INVESTIGATION OF HEMATOLOGIC DISORDERS

An investigation of the hematopoietic system often begins with a complete blood count (CBC). The number of any cell type in the blood is determined by the balance of cell production and release from the bone marrow and removal from the circulation by loss, destruction, or extravasation. All blood cells originate from a common pluripotent stem cell, and a schematic representation of hematopoiesis can be found in Fig. 15.1.

Erythrocytes

Physiology

Erythropoiesis, the development and maturation of red blood cells (RBCs), is dependent on the presence of erythropoietin, a glycoprotein hormone synthesized by the kidney in response to renal hypoxia. Absence, such as in cases of chronic renal disease,[1,2] or excess of erythropoietin can cause severe nonregenerative anemia or erythrocytosis, respectively. Similar to people, attempts to manipulate erythropoiesis to enhance performance is an increasing concern in equine athletes. Human recombinant erythropoietin and cobalt have been abused as doping agents in racehorses. Cobalt is a chemical inducer of hypoxia-like responses that include induction of erythropoietin transcription.[3]

In contrast to other species, immature RBCs containing ribonucleic acid (RNA) (reticulocytes) produced in the bone marrow of horses are normally not released into the circulation, even during moderate to severe anemia. Until recently assessment of RBC regeneration in horses was therefore limited to size analysis of RBCs or evaluation of bone marrow biopsies. Macrocytosis (an increase in the mean corpuscular volume [MCV]; Box 15.1) usually correlates with a regenerative anemia. With increasing sensitivity of modern automated hematology analyzers that enumerate RBC-containing nucleic acids by flow cytometry, increased reticulocytes have been detected in the blood of a horse with severe anemia and in horses treated with human recombinant erythropoietin.[4]

Hemoglobin, the component that allows erythrocytes to transport oxygen, is composed of four polypeptide chains (two α- and two β-chains) and a heme moiety. Heme is the functional portion of the molecule and contains an iron atom held in place by the four pyrrole rings of a porphyrin molecule. The iron in hemoglobin must be in the ferrous form (Fe^{2+}) to bind oxygen reversibly. When the heme iron is oxidized to the ferric form (Fe^{3+}), methemoglobin is formed, and the molecule is incapable of oxygen transport. Normal concentration of methemoglobin in blood does not exceed 1% to 2% of the total hemoglobin concentration. Small amounts of methemoglobin with ferric iron are formed under normal conditions but readily converted back to the ferrous form by methemoglobin reductase. Several other cellular enzymes contain iron in their functional reduced state.

Glutathione, an important tripeptide cellular antioxidant, is present in RBCs in high concentrations and assists in maintenance of proteins in their functional, reduced state. This process is energy dependent, which in turn is glucose dependent. Because of the absence of a Krebs cycle in RBCs, glucose is metabolized in these cells via the anaerobic glycolytic Emden-Meyerhof pathway or via the hexose monophosphate shunt. Hereditary deficiency of critical enzymes in these metabolic pathways, glucose-6-phosphate dehydrogenase and flavin adenine dinucleotide, caused hemolytic anemia and hyperbilirubinemia[5] and persistent methemoglobinemia[6] in a colt and an adult horse, respectively.

Normal equine RBCs remain in the circulation for about 150 days before they are removed by mononuclear phagocytes in spleen, liver, and bone marrow. In the liver, heme is converted to biliverdin and then to bilirubin, which is released in an unconjugated form into the circulation.

Hemoglobin released into the blood during intravascular hemolysis is transported to the liver by haptoglobin. If the transport capacity of haptoglobin is saturated, free hemoglobin is present in the plasma. It is filtrated across the renal glomeruli and reabsorbed by renal tubular epithelial cells. Pigment molecules containing heme have nephrotoxic potential due to direct cytotoxic and indirect proinflammatory effects. Heme can oxidize lipids, denature proteins, disrupt the cytoskeleton, impair cytosolic enzyme activity, activate cell-damaging enzymes, and interrupt mitochondrial metabolism.[7]

Evaluation

The erythron is evaluated by measurement of RBC numbers, size, and volume; hemoglobin concentration; and determination of erythrocyte indices (see Box 15.1). These measurements are complemented by subjective evaluation of erythrocyte morphology. Hematocrit and packed cell volume (PCV) measure the percentage of the volume of whole blood that is occupied by RBCs. If both are measured by centrifugation, the terms can be used interchangeably. Hematocrit determined

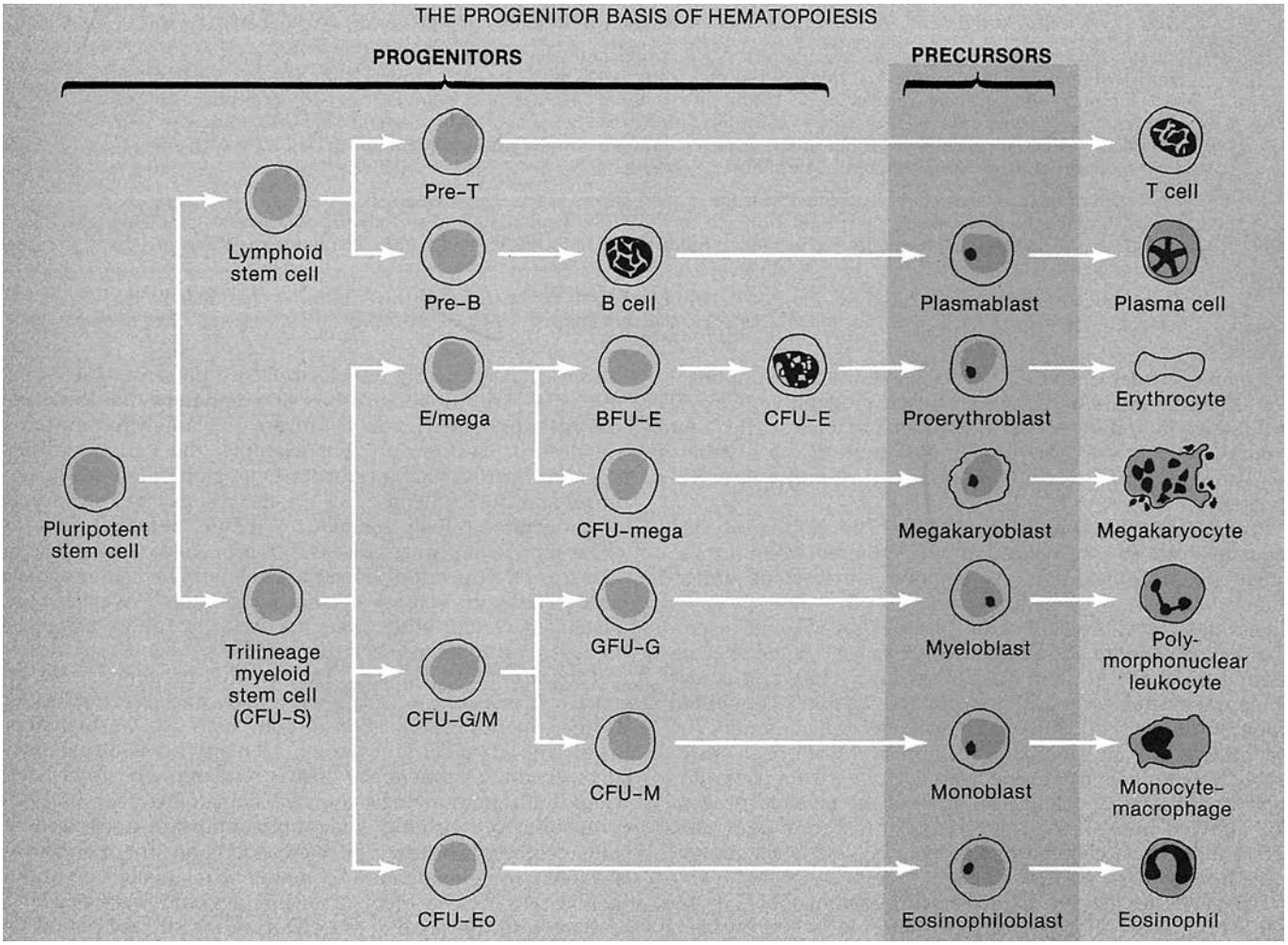


FIG. **15.1** Schematic representation of the progenitor basis of hematopoiesis. Maturation is depicted from left to right with circulating blood cells as the final product on the far right of the drawing. Progressive amplification of progenitors and precursors as they mature and differentiate is not shown. *CFU-S,* Colony-forming unit, spleen; *CFU-G/M,* CFU-granulocyte/macrophage; *CFU-Eo,* CFU-eosinophil; *BFU-E,* burst-forming unit, erythroid; *CFU-mega,* CFU-megakaryocyte; *CFU-G,* CFU-granulocyte; *CFU-M,* CFU-monocyte; *CFU-E,* CFU-erythrocyte. (From Wyngaarden JB, Smith LH, editors: *Cecil textbook of medicine,* ed 18, Philadelphia, 1988, WB Saunders.)

BOX 15.1 Evaluation of Red Blood Cell Indices: Calculation from the Packed Cell Volume, Red Blood Cell Count (Millions), and Hemoglobin (g/dL)

MCV = (PCV × 10)/RBC count

Expressed as femtoliters (fL)
Increased in some horses with regenerative anemia
Decreased with iron deficiency anemia
Increased in older horses

MCV = (Hemoglobin × 10)/RBC count

Expressed as picograms (pg)
Increased with intravascular hemolysis
Decreased with iron deficiency anemia

MCHC = (Hemoglobin × 100)/PCV

Expressed as grams per deciliter (g/dL)
Increased with intravascular hemolysis
Decreased with iron deficiency anemia

by automated analyzers is usually a calculated value (MCV × RBC count/10). Hemoglobin concentration is approximately one third of the PCV; this percentage increases with the presence of free hemoglobin in the bloodstream (hemoglobinemia). Normal values for hematologic parameters in horses of different breeds are given in Table 15.1 and 15.2; calculations for RBC indices can be found in Box 15.1.

Peripheral blood evaluation should include a thorough examination of a stained blood smear to assess erythrocyte morphology. The normal equine erythrocyte is a biconcave disk; however, in contrast to other species, most equine RBCs lack distinct central pallor. Equine RBCs sediment quickly as a result of rapid Rouleaux formation (coinlike stacking of RBCs). This physiologic phenomenon can be differentiated from pathologic autoagglutination (diagnostic for intravascular hemolysis) by dilution of the blood with physiologic saline, which disperses Rouleaux but not autoagglutination.

Occasionally specialized tests of erythrocyte function or stability are indicated. Osmotic fragility tests measure the resistance of erythrocytes to in vitro hemolysis when

TABLE **15.1 Reference Ranges for Equine Hematologic Parameters**[a]

Parameter	Units	Light Horse[b]	Draft Horse[c]	Miniature Horse[d]	Donkey[e]
RBC count	10^6/μL	6.0–10.0	5.5–9.5	4.3–10.3	4.7–9.0
Hemoglobin	g/dL	12.0–17.0	8.0–14.0	9.0–16.0	9.5–16.5
PCV	%	32–50	24–44	24–42	28–47
MCV	fl	42–58	—	38–61	46–67
MCH	μg	15–20	—	14–23	16–23
MCHC	g/dL	32–38	—	33–40	32–36

RBC, Red blood cell; *PCV,* packed cell volume; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration.
[a]Numbers are for comparison only. Each laboratory should establish its own normal values.
[b]North Carolina State University Clinical Pathology Laboratory equine reference values.
[c]Data from Jain N: *Schalm's veterinary hematology,* ed 4, Philadelphia, 1986, Lea & Febiger.
[d]Data from Harvey R, Hambright M, Rowe L: Clinical biochemical and hematologic values of the American Miniature Horse: reference values, *Am J Vet Res*. 1984;45:987.
[e]Data from Zinkl J, Mae D, Merida P, et al: Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys *(Equus asinus), Am J Vet Res*. 1990;51:408.

TABLE **15.2 Influence of Breed on Normal Erythron Values (Mean ± Standard Deviation) in Adult Horses**

Breed	RBC (× 10^6/μL)	HGB (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (%)
Thoroughbred	9.35 ± 1.05	14.8 ± 1.3	41.7 ± 3.8	44.7 ± 3.4	15.9 ± 1.4	35.8 ± 1.4
Standardbred	8.37 ± 1.02	13.6 ± 1.6	38.3 ± 3.5	46.1 ± 4.0	16.3 ± 1.4	35.5 ± 1.6
Quarter Horse	8.26 ± 1.02	13.3 ± 1.6	38.0 ± 4.0	46.2 ± 3.9	16.1 ± 1.7	34.9 ± 1.6
Appaloosa	8.60 ± 1.11	13.3 ± 1.6	38.4 ± 4.7	44.8 ± 4.4	15.5 ± 1.3	34.5 ± 0.8
Arabian	8.41 ± 1.21	13.8 ± 2.1	39.3 ± 5.0	46.9 ± 1.9	16.4 ± 0.9	34.9 ± 1.0
Clydesdale	7.30 ± 0.87	12.4 ± 1.1	33.0 ± 3.0	44.6	—	38.1
Percheron	7.39 ± 1.08	11.7 ± 1.4	—	—	—	—
Mixed cold-blooded	7.76 ± 1.23	—	33.0 ± 7.0	42.3	—	—

From Moms DD: Review of anemia in horses. I. Clinical signs, laboratory findings and diagnosis, *Vet Clin North Am Equine Pract*. 1989;11:27–34; modified from Jain NC, editor: *Schalm's veterinary hematology,* ed 4, Philadelphia, 1986, Lea & Febiger.
RBC, Red blood cell; *Hgb,* hemoglobin; *PCV,* packed cell volume; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration.

incubated in increasingly hypotonic sodium chloride (NaCl) solution. Erythrocytes from horses with immune-mediated hemolytic anemia (IMHA) are frequently more readily lysed in hypotonic solution (more fragile) than are RBCs from normal horses. Direct and indirect Coombs tests are also used to aid in the diagnosis of IMHA. The direct Coombs test detects immunoglobulin (Ig) or complement on the RBC surface by use of antibodies against IgG, IgM, and complement 3 (C3). Incubation at 10°C and 30°C detects cold- and warm-reacting antibodies, respectively. Because agglutination is the endpoint of the direct Coombs test, autoagglutination of blood is considered diagnostic of IMHA. The indirect Coombs test screens the patient's serum for presence of anti-RBC antibodies by incubating it with normal equine RBC and monitoring for agglutination.

Interpretation

Increased (erythrocytosis) or decreased (anemia) RBC numbers are indicative of increased or decreased production in the bone marrow or increased removal from the circulation by acute or chronic blood loss (Boxes 15.2 and 15.3) or intravascular or extravascular destruction (hemolysis; Box 15.4). Hemoglobinemia indicates intravascular hemolysis; extravascular hemolysis is less readily confirmed. A diagnosis of IMHA is supported by an increased osmotic fragility, positive direct or indirect Coombs test, or the presence of autoagglutination.

Morphologic examination of RBCs on a stained blood smear may reveal the following abnormalities:

Poikilocyte: Any abnormally shaped erythrocyte; usually reserved for description of RBCs that exhibit a variety of morphologies rather than one predominating shape.
Anisocytosis: Variability in RBC size, usually associated with an increased RBC distribution width.
Polychromasia: Variability in RBC color, usually caused by a variable hemoglobin and RNA content.
Spherocyte: Spherical erythrocytes might be observed with immune-mediated hemolysis.
Echinocyte: Burr cell with short, regularly spaced spicules projecting from the RBC surface; can be associated with uremia.
Acanthocyte: Spur cell with irregularly shaped spicules extending from the RBC surface; can be associated with liver disease or gastrointestinal malabsorption.
Elliptocyte: Ellipsoid or oval erythrocyte found in animals with iron deficiency or myelophthisic anemia.
Leptocyte: Thin, flat RBC frequently associated with hepatic disease or iron deficiency.
Codocyte: Target cell with a dense central area of hemoglobin surrounded by a pale zone; can be associated with hypochromic anemias or hepatic disease.
Howell-Jolly bodies: Basophilic nuclear remnants seen in the cytoplasm of erythrocytes. Approximately 10 in 10,000 erythrocytes contain Howell-Jolly bodies in the normal horse.

BOX 15.2 Differential Diagnoses for Blood Loss in the Horse

EPISTAXIS
Guttural pouch mycosis
Pulmonary abscess
Exercise-induced pulmonary hemorrhage
Ethmoid hematoma
Paranasal sinus abscess or infection
Traumatic nasogastric intubation
Upper respiratory tract neoplasm
Coagulopathy
Trauma
Rupture of rectus capitis muscle
Pneumonia/pleuritis
Hemothorax
Thoracic trauma
  Fractured rib
  Lacerated heart or vessels
Pulmonary thrombembolism
Ruptured pulmonary abscess
Ruptured great vessel
Neoplasia
Coagulopathy

HEMATURIA
Pyelonephritis
Cystitis/urolithiasis
Neoplasia
Trauma
Urethral ulceration
Coagulopathy

HEMOPERITONEUM
Trauma
  Splenic rupture
  Hepatic rupture
Mesenteric vessel rupture
Verminous arteritis
Uterine artery rupture
Phenylephrine treatment
Abdominal abscess
Neoplasia
Coagulopathy

GASTROINTESTINAL HEMORRHAGE
Ulcerations
Nonsteroidal antiinflammatory drug (NSAID) toxicity
Parasites
  Strongylus vulgaris
  Small strongyles
Granulomatous intestinal disease
  Histoplasmosis
  Tuberculosis
  Granulomatous enteritis
Neoplasia
  Squamous cell carcinoma
  Lymphosarcoma
Coagulopathy

EXTERNAL HEMORRHAGE
Trauma
Surgical complication
Coagulopathy
External parasites

Heinz bodies: Oxidized precipitated hemoglobin indicating oxidative damage to the RBC, usually resulting in intravascular or extravascular hemolysis. One may see Heinz bodies best using new methylene blue stain but may observe them in smears using Wright's stain as round structures protruding from the edge of the RBC membrane.

Iron Metabolism

Physiology

Iron is abundant in the normal horse diet, and its absorption, predominantly occurring in the small intestine, correlates with demand. It increases during iron depletion and decreases during iron abundance. After absorption into the circulation iron combines with apotransferrin forming transferrin. Excess iron is deposited in the storage form of ferritin in hepatocytes and reticuloendothelial cells in the spleen and bone marrow. Excess iron intake, especially in foals, can lead to hemochromatosis, liver cirrhosis, and liver failure.[8-10] However, experimental feeding of adult ponies with 50 mg/kg iron (22.7 mg/lb) as ferrous sulfate containing 20% elemental iron did not lead to adverse clinical signs or histopathologic liver changes.[11]

Iron deficiency is first characterized by low serum ferritin concentration and decreased stainable iron stores in the bone marrow. Once iron concentrations are sufficiently low to affect erythropoiesis, a decreased percentage of plasma transferrin saturation, an increased total iron binding capacity (TIBC), hypochromic erythrocytes (decreased mean corpuscular hemoglobin concentration [MCHC]), and ultimately release of small cells (microcytosis; decreased MCV) are noted. In healthy foals at birth, serum iron and ferritin concentrations are lower than adult levels, followed by a rapid increase in the first 24 hours as a result of absorption of colostral iron.[12] During the next 3 weeks, concentrations fall below concentrations of adults, reaching a nadir at 3 weeks. The PCV of neonatal foals correlates closely with serum iron and ferritin concentrations. In healthy foals, a higher PCV during the first days postpartum is followed by a decrease below the adult reference range and increased production of microcytes after 2 weeks.[13] This relative iron deficiency in healthy foals does not respond to supplementation, the PCV does not decrease below 32%, and morphologic abnormalities are limited to mild microcytosis.[14,15]

Evaluation

Inadequate iron intake or uncompensated loss gives rise to microcytic hypochromic iron deficiency anemia, which can be identified on blood smear evaluation and by calculation of erythrocyte indices. Iron metabolism can be directly evaluated by measuring the amount of iron in the blood

BOX 15.3 Differential Diagnoses for Nonregenerative Anemia in the Horse

IRON DEFICIENCY
Chronic hemorrhage
Nutritional deficiency (rare)

CHRONIC DISEASE
Chronic infection/inflammation
- Pleuritis/pneumonia
- Peritonitis/enteritis
- Bacterial endocarditis
- Internal abscess
- Chronic viral disease (e.g., equine infectious anemia [EIA])

Neoplasia
Endocrine disorders

BONE MARROW FAILURE
Myelophthisis
Myeloproliferative disease
Bone marrow toxins
- Phenylbutazone
- Chloramphenicol

Radiation
Idiopathic pancytopenia

MISCELLANEOUS CONDITIONS
Administration of human recombinant erythropoietin
Chronic hepatic disease
Chronic renal disease
Recent hemorrhage or hemolysis

BOX 15.4 Differential Diagnoses for Hemolysis in the Horse

INFECTIOUS DISEASES
Piroplasmosis
Equine infectious anemia (EIA)
Leptospira
Hemotrophic mycoplasma

IMMUNE-MEDIATED DISEASE
Autoimmune disease
Bacterial infection
- *Clostridium perfringens*
- Streptococcal infections

Viral infection
- EIA

Neoplasia
- Lymphosarcoma

Drug reaction
- Penicillin

Neonatal isoerythrolysis

OXIDATIVE INJURY
Phenothiazine
Onion and garlic consumption
Red maple leaves, silver and sugar maple leaves
Pistacia leaves
Snake or bee envenomation
Familial methemoglobinemia

IATROGENIC CONDITIONS
Hypotonic solutions
Hypertonic saline
Formaldehyde
Dimethyl sulfoxide (DMSO)

MISCELLANEOUS CONDITIONS
Hepatic disease
Hemolytic uremic syndrome
Disseminated intravascular coagulation

OTHER TOXICITIES
Intravenous DMSO
Bacterial toxins *(Clostridium)*
Oak
Burn injury

(73–140 µg/dL), the capacity of the blood to transport iron by measuring transferrin or TIBC (231–455 µg/dL), and the amount of iron storage; ferritin[16,17] TIBC measures all iron carrier proteins in the blood, including transferrin. Because transferrin is the primary iron-binding protein, TIBC can be used as an indirect measurement of transferrin. Transferrin is produced in relationship to the need for iron. When iron stores are low, transferrin levels increase and vice versa. In healthy horses, about 30% to 40% of the binding sites on transferrin are used to transport iron. Unsaturated iron-binding capacity measures the reserve capacity of transferrin, the portion of transferrin that has not yet been saturated. Transferrin saturation represents the percentage of transferrin that is saturated with iron. Serum ferritin provides an index of stored hepatic and splenic iron and measures 152 ± 55 ng/mL in normal horses. Ferritin concentrations decrease during iron deficiency and increase during iron overload, hemolysis, liver disease, inflammation, and colic.[16,18] The effects of different diseases on iron measurements are summarized in Table 15.3.

Leukocytes

Leukocytes are categorized as granulocytes (neutrophils, eosinophils, basophils, and mast cells), monocytes and macrophages, and lymphocytes. They are essential for normal immune function and increased or decreased numbers in the circulation are most commonly secondary to other disease processes. However, on occasion diseases of the bone marrow, neoplasia, or functional abnormalities can give rise to primary disease processes.

Neutrophils

Physiology. Granulocyte-macrophage and granulocyte colony-stimulating factor (GM-CSF and G-CSF, respectively), among several other cytokines, regulate proliferation and differentiation of precursor cells into granulocytes and mononuclear phagocytes. Mature neutrophils are stored in the bone marrow. Once released, their half-life in the circulation is approximately 10.5 hours before they extravasate into peripheral tissues, where they remain active for several days. An increased demand for neutrophils in the periphery leads to release of immature band neutrophils from the bone marrow. The neutrophil pool in the blood is composed of circulating cells and

TABLE **15.3 Indices of Iron Metabolism**

Disease	Plasma Iron	TIBC/Transferrin	% Transferrin Saturation	Ferritin
Iron deficiency	Low	High	Low	Low
Chronic disease	Low	Low	Low	Normal or high
Hemolytic anemia	High	Normal or low	High	High
Iron overload	Normal or high	Normal	High	Normal or high
Normal values (mean ± SD and reference range)[a]	123 ± 37 μg/dL 73–140 μg/dL	343 ± 56 μg/dL 231–455 μg/dL	30%–40%	152 ± 55 ng/mL 43–261 ng/mL

SD, Standard deviation; *TIBC,* total iron binding capacity.
[a]Smith JE, Cipriano JE, DeBowes R, et al: Iron deficiency and pseudo-iron deficiency in hospitalized horses. *J Am Vet Med Assoc*. 1986;188(3):285–287; Kaneko JJ, Harvey JW, Bruss ML, editors: *Clinical biochemistry of domestic animals*. 6th edition, 2008, pp 262–285.

marginated cells that are localized close to the endothelium. Only circulating cells, not marginated, are included in the peripheral cell count, and changes between marginated and circulating states can explain why neutrophil counts can change very rapidly. Selectin-mediated rolling of neutrophils along the endothelium is followed by integrin-mediated adhesion if neutrophils come in contact with activated endothelial cells at sites of injury or inflammation. Stress, exercise, glucocorticoids, and epinephrine mobilize marginated neutrophils. In addition, glucocorticoids increase neutrophil release from the bone marrow and delay extravasation and apoptosis. Defense mechanisms of neutrophils include release of lytic enzymes and reactive oxygen species, phagocytosis, and the more recently described release of deoxyribonucleic acid (DNA) in the form of neutrophil extracellular traps (NETs). These NETs have the ability to bind and kill extracellular microorganisms, and their formation by equine neutrophils has been demonstrated.[19]

Neutrophil function can be affected by age and disease state. Altered hormone concentrations, as they occur in horses with pituitary pars intermedia dysfunction (PPID), decrease neutrophil oxidative burst activity and adhesion, but not phagocytosis, compared with healthy controls. Chemotaxis is greater in healthy aged horses than in young horses or aged horses with PPID. Plasma insulin–to–α-melanocyte-stimulating hormone (MSH) ratio, but not individual hormone concentrations, correlated with neutrophil oxidative burst activity.[20] Phagocytic capacities and oxidative burst activity are impaired in neonatal foals in comparison to adult horses.[21,22] Human recombinant G-CSF products have been used therapeutically in foals with neutropenia[23,24] and experimentally as a preventive agent against disease following long-distance travel (0.23 μg/kg subcutaneously [SC] once).[25] In these reports, the neutrophil count increased markedly following treatment. Interestingly, a smaller increase in platelet count was also noted.[23] Human recombinant G-CSF is also used illegally in racehorses as a doping agent.[26]

Evaluation. Neutrophil numbers and morphology can give an indication of the severity of an insult affecting the body. The presence of band (immature) neutrophils in circulation is termed a *regenerative left shift* and indicates a bone marrow response to an inflammatory stimulus. When band cells outnumber mature neutrophils in the peripheral blood, the condition is referred to as a *degenerative left shift*. Morphologic changes indicative of a toxic insult to the neutrophils (strong inflammatory stimulus) include cytoplasmic basophilia, vacuolation, and Döhle bodies (remnant RNA from rough endoplasmic reticulum, from previous maturation stages). Occasionally, infectious organisms such as *Anaplasma phagocytophilum* can be observed in the cytoplasm of neutrophils. A congenital morphologic abnormality presenting as hyposegmentation of the nucleus (Pelger Huët anomaly) has also been described in Arabian horses.[27,28] Hypersegmentation in other species is seen as an aging process with a prolonged transit time in the blood and has been associated with resolving chronic inflammation, glucocorticoid administration, and hyperadrenocorticism. In horses, it has been described in a case of hemolytic anemia secondary to hepatic disease and as an idiopathic condition in three Quarter Horses.[29-31]

Interpretation. Most changes in neutrophil numbers are secondary to systemic inflammation, infection, stress, or glucocorticoid administration. Unfortunately, the leukocyte count can also be normal despite the presence of severe inflammation or infection. Impressive leukocytosis frequently accompanies chronic bacterial infections such as *Rhodococcus equi* or *Corynebacterium pseudotuberculosis* infection. Experimentally, endotoxin is the best investigated bacterial product that induces neutropenia, sometimes followed by a rebound neutrophilia (Chapter 12). Other microbial or inflammatory mediators probably have similar capacities, and acute intraabdominal inflammation such as may occur with colitis, enteritis, or acute peritonitis is one of the most common causes of leukopenia in horses. Extreme leukopenia and neutropenia can be observed with intestinal rupture and subsequent fecal peritonitis. Neutropenia in foals is a strong indicator for systemic inflammation and sepsis. If changes are persistent or progressive, abnormalities of the bone marrow should be considered.

Eosinophils

Physiology. Eosinophils are primarily tissue-dwelling cells, and only low numbers are present in the circulation. Previously eosinophils were believed to be predominantly associated with innate immunity, particularly with parasitic infections and allergic conditions. However, several other crucial noninflammatory roles are now also recognized. Eosinophils are essential for the long-term survival of plasma cells in the bone marrow and for their maintenance in the lamina propria of the intestinal epithelium. Eosinophils are required to instigate antibody class-switch to IgA and to maintain long-term mucosal IgA plasma cells. They also play a vital role in tissue integrity and tissue remodeling.[32]

Evaluation and Interpretation. Eosinophil numbers in the peripheral blood are usually low, and eosinopenia is therefore

difficult to appreciate. Corticosteroid treatment and stress are often associated with low eosinophil numbers. Eosinophilia in horses is uncommon, but parasitism such as cyathostomiasis or forms of eosinophilic colitis, enteritis, or multisystemic disease should be ruled out in horses with a suitable history and presentation.[33-35] In humans and other species, including the horse, eosinophilia has been observed concurrently or even preceding hematologic and other malignancies, particularly types of lymphomas.[36-38]

Basophils and Mast Cells

Basophils are the least numerous leukocytes in the peripheral blood. Together with mast cells, they are regarded as effector cells of IgE-dependent allergic inflammation, rapidly releasing histamine and inflammatory mediators on cross-linking of receptor FcεR1 by IgE-allergen complexes.[39] Recently, roles in non-IgE–mediated inflammatory conditions have also been elucidated.[39] Mast cells are long-lived granulated cells derived from hematopoietic precursors but only very small numbers of their progenitors are present in the blood. Mast cells complete their differentiation and maturation in the microenvironments of nearly all tissues, often in close contact to potential targets of their mediators.[40]

Lymphocytes

Physiology. Lymphocytes were long considered the primary effector cells of the adaptive immunity, but with increasing knowledge, the borders between innate and adaptive immunity are becoming increasingly blurred. Circulating lymphocytes can be divided into B, T, and natural killer cells with an ever increasing plethora of subpopulations being recognized. Most lymphocytes are localized in the lymphatic tissues distributed throughout the body, including spleen and lymph nodes.

Evaluation and Interpretation. Investigation of lymphocyte numbers, function, and morphology becomes important when immunodeficiencies or lymphoid neoplasia is suspected. Absolute and relative lymphopenias are associated with administration or release of glucocorticoids, viral infection, old age, and severe combined immunodeficiency in Arabian foals and one Caspian and one Fell pony foal.[41-43] Lymphocytosis can be encountered during epinephrine release or administration, exercise, equine herpes virus–2 (EHV-2) infection in foals, and lymphocytic leukemia.[44] Investigation of lymphocyte types and function is discussed in Chapter 1.

Platelets

Physiology. Platelets or thrombocytes are anucleate blood cells derived as cell fragments budding from the boarders of polyploid precursor cells, megakaryocytes, in the bone marrow. In other species, platelet generation also occurs in the peripheral blood, particularly in the pulmonary circulation, which, besides the spleen, is one of the major storage sites for platelets. The daily production of platelets has been estimated to be approximately 35,000/μL. Radiolabeled equine platelets have an average life span of 4 to 5 days before they are removed from the circulation by macrophages residing in liver, spleen, and bone marrow. Although fundamentally similar, equine platelets show some subtle morphologic and functional differences in comparison to other species such as the absence of an open canalicular system and differences in responsiveness to some mediators.[45,46] In recent years it has become increasingly evident that platelets, besides their hemostatic functions, play a vital role in inflammation, immunity, tissue regeneration, and the pathophysiology of many diseases. Platelets from other species not only contain and release a myriad of inflammatory and regenerative mediators but also synthesize new factors such as interleukin-1β by rapid signal-dependent translation of mRNA acquired from the parent megakaryocyte. Recent evidence suggests that platelet generation is a highly sophisticated process. Depending on the circumstances, platelets are equipped with different subsets of mRNA strands during the budding process that later allow synthesis of different mediators. In the circulation, they interact with other cells, particularly leukocytes and endothelial cells, and play an essential part in attracting leukocytes to sites of inflammation or tissue damage. Human platelets are capable of active cell locomotion and gradient-driven chemotaxis in response to bacterial peptides and allergenic stimuli. With increasing interest in equine platelets and more widely available facilities to assess multiple aspects of platelet function, cell surface marker expression and interactions with other cells, knowledge about equine platelets is bound to expand rapidly within the near future.

Evaluation. Platelet aggregation, an indicator of platelet function, is assessed by determining platelet counts before and after stirring of samples using a magnetic stir bar and calculation of the percentage decrease. Alternatively, and more accurately, measures are made by use of aggregometers.[47] Flow cytometry is now widely used in the experimental setting to investigate surface expression of platelet membrane glycoproteins (GPs), including GP IIb-IIIa (CD41/61), GPIV, CD62P (P-selectin), lysosomal integral membrane protein (LIMP), and binding of annexin V and fibrinogen.[48-50] Platelet microparticles and platelet-leukocyte aggregates have also been detected using this technique.[49-51] Methods to detect platelet adhesion to coated microtiter plates under static conditions and to subendothelium under flow conditions have been described.[46,52] Several platelet release products can be measured in plasma and other biologic fluid samples as an indication of platelet activation and degranulation. Growth factors, serotonin, and thromboxane A_2 (TXA_2) are among the most frequently used markers.[53,54] Because of the short half-life of platelet factor 4 (PF4), a β-thromboglobulin (β-TG)/PF4 ratio greater than 2 has been used to distinguish between in vivo and accidental in vitro platelet activation.[55]

Interpretation. Thrombocytosis is frequently defined as a platelet count of greater than 400,000/μL, although counts of up to 500,000/μL might be normal in young male horses.[56] Thrombocytopenia, defined as a platelet count of less than 100,000/μL, is also a relatively rare occurrence affecting approximately 1.5% of hospitalized horses in which a CBC was performed.[57,58] Thrombocytopenia can be associated with systemic diseases and coagulopathies, immune-mediated destruction of platelets, or insufficient production (Box 15.5). Platelet activation often accompanies systemic inflammation and has been demonstrated in recurrent airway obstruction, laminitis, gastrointestinal disease, and EHV-1 infection.[49,52,55,59,60] Congenital defects in platelet function have been described in horses.[61-65] Thrombocytosis, thrombocytopenia, and platelet dysfunction are further discussed under disorders of hemostasis and hematopoiesis later in this chapter. In contrast, pseudothrombocytopenia is a common artificial reduction in the platelet count due to sampling and counting procedures. Automated platelet counts performed on ethylenediaminetetraacetic acid (EDTA)- and, less

BOX 15.5 Differential Diagnoses for Thrombocytopenia in the Horse

DECREASED PLATELET PRODUCTION
Hereditary defects
Myelophthisis
Myeloproliferative disease
Idiopathic pancytopenia
Myelosuppressive drugs
  Phenylbutazone
  Chloramphenicol
  Estrogens
  Trichlorethylene-extracted soybean meal
Irradiation

INCREASED PLATELET USE
Intravascular coagulation
  Disseminated intravascular coagulation
  Localized
  - Hemolytic uremic syndrome
  - Hemangioma/hemangiosarcoma

Hemorrhage
Thrombosis

PLATELET SEQUESTRATION
Splenomegaly

INCREASED PLATELET DESTRUCTION
Infectious diseases
  Equine infectious anemia
  Anaplasma phagocytophilum
Immune-mediated diseases
  Autoimmune disease
  Systemic lupus erythematosus
  Idiopathic disease
  Secondary disease
  Neoplasia (lymphosarcoma)
  Bacterial infection
  Viral infection
  Drugs
  Neonatal alloimmune disease
Drugs or toxins
Snakebites

LABORATORY ERROR
Pseudothrombocytopenia

frequently, heparin- and citrate-anticoagulated blood are often affected. In blood anticoagulated with EDTA, platelets can form aggregates following calcium chelation with subsequent artificially low counts. Inspection of a blood smear along the feathered edge and a manual platelet count can quickly establish an accurate platelet count. Rarely, true pseudothrombocytopenia secondary to EDTA-dependent antibody formation and subsequent platelet clumping are encountered in individual horses.[66,67] Use of a different anticoagulant abolishes platelet clumping.

Bone Marrow

Evaluation of bone marrow is indicated if quantitative, functional, or morphologic abnormalities of blood cells are detected that cannot be explained by an increased demand, increased cell loss, or exogenous influences.

Physiology

Production of the cellular elements of blood—namely, erythrocytes, lymphocytes, monocytes, polymorphonuclear granulocytes (neutrophils, basophils, and eosinophils), and platelets—occurs in the fetus in liver and spleen before shifting to the bone marrow as the main site of hemopoiesis in the adult horse. During aging, productive bone marrow is gradually replaced by fat, leaving the marrow of sternum, ribs, vertebra, skull, pelvis, and epiphyseal marrow of humerus and femur as the main production sites in older animals. Under the influence of not fully elucidated cytokine mediators the self-renewing pluripotent stem cells differentiate into separate cell lines (see Fig. 15.1) and after further differentiation and maturation, functional white and red blood cells and platelets are released into the bloodstream.

Evaluation

Bone marrow aspirates or core biopsies are most commonly obtained from the sternum in adult horses, but tuber coxae or proximal ribs are also possible. In the sedated horse, the cartilaginous junctions between the seventh, sixth (often partially fused), fifth, and fourth sternebrae are identified ultrasonographically. The fifth–sixth intersternebral space is often located immediately caudal to the olecranon in the standing horse, and bone marrow can be aspirated successfully from the sixth, fifth, and occasionally fourth sternebrae (located between the front legs). Marking the intersternebral spaces with clippers or a marker pen aids in identifying the middle of the sternebrae. The area is clipped, a sterile prep is performed, and local anesthetic is injected before making a small stab incision through skin and subcutaneous tissue. A Jamshidi or similar needle that is ≥16 gauge and ≥2 inches long, ideally with a stylet, is advanced until it contacts the bone surface. The index finger is placed 2 cm (¾ inch) from the skin surface, and the needle is then advanced through the bone into the marrow cavity until the tip of the finger touches the skin. This requires considerable force; rotational movements often help with insertion. In a Thoroughbred the dorsoventral diameters of the sixth, fifth, and fourth sternebrae measure approximately 3.5, 4.8, and 6.2 cm, respectively.[68] The apex of the heart is located adjacent to the seventh sternebra and xyphoid.[68] The stylet is removed and marrow aspirated into a 10-mL syringe preloaded with heparin (final concentration of 625 IU/mL bone marrow) and mixed gently as bone marrow coagulates extremely quickly. Slides can be prepared immediately or the bone marrow can be transferred into a Petri dish from which spicules can be transferred onto glass slides for staining and evaluation.

Interpretation

Evaluation includes identification of progenitor cells of different cell lines and estimation of the myeloid-erythroid ratio (M:E; 0.5–3.76 in normal horses). Ratios less than 0.5 are indicative of erythrocyte regeneration or myeloid suppression. Prussian blue staining can be used to evaluate peripheral iron stores. Bone marrow macrophages ingesting RBCs might be present in hemolytic anemia. A reduction in all cellular elements is termed *myelophthisis* and is often due to proliferation of fibrous tissue (myelofibrosis). Myelodysplasia indicates the presence of abnormal cells, often due to myeloproliferative neoplasia.

TABLE **15.4 Coagulation Factors**

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Tissue factor, tissue thromboplastin
IV	Calcium
V	Proaccelerin, labile factor
VI	Not assigned
VII	Proconvertin, stable factor
VIII	Antihemophilic factor
IX	Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor

Hemostasis

Physiology

Repair of microvascular and macrovascular injury is essential for survival during health but even more so at times of disease or injury. Effective hemostasis is a tightly controlled process and relies on the interaction of the vascular wall, particularly the endothelium, platelets, and soluble plasma proteins. In the uninjured vessel, spontaneous coagulation is avoided by the presence of several anticoagulant mechanisms expressed on and released by endothelial cells. The healthy endothelium is covered by a glycocalyx, a complex network of negatively charged macromolecules formed by cell-bound proteoglycans, glycosaminoglycan side chains, and sialoproteins. Further components include loosely attached soluble molecules including albumin, hyaluronic acid, heparan sulfate, thrombomodulin, and antithrombin.[69] The glycocalyx plays a key role in regulating microvascular tone and endothelial permeability, maintaining an oncotic gradient, regulating adhesion and extravasation of leukocytes, and inhibiting intravascular thrombosis.[69] Furthermore, the quiescent state of circulating platelets and presence of the circulating coagulation factors in an inactive zymogen form prevents spontaneous clot formation. Dysregulation of hemostasis, as it occurs with severe systemic inflammation, is a major source of morbidity and mortality in sick horses. The plasma-based coagulation system comprises a number of factors that loosely serve four different functions: coagulation, anticoagulation, fibrinolysis, and antifibrinolysis. Important coagulation factors are listed in Table 15.4. However, a large amount of overlap exists, and one factor might have several functions, depending on circumstances and effects of negative and positive feedback loops. The understanding of clot formation and its biologic opponent, fibrinolysis, is continuously evolving. Clot formation, or a tendency to thrombosis (hypercoagulability), is favored by a procoagulant environment, decreased anticoagulant activity, and inhibition of clot lysis (antifibrinolysis). Inhibition of clot formation, or an increased bleeding tendency (hypocoagulation), is encountered with high antithrombotic and fibrinolytic activities and a decreased activity of coagulation factors. If vascular injury occurs, the first response is intense vasoconstriction to divert blood flow away from the injury. Two main components, primary and secondary hemostasis, then initiate closure of the defect and vascular repair. Primary hemostasis refers to platelet aggregation to form a platelet plug. Platelets are activated (see section on coagulation) and adhere to the site of injury and to each other, closing the defect. Secondary hemostasis refers to the conversion of fibrinogen into fibrin by the proteolytic coagulation cascade and deposition of the insoluble fibrin to form a mesh that incorporates and stabilizes the platelet plug. These two processes happen simultaneously and are mechanistically intertwined.[70] Defects of primary hemostasis are characterized by petechiae, ecchymosis, spontaneous bleeding from mucosal surfaces (including epistaxis and gingival bleeding), hyphema, hematuria, and melena. Defects of secondary hemostasis often present as a tendency to hematoma formation and bleeding into subcutaneous tissues, body cavities, muscles, or joints. Overlap exists and particularly in acquired disorders, such as disseminated intravascular coagulation (DIC), multiple hemostatic defects cause clinical signs compatible with defects in primary and secondary hemostasis. Similarly, mild von Willebrand disease might resemble a primary hemostatic defect, but in severe cases signs of a secondary hemostatic disorder are also present.

The next section describes the four systems and their interaction in more detail. Available tests evaluating different aspects of hemostasis and normal coagulation times are summarized in Tables 15.5 and 15.6.

Coagulation. Earlier cascade or waterfall models of coagulation, dividing the process into an intrinsic and extrinsic pathway, have now been replaced by cell-based models.[71] There is extensive cross-talk between the inflammatory and coagulation systems, and many coagulation factors have proinflammatory or antiinflammatory capacities, but those will not be discussed. A key element of coagulation remains activation of prothrombin to thrombin and the subsequent conversion of fibrinogen into fibrin. The most important activator of coagulation is now thought to be tissue factor (TF). TF is highly expressed in extravascular tissue such as smooth muscle and fibroblasts and becomes exposed on vascular damage. Platelets then adhere to the damaged site, mediated by von Willebrand factor, and aggregate through interactions of platelet receptors with extracellular ligands and soluble proteins. Vascular damage–induced exposure of subendothelial TF generates trace amounts of thrombin with multiple effects on other coagulation factors and platelets. This progression can be divided into three phases: initiation, amplification, and propagation (Fig. 15.2). During the initiation phase (corresponding to the extrinsic pathway in the cascade model) exposed TF binds and activates factor VII (FVII). The TF/FVIIa complex activates FIX and FX into FIXa and FXa, respectively. FXa associates with cofactor FVa to form a prothrombinase complex on TF-expressing cells and convert prothrombin (FII) into thrombin.[71] In the amplification phase the small amounts of thrombin generated in the initiation activate further platelets that have adhered to the injury site. In parallel, thrombin converts platelet-derived FV into FVa, amplifying prothrombinase activity, and converts FVIII into FVIIIa, which acts as a cofactor to FIXa on the surface of activated platelets to support FXa generation. The propagation phase (intrinsic pathway in cascade model) occurs mainly on phospholipid-containing cell surfaces, predominantly

TABLE **15.5 Interpretation of Hemostatic Parameters**

	Hypocoagulation	Hypercoagulation	Fibrinolysis	Platelet Function
Prothrombin time	++	–	–	–
Activated partial thromboplastin time	++	–	–	–
Platelet concentration ↓	+	–	–	+/– (↓↓)
Fibrinogen concentration ↓↑	+/– (↓)	+/– (↑)	–	–
Fibrinogen degradation products ↑	–	+/–	+/–	–
D-dimer concentration ↑	–	++	+/–	–
Tissue factor activity ↑	–	++	–	–
Antithrombin activity ↓	++	+	–	–
Thrombin-antithrombin complexes ↑	–	+	–	–
Protein C activity ↓	–	+	–	–
Plasminogen concentration ↓	–	+/–	+	–
Plasminogen activator inhibitor-1 activity ↑	–	+/–	++	–
Viscoelastography	++	++	++	++

↑, Increased concentration or activity; ↓, decreased concentration or activity.

TABLE **15.6 Reference Ranges for Equine Hemostatic Values**[a]

Parameter	Units	Value	Reference
Platelet count	per μL	75,000–300,000	NCSU[b]
Fibrinogen	g/dL	<400	NCSU
PT	seconds	8.5–9.9	NCSU
aPTT	seconds	30–44	NCSU
FDPs	mg/dL	<20	NCSU
AT III	% PNEP	63–131	Johnstone et al.[c]
AT III	% PNHP	218 ± 18	Bernard et al.[d]
Plasminogen	% PNEP	64.6–155.9	Welles et al.[e]
Protein C	% PNEP	104.5 ± 13.8	Welles et al.[f]

PT, Prothrombin time; *aPTT,* activated partial thromboplastin time; *FDPs,* fibrin degradation products; *AT III,* antithrombin III; *PNEP,* pooled normal equine plasma; *PNHP,* pooled normal human plasma.
[a]These numbers are for reference only. Each laboratory should establish its own normal equine values.
[b]Data from NCSU, North Carolina State University College of Veterinary Medicine Clinical Pathology Laboratory normal equine reference values.
[c]Johnstone IB, Physick-Sheard P, Crane S: Breed, age, and gender differences in plasma antithrombin-III activity in clinically normal young horses, *Am J Vet Res*. 1989;50:1751.
[d]Bernard W, Morris DD, Divers TJ, et al: Plasma antithrombin-III values in healthy horses: effect of sex and/or breed, *Am J Vet Res*. 1987;48:866.
[e]Welles EG, Prasse KW, Duncan A: Chromogenic assay for equine plasminogen, *Am J Vet Res*. 1990;51:1080.
[f]Welles EG, Prasse KW, Duncan A, et al: Antigenic assay for protein C determination in horses, *Am J Vet Res*. 1990;51:1075.

activated platelets. Activated FXIa converts FIX into FIXa, which then associates with thrombin-cleaved FVIII. On phosphatidylserine-exposing cell membranes, the FIXa/FVIIIa complex catalyzes the conversion of FX to FXa, which complexes with FVa complex to form enough thrombin to form fibrin fibers. As a final step, the thrombin-activated FXIIIa catalyzes covalent cross-linking between adjacent fibrin fibers, generating a fibrin clot.[71] Via multiple enforcement loops in the coagulation system and in platelet activation, large amounts of fibrin are formed, stabilizing the earlier formed platelet thrombi.[71] It is now well established that TF is also expressed by intravascular cells, particularly when activated. Endothelial cells, monocytes, platelets, and microparticles are thought to contribute to intravascular coagulation during states of inflammation. Factor VIII deficiency in horses or factor IX deficiency in other species leads to severe bleeding complications (hemophilia A and B, respectively), thus underlining the importance of these coagulation factors for normal hemostasis.

Vitamin K is an essential coenzyme for factors II, VII, IX, and X, and vitamin K deficiency can cause severe and even fatal hemostatic dysfunctions (see acquired disorders of hemostasis). It is necessary for synthesis of four coagulation factors (II, VII, IX, and X) in the liver, as well as the anticoagulation proteins C and S. Vitamin K catalyzes posttranscriptional carboxylation of glutamic acid residues on factors II, VII, IX, and X. This process is essential for their biologic activity and allows binding of calcium and localization on negatively charged phospholipid surfaces with subsequent activation of the coagulation pathway.[72-74] In the absence of carboxylation, vitamin K–dependent factors cannot bind to platelets, leading to hemostatic dysfunctions. Deficits of vitamin K can occur quickly due the short half-life of the vitamin K–dependent coagulation factors. Vitamin K is lipid soluble and present in two types: K_1 (phylloquinone) is found in greens and oils, and K_2 (menaquinone) is produced by the intestinal microflora. Absorption occurs in the intestine in the presence of bile salts.

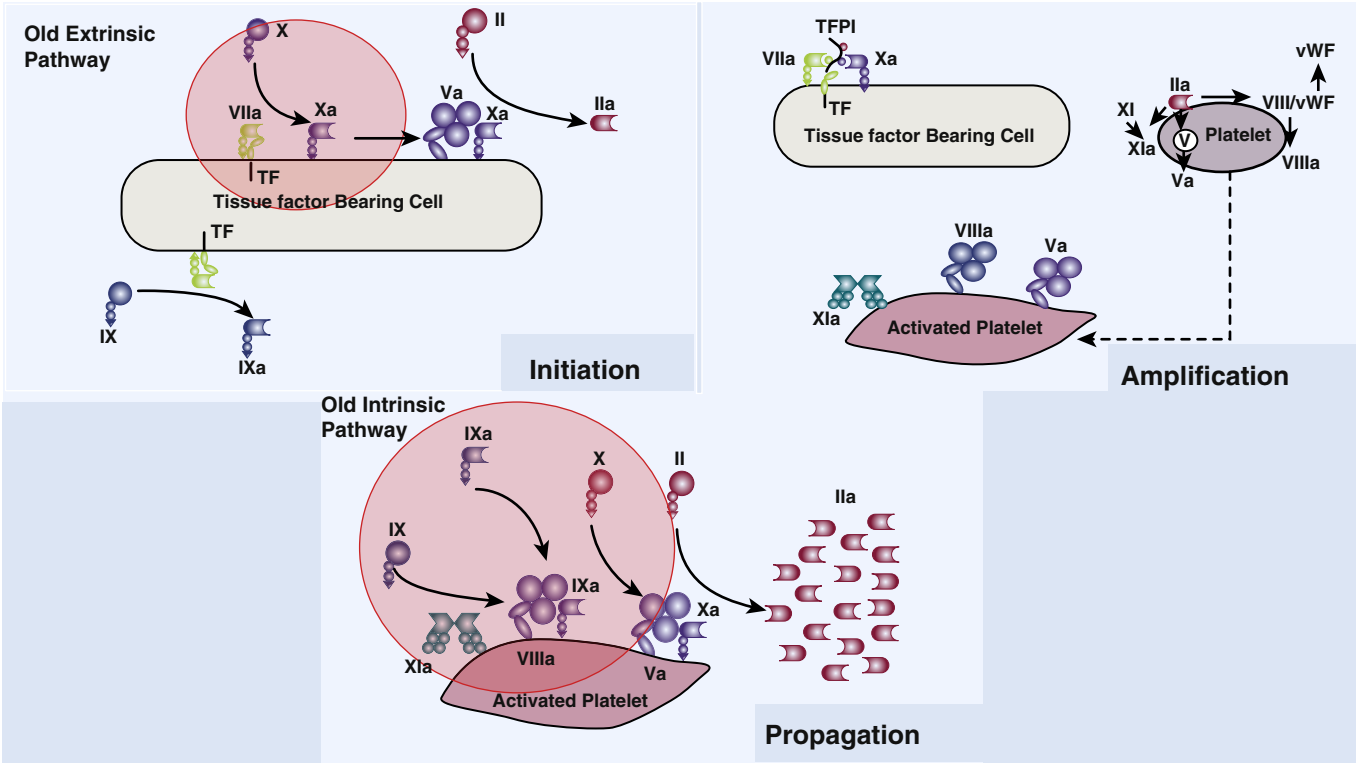


FIG. **15.2** Cell-based coagulation model. Coagulation occurs in three phases: initiation, amplification, and propagation. In the initiation, factor VIIa bound to tissue factor (TF) activates factor (F) IX and also FX. FXa then activates FV on the TF-bearing cell, complexes with factor FVa, and converts a small amount of prothrombin (II) to thrombin (IIa). In the amplification phase, the small amount of IIa activates platelets, causing release of α-granule contents including FV and activates FV, FXI, and FVIII by cleaving it from von Willebrand factor (vWF). In the propagation phase, FIXa generated by FVIIa/TF binds to the activated platelets and subsequently activates FX. This factor IXa is supplemented by factor IXa generated on the platelet surface by factor XIa. FXa and FVa then form the prothrombinase complex catalyzing a burst of thrombin generation. (Modified after Monroe DM, Hoffman M, Roberts HR: Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol*. 2002; 22(9):1381–1389.)

Anticoagulation. Mechanisms of anticoagulation prevent spread of coagulation to unaffected areas and help to retain clot formation at sites of injury. The two best described pathways include the protease inhibitors antithrombin with its cofactor heparin and tissue factor pathway inhibitor (TFPI) and the protein C/protein S pathway. Antithrombin is one of the most important inhibitors of thrombin generation because of its high affinity for FIXa, FXa, and thrombin. The presence of its cofactor heparin antithrombin increases its activity up to 1000-fold. TFPI directly inhibits FXa and interacts with TF/FVIIa/FXa complex. Thrombin itself initiates anticoagulation by binding to thrombomodulin, which enables activation of endothelial-bound protein C. The complex of activated protein C (APC) and its cofactor protein S inactivates FVIIIa and FVa.

Fibrinolysis. The final step in the process of fibrinolysis is the conversion of the circulating zymogen plasminogen into active plasmin. This is mainly achieved by tissue-type plasminogen activator (t-PA) and, to a lesser degree, urokinase. In the presence of fibrin, binding of both plasminogen and t-PA to fibrin increases plasmin generation more than twofold.[75] The colocalization of both factors on fibrin aims to minimize indiscriminate fibrinolysis.

Antifibrinolysis. Plasma inhibitors (α_2-antiplasmin and plasminogen activator inhibitor [PAI]-1 and PAI-2) also limit plasmin activity locally and its generation in the circulation. The recently described thrombin activatable fibrinolysis inhibitor (TAFI), which is activated by thrombin concentrations near recently formed clots, appears to inhibit premature clot lysis by preventing docking of t-PA and plasminogen to fibrin.[75]

Evaluation

An increasing number of available tests enables the clinician to investigate all aspects of the hemostatic system, at least in the experimental setting. Unfortunately, fewer tests are available commercially, and many functional tests need to be performed on fresh blood. Most traditional coagulation profiles include platelet count, prothrombin time (PT; investigates factors V, VII, X, prothrombin, and fibrinogen), activated partial thromboplastin time (aPTT; factors V, VIII, IX, X, XI, XII, prothrombin, and fibrinogen), antithrombin (AT) activity, fibrinogen concentration, and a measure of fibrin breakdown, either fibrinogen degradation products (FDPs) or D-dimer concentrations (see Table 15.5). Although FDPs incorporate

fragments produced by degradation of fibrinogen and fibrin, D-dimers are specific for degradation of fibrin and therefore a better indicator of intravascular coagulation and subsequent fibrinolysis.

On cross-linking, a D-dimer neo-epitope is formed that is recognized by the antibodies in D-dimer assays.[76] Viscoelastic testing offers the advantage of evaluating coagulation in whole blood, taking into account cellular function and interaction with coagulation proteins. In theory, it should provide information about speed of clot formation, clot strength, and rapidity and efficiency of clot lysis as it occurs in the body. The clinician should be able to differentiate between hypocoagulable and hypercoagulable states and increased or decreased fibrinolysis in the patient. The technique has been investigated in numerous experimental and clinical studies in horses, but despite several modifications of the available assays,[77-79] many clinicians still feel that little additional information in comparison to commonly performed coagulation profiles is provided.

Interpretation

Abnormalities of the hemostatic system can be associated with disorders of the vascular wall, platelet numbers or function, and abnormalities of the plasma-based coagulation system. Disorders might be congenital or acquired. Several hereditary abnormalities have been described including deficiencies in von Willebrand factor, FVIII and prekallikrein, and functional platelet defects (see section on abnormalities of the plasma-based coagulation system). Acquired abnormalities are common with severe infectious or inflammatory systemic diseases including neoplasia and conditions causing vasculitis. Recently, acquired FVIII deficiency has also been reported.[80]

TREATMENT OF HEMATOPOIETIC DISORDERS

Hematologic abnormalities are frequently secondary to a different, primary disease, and with successful treatment of the primary process hematologic functions will normalize. However, if the hematologic abnormalities become life threatening, either due to acute blood loss, severe hypocoagulation, or inappropriate clot formation in the microvasculature, direct intervention might be necessary. Transfusion of whole blood or blood components and pharmacologic manipulation of the hemostatic system are discussed in the following section.

Transfusion Medicine

A transfusion can either be comprised of whole blood or blood components, depending on the individual needs of the patient. In equine medicine, blood is usually freshly collected whereas plasma can be prepared from fresh blood by gravity sedimentation or purchased frozen.

Approach to Acute Blood Loss

The total blood volume of a horse depends on the breed, age, and sex, with foals less than 100 days of age and stallions having higher blood volumes than animals greater than 100 days old and geldings and mares, respectively.[81,82] In Thoroughbreds, light breed horses' and draft horses' blood volume is approximately 100 mL/kg, 78 mL/kg, and 61 mL/kg (6%–10% of the body weight), respectively.[83] Horses can tolerate loss of up to 15% of their blood volume (approximately 9–15 mL/kg depending on breed; 4.5–7.5 L in a 500-kg horse) without causing overt effects on physical parameters and up to 25% to 30% (approximately 15–30 mL/kg depending on breed; 7.5–15 L in a 500-kg horse) might be tolerated without need for a transfusion. A whole blood transfusion is indicated when the oxygen-carrying capacity of an animal has decreased to a degree that oxygen delivery to the tissues is compromised. Because 98% of oxygen in the blood is carried by hemoglobin (Box 15.6), acute blood loss is much more likely to result in tissue hypoxia than many respiratory conditions. Colic, cardiac arrhythmias, and increase in cardiac troponin I (cTnI), presumably caused by tissue hypoxia, have all been reported in horses following acute hemorrhage and also secondary to methemoglobinemia.[84] The decision whether or not a transfusion is necessary depends on the amount of blood lost and the time scale over which this loss has occurred (acute blood loss is less well tolerated than chronic anemia). In people, a hemoglobin concentration of 7 g/dL has been used as transfusion threshold. Hypovolemia should be corrected first; however, overly aggressive correction is discouraged to avoid rapid increases in blood pressure and disruption of clot formation, particularly in uncontrolled bleeding. Initial hypotensive resuscitation in form of 20-mL/kg boluses of isotonic fluids or 2 to 4 mL/kg of hypertonic saline and maintaining a blood pressure of approximately 60 mm Hg has been recommended until control of bleeding has been achieved.[85] However, prolonged hypotension or failure to correct hypovolemia can also have deleterious effects, and achieving a balance can be challenging. Hydroxyethyl starch is best avoided due to its hypocoagulatory effects.[86] Commonly used clinicopathologic parameters such as heart and respiratory rate, PCV, and total plasma protein concentration are more suitable for monitoring changes in previously stable patients than for primary assessment of acute blood loss. Clinical parameters are strongly influenced by the primary disease process, whereas interpretation of PCV and plasma protein concentration requires knowledge of baseline values for the individual animal. In addition, values will not be reliable under conditions of acute blood loss as PCV and plasma protein concentration remain unchanged until lost volume is replaced by interstitial fluid. Plasma protein concentration often decreases first, and a normal or only minimally increased PCV and normal or low-normal plasma protein concentration in a clinically severely hypovolemic animal should raise suspicion of acute blood loss. Preexisting anemia, hypoproteinemia or hyperproteinemia, and splenic contraction further complicate an accurate assessment. More objective measures of tissue hypoxia and increased oxygen extraction in the periphery such as lactate concentrations, partial pressure of oxygen in the venous blood (PvO_2), and oxygen extraction ratio might be more helpful.

BOX 15.6 Calculation of Oxygen Content in the Blood

$$\text{Arterial oxygen content } (CaO_2) = (Hg \times 1.34 \times SaO_2) + (0.0031 \times PaO_2)$$

Hg: Hemoglobin (g/dL); 1.34 mL of oxygen carried per gram of hemoglobin; Sa_{O_2}: percentage of hemoglobin saturated with oxygen (%); 0.0031: mL of oxygen dissolved per dL blood per mm Hg (mL/dL/mm Hg); Pa_{O_2}: partial pressure of oxygen in arterial blood. A patient with a hemoglobin concentration of 15 g/dL, Sa_{O_2} of 98%, and Pa_{O_2} of 95 mm Hg has a blood oxygen content of 20 mL/dL.

Allogeneic Whole Blood Transfusion

Horses have seven known blood groups (A, C, D, K, P, Q, U), with each group also having multiple factors. This combination of groups and factors gives rise to approximately 400,000 possible blood types. Anti-Aa and anti-Qa antibodies are most frequently associated with transfusion reaction or neonatal isoerythrolysis, and ideally blood donors should be Aa and Qa negative. Blood donors should also not have had foals (hence a preference for geldings) or blood transfusions to decrease chances of the presence of anti-RBC antibodies. Donkey and mule RBCs carry an antigen called *donkey factor* that is presumed to contribute to the high risk of neonatal isoerythrolysis in mule foals.[87] A similar antigen is also suspected on donkey platelets, predisposing them to neonatal alloimmune thrombocytopenia.[88] Donkeys should therefore not be used as blood donors for horses and only receive horse blood free of anti–donkey factor antibodies. Major (donor's RBCs with recipient's plasma) and minor (donor's plasma with recipient's RBCs) crossmatching should include evaluation for agglutination and hemolysis. Investigation of a hemolytic response requires the addition of rabbit complement and is not carried out by every laboratory. Reassuringly, a recent study showed that incompatibility was always indicated by the agglutination test, whether or not hemolysis was also detected.[89] Because of the low natural occurrence and minimal clinical effects of alloantibodies in horses, a first transfusion is frequently carried out without prior crossmatching. However, use of a matched donor significantly prolongs the life span of transfused RBCs. If blood type is matched and crossmatches indicate compatibility, the mean life span of transfused autologous and allogeneic RBCs is approximately 89 days and 39 days, respectively, with a mean posttransfusion allogeneic RBC half-life of 33.5 days.[89,90] The half-life was reduced to 11 days and 4.7 days, respectively, if evidence of minimal or moderate agglutination was noted on the major crossmatch reaction.[89] This suggests that at least a major agglutination crossmatch should be carried out if several blood donors and time for crossmatching are available. The most antigenic group appears to be Aa, with all Aa-negative horses developing antibodies if transfused with Aa-positive RBCs; those persisted for over a year.[89,91] Antibodies against other RBC antigens develop infrequently, can be detected 1 to 154 weeks after transfusion, and might disappear again after 4 weeks.[91]

Blood donors should be free of infectious diseases, most notably equine infectious anemia (EIA). Approximately 20% of the donor's blood volume can be collected (8 L in a 500-kg horse) every 30 days. Volume replacement with isotonic fluids is recommended if greater than 15% of blood volume is collected. The aim is replacing 25% to 50% of the lost blood to ensure adequate oxygen delivery while the patient's RBCs are regenerated. In horses undergoing experimental blood loss of 15 mL/kg once or 15 mL/kg three times on consecutive days, the daily RBC production rate increased from 54,207 ± 7682 cells/μL per day to 83,620 ± 28,740 and 105,840 ± 19,290 cells/μL per day, respectively,[92] which equates to approximately 0.5% to 1.0% increase in PCV per day. The approximate amount of blood needed can be calculated using the equation in Box 15.7. Blood should be collected through a large-bore catheter (10–12 gauge) inserted in a rostral direction (against the direction of flow) into the jugular vein into blood bags containing 3.2% sodium citrate at a blood-to-anticoagulant ratio of 9:1. If blood is to be stored, citrate-phosphate-dextrose with adenine (CPDA) should be used and blood kept at 4°C. Red blood cells from autologous equine blood stored for 28 days had a half-life of 29 days whereas compatible allogeneic blood stored for 24 hours only had a half-life of 20 days.[90,93] Pretransfusion heart and respiratory rates and rectal temperature should be determined before administration of blood through an inline filter beginning slowly at 0.1 mL/kg for the first 15 minutes (3–4 mL/min for a 500-kg horse), checking vital parameters every 5 minutes. If no adverse reactions are observed, the remaining transfusion can be given at 20 mL/kg/hr, checking vital parameters every 10 to 15 minutes until the transfusion is complete. Adverse reactions occur in approximately 16% of horses[94] and can be as high as 87.5% in crossmatch-incompatible transfusions.[89] Hemolytic reactions can occur immediately or up to 24 hours and longer after the transfusion. Other adverse reactions include fever and type I hypersensitivity reactions ranging from urticaria, pruritus, and piloerection to anaphylactic shock.[95] Depending on severity, antihistamines (hydroxyzine 0.5–1.0 mg/kg PO q12h), corticosteroids (dexamethasone 0.1 mg/kg intravenously [IV]), or epinephrine (0.01–0.02 mL/kg of a 1:1000 solution IV; 5–10 mL for a 500-kg horse) may be indicated.

BOX 15.7 Calculation of Blood Volume Needed to Increase PCV by Desired Percentage

Blood transfusion volume (mL) = body weight (kg) × 80 mL/kg × [(desired PCV − actual PCV) /donor PCV]

If the recipient (500 kg) has a PCV of 12% and a PCV of 20% is desired, 9 L of donor blood with a PCV of 35% needs to be administered: 500 kg × 80 mL/kg × [(20 −12)/35] = 9142 mL = approximately 9 L.

Autologous Blood Transfusion

Autologous blood transfusions can be considered if blood loss is anticipated in advance—for example, during a planned surgery or following major body cavity bleeds, most commonly hemoabdomen. The advantage is a longer RBC half-life if blood is collected in advance and minimal risk of transfusion reaction. The half-life of RBCs obtained from a body cavity has not been investigated in horses. In the past, presence or suspicion of neoplasia has been considered a contraindication to autotransfusion; however, in people no effect on metastasis or prognosis has been established. Whether or not an anticoagulant should be added to blood collected from a body cavity is controversial as blood in contact with serosal surfaces for longer than 1 hour becomes defibrinated. Platelets and coagulation factors are also diminished, making an allogeneic transfusion potentially a better option in cases of hypocoagulation. Recommendations in dogs range from addition of 0.05 to 0.14 mL of anticoagulant per milliliter of blood, whereas in horses a blood-to-anticoagulant ratio of 15:1 (0.07 mL/mL of blood) has been suggested.[96,97] In horses, blood from the abdomen can be collected using a 30-Fr Foley catheter or 28-Fr chest drain, inserted into the abdomen through a sterile stab incision and connected to a blood collection bag. Blood can then be directly administered through an intravenous blood administration set.[97,98]

Alternative Oxygen Carriers

Hemoglobin-based oxygen carriers have been investigated experimentally and used clinically in horses (11–16 mL/kg)

and a foal (22 mL/kg).[99-102] The half-life is short (1.3–12 hours depending on size of aggregates), making it only suitable as rescue therapy until a suitable blood donor has been localized.[103] Availability of the product has been limited in recent years.

Platelet Transfusion

Platelet transfusions might be indicated in horses with severe thrombocytopenia or in horses with known functional platelet abnormalities that have sustained a trauma or are to undergo surgery. The risk of hemorrhage is dependent on platelet numbers, platelet function, and the activation state of the plasma-based coagulation and fibrinolysis system and the endothelium and vascular wall. In horses, a platelet count less than 30,000 to 50,000/μL is likely to be associated with clinical signs such as petechiae or epistaxis.[57,104,105] If the platelet count decreases below 10,000/μL, active hemorrhage such as prolonged bleeding after venipuncture or hematoma formation frequently occurs.[105-107] Clinical signs often stabilize or disappear once the platelet counts increase above 15,000 to 30,000/μL, particularly in animals with few or no comorbidities. In horses with thrombocytopenia secondary to neoplastic conditions but rarely also primary immune-mediated thrombocytopenia (ITP), clinical signs can be observed over a much wider range of platelet numbers from 20,000 to 91,000/μL, suggesting that other factors also influence the tendency to bleed. A platelet transfusion is indicated if prolonged bleeding is observed or suspected (e.g., due to a sudden decrease in PCV).[105,108] Most equine hospitals lack the ability for apheresis or to concentrate platelets, but a platelet transfusion can still be prepared if a donor horse is available. In people, six platelet antigen systems have been described to date, contributing to neonatal alloimmune thrombocytopenia, transfusion refractoriness, and transfusion reactions.[109] Potential platelet alloantigens have been identified in equids, but their clinical significance is yet unknown.[88,110] It seems likely that equine platelet antigens function similarly to their human counterparts. Citrate-based anticoagulant solutions are recommended for most platelet preparations, and care should be taken not to underfill containers because platelet function is affected by increasing citrate concentrations.[111] Glass bottles are not suitable for collection as glass surfaces activate platelets. Because of their small size, platelets remain in suspension when RBCs are allowed to settle by gravity as is commonly done for preparation of fresh equine plasma. The harvested plasma therefore contains the vast majority of the blood platelets (and leukocytes) and can be used as a platelet transfusion. If not administered immediately, the platelet-rich plasma should be stored at room temperature (22°C) as exposure to temperatures below 59°F (15°C) induces irreversible changes and significantly decreases platelet function. Platelets can be stored under constant agitation for up to 5 days, but the risk of bacterial contamination is considered to be higher at this storage temperature. In dogs, a fresh whole blood transfusion of 10 mL/kg of body weight is expected to increase the recipient's platelet count by a maximum of 10,000/μL, whereas giving 8×10^{10} platelets per 10 kg of body weight results in a maximum increase of 40,000/μL.[112] No guidelines are available for horses but similar to slightly lower increases might be expected, depending on the donor's platelet count. Administration of drugs that negatively influence platelet function (acetylsalicylic acid, clopidogrel, heparin) is probably contraindicated. Other nonsteroidal antiinflammatory drugs (NSAIDs) such as flunixin meglumine and phenylbutazone have minimal effects on in vitro platelet function, and their benefits might outweigh any theoretical negative effects.[113-115] Whether drugs that enhance platelet function or decrease fibrinolysis such as etamsylate, tranexamic, or ε-aminocaproic acid are beneficial remains to be established.[78,116,117]

Plasma Transfusion

Plasma transfusions might be indicated in horses with low oncotic pressure due to protein-losing diseases, to combat systemic inflammation, in foals with failure of passive transfer or in need of *Rhodococcus equi* prophylaxis, and in horses with coagulopathies to provide essential coagulation factors. Frozen equine plasma is commercially available or can be prepared fresh by gravity sedimentation from collected blood. Fresh frozen plasma refers to plasma separated from blood within 8 hours of collection and stored at −0.4°F (−18°C) for up to 1 year. If plasma is separated after 8 hours or stored for longer than 1 year it is referred to as *frozen plasma*. Following preparation of plasma from whole blood by gravity sedimentation over 48 hours at 41°F (5°C), clotting factors retain 66% to 95% of their activity, and all activities remain within the laboratory reference ranges. Activities for all factors except FX remain within normal ranges after storage at −4°F (−20°C) for 90 days.[118] The amount of plasma needed to increase plasma protein concentrations can be calculated as follows: Volume (L) = ([TPTarget − TPPatient] × 0.05 × Body weight [kg]) ÷ TPDonor. A 500-kg horse will require approximately 4 to 5 L of plasma to increase the plasma protein concentration by 1 g/dL. Administration of plasma follows the same guidelines as blood administration, using a filter and beginning slowly, while closely monitoring for adverse reactions. Reactions to commercial plasma have a reported incidence of 8.7% in foals and 0% in adults, while the incidence with noncommercial plasma is 10%.[118,119] In case of a reaction, the plasma infusion should immediately be discontinued. Depending on severity, treatments as outlined in the discussion on whole blood transfusions can be initiated. Frequently, administration of an NSAID such as flunixin meglumine (1.0 mg/kg IV) is sufficient to control symptoms, and the transfusions can be continued at a very slow rate once clinical signs have returned to pretransfusion values.

Pharmacologic Treatment of Hemostatic Dysfunctions

Pharmacologic options for management of hemostatic dysfunctions can be divided into procoagulants and anticoagulants, fibrinolytic drugs, and those modifying platelet function, with some drugs belonging to more than one class.

Procoagulants and Drugs That Enhance Platelet Function

Drugs promoting hemostasis include the synthetic lysine analogues ε-aminocaproic acid and tranexamic acid. Both act by reversibly blocking the lysine binding sites on plasminogen. This prevents plasmin formation and improves clot stability and longevity by decreasing fibrinolysis. Indications in horses include trauma, rupture of major vessels, and bleeding into body cavities. Anecdotally, tranexamic acid has been used in a horse with Glanzmann's thrombasthenia with apparent clinical

success. The currently suggested doses for ε-aminocaproic acid (3.5 mg/kg/min for 15 minutes followed by 0.25 mg/kg/min IV) in horses target therapeutic plasma concentration required in humans. It has been suggested that in horses, due to a lower fibrinolytic activity and higher threshold for induction of fibrinolysis, 20 times lower plasma concentration might be sufficient to achieve the desired effect.[78] Lower doses have not yet been examined clinically in horses. Pharmacokinetic data for tranexamic acid are not available for horses, but doses of 5 to 25 mg/kg PO q6–12hr or 10 mg/kg IV q 12 h have been suggested. Formaldehyde (10–150 mL of 10% buffered formalin in 1 L of isotonic fluids IV or 0.37% formaldehyde) has long been used in desperate situations. It has been suggested to enhance primary hemostasis in humans and goats by activating platelets, but experimental investigations showed no effect on coagulation parameters in horses. Use of higher doses (1 L of 0.74%–1.0% formaldehyde in lactated Ringer's solution IV) results in adverse reactions including muscle tremor, tachycardia, tachypnea, nasal and ocular discharge, restlessness, and mild colic signs. Signs resolve quickly after discontinuation of the infusion.[120] Administration of higher concentrations has been associated with hemolysis. Etamsylate, an antihemorrhagic, nonthrombotic drug promoting platelet adhesion by increasing P-selectin expression on platelets, has no effects on bleeding time in healthy horses when used at 12.5 mg/kg.[116] Tramadol, used at concentrations expected to be achieved in vivo, enhances adenosine diphosphate (ADP)-induced aggregation of platelets from fed, but not fasted, horses in vitro. The potential proaggregatory effects of tramadol need to be investigated further in the experimental and clinical setting.[121] Conjugated estrogens (0.05–0.1 mg/kg IV q 12–24 h) have been used in mares with postpartum hemorrhage.[122]

Anticoagulants and Drugs That Decrease Platelet Function

Treatment with either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) might prevent clot formation in the microcirculation. LMWH has fewer side effects than UFH, and significantly fewer jugular vein changes were noted in horses treated with LMWH compared with UFH in surgical colic cases, suggesting that it may have superior anticoagulant properties.[123] In a study investigating fibrin deposition in the microcirculation of horses with gastrointestinal disease, fibrin deposits were detected in 40% of nonsurviving horses overall but only in 1 out of 8 horses (12.5%) receiving 50 IU/kg LMWH once daily for 4 days.[124] Viscoelastic testing appears to be the best way for monitoring LMWH therapy: aPTT has a weak correlation with anti–factor Xa activity whereas thromboelastography and Sonoclot variables are significantly, albeit modestly, correlated with anti–factor Xa activity. Absolute and relative changes in the thromboelastography parameter *R* (ΔR and $\%\Delta R$) predict anti–factor Xa activity below the suggested thromboprophylactic level best, at least in healthy horses.[125] LMWH and UFH also have a dose-dependent inhibitory effect on equine myeloperoxidase (MPO) in vitro and are able to suppress MPO cell-bound activity in equine digital endothelial cells, indicating some antiinflammatory properties.[126] Recommended doses for enoxaparin are 0.35 mg/kg SC q 24 h and for dalteparin 50 IU/kg SC q 24 h in adult horses and 100 IU/kg SC q 24 h in neonatal foals.[123,127,128] Fresh or fresh frozen plasma with or without the addition of heparin (anecdotally 1500 U/L UFH) could also be used in hypercoagulation as antithrombin concentrations, one of the main anticoagulants, are frequently low in horses with systemic inflammation. Acetylsalicylic acid (aspirin) has been suggested as an antithrombotic agent in the horse because of its ability to decrease platelet aggregation in human patients and thromboxane production in horses.[54,129] However, it has a short plasma half-life in horses, and, in contrast to human platelets, the effects are largely limited to collagen-induced aggregation, which probably only plays a very minor role in inflammation-associated coagulopathies.[129,130] Nevertheless, aspirin reduces endotoxin-induced thromboxane formation and digital hypoperfusion in the experimental setting.[131] Doses vary widely, and 10 to 100 mg/kg PO q 24–48 h have been suggested. In people, up to 30% to 40% of patients do not respond to aspirin (nonresponders).[132] A nonresponse to aspirin has also been identified in several equine studies and appears to be of a similar magnitude.[54,129] Clopidogrel is an antiplatelet medication acting by inhibiting platelet ADP receptors. Experimentally, it inhibits some platelet functions in horses, such as ADP- and arachidonic acid–induced aggregation, within hours of administration of a loading dose (6.5 mg/kg PO once). This effect can be maintained on a low dose (1.4 mg/kg PO q 24 h) for the duration of treatment, and platelet function is restored within 6 days after the end of therapy.[47] Other measures of platelet function and activation, such as collagen-induced aggregation, P-selectin expression, platelet-neutrophil aggregate formation, modified thromboelastography, and flow cytometric tests for platelet fibrinogen binding and phosphatidylserine externalization, are not affected by clopidogrel (2 mg/kg PO q 24 h) or aspirin (5 mg/kg PO q 24 h).[133,134] Following experimental lipopolysaccharide (LPS) administration, decreases in ADP-induced platelet aggregation and decreased heart rates were noted in clopidogrel-treated horses. However, no significant differences in other hematologic and hemostatic variables were detected, making its clinical efficacy in equine inflammatory conditions doubtful.[50] In addition, no response to clopridogrel was seen in two of six horses in one study, potentially suggesting a nonresponder state similar to that observed with aspirin. Other treatments, including pentoxifylline and aminophylline, have no or only very limited effects on equine platelet function, and coagulation and clinical effects on the coagulation system still need to be established.[121]

Fibrinolytic Drugs

Recombinant human tissue plasminogen activator (rt-PA) has been used locally to remove intraocular and intrapleural fibrin accumulations in horses with uveitis and pleuropneumonia, respectively.[135,136] Anecdotally, it has also been used systemically in cases of suspected neonatal stroke (0.2 mg/kg IV once within 4 hours of onset of neurologic signs; Kevin Corley, personal communication, November 2015). Pharmacokinetics in adult horses have been described; 1 mg/kg (given as an infusion with a final concentration of 2 mg/mL IV over 30 minutes) is well tolerated and results in plasma levels similar to those required for thrombolysis in people.[137] Regional treatment of digital artery thrombosis with urokinase (approximately 80 U/kg/min for 60 minutes) was unsuccessfully attempted in a foal.[138] In contrast to rt-PA and urokinase, streptokinase, a human plasminogen activating enzyme secreted by streptococci species, shows experimentally minimal ability to activate equine plasminogen.[139] However, regional bolus

administration of 100 mL of streptokinase solution (7500 U/mL in 0.9% sodium chloride) reportedly showed thrombolytic effects but could not recanalize experimentally thrombosed jugular veins in horses permanently.[140] In a different report, infiltration of a thrombosed jugular vein using 6 mL of 125,000 U/mL streptokinase in 0.9% sodium chloride q 24 h for 3 days resulted in some thrombolysis.[141]

DISORDERS ASSOCIATED WITH ACUTE AND CHRONIC BLOOD LOSS

Acute blood loss from an external wound or injury is usually obvious and does not represent a diagnostic challenge, whereas bleeding into body cavities or the source of epistaxis can be more difficult to establish. With a combination of thorough history taking, physical and laboratory examination, and diagnostic imaging, particularly ultrasonography and endoscopy, the origin of blood loss can usually be identified. Box 15.2 lists possible causes of blood loss in the horse.

Hemoperitoneum

Hemoperitoneum (or hemoabdomen) is one of the more common causes of acute internal blood loss. It affects horses of all ages and breeds, but the risk might be increased in Thoroughbreds, Arabians, and mature and aged horses.[142] The clinical signs can resemble acute colic as horses are markedly hypovolemic with high heart and respiratory rates, pale to pale-pink mucous membranes with prolonged capillary refill times, and profuse sweating, and often show signs of pain or muscle fasciculations. Depression, abdominal distention, reluctance to move, weakness, and ataxia can also be noted. Laboratory analysis might show anemia and hypoproteinemia, but with acute blood loss changes in both parameters can be mild or absent or even increased.[142] The white cell count can be low, normal, or high, depending on severity and underlying cause, and the platelet count is frequently reduced. Lactate concentrations are commonly high, corresponding with a low venous oxygen tension (Pvo_2). Coagulation parameters remain initially unchanged unless the bleeding is caused by an underlying coagulopathy. Definitive diagnosis can be established ultrasonographically if free echoic fluid with the characteristic swirling appearance is observed. Abdominocentesis is rarely necessary to confirm the presence of blood and is discouraged because of the risk of introduction of an infection. The recovered fluid will resemble blood with a high PCV and protein concentration. Causes of hemoperitoneum include splenic, hepatic, or mesenteric vessel rupture, neoplasm, intraabdominal abscess with necrosis of associated vessels, complications of pregnancy, foaling and castration, pelvic injury or fracture, and coagulopathies.[142-144] Ovarian hematomas associated with hemoperitoneum have been identified in miniature horse breeds.[99,142,145] Hemoperitoneum has been observed after treatment with warfarin, and sudden death secondary to hemoperitoneum has been reported in racehorses in which traces of rodenticide anticoagulants were found.[146] Abdominal ultrasonography might help in establishing the underlying cause, but in 25% to 42% of cases an etiology cannot be established.[143,144] Management is primarily aimed at stabilizing the patient hemodynamically, using intravenous fluids and transfusions as necessary. Close observation is necessary as tissue hypoxia can predispose horses to arrhythmias, especially ventricular tachycardia, and secondary colic.[84] Celiotomy might be necessary if bleeding from a mesenteric vessel in conjunction with a surgical intestinal lesion is suspected or if bleeding continues or recurs despite medical management. In one retrospective study, the source of the bleeding could be addressed during surgery in 19% of surgically managed cases whereas in 35% the origin of the hemorrhage could not be identified.[143] Overall, 42% of surgically managed horses survived in this study and 57% in another.[142,143] Laparoscopy is probably of limited value as visualization is obscured by blood.[143] The overall prognosis for hemoperitoneum depends on the severity of hemorrhage and the underlying cause, with survival rates of 39% to 75% having been reported.[144]

Hemothorax

In foals, rib fractures are a common cause of hemothorax whereas in adult horses trauma, neoplasm, iatrogenic injury during pulmonary wedge and core biopsies, and strenuous treadmill exercise have been reported.[147-150]

Intestinal Blood Loss

Severe and potentially fatal postoperative blood loss has been reported following enterotomies and intestinal anastomoses in horses. Signs are compatible with acute blood loss, and melena might be noticed within 72 hours of surgery. Continued bleeding necessitated a second celiotomy in two of six horses in one study.[151] Mild anemia with PCV between 25% and 30% and occasionally positive occult fecal blood tests have been reported in horses with right dorsal colitis.[152,153] Fecal occult blood tests are not well validated in horses. It has previously been stated that 1 to 2 L of blood loss into the intestinal tract is necessary to produce a positive fecal occult blood test. Newer tests seem to be more sensitive. A guaiac-based fecal occult blood test reportedly has a sensitivity and specificity of 64% and 100% (any gastrointestinal ulceration), 70% and 53% (colonic ulceration), and 64% and 52% (gastric ulceration).[154] A carbonic anhydrase I (present in erythrocytes but not intestinal tract) and II isoenzyme enzyme-linked immunosorbent assay (ELISA) with the ability to detect 50 µg of hemoglobin per gram of feces has been investigated as a marker for occult blood in horses. An increase in carbonic anhydrase I was noted after oral administration of 200 mL of blood to healthy horses, but variation was large.[155] A new lateral flow immunoassay (SUCCEED Equine Fecal Blood Test) detecting equine hemoglobin at concentrations of 5 to 10 µg/g of feces is being investigated. At this sensitivity, rectal examinations cause positive results for 24 to 48 hours. A negative result probably rules out significant hemorrhage, but the clinical relevance of a positive result still needs to be established (N. Kerbyson, personal communication, November 2015).

Pulmonary Hemorrhage

Pulmonary hemorrhage secondary to *Leptospira* spp. infection causing mild to moderate anemia has been documented in foals and proposed in adult horses.[156,157] Clinical observations suggest that in light-breed horses with lone atrial fibrillation epistaxis is occasionally encountered, whereas draft horses with atrial fibrillation can experience severe exercise-induced pulmonary hemorrhage. Small thrombi forming or lodging in the pulmonary vasculature are unlikely to be noticed clinically. In contrast, massive pulmonary thromboembolism, defined as affecting more than two thirds of a lung and occluding greater than 50% of the pulmonary vasculature, or embolization in both lungs can lead rapidly to right-sided heart failure,

cardiogenic shock, reflex bronchoconstriction, and death. Clinical signs include tachypnea, epistaxis, and hemoptysis, and sudden death occurred in three of six horses.[158] Inhalation of foreign bodies can also be associated with recurrent pulmonary hemorrhage. Causes of epistaxis in the horse are listed in Box 15.2.

Phenylephrine-Associated Bleeding

Epistaxis, hemoptysis, hemothorax, hemoperitoneum, hemopericardium, and rupture of the middle uterine artery in a nonpregnant mare have been reported in five horses after phenylephrine administration for correction of nephrosplenic entrapment. Four of the five horses died, and a 64-times higher risk for phenylephrine-associated bleeding was identified for horses ≥15 years old. A mild and transient episode of epistaxis or hemoptysis immediately after administration of phenylephrine might be a prodromal sign. Horses should be monitored closely for internal hemorrhage, and preparation for a blood transfusion should be initiated immediately if any evidence of bleeding is noted, even if the amount of external blood loss initially appears inconsequential.[159]

Periparturient Blood Loss in Mares

Periparturient bleeding from the major uterine arteries or, less frequently, the ovarian, external iliac, vaginal, and adrenal arteries is a well-recognized cause of urogenital blood loss in mares. Arterial injuries occur most frequently in the proximal uterine artery, and atrophy of smooth muscle cells with fibrosis of the arterial wall is a predisposing factor.[160] Less common locations of blood loss include the placental attachment of the uterus, intrauterine or vaginal bleeding, and intramural hematomas. Hemorrhage is estimated to affect 2% to 3% of broodmares and comprises 17% of postpartum emergencies admitted to a hospital.[122,161] It also accounts for close to 40% of postpartum deaths in mares.[162] Bleeding often develops within 48 hours after foaling, but antepartum bleeding as early as during the fifth month of gestation and up to 4 months after parturition is possible.[122] Predisposing factors include older age, multiparity, and dystocia, but bleeding can occur in mares of any age or parity.[161] Mares present with signs of abdominal pain and hypovolemic shock; vaginal bleeding, ataxia, and recumbency are also reported. Laboratory evaluation is consistent with recent blood loss with a median PCV of 39% and a total protein concentration of 5.0 g/L in one report. Hemorrhage is often suspected based on history and presenting clinical and laboratory investigations, but confirmation of the diagnosis can be difficult. Transrectal palpation and rectal and transabdominal ultrasonography are considered most useful. In the past, clinicians have advised against rectal examinations due to fears of disrupting hematoma formation and worsening bleeding. A recent study found no association between rectal palpation and outcome, but a cautious approach is still advisable.[122] Prompt medical treatment aimed at cardiovascular stabilization with or without the addition of procoagulant drugs and pain management is of utmost priority. Antiinflammatory drugs and, depending on the case, antimicrobials might also be indicated. Most clinicians advise against surgical exploration if bleeding is confirmed, as surgical access to the urogenital vasculature is limited and the anesthetic risk high. However, intestinal lesions are also common in postpartum mares and concurrent presence of intestinal lesions and hemorrhage has been reported.[122,161] Abdominal exploration might become necessary if this is suspected.[122] Complications are encountered in close to 80% of surviving mares and include fever, retained fetal membranes, laminitis, arrhythmias, colic, colitis, and peritonitis in decreasing frequency. Reports on outcome vary widely, with survival ranging from 0% to 88%.[142] Anecdotally, bleeding into the broad ligament, in contrast to development of hemoperitoneum, has been suggested to be more favorable, and two reports have associated antepartum hemorrhage with a poor prognosis with no survivors.[142,143] This was not confirmed in the largest study done so far; the only factor associated with prognosis was tachycardia at admission and during hospitalization and vaginal hemorrhage, although only two mares presented with this condition.[122] Little is known about effects of periparturient hemorrhage on future reproductive performance. Arnold et al.[122] reported that 49% of surviving mares for which follow-up information was available produced at least one foal.

Renal and Urethral Hemorrhage

Renal hemorrhage severe enough to cause anemia has been reported in horses with pyelonephritis (four of seven horses were Arabians), idiopathic hematuria, and renal pseudoaneurysm in an Arabian colt.[163-165] In male horses, bleeding from urethral rents might occur, but this is rarely associated with significant blood loss. These conditions are discussed in detail in Chapter 14.

DISORDERS ASSOCIATED WITH HEMOLYSIS

Hemolysis refers to the intravascular or extravascular destruction of erythrocytes. Destruction can be immune mediated or secondary to infection or oxidative injury; a list of differential diagnoses for hemolysis in the horse is given in Box 15.4.

Immune-Mediated Hemolysis

Immune-Mediated Hemolytic Anemia

Immune-mediated hemolytic anemia (IMHA) can occur independently or concurrently with immune-mediated thrombocytopenia (IMT) in horses. Both conditions are caused by antibody-mediated destruction of RBCs and platelets, respectively. Antibodies can be directed against the unaltered cell surface (primary or idiopathic; examples include autoimmune disease, neonatal isoerythrolysis, or hemolysis following transfusion) or against a surface that has been antigenically altered by secondary causes.[166] Secondary IMHA in horses has been reported with neoplasia, *Rhodococcus equi* infection in foals, clostridial myositis, abscess formation, and drug administration.[166-172] Penicillin is one of the best established drugs causing IMHA in horses, and low titers of anti–penicillin antibodies of the immunoglobulin M (IgM) class were present in 77% of horses tested.[173-176] Hemolysis is thought to be associated with IgG antibodies and high titers of IgG antibody that agglutinated penicillin-coated equine RBCs are found in horses with penicillin-induced IMHA. Titers of anti–penicillin antibody decrease rapidly once penicillin is discontinued but remain detectable for months. Anemia might not reoccur after challenge with penicillin, but usage should probably still be discouraged in horses that have experienced penicillin-induced IMHA.[175] Trimethoprim-sulfamethoxazole has also been suspected as a cause of IMHA.[177] In addition, IMHA and increased hepatic enzyme activities were reported in four foals

17 to 20 days after initiation of treatment with doxycycline and rifampin against *R. equi* infection.[178] Treatment for IMHA includes immunosuppressive drugs, such as dexamethasone (0.1–0.2 mg/kg IV q 24 h), prednisolone (1.0–2.0 mg/kg IV, IM, or PO q 24 h), or, in refractory cases, azathioprine (3.0 mg/kg PO q 24 h).

Neonatal Isoerythrolysis

Neonatal isoerythrolysis is a common IMHA caused by an incompatibility between the mare's plasma and foal's RBCs. It is discussed in detail in Chapter 20.

Alloimmune Neonatal Neutropenia

Immune-mediated destruction of neutrophils in foals has been reported in a Thoroughbred and an Arabian colt.[24,179] A further report describes three foals of varying breeds, but sepsis was not convincingly ruled out.[180] The pathophysiology is presumed to be similar to neonatal isoerythrolysis, but the disease appears to be comparatively rare. Administration of a single injection of G-CSF (3.5–6 μg/kg SC) resulted in a considerable increase in peripheral neutrophils, but one foal required repeated injections every 3 to 4 days (four injections total). Both foals recovered completely.[24,179]

Evans Syndrome

Evans syndrome is characterized by the simultaneous presence of IMHA, thrombocytopenia, and/or leukopenia in the absence of a known underlying cause. Specific cross-reacting autoantibodies (usually IgG) are directed against red cells and platelets causing simultaneous or sequential development of hemolytic anemia and thrombocytopenia. Evans syndrome is rare, and few cases have been published in horses.[181-184]

Infectious Causes of Hemolysis

Equine Infectious Anemia

The equine infectious anemia (EIA) virus is a blood-borne RNA virus with a worldwide distribution belonging to the family of Retroviridae, genus *Lentivirus*. The disease is also referred to as *swamp fever*. The virus utilizes two enzymes, reverse transcriptase and integrase, to generate and integrate viral DNA into the host-cell genome. Virus replication occurs in monocytes, dendritic cells, tissue macrophages, and endothelial cells.[185] Once infected, horses remain lifelong carriers. Transmission occurs naturally via blood-feeding insects, particularly Tabanidae (horse and deer flies) or blood-contaminated instruments or needles. In an outbreak in Ireland, introduction of the virus into the country was traced to contaminated blood products, and aerosol spread between horses in a barn was strongly suspected.[186,187] Three distinct phases (acute, chronic, and inapparent) have been described, and clinical signs differ depending on the stage of the disease. Following an incubation period of 1 to 4 weeks, once viral burdens reach a threshold level, proinflammatory cytokine release initiates clinical signs. Fever and thrombocytopenia are considered to be the most reliable clinical indicators but are far from pathognomonic for EIA. Interleukin-6 (IL-6) and tumor necrosis factor (TNF) are thought to induce a febrile response. Thrombocytopenia is caused by a combination of immune-mediated destruction; suppression of megakaryocyte colony growth in the bone marrow by TNF and transforming growth factor–β (TGF-β); and possibly increased release of platelet agonists including thrombin, plasmin, and serotonin, triggering platelet aggregation.[188] Platelet function is also compromised, contributing to bleeding tendencies.[189] Intravascular and extravascular hemolysis and downregulation of erythropoiesis by TNF cause anemia. Depression, weakness, hemorrhage, peripheral edema, and weight loss are commonly seen in the chronic form but can also accompany the acute stages.[186,187] Inapparent carriers show no clinical signs, but periods of stress, immunosuppression, or treatment with corticosteroids can induce recrudescence. Hyperglobulinemia and glomerulonephritis can also be present. Diagnosis currently still relies on serology. Because EIA virus causes lifelong infection, presence of antibodies implies presence of virus, not mere exposure. The agar gel immunodiffusion assay (AGID; Coggins test) is still the most widely used test and detects antibodies against the major core protein p26. Other U.S. Department of Agriculture (USDA)-approved tests include several ELISA kits. In comparison to the AGID, ELISAs are considered to be less prone to human error and more sensitive, albeit less specific. A positive ELISA result therefore needs to be confirmed by AGID. Immunoblot is not an approved test but can be used to resolve equivocal results. One ELISA kit also detects glycoprotein 45 (gp45), and immunoblot can detect proteins gp90 and gp45 in addition to p26. Time from infection to seroconversion varies widely depending on viral dose, virus strain, and test used (between 21 and 180 days).[190] Proviral DNA integrated into the host cell can be detected by polymerase chain reaction (PCR) whereas viral RNA can be detected by reverse transcriptase–PCR (RT-PCR). The high variability of the viral genome and the lack of a confirmed conserved genomic region suitable as a primer binding sequence have hampered widespread implementation of this technique. In an outbreak in Ireland specific PCR and RT-PCR assays were developed for the particular strain of the virus present. These assays detected viral RNA and DNA in all tissues, nasal secretions, and genital swabs, and RNA was also detected in saliva. Viral DNA was detected in the plasma of all the seropositive horses tested, but viral RNA was detected in only 52% of these samples.[191,192]

Piroplasmosis/Babesiosis

Equine piroplasmosis or babesiosis is a tick-borne disease caused by two intra–erythrocytic apicomplexan equine-specific hemoprotozoa, *Babesia caballi* and *Theileria* (previously *Babesia*) *equi.* Although North America and Canada are considered free of the disease, the parasites and their tick vectors are endemic to most countries with tropical, subtropical, and occasional temperate climates. Central and South America, Cuba, at least parts of Mexico, Southern Europe, Asia, and Africa are considered endemic, and outbreaks in the United States have repeatedly been reported.[193,194] The fact that horses recovering from the disease remain inapparent carriers and a potential reservoir strongly affects management in endemic and nonendemic or free areas. Carrier state in untreated horses is lifelong for *T. equi* whereas *B. caballi* can be cleared from the horse. Virulence varies strongly depending on geographic location and potentially genetic differences.[195] Natural vectors for *T. equi* in North America include *Dermacentor variabilis, Boophilus (Rhiphicephalus) microplus,* and *Amblyomma cajennense* and *Dermacentor nitens* and *Dermacentor albipictus* for *B. caballi.* Transmission from dam to fetus has also been reported. Iatrogenic transmission via contaminated blood transfusions, but also tick transmission, caused recent outbreaks in Florida and southern Texas.[194,196] The life cycle of *B. caballi* and *T. equi*

differs. *B. caballi* directly invades erythrocytes whereas the first developmental stage of *T. equi* takes place in leukocytes. Intravascular and extravascular hemolysis ensues, frequently accompanied by thrombocytopenia and coagulopathies, leading to clinical signs of lethargy, fever, petechiae, edema, and hemoglobinuria. Colic, respiratory, and neurologic signs are also possible. Signs are milder for *B. caballi* compared with *T. equi* and develop within 10 to 30 days and 12 to 19 days after infestation by ticks, respectively. After intravenous transmission signs can occur as early as 5 days. Surviving horses show few or mild signs, but recrudescence under stressful conditions has been reported for *T. equi.* Horses infected with *T. equi* develop antibodies against equi merozoite antigens (EMAs) within 7 to 11 days; the immune response against *B. caballi*'s apical merozoite protein rhoptry (RAP) 1 is less well understood. Carrier status protects horses from reinfection, but coinfection with both parasites is possible. Diagnosis on microscopic identification of parasites by careful inspection of a blood smear is feasible in the acute stages. In the chronic stages, parasitemia is too low to be detected visually, making serologic tests, including complement fixation (CFT), indirect immunofluorescence assay (IFA), Western blot, and competitive enzyme-linked immunosorbent assay (cELISA), the diagnostic methods of choice. Complement fixation is very specific but not sensitive enough for detection of nonsymptomatic carriers, and cross-reactivity between both parasites occurs. The most sensitive test for chronic infection is the EMA 1 and 2 detecting cELISA; a cELISA detecting RAP-1 is also available. Immunofluorescence tests are often used as adjunctive test. A Western blot and nested PCR test are under development. Treatment during the acute stages is largely supportive. As the carrier status protects against active disease and reinfection, elimination of the parasite is not desired in endemic areas. In nonendemic areas, elimination of all transmission risk to other animals is paramount, with options being enrollment in an approved treatment program, lifelong quarantine, or euthanasia. Imidocarb diproprionate (2.2–4.4 mg/kg IM once or q 72 h for 4 doses) is the treatment of choice for parasite clearance. The anticholinesterase activity of the drug can be counteracted by preadministration of *n*-butylscopolamine (0.3 mg/kg IV). Injection site reactions are common, and increased sensitivity to side effects and even fatal reactions have been reported in donkeys and mules. Clearance of the parasite is confirmed by serial negative PCR or serology results. Control strategies vary among countries but frequently involve negative serologic results. A positive test result in the United States must be reported to federal and state regulatory officials.

Leptospirosis

Leptospirosis is a zoonosis with worldwide distribution. The seroprevalence and predominant serovar differ widely among geographic locations. Infection can be asymptomatic or present with mild and nonspecific signs. Apart from *Leptospira*-associated equine recurrent uveitis, abortion, stillbirth or premature birth, neonatal disease, and renal failure represent the most common clinical manifestations.[197-199] Anemia, epistaxis, petechiae, and ecchymotic hemorrhages were described in 4- to 12-week-old foals with *Leptospira* infection, but this appears to be a very rare manifestation.[200] Pulmonary hemorrhage in foals and adult horses is possible.[156,157]

Hemotropic *Mycoplasma* Infection

Hemotropic mycoplasmas or hemoplasmas (previously classified as Eperythrozoon and *Haemobartonella*) are bacteria that parasitize erythrocytes of a variety of species including pigs, cattle, cats, and dogs.[201,202] Infection in horses has been reported in Germany and potentially in Nigeria, but very little is known about the clinical significance and presentation of infection.[203] In other species, hemolytic anemia, ill thrift, and infertility are observed. Reported clinical signs in horses include weight loss, depression, and mild anemia, which might be worse in animals less than 1 year old.[201,202] Microscopic identification lacks sensitivity and specificity, making PCR the preferred diagnostic tool. Treatment in other species includes tetracyclines or fluoroquinolones.

Hemolysis Secondary to Oxidative Injury

Equine erythrocytes are more susceptible to oxidant-induced damage than human RBCs and experience a greater extent of methemoglobin formation, alteration of aggregation, and reduction of cellular deformability when exposed to superoxide anions.[204] Oxidants can damage erythrocytes by oxidizing the heme iron in hemoglobin from Fe^{2+} to Fe^{3+}, forming methemoglobin. Horses reduce methemoglobin via a lactate-dependent pathway, which is less efficient than the glucose-dependent pathway of methemoglobin reduction favored by erythrocytes of other species.[205,206] This, in addition to the greater methemoglobin formation, is thought to make horses more vulnerable to clinically relevant methemoglobinemia. Other forms of damage include oxidation of sulfhydryl groups in the globin part of hemoglobin leading to protein denaturation and Heinz body formation. The oxidation of sulfhydryl groups and unsaturated lipids also compromises membrane integrity, leading to increased fragility and early removal from the circulation. Reduced glutathione (GSH) is a potent antioxidant system; however, horses have a decreased GSH reduction rate, compared with humans and other species, again leaving them more predisposed to oxidative injury.[207,208]

Red Maple Leaf Toxicity

Ingestion of wilted red maple *(Acer rubrum)* leaves is a well-documented cause of hemolytic anemia and methemoglobinemia in horses. Similarly, wilted leaves of silver and sugar maple (*Acer saccharinum* and *Acer saccharum,* respectively) are also potentially toxic to horses.[209]

Most cases occur in summer and fall, often following adverse weather conditions. Fresh leaves are not toxic to ponies in the experimental setting, but dried leaves fed at a dose of 3.0 g/kg produce fatal toxicities. Leaves remained toxic for at least 30 days, and horses consuming more than 1.5 g/kg body weight are likely to show symptoms.[210] Depending on the time of year, either signs of hemolysis (before mid-September) or methemoglobinemia (after mid-September) might predominate. Gallic acid and a yet unidentified compound are thought to be responsible for the oxidative damage observed.[209] In addition, gallotannins and gallic acid present in leaves can be metabolized by *Klebsiella pneumonia* and *Enterobacter cloacae,* found in the equine ileum, to form pyrogallol, an even more potent oxidizing agent.[211] Clinical signs relate to tissue hypoxia caused by anemia and methemoglobinemia, including lethargy, tachycardia, tachypnea, fever, hemoglobinuria, and frequently also colic, renal insufficiency, and laminitis. Mucous membranes are often discolored, descriptions ranging from muddy to cyanotic and chocolate brown, while the sclera might be icteric. Laboratory analysis confirms anemia, renal compromise, and often systemic inflammation with leukopenia or leukocytosis. Total methemoglobin concentrations

of 0 to 4.08 g/dL (normal, less than 1.77 g/dL) have been measured, corresponding to up to 44% of the total hemoglobin concentration (normal, less than 3%).[212] Treatment is largely supportive including intravenous fluids, pain management, and blood transfusions if necessary. Mineral oil to reduce further toxin absorption and ascorbic acid as an antioxidant have been advocated. Vitamin C (30–50 mg/kg IV q 12 h diluted in 0.9% sodium chloride continued until hemolysis ceases; oral supplementation will take longer to achieve tissue levels) has been used in the clinical setting.[213,214] Experimentally, daily feeding of 9000 mg/day for 12 weeks significantly reduced hemolysis and methemoglobin formation in response to leaf extract, whereas 8000 U/kg α-tocopherol daily only reduced hemolysis.[215] The use of sodium bicarbonate to prevent pigment nephropathy is controversial in humans.[216] The aim of urinary alkalinization is to prevent tubular obstruction caused by cast formation by reducing the conversion of hemoglobin to methemoglobin, to reduce uptake of hemoglobin by tubular cells, lowering production of hydroxyl radicals and promoting free radical scavenging.[217] Corticosteroids are often used in an attempt to decrease RBC destruction. Complications are frequent; 44% of horses develop fever, 43% experience colic, 32% laminitis, 7% DIC, and 6% cecal impactions. The prognosis is guarded, with 41% of horses surviving in the largest retrospective study. Factors associated with mortality included lack of pyrexia on admission and corticosteroid administration whereas PCV and percentage methemoglobin were not associated with outcome.[212]

Pistacia Leaf Toxicity

Hemolytic anemia, methemoglobinemia, renal failure, and death have been reported in horses following ingestion of *Pistacia* leaves. Although not native to North America, *Pistacia atlantica, P. terebinthus,* and *P. chinensis* are found in several states ranging from the Southwest to the Southeast of the United States. *Pistacia* leaves contain gallic acid, and the clinical picture closely resembles red maple leaf intoxication.[218,219]

Garlic and Onion Toxicity

Experimental feeding at the maximal voluntary intake of garlic (0.25 g/kg PO q 12 h) resulted in mild anemia (decrease of PCV from mean 36% to 28%) with increased Heinz body formation. Excessive sweating was also observed. Signs were noted 4 days after initiation of feeding, and recovery from anemia was completed within 5 weeks after discontinuation of garlic feeding.[220] Hemolytic anemia has also been reported following natural and experimental ingestion of wild and domestic onions (*Allium canadense* and *Allium cepa*). Wild onions are only eaten by horses once all other forage has been consumed and in the experimental setting, feeding of 1.0 to 1.8 kg/day (2–4 lb/day) is necessary to create clinical and laboratory signs. Horses recover completely once ingestion stops.[221]

Envenomation by Bees or Snakes

Envenomation by snakebites and, less commonly, multiple bee stings can cause intravascular and extravascular hemolysis, thrombocytopenia, and profound coagulopathies with increased bleeding and/or excessive thrombosis, often compatible with DIC.[222,223] Effects are largely mediated by enzymatic and nonenzymatic proteins and proteases contained in the venom. Apart from antivenom, treatment is largely supportive.

Other Causes of Hemolysis

Hemolytic-Uremic Syndrome

Hemolytic uremic syndrome in humans is described as a form of thrombotic microangiopathy. The clinical picture is characterized by the combination of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. It is often caused by exposure to Shiga-toxin producing *E. coli,* but noninfectious causes including inherited mutations of complement-regulating proteins, and disorders associated with endothelial damage also occur in people. Subsequent activation of the inflammatory system and particularly the complement system causes vascular wall thickening and deposition of fibrin- and thrombocyte-rich thrombi in the microcirculation. This compromises end-organ function, often affecting kidney or brain. Schistocyte formation and hemolysis ensue when erythrocytes are exposed to increased shear stress across vessels that are partially obstructed by fibrin. Thrombocytopenia is caused by platelet consumption caused by excessive coagulation and immune-mediated destruction.[224] Coagulation times are usually normal, differentiating the condition from DIC. In horses, the syndrome has been described in a postpartum mare with metritis and in three horses without apparent underlying cause. Clinical signs are compatible with systemic inflammation, hemolysis, thrombocytopenia, and renal failure including hemoglobinuria, epistaxis, oliguria or anuria, peripheral edema, lymphadenopathy, and altered neurologic status. Supportive treatment has been unsuccessful in all reported cases.[225-227]

Congenital Enzyme Defect

Severe deficiency of glucose-6-phosphate dehydrogenase caused persistent hemolytic anemia, hyperbilirubinemia, and Howell-Jolly bodies in a Saddlebred colt and his dam.[5] Persistent methemoglobinemia, eccentrocytosis, and pyknocytosis in a Spanish mustang mare were attributed to deficiency of flavin adenine dinucleotide (FAD) resulting from a defect in erythrocyte riboflavin metabolism.[6] Horses with erythrocyte FAD deficiency have both eccentrocytosis (attributable to severe deficiency in glutathione reductase activity) and methemoglobinemia (attributable to cytochrome b5 reductase deficiency); the dual enzyme deficiency occurs because FAD is a required cofactor for both enzymes.[228] A familial decrease in erythrocyte glutathione reductase and glutathione was suspected in a trotter mare and her dam, causing poor performance secondary to persistent methemoglobinemia and hemolytic anemia.[229]

Miscellaneous

A rare fulminant intravascular hemolytic syndrome with prominent hemoglobinuria has been reported in horses in the end stages of liver failure but also in one horse that subsequently recovered from an acute hepatic insult. This horse also had markedly decreased erythrocyte reduced glutathione (GSH) content and showed marked neutrophil hypersegmentation. The exact mechanism of hemolysis is unknown, but increased concentrations of bile acids and their salts have been suspected to play a role.[29,230] Hemolytic anemia has repeatedly occurred in horses receiving predominantly high, but also recommended (20–30 g/500-kg horse), doses of phenothiazine. Horses are reportedly one of the most sensitive species, and signs are seen 6 to 13 days after administration. Heinz bodies are a frequent finding.[231,232] Experimental oral administration of L-tryptophan causes hemolysis in ponies.[233] Selenosis

in horses predominantly manifests with skin and hoof abnormalities and neurologic symptoms, but hemolytic anemia following acute poisoning has been described in other species.

DISORDERS OF HEMOSTASIS

Hemostatic dysfunction can be caused by abnormalities affecting the vascular wall, most commonly in the form of a vasculitis, changes in platelet numbers or function, and abnormalities of the plasma-based coagulation system.

Vasculitis

Vasculitis is a pathologic term describing inflammation and often necrosis of blood vessels. It can be immune mediated, secondary to severe systemic inflammation, due to direct effects of infectious organisms on the vascular endothelium, or a combination of these mechanisms. Infectious organisms frequently implicated in the development of vasculitis include *Streptococcus equi* subsp. *equi* (strangles), equine viral arteritis virus, *Anaplasma phagocytophila, Corynebacterium pseudotuberculosis,* and, occasionally, equine infectious anemia virus.

Immune-Mediated Vasculitis/Purpura Hemorrhagica

The term *purpura hemorrhagica* refers to immune-mediated vasculitis that is frequently, but not always, secondary to *S. equi* subsp. *equi* infection. The process can be triggered by multiple factors, affect one or multiple organ systems, and vary dramatically in severity. In horses, immune-mediated vasculitis is usually secondary to another disease process, frequently exposure to an infectious organism. *S. equi* subsp. *equi* infection is the best described cause of immune-mediated vasculitis. Signs are first noticed within 2 to 4 weeks of infection, but development within 2 to 6 days has occurred in previously vaccinated horses.[234] It can also be observed secondary to other viral or respiratory tract infections, drug administration, open wounds, abscesses, vaccination against strangles, and neoplasia. Severe and frequently fatal immune-mediated vasculitis occurred in Miniature Horses following vaccination with a live spore anthrax vaccine.[235] Not infrequently, the underlying cause cannot be detected.[236] The disease can affect horses of any age ranging from 6 months to 20 years and older.[236] It is presumed that deposition of complexes of antigen and immunoglobulins in the walls of the capillaries and other small blood vessels leads to a type III hypersensitivity reaction. In horses with previous *S. equi* exposure, an association between the development of vasculitis and IgA antibodies has been suggested. IgA titers to both M-like protein and culture supernatant proteins were higher in horses with vasculitis than in either horses recently infected with *S. equi* or with no known history of exposure.[237] In addition, immune complexes with IgA and M-like proteins have been found in the sera of horses with vasculitis.[238] Microscopically, lesions are characterized by a leukocytoclastic vasculitis.[238,239] The increase in vascular permeability accounts for clinical signs of edema, petechial or ecchymotic hemorrhages in mucosal and subcutaneous tissues, serum oozing, and, less commonly, epistaxis. Edema is often well demarcated and most commonly noticed in the limbs followed by ventrum and head. Fever, tachycardia, tachypnea, anorexia, and depression are frequently present. Depending on organ systems involved, colic, reluctance to move, lameness, or neurologic signs might be noted.[240] Renal dysfunction and glomerulonephritis, multiple small intestinal intussusceptions, and muscular infarcts have also been reported secondary to presumed strangle-induced vasculitis.[241,242] Laboratory abnormalities include leukopenia or leukocytosis, anemia, thrombocytopenia, hypergammaglobulinemia, increased acute phase proteins, and increased muscle enzyme activities. A presumptive diagnosis can usually be made based on history, clinical picture, and exclusion of other causes of edema and/or hemorrhagic diasthesis. Very high SeM-specific antibody titers (greater than 1:12,800) are strongly suggestive of vasculitis secondary to strangles vaccination or exposure.[243] Skin biopsies can confirm the presence of a leukocytoclastic or nonleukocytoclastic vasculitis, but histologic evidence can be absent in horses previously treated with corticosteroids.[236] Secondary complications such as laminitis and thrombophlebitis are not uncommon; rarely, massive and even fatal hemorrhage can occur.[236] Treatment is based on removal of the underlying cause if possible and immunosuppressive treatment with corticosteroids (dexamethasone 0.1–0.2 mg/kg IV, IM, or PO q 24 h or prednisolone 0.5–1.0 mg/kg PO q 24 h). Azathioprine (3.0 mg/kg PO q 24 h) has also been used anecdotally. Supportive care, including hydrotherapy, limb bandages, mild exercise, and intravenous fluids, can be helpful. Once clinical signs are improving, the corticosteroid dose should gradually be reduced while monitoring for recurrence. Relapse of symptoms is common if doses are decreased or treatment is discontinued too quickly. Almost 50% of horses in one study required treatment for more than 21 days. The prognosis is good if no severe complications arise, with 92% of horses surviving in the largest case series.[236]

Anaplasma phagocytophila

Anaplasma phagocytophila (formerly *Ehrlichia equi*) is an obligate intracellular infection infecting neutrophils and occasionally eosinophils of several mammalian species.[244] The organism is transmitted by *Ixodes* ticks but *Dermacentor* spp., *Rhipicephalus* spp., *Hyalomma* spp., and *Haemaphysalis* spp. are also potential vectors. The organism has a worldwide distribution in the Northern Hemisphere where vectors are endemic. Recent studies suggest that multiple strains with different host tropism and pathogenicity might be circulating in wild and domestic animal populations. The organism is transmitted transstadially, not transovarially, making a mammalian reservoir necessary to infect the ticks.[245] Disease transmission from ticks to a sensitive host occurs within 2 to 36 hours while the tick is feeding on the surface of the host's body. Coinfections with other tick-borne pathogens can occur. The incubation period is approximately 10 days, and signs vary from subclinical infection to high fever (100°–106.9°F [37.7°–41.6°C] with a mean of 104.2°F [40.1°C] in one study), depression, inappetence, petechiae, reluctance to move, and distal limb edema.[244,246] On occasion, high fever with or without peripheral edema can be the only clinical sign, with horses being remarkably bright and otherwise normal. Neurologic signs including ataxia and recumbency have been reported infrequently. Neurologic signs resolved quickly following therapy with oxytetracycline in two cases but euthanasia was necessary in another that failed to improve despite treatment.[247-249] Some reports state that younger (less than 4 years old) animals experience milder clinical signs, but this has not been confirmed by others.[244,249,250] Laboratory abnormalities include often marked thrombocytopenia (less than 10,000–100,000/μL; presumed to be at least partially due

to bone marrow suppression), mild anemia (PCV usually greater than 20%), lymphopenia, and neutropenia followed by rebound leukocytosis.[246,249] The disease can be self-limiting if left untreated, and clinical signs usually last from 7 to 14 days. Relapse has been reported.[244] Diagnosis relies on identification of granulocytic inclusions, either morulae or initial bodies, in Wright-Giemsa– or hematoxylin and eosin–stained blood smears. Inclusion bodies in peripheral blood are best observed 3 to 5 days after the onset of fever and might be present in 30% to 50% of neutrophils; much lower percentages (1%–8%) of infected cells were noted in other studies.[244,251] In the later stages or in horses that have received treatment, molecular techniques such as PCR or serology demonstrating increasing antibody titers might be necessary to confirm the infection. The organism is very sensitive to treatment with oxytetracycline (7 mg/kg IV q 24 h for 3–7 days), and clinical improvement is often seen within 12 to 24 hours.[244] Doxycycline (10 mg/kg PO q 12 h) has also been used. Treatment with dexamethasone in experimental infection resulted in a higher total bacterial burden but ameliorated clinical signs slightly. Hematologic parameters did not differ between dexamethasone-treated and untreated animals.[252] The prognosis is excellent with 96% of horses recovering in one study. In this study, no abortions were observed in pregnant mares and no horse developed laminitis. Fatal cases have been reported, secondary to injury due to ataxia, failure to recover from the neurologic form, secondary infections, widespread hemorrhage, and vasculitis and thrombosis consistent with DIC.[244,249,253]

Equine Viral Arteritis

Equine viral arteritis (EVA) virus is covered in detail in Chapters 8 and 19, but infection remains an important differential diagnosis for horses displaying signs of vasculitis. Equine viral arteritis virus, a single-stranded RNA virus belonging to the family Arteriviridae, genus *Arterivirus,* has a worldwide distribution and can affect horses of all ages. Transmission occurs by venereal or respiratory routes. Semen from persistently infected stallions and aerosol from contaminated body fluids are major sources of infection. The incubation period ranges between 2 and 14 days. Most infections are clinically silent or result only in an increased temperature, but severe infection can be seen in foals and immunologically compromised animals. The virus targets mononuclear cells and endothelial cells, and clinical signs are mostly associated with vasculitis. Distinct EVA isolates vary markedly in the severity of the clinical signs they induce, and it is generally thought that the American strains are more virulent than the European isolates. Depending on the strain, extensive necrosis of small muscular arteries in multiple tissues, mild infection of terminal capillaries, or little endothelial involvement at all can occur.[254-256] Fever and leukopenia are most consistently reported, but depression, periorbital and supraorbital or dependent edema, conjunctivitis, lacrimation, petechiae, respiratory signs, and colic or diarrhea might ensue. In pregnant mares, abortion between the third and tenth months or birth of compromised foals if infected in late pregnancy is common.[257]

Platelet Disorders

Thrombocytopenia

Box 15.5 contains a list of differential diagnoses for thrombocytopenia. Thrombocytopenia secondary to decreased production in the bone marrow is discussed under disorders of hematopoiesis.

Thrombocytopenia Secondary to Systemic Disease. The most common cause of thrombocytopenia in hospitalized horses is increased utilization or destruction of platelets secondary to a primary infectious, inflammatory, or neoplastic disease process. Thrombocytopenia is also common in strangulating/ischemic gastrointestinal disorders, and platelet counts as low as 4000/μL might be encountered. Accompanying laboratory changes reflect systemic inflammation and include increased PCV, percentage of band neutrophils, leukopenia or leukocytosis, and decreased plasma protein concentrations.[58] Thrombocytopenia is often present in horses with subclinical or clinical DIC and is regarded as a sensitive indicator for the presence of DIC.[258] An association between thrombocytopenia and a poor prognosis has been demonstrated in hospitalized horses in general and in horses with colon torsion.[58,259] Thrombocytopenia is also frequently found in other diseases such as equine infectious anemia (a combined effect of immune-medicated destruction and decreased platelet production), *A. phagocytophilum* infection (presumed to be at least partially due to bone marrow suppression), babesiosis/piroplasmosis (mechanisms not well understood), neoplasia (presumed to be immune mediated), and snake or bee envenomation (a combined effect of sequestration, increased aggregation, and consumption, frequently associated with DIC).[222,260] Thrombocytopenia secondary to sepsis and also cystic hematomas has been reported in neonatal foals.[261,262]

Immune-Mediated Thrombocytopenia. Immune-mediated thrombocytopenia (IMT) is an uncommon disease in adult horses, and the diagnosis is based on exclusion of other causes. The inappropriate immune response can be directed against intrinsic platelet antigens, antigens/substances bound to the platelet membrane, or infectious agents. The term *immune thrombocytopenic purpura* is occasionally used if autoantibodies are responsible for platelet destruction. However, clinically the differentiation between a primary and secondary immune response can be difficult. Administration of penicillin and trimethoprim-sulfadoxine has been suspected to be involved in IMT, but this was not confirmed.[105] The suspicion of IMT is strengthened by detection of antibodies on the platelet membrane or on megakaryocytes by flow cytometry.[105] Platelet dysfunction secondary to bound antibodies can also be expected and bleeding can occur at relatively high platelet concentrations if function is severely affected. Clinically, a positive response to immunosuppressive therapy including dexamethasone (0.1–0.2 mg/kg IV q 24 h) or, in refractory cases, azathioprine (3.0 mg/kg PO q 24 h) is also strongly supportive.[263,264] Vincristine at the recommended human dose (1.4 mg/m^2 IV every 7–10 days; 8.4 mg in 500 mL of 0.9% NaCl) was used in a stallion with relapsing thrombocytopenia. Treatment appeared to be successful and was tolerated well with the exception of development of symmetric alopecia on head, neck, and inner thighs.[263] Relapse is not uncommon, and horses should be carefully monitored for recurrence of signs.[265,266]

Neonatal Alloimmune Thrombocytopenia. Immune-mediated destruction of platelets following ingestion of preformed antiplatelet antibodies with the colostrum has been reported in horse and mule foals and is suspected to be more common in the latter. Thrombocytopenia can be the sole abnormality or can occur in conjunction with neonatal isoerythrolysis. In mule foals, platelet destruction is suspected to be IgG mediated, targeting the collagen receptor (GP Ia IIa). Platelet dysfunction secondary to adhering antibodies has been described in these foals, highlighting the fact that

risk of bleeding is not only determined by platelet numbers but also by their functional state. A syndrome of ulcerative dermatitis, thrombocytopenia, and neutropenia has been described in six neonatal foals of varying breeds. Platelet counts varied between 0 and 30,000/μL, and four of six foals had petechiae or ecchymosis. All foals recovered completely, and thrombocytopenia appeared to be responsive to corticosteroid administration, suggesting an immune-mediated component. Although the exact etiology was not established, acquisition of antibodies or other factors via the colostrum was strongly suspected.[23]

Changes in Platelet Function

Glanzmann's Thrombasthenia. Glanzmann's thrombasthenia is a quantitative deficiency or a qualitative defect of the platelet fibrinogen receptor (also referred to as GP IIb/IIIa, $\alpha_{IIb}\beta_3$ integrin, or CD41/61). It is an autosomal recessive disorder, and the genetic defect has been localized to the gene encoding GP IIb (also referred to as α_{IIb}).[61,267,268] The disease has been reported in Quarter Horses, Thoroughbreds, Standardbreds, Warmbloods, and a Peruvian Paso.[62,269,270] Prolonged or recurrent epistaxis appears to be the most common clinical sign whereas the presence of petechiae or ecchymosis is more variable. The diagnosis is based on confirmation of a normal platelet count and coagulation profile including PT, aPTT, and, ideally, measurement of von Willebrand factor activity. Other simple clinical tests such as demonstration of a prolonged bleeding time and prolonged clot retraction are suggestive of a platelet function disorder but not diagnostic. Equally, an abnormal viscoelastic coagulation profile indicating reduced clot strength or a decreased aggregation response confirms a functional platelet defect. A definitive diagnosis requires demonstration of reduced CD41/61 expression on the platelet surface using flow cytometry. Alternatively, absent fibrinogen binding in response to stimulation might also be used. No effective treatment is available, but anecdotally, administration of tranexamic acid has decreased the frequency and severity of epistaxis in one horse. To the author's knowledge the pharmacokinetics of tranexamic acid have not been established in horses, but dosages of 10 g/kg IV q 12 h or 5 to 25 mg/kg PO q 6–12 h have been suggested. Theoretically, ε-aminocaproic acid might achieve a similar effect.

Atypical Equine Thrombasthenia/Reduced Fibrinogen Binding in Thoroughbreds. A second genetic platelet defect associated with reduced fibrinogen binding but distinct from Glanzmann's thrombasthenia has recently been identified in Thoroughbreds. Affected horses have normal fibrinogen binding in response to adenosine diphosphate (ADP) and normal levels of fibrinogen receptor (also referred to as CD41/61, GP IIb/IIIa, or $\alpha_{IIb}\beta_3$ integrin) but show decreased fibrinogen binding in response to thrombin. The defect is thought to cause an inability to generate thrombin following activation due to defective secretion of factor V from α-granules.[63] A prevalence of 0.7% in the Thoroughbred population has been estimated based on examination of 444 animals. Clinically, severe bleeding after trauma and prolonged template bleeding times were noted.[63-65,271] Laboratory abnormalities include a prolonged lag time in the aggregation response to collagen and thrombin. In contrast to horses with Glanzmann's thrombasthenia, normal quantities of CD41/61 can be demonstrated by flow cytometry and fibrinogen binding after stimulation with thrombin is only decreased, not absent.

Altered Platelet Function with Systemic Disease. The contribution of platelets to many disease processes has only been recognized in recent years. With the increasing awareness and more widespread availability of platelet function assays and viscoelastography, changes in platelet function during systemic disease are gradually elucidated. Platelet activation has been demonstrated in horses with airway inflammation, laminitis, and strangulating intestinal lesions and in response to equine herpesvirus 1.[49,52,60,272,273] Evidence linking decreased platelet function to decreased survival is beginning to accumulate in horses with acute gastrointestinal disease and in critically ill foals.[274,275] Further work is needed to delineate the contribution of platelets to the pathophysiology of equine diseases.

Abnormalities of the Plasma-Based Coagulation System

Hereditary Disorders

Von Willebrand's Disease. Von Willebrand's disease is caused by quantitative and/or qualitative defects of von Willebrand factor (vWF), a multimeric glycoprotein synthesized by endothelial cells and megakaryocytes. The factor mediates platelet adhesion/aggregation and stabilizes factor VIII (FVIII) in the circulation.[276] Without vWF platelet binding to collagen is reduced and primary hemostasis disrupted. vWF is an acute phase reactant, and natural fluctuations occur. Concentrations in mares increase from midgestation to reach near-maximal activity at parturition,[277] and changes in vWF and FVIII have been observed in strenuously exercised Standardbreds and Thoroughbreds.[278] Von Willebrand's disease can be congenital or acquired. In humans, six different types (1, 3, 2A, 2B, 2M, 2N) of the congenital form are currently differentiated. A partial quantitative defect defines type 1, whereas type 3 is characterized by the nearly complete absence of vWF in plasma and platelets. Type 2 consists of malfunctioning vWF and is divided into four subtypes. Types 2A and 2B are marked by the absence of high-molecular-weight vWF multimers, but in type 2B there is also an increased affinity of vWF for its platelet receptor, the glycoprotein Ib alpha.[276] Von Willebrand's disease is rare in horses, and so far only congenital deficiencies corresponding to human type 2A and one suspected case of type 1 have been reported.[279-281] Clinical signs in horses include bleeding from mucosal surfaces, epistaxis, hematoma formation, hemarthrosis, and prolonged bleeding from venipuncture sites, from injuries, or after surgery. Diagnosis can be achieved by measuring plasma von Willebrand antigen using an ELISA; results will be low or normal, depending on type. vWF ristocetin cofactor assay measures the ability of a patient's plasma to agglutinate platelets in the presence of the antibiotic ristocetin. Platelet agglutination caused by ristocetin can occur only in the presence of vWF multimers. Lack of agglutination indicates absence of the factor or its receptor, and the test aids in identifying types 2A, B, and M. Measurement of FVIII coagulant activity is also helpful and is necessary to identify type 2N. Without the stabilizing effect of vWF, FVIII is cleared rapidly and concentrations are frequently low. Bleeding times are prolonged whereas platelet counts and PT are normal; aPTT can be normal or increased if FVIII is deficient. Treatment in other species includes plasma transfusion or administration of desmopressin (deamino-8-D-arginine vasopressin [DDAVP]). Desmopressin is a synthetic vasopressin analog that increases release of vWF

from endothelial cells, particularly effective in type 1 cases.[282] In healthy dogs, both vWF and factor VIII are increased following DDAVP administration. In humans, the acquired form is associated with hypothyroidism, myeloproliferative and lymphoproliferative disorders, and autoimmune disorders but also with ciprofloxacin or hetastarch administration.[283]

Prekallikrein Deficiency. Prekallikrein deficiency has been diagnosed in a family of Belgian and Miniature Horses.[284,285] Excessive hemorrhage and a markedly prolonged aPTT prompted further investigation, which led to the diagnosis. It was presumed that animals with low prekallikrein activity might be homozygous for prekallikrein deficiency, whereas close relatives with values closer to normal might be heterozygous for the genetic defect.

Hemophilia A (Factor VIII Deficiency). Hereditary hemophilia A has been reported in Thoroughbreds, Standardbreds, and Quarter Horses and in an Arabian colt with multiple clotting defects including factors VIII, IX, and X and to a lesser degree factor VII and prothrombin.[286-291] The disorder is transmitted via an X-linked recessive trait, and affected colts generally suffer repeated bleeding episodes. A milder form has also been described in a Thoroughbred gelding that was 3 years old at diagnosis and had been successfully castrated.[292]

Acuired Disorders

Acquired Hemophilia A. A 23-year-old Thoroughbred was diagnosed with temporary, acquired plasma factor VIII inhibitory activity following evaluation of a coagulopathy causing hemoperitoneum, hematomas, and anemia. Coagulation testing revealed prolonged aPTT, factor VIII deficiency, and increased factor VIII inhibitory antibody activity using a Bethesda assay.[293] Treatment included transfusions and immunosuppressive therapy (dexamethasone and azathioprine). Factor VIII deficiency and signs of coagulopathy resolved, and the inhibitory antibody titer decreased. The mare remained healthy with no relapse for at least 1 year after treatment.[80]

Vitamin K Deficiency Bleeding in Neonatal Foals. Vitamin K deficiency bleeding (VKDB) has been suspected in a 4-week-old colt presenting with hemarthrosis, spontaneous bleeding from the gingiva, blood covering of feces, and hematoma formation after venipuncture. Activities of vitamin K–dependent factors II, VII, IX, and X was markedly decreased and improved following supplementation with vitamin K_1 (2 mg/kg SC q 12 h for 2 days, then 0.5 mg/kg PO q 12 h for 17 days). Subcutaneous administration is preferred to avoid hematoma formation and risk of anaphylactoid reactions after intramuscular and intravascular administration, respectively.[72] The condition was also suspected in a half-sibling colt but not further investigated. It is therefore unclear whether the condition was inherited or acquired. VKDB, formerly known as hemorrhagic disease of the newborn, is a bleeding disorder caused by low levels of vitamin K–dependent clotting factors. Acquired VKDB is more common in human neonates, which are very susceptible to vitamin K deficiency because of low stores at birth, poor placental transfer, low concentrations in milk, and lack of production by an immature intestinal microflora. Routine prophylactic administration of vitamin K has made human VKDB a rarity in most developed countries. Heritable combined deficiency of vitamin K–dependent clotting factors has been described in cats and sheep; in the sheep, defective γ-glutamyl carboxylase activity was identified as a cause of the deficiency.[294]

Warfarin/Anticoagulant Rodenticide Toxicity. Horses might come in contact with anticoagulant rodenticides when used for treatment of thrombotic diseases or by accidental consumption. Available anticoagulant rodenticides include first-generation (warfarin, diphacinone, and chlorophacinone) and second-generation (brodifacoum, bromadiolone, difethialone, and difenacoum) compounds. Second-generation rodenticides are more toxic and have longer half-lives than the first generation. Both act by competitively inhibiting vitamin K_1 epoxide reductase activity in the liver, thereby reducing circulating vitamin K–dependent clotting factors II, VII, IX, and X.[146] Brodifacoum is highly potent in horses with an LD_{50} of 0.1 to 0.2 mg/kg (approximately 1–2 kg of commercial bait per horse) and administration of 0.125 mg/kg resulting in clinical and laboratory signs of a coagulopathy.[295,296] Onset of clinical signs occurs within 3 to 7 days after exposure but might be as quick as 24 hours.[297] The half-life in horses is 1.22 days, but normalization of coagulation times can take up to 23 days following ingestion of 0.125 mg/kg.[296] Diagnosis relies on history of exposure, evidence of bleeding, and abnormal coagulation times. Large-vessel hemorrhagic diathesis including hematoma formation, epistaxis, body cavity bleeds, pulmonary hemorrhages, hyphema, melena, and hemopericardium are typical, but ecchymosis and subcutaneous bleeding have also been reported.[146,297] Due to the shorter half-life of factor VII, PT becomes prolonged before other coagulation times, and a prolongation of PT by 1.5 to 2 times has been used as target for warfarin treatment. Platelet counts are at least initially normal, differentiating the condition from DIC. Toxicity can be enhanced by hepatic dysfunction, concurrent administration of highly protein-bound substances that displace protein-bound toxin (e.g., phenylbutazone), and hypoproteinemia.[298] Corticosteroids and thyroxin also increase toxicity by increasing receptor affinity and coagulation factor catabolism. Increased microsomal enzyme activity in the liver, as it might be induced by rifampin, chloramphenicol, or barbiturates, decreases the half-life of warfarin in the blood. Specific treatment includes administration of vitamin K_1 (phylloquinone, phytomenadione, or phytonadione; 0.5–1.0 mg/kg SC q 6 h until normalization of PT). Because of the long half-life of second-generation rodenticides, treatment might need to be continued for weeks. Vitamin K_3 (menadione), a synthetic vitamin, should not be used as injection because of nephrotoxicity observed after intravenous and intramuscular administration in horses.[299,300] Poor therapeutic action when given orally had also been reported previously, but recent studies showed increased menaquinone-4 concentrations after oral supplementation with menadione but not with supplementation of vitamin K_2 (menaquinone).[301,302] A plasma or whole blood transfusion can provide coagulation factors and RBCs in cases of life-threatening hemorrhage. Activated charcoal might be indicated to decrease toxin absorption if ingestion has been recent.

Mouldy Sweet Clover (*Melilotus* spp.) Toxicity. Coumarins naturally present in sweet clover are converted into dicoumarol when hay or silage is improperly cured and mold develops. Disease is more common in cattle but can occur in horses.[303] Clinical signs, diagnosis, and treatment are as described for warfarin toxicity.

Liver Disease. Circulating coagulation factors are largely produced in the liver, and coagulopathies are not infrequently observed in horses with significant hepatic compromise.

Abnormalities of at least one coagulation parameter were observed in 58% of horses with confirmed hepatic disease, but complications secondary to percutaneous liver biopsy were only observed in four horses, and complications did not correlate with coagulation profile abnormalities.[304]

Disseminated Intravascular Coagulation. Activation of the coagulation cascade accompanies a variety of primary inflammatory, traumatic, infectious, or neoplastic conditions. In most cases, this does not lead to clinical complications because of the body's ability to maintain a balance between procoagulant and anticoagulant tendencies. However, if activation of the coagulation cascade is strong, as it frequently occurs in critically ill patients with systemic inflammation, homeostasis of the coagulation system is disrupted and the resulting coagulopathy can significantly increase morbidity and mortality. In severe cases, the coagulopathy may progress to its most extreme form, *disseminated intravascular coagulation* (DIC).[305] Subclinical or clinical DIC is characterized by dysregulation of coagulation, anticoagulation, and fibrinolysis.[306] Classically, DIC manifests first as a hypercoagulable state due to increased levels of circulating inflammatory mediators and tissue factor (TF). TF, in conjunction with coagulation factor VII (FVIIa), is now thought to be the main activator of the coagulation cascade. In addition, all major anticoagulant pathways (antithrombin III [AT], the protein C system, and TF pathway inhibitor) are substantially impaired as a result of consumption, degradation, and impaired synthesis. Moreover, the fibrinolytic system is widely suppressed during the time of maximal activation of coagulation, supporting fibrin deposition and clot formation in the microcirculation, which can lead to significant end-organ compromise.[305] Ongoing activation of the coagulation cascade causes thrombocytopenia, functional platelet abnormalities, and depletion of coagulation factors, resulting ultimately in profound hypocoagulation.[307,308] The progression of DIC is not always linear, and hypercoagulable and hypocoagulable tendencies may coexist in the same patient. Coagulopathies and subclinical or clinical DIC are common in critically ill equine patients, particularly those with gastrointestinal disease.[124,258,259,309-313] Deposition of fibrin in the microvasculature can lead to the development of ischemia, multiorgan failure, and death. In one study, fibrin deposits were detected in the kidneys, lung, or liver in 40% of nonsurviving horses with enteritis, peritonitis, and ischemic colic and in 88% of septic equine neonates, with the lungs being the most frequently affected organ.[124,312] Organ failure, although statistically not associated with fibrin deposition, occurred in 56% of these foals, and respiratory failure accounted for 25% of the organ failure cases.[312] These findings suggest that intravascular fibrin formation is a significant complication in critically ill equine patients. No universally recognized definition of DIC in horses exists, but abnormalities in ≥3 coagulation tests with (clinical DIC) or without (subclinical DIC) clinical signs is widely accepted.[258,313,314] Unfortunately, comparison of antemortem-identified coagulopathies and postmortem diagnosis of DIC found no association between the two, suggesting that our ability to identify horses in need of treatment is severely limited by the tests available.[315] Treatment apart from eliminating the underlying cause is further complicated by the coexistence of hypocoagulation and hypercoagulation. In humans, LMWH is recommended until bleeding is observed, and platelet transfusions are used to maintain a minimal platelet count between 20,000 and 50,000/μL.[316] Some investigations suggest that heparin and LMWH might be beneficial in horses with coagulopathies, but controlled studies have not been carried out.[124,314] The prognosis for horses with subclinical DIC is fair to guarded with survival of 58% reported, and studies including horses with clinical DIC stated a positive outcome in 0% to 34% of cases.[258,314,317]

Thrombosis. Thrombosis arises from an imbalance of procoagulant and anticoagulant mediators in the body when the balance is tipped toward a hypercoagulant environment. In systemically ill horses, both large-vessel and microvascular thrombi occur frequently and contribute to patient morbidity and mortality. Microvascular thrombi are discussed in the section describing DIC. Thrombosis describes the formation of a blood clot within the vascular lumen whereas thrombophlebitis includes inflammation of the surrounding vein, which in most cases triggered thrombus formation. If catheterization is the cause, infection of the thrombus might also be present.

LARGE-VESSEL THROMBOSIS. In most cases, large-vessel thrombosis is, at least partially, an iatrogenic problem caused by venipuncture or catheterization of a large peripheral vein. Cases of spontaneous thrombosis without external injury to the vessel is reported in foals and adult horses as a sequela of sepsis and systemic coagulopathies, discussed in the previous section. Thrombi involved cutaneous and limb arteries, the caudal vena cava, the right ventricle, the aortoiliac artery, and the pulmonary vasculature.[158,318-323] Aortoiliac thrombosis is discussed in Chapters 9 and 10. The incidence of catheter-associated thrombophlebitis overall varies greatly. In a mixed surgical and medical hospital population, clinically noticeable thrombophlebitis was seen in 1% of catheters placed and between 8.3% and 18% in horses undergoing emergency colic surgery.[324-326] The etiology of catheter-associated thrombophlebitis is multifactorial and influenced by the skill and technique of the person inserting and managing the catheter, substances administered through the catheter, and also to a large degree by patient-related factors. Solutions with a high osmolarity, such as parenteral nutrition, are irritating to the vascular wall and should be diluted or given in conjunction with intravascular fluids. Intravenous dextrose up to 25% (1330 mOsmol/L) has been given to horses without development of phlebitis.[327] Catheter-related complications associated with administration of parenteral nutrition range between 6% and 15% in foals and at least 5% in adult horses.[328-330] Any primary disease process associated with systemic inflammation and clinical or subclinical coagulopathies greatly increases the risk. The odds for developing catheter-associated thrombophlebitis are 4, 5, 18, and 68 times higher for horses with large intestinal disease, hypoproteinemia, endotoxemia, and salmonellosis, respectively, compared to horses without these findings.[331] Another study found a 4-times increased risk in horses with a rectal temperature of ≥101.3°F (38.5°C) when the catheter was inserted.[326] Clinical signs of thrombophlebitis can develop within minutes to hours of venipuncture or catheterization. Early signs include a subtle thickening of the vascular wall and perivenous tissues that can be appreciated by palpation or with ultrasonography. A change in the filling pattern when the vein is occluded distally to the catheter tip might also be appreciated. Changes often start at the catheter insertion site or at the distal end of the catheter where the tip of the catheter can come in contact with the endothelium.[326] Once a thrombus has formed noncompressible distention of the vessel with a palpable cord in the vascular lumen can be noted. Engorgement of the facial veins or unilateral face swelling can be observed with complete occlusion but is more common with

bilateral obstruction. Ultrasonography is more sensitive than palpation in early and subtle cases and also useful to establish the extent of the lesion. Ultrasonographically, the normal vein wall measures less than 1 mm in diameter, and thrombi appear as irregularly shaped hyperechoic luminal masses with homogeneous echogenicity or pockets of hyperechogenicity and hypoechogenicity.[326,332] In a recent study, clinically evident thrombophlebitis was noticed in 1% of cases but ultrasonographically, thickening of the vein wall was noted in 28% (31.4% of first catheter inserted) and thrombi were found in 16% of catheterized veins.[326] The intravenous catheter should be removed immediately once any sign of beginning phlebitis is observed; however, not infrequently thrombosis progresses even after removal. Ideally, the tip and a piece of catheter adjacent to the hub should be cultured and the vein not used until signs have completely resided.[326] Local increase in temperature and signs of pain on palpation might suggest the presence of an infection. In cases of infectious thrombophlebitis, aspiration of a fluid pocket under ultrasonographic guidance and culture of the retrieved material can aid in choosing the appropriate antimicrobials. Bilateral jugular vein thrombosis can lead to severe head and nasal passage edema and respiratory obstruction, necessitating tracheostomy. For this reason, many clinicians prefer not to use the opposite jugular vein for catheterization if one is already thrombosed. Lateral thoracic and cephalic veins are obvious alternatives, but on occasion, even the saphenous vein has been used. Other potentially serious complications include endocarditis and laryngeal hemiplegia.[333,334] Treatment includes hot-packing and application of topical DMSO, nonsteroidal, and/or heparin-containing gels. Systemic use of aspirin (10–100 mg/kg PO q 24–48 h), LMWH (enoxaparin 0.35 mg/kg SC q 24 h or dalteparin 50 IU/kg SC q 24 h for adult horses; dalteparin 100 IU/kg SC q 24 h for neonatal foals), or unfractionated heparin (30–40 U/kg SC q 12 h) will not resolve an existing thrombus but potentially prevent further growth. None of these treatments has been critically evaluated. Fibrinolytic therapies have occasionally been attempted. Regional bolus administration of 100 mL of streptokinase solution (7500 U/mL in 0.9% sodium chloride) reportedly showed thrombolytic effects but could not recanalize experimentally thrombosed jugular veins in horses permanently.[140] In a different report, infiltration of a thrombosed jugular vein using 6 mL of 125,000 U/mL streptokinase in 0.9% sodium chloride q 24 h for 3 days resulted in some thrombolysis.[141] Fibrinolysis of infected thrombi should probably not be attempted. In cases of septic thrombophlebitis antimicrobial treatment is indicated, ideally guided by culture and sensitivity. A variety of bacteria including *S. equi* subsp. *zooepidemicus, E. coli, Actinobacillus, Pseudomonas, Enterobacter,* and *Enterococcus* have been cultured.[326,333] Surgical treatment has been used in refractory cases. This can involve thrombectomy with removal of infected clot material, drainage of abscess material, and subsequent lavage. A prerequisite for the procedure is complete occlusion of the vein proximal and distal to the planned incision site. The prognosis depends greatly on the underlying etiology. The prognosis for horses that develop large vessel thrombosis secondary to sepsis is heavily influenced by the primary disease process and at best guarded to poor. Horses with catheter-associated thrombosis, in contrast, have a good prognosis for life and future performance. Over time, the vein frequently recanalizes with complete resolution of the thrombus. Athletic performance of non–racing horses was not affected whereas racing Standardbreds had a decreased chance of return to racing; however, in those that raced again, performance was not impaired.[335] Prevention of thrombophlebitis is difficult and not well investigated. Meticulous attention to sterility and atraumatic placement, use of the smallest gauge catheter possible, and removal as soon as intravascular access is no longer needed will help to reduce the risk. Any irritation of a catheter-containing vein is best avoided, and many hospitals have a policy of not taking blood from catheterized veins. Administration of NSAIDs through a catheter reportedly decreases the risk of thrombophlebitis but should only be used if clinically indicated.[326]

DISORDERS OF HEMATOPOIESIS

Nonregenerative/Hypoproliferative Anemia

Iron Deficiency Anemia

Iron deficiency is a rare cause of anemia in horses as iron intake via forage is usually more than sufficient to meet their needs. However, with chronic external blood loss deficiencies might be noted. Nonregenerative anemia secondary to iron deficiency is characterized by low serum ferritin concentration, decreased stainable iron stores in the bone marrow, decreased plasma transferrin saturation, normal or increased total iron binding capacity (TIBC), hypochromic erythrocytes (decreased MCHC), and microcytosis (decreased MCV) (see Box 15.1 and Table 15.3).[14] Treatment should be aimed at addressing the underlying problem. If iron supplementation is necessary, oral products containing ferrous sulfate (2.0 mg/kg) are probably safest. Alternatively, iron cacodylate (2.0 mg/kg can be given slowly IV, but caution is warranted as anaphylactic reactions might occur). Fatalities after intramuscular administration of iron dextran have been reported.[336] Iron deficiency anemia develops more rapidly in foals as forage consumption is absent or low during the neonatal period. Deficiency can develop as a result of limited storage of body iron, increased iron demand during growth, and low concentration of iron in the dam's milk.[14] Iron deficiency anemia has been reported in foals without access to pasture and in a hospitalized foal treated for presumed sepsis.[14,337] In the latter foal, microcytosis and hypochromasia were absent, but multiple erythrocyte abnormalities including echinocytosis, keratocytosis, and schistocytosis were noted.[14] In neonatal foals with confirmed iron deficiency anemia, treatment with ferric sulfate (2 mg/kg PO q 12 h) should be implemented. Prophylactic iron supplementation in healthy foals is not recommended, and acute and fatal hepatic failure has been reported following administration of products containing ferric fumarate (16 mg/kg of ferric fumarate) to neonatal foals within the first 48 hours of life. Foals with low levels of vitamin E and selenium are thought to be at particular risk. Colostrum provides large quantities of vitamin E, making administration before colostrum intake particularly perilous.[8,338-340]

Anemia Secondary to Other Deficiencies

Persistent anemia and leukopenia secondary to gelatinous bone marrow transformation, also referred to as *serous atrophy,* occurs in humans and other veterinary species and has also been reported in a malnourished pony.[341] Reasons are poorly understood, but malnutrition or maldigestion, cancer, infection, heart failure, and endocrine disorders play a role in other species. Weight loss is frequently but not always present. It is suspected that an unfavorable environment in combination

with inadequate hematopoietic substrate availability, lack of stimulation from adipocytes or fibrocytes, and deficiency of colony-stimulating factors also contributes.[341] The magnetic resonance characteristics of serous atrophy in the distal limbs of emaciated horses has been described.[342]

Anemia of Inflammation/Chronic Disease

Anemia of inflammation or chronic disease is a mild, normocytic, normochromic functional iron deficiency anemia associated with altered iron homeostasis during disease. Hypoferremia is thought to be part of the innate immune system, limiting iron availability to invading microorganisms. Anemia is caused by inflammation-driven retention of iron in macrophages, making the metal unavailable for heme synthesis. Concurrent impaired biologic activity of erythropoietin and a reduced proliferative capacity of erythroid progenitor cells further compound erythropoiesis.[343] Hepcidin, a liver-derived peptide, is thought to be the central iron-regulatory hormone and key regulator of this process. It can be induced by iron loading but also by inflammatory cytokines including interleukin-1 (IL-1) and IL-6. Hepcidin acts by binding to the iron exporter ferroportin. Subsequent ferroportin internalization and degradation inhibit the iron influx into plasma by reducing uptake of dietary iron from enterocytes. Furthermore, iron recycling by macrophages is impaired, which delivers approximately 95% of the daily iron needs for erythropoiesis.[343] Hepcidin also acts as an acute-phase protein in horses,[344] and hepatic hepcidin mRNA expression is upregulated following experimental LPS administration, resulting in hypoferremia. Laboratory findings suggestive of anemia of chronic disease include low serum iron concentrations, decreased transferrin saturation, normal to increased serum ferritin concentration, normal to decreased TIBC, and increased iron stores in the reticuloendothelial system. Anemia is generally mild, and treatment with blood products or iron supplementation is not indicated. In cases of severe anemia, other causes are likely to contribute and should be carefully ruled out.

Anemia Secondary to Recombinant Human Erythropoietin Administration

Severe anemia with PCVs as low as 4.1% has been reported in racehorses treated with recombinant human erythropoietin (rhEPO).[4,345,346] It is presumed that equine antibodies build against rhEPO and cross-react with equine erythropoietin, inhibiting its binding to erythropoietin receptors on bone marrow erythroid precursors.[345,346] Bone marrow suppression can be temporary, with horses returning to full function, or permanent and ultimately fatal.[346] Diagnosis relies on history and on identification of erythroid hypoplasia on bone marrow evaluation. Several tests exist for detection of rhEPO in equine plasma and urine within days after administration and detection of antibodies against rhEPO in the plasma.[347]

Aplastic Anemia and Pancytopenia

Aplastic anemia is a bone marrow failure syndrome characterized by bone marrow aplasia and peripheral blood pancytopenia. In humans, most cases are acquired and often idiopathic but can also be secondary to a number of causes, including bacterial or viral infections, chronic renal or hepatic disease, neoplasia, and drug therapy.[348] Pancytopenia can also be the result of *myelophthisis,* a term used when normal marrow space is infiltrated and replaced by nonhematopoietic or abnormal cells. Causes include metastatic neoplasia, myeloproliferative disorders, and marrow fibrosis, which can also be secondary to the neoplastic conditions.[349] Early clinical signs are representative of thrombocytopenia, including petechiae, epistaxis, hematoma formation, and bleeding from injection sites.[349,350] With progressive leukopenia, a propensity for infections might be noticed. Diagnosis is achieved by examination of bone marrow aspirates or biopsies; multiple samples might be necessary. Pancytopenia secondary to drug administration has been reported in horses. One animal had been treated with penicillin, oxytetracycline, trimethoprim-sulfadiazine, phenylbutazone, dipyrone, flunixin meglumine, and isoxsuprine, but the offending agent was not definitively identified.[349] The horse responded to treatment with corticosteroids, androgens, and withdrawal of the suspected drugs. Pancytopenia secondary to administration of potentiated sulfonamides, often in combination with oral folic acid supplementation, has been observed in adult horses and in foals born to treated mares. Two out of three adult horses, but none of the foals, survived.[351-353] Although one of the cases was thought to be an immune-mediated reaction,[351] the others were speculated to be secondary to folic acid deficiency. In addition to the effects of potentiated sulfonamides on folate synthesis, oral administration of folic acid in other species competitively reduces the absorption of the active form of folic acid. This paradoxically worsens folic acid deficiency and also teratogenic effects of pyrimethamine. Administration of folinic acid can prevent these complications but might counteract the antimicrobial properties of potentiated sulfonamides.[352,353] Pancytopenia responsive to corticosteroid treatment occurred in a horse with equine multinodular pulmonary fibrosis. Equine herpesvirus 5 inclusion bodies and DNA were identified in the bone marrow, and an immune-mediated cell destruction was suspected.[354] Mild anemia (PCV 25%–30%) has been observed in horses with chronic renal failure.[2] Other causes of pancytopenia in horses include fatal myelofibrosis of unknown etiology,[355] and transient pancytopenia associated with a presumed splenic hematoma was described in a donkey.[356] In several cases an underlying cause cannot be established.[350,357,358] In contrast to cattle, horses do not develop aplastic anemia after acute bracken fern toxicosis but rather present with neurologic signs.[359] Treatment is often unrewarding unless pancytopenia is immune mediated. Possible offending medications and supplements should be discontinued, and treatment with corticosteroids and androgens (nandrolone decanoate 250 mg/500-kg horse IM) can be attempted.[349]

Familial Myeloid and Megakaryocytic Hypoplasia in Standardbreds

Moderate to severe neutropenia in combination with intermittent thrombocytopenia due to myeloid and megakaryocytic bone marrow hypoplasia was diagnosed in eight Standardbred horses. All were sired by the same stallion, making a familial basis likely. Horses were between 4 months and 3 years old on first examination and presented for persistent fever, malaise, and nonhealing wounds. Evaluation of serial neutrophil counts in two horses suggested cyclic variations in neutrophil numbers with increased lymphocyte counts and decreased platelet counts when neutrophil counts were low. Myeloid progenitor cells were present and able to respond to exogenous

growth factors, suggesting that a bone marrow microenvironment or growth factor defect was responsible for the observed changes. Seven of the eight horses died or were euthanized; the surviving horse had moderate neutropenia and a normal platelet count and raced for 3 years.[360]

Erythrocytosis

Erythrocytosis describes an increase in RBC numbers in the circulation and can be relative (following hemoconcentration and/or splenic contraction) or absolute (true increase).

Primary Erythrocytosis/Polycythemia Vera

Primary absolute erythrocytosis, sometimes referred to as polycythemia vera, is caused by proliferation of cells of the erythroid lineage without an increase in erythropoietin. Polycythemia vera in people is a myeloproliferative neoplasm associated with a genetic mutation of the *JAK2* gene causing erythrocytosis (with or without an increase in erythropoietin); other cell types might also be increased. The disease is slowly progressive with long survival times but carries the risk of leukemic or fibrotic transformation.[361] Primary absolute erythrocytosis was diagnosed in one and suspected in another horse. The former horse was initially treated with a combination of repeated phlebotomy, hydroxyurea (up to 29 mg/kg q 24 h), and dexamethasone. Eventually, all treatment was discontinued, and the horse lived with a PCV between 65% and 70% without apparent ill effects during a 2-year follow-up period.[362] In the other case, a granular cell myoblastoma was also identified, and therefore secondary polycythemia could not conclusively be ruled out.[363]

Secondary Erythrocytosis

Secondary absolute erythrocytosis is triggered by a physiologic or pathologic increase in erythropoietin concentrations. Secondary erythrocytosis can be congenital, due to genetic abnormalities (not reported in horses) causing hypersensitivity of erythroid progenitors to erythropoietin, or acquired.[364] Secondary acquired erythrocytosis can be driven by hypoxia and was previously referred to as secondary appropriate erythrocytosis. Chronic pulmonary disease, high altitude, congenital cardiac anomalies, and local renal hypoxia are common causes.[365] In horses, erythropoietin concentrations only increased transiently at 3800 m, whereas increase in PCV at the same altitude was variable but longer lasting.[366-368] Overall, altitude ≤3800 m probably does not contribute to significant resting erythrocytosis in horses. The degree of hypoxia necessary to induce erythrocytosis in horses is not known, but Pao_2 of less than 60 to 80 mm Hg (Sao_2 less than 90%–92%) has been suggested. Low-dose rhEPO (50 IU/kg body weight rhEPO/3 mL saline 3 times/week for 3 weeks) administration increases RBC volume and aerobic capacity without altering anaerobic power.[369] Pathologic erythropoietin production (also called inappropriate secondary erythrocytosis) is caused by release of erythropoietin or erythropoietin-like substances without underlying physiologic demand and is often associated with neoplasia, particularly hepatoblastoma and hepatocellular carcinoma.[370-374] Administration of performance-enhancing drugs such as human recombinant erythropoietin might cause erythrocytosis in horses. Cobalt is used as a doping agent in human and equine athletes and in the treatment of anemia in people. Cobalt acts by preventing degradation of hypoxia-inducible factor 1α (HIF1α), which regulates expression of erythropoietin. Administration of a single dose of cobalt (109 mg cobalt chloride or 169 mg cobalt gluconate) did not increase erythropoietin or RBC numbers in horses, but a cumulative effect cannot be ruled out.[375]

Thrombocytosis

Primary or Clonal Thrombocytosis

Primary (also referred to as essential or clonal) thrombocytosis is a rare chronic myeloproliferative disorder that has been suspected in one horse with a platelet count of 1,104,000/μL.[56] The condition is associated with sustained megakaryocyte proliferation causing increased numbers of circulating platelets. Other cell types might also be increased. In humans, the disease is slowly progressive with long survival times but, similar to polycythemia vera, carries the risk of leukemic or fibrotic transformation.[361] Because of an increased risk of thrombosis, treatment with platelet-suppressing drugs is often implemented. Interestingly, in the suspected equine case pulmonary thrombosis and laminitis were noted, raising the suspicion that a similar risk might also be present.[56]

Reactive or Secondary Thrombocytosis

Secondary thrombocytosis with platelet counts of 400,000 to 839,000/μL is rare and affects approximately 1% of hospitalized horses in which a platelet count is performed.[56] Thrombocytosis is associated with young age (less than 3 years), intact male gender, tachycardia, pyrexia, and infectious or inflammatory conditions and is often accompanied by hyperfibrinogenemia, leukocytosis, hypoproteinemia, and anemia. A relation between thrombocytosis and *Rhodococcus equi* infection in foals has been suggested but not confirmed.[376,377] Excessive cytokine release (IL-1, IL-4, IL-6, TNF-α, thrombopoietin, etc.) is thought to activate cytokine-mediated megakaryocyte proliferation. Although a statistically significant link to decreased survival could not be established, 33% of horses with thrombocytosis in one study did not survive. Much smaller increases in platelet numbers have been reported in pregnant mares toward the time of parturition. It has been speculated that this is part of a protective hypercoagulable response.[378]

Lymphoproliferative Disorders

The lymphomas (malignant lymphoma or lymphosarcoma) are a diverse group of neoplasms that originate from lymphoreticular cells. They usually arise in lymphoid tissues such as lymph nodes and spleen but can originate from almost any tissue in the body. This is in contrast to primary leukemias, which arise in the bone marrow and/or peripheral blood from lymphoid, myeloid, monocytic, or erythroid cells, often with the presence of neoplastic cells in the circulation. The term *secondary leukemia* is used if the bone marrow is invaded by metastases from another site (e.g., multicentric lymphosarcoma). Lymphosarcoma and leukemia of the same cell type are regarded by some as different manifestations of the same disease process.[379]

Lymphosarcoma/Lymphoma

Lymphosarcoma is the most common equine hematopoietic tumor affecting horses of all ages, even fetal stages. Human classification systems are not readily applied to animals, and the nomenclature can be confusing. In horses, immunophenotypes including B cells, T cells, T-cell–rich B cells, and potentially natural killer cells have been described. Further classifications include large, small, and mixed cell types. Based on localization, multicentric (generalized); alimentary;

mediastinal, thymic, and thoracic; subcutaneous and cutaneous; hepatosplenic and splenic; nasal, paranasal, and nasopharyngeal; nervous system; and ocular lymphosarcoma can be differentiated.[379] Clinical signs are determined by localization and extent of the tumor. Common laboratory findings include anemia, presumably caused by immune-mediated hemolysis, chronic inflammation, myelophthisis, blood loss, neutrophilia, hypoalbuminemia, hypogammaglobulinemia with decrease/absence of IgM, hypergammaglobulinemia (with polyclonal or monoclonal gammopathy), hyperfibrinogenemia, and hypercalcemia. Paraneoplastic syndromes described in horses with lymphosarcoma include alopecia and pruritus,[380] hypercalcemia,[381] pseudohyperparathyroidism,[382] immune-mediated hemolytic anemia and/or thrombocytopenia,[168] polycythemia due to erythropoietin gene expression,[371] and hypereosinophilia.[38] The prognosis depends on location and extent of the tumor and whether or not metastasis has occurred. Complete surgical excision, if possible, can be curative, and in rare cases, successful chemotherapy has also been described.[383,384]

Leukemia

The term *leukemia* describes abnormal proliferation and development of one or more cell lines in the bone marrow. Classifications of leukemias involve cell type and stage of differentiation. Acute leukemias are often rapidly progressive and poorly differentiated forms, whereas in chronic leukemias slowly progressive accumulation (rather than proliferation) of cells in the bone marrow and peripheral tissues predominates.[379] Replacement of the normal hematopoietic tissue in the bone marrow by tumor cells can occur before the onset of overt leukemia. Clinically, immunosuppression and susceptibility to secondary infection might be noted, and in laboratory investigations, a degree of bone marrow failure such as anemia, thrombocytopenia, neutropenia, and/or lymphopenia is evident.[379,385-387] Bone marrow necrosis secondary to T-cell lymphoma has also been reported.[387] Leukemias for every white cell line present in the bone marrow are described. Myeloid leukemias reported in horses include acute myelomonocytic leukemia with concurrent proliferation of neutrophilic and monocytic cell lines, acute monocytic leukemia, eosinophilic myeloproliferative disorder, and acute erythroid leukemia (erythemic myelosis) in a 10-week-old foal.[386,388-392] Lymphoid leukemias of B- and T-cell origin have been described with potentially T-cell forms predominating in horses.[393-395]

Multiple Myeloma

Multiple myeloma is a highly malignant neoplasm derived from plasma cells or plasmacytoid lymphocytes (large B lymphocytes with some resemblance to plasma cells) in the bone marrow. Monoclonal gammopathy, hyperviscosity of the blood, osteolysis, bone pain, and myelophthisis are common characteristics.[379,396,397] Light-chain amyloidosis secondary to multiple myeloma developed in a Quarter Horse gelding secondary to multiple myeloma.[398]

VASCULAR TUMORS

In humans, the nomenclature of vascular anomalies has been defined by the International Society for the Study of Vascular Anomalies (ISSVA). Vascular tumors are differentiated from vascular malformations based on their clinical appearance, radiologic and pathologic features, and biologic behavior. Vascular tumors can arise from blood or lymphatic vessels and grow by endothelial hyperplasia, whereas vascular malformations have a quiescent endothelium and are considered to be localized defects of vascular morphogenesis.

Hemangiosarcoma and Hemangioma

Hemangiosarcomas often present as a disseminated malignancy involving the spleen, heart, lung, liver, and soft tissues of the trunk and extremities, whereas hemangiomas are typically benign, solitary, deep dermal tumors.[399] Hemangiosarcoma, a malignant neoplasm of vascular endothelial cells also known as malignant hemangioendothelioma or angiosarcoma, is rare, but Thoroughbreds might be overrepresented.[400,401] It occurs mainly in middle-aged horses, but young animals and even neonatal foals can be affected.[400-402] Solar radiation might play a role in development of the neoplasm.[403] Hemangiosarcomas can present as cutaneous, locally invasive, or disseminated forms and can affect any organ system or location. Local invasions of the vertebrae and spinal cord, oral cavity, eye, third eyelid, tarsal sheath, and frontal sinus, as well as disseminated disease, have been reported.[402,404-406] Because of the varying locations, a wide range of clinical findings can be encountered. In adult horses, lungs, pleura, spleen, and skeletal muscle are most commonly affected, whereas in young animals skin and limbs are frequently involved.[400,401] Subcutaneous or muscular swellings, tachypnea, epistaxis, and lameness often prompt examination, and not infrequently the tumor is initially mistaken for a nonresolving hematoma or continuously bleeding wound.[400,407] Progression can be extremely rapid, with new masses occurring within days with a median time between onset of clinical signs and euthanasia of 17 days (range 0 days to 4 years) or more prolonged over several months.[400,401] Laboratory examination might identify anemia, thrombocytopenia, leukocytosis, and hyperfibrinogenemia or hypofibrinogenemia. More profound hematologic changes than what would be expected with simple trauma can alert the clinician to the presence of a hemangiosarcoma. In advanced cases, coagulation abnormalities or disseminated intravascular coagulation might be observed. Results of diagnostic imaging are often nonspecific, with an ultrasonographic appearance resembling tissue disruption and hematoma formation. Cytology from aspirates or paracentesis is usually consistent with blood.[401,408] Histology is required to obtain a definitive diagnosis, and multiple biopsies might be necessary before a clinical suspicion can be confirmed. Sample collection can be hampered by massive hemorrhage. Although the disseminated form in adults appears to be uniformly fatal, curative excision of cutaneous or locally invasive masses and even spontaneous regression in horses less than 3 years old have been reported.[400]

Hemangiomas have largely been described in the skin of young animals (median age of 8 months in one study), but cases in adult horses also occur.[399] Lesions are either present at birth or develop over weeks to months with progressive enlargement.[409,410] The masses frequently affect the extremities, head, or neck, but synovial involvement has also been reported. Resection is often curative, but repeated surgeries might be required.[409,411]

Lymphangioma and Lymphangiosarcoma

Lymphangiomas and lymphangiosarcomas are very rare in horses. Lymphangiosarcomas display infiltrative behavior, and differentiation between tumors originating from lymphatic or blood vessels can be difficult because of the overlapping morphologic features. Confirmation relies on a combination of clinical, gross, microscopic, immunohistochemical, and

ultrastructural findings.[412] Lymphangiosarcoma affecting the forelimb, third eyelid, and internal organs in adult horses and cutaneous lymphangiomas in young horses involving limbs and neck have been described.[412-418]

REFERENCES

1. Zicker SC, Marty GD, Carlson GP, et al. Bilateral renal dysplasia with nephron hypoplasia in a foal. *J Am Vet Med Assoc*. 1990;196:2001–2005.
2. Schott HC, Patterson KS, Fitzgerald SD, et al. Chronic renal failure in 99 horses. *Proceedings of the 43rd Annual Convention of the American Association of Equine Practitioners*. 1997:345–346.
3. Ho EN, Chan GH, Wan TS, et al. Controlling the misuse of cobalt in horses. *Drug Test Anal*. 2015;7:21–30.
4. Cooper C, Sears W, Bienzle D. Reticulocyte changes after experimental anemia and erythropoietin treatment of horses. *J Appl Physiol* (1985). 2005;99(3):915–921.
5. Stockham SL, Harvey JW, Kinden DA. Equine glucose-6-phosphate dehydrogenase deficiency. *Vet Pathol*. 1994;31:518–527.
6. Harvey JW, Stockham SL, Scott MA, et al. Methemoglobinemia and eccentrocytosis in equine erythrocyte flavin adenine dinucleotide deficiency. *Vet Pathol*. 2003;40:632–642.
7. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. *J Am Soc Nephrol*. 2007;18:414–420.
8. Mullaney TP, Brown CM. Iron toxicity in neonatal foals. *Equine Vet J*. 1988;20:119–124.
9. Pearson EG, Hedstrom OR, Poppenga RH. Hepatic cirrhosis and hemochromatosis in three horses. *J Am Vet Med Assoc*. 1994;204:1053–1056.
10. Lavoie JP, Teuscher E. Massive iron overload and liver fibrosis resembling haemochromatosis in a racing pony. *Equine Vet J*. 1993;25:552–554.
11. Pearson EG, Andreasen CB. Effect of oral administration of excessive iron in adult ponies. *J Am Vet Med Assoc*. 2001;218:400–404.
12. Harvey JW, Asquith RL, Sussman WA, et al. Serum ferritin, serum iron, and erythrocyte values in foals. *Am J Vet Res*. 1987;48:1348–1352.
13. Harvey JW, Asquith RL, McNulty PK, et al. Haematology of foals up to one year old. *Equine Vet J*. 1984;16:347–353.
14. Fleming KA, Barton MH, Latimer KS. Iron deficiency anemia in a neonatal foal. *J Vet Intern Med*. 2006;20:1495–1498.
15. Kohn CW, Jacobs RM, Knight D, et al. Microcytosis, hypoferremia, hypoferritemia, and hypertransferrinemia in Standardbred foals from birth to 4 months of age. *Am J Vet Res*. 1990;51:1198–1205.
16. Smith JE, Cipriano JE, DeBowes R, et al. Iron deficiency and pseudo-iron deficiency in hospitalized horses. *J Am Vet Med Assoc*. 1986;188:285–287.
17. Kaneko JJ, Harvey JW, Bruss ML, editors. *Clinical biochemistry of domestic animals*. 6th edition, 2008, pp 262–285.
18. Dondi F, Lukacs RM, Gentilini F, et al. Serum amyloid A, haptoglobin, and ferritin in horses with colic: association with common clinicopathological variables and short-term outcome. *Vet J*. 2015;205:50–55.
19. Rebordao MR, Carneiro C, Alexandre-Pires G, et al. Neutrophil extracellular traps formation by bacteria causing endometritis in the mare. *J Reprod Immunol*. 2014;106:41–49.
20. McFarlane D, Hill K, Anton J. Neutrophil function in healthy aged horses and horses with pituitary dysfunction. *Vet Immunol Immunopathol*. 2015;165:99–106.
21. McTaggart C, Yovich JV, Penhale J, et al. A comparison of foal and adult horse neutrophil function using flow cytometric techniques. *Res Vet Sci*. 2001;71:73–79.
22. Demmers S, Johannisson A, Grondahl G, et al. Neutrophil functions and serum IgG in growing foals. *Equine Vet J*. 2001;33:676–680.
23. Perkins GA, Miller WH, Divers TJ, et al. Ulcerative dermatitis, thrombocytopenia, and neutropenia in neonatal foals. *J Vet Intern Med*. 2005;19:211–216.
24. Wong DM, Alcott CJ, Clark SK, et al. Alloimmune neonatal neutropenia and neonatal isoerythrolysis in a Thoroughbred colt. *J Vet Diagn Invest*. 2012;24:219–226.
25. Endo Y, Hobo S, Korosue K, et al. Effects of low-dose G-CSF formulation on hematology in healthy horses after long-distance transportation. *J Vet Med Sci*. 2015;77:507–509.
26. Ho EN, Kwok WH, Lau MY, et al. Doping control analysis of filgrastim in equine plasma and its application to a co-administration study of filgrastim and recombinant human erythropoietin in the horse. *J Chromatogr A*. 2014;1338:92–101.
27. Grondin TM, DeWitt SF, Keeton KS. Pelger-Huet anomaly in an Arabian horse. *Vet Clin Pathol*. 2007;36:306–310.
28. Gill AF, Gaunt S, Sirninger J. Congenital Pelger-Huet anomaly in a horse. *Vet Clin Pathol*. 2006;35:460–462.
29. Ramaiah SK, Harvey JW, Giguere S, et al. Intravascular hemolysis associated with liver disease in a horse with marked neutrophil hypersegmentation. *J Vet Intern Med*. 2003;17:360–363.
30. Prasse KW, George LW, Whitlock RH. Idiopathic hypersegmentation of neutrophils in a horse. *J Am Vet Med Assoc*. 1981;178:303–305.
31. Tvedten H, Riihimaki M. Hypersegmentation of equine neutrophils. *Vet Clin Pathol*. 2007;36:4–5.
32. Berek C. Eosinophils: important players in humoral immunity. *Clin Exp Immunol*. 2015;183:57–64.
33. Corning S. Equine cyathostomins: a review of biology, clinical significance and therapy. *Parasit Vectors*. 2009;2(suppl 2):S1.
34. Wong DM, Crisman MV. Eosinophilic enterocolitis in a 4-year- old miniature horse stallion. *Can Vet J*. 2004;45:73–75.
35. Latimer KS, Bounous DI, Colatos C, et al. Extreme eosinophilia with disseminated eosinophilic granulomatous disease in a horse. *Vet Clin Pathol*. 1996;25:23–26.
36. Jin JJ, Butterfield JH, Weiler CR. Hematologic malignancies identified in patients with hypereosinophilia and hypereosinophilic syndromes. *J Allergy Clin Immunol Pract*. 2015;3:920–925.
37. Marchetti V, Benetti C, Citi S, et al. Paraneoplastic hypereosinophilia in a dog with intestinal T-cell lymphoma. *Vet Clin Pathol*. 2005;34:259–263.
38. Duckett WM, Matthews HK. Hypereosinophilia in a horse with intestinal lymphosarcoma. *Can Vet J*. 1997;38:719–720.
39. Sarfati M, Wakahara K, Chapuy L, et al. Mutual interaction of basophils and T cells in chronic inflammatory diseases. *Front Immunol*. 2015;6:399.
40. Galli SJ, Tsai M, Marichal T, et al. Approaches for analyzing the roles of mast cells and their proteases in vivo. *Adv Immunol*. 2015;126:45–127.
41. McFarlane D, Sellon DC, Gibbs SA. Age-related quantitative alterations in lymphocyte subsets and immunoglobulin isotypes in healthy horses. *Am J Vet Res*. 2001;62:1413–1417.
42. Larson J, Buechner-Maxwell V, Crisman MV, et al. Severe combined immunodeficiency in a Caspian filly. *J Vet Intern Med*. 2011;25:954–958.
43. Jelinek F, Faldyna M, Jasurkova-Mikutova G. Severe combined immunodeficiency in a Fell pony foal. *J Vet Med A Physiol Pathol Clin Med*. 2006;53:69–73.
44. Brault SA, Blanchard MT, Gardner IA, et al. The immune response of foals to natural infection with equid herpesvirus-2 and its association with febrile illness. *Vet Immunol Immunopathol*. 2010;137:136–141.
45. Weiss DJ, Evanson OA, Wells RE. Evaluation of arginine-glycine-aspartate-containing peptides as inhibitors of equine platelet function. *Am J Vet Res*. 1997;58:457–460.
46. Segura D, Monreal L, Perez-Pujol S, et al. Assessment of platelet function in horses: ultrastructure, flow cytometry, and perfusion techniques. *J Vet Intern Med*. 2006;20:581–588.

47. Roscher KA, Failing K, Moritz A. Inhibition of platelet function with clopidogrel, as measured with a novel whole blood impedance aggregometer in horses. *Vet J*. 2015;203:332–336.
48. Kingston JK, Bayly WM, Sellon DC, et al. Measurement of the activation of equine platelets by use of fluorescent-labeled annexin V, anti-human fibrinogen antibody, and anti-human thrombospondin antibody. *Am J Vet Res*. 2002;63:513–519.
49. Dunkel B, Rickards KJ, Werling D, et al. Neutrophil and platelet activation in equine recurrent airway obstruction is associated with increased neutrophil CD13 expression, but not platelet CD41/61 and CD62P or neutrophil-platelet aggregate formation. *Vet Immunol Immunopathol*. 2009;131:25–32.
50. Watts AE, Ness SL, Divers TJ, et al. Effects of clopidogrel on horses with experimentally induced endotoxemia. *Am J Vet Res*. 2014;75:760–769.
51. Springer NL, Smith E, Brooks MB, et al. Flow cytometric detection of circulating platelet-derived microparticles in healthy adult horses. *Am J Vet Res*. 2014;75:879–885.
52. Dunkel B, Rickards KJ, Page CP, et al. Platelet activation in ponies with airway inflammation. *Equine Vet J*. 2007;39:557–561.
53. Dunkel B, Bolt DM, Smith RK, et al. Stimulus-dependent release of tissue-regenerating factors by equine platelets. *Equine Vet J*. 2012;44:346–354.
54. Brainard BM, Epstein KL, LoBato D, et al. Effects of clopidogrel and aspirin on platelet aggregation, thromboxane production, and serotonin secretion in horses. *J Vet Intern Med*. 2011;25:116–122.
55. Delesalle C, van de Walle GR, Nolten C, et al. Determination of the source of increased serotonin (5-HT) concentrations in blood and peritoneal fluid of colic horses with compromised bowel. *Equine Vet J*. 2008;40:326–331.
56. Sellon DC, Levine JF, Palmer K, et al. Thrombocytosis in 24 horses (1989–1994). *J Vet Intern Med*. 1997;11:24–29.
57. Sellon DC. Thrombocytopenia in horses. *Equine Vet Educ*. 1998;10:133–139.
58. Sellon DC, Levine J, Millikin E, et al. Thrombocytopenia in horses: 35 cases (1989–1994). *J Vet Intern Med*. 1996;10:127–132.
59. Bailey SR, Adair HS, Reinemeyer CR, et al. Plasma concentrations of endotoxin and platelet activation in the developmental stage of oligofructose-induced laminitis. *Vet Immunol Immunopathol*. 2009;129:167–173.
60. Stokol T, Yeo WM, Burnett D, et al. Equid herpesvirus type 1 activates platelets. *PLoS One*. 2015;10:e0122640.
61. Macieira S, Lussier J, Bedard C. Characterization of the cDNA and genomic DNA sequence encoding for the platelet integrin alpha IIB and beta III in a horse with Glanzmann thrombasthenia. *Can J Vet Res*. 2011;75:222–227.
62. Macieira S, Rivard GE, Champagne J, et al. Glanzmann thrombasthenia in an Oldenbourg filly. *Vet Clin Pathol*. 2007;36:204–208.
63. Norris JW, Pombo M, Shirley E, et al. Association of factor V secretion with protein kinase B signaling in platelets from horses with atypical equine thrombasthenia. *J Vet Intern Med*. 2015;29:1387–1394.
64. Norris JW, Pratt SM, Auh JH, et al. Investigation of a novel, heritable bleeding diathesis of Thoroughbred horses and development of a screening assay. *J Vet Intern Med*. 2006;20:1450–1456.
65. Norris JW, Pratt SM, Hunter JF, et al. Prevalence of reduced fibrinogen binding to platelets in a population of Thoroughbreds. *Am J Vet Res*. 2007;68:716–721.
66. Hinchcliff KW, Kociba GJ, Mitten LA. Diagnosis of EDTA-dependent pseudothrombocytopenia in a horse. *J Am Vet Med Assoc*. 1993;203:1715–1716.
67. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. *Clin Chem Lab Med*. 2012;50:1281–1285.
68. Kasashima Y, Ueno T, Tomita A, et al. Optimisation of bone marrow aspiration from the equine sternum for the safe recovery of mesenchymal stem cells. *Equine Vet J*. 2011;43:288–294.
69. Chelazzi C, Villa G, Mancinelli P, et al. Glycocalyx and sepsis-induced alterations in vascular permeability. *Crit Care*. 2015;19:26.
70. Gale AJ. Continuing education course #2: current understanding of hemostasis. *Toxicol Pathol*. 2011;39:273–280.
71. Versteeg HH, Heemskerk JW, Levi M, et al. New fundamentals in hemostasis. *Physiol Rev*. 2013;93:327–358.
72. McGorum BC, Henderson IS, Stirling D, et al. Vitamin K deficiency bleeding in a Standardbred colt. *J Vet Intern Med*. 2009;23:1307–1310.
73. Burke CW. Vitamin K deficiency bleeding: overview and considerations. *J Pediatr Health Care*. 2013;27:215–221.
74. Ardell S, Offringa M, Soll R. Prophylactic vitamin K for the prevention of vitamin K deficiency bleeding in preterm neonates. *The Cochrane Library*. 2000;(4):CD002776.
75. Draxler DF, Medcalf RL. The fibrinolytic system—more than fibrinolysis? *Transfus Med Rev*. 2015;29:102–109.
76. Stang LJ. D-dimer and fibrinogen/fibrin degradation products. *Methods Mol Biol*. 2013;992:415–427.
77. Rossi TM, Smith SA, McMichael MA, et al. Evaluation of contact activation of citrated equine whole blood during storage and effects of contact activation on results of recalcification-initiated thromboelastometry. *Am J Vet Res*. 2015;76:122–128.
78. Fletcher DJ, Brainard BM, Epstein K, et al. Therapeutic plasma concentrations of epsilon aminocaproic acid and tranexamic acid in horses. *J Vet Intern Med*. 2013;27:1589–1595.
79. Epstein KL, Brainard BM, Giguere S, et al. Serial viscoelastic and traditional coagulation testing in horses with gastrointestinal disease. *J Vet Emerg Crit Care (San Antonio)*. 2013;23:504–516.
80. Winfield LS, Brooks MB. Hemorrhage and blood loss–induced anemia associated with an acquired coagulation factor VIII inhibitor in a Thoroughbred mare. *J Am Vet Med Assoc*. 2014;244:719–723.
81. Persson SG, Ullberg LE. Blood volume and rate of growth in Standardbred foals. *Equine Vet J*. 1981;13:254–258.
82. Persson SG, Funkquist P, Nyman G. Total blood volume in the normally performing Standardbred trotter: age and sex variations. *Zentralbl Veterinarmed A*. 1996;43:57–64.
83. Marcilese NA, Valsecchi RM, Figueiras HD, et al. Normal blood volumes in the horse. *Am J Physiol*. 1964;207:223–227.
84. Navas de Solis C, Dallap Schaer BL, Boston R, et al. Myocardial insult and arrhythmias after acute hemorrhage in horses. *J Vet Emerg Crit Care (San Antonio)*. 2015;25:248–255.
85. Mudge MC. Acute hemorrhage and blood transfusions in horses. *Vet Clin North Am Equine Pract*. 2014;30:427–436.
86. Epstein KL, Bergren A, Giguere S, et al. Cardiovascular, colloid osmotic pressure, and hemostatic effects of 2 formulations of hydroxyethyl starch in healthy horses. *J Vet Intern Med*. 2014;28:223–233.
87. McClure JJ, Koch C, Traub-Dargatz J. Characterization of a red blood cell antigen in donkeys and mules associated with neonatal isoerythrolysis. *Anim Genet*. 1994;25:119–120.
88. Ramirez S, Gaunt SD, McClure JJ, et al. Detection and effects on platelet function of anti-platelet antibody in mule foals with experimentally induced neonatal alloimmune thrombocytopenia. *J Vet Intern Med*. 1999;13:534–539.
89. Tomlinson JE, Taberner E, Boston RC, et al. Survival time of cross-match incompatible red blood cells in adult horses. *J Vet Intern Med*. 2015;29:1683–1688.
90. Mudge MC, Walker NJ, Borjesson DL, et al. Post-transfusion survival of biotin-labeled allogeneic RBCs in adult horses. *Vet Clin Pathol*. 2012;41:56–62.
91. Wong PL, Nickel LS, Bowling AT, et al. Clinical survey of antibodies against red blood cells in horses after homologous blood transfusion. *Am J Vet Res*. 1986;47:2566–2571.
92. Radin MJ, Eubank MC, Weiser MG. Electronic measurement of erythrocyte volume and volume heterogeneity in horses during erythrocyte regeneration associated with experimental anemias. *Vet Pathol*. 1986;23:656–660.

93. Owens SD, Johns JL, Walker NJ, et al. Use of an in vitro biotinylation technique for determination of posttransfusion survival of fresh and stored autologous red blood cells in Thoroughbreds. *Am J Vet Res*. 2010;71:960–966.
94. Hurcombe SD, Mudge MC, Hinchcliff KW. Clinical and clinicopathologic variables in adult horses receiving blood transfusions: 31 cases (1999–2005). *J Am Vet Med Assoc*. 2007;231:267–274.
95. Mudge MC. Acute hemorrhage and blood transfusions in horses. *Vet Clin North Am Equine Pract*. 2014;30:(9) 427–436.
96. Higgs VA, Rudloff E, Kirby R, et al. Autologous blood transfusion in dogs with thoracic or abdominal hemorrhage: 25 cases (2007–2012). *J Vet Emerg Crit Care (San Antonio)*. 2015;25(6):731–738.
97. Finding EJT, Eliashar E, Johns IC, et al. Autologous blood transfusion following an allogenic transfusion reaction in a case of acute anaemia due to intra-abdominal bleeding. *Equine Vet Educ*. 2011;23:339–342.
98. Waguespack R, Belknap J, Williams A. Laparoscopic management of postcastration hemorrhage in a horse. *Equine Vet J*. 2001;33:510–513.
99. Maxson AD, Giger U, Sweeney CR, et al. Use of a bovine hemoglobin preparation in the treatment of cyclic ovarian hemorrhage in a miniature horse. *J Am Vet Med Assoc*. 1993;203:1308–1311.
100. Vin R, Bedenice D, Rentko VT, et al. The use of ultrapurified bovine hemoglobin solution in the treatment of two cases of presumed red maple toxicosis in a miniature horse and a pony. *J Vet Emergency and Critical Care*. 2002;12:169–175.
101. Belgrave RL, Bayly WM, Hines MT, et al. Effects of oxyglobin administered to ponies with normovolemia anemia. *J Vet Intern Med*. 2001;15:289.
102. Perkins GA, Divers TJ. Polymerized hemoglobin therapy in a foal with neonatal isoerythrolysis. *J Vet Emerg Crit Care*. 2001;11:141–146.
103. Soma LR, Uboh CE, Guan F, et al. The pharmacokinetics of hemoglobin-based oxygen carrier hemoglobin glutamer-200 bovine in the horse. *Anesth Analg*. 2005;100:1570–1575.
104. Byars TD, Greene CE. Idiopathic thrombocytopenic purpura in the horse. *J Am Vet Med Assoc*. 1982;180:1422–1424.
105. McGurrin MK, Arroyo LG, Bienzle D. Flow cytometric detection of platelet-bound antibody in three horses with immune-mediated thrombocytopenia. *J Am Vet Med Assoc*. 2004;224:83–87. 53.
106. Larson VL, Perman V, Stevens JB. Idiopathic thrombocytopenic purpura in two horses. *J Am Vet Med Assoc*. 1983;183:328–330.
107. Buechner-Maxwell V, Scott MA, Godber L, et al. Neonatal alloimmune thrombocytopenia in a quarter horse foal. *J Vet Intern Med*. 1997;11:304–308.
108. Dunkel B. Platelet transfusion in thrombocytopenic horses. *Equine Vet Educ*. 2013;25:359–362.
109. Cooper N, Bein G, Heidinger K, et al. A bead-based assay in the work-up of suspected platelet alloimmunization. *Transfusion*. 2015;56:115–118.
110. Boudreaux MK, Humphries DM. Identification of potential platelet alloantigens in the Equidae family by comparison of gene sequences encoding major platelet membrane glycoproteins. *Vet Clin Pathol*. 2013;42:437–442.
111. Dunkel B, Chan D, Monreal L. Influence of citrate concentration and material of blood tubes on thromboelastographic parameters in horses. *J Vet Emerg Crit Care*. 2009;19:A14.
112. Ohto H, Nollet KE. Overview on platelet preservation: better controls over storage lesion. *Transfus Apher Sci*. 2011;44:321–325.
113. Meyers KM, Lindner C, Grant B. Characterization of the equine platelet aggregation response. *Am J Vet Res*. 1979;40:260–264.
114. Johnstone IB. Comparative effects of phenylbutazone, naproxen and flunixin meglumine on equine platelet aggregation and platelet factor 3 availability in vitro. *Can J Comp Med*. 1983;47:172–179.
115. Kopp KJ, Moore JN, Byars TD, et al. Template bleeding time and thromboxane generation in the horse: effects of three nonsteroidal anti-inflammatory drugs. *Equine Vet J*. 1985;17:322–324.
116. Segura D, Monreal L, Perez-Pujol S, et al. Effects of etamsylate on equine platelets: in vitro and in vivo studies. *Vet J*. 2007;174:325–329.
117. Heidmann P, Tornquist SJ, Qu A, et al. Laboratory measures of hemostasis and fibrinolysis after intravenous administration of epsilon-aminocaproic acid in clinically normal horses and ponies. *Am J Vet Res*. 2005;66:313–318.
118. Wilson EM, Holcombe SJ, Lamar A, et al. Incidence of transfusion reactions and retention of procoagulant and anticoagulant factor activities in equine plasma. *J Vet Intern Med*. 2009;23:323–328.
119. Hardefeldt LY, Keuler N, Peek SF. Incidence of transfusion reactions to commercial equine plasma. *J Vet Emerg Crit Care (San Antonio)*. 2010;20:421–425.
120. Taylor EL, Sellon DC, Wardrop KJ, et al. Effects of intravenous administration of formaldehyde on platelet and coagulation variables in healthy horses. *Am J Vet Res*. 2000;61:1191–1196.
121. Casella S, Giannetto C, Giudice E, et al. ADP-induced platelet aggregation after addition of tramadol in vitro in fed and fasted horses plasma. *Res Vet Sci*. 2013;94:325–330.
122. Arnold CE, Payne M, Thompson JA, et al. Periparturient hemorrhage in mares: 73 cases (1998–2005). *J Am Vet Med Assoc*. 2008;232:1345–1351.
123. Feige K, Schwarzwald CC, Bombeli T. Comparison of unfractioned and low molecular weight heparin for prophylaxis of coagulopathies in 52 horses with colic: a randomised double-blind clinical trial. *Equine Vet J*. 2003;35:506–513.
124. Cotovio M, Monreal L, Navarro M, et al. Detection of fibrin deposits in tissues from horses with severe gastrointestinal disorders. *J Vet Intern Med*. 2007;21:308–313.
125. Tennent-Brown BS, Epstein KL, Whelchel DD, et al. Use of viscoelastic coagulation testing to monitor low molecular weight heparin administration to healthy horses. *J Vet Emerg Crit Care (San Antonio)*. 2013;23:291–299.
126. de la Rebiere G, Franck T, Deby-Dupont G, et al. Effects of unfractionated and fractionated heparins on myeloperoxidase activity and interactions with endothelial cells: possible effects on the pathophysiology of equine laminitis. *Vet J*. 2008;178:62–69.
127. de la Rebiere de Pouyade G, Grulke S, Detilleux J, et al. Evaluation of low-molecular-weight heparin for the prevention of equine laminitis after colic surgery. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:113–119.
128. Armengou L, Monreal L, Delgado MA, et al. Low-molecular-weight heparin dosage in newborn foals. *J Vet Intern Med*. 2010;24:1190–1195.
129. Cambridge H, Lees P, Hooke RE, et al. Antithrombotic actions of aspirin in the horse. *Equine Vet J*. 1991;23:123–127.
130. Heath MF, Evans RJ, Poole AW, et al. The effects of aspirin and paracetamol on the aggregation of equine blood platelets. *J Vet Pharmacol Ther*. 1994;17:374–378.
131. Menzies-Gow NJ, Sepulveda MF, Bailey SR, et al. Roles of thromboxane A_2 and 5-hydroxytryptamine in endotoxin-induced digital vasoconstriction in horses. *Am J Vet Res*. 2008;69:199–207.
132. Grundmann K, Jaschonek K, Kleine B, et al. Aspirin non-responder status in patients with recurrent cerebral ischemic attacks. *J Neurol*. 2003;250:63–66.
133. Brooks MB, Divers TJ, Watts AE, et al. Effects of clopidogrel on the platelet activation response in horses. *Am J Vet Res*. 2013;74:1212–1222.
134. Brainard BM, Epstein KL, LoBato DN, et al. Treatment with aspirin or clopidogrel does not affect equine platelet expression of P selectin or platelet-neutrophil aggregates. *Vet Immunol Immunopathol*. 2012;149:119–125.

135. Tomlinson JE, Byrne E, Pusterla N, et al. The use of recombinant tissue plasminogen activator (rTPA) in the treatment of fibrinous pleuropneumonia in horses: 25 cases (2007–2012). *J Vet Intern Med*. 2015;29:1403–1409.
136. Clark-Price SC, Cox JH, Bartoe JT, et al. Use of dapsone in the treatment of *Pneumocystis carinii* pneumonia in a foal. *J Am Vet Med Assoc*. 2004;224(3):407–410, 371.
137. Baumer W, Herrling GM, Feige K. Pharmacokinetics and thrombolytic effects of the recombinant tissue-type plasminogen activator in horses. *BMC Vet Res*. 2013;9:158.
138. Forrest LJ, Cooley AJ, Darien BJ. Digital arterial thrombosis in a septicemic foal. *J Vet Intern Med*. 1999;13:382–385.
139. Yakovlev SA, Rublenko MV, Izdepsky VI, et al. Activating effect of the plasminogen activators on plasminogens of different mammalia species. *Thromb Res*. 1995;79:423–428.
140. Dias DPM. *Avaliação hemodinâmica de equinos com oclusão jugular por trombose induzida submetidos a exercício físico e teste da terapia com estreptoquinase*. Sao Paulo, Brazil: Doutor em Cirurgia Veterinária: Unesp, Câmpus de Jaboticabal; 2011.
141. des Lions JA, Carette O, de Broucker C-A, et al. Utilisation de la streptokinase dans le traitement des thrombophlebites de la jugulair chez le cheval: a propos de 2 cas cliniques. *Bull Soc Vet Prat de France*. 2008;92:17–22.
142. Dechant JE, Nieto JE, Le Jeune SS. Hemoperitoneum in horses: 67 cases (1989–2004). *J Am Vet Med Assoc*. 2006;229:253–258.
143. Conwell RC, Hillyer MH, Mair TS, et al. Haemoperitoneum in horses: a retrospective review of 54 cases. *Vet Rec*. 2010;167:514–518.
144. Pusterla N, Fecteau ME, Madigan JE, et al. Acute hemoperitoneum in horses: a review of 19 cases (1992–2003). *J Vet Intern Med*. 2005;19:344–347.
145. Sedrish SA, Johnson PJ. Theriogenology question of the month. Excessive hemorrhaging from ovarian hematomas on both ovaries. *J Am Vet Med Assoc*. 1997;210:179–180.
146. Carvallo FR, Poppenga R, Kinde H, et al. Cluster of cases of massive hemorrhage associated with anticoagulant detection in race horses. *J Vet Diagn Invest*. 2015;27:112–116.
147. Lugo J, Stick JA, Peroni J, et al. Safety and efficacy of a technique for thoracoscopically guided pulmonary wedge resection in horses. *Am J Vet Res*. 2002;63:1232–1240.
148. Perkins G, Ainsworth DM, Yeager A. Hemothorax in 2 horses. *J Vet Intern Med*. 1999;13:375–378.
149. Gruys E, Kok HA, Van Der Werff YD. [Dyspnoea due to intrathoracic hemorrhage and haemangiosarcoma in a horse (author's transl)]. *Tijdschr Diergeneeskd*. 1976;101:310–312.
150. Hassel DM. Thoracic trauma in horses. *Vet Clin North Am Equine Pract*. 2007;23:67–80.
151. Doyle AJ, Freeman DE, Rapp H, et al. Life-threatening hemorrhage from enterotomies and anastomoses in 7 horses. *Vet Surg*. 2003;32:553–558.
152. Cohen ND, Carter GK, Mealey RH, et al. Medical management of right dorsal colitis in 5 horses: a retrospective study (1987–1993). *J Vet Intern Med*. 1995;9:272–276.
153. Galvin N, Dillon H, McGovern F. Right dorsal colitis in the horse: minireview and reports on three cases in Ireland. *Ir Vet J*. 2004;57:467–473.
154. Pellegrini FL. Results of a large-scale necroscopic study of equine colonic ulcers. *J Equine Vet Sci*. 2005;25:113–117.
155. Nishita T, Anezaki R, Matsunaga K, et al. Measurement of carbonic anhydrase I and II isozymes in feces as a marker of occult blood in horses with intestinal tract bleeding. *J Equine Sci*. 2013;24:57–62.
156. Broux B, Torfs S, Wegge B, et al. Acute respiratory failure caused by *Leptospira* spp. in 5 foals. *J Vet Intern Med*. 2012;26:684–687.
157. Hamond C, Martins G, Lilenbaum W. Pulmonary hemorrhage in horses seroreactive to leptospirosis in Rio de Janeiro. *Brazil. J Vet Intern Med*. 2012;26:1237; author reply 1238.
158. Norman TE, Chaffin MK, Perris EE, et al. Massive pulmonary thromboembolism in six horses. *Equine Vet J*. 2008;40:514–517.
159. Frederick J, Giguere S, Butterworth K, et al. Severe phenylephrine-associated hemorrhage in five aged horses. *J Am Vet Med Assoc*. 2010;237:830–834.
160. Ueno T, Nambo Y, Tajima Y, et al. Pathology of lethal peripartum broad ligament haematoma in 31 Thoroughbred mares. *Equine Vet J*. 2010;42:529–533.
161. Dolente BA, Sullivan EK, Boston R, et al. Mares admitted to a referral hospital for postpartum emergencies: 163 cases (1992–2002). *J Vet Emerg Crit Care*. 2005;15:193–200.
162. Dwyer R. Postpartum deaths in mares. *Equine Dis Q*. 1993;2:5.
163. Kisthardt KK, Schumacher J, Finn-Bodner ST, et al. Severe renal hemorrhage caused by pyelonephritis in 7 horses: clinical and ultrasonographic evaluation. *Can Vet J*. 1999;40:571–576.
164. Larsdotter S, Ley C, Pringle J. Renal pseudoaneurysm as a cause of hematuria in a colt. *Can Vet J*. 2009;50:759–762.
165. Vits L, Araya O, Bustamante H, et al. Idiopathic renal haematuria in a 15-year-old Arabian mare. *Vet Rec*. 2008;162:251–252.
166. Mair TS, Taylor FG, Hillyer MH. Autoimmune haemolytic anaemia in eight horses. *Vet Rec*. 1990;126:51–53.
167. McGovern KF, Lascola KM, Davis E, et al. T-cell lymphoma with immune-mediated anemia and thrombocytopenia in a horse. *J Vet Intern Med*. 2011;25:1181–1185.
168. Reef VB, Dyson SS, Beech J. Lymphosarcoma and associated immune-mediated hemolytic anemia and thrombocytopenia in horses. *J Am Vet Med Assoc*. 1984;184:313–317.
169. Johns IC, Desrochers A, Wotman KL, et al. Presumed immune-mediated hemolytic anemia in two foals with *Rhodococcus equi* infection. *J Vet Emerg Crit Care (San Antonio)*. 2011;21:273–278.
170. Weiss DJ, Moritz A. Equine immune-mediated hemolytic anemia associated with *Clostridium perfringens* infection. *Vet Clin Pathol*. 2003;32:22–26.
171. Anderson FL, Secombe CJ, Lester GD. Clostridial myonecrosis, haemolytic anaemia, hepatopathy, osteitis and transient hypertrophic cardiomyopathy after intramuscular injection in a Thoroughbred gelding. *Aust Vet J*. 2013;91:204–208.
172. Reef VB. *Clostridium perfringens* cellulitis and immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc*. 1983;182:251–254.
173. McConnico RS, Roberts MC, Tompkins M. Penicillin-induced immune-mediated hemolytic anemia in a horse. *J Am Vet Med Assoc*. 1992;201:1402–1403.
174. Step DL, Blue JT, Dill SG. Penicillin-induced hemolytic anemia and acute hepatic failure following treatment of tetanus in a horse. *Cornell Vet*. 1991;81:13–18.
175. Blue JT, Dinsmore RP, Anderson KL. Immune-mediated hemolytic anemia induced by penicillin in horses. *Cornell Vet*. 1987;77:263–276.
176. Robbins RL, Wallace SS, Brunner CJ, et al. Immune-mediated haemolytic disease after penicillin therapy in a horse. *Equine Vet J*. 1993;25:462–465.
177. Thomas HL, Livesey MA. Immune-mediated hemolytic anemia associated with trimethoprim-sulphamethoxazole administration in a horse. *Can Vet J*. 1998;39:171–173.
178. Venner M, Credner N, Lammer M, et al. Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with *Rhodococcus equi*. *Vet Rec*. 2013;173:397.
179. Davis EG, Rush B, Bain F, et al. Neonatal neutropenia in an Arabian foal. *Equine Vet J*. 2003;35:517–520.
180. Leidl W, Cwik S, Schmid DO. [Neonatal isoimmune leukopenia in foals]. *Berl Munch Tierarztl Wochenschr*. 1980;93:141–144.
181. Väänänen L, Shivo H-K, Hewetson M. Cerebral hemorrhage in a pregnant Standardbred mare with Evan's syndrome. *Equine Vet Educ*. 2013;27:353–358.
182. Sockett DC, Traub-Dargatz J, Weiser MG. Immune-mediated hemolytic anemia and thrombocytopenia in a foal. *J Am Vet Med Assoc*. 1987;190:308–310.

183. Jahn P, Doubek J, Doubek M, et al. Idiopathic thrombocytopenic purpura and anaemia in a pregnant mare. *Equine Vet Educ*. 2006;18:80–83.
184. Lubas VL, Ciattini F, Gavazza A. Immune-mediated thrombocytopenia and haemolytic anaemia (Evan's syndrome) in a horse. *Equine Pract*. 1997;19:27–32.
185. Maury W, Oaks JL, Bradley S. Equine endothelial cells support productive infection of equine infectious anemia virus. *J Virol*. 1998;72:9291–9297.
186. More SJ, Aznar I, Bailey DC, et al. An outbreak of equine infectious anaemia in Ireland during 2006: investigation methodology, initial source of infection, diagnosis and clinical presentation, modes of transmission and spread in the Meath cluster. *Equine Vet J*. 2008;40:706–708.
187. More SJ, Aznar I, Myers T, et al. An outbreak of equine infectious anaemia in Ireland during 2006: the modes of transmission and spread in the Kildare cluster. *Equine Vet J*. 2008;40:709–711.
188. Issel CJ, Cook RF, Mealey RH, et al. Equine infectious anemia in 2014: live with it or eradicate it? *Vet Clin North Am Equine Pract*. 2014;30:561–577.
189. Russell KE, Perkins PC, Hoffman MR, et al. Platelets from thrombocytopenic ponies acutely infected with equine infectious anemia virus are activated in vivo and hypofunctional. *Virology*. 1999;259:7–19.
190. Cook RF, Leroux C, Issel CJ. Equine infectious anemia and equine infectious anemia virus in 2013: a review. *Vet Microbiol*. 2013;167:181–204.
191. Cullinane A, Quinlivan M, Nelly M, et al. Diagnosis of equine infectious anaemia during the 2006 outbreak in Ireland. *Vet Rec*. 2007;161:647–652.
192. Quinlivan M, Cook RF, Cullinane A. Real-time quantitative RT-PCR and PCR assays for a novel European field isolate of equine infectious anaemia virus based on sequence determination of the gag gene. *Vet Rec*. 2007;160:611–618.
193. Beard LA, Pelzel AM, Rush BR, et al. *Babesia equi*–induced anemia in a Quarter Horse and subsequent regulatory response. *J Am Vet Med Assoc*. 2013;242:992–996.
194. Short MA, Clark CK, Harvey JW, et al. Outbreak of equine piroplasmosis in Florida. *J Am Vet Med Assoc*. 2012;240:588–595.
195. Wise LN, Pelzel-McCluskey AM, Mealey RH, et al. Equine piroplasmosis. *Vet Clin North Am Equine Pract*. 2014;30:677–693.
196. Ueti MW, Mealey RH, Kappmeyer LS, et al. Re-emergence of the apicomplexan *Theileria equi* in the United States: elimination of persistent infection and transmission risk. *PLoS One*. 2012;7:e44713.
197. Leon A, Pronost S, Tapprest J, et al. Identification of pathogenic *Leptospira* strains in tissues of a premature foal by use of polymerase chain reaction analysis. *J Vet Diagn Invest*. 2006;18:218–221.
198. Frazer ML. Acute renal failure from leptospirosis in a foal. *Aust Vet J*. 1999;77:499–500.
199. Hogan PM, Bernard WV, Kazakevicius PA, et al. Acute renal disease due to *Leptospira interrogans* in a weanling. *Equine Vet J*. 1996;28:331–333.
200. van den Ingh TS, Hartman EG, Bercovich Z. Clinical *Leptospira interrogans* serogroup Australis serovar lora infection in a stud farm in The Netherlands. *Vet Q*. 1989;11:175–182.
201. Dieckmann SM, Winkler M, Groebel K, et al. Haemotrophic *Mycoplasma* infection in horses. *Vet Microbiol*. 2010;145:351–353.
202. Dieckmann SM, Hoelzle K, Dieckmann MP, et al. Occurrence of hemotrophic mycoplasmas in horses with correlation to hematological findings. *Vet Microbiol*. 2012;160:43–52.
203. Gretillat S. L'he´mobartonellose equine au Niger. *Bull Acad Vet Fr*. 1978;51:351–358.
204. Baskurt OK, Meiselman HJ. Susceptibility of equine erythrocytes to oxidant-induced rheologic alterations. *Am J Vet Res*. 1999;60:1301–1306.
205. Robin H, Harley JD. Regulation of methaemoglobinaemia in horse and human erythrocytes. *Aust J Exp Biol Med Sci*. 1967;45:77–88.
206. Medeiros LO, Nurmberger Jr R, Medeiros LF. The special behavior of equine erythrocytes connected with the methemoglobin regulation. *Comp Biochem Physiol B*. 1984;78:869–871.
207. Mahaffey E, Smith JE. Species differences in erythrocyte glutathione reduction rates after oxidation with t-butyl hydroperoxide. *Int J Biochem*. 1975;6:853–854.
208. Smith JE, Kiefer S, Lee M. Glutathione reduction and other enzyme activities in equine erythrocytes. *Comp Biochem Physiol B*. 1972;43:413–417.
209. Boyer JD, Breeden DC, Brown DL. Isolation, identification, and characterization of compounds from *Acer rubrum* capable of oxidizing equine erythrocytes. *Am J Vet Res*. 2002;63:604–610.
210. George LW, Divers TJ, Mahaffey EA, et al. Heinz body anemia and methemoglobinemia in ponies given red maple (*Acer rubrum* L.) leaves. *Vet Pathol*. 1982;19:521–533.
211. Agrawal K, Ebel JG, Altier C, et al. Identification of protoxins and a microbial basis for red maple *(Acer rubrum)* toxicosis in equines. *J Vet Diagn Invest*. 2013;25:112–119.
212. Alward A, Corriher CA, Barton MH, et al. Red maple *(Acer rubrum)* leaf toxicosis in horses: a retrospective study of 32 cases. *J Vet Intern Med*. 2006;20:1197–1201.
213. McConnico RS, Brownie CF. The use of ascorbic acid in the treatment of 2 cases of red maple *(Acer rubrum)*–poisoned horses. *Cornell Vet*. 1992;82:293–300.
214. Witonsky SG, Grubbs ST, Andrews FM. A case of red maple *(Acer rubrum)* toxicity associated with fallen branches. *Equine Vet Educ*. 2001;13:119–123.
215. O'Callaghan DK, Schall SA, Brimingham SSW, et al. Protective effects of ascorbic acid and α-Tocopherol on the in vitro oxidation of equine erythrocytes caused by extracts of wilted red maple leaves. *J Equine Vet Sci*. 2015;35:940–946.
216. Schiffl H. Sodium bicarbonate infusion for prevention of acute kidney injury: no evidence for superior benefit, but risk for harm? *Int Urol Nephrol*. 2015;47:321–326.
217. Wetz AJ, Brauer A, Quintel M, et al. Does sodium bicarbonate infusion really have no effect on the incidence of acute kidney injury after cardiac surgery? A prospective observational trial. *Crit Care*. 2015;19:183.
218. Bozorgmanesh R, Magdesian KG, Rhodes DM, et al. Hemolytic anemia in horses associated with ingestion of *Pistacia* leaves. *J Vet Intern Med*. 2015;29:410–413.
219. Walter KM, Moore CE, Bozorgmanesh R, et al. Oxidant-induced damage to equine erythrocytes from exposure to *Pistacia atlantica, Pistacia terebinthus*, and *Pistacia chinensis*. *J Vet Diagn Invest*. 2014;26:821–826.
220. Pearson W, Boermans HJ, Bettger WJ, et al. Association of maximum voluntary dietary intake of freeze-dried garlic with Heinz body anemia in horses. *Am J Vet Res*. 2005;66:457–465.
221. Pierce KR, Joyce JR, England RB, et al. Acute hemolytic anemia caused by wild onion poisoning in horses. *J Am Vet Med Assoc*. 1972;160:323–327.
222. Lewis N, Racklyeft DJ. Mass envenomation of a mare and foal by bees. *Aust Vet J*. 2014;92:141–148.
223. Dickinson CE, Traub-Dargatz JL, Dargatz DA, et al. Rattlesnake venom poisoning in horses: 32 cases (1973–1993). *J Am Vet Med Assoc*. 1996;208:1866–1871.
224. Conway EM. HUS and the case for complement. *Blood*. 2015;126:2085–2090.
225. Dickinson CE, Gould DH, Davidson AH, et al. Hemolytic-uremic syndrome in a postpartum mare concurrent with encephalopathy in the neonatal foal. *J Vet Diagn Invest*. 2008;20:239–242.
226. Morris CF, Robertson JL, Mann PC, et al. Hemolytic uremic-like syndrome in two horses. *J Am Vet Med Assoc*. 1987;191:1453–1454.

227. MacLachlan NJ, Divers TJ. Hemolytic anemia and fibrinoid change of renal vessels in a horse. *J Am Vet Med Assoc*. 1982;181:716–717.
228. Harvey JW. Pathogenesis, laboratory diagnosis, and clinical implications of erythrocyte enzyme deficiencies in dogs, cats, and horses. *Vet Clin Pathol*. 2006;35:144–156.
229. Dixon PM, McPherson EA, Muir A. Familial methaemoglobinaemia and haemolytic anaemia in the horse associated with decreased erythrocytic glutathione reductase and glutathione. *Equine Vet J*. 1977;9:198–201.
230. Tennant BC, Evans CD, Kaneko JJ, et al. Intravascular hemolysis associated with hepatic failure in the horse. *Calif Vet*. 1972;27:15–18.
231. McSherry BJ, Roe CK, Milne FJ. The hematology of phenothiazine poisoning in horses. *Can Vet J*. 1966;7:3–12.
232. Baird JD, Hutchins DR, Lepherd EE. Phenothiazine poisoning in a Thoroughbred horse. *Aust Vet J*. 1970;46:496–499.
233. Paradis MR, Breeze RG, Bayly WM, et al. Acute hemolytic anemia after oral administration of L-tryptophan in ponies. *Am J Vet Res*. 1991;52:742–747.
234. Sweeney CR, Whitlock RH, Meirs DA, et al. Complications associated with *Streptococcus equi* infection on a horse farm. *J Am Vet Med Assoc*. 1987;191:1446–1448.
235. Wobeser BK. Anthrax vaccine associated deaths in miniature horses. *Can Vet J*. 2015;56:359–360.
236. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118–121.
237. Heath SE, Geor RJ, Tabel H, et al. Unusual patterns of serum antibodies to *Streptococcus equi* in two horses with purpura hemorrhagica. *J Vet Intern Med*. 1991;5:263–267.
238. Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. *J Immunol*. 1985;135:3134–3137.
239. Roberts MC, Kelly WR. Renal dysfunction in a case of purpura haemorrhagica in a horse. *Vet Rec*. 1982;110:144–146.
240. Gunson DE, Rooney JR. Anaphylactoid purpura in a horse. *Vet Pathol*. 1977;14:325–331.
241. Dujardin CL. Multiple small-intestine intussusceptions: a complication of purpura haemorrhagica in a horse. *Tijdschr Diergeneeskd*. 2011;136:422–426.
242. Kaese HJ, Valberg SJ, Hayden DW, et al. Infarctive purpura hemorrhagica in five horses. *J Am Vet Med Assoc*. 2005;226(11):1893–1898, 1845.
243. Sweeney CR, Timoney JF, Newton JR, et al. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med*. 2005;19:123–134.
244. Madigan JE, Gribble D. Equine ehrlichiosis in northern California: 49 cases (1968–1981). *J Am Vet Med Assoc*. 1987;190: 445–448.
245. Dziegiel B, Adaszek L, Kalinowski M, et al. Equine granulocytic anaplasmosis. *Res Vet Sci*. 2013;95:316–320.
246. Reubel GH, Kimsey RB, Barlough JE, et al. Experimental transmission of *Ehrlichia equi* to horses through naturally infected ticks *(Ixodes pacificus)* from Northern California. *J Clin Microbiol*. 1998;36:2131–2134.
247. Gussmann K, Czech C, Hermann M, et al. *Anaplasma phagocytophilum* infection in a horse from Switzerland with severe neurological symptoms. *Schweiz Arch Tierheilkd*. 2014;156:345–348.
248. Nolen-Walston RD, D'Oench SM, Hanelt LM, et al. Acute recumbency associated with *Anaplasma phagocytophilum* infection in a horse. *J Am Vet Med Assoc*. 2004;224(12):1964–1966, 1931.
249. Butler CM, Nijhof AM, Jongejan F, et al. *Anaplasma phagocytophilum* infection in horses in the Netherlands. *Vet Rec*. 2008;162:216–217.
250. Ziemer EL, Keenan DP, Madigan JE. *Ehrlichia equi* infection in a foal. *J Am Vet Med Assoc*. 1987;190:199–200.
251. Berrington A, Moats R, Lester S. A case of *Ehrlichia equi* in an adult horse in British Columbia. *Can Vet J*. 1996;37:174–175.
252. Davies RS, Madigan JE, Hodzic E, et al. Dexamethasone-induced cytokine changes associated with diminished disease severity in horses infected with *Anaplasma phagocytophilum*. *Clin Vaccine Immunol*. 2011;18:1962–1968.
253. Franzen P, Berg AL, Aspan A, et al. Death of a horse infected experimentally with *Anaplasma phagocytophilum*. *Vet Rec*. 2007;160:122–125.
254. Szeredi L, Hornyak A, Denes B, et al. Equine viral arteritis in a newborn foal: parallel detection of the virus by immunohistochemistry, polymerase chain reaction and virus isolation. *J Vet Med B Infect Dis Vet Public Health*. 2003;50:270–274.
255. Vairo S, Favoreel H, Scagliarini A, et al. Identification of target cells of a European equine arteritis virus strain in experimentally infected ponies. *Vet Microbiol*. 2013;167:235–241.
256. Vairo S, Saey V, Bombardi C, et al. The recent European isolate (08P178) of equine arteritis virus causes inflammation but not arteritis in experimentally infected ponies. *J Comp Pathol*. 2014;151:238–243.
257. Balasuriya UB. Equine viral arteritis. *Vet Clin North Am Equine Pract*. 2014;30:543–560.
258. Dolente BA, Wilkins PA, Boston RC. Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. *J Am Vet Med Assoc*. 2002;220:1034–1038.
259. Dallap BL, Dolente B, Boston RC. Coagulation profiles in 27 horses with large colon volvulus. *J Vet Emerg Crit Care*. 2003;13:215–225.
260. Fielding CL, Pusterla N, Magdesian KG, et al. Rattlesnake envenomation in horses: 58 cases (1992–2009). *J Am Vet Med Assoc*. 2011;238:631–635.
261. Arnold CE, Chaffin MK, Rush BR. Hematuria associated with cystic hematomas in three neonatal foals. *J Am Vet Med Assoc*. 2005;227(5):778–780, 741.
262. Bentz AI, Wilkins PA, MacGillivray KC, et al. Severe thrombocytopenia in 2 thoroughbred foals with sepsis and neonatal encephalopathy. *J Vet Intern Med*. 2002;16:494–497.
263. Fey K, Sasse HHL. Relapsing immune-mediated thrombocytopenia of unknown origin in a stallion. *Equine Vet Educ*. 1998;10:127–132.
264. Humber KA, Beech J, Cudd TA, et al. Azathioprine for treatment of immune-mediated thrombocytopenia in two horses. *J Am Vet Med Assoc*. 1991;199:591–594.
265. Eberspacher E, Stanley SD, Rezende M, et al. Pharmacokinetics and tolerance of transdermal fentanyl administration in foals. *Vet Anaesth Analg*. 2008;35:249–255.
266. Morris DD, Whitlock RH. Relapsing idiopathic thrombocytopenia in a horse. *Equine Vet J*. 1983;15:73–75.
267. Christopherson PW, Insalaco TA, van Santen VL, et al. Characterization of the cDNA encoding alphaIIb and beta3 in normal horses and two horses with Glanzmann thrombasthenia. *Vet Pathol*. 2006;43:78–82.
268. Christopherson PW, van Santen VL, Livesey L, et al. A 10-base-pair deletion in the gene encoding platelet glycoprotein IIb associated with Glanzmann thrombasthenia in a horse. *J Vet Intern Med*. 2007;21:196–198.
269. Sanz MJ, Alvarez A, Piqueras L, et al. Rolipram inhibits leukocyte-endothelial cell interactions in vivo through P- and E-selectin downregulation. *Br J Pharmacol*. 2002;135:1872–1881.
270. Livesey L, Christopherson P, Hammond A, et al. Platelet dysfunction (Glanzmann's thrombasthenia) in horses. *J Vet Intern Med*. 2005;19:917–919.
271. Fry MM, Walker NJ, Blevins GM, et al. Platelet function defect in a thoroughbred filly. *J Vet Intern Med*. 2005;19:359–362.
272. Weiss DJ, Evanson OA, McClenahan D, et al. Effect of a competitive inhibitor of platelet aggregation on experimentally induced laminitis in ponies. *Am J Vet Res*. 1998;59:814–817.
273. Weiss DJ, Evanson OA, McClenahan D, et al. Evaluation of platelet activation and platelet-neutrophil aggregates in ponies with alimentary laminitis. *Am J Vet Res*. 1997;58: 1376–1380.

274. Epstein KL, Brainard BM, Gomez-Ibanez SE, et al. Thrombelastography in horses with acute gastrointestinal disease. *J Vet Intern Med*. 2011;25:307–314.
275. Dallap Schaer BL, Bentz AI, Boston RC, et al. Comparison of viscoelastic coagulation analysis and standard coagulation profiles in critically ill neonatal foals to outcome. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:88–95.
276. Federici AB. Clinical and laboratory diagnosis of VWD. *Hematology Am Soc Hematol Educ Program*. 2014;(1):524–530.
277. Gentry PA, Feldman BF, O'Neill SL, et al. Evaluation of the haemostatic profile in the pre- and post parturient mare, with particular focus on the perinatal period. *Equine Vet J*. 1992;24: 33–36.
278. Smith JM, Meyers KM, Barbee DD, et al. Plasma von Willebrand factor in thoroughbreds in response to high-intensity treadmill exercise. *Am J Vet Res*. 1997;58:71–76.
279. Brooks M, Leith GS, Allen AK, et al. Bleeding disorder (von Willebrand disease) in a quarter horse. *J Am Vet Med Assoc*. 1991;198:114–116.
280. Laan TT, Goehring LS. Sloet van Oldruitenborgh-Oosterbaan MM: Von Willebrand's disease in an eight-day-old quarter horse foal. *Vet Rec*. 2005;157:322–324.
281. Rathgeber RA, Brooks MB, Bain FT, et al. Clinical vignette. Von Willebrand disease in a Thoroughbred mare and foal. *J Vet Intern Med*. 2001;15:63–66.
282. Neff AT. Current controversies in the diagnosis and management of von Willebrand disease. *Ther Adv Hematol*. 2015;6: 209–216.
283. Budde U, Scheppenheim S, Dittmer R. Treatment of the acquired von Willebrand syndrome. *Expert Rev Hematol*. 2015;8:799–818.
284. Geor RJ, Jackson ML, Lewis KD, et al. Prekallikrein deficiency in a family of Belgian horses. *J Am Vet Med Assoc*. 1990;197:741–745.
285. Turrentine MA, Sculley PW, Green EM, et al. Prekallikrein deficiency in a family of Miniature Horses. *Am J Vet Res*. 1986;47:2464–2467.
286. Hinton M, Jones DR, Lewis IM, et al. A clotting defect in an Arab colt foal. *Equine Vet J*. 1977;9:1–3.
287. Henninger RW. Hemophilia A in two related quarter horse colts. *J Am Vet Med Assoc*. 1988;193:91–94.
288. Littlewood JD, Bevan SA, Corke MJ. Haemophilia A (classic haemophilia, factor VIII deficiency) in a Thoroughbred colt foal. *Equine Vet J*. 1991;23:70–72.
289. Archer RK, Allen BV. True haemophilia in horses. *Vet Rec*. 1972;91:655–656.
290. Sanger VL, Mairs RE, Trapp AL. Hemophilia in a foal. *J Am Vet Med Assoc*. 1964;144:259–264.
291. Nossel HL, Archer RK, Macfarlane RG. Equine haemophilia: report of a case and its response to multiple infusions of heterospecific AHG. *Br J Haematol*. 1962;8:335–342.
292. Mills JN, Bolton JR. Haemophilia A in a 3-year-old Thoroughbred horse. *Aust Vet J*. 1983;60:63–64.
293. Duncan E, Collecutt M, Street A. Nijmegen-Bethesda assay to measure factor VIII inhibitors. *Methods Mol Biol*. 2013;992: 321–333.
294. Johnson JS, Soute BA, Olver CS, et al. Defective gamma-glutamyl carboxylase activity and bleeding in Rambouillet sheep. *Vet Pathol*. 2006;43:726–732.
295. McConnico RS, Copedge K, Bischoff KL. Brodifacoum toxicosis in two horses. *J Am Vet Med Assoc*. 1997;211:882–886.
296. Boermans HJ, Johnstone I, Black WD, et al. Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. *Can J Vet Res*. 1991;55:21–27.
297. Ayala I, Rodriguez MJ, Martos N, et al. Fatal brodifacoum poisoning in a pony. *Can Vet J*. 2007;48:627–629.
298. Thijssen HH, van den Bogaard AE, Wetzel JM, et al. Warfarin pharmacokinetics in the horse. *Am J Vet Res*. 1983;44:1192–1196.
299. Rebhun WC, Tennant BC, Dill SG, et al. Vitamin K_3–induced renal toxicosis in the horse. *J Am Vet Med Assoc*. 1984;184: 1237–1239.
300. Maxie G, van Dreumel T, McMaster D, et al. Ontario. Menadione (vitamin K(3)) toxicity in six horses. *Can Vet J*. 1992;33:756–757.
301. Skinner JE, Cadwell-Smith AJ, Biffen JR, et al. Intestinal absorption of different vitamin K compounds in the horse. *J Equine Vet Sci*. 2015;35:387.
302. Terachi T, Inoue Y, Ashihara N, et al. Plasma vitamin K concentration in horses supplemented with several vitamin K homologs. *J Anim Sci*. 2011;89:1056–1061.
303. McDonald GK. Moldy sweetclover poisoning in a horse. *Can Vet J*. 1980;21:250–251.
304. Johns IC, Sweeney RW. Coagulation abnormalities and complications after percutaneous liver biopsy in horses. *J Vet Intern Med*. 2008;22:185–189.
305. Levi M. Disseminated intravascular coagulation. *Crit Care Med*. 2007;35:2191–2195.
306. Bastarache JA, Ware LB, Bernard GR. The role of the coagulation cascade in the continuum of sepsis and acute lung injury and acute respiratory distress syndrome. *Semin Respir Crit Care Med*. 2006;27:365–376.
307. Donahue SMO, Cynthia M. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. *J Vet Emerg Crit Care*. 2005;15:9–16.
308. de Laforcade AM, Freeman LM, Shaw SP, et al. Hemostatic changes in dogs with naturally occurring sepsis. *J Vet Intern Med*. 2003;17:674–679.
309. Dallap BL. Coagulopathy in the equine critical care patient. *Vet Clin North Am Equine Pract*. 2004;20:231–251.
310. Dallap Schaer BL, Wilkins PA, Boston R, et al. Preliminary evaluation of hemostasis in neonatal foals using a viscoelastic coagulation and platelet function analyzer. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:81–87.
311. Armengou L, Monreal L, Tarancon I, et al. Plasma D-dimer concentration in sick newborn foals. *J Vet Intern Med*. 2008;22:411–417.
312. Cotovio M, Monreal L, Armengou L, et al. Fibrin deposits and organ failure in newborn foals with severe septicemia. *J Vet Intern Med*. 2008;22:1403–1410.
313. Monreal L, Angles A, Espada Y, et al. Hypercoagulation and hypofibrinolysis in horses with colic and DIC. *Equine Vet J Suppl*. 2000:19–25.
314. Welch RD, Watkins JP, Taylor TS, et al. Disseminated intravascular coagulation associated with colic in 23 horses (1984–1989). *J Vet Intern Med*. 1992;6:29–35.
315. Cesarini C, Cotovio M, Rios J, et al. Association between necropsy evidence of disseminated intravascular coagulation and hemostatic variables before death in horses with colic. *J Vet Intern Med*. 2015;30:269–275.
316. Squizzato A, Hunt BJ, Kinasewitz GT, et al. Supportive management strategies for disseminated intravascular coagulation. An international consensus. *Thromb Haemost*. 2015;115:896–904.
317. Morris DD, Beech J. Disseminated intravascular coagulation in six horses. *J Am Vet Med Assoc*. 1983;183:1067–1072.
318. Banse H, Holbrook TC, Gilliam L, et al. Right ventricular and saphenous vein thrombi associated with sepsis in a Quarter Horse foal. *J Vet Intern Med*. 2012;26:178–182.
319. Brianceau P, Divers TJ. Acute thrombosis of limb arteries in horses with sepsis: five cases (1988–1998). *Equine Vet J*. 2001;33:105–109.
320. Duggan VE, Holbrook TC, Dechant JE, et al. Diagnosis of aorto-iliac thrombosis in a quarter horse foal using Doppler ultrasound and nuclear scintigraphy. *J Vet Intern Med*. 2004;18:753–756.
321. Moore LA, Johnson PJ, Bailey KL. Aorto-iliac thrombosis in a foal. *Vet Rec*. 1998;142:459–462.
322. Ness SL, Kennedy LA, Slovis NM. Hyperammonemic encephalopathy associated with portal vein thrombosis in a thoroughbred foal. *J Vet Intern Med*. 2013;27:382–386.
323. Schoster A, Anderson ME. Caudal vena cava thrombosis-like syndrome in a horse. *Can Vet J*. 2010;51:891–894.

324. Mair TS, Smith LJ. Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 2: Short-term complications. *Equine Vet J*. 2005;37:303–309.
325. Lankveld DP, Ensink JM, van Dijk P, et al. Factors influencing the occurrence of thrombophlebitis after post-surgical long-term intravenous catheterization of colic horses: a study of 38 cases. *J Vet Med A Physiol Pathol Clin Med*. 2001;48:545–552.
326. Geraghty TE, Love S, Taylor DJ, et al. Assessment of subclinical venous catheter-related diseases in horses and associated risk factors. *Vet Rec*. 2009;164:227–231.
327. Spurlock SL. Providing parenteral nutritional support for equine patients. *Vet Med*. 1990;85:883–890.
328. Myers CJ, Magdesian KG, Kass PH, et al. Parenteral nutrition in neonatal foals: clinical description, complications and outcome in 53 foals (1995–2005). *Vet J*. 2009;181:137–144.
329. Krause JB, McKenzie 3rd HC. Parenteral nutrition in foals: a retrospective study of 45 cases (2000–2004). *Equine Vet J*. 2007;39:74–78.
330. Lopes MA, White 2nd NA. Parenteral nutrition for horses with gastrointestinal disease: a retrospective study of 79 cases. *Equine Vet J*. 2002;34:250–257.
331. Dolente BA, Beech J, Lindborg S, et al. Evaluation of risk factors for development of catheter-associated jugular thrombophlebitis in horses: 50 cases (1993–1998). *J Am Vet Med Assoc*. 2005;227:1134–1141.
332. Gardner SY, Reef VB, Spencer PA. Ultrasonographic evaluation of horses with thrombophlebitis of the jugular vein: 46 cases (1985–1988). *J Am Vet Med Assoc*. 1991;199:370–373.
333. Russell TM, Kearney C, Pollock PJ. Surgical treatment of septic jugular thrombophlebitis in nine horses. *Vet Surg*. 2010;39:627–630.
334. Gilbert GH. Laryngeal hemiplegia following jugular injury. *J Am Vet Med Assoc*. 1972;161:1686–1687.
335. Moreau P, Lavoie JP. Evaluation of athletic performance in horses with jugular vein thrombophlebitis: 91 cases (1988–2005). *J Am Vet Med Assoc*. 2009;235:1073–1078.
336. Wagenaar G. [Iron dextran administered to horses (author's transl)]. *Tijdschr Diergeneeskd*. 1975;100:562–563.
337. Brommer H. Sloet van Oldruitenborgh-Oosterbaan MM: Iron deficiency in stabled Dutch Warmblood foals. *J Vet Intern Med*. 2001;15:482–485.
338. Divers TJ, Warner A, Vaala WE, et al. Toxic hepatic failure in newborn foals. *J Am Vet Med Assoc*. 1983;183:1407–1413.
339. Swerczek TW, Crowe MW. Hepatotoxicosis in neonatal foals. *J Am Vet Med Assoc*. 1983;183:388.
340. Mullaney TP, Brown CM, Watson GL, et al. Suspected hepatotoxicity in neonatal foals: preliminary report of an emerging syndrome. *Vet Rec*. 1984;114:115–117.
341. Beeler-Marfisi J, Gallastegui Menoyo A, Beck A, et al. Gelatinous marrow transformation and hematopoietic atrophy in a miniature horse stallion. *Vet Pathol*. 2011;48:451–455.
342. Sherlock CE, Mair TS, Murray RC, et al. Magnetic resonance imaging features of serous atrophy of bone marrow fat in the distal limb of three horses. *Vet Radiol Ultrasound*. 2010;51:607–613.
343. Weiss G. Anemia of chronic disorders: new diagnostic tools and new treatment strategies. *Semin Hematol*. 2015;52:313–320.
344. Oliveira-Filho JP, Badial PR, Cunha PH, et al. Lipopolysaccharide infusion up-regulates hepcidin mRNA expression in equine liver. *Innate Immun*. 2012;18:438–446.
345. Piercy RJ, Swardson CJ, Hinchcliff KW. Erythroid hypoplasia and anemia following administration of recombinant human erythropoietin to two horses. *J Am Vet Med Assoc*. 1998;212:244–247.
346. Woods PR, Campbell G, Cowell RL. Nonregenerative anaemia associated with administration of recombinant human erythropoietin to a Thoroughbred racehorse. *Equine Vet J*. 1997;29:326–328.
347. Lonnberg M, Bondesson U, Cormant F, et al. Detection of recombinant human EPO administered to horses using MAIIA lateral flow isoform test. *Anal Bioanal Chem*. 2012;403:1619–1628.
348. Zeng Y, Katsanis E. The complex pathophysiology of acquired aplastic anaemia. *Clin Exp Immunol*. 2015;180:361–370.
349. Lavoie JP, Morris DD, Zinkl JG, et al. Pancytopenia caused by bone marrow aplasia in a horse. *J Am Vet Med Assoc*. 1987;191:1462–1464.
350. Berggren PC. Aplastic anemia in a horse. *J Am Vet Med Assoc*. 1981;179:1400–1402.
351. Cudmore L, Jalim S, Williamson MM, et al. Pancytopenia in two horses following administration of potentiated sulfonamide antimicrobials. *Equine Vet Educ*. 2015;27:519–523.
352. Toribio RE, Bain FT, Mrad DR, et al. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc*. 1998;212:697–701.
353. Piercy RJ, Hinchcliff KW, Reed SM. Folate deficiency during treatment with orally administered folic acid, sulphadiazine and pyrimethamine in a horse with suspected equine protozoal myeloencephalitis (EPM). *Equine Vet J*. 2002;34:311–316.
354. Hart KA, Barton MH, Williams KJ, et al. Multinodular pulmonary fibrosis, pancytopenia and equine herpesvirus-5 infection in a Thoroughbred gelding. *Equine Veterinary Education*. 2008;20:470–476.
355. Angel KL, Spano JS, Schumacher J, et al. Myelophthisic pancytopenia in a pony mare. *J Am Vet Med Assoc*. 1991;198:1039–1042.
356. Ayala I, Rodriguez MJ, Martos N, et al. Nonfatal splenic haematoma and pancytopenia in an ass. *Aust Vet J*. 2004;82:479–480.
357. Archer RK, Miller WC. A case of idiopathic hypoplastic anaemia in a two-year-old Thoroughbred filly. *Vet Rec*. 1965;77:538–540.
358. Milne EM, Pyrah ITG, Smith KC, et al. Aplastic anemia in a Clydesdale foal: a case report. *J Equine Vet Sci*. 1995;15:129–131.
359. Vetter J. A biological hazard of our age: bracken fern [*Pteridium aquilinum* (L.) Kuhn]—a review. *Acta Vet Hung*. 2009;57:183–196.
360. Kohn CW, Swardson C, Provost P, et al. Myeloid and megakaryocytic hypoplasia in related Standardbreds. *J Vet Intern Med*. 1995;9:315–323.
361. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2015;90:162–173.
362. McFarlane D, Sellon DC, Parker B. Primary erythrocytosis in a 2-year-old Arabian gelding. *J Vet Intern Med*. 1998;12:384–388.
363. Steiger R, Feige K. [Case report: polycythemia in a horse]. *Schweiz Arch Tierheilkd*. 1995;137:306–311.
364. McMullin MF. The classification and diagnosis of erythrocytosis. *Int J Lab Hematol*. 2008;30:447–459.
365. Belli CB, Baccarin RY, Ida KK, et al. Appropriate secondary absolute erythrocytosis in a horse. *Vet Rec*. 2011;169:609.
366. McKeever KH. The endocrine system and the challenge of exercise. *Vet Clin North Am Equine Pract*. 2002;18:321–353. vii.
367. Greene HM, Hurson MJ, Wickler SJ. Haematological and respiratory gas changes in horses and mules exercised at altitude (3800 m). *Equine Vet J Suppl*. 2006:551–556.
368. Wickler SJ, Anderson TP. Hematological changes and athletic performance in horses in response to high altitude (3,800 m). *Am J Physiol Regul Integr Comp Physiol*. 2000;279:R1176–R1181.
369. McKeever KH, Agans JM, Geiser S, et al. Low dose exogenous erythropoietin elicits an ergogenic effect in Standardbred horses. *Equine Vet J Suppl*. 2006:233–238.
370. Lennox TJ, Wilson JH, Hayden DW, et al. Hepatoblastoma with erythrocytosis in a young female horse. *J Am Vet Med Assoc*. 2000;216:718–721. 685.
371. Koch TG, Wen X, Bienzle D. Lymphoma, erythrocytosis, and tumor erythropoietin gene expression in a horse. *J Vet Intern Med*. 2006;20:1251–1255.
372. Axon JE, Russell CM, Begg AP, et al. Erythrocytosis and pleural effusion associated with a hepatoblastoma in a Thoroughbred yearling. *Aust Vet J*. 2008;86:329–333.

373. Cook G, Divers TJ, Rowland PH. Hypercalcaemia and erythrocytosis in a mare associated with a metastatic carcinoma. *Equine Vet J*. 1995;27:316–318.
374. Roby KA, Beech J, Bloom JC, et al. Hepatocellular carcinoma associated with erythrocytosis and hypoglycemia in a yearling filly. *J Am Vet Med Assoc*. 1990;196:465–467.
375. Knych HK, Arthur RM, Mitchell MM, et al. Pharmacokinetics and selected pharmacodynamics of cobalt following a single intravenous administration to horses. *Drug Test Anal*. 2015;7:619–625.
376. Leadon D, Farrelly B, Fogarty U, et al. Platelet counting in diagnosis of *Rhodococcus equi*. *Vet Rec*. 1988;123:279.
377. Knottenbelt DC. *Rhodococcus equi* infection in foals: a report of an outbreak on a Thoroughbred stud in Zimbabwe. *Vet Rec*. 1993;132:79–85.
378. Bazzano M, Giannetto C, Fazio F, et al. Hemostatic profile during late pregnancy and early postpartum period in mares. *Theriogenology*. 2014;81:639–643.
379. Haematopoietic (round cell) neoplasms. In: Knottenbelt DC, Patterson-Kane JC, Snalune KL, eds. *Clinical equine oncology*. Elsevier Ltd. 2015:342–362.
380. Finley MR, Rebhun WC, Dee A, et al. Paraneoplastic pruritus and alopecia in a horse with diffuse lymphoma. *J Am Vet Med Assoc*. 1998;213:102–104.
381. Mair TS, Yeo SP, Lucke VM. Hypercalcaemia and soft tissue mineralisation associated with lymphosarcoma in two horses. *Vet Rec*. 1990;126:99–101.
382. Marr CM, Love S, Pirie HM. Clinical, ultrasonographic and pathological findings in a horse with splenic lymphosarcoma and pseudohyperparathyroidism. *Equine Vet J*. 1989;21:221–226.
383. Saulez MN, Schlipf Jr JW, Cebra CK, et al. Use of chemotherapy for treatment of a mixed-cell thoracic lymphoma in a horse. *J Am Vet Med Assoc*. 2004;224:733–738. 699.
384. Finding EJT, Ciasca T, Pinilla MJ, et al. Diagnosis, treatment and outcome of spinal lymphoma in a pony. *Equine Vet Educ*. 2014;26:303–305.
385. Lester GD, Alleman AR, Raskin RE, et al. Pancytopenia secondary to lymphoid leukemia in three horses. *J Vet Intern Med*. 1993;7:360–363.
386. Morris DD, Bloom JC, Roby KA, et al. Eosinophilic myeloproliferative disorder in a horse. *J Am Vet Med Assoc*. 1984;185:993–996.
387. Kelton DR, Holbrook TC, Gilliam LL, et al. Bone marrow necrosis and myelophthisis: manifestations of T-cell lymphoma in a horse. *Vet Clin Pathol*. 2008;37:403–408.
388. Clark P, Cornelisse CJ, Schott HC, et al. Myeloblastic leukaemia in a Morgan horse mare. *Equine Vet J*. 1999;31:446–448.
389. Boudreaux MK, Blue JT, Durham SK, et al. Intravascular leukostasis in a horse with myelomonocytic leukemia. *Vet Pathol*. 1984;21:544–546.
390. Spier SJ, Madewell BR, Zinkl JG, et al. Acute myelomonocytic leukemia in a horse. *J Am Vet Med Assoc*. 1986;188:861–863.
391. Burkhardt E, von Saldern F, Huskamp B. Monocytic leukemia in a horse. *Vet Pathol*. 1984;21:394–398.
392. Forbes G, Feary DJ, Savage CJ, et al. Acute myeloid leukaemia (M6B: pure acute erythroid leukaemia) in a Thoroughbred foal. *Aust Vet J*. 2011;89:269–272.
393. Rendle DI, Durham AE, Thompson JC, et al. Clinical, immunophenotypic and functional characterisation of T-cell leukaemia in six horses. *Equine Vet J*. 2007;39:522–528.
394. Dascanio JJ, Zhang CH, Antczak DF, et al. Differentiation of chronic lymphocytic leukemia in the horse. A report of two cases. *J Vet Intern Med*. 1992;6:225–229.
395. Roberts MC. A case of primary lymphoid leukaemia in a horse. *Equine Vet J*. 1977;9:216–219.
396. Pusterla N, Stacy BA, Vernau W, et al. Immunoglobulin A monoclonal gammopathy in two horses with multiple myeloma. *Vet Rec*. 2004;155:19–23.
397. Barton MH, Sharma P, LeRoy BE, et al. Hypercalcemia and high serum parathyroid hormone-related protein concentration in a horse with multiple myeloma. *J Am Vet Med Assoc*. 2004;225:409–413. 376.
398. Kim DY, Taylor HW, Eades SC, et al. Systemic AL amyloidosis associated with multiple myeloma in a horse. *Vet Pathol*. 2005;42:81–84.
399. Warren AL, Summers BA. Epithelioid variant of hemangioma and hemangiosarcoma in the dog, horse, and cow. *Vet Pathol*. 2007;44:15–24.
400. Johns I, Stephen JO, Del Piero F, et al. Hemangiosarcoma in 11 young horses. *J Vet Intern Med*. 2005;19:564–570.
401. Southwood LL, Schott 2nd HC, Henry CJ, et al. Disseminated hemangiosarcoma in the horse: 35 cases. *J Vet Intern Med*. 2000;14:105–109.
402. Dunkel BM, Del Piero E, Kraus BM, et al. Congenital cutaneous, oral, and periarticular hemangiosarcoma in a 9-day-old Rocky Mountain horse. *J Vet Intern Med*. 2004;18:252–255.
403. Gumber S, Baia P, Wakamatsu N. Vulvar epithelioid hemangiosarcoma with solar elastosis in a mare. *J Vet Diagn Invest*. 2011;23:1033–1036.
404. Sansom J, Donaldson D, Smith K, et al. Haemangiosarcoma involving the third eyelid in the horse: a case series. *Equine Vet J*. 2006;38:277–282.
405. Arenas-Gamboa AM, Mansell J. Epithelioid haemangiosarcoma in the ocular tissue of horses. *J Comp Pathol*. 2011;144:328–333.
406. Ladd SM, Crisman MV, Duncan R, et al. Central nervous system hemangiosarcoma in a horse. *J Vet Intern Med*. 2005;19:914–916.
407. Cian F, Stewart J, Minshall GJ, et al. What is your diagnosis? Swelling of the left antebrachium and carpus in a horse. *Vet Clin Pathol*. 2015;44(4):609–610.
408. Recknagel S, Nicke M, Schusser GF. [Diagnostic assessment of peritoneal fluid cytology in horses with abdominal neoplasia]. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2012;40:85–93.
409. Johnson GC, Miller MA, Floss JL, et al. Histologic and immunohistochemical characterization of hemangiomas in the skin of seven young horses. *Vet Pathol*. 1996;33:142–149.
410. Hargis AM, McElwain TF. Vascular neoplasia in the skin of horses. *J Am Vet Med Assoc*. 1984;184:1121–1124.
411. Holzhausen L, Nowak M, Junginger J, et al. Synovial hemangioma in an adult horse. *J Vet Diagn Invest*. 2012;24:427–430.
412. Junginger J, Rotting A, Staszyk C, et al. Identification of equine cutaneous lymphangioma by application of a lymphatic endothelial cell marker. *J Comp Pathol*. 2010;143:57–60.
413. Sanchez B, Nieto A, de Ruiz Leon MA, et al. Metastatic lymphangiosarcoma in a horse. *Vet Pathol*. 2002;39:266–268.
414. Puff C, Herder V, Philipp A, et al. Lymphangiosarcoma in the nictitating membrane of a horse. *J Vet Diagn Invest*. 2008;20:108–110.
415. Ijzer J, van den Ingh TS. Lymphangiosarcoma in a horse. *J Comp Pathol*. 2000;122:312–316.
416. Turk JR, Gallina AM, Liu IM, et al. Cystic lymphangioma in a colt. *J Am Vet Med Assoc*. 1979;174:1228–1230.
417. Gehlen H, Wohlsein P. Cutaneous lymphangioma in a young Standardbred mare. *Equine Vet J*. 2000;32:86–88.
418. Platt H. Vascular malformations and angiomatous lesions in horses: a review of 10 cases. *Equine Vet J*. 1987;19:500–504.

CHAPTER 16

Disorders of the Endocrine System

Disorders of Calcium and Phosphorus

Ramiro E. Toribio

Calcium and phosphorus have structural (mechanical) and nonstructural (ionic) functions, and their concentrations in the extracellular and intracellular compartments are influenced by physiologic and pathologic conditions. Disorders of calcium and phosphorus metabolism are frequent in horses and foals with various diseases, infectious and noninfectious, acute and chronic, and in many instances their blood concentrations have been associated with disease severity and outcome.[1] Progress in elucidating how equine pathologies alter calcium and phosphorus homeostasis has been made[1-7]; however, major knowledge gaps still exist. These minerals are highly interactive with other ions (Mg^{2+}, Na^{+}, K^{+}) and analytes (glucose), and imbalances should be assessed in the context of laboratory and clinical findings.

CALCIUM

Calcium is essential for physiologic processes such as muscle contraction, neuromuscular excitability, blood coagulation, enzyme activation, hormone secretion, cell division, and cell membrane stability.[8] It is also involved in processes leading to cell injury and cell death, including free radical production, protease activation, vasoconstriction, and apoptosis. Because of the importance of calcium in intracellular and extracellular processes, maintenance of steady calcium concentrations is essential.[9]

Distribution and Physical Properties of Calcium

Calcium is found in three main compartments: the *skeleton,* the *soft tissues,* and the *extracellular fluid.* The skeleton contains approximately 99% of the total body calcium as hydroxyapatite crystals ($Ca10(PO^4)6(OH)^2$). As part of the skeleton (structural function), calcium provides support against gravity, protection of vital internal organs (brain, spinal cord, thoracic organs), and a niche for blood-forming elements. The skeleton also acts as a reservoir for calcium. The nonstructural functions are related to calcium as a regulatory ion. The remaining 1% of total body calcium is present in cell membranes, mitochondria, endoplasmic reticulum (0.9%), and extracellular fluid (0.1%).[8] In blood, virtually all calcium is in plasma, and within plasma calcium exists as a free (or ionized) form, is bound to proteins (40%–45%), or is complexed to anions such as citrate, bicarbonate, phosphate, and lactate (5%–10%) (Fig. 16.1).[8,10] In horses, serum ionized calcium represents 50% to 58% of the total serum calcium concentration.[4,11,12] Free, unbound, or ionized calcium (Ca^{2+}) is the biologically active form of calcium. Of the protein-bound calcium, approximately 80% is associated with albumin and 20% with globulins.

Plasma Ca^{2+} binds to anionic proteins (albumin), and its affinity for negatively charged sites is pH dependent. During acidosis, Ca^{2+} binding to albumin is reduced due to increased H^{+} concentrations, leading to higher Ca^{2+} concentrations; during alkalosis, Ca^{2+} concentrations are lower. Total calcium concentrations remain unchanged. Hypoalbuminemia results in total hypocalcemia (pseudohypocalcemia), with Ca^{2+} concentrations remaining within the normal range unless the primary cause of hypoabuminemia is also altering calcium homeostasis (e.g., as with sepsis or gastrointestinal disease). Plasma and serum calcium concentrations are lower in foals than in adult horses.[13]

Cytosolic Ca^{2+} (Ca^{2+}_i) concentrations are very low (10^{-6}–10^{-7} M) compared with extracellular Ca^{2+} concentrations (10^{-3} M). This 10,000-fold Ca^{2+} gradient between extracellular and intracellular compartments increases the utility of calcium for signal transduction. Approximately 95% of Ca^{2+}_i is sequestered within the endoplasmic reticulum and mitochondria (see Fig. 16.1). Mechanisms that control changes in Ca^{2+}_i concentrations include Ca^{2+} channels in the plasma membrane (PM), the endoplasmic reticulum (ER), and the sarcoplasmic reticulum (SR). Some of the mechanisms that remove Ca^{2+}_i from the cytosol include Ca^{2+} ATPases and Na^{+}/Ca^{2+} exchangers in the PM, ER, and SR. The mitochondria are also important in regulating Ca^{2+}_i by providing energy for active calcium transport and by storing Ca^{2+}_i.

The release of calcium from intracellular stores depends on messenger-activated channels present in the ER, SR, and mitochondria. For example, by interacting with specific cell membrane receptors, hormones may activate phospholipase C to hydrolyze phosphatidylinositol 4,5-biphosphate to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. IP_3 is highly diffusible and interacts with IP_3 receptors on the ER and SR, opening calcium channels and increasing Ca^{2+}_i concentrations. The ryanodine receptors are intracellular calcium channels present in excitable cells (myocytes, neurons), and their activation mediates calcium-induced calcium release (CICR) from the sarcoplasmic reticulum.

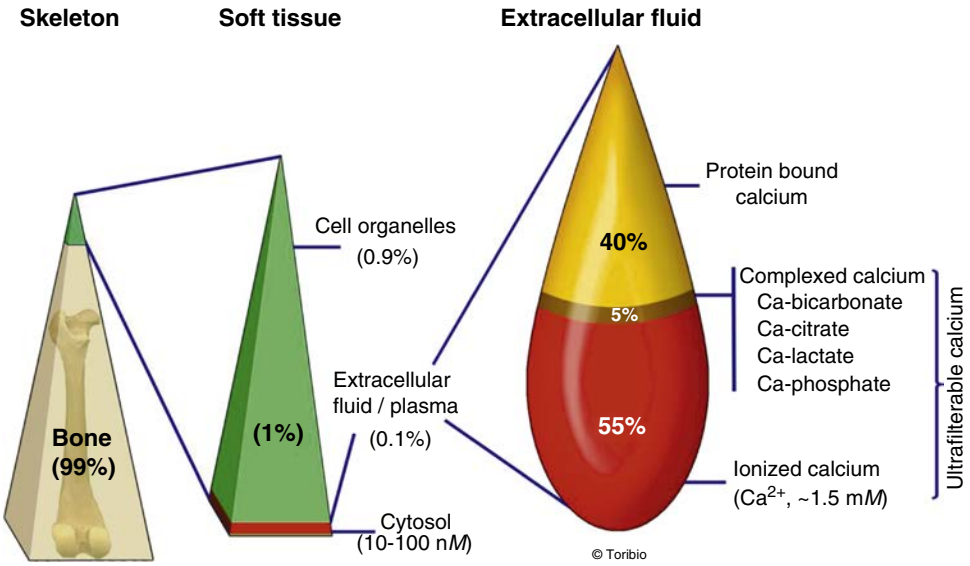


FIG. **16.1** Calcium distribution in the body. The skeleton contains approximately 99% of the total body calcium. The remaining 1% of calcium is present in the cell membrane, mitochondria, endoplasmic reticulum, and extracellular fluid. In blood, calcium exists as a free or ionized form (Ca^{2+}), bound to proteins, and complexed to anions such as citrate, bicarbonate, phosphate, and lactate. In horses, serum ionized calcium represents 50% to 58% of the total serum calcium concentration.[4,11,12] (Courtesy Dr. R. E. Toribio, The Ohio State University.)

Ca^{2+} channels (slow channels) are slightly permeable to Na^+ and are abundant in cardiac and smooth muscle where they regulate action potentials. Because Ca^{2+} channel ion kinetics are slow, depolarization in smooth muscle cells is slow and Ca^{2+} dependent. In contrast, Na^+ channels (fast channels) are abundant in skeletal muscle and neurons where they regulate neurotransmitter release and rapid cell depolarization. During hypocalcemia, fast Na^+ channels open with small changes in the membrane potential, rendering nerve and muscle fibers very excitable. This is associated with hyperexcitability, muscle fasciculations, and tetany. Extracellular Ca^{2+} binds to Na^+ channels to reduce Na^+ entry, increasing the depolarization threshold, thus stabilizing the cell membrane. This is the mechanism by which Ca^{2+} antagonizes the effects of hyperkalemia.

In skeletal muscle, almost all Ca^{2+} required for excitation and contraction comes from the SR. In contrast, in the smooth muscle cell, the SR is a rudimentary organelle, and most of the Ca^{2+} required for contraction comes from the extracellular fluid. This has clinical implications and explains why hypocalcemia frequently results in ileus and hypotension and can contribute to conditions such as retained fetal membranes.

Ca^{2+} (factor IV) is necessary for the process of coagulation. Ca^{2+} is a cofactor for factors II, VII, IX, X, XI, XII, and XIII (procoagulant) and proteins C, S, and Z (anticoagulant). Of interest, vitamin K–dependent factors (II, VII, IX, X, proteins C, S, Z) contain γ-carboxylated glutamic acid (Gla) residues that allow them to bind Ca^{2+} to perform their specific functions. The Gla residues of osteocalcin, a bone protein secreted by osteoblasts, bind to calcium in hydroxyapatite crystals to facilitate conformational changes and bone formation. The Ca^{2+} concentrations required for coagulation are minimal, and low Ca^{2+} concentrations do not seem to interfere with the clotting process. However, prolonged or severe hypercalcemia may reduce coagulation times.[14]

TABLE **16.1** Calcium and Phosphorus Requirements in Horses[15]

	Percent in Diet		Daily (g)	
	Ca	P	Ca	P
Foals (less than 6 months)	0.80	0.55	33	20
Weanlings	0.60	0.45	34	25
Yearlings	0.50	0.35	31	22
Two-year-old	0.40	0.30	25	17
Mare, late pregnancy	0.45	0.30	34	23
Mare, lactation	0.45	0.30	50	34
Mature horses	0.30	0.20	23	14

Adapted from Schryver HF, Hintz HF Minerals. In Robinson NE (ed.): *Current Therapy in Equine Medicine*, 2nd ed. Philadelphia: W.B. Saunders, 1987: 393-405.[15]

Requirements

Calcium and phosphorus requirements in horses and ponies depend on the age, physiologic status, and amount of work or exercise performed (Table 16.1). Horses do not have a nutritional drive to meet their calcium needs, which are highly dependent on dietary intake. However, there appears to be a nutritional drive for phosphorus, as animals with phosphorus deficiency will eat or lick foreign materials (dirt, rocks, and bones) in a condition known as pica. Because extracellular Ca^{2+} is under the homeostatic control of several factors, serum Ca^{2+} concentration is not a reliable indicator of dietary calcium intake. An acceptable diet for horses must have 0.15% to 1.5% of calcium and 0.15% to 0.6% of phosphorus in feed dry matter (DM) (Table 16.2). A calcium:phosphorus ratio less than 1:1 may have negative effects on calcium absorption and skeletal development, whereas calcium:phosphorus ratios as high as 6:1 for growing equids may not be detrimental if

TABLE **16.2 Acceptable Ranges of Minerals and Vitamins in Feed of Horses**[16,17,36,37,51]

	In Dry Matter
Ca (%)	0.25–1.5
P (%)	0.15–0.6
Mg (%)	0.08–0.16 (14 mg/kg body weight)
Vitamin D (IU/kg)	300–800

TABLE **16.3 Calcium and Phosphorus Content (%) in Some Mineral Supplements**[1,15,23,25,43,46,214]

	Ca (%)	P (%)
Bone meal	30	13
Calcium carbonate	39	0
Defluorinated phosphate	32	18
Dicalcium phosphate	22	19
Ground limestone	34	0
Monocalcium phosphate	16	22
Monobasic sodium phosphate[a]	0	23
Dibasic sodium phosphate[a]	0	22
Monobasic potassium phosphate[a]	0	23
Dibasic potassium phosphate[a]	0	18
Calcium gluconate 23%[b]	2.14	0

Phosphorus bioavailability for these supplements is 40%–50%.
[a]Phosphorus content based on chemical-grade anhydrous salts. Sodium phosphate enemas contain monobasic and dibasic sodium phosphate.
[b]Elemental calcium calculated on the basis of molecular weight of calcium gluconate hemicalcium salt. Each mL of the 23% parenteral solution contains 21.4 mg of elemental calcium.

phosphorus intake is adequate.[18] Adult horses should receive approximately 40 mg of calcium/kg of body weight (BW) per day. These requirements depend on the physiologic status of the animal. Pregnant mares require around 50 to 60 g of calcium/day, and lactating mares and growing horses may require 50 to 75 g of calcium/day.

The daily calcium requirements of the lactating mare at the onset of lactation are double the maintenance amount. Mares' milk calcium concentration ranges from 1.3 g/L of fluid milk during the first 2 weeks of lactation to 0.8 g/L of milk during weeks 15 to 17.[19,20] For a 500-kg mare producing 15 L of milk/day with a calcium concentration of 1.2 g/L of milk, this represents a demand of 36 g of calcium (15 kg of milk × 1.2 g of calcium × 50% absorption) a day, in addition to her maintenance needs.

It has been estimated that the average horse must absorb 20 to 25 mg of calcium and 10 to 12 mg of phosphorus/kg BW/day to balance losses.[21-23] According to the American National Research Council (NRC),[24] the maximum tolerable content of dietary phosphorus in horses fed adequate amounts of calcium is 1%. Calcium and phosphate content in some mineral supplements and equine feeds are presented in Tables 16.3 and 16.4.

Absorption

Horses absorb a larger proportion of dietary calcium compared with other species.[26] Horses absorb calcium and phosphorus with high efficiency and with little effect of age.[23,26] Horses fed

TABLE **16.4 Mineral Composition of Some Equine Feeds on a Dry Matter Basis**[16]

Source	Ca (%)	P (%)	Mg (%)
Alfalfa	1.71	0.30	0.36
Alfalfa hay	1.41	0.21	0.34
Timothy	0.40	0.26	0.16
Timothy hay	0.51	0.29	0.13
Bluegrass	0.50	0.4	0.18
Oat hay	0.32	0.25	0.29
Orchard grass	0.25	0.39	0.31
Barley	0.05	0.37	0.15
Corn	0.05	0.60	0.03
Oats	0.09	0.38	0.16
Wheat	0.05	0.42	0.14
Cottonseed meal	0.18	1.22	0.59
Linseed meal	0.43	0.90	0.67
Skim milk	1.36	1.09	0.13
Soybean meal	0.40	0.71	0.31
Molasses, cane	1.10	0.15	0.47
Wheat bran	0.14	1.27	0.63

Adapted from the National Academy of Sciences: *Nutrient Requirements of Horses,* 5th ed. Washington, DC: National Research Council, 1989.[16]

TABLE **16.5 Availability (%) of Calcium and Phosphorus in Some Equine Feeds and Supplements***

Source	Ca	P
Corn	—	38
Timothy hay	70	42
Alfalfa hay	77	38
Milk products	77	57
Wheat bran	—	34
Limestone	67	—
Dicalcium phosphate	73	44
Bone meal	71	46
Monosodium phosphate	—	47

Adapted from Schryver HF, Hintz HF Minerals. In Robinson NE (ed.): *Current Therapy in Equine Medicine* 2nd ed. Philadelphia: W.B. Saunders, 1987: 393-405.[15]

diets with adequate amounts of calcium absorb 50% to 75% of the calcium and less than half of the phosphorus present.[23,27] Calcium in most forages for horses is more than 50% digestible (Table 16.5). The efficiency of calcium absorption is inversely related to the calcium content of the diet. The proximal half of the small intestine is the main site for calcium absorption in the horse, followed by the distal small intestine and the dorsal colon (Fig. 16.2).[23,28] The amount of calcium absorbed in the dorsal colon is minimal, and the cecum and ventral colon are mainly secretory sites for calcium (see Fig. 16.2). Intestinal absorption of calcium is paracellular and transcellular. Paracellular absorption is passive and driven by high luminal calcium concentrations. Transcellular calcium absorption is an active process mediated by epithelial calcium channels for apical uptake, calcium-binding proteins (calbindins) to move calcium across the cell, and basolateral proteins for extrusion.

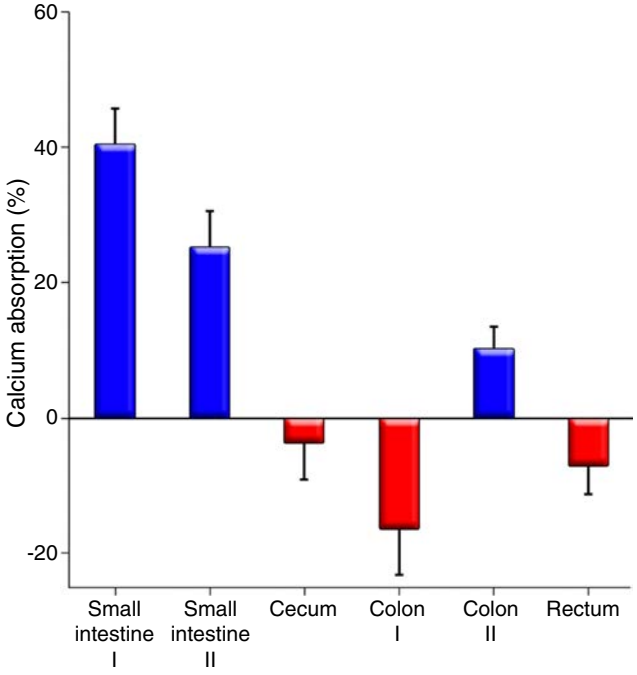


FIG. **16.2** Net absorption of calcium from the intestine of the horse. The intestine was divided into six regions: proximal (small intestine I) and distal (small intestine II) halves of the small intestine, cecum, proximal (colon I) and distal (colon II) large colon, and transverse colon and rectum. (Adapted from Schryver HF, Craig PH, Hintz HF, Hogue DE, Lowe JE. The site of calcium absorption in the horse. *J Nutr.* 1970; 100:1127-1131.[28])

Transcellular absorption is regulated by $1,25(OH)_2D$. For specific mechanisms involved in transepithelial calcium transport see the section on the physiologic effects of parathyroid hormone in the kidney. High dietary phosphate or phytate content inhibits calcium absorption,[29] although a high dietary calcium content has minimal effect on phosphorus absorption.[30] Dietary oxalate reduces calcium absorption, with a 1% oxalate content in an equine diet reduced calcium absorption by 66%.[31] Oxalate content in the diet higher than 0.5% or a calcium:oxalate ratio below 0.5 can result in a negative calcium balance.[32] Some plants containing harmful amounts of oxalate are listed in Table 16.6.

The dietary cation-anion balance (DCAB) affects Ca^{2+} absorption—anionic diets with a low DCAB increase intestinal Ca^{2+} absorption and serum Ca^{2+} concentrations,[33] whereas a high-DCAB diet has the opposite effect.

Glucocorticoids affect the calcium metabolism in the horse. Dexamethasone decreases intestinal absorption of calcium, decreases bone resorption, and increases urinary excretion of calcium.[34,35]

There is limited information on the effect of magnesium on calcium and phosphorus absorption from the gut in the horse. [36,37] No changes in serum calcium and phosphorus concentrations were detected in foals fed a magnesium-deficient diet, although mineralization of the aorta was present.[36]

Elimination

Calcium is eliminated through urine, milk, sweat, feces, and the fetus. Because horses absorb a greater proportion of dietary calcium, fecal calcium content is lower than in ruminants when fed equivalent diets.[23,26] Endogenous losses of calcium in horses are estimated to be 20 to 25 mg/kg BW/day.[21]

TABLE **16.6 Plants Containing Harmful Amounts of Oxalate**[a]

Scientific Name	Common Name
Amaranthus spp.	Red-rooted pigweed[b]
Bassia (Kochia) scoparia	Summer cypress, Mexican fireweed[b]
Beta vulgaris	Sugar beet
Brachiaria spp.	Para grass
Cenchrus ciliaris	Buffel grass
Chenopodium spp.	Lambsquarter
Cynodon dactylon	Bermuda grass
Digitaria eriantha	Pangola
Halogeton glomeratus	Halogeton[b]
Oxalis spp.	Soursob, Shamrock[b]
Panicum spp.	Panicgrass, kleingrass
Paspalum dilatatum	Dallisgrass, Dallas grass
Panicum virgatum	Switchgrass
Panicum (Megathyrsus) maximum	Guineagrass
Pennisetum clandestinum	Kikuyu, kikuyo
Pennisetum polystachion	Mission grass
Pennisetum purpureum	Napier grass
Pennisetum typhoides	Pearl millet
Phytolacca americana	Pokeberry
Portulaca oleracea	Purslane, pigweed, verdolaga
Rheum rhaponticum	False rhubarb, rhapontic rhubarb
Rumex spp.	Sorrel, curly dock[b]
Salsola spp.	Russian thistle, tumbleweed
Sarcobatus vermiculatus	Greasewood[b]
Setaria incrassate	Purple pigeon grass
Setaria sphacelata	Setaria, pigeon grass, bristle grass[b]
Setaria spp.	Foxtail grass

[a]These plants have an oxalate content higher than 0.5% dry matter (DM) or a calcium:oxalate ratio less than 0.5.
[b]Oxalate content in these plants is higher than 5% DM. Their ingestion can result in calcium deficiency and clinical signs consistent with nutritional secondary hyperparathyroidism. Some of these plants may also cause gastrointestinal irritation, diarrhea, renal injury, and signs of acute hypocalcemia.

Assuming a 50% calcium digestibility, a 500-kg horse would require 20 g of calcium to replace losses, or 40 mg/kg BW/day. Growing and lactating horses can double these requirements. Calcium elimination depends on physiologic status, amount of calcium ingested, presence of substances that interfere with calcium absorption (phosphates, oxalates, phytates), and diseases. The urinary fractional excretion of calcium and the ratio of urinary to serum calcium and phosphorus have been proposed as methods to estimate calcium intake.[38] However, interpretation of calcium excretion is difficult due to the large amounts of calcium eliminated in the equine urine.[39] A large proportion of the ultrafiltered calcium is reabsorbed in the proximal convoluted tubules (PCTs), cortical thick ascending loop of Henle (CTAL), and distal convoluted tubules (DCTs) by different mechanisms. The principles of intestinal calcium absorption (paracellular and transcellular mechanisms) apply to renal reabsorption of calcium.

TABLE **16.7 Normal Serum Concentrations of Calcium, Phosphorus, Magnesium, Vitamin D, and PTH and Urinary Excretion of Calcium, Phosphorus, and Magnesium in Horses**

Total calcium (mg/dL)[a]	11.1–13.0
Ionized calcium (mg/dL)[a]	6.0–7.0
Phosphorus (mg/dL)[b]	2.0–4.8
Total magnesium (mmol/L)	0.53–0.91
Ionized magnesium (mmol/L)	0.46–0.66
PTH (pmol/L)	<4.0 (<40 pg/mL)
Calcitonin (pg/mL)	<20
FCa (%)	3%–10%[4,6,7]
FP (%)	<0.5%[4,6,7]
FMg (%)	15%–35%[6,7,40]
$25(OH)D_3$[a]	<2.0 ng/mL[113] 4.7 ± 1.0 ng/mL[113c] 1.90 ± 0.23 ng/mL—winter[110] 2.43 ± 0.09 ng/mL—summer[110] 4.2 ± 0.34 µg/L—winter[111d] 6.2 ± 0.36 µg/L—summer[111d] 11.42 ± 3.26 ng/mL[114] 14.3–37.2 ng/mL[115] 9.5–19.2 ng/mL[3,115a]
$1,25(OH)_2D_3$	18.6 ± 7.3 ng/L—winter[111c] 18.7 ± 8.0 ng/L—summer[111c] 55.0 ± 24.0 pmol/L[107] 7.1–16.5 pmol/L[3c] 8.6 ± 4.7 pg/mL[120] 13.4 ± 5.9 pg/mL[120e]

Values from the laboratory at the College of Veterinary Medicine, The Ohio State University.
$25(OH)D_3$ = 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$ = 1,25-dihydroxyvitamin D_3.
[a]Serum/plasma values are lower in foals than adults.
[b]Serum/plasma values are higher in foals.
[c]D_2 metabolite.
[d]Includes D_2 and D_3 metabolites.
[e]Values from donkeys.
Calcium conversion: mg/dL = mmol/L × 4; mmol/L = mg/dL × 0.25
Magnesium conversion: mg/dL = mmol/L × 2.43; mmol/L = mg/dL × 0.415
Phosphorus conversion: mg/dL = mmol/L × 3.1; mmol/L = mg/dL × 0.32
PTH conversion: pg/mL = pmol/L × 0.105; pmol/L = pg/mL × 9.5
Calcitonin conversion: pg/mL = pmol/L × 3.42; pmol/L = pg/mL × 0.29
$1,25(OH)_2D$ conversion: pg/mL = pmol/L × 2.4; pmol/L = pg/mL × 0.42
25(OH)D conversion: ng/mL = nmol/L × 2.5; nmol/L = ng/mL × 0.40

The urinary fractional excretion of calcium or phosphorus can be calculated using the following formula.

$$\left(\text{Urine } Ca^{2+} \text{or P/Serum } Ca^{2+} \text{or P}\right) \times \left(\text{Serum creatinine/Urine creatinine}\right) \times 100$$

The fractional excretion of calcium and phosphorus in healthy horses is 3% to 10% and less than 0.5%, respectively (Table 16.7).[4,6,7] For most healthy horses the clearance of phosphorus is less than 0.2%.[7] Recent feeding can influence these values to some extent.

Deficiency

Calcium deficiency can be acute or chronic. Signs of acute calcium deficiency are usually associated with increased neuromuscular excitability, whereas chronic calcium deficiency presents as abnormal cartilage and bone development, developmental orthopedic disease (DOD), and lameness. However, neuromuscular signs can develop in chronic cases. When calcium deficiency is suspected, feed analysis is recommended to determine whether dietary calcium and phosphorus content are adequate.

PHOSPHORUS

Phosphorus (or phosphate) represents approximately 1% of the body weight, with most (85%) located in the bone matrix (hydroxyapatite), 15% in blood and soft tissues, and less than 0.1% in the extracellular fluid. In blood, phosphate exists in organic (70%) and inorganic (30%) forms. Most phosphate in the extracellular fluid is inorganic. Organic phosphate is bound to lipids, proteins, and blood cells; however, only inorganic phosphate (PO_4; P_i) is measured. In plasma, PO_4 exists as ionized PO_4 (40%–50%), is complexed to cations (Na^+, Ca^{2+}, Mg^{2+}; 30%–40%), and bound to proteins (1%–15%) (Fig. 16.3). There are four forms of PO_4 in the extracellular compartment: H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_3^{2-}. However, at physiologic pH only divalent (HPO_4^{2-}) and monovalent ($H_2PO_4^-$) PO_4 are present at relevant concentrations in a 4:1 ratio. During acidosis this ratio might be as low as 1:1, whereas in alkalosis it can be as high as 9:1.[41] Like intracellular Ca^{2+}, free cytosolic PO_4 concentration is very low. Phosphate is the main intracellular anion existing in organic (phospholipids, nucleic acids, phosphoproteins, creatine phosphate, adenosine triphosphate [ATP], guanosine triphosphate [GTP], cyclic adenosine monophosphate [cAMP], nicotinamide adenine dinucleotide phosphate [$NADP^+$], thiamine pyrophosphate, 2,3-diphosphoglycerate [2,3-DPG or 2,3-BPG], inositol triphosphate, DNA, RNA) and inorganic forms. Therefore phosphorus is important for nerve function, muscle contraction, electrolyte transport, oxygen transport, enzyme activity, and gene transcription; metabolism of proteins, carbohydrates, and fats; and cell proliferation. The regulation of extracellular PO_4 is closely associated with Ca^{2+} homeostasis.

Phosphorus requirements depend on age, physiologic status, and physical activity (see Table 16.1). In horses, phosphorus absorption ranges from 20% to 55% and takes place in the small and large intestines (Fig. 16.4).[23,42-47] The apparent phosphorus digestibility is higher for yearlings than mature horses.[45] True phosphorus digestibility for growing horses is estimated at 45%,[45] whereas for mature horses it is around 25% to 35%.[45-47] Phytate (organic phosphorus; phytic acid; inositol polyphosphate) has low digestibility, is the main form of phosphorus present in grains and forages, and requires bacterial phytase in the equine large colon to liberate phosphorus and increase its absorption.[48] Age does not influence phytate digestibility, and yearlings can utilize organic phosphorus (phytate) at a similar level to adults, suggesting that diets without inorganic phosphorus may be acceptable for growing equids.[45] Horses have a good capacity to degrade phytate[45,49,50]; however, it is unclear how much of the released phosphate is absorbed. It is assumed that most phosphate from phytate degradation is excreted in feces.[50] Results on the beneficial effect of supplementing equine diets with phytase to increase phosphate absorption have been controversial.[50] In one study, phytase supplementation had more influence on calcium than PO_4 absorption, suggesting that it could be useful in horses fed phytate-rich diets.[47] However, digestibility results from a recent study in yearlings and adult horses indicate that phytase supplementation may not be necessary

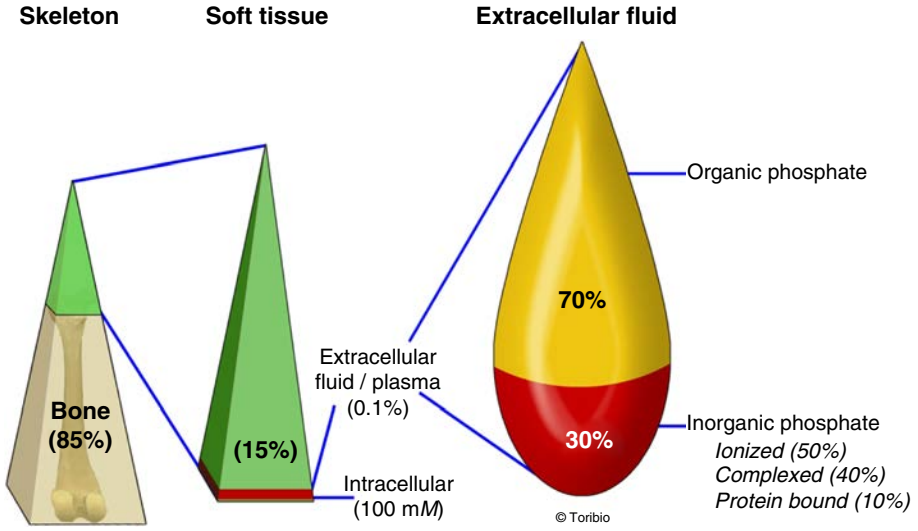


FIG. **16.3** Phosphorus distribution in the body. The skeleton contains approximately 85% of the total body phosphorus. The remaining 15% of phosphorus is present in soft tissues and the extracellular fluid. In blood, phosphorus exists in organic (70%) and inorganic (30%) forms. Inorganic phosphorus, which is measured clinically, is present in an ionized form, complexed to cations such as Na^+, Ca^{2+}, and Mg^{2+}, and bound to proteins. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

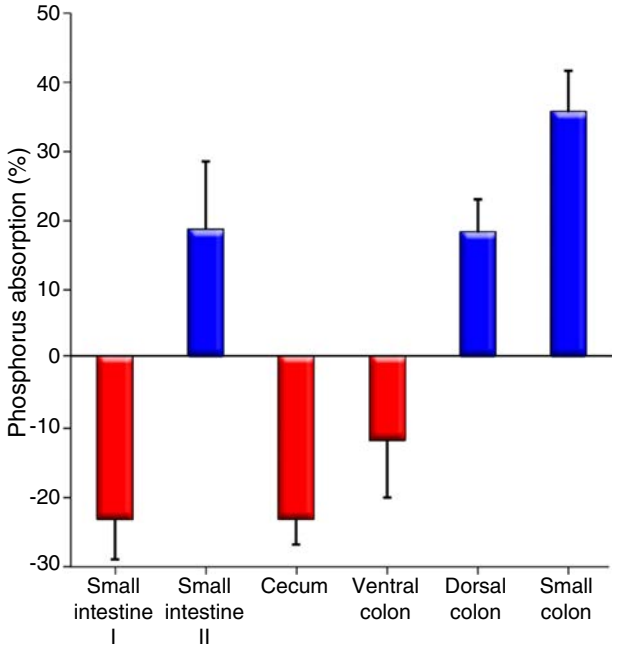


FIG. **16.4** Net absorption of phosphorus from the intestine of ponies fed an alfalfa diet. (Adapted from Schryver HF, Hintz HF, Craig PH, Hogue DE, Lowe JE. Site of phosphorus absorption form the intestine of the horse. *J Nutr.* 1972; 102:143-148.[42])

as they can digest most of the phytate in the diet.[45] Unlike other species, excess dietary aluminum or magnesium does not appear to interfere with PO_4 absorption in horses.[48,51,52] Most renal PO_4 reabsorption (greater than 80%) occurs in the proximal tubules by Na^+-dependent mechanisms (sodium-dependent phosphate transport protein [Na^+/P_i cotransporter; NPT2 cotransporter), with minimal reabsorption in the distal nephron, and the urinary fractional excretion of PO_4 in horses is low (less than 0.5%). Chronic excess of dietary phosphorus is associated with clinical signs of calcium deficiency, which include lameness, abnormal cartilage and bone development, fractures, and osteodystrophia fibrosa (nutritional secondary hyperparathyroidism). Chronic phosphorus deficiency is manifest as weight loss, weakness, depraved appetite (pica), lameness, and DOD. Serum PO_4 concentrations are more indicative of dietary phosphorus intake and status than serum calcium concentrations because the homeostatic control for PO_4 is not as precise as that of Ca^{2+}.

Serum PO_4 concentrations are higher in foals (up to 9.0 mg/dL; 2.9 mmol/L) than horses (2.5–5.0 mg/dL; 0.8–1.6 mmol/L) as a result of increased intestinal absorption and renal reabsorption of PO_4 to supply the growing skeleton and other tissues. Alkaline phosphatase activity is also higher in foals from increased osteoblastic activity and bone formation. Normal PO_4 concentrations are presented in Table 16.7.

The atomic weight of phosphorus is 31. Therefore for unit conversion, 1 mmol/L = 3.1 mg/dL (1 mmol = 31 mg); mg/dL × 0.32 = mmol/L (1 mg/dL = 0.32 mmol/L; 1 mg = 0.032 mmol); 1 mmol/L = 1.8 mEq/L.

Acute renal failure and hypoparathyroidism are associated with hyperphosphatemia. Serum PO_4 concentrations in horses with chronic renal failure are variable.

Conditions that result in cell lysis (hemolysis, rhabdomyolysis, tumor necrosis) and acidemia may cause acute hyperphosphatemia. Hyperphosphatemia is also a common finding in hospitalized foals from acid-base and endocrine disorders. Conditions associated with hypophosphatemia include inadequate intake, decreased intestinal absorption, renal waste, hyperparathyroidism, sepsis, and PO_4 shift to the intracellular compartment (insulin, alkalosis). PO_4 translocation to the intracellular compartment is a frequent cause of hypophosphatemia in critically ill humans, and occasionally occurs in horses with starvation, refeeding syndrome, parenteral nutrition (hyperglycemia, hyperinsulinemia), and hyperlipemia (hypertriglyceridemia). Acute hypophosphatemia can lead to cell membrane fragility and lysis (hemolysis, rhabdomyolysis).

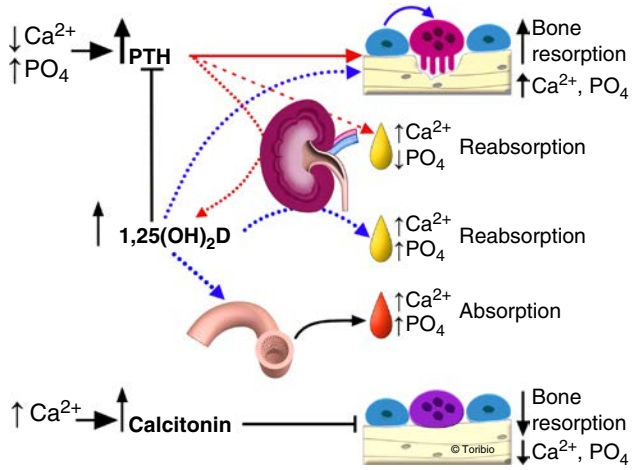


FIG. **16.5** Calcium and phosphate homeostasis. A decrease in calcium (Ca^{2+}) or increase in phosphate (PO_4) concentrations will stimulate PTH secretion. PTH increases renal reabsorption of Ca^{2+}, excretion of PO_4, and activation of vitamin D to $1,25(OH)_2D$. In bone, PTH promotes osteoclastic differentiation, bone resorption, and subsequent release of Ca^{2+} and PO_4 into systemic circulation. After activation, $1,25(OH)_2D$ increases intestinal absorption and renal reabsorption of Ca^{2+} and PO_4, facilitates bone remodeling, and inhibits PTH secretion. Elevations in Ca^{2+} concentrations stimulate the thyroid gland C cells to release calcitonin that will suppress osteoclastic bone resorption. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

Treatment of hypophosphatemia should be based on the extracellular deficit. This deficit should be overestimated as PO_4 is a major intracellular anion, and cell depletion is likely to be present in animals with hypophosphatemia. A preferred parenteral solution is potassium phosphate, although sodium phosphate is a good alternative. Critically ill horses can also be supplemented with oral potassium phosphate or sodium phosphate (chemical-grade or phosphate-based enema solutions). Hypokalemia and hypomagnesemia are frequent findings in horses with hypophosphatemia due to their intricate interactions. For chronic phosphate deficiency oral supplementation with dicalcium phosphate is a good option (see Table 16.3).

Calcium and Phosphorus Homeostasis

Extracellular calcium concentrations are regulated by a complex homeostatic system that includes three major hormones: parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$, or calcitriol).[4,10,53] Parathyroid hormone–related protein (PTHrP) shares considerable homology with PTH, binds and activates the PTH-1 receptor, and is important for calcium homeostasis in the fetus (but not in the adult).[54,55] PTH increases during hypocalcemia whereas CT increases during hypercalcemia (Fig. 16.5). Fibroblast growth factor 23 (FGF23) and klotho (FGF23/klotho axis) play central roles in phosphorus, calcium, PTH, and vitamin D regulation.

Parathyroid Hormone

The main function of PTH is to maintain calcium concentrations within narrow physiologic limits. PTH is secreted by the chief cells of the parathyroid gland in response to hypocalcemia. The relationship between serum Ca^{2+} concentrations and PTH secretion in horses is inverse and sigmoidal, which allows the parathyroid gland to rapidly respond to minimal changes in Ca^{2+} concentrations.[4,9,56-59] Extracellular Ca^{2+} concentrations are detected by a Ca^{2+}-sensing G protein–linked cation receptor (CaSR) in the parathyroid cells.[60]

Horses have four parathyroid glands. The parathyroid glands IV (cranial or upper) are usually located in the fat dorsolaterally to the cranial pole of the thyroid gland and along the thyroid artery; however, they can be found in any location around the thyroid gland. A number of horses may also have parathyroid gland tissue embedded within the thyroid gland. The parathyroid glands III (lower, caudal) can be located at the bifurcation of the bicarotid trunk, at the cranial pole of the thymus, or within the thymus (young horses), and in general are twice the size of the upper parathyroid glands. Parathyroid glands consist of chief cells, oxyphil cells, and clear cells, which represents different morphologic and metabolic stages of the same parenchymal cells. The chief cells are considered the most active.

Mature equine intact PTH is a straight chain of 84 amino acids (molecular weight [MW]: 9393 Daltons in the horse; MW: 9500 Daltons in humans).[61] Other forms of PTH (amino- and carboxy-terminal peptides) can be found in circulation following cleavage in different organs.[62] From 50% to 90% of PTH immunoreactivity in blood is from carboxy-terminal fragments.[63-65] The plasma half-life of PTH in various species is approximately 2 minutes.[65-68] However, the half-life of equine PTH is unknown. The rapid metabolism of PTH, together with the fast response of the parathyroid chief cells to changes in Ca^{2+} concentrations, ensures that PTH concentrations can rapidly adjust to changes in Ca^{2+}. Approximately 60% to 70% of PTH is removed in the liver, about 25% by the kidney, and the rest by other organs.[65,66,68]

PTH gene expression and secretion are under the influence of extracellular Ca^{2+}, PO_4, $1,25(OH)_2D_3$, and fibroblast growth factor 23 (FGF23) concentrations. The role of vitamin D metabolites on equine parathyroid gland function and calcium regulation remains unclear, although it is well accepted that $1,25(OH)_2D_3$ decreases PTH gene expression and secretion.[69] This concept is important in order to understand the role of the kidney in vitamin D metabolism and parathyroid gland physiology. In the chief cells, vitamin D binds to the vitamin D receptor, which acts as a transcription factor to decrease PTH gene transcription and secretion. Unlike vitamin D, the effects of Ca^{2+} and phosphorus in the regulation of PTH secretion are not only mediated by gene transcription but also by altering PTH mRNA stability and translation.[70] Low Ca^{2+} concentrations increase PTH mRNA expression in equine parathyroid chief cells.[5]

Physiologic Actions of Parathyroid Hormone

Kidney. The kidney is the main target organ for PTH, where it increases Ca^{2+} reabsorption, reduces phosphate reabsorption, and promotes $1,25(OH)_2D_3$ synthesis (see Fig. 16.5). These effects are mediated by the PTH-1 receptor (PTH1R), which is widely distributed throughout the nephron.[71] The PTH1R is coupled to G proteins to activate adenylate cyclase and increase intracellular cAMP. PTH also activates phospholipases (A_2, C, D), protein kinase A, and protein kinase C.[71,72]

a. Effect on Ca^{2+} and Mg^{2+} Reabsorption. It has been estimated that 60% of the filtered calcium and 20% of the filtered magnesium are reabsorbed in the renal proximal tubules (the PCTs) by paracellular passive processes driven by the high luminal concentrations of Ca^{2+} and Mg^{2+}.[73,74] In the cortical thick

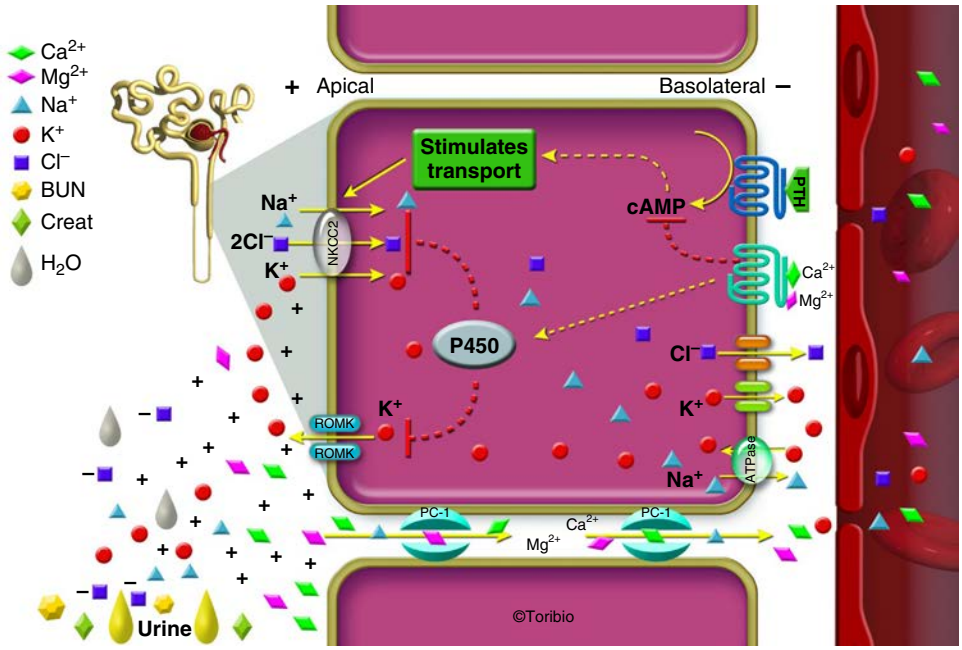


FIG. **16.6** Schematic representation of renal paracellular transport of Ca^{2+} and Mg^{2+} in the cortical thick ascending loop of Henle (CTAL). The CTAL lumen is positively charged and the electroneutral $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) is the main force for the paracellular movement of Ca^{2+}, Mg^{2+}, and Na^+. Most K^+ that enters the tubular cells via NKCC2 returns to the lumen via the renal outer medullary K^+ channel (ROMK), hyperpolarizing the apical cell membrane. The basolateral Na^+/K^+-ATPase indirectly drives luminal NKCC2. PTH, via cyclic adenosine monophosphate (cAMP), enhances NKCC2 and ROMK activity and paracellular Ca^{2+} and Mg^{2+} transport, while extracellular Ca^{2+} (and to a lesser extent Mg^{2+}) through the calcium-sensing receptor inhibits NKCC2 and ROMK activity, decreasing Ca^{2+} and Mg^{2+} reabsorption.[74,76] Paracellin-1 (PC-1) facilitates paracellular permeability to Ca^{2+} and Mg^{2+}. PTH also increases renal vitamin D activation (not shown). (Courtesy Dr. R. E. Toribio, The Ohio State University.)

ascending loop of Henle (CTAL), Ca^{2+} and Mg^{2+} transport is also paracellular, but in this segment there is more permeability to Mg^{2+} (60% for Mg^{2+} vs. 20% for Ca^{2+}). The CTAL lumen is positively charged, and the main force for paracellular movement of Ca^{2+}, Mg^{2+}, and Na^+ is the transepithelial voltage gradient generated by the electroneutral $Na^+/K^+/2Cl^-$ cotransporter (NKCC2).[75] Most of the K^+ that enters the tubular cells via NKCC2 returns to the lumen via the renal outer medullary K^+ channel (ROMK), hyperpolarizing the apical membrane.[76] The Na^+/K^+-ATPase at the basolateral drives the NKCC2. In addition, basolateral Cl^- movement enhances the voltage gradient. PTH, through the PTH1R and cAMP, enhances NKCC2 and ROMK activity, while extracellular Ca^{2+} (to a lesser extend Mg^{2+}) via the calcium-sensing receptor (CaSR) inhibits NKCC2 and ROMK activity, as well as paracellular reabsorption of Ca^{2+} and Mg^{2+}[74,76] (Fig. 16.6). Hypercalcemia induces diuresis in horses because NKCC2, the target for loop diuretics, is also blocked by Ca^{2+}.[7] Hypercalcemia also promotes diuresis by blunting vasopressin-mediated apical insertion of aquaporin channels and water reabsorption in the collecting ducts. Paracellin-1 (claudin-16) is an essential protein for paracellular permeability of Ca^{2+} and Mg^{2+} in the CTAL.[76,77] PTH has minimal effect in the DCTs where $1,25(OH)_2D$ seems to be the main calcium-regulating hormone. Unlike the PCT and CTAL where calcium transport is paracellular, calcium transport in the DCT is transcellular, mediated by epithelial calcium channels (transient receptor potential cation channel subfamily V member 5 and 6 [TRPV5; TRPV6]), calcium-binding proteins (calbindin D_{9k}, calbindin D_{28k}), basolateral proteins (plasma membrane calcium ATPase [PMCA], Na^+/Ca^{2+} exchanger [NCX]), and regulated by $1,25(OH)_2D$ (Fig. 16.7).[78,79] TRPV5 is the main renal epithelial calcium channel, and TRPV6 is expressed in the intestine, kidney, and other epithelia in multiple species, including the horse.[80,80a] Calbindin D_{9k} is more important for intestinal calcium transport than calbindin D_{28k}, while both calbindins participate in renal calcium movement.[80] In horses, renal expression of calbindin D_{28k} is higher than calbindin D_{9k}.[80]

b. Effects on Phosphorus. The movement of PO_4 in the PCT occurs against electrochemical and concentration gradients and is mediated by Na^+/P_i cotransporters (NPT2 cotransporters). PTH lowers Na^+ and PO_4 transport across the brush border by enhancing the degradation of Na^+/P_i cotransporters.[71,81,82] The end result is decreased PO_4 reabsorption and phosphaturia. Low PTH concentrations will lead to hyperphosphatemia, whereas the opposite occurs with high PTH.

c. Effects on Vitamin D Metabolism. PTH, through cAMP, increases renal $1,25(OH)_2D_3$ synthesis by enhancing 1α-hydroxylase activity and suppressing 24-hydroxylase, the enzyme that inactivates $1,25(OH)_2D_3$.[71]

Bone

Bone is a very active organ that is in a continuous process of formation (osteoblasts) and resorption (osteoclasts). In adult vertebrates, formation is in equilibrium (coupled) with resorption. However, in growing animals, bone formation exceeds bone resorption, whereas in old animals and humans

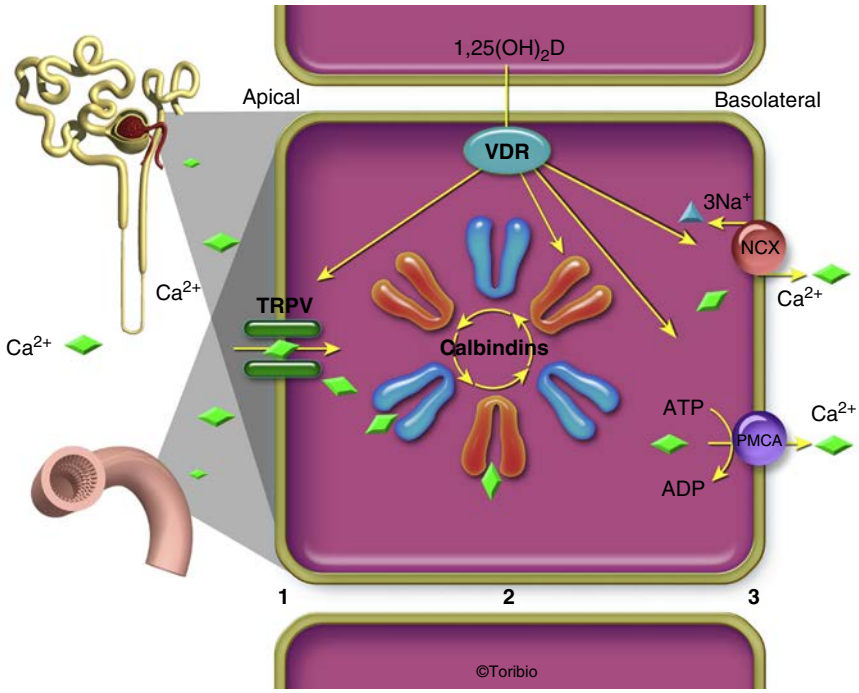


FIG. **16.7** Schematic representation of transcellular transport of calcium (Ca^{2+}) in the small intestine and kidney. Transcellular Ca^{2+} transport is considered a three-step process in which calcium (1) enters the cell passively via epithelial calcium channels (transient receptor potential cation channel subfamily V [TRPV]), (2) diffuses through the cytosol facilitated by calcium-binding proteins (calbindins), and (3) at the basolateral membrane calcium is extruded by a plasma membrane Ca^{2+} ATPase (PMCA) and a Na^{+}-Ca^{2+} exchanger (NCX). Through the vitamin D receptor, $1,25(OH)_2D$ increases all three steps of transcellular calcium transport. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

the opposite occurs. Of interest, higher PTH concentrations were measured in older compared with younger mares in late pregnancy.[83] There are no PTH receptors in osteoclasts but there are in osteoblasts. Thus the effect of PTH on osteoclast activation is mediated by the osteoblast. On PTH stimulation, osteoblasts secrete a series of factors, including macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B (RANK) ligand (RANKL).[84] These two factors (M-CSF and RANKL) are essential for osteoclast function (Fig. 16.8). Monocytes and preosteoclasts express RANK, the membrane receptor for RANKL, and the interaction of RANKL with RANK and M-CSF with its receptor results in osteoclast recruitment and activation. To keep formation and resorption in balance, the osteoblasts also release osteoprotegerin (OPG), a decoy receptor for RANKL that blocks its action on osteoclasts.[85] Other factors that stimulate osteoclast differentiation include $1,25(OH)_2D$, tumor necrosis factor–alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin 11 (IL-11). Osteoclasts create resorption pits (Howship's lacunae) that are sealed by $\alpha_v\beta_3$ integrin to conserve the acid conditions necessary for mineral resorption. Through the ruffled border, osteoclasts release H^+ ($H_2O + CO_2$ → carbonic anhydrase → HCO_3^- + H^+) and Cl^- (exchanged with HCO_3^-) into the pit, lowering the pH, dissolving mineralized bone matrix, and releasing Ca^{2+}, PO_4, and other products. Cathepsin K is a key protease that degrades extracellular matrix proteins including type I collagen. Matrix metalloproteinases (MMPs) are zinc-containing endopeptidases that facilitate bone resorption. Tartrate-resistant acid phosphatase (TRAP) is necessary for osteoclast activity, dephosphorylates osteopontin and bone sialoprotein to allow osteoclast migration, and generates reactive oxygen species that participate in bone degradation (see Fig. 16.8).

Markers of bone formation in horses include serum osteocalcin, N-terminal peptide of type I procollagen, and bone-specific alkaline phosphatase.[86-88] Markers of bone resorption include urinary or serum collage type 1 cross-linked carboxy-terminal telopeptide (CTX), urinary collagen type 1 cross-linked N-terminal telopeptide (NTX), urinary hydroxyproline, urinary total pyridinoline, and serum TRAP 5b.[86,88]

Intestine

PTH has minimal direct actions on intestinal absorption of calcium and phosphorus. However, indirect effects are mediated by $1,25(OH)_2D$.

Vitamin D

Vitamin D plays important roles in calcium and phosphate homeostasis and to a lesser extent in magnesium metabolism. Vitamin D derives from dietary sources (vitamin D_2 or ergocalciferol from fungi and plants; vitamin D_3 or cholecalciferol from animal-based diets) and from cutaneous activation of 7-dehydrocholesterol into cholecalciferol. In mammals, ultraviolet light (290–315 nm) photolytic cleavage of 7-dehydrocholecalciferol results in previtamin D_3, which, after thermal isomerization, forms vitamin D_3 (cholecalciferol).[89] From the skin, vitamin D_3 is translocated to the liver by a vitamin D binding protein (DBP) where it is hydroxylated at carbon 25 by a mixed function P_{450} oxidase (25-hydroxylase) to form 25-hydroxyvitamin D_3 ($25[OH]D_3$; calcidiol). Other tissues

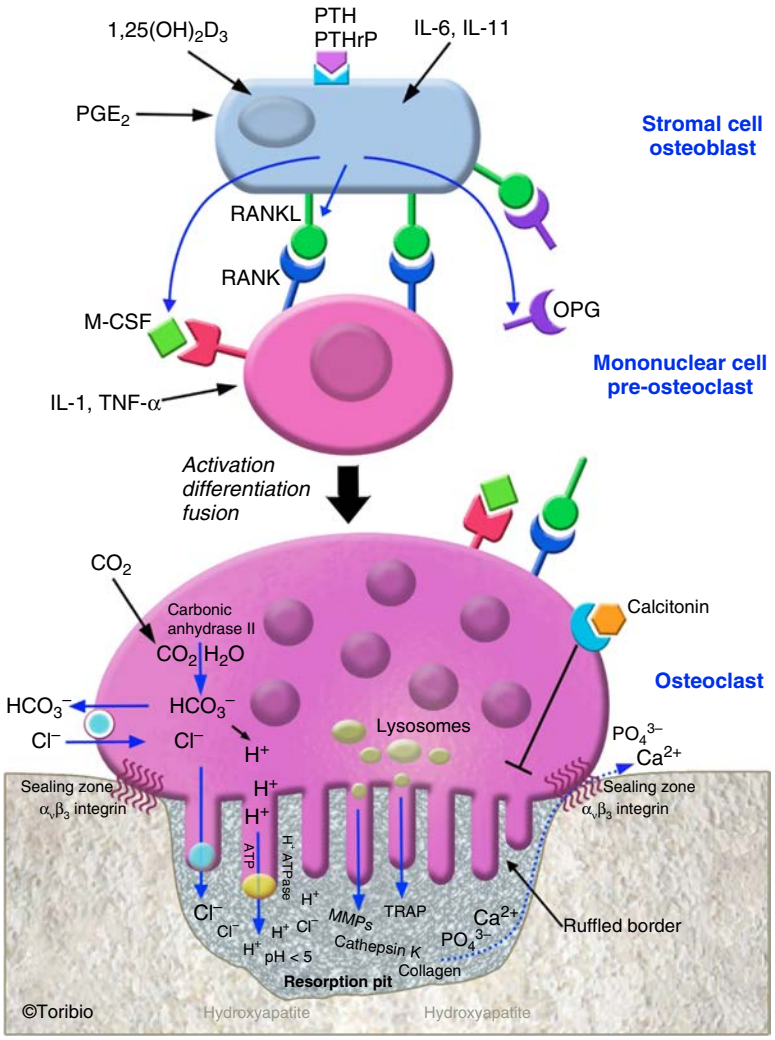


FIG. **16.8** Representation of osteoclastic activation and bone resorption. Factors that indirectly through osteoblasts stimulate osteoclast differentiation include parathyroid hormone (PTH), PTH-related protein (PTHrP), 1,25(OH)$_2$D, IL-6, IL-11, and prostaglandin E_2 (PGE_2). In response, osteoblasts secrete macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B (NF-κB) (RANK) ligand (RANKL). Mononuclear cells, osteoclast precursors, and osteoclasts express RANK (receptor for RANKL) and M-CSF receptors. Osteoblasts also release osteoprotegerin (OPG), which is a soluble decoy receptor for RANKL. IL1 and TNF-α promote osteoclast differentiation. Calcitonin inhibits osteoclast activity. Osteoclasts create a resorption pit that is sealed by $\alpha_v\beta_3$ integrin to facilitate an acid environment for mineral resorption. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

can also activate vitamin D_3, in particular under pathologic conditions. The conversion of vitamin D_3 into 25(OH)D_3 is a poorly regulated reaction, and therefore its plasma concentrations are an indicator of the vitamin D status.

From the liver, 25(OH)D is transported to the kidney by DBP where it is converted by another cytochrome P_{450} mixed function oxidase, 25(OH)D-1α-hydroxylase (1α-hydroxylase), to the active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2D_3$; calcitriol). The kidney is considered the main site for 1,25(OH)$_2D_3$ synthesis, although other organs (placenta, skin, monocytes) also have 1α-hydroxylase activity.

In the case of ergocalciferol, the skin steps do not occur. Instead, after intestinal absorption ergocalciferol is converted to 25-hydroxyvitamin D_2 (25[OH]D_2) in the liver and subsequently to 1,25-dihydroxyvitamin D_2 (1,25[OH]$_2D_2$) in the kidneys, by the same enzymes.

Unlike 25-hydroxylase, 1α-hydroxylase is a tightly regulated enzyme (PTH, PO_4, 1,25[OH]$_2D_3$, FGF23). Mutations of the 1α-hydroxylase gene in humans and laboratory animals results in vitamin D–dependent type I rickets.[90] We will refer to both vitamin D active metabolites, 1,25(OH)$_2D_2$ and 1,25(OH)$_2D_3$, as 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). In blood, 1,25(OH)$_2$D concentrations are 1/1000 the concentrations of 25(OH)D. The half-life of 25(OH)D can be measured in weeks, whereas the half-life of 1,25(OH)$_2$D is measured in a few hours. Both 25(OH)D and 1,25(OH)$_2$D are inactivated at the target organs by 24-hydroxylase.

Regulation of Vitamin D Metabolism

Blood concentrations of 1,25(OH)$_2$D are regulated by PTH, Ca^{2+}, PO_4, 1,25(OH)$_2$D, and FGF23. Low PO_4 and PTH increase, whereas high Ca^{2+}, PO_4, 1,25(OH)$_2$D, and FGF23

concentrations suppress renal 1α-hydroxylase activity and therefore $1,25(OH)_2D_3$ synthesis.

The role of calcitonin on vitamin D metabolism remains unclear. The FGF23/klotho axis is a recently discovered homeostatic mechanism for vitamin D synthesis.[91,92] FGF23 secreted by osteocytes binds to its receptor and klotho (coreceptor) in the renal tubular cells to suppress 1α-hydroxylase activity and $1,25(OH)_2D_3$ synthesis.[91,92] It also inhibits Na^+/P_i cotransporters to promote phosphaturia (phosphatonin action).

Actions of Vitamin D

The actions of vitamin D are mediated by the vitamin D receptor (VDR) that is found in organs involved in mineral homeostasis and bone health (intestine, bone, kidney, parathyroid gland — classic actions of vitamin D), as well as in organs not involved in mineral regulation (skin, pancreas, the immune system, and reproductive organs — nonclassic actions of vitamin D).[93] The vitamin D–activated VDR forms a heterodimer with the retinoid-X receptor to bind specific DNA sequences (vitamin D response element [VDRE]) in the promoter region of target genes to increase or decrease their expression depending on the organ or cell type.

In the intestinal tract and kidney, $1,25(OH)_2D$ promotes transcellular Ca^{2+} transport in a three-step process: (1) calcium entry by epithelial calcium channels (TRPV5/6), (2) calcium diffusion across the cytosol that is facilitated by calcium-binding proteins (calbindins), and (3) calcium extrusion at the basolateral side by plasma membrane Ca^{2+} ATPases (PMCA) and Na^+-Ca^{2+} exchangers (NCX) (Fig. 16.7).[94,95] Vitamin D increases the expression of these proteins. In addition to its effects on intestinal and renal calcium transport, $1,25(OH)_2D$ also promotes PO_4 transport by increasing the number of Na^+/P_i cotransporters.

In the kidney, $1,25(OH)_2D$ stimulates Ca^{2+} reabsorption (DCT) and phosphorus reabsorption (PCT).[93] In the skeleton, $1,25(OH)_2D$ is essential for bone remodeling; both bone formation and resorption are vitamin D–dependent processes. Vitamin D stimulates FGF23 secretion by the osteocytes, which in turn will suppress renal 1α-hydroxylase activity, creating a bone-kidney feedback system. Vitamin D deficiency results in rickets in growing animals and osteomalacia in adults. No cases of rickets or osteomalacia have been documented in horses.

As previously mentioned, PTH increases renal 1α-hydroxylase activity, increasing $1,25(OH)_2D_3$ secretion, which will suppress parathyroid chief cell proliferation and PTH secretion.[96] This is the explanation for the development of parathyroid hyperplasia and hyperparathyroidism in humans and animals with chronic kidney disease and vitamin D deficiency.

Vitamin D also has nonclassical functions. Anemia, predisposition to infections from impaired immune function, skeletal muscle weakness, cardiomegaly, reduced pancreatic glucose-mediated insulin secretion, infertility, and critical illness are among conditions reported with vitamin D deficiency.[93,97-99] Vitamin D is important in skin and hair development, modulates cell proliferation and differentiation, and has antibacterial properties through cathelicidins and defensins.[97-101] Because of these properties, several vitamin D–based compounds with antiproliferative and prodifferentiation properties have been developed to treat a number of pathologic conditions in humans and small animals (seborrhea, psoriasis, secondary hyperparathyroidism, breast cancer, prostate cancer, leukemia).[93] Reduced vitamin D concentrations have been associated with a multitude of human pathologies,[98,102-106] and more recently were linked to sepsis and mortality in critically ill newborn foals.[3]

Vitamin D Metabolism in the Horse

There is limited information on vitamin D in equids. Compared with other domestic animals (except rabbits), horses have high serum total and ionized calcium concentrations, high intestinal absorption of calcium, high urinary fractional excretion of calcium, a high Ca^{2+} set point, and low serum concentrations of vitamin D metabolites ($25[OH]_2D$ and $1,25[OH]_2D$).[3,4,23,107-115] Despite the low vitamin D concentrations, rickets or osteomalacia have not been documented in horses.[116] Horses with limited access to sunlight and fed a vitamin D–deficient diet had decreased growth, lameness, and lower bone density.[116,116] In northern latitudes, the synthesis of $25(OH)D_3$ and $1,25(OH)_2D_3$ in horses during winter months is low.[110,111] A similar phenomenon was seen in horses in the Southern Hemisphere where 25(OH)D, $25(OH)D_2$, and $1,25(OH)_2D_3$ concentrations were higher in spring and summer compared with winter months.[117] This increase in serum $25(OH)D_2$ concentrations during warmer months could be an effect of daylight on the synthesis of ergocalciferol by plants. Diurnal variations in $25(OH)D_3$ concentrations were reported in horses with peaks in the early afternoon.[118] A circadian rhythmicity for $1,25(OH)_2D$ occurred during the longest days of the year in horses in New Zealand.[119] One study in which horses were covered with blankets year around found no differences in serum $25(OH)D_2$, $25(OH)D_3$, and $1,25(OH)_2D$ concentrations between covered and uncovered horses.[117] Of interest, serum $25(OH)D_3$ concentrations were undetectable, but $25(OH)D_2$ were not. The authors concluded that horses produce minimal amounts of vitamin D_3, and that vitamin D_2, not vitamin D_3, is the main form of vitamin D in equids.[117] As previously stated, other studies have measured both vitamin D metabolites, opening the question on whether these discrepancies are statistical (sample size, study population), analytical, or vitamin D_2 is more important in horses.

Plasma 25(OH)D and $1,25(OH)_2D$ concentrations were very low or undetectable in healthy horses and ponies compared with vitamin D metabolite concentrations from other species.[107] In the same study, renal 1α-hydroxylase activity could not be detected. Serum 25(OH)D and $1,25(OH)_2D$ concentrations are lower in healthy foals than horses.[3,115] Another study found higher calcitriol concentrations in donkeys than horses[120]; however, these values were similar to concentrations measured in horses by other groups.[107,111] Both vitamin D metabolites were lower in hospitalized foals and linked to severity of disease and mortality.[3] This information supports a potential role for $1,25(OH)_2D_3$ as a modulatory factor in immunity, inflammation, and metabolism. One study found that physical activity reduced vitamin D_3 concentrations in horses.[121]

The fact that vitamin D is important for immune modulation and indirectly has antimicrobial properties suggests that vitamin D deficiency contributes to a proinflammatory state and immune deficiency in critically ill foals.[3] In the same foals of this study, low concentrations were inversely associated with PTH concentrations, supporting the importance of $1,25(OH)_2D$ in regulating parathyroid chief cell function and PTH secretion in sick foals and, probably, in adult horses. We have proposed that hypovitaminosis D could be a

potential explanation for the high PTH concentrations measured in critically ill foals and horses and also in other species. For additional information on this topic see the phosphorus section in which a potential role of the FGF23/klotho axis is incorporated into the discussion of hypovitaminosis D and increased PTH secretion.

Normal vitamin D metabolite concentrations in horses, foals, and donkeys are listed in Table 16.7.

Calcitonin

Calcitonin (thyrocalcitonin; CT) is a 32-amino-acid peptide that inhibits osteoclast function. It is secreted by the parafollicular cells (C cells) of the thyroid gland in response to hypercalcemia. Equine CT has 90% homology to human calcitonin.[122] Secretion of CT is controlled by several factors, Ca^{2+} and gastrin being the most important. The C cells sense changes in extracellular Ca^{2+} concentrations by the same calcium-sensing receptor (CaSR) present in parathyroid chief cells and renal tubular cells.[123] CT decreases plasma Ca^{2+} and PO_4 concentrations by suppressing osteoclastic bone resorption and increasing urinary Ca^{2+} and PO_4 excretion. Despite these effects, the importance of CT on mammalian Ca^{2+} homeostasis remains unclear. It has been proposed that CT may be protective against extreme hypercalcemia, but that in the day-to-day Ca^{2+} variations it may not be as relevant.[10,124] Based on recent research, it seems the most important CT function in mammalians is to prevent excessive bone resorption during lactation.[125] Postprandial increases in serum CT concentrations are mediated by gastrin, and this is likely a preemptive mechanism to avoid hypercalcemia after a calcium-rich meal. Few studies have evaluated CT in the horse.[80,126-129] Equine C cells are highly responsive to hypercalcemia.[80]

Unlike terrestrial vertebrates, CT is important to regulate extracellular Ca^{2+} concentrations in saltwater fish, where ambient calcium concentrations are extremely high (10 mM; 40 mg/dL).

Parathyroid Hormone–Related Protein

Parathyroid hormone–related protein (PTHrP), first discovered as the factor responsible for humoral hypercalcemia of malignancy (HHM),[54] has important physiologic functions in skeletal development. PTH and PTHrP share homology in their first 13 amino acids and act through the same receptor (PTH1R). Unlike PTH, which is exclusively synthesized by the parathyroid glands, PTHrP is produced by almost every tissue and has a broad range of functions.[53,55] The *physiologic* functions of PTHrP are considered to be paracrine, autocrine, and intracrine, whereas the *endocrine* actions of PTHrP are considered pathologic (HHM). PTHrP is central to fetal calcium regulation, but it has minimal effect on calcium homeostasis in the adult. Depending on the protein domain involved, PTHrP has varying functions: the amino-terminus interacts with the PTH1R, initiating PTH-like actions (bone resorption, renal Ca^{2+} reabsorption; PO_4 excretion); the midregion PTHrP participates in transplacental Ca^{2+} transport; and the carboxy-terminal inhibits osteoclastic bone resorption.[55] PTHrP is essential for mammary development and lactation; transgenic animals lacking the PTHrP gene do not develop mammary tissue.[130] High PTHrP concentrations are found in the milk of lactating humans and animals, including mares.[131] Although the functions of PTHrP in milk are unclear, PTHrP enhances Ca^{2+} transport into milk and perhaps promotes intestinal Ca^{2+} absorption in the newborn. It has been proposed that during lactation, PTHrP release into the systemic circulation is necessary to promote osteoclastic activity to meet the calcium demands from milk production.[130] Increased circulating PTHrP concentrations have been correlated with decreased bone mass in women and rodents,[130] but information in mares is lacking.

HHM, a paraneoplastic syndrome associated with excessive secretion of PTHrP by some tumors, has been reported in horses with lymphoma, gastric and preputial squamous cell carcinoma, ameloblastoma, and multiple myeloma.[132-136]

Fibroblast Growth Factor 23 and Klotho Axis

Fibroblast growth factor 23 (FGF23) and klotho play central functions in calcium, phosphorus, vitamin D, and PTH regulation. FGF23 is considered the main PO_4-regulating hormone and is secreted by osteocytes and osteoblasts in response to $1,25(OH)_2D$, PTH, and PO_4 concentrations.[91,137,138] It promotes renal PO_4 excretion *(phosphatonin)* directly by decreasing renal Na^+/P_i cotransporter number and indirectly by suppressing 1α-hydroxylase activity ($1,25[OH]_2D$ synthesis).[137-141] FGF23 also inhibits PTH synthesis and secretion.[137-141] Klotho is a transmembrane protein that functions as the coreceptor for FGF23 to promote renal phosphorus excretion, reduce $1,25(OH)_2D$ synthesis, and decrease PTH synthesis.[91,138,142] Abnormalities in FGF23 and klotho could lead to disorders in multiple body systems.[143]

CALCIUM DISORDERS IN THE HORSE

Equine calcium dysregulation is associated with hypocalcemic or hypercalcemic disorders. Conditions associated with abnormal calcium homeostasis include foal hypocalcemia,[144] hypoparathyroidism,[145,146] primary hyperparathyroidism,[147-149] nutritional secondary hyperparathyroidism,[150] hypercalcemia of malignancy,[134-136] vitamin D toxicity,[151] renal failure,[152] exercise-induced hypocalcemia,[56,153] and systemic inflammatory response syndrome (SIRS)/sepsis.[4,154,155] Normal calcium concentrations of horses are presented in Table 16.7.

Hypocalcemia

Different pathologies are associated with hypocalcemia in the horse (Table 16.8). Clinical signs of acute hypocalcemia result from increased neuromuscular excitability and decreased smooth muscle cell contractility (Table 16.9). Extracellular

TABLE **16.8 Conditions in Which Hypocalcemia Has Been Reported in Horses and Foals**

Colic	Pancreatitis
Enterocolitis	Furosemide administration
Sepsis/septicemia	Excessive administration of $NaHCO_3$
Endotoxemia	Oxalate ingestion
After endurance exercise	Primary hypoparathyroidism
Late pregnancy	Hypomagnesemia
During lactation (lactation tetany)	Cantharidin toxicosis
During transport (transit tetany)	Liver disease
Acute renal failure	Dystocia
Chronic renal failure	Malignant hyperthermia
Rhabdomyolysis	Magnesium toxicosis
Pleuropneumonia	Retained placenta
Heat stroke	Postoperative myopathy

Ca^{2+} concentrations affect the voltage at which Na^+ channels in nerve and muscle fibers are activated; Ca^{2+} decreases Na^+ permeability and raises the depolarization threshold. Thus Ca^{2+} can be seen as a natural Na^+ channel blocker. When Ca^{2+} concentrations are low, Na^+ channels are easily activated, leading to nerve and muscle fiber hyperexcitability, muscle fasciculations, tremors, and tetany. Tachycardia and cardiac arrhythmias may be present during hypocalcemia, although bradycardia may develop with severe hypocalcemia (probably from decreased cardiac muscle contractility). Depending on the severity, clinical signs of hypocalcemia may include anxiety, depression, synchronous diaphragmatic flutter (see discussion that follows), hyperexcitability, ataxia, stiff gait, tetany, muscle fasciculations and tremors, tachypnea with flared nostrils, upper airway stridor, dyspnea, dysphagia, hypersalivation, hyperhidrosis, ileus, seizures, hypotension, recumbency/collapse, and death (see Table 16.8).

Synchronous Diaphragmatic Flutter

Synchronous diaphragmatic flutter (SDF; thumps, hiccups) refers to a rhythmic movement on the flank resulting from diaphragmatic contractions that are synchronous with the heartbeat. SDF results from ionized hypocalcemia and/or hypomagnesemia, and it has been reported in horses with gastrointestinal disease,[4,156] lactation tetany (eclampsia),[157] thoracic hematoma,[158] blister beetle toxicosis,[159] urethral obstruction,[160] endurance exercise,[161] hypoparathyroidism,[146,162] idiopathic hypocalcemia,[144] and sepsis.[4] Depolarization of the right atrium stimulates action potentials in the hyperexcitable phrenic nerve as it crosses over the surface of the heart. This condition is frequent in horses after prolonged exercise, in which significant amounts of electrolytes (Ca^{2+}, Na^+, K^+, Mg^{2+}, Cl^-) are lost in sweat.[163,164] During alkalosis there is increased binding of Ca^{2+} and Mg^{2+} to plasma anions, in particular albumin, lowering their concentrations. Exercising horses often develop alkalosis from hyperventilation (respiratory alkalosis) and sweat loss of Cl^- (metabolic hypochloremic alkalosis).[158] Proximal gastrointestinal disorders associated with Cl^- loss (duodenitis/jejunitis) can also cause metabolic alkalosis. Calcium is a natural Na^+ channel blocker, and a reduction in Ca^{2+} concentrations increases Na^+ permeability, decreasing the cell membrane depolarization threshold and increasing neuromuscular excitability. Serum Mg^{2+} concentrations are often low in horses with SDF, and similar to hypocalcemia, hypomagnesemia also increases neuromuscular excitability. Low Mg^{2+} concentration facilitates Na^+ channel conductance. Hypokalemia is common with alkalosis (K^+-H^+ exchange) and may contribute to SDF. Hypokalemia raises the resting cell membrane potential and increases neuromuscular excitability, likely by keeping Na^+ channels open longer. Hypomagnesemia should always be included in the differential diagnosis of hypocalcemia and SDF.

TABLE **16.9 Clinical Signs Reported in the Literature of Horses with Hypocalcemia**

Anxiety	Hypersalivation
Asphyxia	Hyperthermia
Ataxia	Ileus
Bruxism	Laryngeal spasm
Cardiac arrhythmias	Muscle fasciculations
Colic	Seizures
Convulsions	Stiff gait
Death	Synchronous diaphragmatic flutter
Depression	Tachycardia
Dysphagia	Tachypnea
Dyspnea	Tetany
Excitation	Tremors
Hyperhidrosis	Trismus

Tetany

Low Ca^{2+} concentrations increase cell membrane excitability, and some horses may develop excessive and sustained skeletal muscular contractions or tetany. Lactation tetany occurs in mares from 2 weeks before foaling up to few days after weaning. The predisposing cause of lactation tetany is the calcium losses in milk. Mares producing large amounts of milk and eating a low-calcium diet, and performing physical work (draft mares), are at higher risk. Some horses transported for long distances may develop hypocalcemia and transit tetany.[157] Hypomagnesemia, which is often present, can worsen the signs of hypocalcemia.

Hypocalcemic Seizures

As for peripheral nerves, a drop in extracellular Ca^{2+} concentrations in the central nervous system (CNS) will increase neuroexcitability. Hypocalcemic seizures have been reported in foals and horses with hypocalcemia, sepsis, and hypoparathyroidism.[144] Clinical signs usually improve with calcium supplementation, although some animals may require repeated treatments with calcium salts. Others, in addition to calcium supplementation, also require magnesium therapy. Horses with hypocalcemic seizures have a poor prognosis for recovery.

Ileus

Smooth muscle cells have more voltage-gated Ca^{2+} channels and fewer voltage-gated Na^+ channels than skeletal muscle fibers, and therefore Na^+ is less important for the action potential and muscle contraction. This results in slower and more sustained contractions (Ca^{2+} channels are slow channels). In skeletal muscle almost all Ca^{2+} required for contraction comes from the sarcoplasmic reticulum, but in smooth muscle cells the sarcoplasmic reticulum is a rudimentary organelle; these cells depend on extracellular Ca^{2+} for contraction. Therefore any pathologic condition that reduces Ca^{2+} affects smooth muscle contractility. This is evident in horses that develop ileus and colic secondary to hypocalcemia (e.g., after exercise, transport, or sepsis). A recent study found that low ionized calcium concentrations were associated with colic in pregnant mares.[165] Treatment with calcium gluconate may restore gastrointestinal motility.

Retained Placenta

The incidence of retained fetal membranes in mares has been reported to be up to 10% of foalings.[166] Low serum total and ionized calcium concentrations are frequently seen in mares with retained placenta and acute endometritis. As for gastrointestinal motility, it is likely that decreased uterine tone and contractility may result from a similar mechanism as ileus. One study found that mares with retained placenta had statistically lower serum total calcium concentrations than mares without retained placenta.[167] Furthermore, 64% of mares treated with a combination of oxytocin in a calcium/magnesium

borogluconate solution responded to treatment compared with 44% of the mares treated with oxytocin in saline solution. No differences in serum magnesium concentrations were found in this study.

Treatment of Hypocalcemia

When treating hypocalcemia, consider calcium deficit, maintenance, losses, and sequestration. However, because the skeleton is a major calcium reservoir, therapy should be focused on correcting the deficit. Calcium therapy is essential in horses that develop rapid hypocalcemia and in horses with impaired parathyroid gland function that cannot restore normocalcemia. The decision to treat horses with hypocalcemia should be based on the existence of hypocalcemia and not necessarily on the presence of signs of hypocalcemia. Most horses with ionized hypocalcemia do not show signs of hypocalcemia, or signs are very subtle and difficult to detect and delaying therapy may result in additional complications (e.g., ileus). Horses with mild hypocalcemia in general restore normocalcemia without calcium administration. However, calcium administration is unlikely to be harmful, may hasten recovery, and therefore should be considered. Horses with functional kidneys can rapidly eliminate large amounts of calcium, and hypercalcemia from excessive intravenous calcium administration is rare, particularly if the horse is receiving fluid therapy.

The use of standard formulas to calculate electrolyte deficits based on extracellular fluid and body weight do not apply to calcium. Calcium can rapidly be eliminated or sequestered in different compartments, and larger doses of calcium are often required.

To calculate Ca^{2+} deficit the following formula is used:

$$([6.5 - Ca^{2+}][0.3][BW][10])/Ca^{2+}\ \text{ratio} = Ca^{2+}\ \text{deficit}$$

In this formula, the difference between the measured Ca^{2+} and normal Ca^{2+} (6.5 mg/dL) is multiplied by the extracellular fluid volume, the body weight (kg), and a factor of 10 (Ca^{2+} is expressed in dL, but body weight is in kg = L) and then divided by the Ca^{2+} ratio (Ca^{2+}/total calcium). For a 450-kg horse with a serum Ca^{2+} concentration of 4.5 mg/dL and a total calcium of 10 mg/dL, the estimated Ca^{2+} deficit will be 6000 mg:

$$([6.5 - 4.5][0.3][450\ \text{kg}][10]) = 6000\ \text{mg}$$

This is a deficit of elemental calcium, and calcium gluconate or borogluconate are the salts of choice for parenteral treatment. Calcium gluconate contains 9.3% of elemental calcium; in other words, every 100 mL of calcium gluconate 23% solution contains 2.14 g of elemental calcium or 21.4 mg/mL. The horse in this example would require approximately 300 mL of calcium gluconate 23% solution over 24 hours to replace the Ca^{2+} deficit. Ca^{2+} conversion factors are mg/dL = mmol/L × 4; mmol/L = mg/dL × 0.25.

Most veterinary laboratories do not measure Ca^{2+} concentrations; however, total calcium concentrations can also be used to calculate the overall deficit. By replacing measured and normal total calcium (12.0 mg/dL) in the same formula, and dividing by a 0.5 Ca^{2+} ratio, this formula gives a close approximation of the calcium deficit. It is important to mention that total calcium has more variability and is less tightly controlled than Ca^{2+} concentration. A horse may have total hypocalcemia, but serum Ca^{2+} concentrations may be within the normal range and in such a case calcium administration may not be necessary.

Frequent monitoring of calcium concentrations is recommended to adjust dosing. Some horses with severe gastrointestinal disease and sepsis remain hypocalcemic despite aggressive calcium therapy. In horses with colic, the response to calcium therapy was associated with outcome—survivors showed a steady return to normal values that was not seen in nonsurvivors.[168] Rapid administration of calcium may result in cardiovascular complications, particularly in septic animals, which may be more vulnerable to the cytotoxic effects of calcium. Fortunately, horses can handle large calcium dosages.[7,169] Based on experience treating critically ill horses, calcium doses of 2 mg/kg/hr are safe in horses. This represents approximately 50 mL of calcium gluconate 23% solution for a 500-kg horse per hour, while receiving fluid therapy. In horses with signs of hypocalcemia, higher doses are indicated.

Calcium chloride may be another option to treat hypocalcemia; however, it is not available in large volumes, it is more expensive, and it may cause irritation at the administration site. Do not add calcium salts to fluid solutions containing bicarbonate because calcium carbonate complexes may form and precipitate.

Oral treatment with calcium salts is feasible in horses with non–life-threatening hypocalcemia. Dicalcium phosphate and calcium carbonate (limestone) can be used safely (see Table 16.3). The management of horses with reduced calcium intake, which typically develop signs of orthopedic diseases, is different and directed to increase dietary calcium. This typically occurs in horses ingesting high phosphate or oxalate diets.

There is no clear evidence that vitamin D therapy affects outcome in foals or horses with refractory hypocalcemia. However, it should be considered in situations where clinical signs are persistent and the animal remains refractory to routine therapies. Based on recent work from our group showing that hypovitaminosis D is highly prevalent in critically ill equine patients,[3] if a decision to use vitamin D is made for an animal refractory to calcium therapy, it is recommended to use calcitriol ($1,25[OH]_2D_3$) rather than precursors because it displays rapid onset of action, renal activation is not required, and it has a short half-life (less potential for toxicity). The calcitriol dose for equids is unknown. Daily recommended calcitriol dose for small animals is 0.025 to 0.06 μg/kg. For children over 6 years of age and adults with hypoparathyroidism, the oral dose is 0.5 to 2 μg/day, and the intravenous (IV) dose is 1 to 2 μg 2 to 3 times weekly.

Hypocalcemic Disorders

Hypoparathyroidism

Hypoparathyroidism is a condition characterized by hypocalcemia, hyperphosphatemia, and decreased serum PTH concentrations. Hypomagnesemia may be present. Primary hypoparathyroidism results from decreased synthesis and secretion of PTH, whereas secondary hypoparathyroidism most commonly results from hypomagnesemia or sepsis. Mg^{2+} is required for PTH secretion and PTH1R activation; therefore Mg^{2+} depletion impairs PTH release and action.

Primary Hypoparathyroidism

This condition has been reported in horses.[146,162] Horses present with clinical signs of hypocalcemia, including ataxia, seizures, hyperexcitability, SDF, tachycardia, tachypnea, muscle fasciculations, stiff gait, recumbency, ileus, and colic. The diagnosis is based on the determination of serum concentrations

of Ca^{2+}, Mg^{2+}, phosphorus, and PTH. Hypocalcemia, hyperphosphatemia, hypomagnesemia, and low serum PTH concentrations are the features of primary hypoparathyroidism.[146,162] Low PTH leads to hypocalcemia and reduced renal PO_4 excretion and contributes to hypomagnesemia because PTH also promotes Mg^{2+} reabsorption in the distal nephron. Horses with primary hypoparathyroidism may benefit from $MgSO_4$ or $MgCl_2$ administration.

Secondary Hypoparathyroidism

Secondary hypoparathyroidism as a pathologic entity has not been described in horses; however, septic horses with hypocalcemia and impaired parathyroid gland function most likely have a form of secondary hypoparathyroidism from hypomagnesemia and increased concentrations of inflammatory mediators. This has been shown to be the case in horses with acute gastrointestinal disease.[4] It also has been demonstrated that inflammatory mediators known to be increased in critically ill horses (IL-1, IL-6, TNF-α) impair PTH secretion.[4-6] A number of foals with hypocalcemia do not have the expected increase in intact PTH concentrations, suggesting hypoparathyroidism.[2,3,144] Of interest, most hospitalized foals with hypocalcemia have an appropriate PTH response to hypocalcemia.[2,3] Secondary hypoparathyroidism with hypercalcemia was reported in an 11-year-old Andalusian gelding with chronic renal failure and polycystic kidney disease.[145]

Sepsis and Systemic Inflammation

For reasons not yet clearly understood, hypocalcemia is a frequent finding in critically ill humans and animals.[2-4,154,155,170-172] Sepsis is the most common cause of hypocalcemia in equine patients,[4,168] particularly in hospitalized newborn foals[2,3] and horses with severe gastrointestinal disease,[4,154,155,168,173] and the hypocalcemia can contribute to the patient's death. Total and ionized hypocalcemia are highly prevalent in horses with enterocolitis with 75% of these patients having total hypocalcemia, 80% exhibiting ionized hypocalcemia, and 70% having ionized hypomagnesemia.[4] Similar prevalence of hypocalcemia was documented in horses with colic.[168] Horses with severe hypocalcemia were more likely to die than those with normocalcemia.[168]

The mechanisms responsible for the development of hypocalcemia during critical illness are poorly understood. It is believed that in most acutely ill horses and foals, endotoxemia is the trigger leading to hypocalcemia.[1] Horses with gastrointestinal disease may have detectable endotoxin in plasma,[174-176] and parenteral administration of endotoxin to healthy horses has been shown to cause hypocalcemia.[6,177] Possible causes of hypocalcemia include renal loss of calcium,[178] calcium sequestration in the gastrointestinal lumen,[179] intracellular calcium accumulation,[180] impaired calcium mobilization,[181-183] tissue sequestration,[170,178] reduced calcium release in response to PTH,[178] failure to synthesize $1,25(OH)_2D$,[171] and parathyroid gland dysfunction.[4] Renal loss of calcium seems unlikely, as horses with endotoxemia, enterocolitis, and hypocalcemia have been shown to have low fractional urinary excretion of calcium.[4,6]

Some horses with evidence of SIRS and hypocalcemia have inappropriately low serum concentrations of PTH, indicating parathyroid gland dysfunction.[4] Most hospitalized foals with hypocalcemia have an adequate PTH response.[2] However, a number of them remain hypocalcemic, with PTH concentrations below or in the normal range, which is consistent with functional hypoparathyroidism secondary to critical illness. In some cases, low Mg^{2+} concentrations could explain the low PTH, but in other instances Mg^{2+} concentrations are in the normal range. It is likely that inflammatory byproducts, endotoxins, and cytokines (IL1-β, IL-6, and TNF-α) disrupt parathyroid chief cell function.[6] It has been shown that in endotoxemia, IL-1β and IL-6 could decrease PTH mRNA transcription and secretion by equine parathyroid chief cells.[5] It is well documented that these cytokines are increased in foals and horses with systemic inflammation.[6]

Hypocalcemia in Foals

Sepsis, SIRS, hypomagnesemia, acute renal injury, pancreatitis, and acute muscle injury have been linked to hypocalcemia in newborn foals. *Idiopathic hypocalcemia* is a broad term used to describe a hypocalcemic condition that occurs in young foals for reasons poorly understood. From experience, idiopathic hypocalcemia likely represents multiple disorders, due to increased inflammatory cytokines, abnormal parathyroid gland function, hypomagnesemia, and calcium shifts to different compartments. It is also possible that genetic disorders may explain this disorder in some foals.

Exercise-Induced Hypocalcemia

Electrolyte and acid-base abnormalities are frequent in horses undergoing intense physical activity. Unlike human athletes, who develop either ionized hypocalcemia or hypercalcemia, ionized hypocalcemia is more common in exercising horses.[153,184,185] Hypocalcemia in these horses could be explained by sweat loss of calcium,[163,186] calcium shift to the intracellular compartment,[187] and increased Ca^{2+} binding to albumin due to alkalosis.[182] Exercising horses develop respiratory alkalosis from hyperventilation, metabolic alkalosis from Cl^- loss in sweat (hypochloremic), or a combined alkalosis. Serum Ca^{2+} decreased while serum PTH increased in jumping horses, suggesting that parathyroid gland dysfunction as the cause of hypocalcemia was unlikely.[56] One study in endurance horses found that serum PTH concentrations did not increase in all horses that developed hypocalcemia, indicating that in some horses impaired parathyroid gland function could be a contributory factor.[153] Renal loss of calcium as a cause of hypocalcemia during exercise is unlikely because urinary calcium concentrations decrease in performance horses.[164] Dietary calcium and phosphorus supplementation did not impair PTH secretion in exercising horses.[185]

Common clinical signs in horses with exercise-induced hypocalcemia include SDF, muscle weakness, muscle fasciculation, ileus, colic, excitation, and cardiac arrhythmias.

Cantharidiasis

Equine cantharidiasis (blister beetle toxicosis) is a condition reported in the central, southern, and midwestern United States and is produced by the ingestion of alfalfa contaminated with blister beetles (*Epicauta* spp.), which produce cantharidin (cantharidic acid).[159,188,189] Cantharidin is such a caustic compound that it is being used in human dermatology to treat recalcitrant warts and molluscum contagiosum.[190] Horses are very susceptible to the irritant effects of this toxin on mucosal surfaces (gastrointestinal and urinary tracts). Cantharidin causes necrosis and sloughing of the intestinal mucosa, acute tubular necrosis/renal failure, and myocardial necrosis. Laboratory abnormalities are a consequence of gastrointestinal, renal, and myocardial dysfunction. Acute hypocalcemia

and hypomagnesemia are consistent findings. Clinical signs include SDF, muscle fasciculations, ataxia, dyspnea, laryngeal spasm, cardiac dysrhythmias, colic, diarrhea, endotoxemia, dehydration, hypotension, hematuria, stranguria, pollakiuria, and sudden death.[1,159,188,189] Additional systemic signs are the result of multiple abnormalities, including acid-base, electrolyte, proinflammatory, and hemodynamic changes.

Acute Renal Failure

Hypocalcemia and hypomagnesemia are common findings in horses with acute renal injury. Reabsorption of Ca^{2+} and Mg^{2+} by the nephron is highly dependent on functional epithelial cells, which are very susceptible to various insults (hypoxia, toxins). Damage to tubular epithelial cells leads to reduced absorptive capacity for Ca^{2+} and Mg^{2+}. Additional details are provided in Chapter 14.

Exertional Rhabdomyolysis

The pathogenesis of hypocalcemia in exertional rhabdomyolysis is unknown. Speculations include muscle fiber damage during intense exercise resulting in Ca^{2+} influx and sequestration into the sarcoplasm and SR. A several-fold increase in sarcoplasmic $Ca^{2+}{}_i$ concentration has been reported in horses with exertional rhabdomyolysis,[191] indicating that elevations in $Ca^{2+}{}_i$ concentrations may be important in the pathogenesis of ER. It is also possible that alterations in the acid-base status during exercise, alkalosis in particular, could contribute to the development of hypocalcemia in some horses. See Chapter 10 for additional information on this topic.

Pancreatitis

Pancreatitis is a well-recognized cause of hypocalcemia in people and small animals; however, there are few reports of pancreatic disease in foals or horses.[192-195] Pancreatitis is rare in horses and when present is often associated with gastrointestinal disease.[193] Hypocalcemia associated with pancreatitis and seizures was reported in a newborn foal.[195] The foal had diarrhea, abdominal distention, peritoneal effusion, increased lipase and amylase activities, hypoglycemia, and hypertriglyceridemia. It was unclear whether the hypocalcemia was a consequence of pancreatic disease or SIRS. Pancreatitis should be in the list of differentials in foals and horses with hypocalcemia and acute abdominal disease, and assessing lipase and amylase activities and triglyceride concentrations should be considered in animals without a clear diagnosis. Low calcium concentrations were reported in horses with pancreatic disease, many of which also had large intestinal pathologies.[194]

Oxalate Toxicity

The rapid ingestion of oxalate-containing plants (see Table 16.6) can cause signs of acute hypocalcemia. More commonly, one sees chronic oxalate toxicity from prolonged consumption of these plants. The clinical presentation is associated with orthopedic problems (lameness, fractures) as a consequence of secondary hyperparathyroidism and reduced bone mass from (see Nutritional Secondary Hyperparathyroidism section later in this chapter). Some of these animals may develop neuromuscular signs from hypocalcemia.

Other causes of hypocalcemia include excessive administration of tetracyclines, furosemide, and bicarbonate. Hypovitaminosis D remains to be documented as a cause of hypocalcemia in horses.

Hypercalcemic Disorders

Hypercalcemic disorders can be divided in two categories: (1) hypercalcemia associated with parathyroid gland dysfunction (parathyroid-dependent hypercalcemia) and (2) hypercalcemia independent of the parathyroid gland function (hypercalcemia develops despite parathyroid gland suppression). Parathyroid-dependent hypercalcemia in the horse is limited to primary hyperparathyroidism, whereas parathyroid-independent hypercalcemia occurs from various conditions (secondary hyperparathyroidism, chronic renal failure, cancer, vitamin D intoxication).

Primary Hyperparathyroidism

Primary hyperparathyroidism (PHPT) is an endocrinopathy in which the chief cells of the parathyroid glands autonomously secrete excessive amounts of PTH and do not respond to the negative feedback of Ca^{2+}. In horses, PHPT results from parathyroid adenomas or parathyroid hyperplasia.[148,149,196] Parathyroid carcinoma has not been reported in the horse. In the kidneys, PTH will increase Ca^{2+} reabsorption (hypocalciuria), reduce phosphorus reabsorption (hyperphosphaturia), and promote $1,25(OH)_2D_3$ synthesis, whereas in bone it will enhance osteoclastic activity. Thus hypercalcemia, hypophosphatemia, and increased intact PTH concentrations are typical laboratory findings of PHPT. Osteodystrophia fibrosa (also known as "rubber jaw" or "big head") from excessive bone loss may ensue. Primary hyperparathyroidism associated with osteodystrophia fibrosa has been reported in ponies and horses.[147,197-200] These horses may have enlargement of the facial bones, lameness, and a poor body condition. Radiographic findings include reduced bone density, particularly in flat bones, and loss of the lamina dura dentes surrounding molars and premolars.[147] Endoscopic examination may reveal narrowing of the nasal passages. Previous attempts to identify abnormal equine parathyroid tissue by nuclear scintigraphy failed.[196] However, nuclear scintigraphy and ultrasonography were successfully used to detect functional parathyroid adenomas in horses.[148, 148a] Ultrasonography alone was useful to identify parathyroid tissue (adenoma) within the thyroid gland of a horse with clinical and laboratory findings consistent with PHPT, including hypercalcemia, hypophosphatemia, increased serum PTH concentration, and low urinary fractional excretion of calcium.[149]

Tests to rule out other conditions associated with hypercalcemia include assessment of renal function, urinary excretion of electrolytes, and measurement of PTHrP and vitamin D metabolite concentrations.

Postmortem findings in horses with PHPT include enlargement of the maxilla and mandible, stenosis of the nasal passages, and loosening of premolars and molars. Histologic examination of the parathyroid gland is important to confirm the diagnosis of PHPT (chief cell hyperplasia); however, finding the parathyroid glands in horses can be challenging due to their small size and variable location.

Secondary Hyperparathyroidism

In secondary hyperparathyroidism, excessive PTH secretion is the response of the parathyroid gland to hypocalcemia and/or hyperphosphatemia and/or hypovitaminosis D from renal disease or from nutritional imbalances.

Renal Secondary Hyperparathyroidism

In the case of renal secondary hyperparathyroidism in small animals and humans, phosphorus retention from renal disease

and decreased $1,25(OH)_2D$ synthesis stimulate PTH secretion.[201] In addition, hyperphosphatemia lowers serum Ca^{2+} concentrations and inhibits renal 1α-hydroxylase, which is required for $1,25(OH)_2D$ synthesis. Reduced concentrations of $1,25(OH)_2D$ lower intestinal Ca^{2+} absorption, further contributing to hypocalcemia and PTH release. Hypercalcemia is a consistent finding in horses with chronic renal failure (CRF); however, phosphorus concentrations are variable. Of interest, hypercalcemia in horses with CRF is not the result of high PTH concentrations (hyperparathyroidism) but rather a consequence of calcium intake and retention. Serum PTH concentrations in horses with CRF and hypercalcemia are often in the normal range.[145,202] Because equine intestinal calcium absorption is high and poorly regulated, normal renal function is crucial to eliminate excess calcium. Any reduction in glomerular filtration from chronic renal injury will impair excretion, resulting in hypercalcemia.

Nutritional Secondary Hyperparathyroidism

Nutritional secondary hyperparathyroidism (NSHPT) is the result of increased PTH secretion (hyperparathyroidism) from reduced intestinal calcium absorption rather than a hypercalcemic disorder per se. If anything, it can be classified as a hypocalcemic disorder because, unlike other forms of hyperparathyroidism, hypercalcemia is not a feature of NSHPT. Disorders resembling NSHPT appeared in the peer-reviewed literature in the nineteenth century[198,203]; however, there are reports as early as the fourth century of a condition described by Vegetius as animal osteomalacia,[198] which can be assumed to be NSHPT.

Horses fed a diet low in calcium, high in phosphorus, or high in oxalate may develop NSHPT. This condition, also known as bran disease, miller's disease, big head, osteodystrophia fibrosa, fibrous osteodystrophia, osteitis fibrosa, and equine osteoporosis, can affect one or many animals in a herd. Balanced equine diets should have a calcium:phosphorus ratio greater than 1.5:1 to meet daily requirements. Diets with a calcium:phosphorus ratio less than 1:3 predispose horses to NSHPT.[150,204] This condition is uncommon in developed countries; however, a young horse may occasionally present with signs of NSHPT, including enlargement of facial bones, upper respiratory noise, and lameness. The condition is endemic in some regions of Latin America, North America, Africa, Asia, Australia, and Europe.[32,150,205-211] In old horses, facial bone enlargement is variable but in general not as evident as in young animals. In the past, NSHPT was associated with grain-rich diets (high phytate-PO_4 content); however, with improvements in animal nutrition, NSHPT is rarely associated with excessive grain feeding. Rather it is more commonly linked to the ingestion of plants containing large amounts of oxalate (oxalic acid) (see Table 16.6), which bind dietary calcium to form insoluble calcium oxalate (CaC_2O_4), thus decreasing calcium absorption (Fig. 16.9).[206-212] A 1% oxalate content in the equine diet can reduce calcium absorption by about 67%.[31] Diets with greater than 0.5% oxalate or a calcium:oxalate ratio less than 0.5 are detrimental to the calcium balance in horses and ponies.[32] Ruminants are more tolerant to oxalate toxicity than monogastric animals because rumen bacteria degrade a major part of oxalate into formic acid and carbon dioxide.[213] Some plants containing harmful amounts of oxalate are listed in Table 16.6. Many of these plants can contain up to 20% of their dry weight as soluble and insoluble oxalates.[211,212] Oxalate also binds magnesium, but the resulting complex (MgC_2O_4) is more soluble

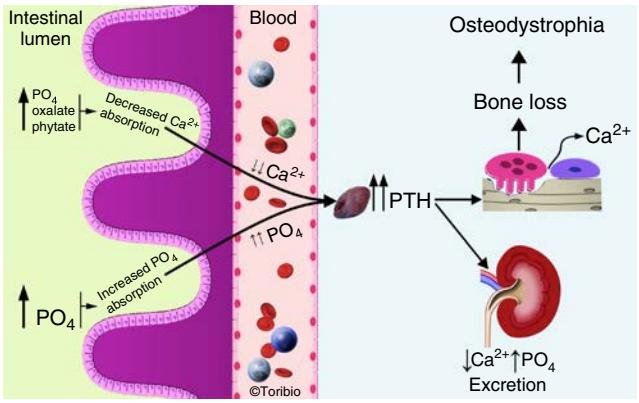


FIG. **16.9** Pathogenesis of nutritional secondary hyperparathyroidism. The ingestion of diets rich in phytates, phosphates (PO_4), or oxalates that interfere with calcium (Ca^{2+}) absorption stimulate the parathyroid gland to release excessive amounts of parathyroid hormone (PTH). In the kidneys PTH will promote Ca^{2+} reabsorption and PO_4 excretion, whereas in bone PTH will increase osteoclastic bone resorption leading to bone loss that will be replaced with unmineralized fibrous tissue. This results in signs consistent with nutritional secondary hyperparathyroidism (see Figs. 16.10 and 16.11). (Courtesy Dr. R. E. Toribio, The Ohio State University.)

than CaC_2O_4 and more likely to be absorbed. If dietary calcium is low, soluble oxalate can be absorbed and combine with calcium or magnesium in circulation to form crystals that precipitate in the kidneys, causing renal failure. However, this is not as widely a recognized problem in horses as in other species.

Excessive dietary phosphorus reduces calcium absorption, leading to hypocalcemia, hyperphosphatemia, and hyperparathyroidism (see Fig. 16.9).[198] Hyperphosphatemia stimulates PTH secretion but inhibits renal $1,25(OH)_2D$ synthesis. Given that $1,25(OH)_2D$ is a suppressor of parathyroid cell function, low $1,25(OH)_2D$ concentrations will further contribute to parathyroid cell hyperplasia and PTH secretion. As previously mentioned, PTH will increase osteoclastic activity, bone resorption, and bone loss.[214] Facial bone loss and excessive accumulation of unmineralized bone matrix (osteodystrophia fibrosa) results in facial enlargement (big head) (Fig. 16.10). Because this is a slowly progressive condition, the homeostatic mechanisms that regulate extracellular Ca^{2+} concentrations (PTH, vitamin D, calcitonin) may be effective at maintaining Ca^{2+} close to or within the normal range. Thus these animals rarely have abnormal calcium concentrations.

Clinical signs of NSHPT may include intermittent, shifting lameness, and a stiff gait.[211,215,216] In most cases there is swelling of the facial bones. Young animals may develop physeal enlargement and limb deformities.[204] Bone resorption around the lamina dura dentes of molars and premolars may cause masticatory problems. These animals are weak and may be in poor body condition. In severe cases, teeth may become loose, and spontaneous fractures may occur. Vertebral abnormalities and compressive neurologic signs may develop.[211] Epiphora may be present from obstruction of the nasolacrimal duct. Upper airway obstruction from deformation of nasal passages and turbinates may impair airflow, causing stridor and dyspnea.[205,211,217] Signs of acute hypocalcemia may develop in some animals as a consequence of high dietary oxalate content combined with a low-calcium diet.[211] In addition, absorption of soluble oxalate can chelate calcium in the circulation (and to lesser extent, magnesium), leading to hypocalcemia.



FIG. **16.10** Belgian yearling presented to The Ohio State University Veterinary Medical Center with clinical signs consistent with NSHPT, including enlargement of facial bones and upper respiratory noise. The horse was fed excessive amounts of grain. Narrowing of the nasal passages, loss of bone mass, and excessive accumulation of unmineralized bone matrix (osteodystrophia fibrosa) was evident. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

Differential diagnoses linked to excessive bone loss include NSHPT, PHPT, HHM, and bone fragility syndrome (silica-associated osteoporosis). However, there are unique clinical, laboratory, and epidemiologic features characteristic of these conditions. PHPT and HHM affect single animals and are characterized by hypercalcemia and hypophosphatemia, with either increased PTH but low PTHrP (i.e., PHPT) or increased PTHrP and low PTH (i.e., HHM). Body condition tends to be better with NSHPT and PHPT than HHM and bone fragility syndrome.

Typical laboratory findings during active disease include mild hypocalcemia and hyperphosphatemia although these values may be within the normal range.[218,219] Depending on the inciting cause, serum phosphorus concentrations could be increased (high dietary phosphate), low, or within normal limits (oxalate toxicity). Serum PTH concentrations may be increased.[209,215] Urinary excretion of calcium is low (normal 3%–10%) and of phosphorus is high (normal less than 0.5%).[209] Calculating the urinary fractional excretion of phosphorus may be useful to assess phosphorus intake and PTH activity. Phosphaturia is a consequence of hyperphosphatemia when dietary phosphate is high but also reflects the phosphaturic actions of PTH. In the case of oxalate toxicity, phosphaturia is mostly mediated by high PTH concentrations. Serum alkaline phosphatase activity may be increased. Markers of bone resorption will likely increase, but they have not been investigated in horses with this condition.

Radiologic findings include decreased bone density[215,219]; however, bone mass must be decreased 30% before it is radiographically detectable.[219,220] Resorption of the dental alveolar sockets may be present before any other radiographic changes are evident. Radiolucency, diffuse osteolysis, and osteopenia may be detected in long bones, vertebrae, and ribs early in the process and can progress to a patchy osteoporotic pattern from excessive bone resorption, particularly with chronic oxalate toxicity (Fig. 16.11). Taking radiographs with similar settings from age-matched healthy animals for comparison purposes is recommended. This is also useful for radiographic follow-up in response to therapy.

Pathology findings include facial bone enlargement, in particular maxillae and mandible, weak bones, reduced cortical thickness, fractures, and deformities in appendicular and axial skeleton.[198,211] The kidneys are in general normal, but there could be mineralization. Histologically, there is thin porous cortical and trabecular bone, fibrous periosteal thickening, and active bone remodeling with increased osteoclast number.[211] There is parathyroid gland hypertrophy with chief cell hyperplasia, consistent with chronic hypocalcemia or hyperphosphatemia.[198,211]

Dietary supplementation with calcium carbonate (limestone; $CaCO_3$) or dicalcium phosphate (dibasic calcium phosphate; $CaHPO_4$) has resulted in clinical improvement.[150] An affected animal may require a total of 100 to 300 grams/day, and the diet should have a Ca:P ratio of 3 to 4:1. Limestone may decrease feed palatability, and adding molasses can improve palatability. Oxalate-rich plants with high calcium content (e.g., legumes) are considered safe for equids.[213,221] The addition of alfalfa hay and a reduction in grain intake may be helpful. Confinement of severely affected horses is advised. The use of nonsteroidal antiinflammatory drugs (NSAIDs) may be indicated in horses and ponies with severe pain. Supplementation with vitamin D has been proposed. Young horses may require up to 12 months for complete recovery. For animals consuming feeds with high oxalate content, 20 additional mg of calcium/kg and 10 mg of phosphorus/kg BW per day may be necessary.[32]

Prognosis for NSHPT varies depending on age, duration, severity of clinical signs, inciting cause (phosphorus vs. oxalate ingestion), and response to treatment. Young animals have a fair to good prognosis if treated early. Animals with mild clinical signs have a good prognosis, whereas those with severe facial deformation, dental loss, orthopedic complications, and

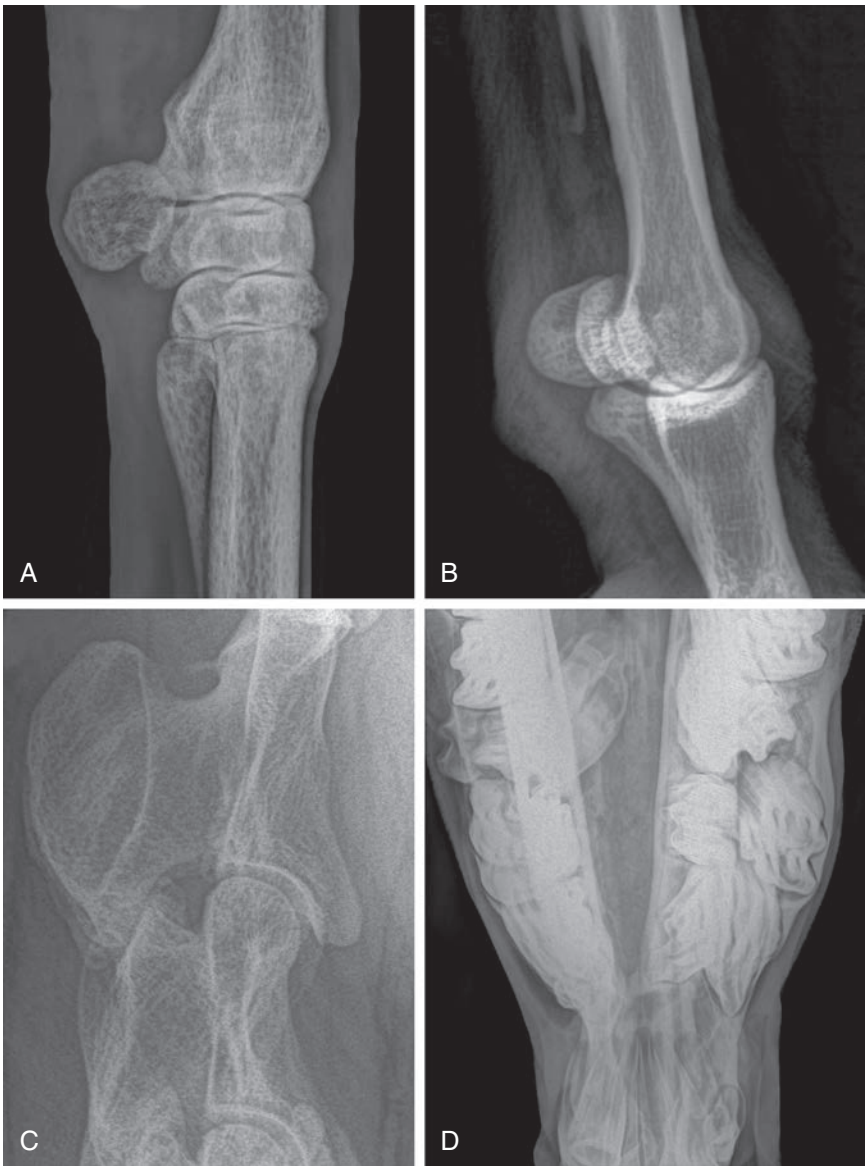


FIG. **16.11** Radiographs of adult miniature horses with nutritional secondary hyperparathyroidism associated with oxalate toxicity from chronic ingestion of soursob *(Oxalis pes-caprae).* Lateral views of the carpus (A) and fetlock (B) show diffuse and excessive bone loss and osteolysis, reduced density (osteopenia), widespread radiolucency (evident in accessory carpal and sesamoid bones), and thinning of cortical bone. A similar pattern is present in the cervical vertebrae (C). Dorsoventral view of the skull reveals reduced bone density, dental displacement, and loss of the laminae dura (D). (Courtesy Dr. E. W. Herbert, Adelaide Plains Equine Clinic, Gawler, Australia.)

neurologic signs have a guarded to poor prognosis. Signs tend to be more severe with oxalate toxicity.

Hypervitaminosis D

The ingestion or administration of ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) causes disturbances of calcium and phosphorus homeostasis in domestic animals.[151,222-229] Intoxication with both forms of vitamin D have been reported in horses.[151,223,229] Ingestion of plants containing 1,25$(OH)_2$D-like compounds results in clinical signs of vitamin D toxicity.[224,226-228,230] *Solanum glaucophyllum (S. malacoxylon)* is associated with a condition known as "enteque seco" in Argentina and "espichamento" in Brazil.[222,228,230] In Hawaii, the ingestion of *S. sodomaeum* and *S. torvum* results in hypercalcemia.[230] *Nierembergia* spp. have also been linked to hypervitaminosis D in Brazil and Uruguay.[230] Jessamine *(Cestrum diurnum),* a shrub widely distributed in the southern United States (from Florida to California), Jamaica, and Cuba may cause hypervitaminosis D.[224,230] In Europe, *Trisetum flavescens* has been linked to enzootic calcinosis in ruminants and horses.[230-233] Hyperphosphatemia is the most consistent and early laboratory finding in horses with vitamin D intoxication.[223] Serum calcium concentrations may be within the normal range or increased.[151,223,227,229] Because vitamin D is a negative regulator of parathyroid cell proliferation, parathyroid cell atrophy and low PTH concentrations may be present.[225]

Clinical signs include anorexia, weight loss, polyuria, polydipsia, lameness, and reluctance to move.[223,229,233] Acute death from severe cardiovascular mineralization has been reported.[151] Renal mineral deposition may precede mineralization elsewhere, leading to renal failure, uremia, hyposthenuria, polyuria, and polydipsia.[151] Lameness is probably due to calcification of ligaments and tendons. Radiographically, these horses have increased bone density, reduced medullary cavity lumen, and increased calcification of soft tissues.

The prognosis for horses with hypervitaminosis D is poor. Treatment may include reducing dietary calcium intake. The use of calcium-binding agents such as sodium phytate, which is high in many cereals, has been proposed.[229] Glucocorticoids are used in humans with hypervitaminosis D, with the rationale that they may inhibit the vitamin D–mediated intestinal calcium absorption. Dexamethasone administration to pony foals decreased intestinal calcium absorption, reduced bone resorption, and increased urinary excretion of calcium.[34,35] Dexamethasone has been administered to horses with hypervitaminosis D with inconclusive results.[229]

Postmortem examination may reveal mineralization of soft tissues. Extensive cardiovascular mineralization of the endothelium of the aorta and pulmonary vessels, as well as of the endocardium of the atrium and ventricles, is frequent. Mineralized plaques may be present in the endothelium and endocardium. Mineralization may be found in the kidney, liver, lymph nodes, lungs, ligaments, and tendons. Osteopetrosis of the epiphyses and metaphyses may be present. Atrophy of the parathyroid chief cells may be severe.[225]

Hypercalcemia of Malignancy

Humoral hypercalcemia of malignancy (HHM) refers to a paraneoplastic condition in which tumors secrete PTHrP (pseudohyperparathyroidism) leading to hypercalcemia. In horses, HHM has been described with gastric, vulvar, and preputial squamous cell carcinomas, adrenocortical carcinoma, lymphosarcoma, multiple myeloma, and ameloblastoma.[132-136,234,235] These animals have hypercalcemia, hypophosphatemia, low to normal PTH concentrations, reduced urinary excretion of calcium, increased urinary excretion of PO_4, and, over time, reduced bone density. Most clinical signs are the result of the primary problem rather than hypercalcemia. Severe hypercalcemia can potentially reduce gastrointestinal motility, induce diuresis, and disrupt neurologic function.

Neonatal Hypercalcemia and Asphyxia

Clinical observations indicate that a number of critically ill newborn foals develop hypercalcemia that is not associated with renal failure or excessive PTH secretion. These foals are usually recumbent, have severe hypotension, and often die of asphyxia. Factors to consider in the pathogenesis of this condition include high PTHrP and $1,25(OH)_2D$ concentrations; however, this remains to be investigated.

Idiopathic Systemic Granulomatous Disease

This appears to be an immune-mediated disorder that has been documented in horses and ponies (equivalent to human sarcoidosis) in which crusty and exudative skin lesions develop in different locations, as well as along the coronary bands and mucocutaneous junctions.[236-238] In horses, cattle, and human beings with systemic granulomatous disease, there is perivascular granulomatous and lymphoplasmacytic inflammation in multiple organs, including lungs, liver, gastrointestinal tract, kidneys, and skin.[236,239,240] Some animals may develop hypercalcemia.[236,239,240] It has been proposed that excessive production of vitamin D or PTHrP by granulomatous cells leads to hypercalcemia in these animals.[1] At least in one case, increased expression of PTHrP in mononuclear cells was documented in a pony with this condition.[236] In horses, lesion distribution may resemble that of multisystemic eosinophilic epitheliotropic disease. In cattle, hairy vetch *(Vicia villosa)* toxicity has been linked to infiltration of many organs and skin by monocytes, lymphocytes, eosinophils, and multinucleated cells.[241] These animals have dermatitis, pruritus, diarrhea, and wasting.[241] A similar disorder has been recognized in horses grazing hairy vetch,[237,238] which showed crusting dermatitis, alopecia, conjunctivitis, lymphadenopathy, ventral edema, diarrhea, and weight loss.[236-238]

Calcinosis

Calcification of soft tissues (liver, heart, kidney, lungs, vessels, muscle), hyperphosphatemia, elevation of the calcium × phosphorus product, and evidence of calciphylaxis were reported in a number of horses with various pathologies.[242] Calcinosis has been defined as mineralization or calcium deposition in soft tissues. This disorder is different from enzootic calcinosis due to ingestion of plants with vitamin D–like activity. Calciphylaxis is a form of extraskeletal calcification (as calcinosis) but more specifically refers to calcification and necrosis of vascular structures leading to organ damage. In people, calciphylaxis mostly occurs with advanced chronic kidney disease. The underlying mechanisms by which these horses developed calcinosis and hyperphosphatemia are unclear. However, calcium phosphate precipitation due to a high calcium × phosphorus product can be seen simply as a consequence.

Equine Bone Fragility Syndrome (Osteoporosis, Silicosis)

The equine bone fragility syndrome (BFS) or silica-associated osteoporosis (SAO) is a progressive and chronic disorder of horses characterized by exercise intolerance, increased respiratory efforts, skeletal deformation, lameness, stiffness, and fractures.[243,244] Lordosis, bowing of appendicular bones (scapula in particular), and neurologic signs as a result of vertebral deformation and spinal cord compression also occur.[243,244] Horses of any age are affected, with median ages of 9 and 14 years (4–25 years).[243,245] The condition has been documented in animals as young as 6 months old.[244]

The pathogenesis of this condition is unclear, but there is a strong association with pulmonary silicosis (a form of pneumoconiosis). Radiographically, in the skeleton there is osteopenia whereas in the respiratory tract the pattern is consistent with silicosis. Cytologic examination of bronchoalveolar cells reveals the presence of silica crystals. Histologically, there is excessive osteoclastic activation and bone resorption. In the respiratory tract there is pulmonary granuloma formation, fibrosis, and lymphadenitis.[246] Intracellular crystals within pulmonary and lymphoid macrophages and dystrophic calcification are consistent findings in horses with SAO.[246] These crystals have been identified as cytotoxic silica dioxide polymorphs.[246] Of interest, high concentrations of a cytotoxic and fibrogenic form of silicate (cristobalite) are found in regions of California where the condition is more common (Monterey shale soil).[243,244,246,247]

The precise mechanisms leading to excessive bone loss in these animals, in which initial manifestations tend to be

respiratory signs, remain to be elucidated. It has been proposed that inflammatory cytokines known to be produced in excess in human pulmonary silicosis (IL-1, IL-6, TNF-α), as well as the release of RANKL, may be activating osteoclastogenesis.[246] The role of the calcium and phosphorus homeostatic system in the development of BFS remains to be determined. Some horses could have increased PTH concentrations, but this is an inconsistent finding.[246]

Some of the bone multifocal and lytic distribution in BFS resembles Paget's disease of bone in people, in which there are pathologic fractures, skeletal deformation, excessive osteoclastic activity, and increased cortical thickness. However, BFS is progressive and affects horses of any age, whereas Paget's disease, once it develops, is more static and mainly affects the elderly.

Even though certain findings are shared with secondary hyperparathyroidism (lameness, lordosis, fractures, high PTH), others are different. For example, the association with respiratory disease, normal skull morphology, increased cortical bone width, and lack of parathyroid hyperplasia are consistent with BFS. Whether a mechanism similar to hypertrophic pulmonary osteopathy (Marie's disease) contributes to skeletal disease with this condition remains to be documented. At the end, evidence indicates that this is a disorder secondary to pulmonary silicosis rather than a primary bone disease. This is further supported by lack of measurable silicon levels in bone tissue from affected horses.[246]

The diagnosis of BFS is made based on clinical history, clinical findings, geography, and imaging (ultrasonography, radiography, and scintigraphy). Scintigraphy remains the best diagnostic modality, although scapular ultrasonography can be diagnostic in animals with severe disease.[245] Serum chemistry and markers of bone remodeling do not appear to have diagnostic value.[245]

The management of these horses depends on predominant clinical signs and prevention. Nonsteroidal antiinflammatory drugs (e.g., phenylbutazone) should be considered in animals with orthopedic pain, keeping in mind that excessive ambulation can increase the risk of fractures. There must be a good justification for the use of glucocorticoids, perhaps for pulmonary disease, as they also favor bone loss. This is one of the few equine conditions in which bisphosphonates may be indicated. Data from one study showed benefits in most horses with BFS 6 months after zoledronate administration.[248] However, no controls were included in that study to provide an objective assessment of the results. Reduction in physical activity and dietary manipulation are indicated.

Hypertrophic pulmonary osteopathy, or Marie's disease, is a condition described in humans and domestic animals associated with intrathoracic space-occupying lesions (neoplasia, abscesses, chronic pneumonia), but less frequently with abdominal masses, that results in periosteal proliferation and mineralization of appendicular bones. Involvement of the calcium-regulating endocrine system in the pathogenesis of this condition has not been demonstrated. However, it should be considered as a differential diagnosis in endocrine, metabolic, and paraneoplastic syndromes of horses that alter bone mass and structure.

Treatment of Hypercalcemia

Hypercalcemia is rarely presented as an equine emergency; however, the differential diagnosis of hypercalcemia is important in the treatment of this disorder. Mild to moderate hypercalcemia in general is not life threatening, and treatment should be directed to the primary cause (primary hyperparathyroidism, chronic renal failure). Hypervitaminosis D can be fatal when there is mineralization of vital organs. Surgical removal of epithelial tumors may be a successful treatment in some patients. In cases of severe hypercalcemia that require treatment, initial therapy should include the administration of 0.9% saline solution and loop diuretics. Furosemide is the diuretic of choice because it inhibits the $Na^+/K^+/2Cl^-$ cotransporter in the distal nephron, increasing urinary excretion of calcium. Thiazide diuretics are contraindicated because they stimulate calcium reabsorption. Glucocorticoid administration should be considered.

PHOSPHORUS DISORDERS IN THE HORSE

Disorders of phosphorus homeostasis can be acute or chronic, resulting in hypophosphatemia or hyperphosphatemia. In critically ill equids hypophosphatemia is more frequent whereas in foals hyperphosphatemia is more common.[3] Genetic conditions leading to hypophosphatemia or hyperphosphatemia remain to be documented in horses.

Hypophosphatemia

Hypophosphatemia develops from three mechanisms: (1) reduced intestinal PO_4 absorption, (2) increased urinary PO_4 excretion, and (3) intracellular shift of PO_4 (redistribution). Acute hypophosphatemia could reflect PO_4 redistribution between intracellular and extracellular compartments and might not necessarily imply total body PO_4 depletion. However, in horses that have been sick for many days one should assume that PO_4 depletion may be present. Based on clinical experience, hypophosphatemia occurs more often in Miniature Horses, ponies, and donkeys than in horses, usually associated with hyperlipemia and parenteral nutrition.

Absorption

Intestinal absorption of PO_4 is decreased by gastrointestinal pathologies (diarrhea, inflammatory bowel disease, enteritis), hypovitaminosis D, diets deficient in PO_4, and interfering substances. Hypovitaminosis D can result in hypophosphatemia for multiple reasons. Vitamin D promotes intestinal absorption and renal reabsorption of calcium and PO_4. A reduction in $1,25(OH)_2D$ leads to hypocalcemia, which increases PTH secretion, and subsequently enhances urinary PO_4 excretion. In addition, low $1,25(OH)_2D$ concentrations exacerbate PTH secretion as $1,25(OH)_2D$ is a negative regulator of parathyroid chief cell function and PTH secretion.

Excretion

The urinary excretion of PO_4 is increased with hyperparathyroidism, hypovitaminosis D, tumors, renal failure and drugs (aminoglycosides, diuretics). Hyperparathyroidism (primary and secondary) was previously discussed. Hypophosphatemia from cancer is mainly the result of increased urinary PO_4 excretion. Cancer-induced hypophosphatemia occurs by two mechanisms: (1) phosphaturia from increased PTHrP concentrations (PTHrP activates PTH receptors in association with HHM) and (2) phosphatonin-mediated phosphaturia.[1] Data on phosphatonin-mediated hypophosphatemia are lacking in domestic animals.

Hyperphosphatemia and hypocalcemia are the hallmarks of acute renal failure in horses, whereas chronic renal failure is characterized by hypercalcemia and hypophosphatemia.[1] Diuretics increase the urinary excretion of PO_4, with those acting in the proximal tubules (carbonic anhydrase inhibitors) having the most phosphaturic effects, as this is the main site for PO_4 reabsorption.[249,250] Neurogenic and nephrogenic diabetes insipidus may be associated with hypophosphatemia from renal loss as well. In other species, renal distal tubular acidosis, metabolic acidosis, and Fanconi syndrome can result in hypophosphatemia as additional causes of decreased renal reabsorption of PO_4.[249,251]

Redistribution

Phosphate redistribution is the main cause of hypophosphatemia in critically ill human patients.[252-254] Redistribution is likely to be important in the development of hypophosphatemia in critically ill horses, ponies, and foals. Causes of PO_4 redistribution include insulin-mediated shift of PO_4, increased catecholamine concentrations, and respiratory alkalosis.[252-254] Sepsis, high-carbohydrate diets, hyperglycemia, insulin administration, starvation, refeeding syndrome, hyperlipemia, and parenteral nutrition (hyperglycemia, hyperinsulinemia) all decrease serum PO_4 due to insulin-mediated intracellular shift of PO_4 and enhanced glycolysis. Hypophosphatemia from these disorders is well documented in critically ill humans and small animals.[249] Clinical experience indicates that hypophosphatemia is associated with similar conditions in acutely ill equine patients. Catecholamines, similar to insulin, stimulate the activity of the Na^+/K^+ ATPase and the requirements for PO_4. In respiratory alkalosis, as blood and intracellular CO_2 decrease, the pH rises. Alkalosis stimulates phosphofructokinase activity (glycolysis), and the need for PO_4 increases to synthesize ATP. As a result, PO_4 from the extracellular compartment moves into the cell, leading to hypophosphatemia. Hyperventilation (respiratory alkalosis) is considered the main cause of hypophosphatemia in human intensive care units (ICUs).[254,255] Even though diabetes mellitus is rare in horses, it has been linked to hypophosphatemia in other species.[249] Diabetic individuals develop hypophosphatemia from reduced insulin concentrations (type 1 diabetes), insulin resistance (type 2 diabetes), and polyuria leading to PO_4 wasting. Insulin therapy can induce or worsen hypophosphatemia, a factor to consider when treating equine patients with energy dysregulation. This is typically seen in ponies or miniature horses with hyperlipemia. Ketoacidosis is not a common consideration in equine practice.

In horses with a history of malnutrition, close monitoring of serum PO_4 concentrations is highly indicated once caloric intake is increased as they may develop refeeding syndrome.[256] The shift from a catabolic to an anabolic state increases the intracellular needs for PO_4. Insulin secretion contributes to the intracellular redistribution of PO_4 during refeeding syndrome.

Hypophosphatemia can be spurious from laboratory error or improper sample handling (long storage at room temperature). High doses of mannitol can cause pseudohypophosphatemia.[257]

Clinical Signs of Hypophosphatemia

The clinical signs of acute hypophosphatemia are a consequence of skeletal and smooth muscle as well as neurologic dysfunction, whereas signs of chronic hypophosphatemia are mainly linked to behavior and the skeleton. Hypophosphatemia can be classified as severe (less than 1.0 mg/dL; 0.32 mmol/L), moderate (1.0–2.0 mg/dL; 0.32–0.65 mmol/L), and mild (2.1–2.5 mg/dL; 0.66–0.8 mmol/L).

Clinical signs of hypophosphatemia in horses often go unnoticed, and when present, serum PO_4 concentration is usually less than 1.0 mg/dL. These signs relate to PO_4 regulatory functions on energy metabolism, cell membrane stability, and ion transport.[1] These include muscle weakness, fasciculations, dysrhythmias, neuromuscular excitability, ileus, and cell membrane fragility and lysis (hemolysis, rhabdomyolysis) from impaired glucose use, decreased ATP synthesis, and altered cell membrane potential.[249,254,258] Signs associated with mild or moderate hypophosphatemia are difficult to document. Hypophosphatemia can potentially result in tissue hypoxia from reduced erythrocyte content of 2,3-bisphosphoglycerate (2,3-BPG; or 2,3-diphosphoglycerate [2,3-DPG]), shifting the hemoglobin dissociation curve to the left and impairing oxygen release. In humans, ruminants, and dogs, hypophosphatemia has been associated with muscle weakness, rhabdomyolysis, myocardial dysfunction, dysrhythmias, hemolysis, seizures, and pontine myelinolysis.[249,252,259] Hypophosphatemia can also predispose to infections from impaired leukocyte phagocytosis.[254]

Chronic hypophosphatemia is rare in horses and manifests as weight loss, weakness, depraved appetite (pica), reduced bone density, DOD, lameness, and hemolysis.[1] Rickets from PO_4 deficiency or hypovitaminosis D is poorly documented in growing equids.

Laboratory Findings

Laboratory findings associated with hypophosphatemia could include hyperglycemia, hyperinsulinemia, hypokalemia, and hypomagnesemia. Hemolysis has been reported in small animals, ruminants, and humans.[254,259,260-262] Muscle enzyme activity may be increased.

Treatment of Hypophosphatemia

Information on the therapeutic management of hypophosphatemia in horses is minimal.[263] Phosphate-containing supplements are listed in Table 16.3. Critically ill equids are rarely treated for hypophosphatemia, but considering the myriad of processes that depend on PO_4 as a regulatory ion, therapy should be considered. Hypokalemia and hypomagnesemia are frequent abnormalities in horses, ponies, donkeys, and foals with hypophosphatemia, and their serum concentrations should be assessed as part of the therapeutic management of hypophosphatemia.

Most PO_4 in the body (similar to K^+ and Mg^{2+}) is intracellular, and deficit calculations based on serum concentrations do not reflect the total body PO_4 status. The PO_4 deficit can be calculated from standard electrolyte formulas, and if anything, this amount should be overestimated, particularly during prolonged hypophosphatemia because cellular depletion is likely to be present. In people, hypophosphatemia from respiratory alkalosis does not require PO_4 supplementation.[255]

Injectable and oral products formulated for other species to treat hypophosphatemia (Tables 16.10 and 16.11) can be used in horses. Potassium phosphate is a good parenteral option for acute hypophosphatemia, in particular when hypokalemia is also present; however, sodium phosphate is also available and a good alternative. Sick horses and foals can also be supplemented via nasogastric intubation with chemical-grade potassium or sodium phosphate salts. Products formulated for cattle that contain Ca^{2+}, Mg^{2+}, and K^+ should be contemplated. Sodium phosphate enemas containing monobasic and dibasic sodium phosphate given via nasogastric intubation may be useful. There are anecdotal reports of their use intravenously without complications, but this should only be considered

TABLE **16.10 Products Used for Parenteral Supplementation of Phosphate**

Product	Composition/mL	Phosphorus/mL	Potassium/mL	Sodium/mL	Presentation	Company
Potassium phosphate[a]	236 mg of K_2HPO_4 224 mg of KH_2PO_4	3 mM or 93 mg	4.4 mM or 170 mg	0	5, 15, 50 mL	American Reagents, Inc.
Sodium phosphate[a]	142 mg of Na_2HPO_4 276 mg of NaH_2PO_4	3 mM or 93 mg	0	4 mM or 92 mg	5, 15, 50 mL	American Reagents, Inc. Hospira, Inc.
Phosphaid[b,c]	200 mg of NaH_2PO_2	2 mM or 60 mg	0	46 mg	100 mL	Vedco, Inc.
Phos-Aid[b,c]	200 mg of NaH_2PO_2	2 mM or 60 mg	0	46 mg	100 mL	Neogen, Inc.
Phos P 200[b,c]	200 mg of NaH_2PO_2	2 mM or 60 mg	0	46 mg	100 mL	Phoenix, Inc.
CMPK[b,d]	5 mg of NaH_2PO_2 16 mg of KCl	1.5 mg	0.4 mM or 16 mg	1.1 mg	500 mL	Vedco, Inc.

[a]For human use.
[b]Approved for cattle. Contains phosphinic acid (hypophosphorous acid [H_3PO_2]; sodium hypophosphite [NaH_2PO_2]).
[c]Dose for adult cattle ranges from 1 mL/25–50 kg (50–100 lb) of body weight.
[d]To treat hypocalcemia and hypomagnesemia in cattle. Suggested dose for adult cattle is 500 mL/360–450 kg (800–1000 lb) of body weight. Also contains magnesium, calcium, and potassium.
Other formulations that contain phosphorus include Norcalciphos (Zoetis, Inc.) and Cal-Phos (Vedco, Inc.).
K_2HPO_4 = dibasic potassium phosphate/KH_2PO_4 = monobasic potassium phosphate.
Na_2HPO_4 = dibasic potassium phosphate/NaH_2PO_4 = monobasic potassium phosphate.

TABLE **16.11 Phosphate-Containing Products Used For Enteral Supplementation in Humans and Small Animals[a,b]**

Product	Phosphorus/mL	Potassium/mL	Sodium/mL	Presentation	Company
Neutra-Phos	250 mg (8.1 mM)	278 mg (7.1 mM)	164 mg (7.1 mM)	Per tablet or 75-mL solution	Baker Norton
Neutra-Phos-K	250 mg (8.1 mM)	566 mg (14.2 mM)	0	Per tablet or 75-mL solution	Baker Norton
K-Phos-Neutral	250 mg (8.1 mM)	45 mg (1.1 mM)	298 mg (13 mM)	Per tablet	Beach
Fleet Phospho-soda	129 mg (4.15 mM)	0	110 mg (4.8 mM)	Per 45-mL oral solution	Fleet
Fleet Enema[c,d]	43 mg (1.38 mM)	0	37 mg (1.6 mM)	Per 118 mL	Fleet
Fleet Enema Extra[c,d]	25 mg (0.8 mM)	0	23 mg (1 mM)	Per 197 mL	Fleet

[a]Other formulations are available on the market.
[b]Sodium and potassium phosphate salts (chemical grade) can be used via nasogastric intubation in horses.
[c]Phosphate enemas contain monobasic (19 g) and dibasic (7 g) sodium phosphate in 118 mL or monobasic (19 g) and dibasic (7 g) sodium phosphate in 197-mL solution.
[d]Phosphate enemas are often used intravenously in ruminants to treat hypophosphatemia. There are anecdotal reports of their used in horses.

when other parenteral products are not available. It is important to verify their phosphate content as strength varies.

The rate of phosphate administration is determined by the duration and severity of hypophosphatemia (deficit, depletion) and the presence of clinical signs. Phosphate can be replaced within a short period of time (hours) in animals showing clinical signs. Data on enteral or parenteral rates of PO_4 supplementation for horses is minimal. Rates of 0.01 to 0.06 mmol/kg/hr are considered safe in small animals.[249] In people, rates of 0.08 to 0.16 mmol/kg/hr are recommended.[254] However, higher doses (0.2–0.6 mmol/kg/hr) are indicated for patients with severe hypophosphatemia.[252,264,265] Recumbent cows with severe hypophosphatemia can be treated with a drench of 200 g of sodium phosphate or with 23 g of sodium phosphate intravenously in saline solution.[266] In critically ill people, there is evidence that rapid correction of hypophosphatemia may be beneficial.[267]

During rapid PO_4 replacement therapy it is important to monitor serum calcium concentrations because high PO_4 doses could cause hypocalcemia.

Hyperphosphatemia

Hyperphosphatemia, defined as a serum PO_4 concentration greater than 5.0 mg/dL (1.6 mmol/L) in horses (greater than 8.5 mg/dL [2.75 mmol/L] in foals), results from increased PO_4 absorption, renal injury, hypoparathyroidism, vitamin D toxicity, metabolic acidosis, cell lysis (hemolysis, rhabdomyolysis, tumor necrosis), or iatrogenic causes.[249,1] Lactic acidosis and ketoacidosis may cause hyperphosphatemia by shifting PO_4 into the extracellular compartment. Metabolic acidosis and high intracellular ATP levels inhibit phosphofructokinase, a key enzyme in glycolysis and PO_4 use. In newborn foals, overuse of phosphate-based enemas can cause hyperphosphatemia.

Improper sample handling can result in spurious hyperphosphatemia, often associated with hyperkalemia. Hemolysis, hyperbilirubinemia, hyperlipemia (hypertriglyceridemia), and hyperproteinemia can falsely increase PO_4 concentrations (pseudohyperphosphatemia).[257] Multiple myeloma can also cause pseudohyperphosphatemia due to immunoglobulins interfering with PO_4 determination.[257,268,269] These samples require deproteinization for correct PO_4 measurement.

Acute hyperphosphatemia may result in hypocalcemia due to the interaction between PO_4 and Ca^{2+} (mass law) and reduced renal synthesis of $1,25(OH)_2D_3$. In other species, hyperphosphatemia and a serum calcium × PO_4 product greater than 70 are risk factors for soft tissue mineralization.[249,270] However, there is no evidence that this applies to horses, except for those with vitamin D toxicity.[223] Soft tissue calcification and hyperphosphatemia have been documented in horses with a number of diseases, including enterocolitis, respiratory distress, and muscle necrosis.[243] The definitive cause of calcinosis in these animals remains unclear. Hyperphosphatemia is common in critically ill foals.[2,3] There are reports of humans developing hyperphosphatemia from bisphosphonate therapy, and blood work should be monitored in horses undergoing similar treatment.[271]

For the pathogenesis of chronic hyperphosphatemia see nutritional secondary hyperparathyroidism in this chapter.

Clinical Signs of Hyperphosphatemia

The signs of acute hyperphosphatemia are those of acute hypocalcemia and include tetany, hyperexcitability, muscle fasciculation, colic, and dysrhythmias. Signs of chronic hyperphosphatemia are those of calcium deficiency including lameness, orthopedic pathologies, fractures, and osteodystrophia fibrosa (nutritional secondary hyperparathyroidism).[258] Developmental orthopedic diseases can be a problem in growing animals. Soft tissue mineralization (calcinosis) can be present, although it is rare.

Treatment of Hyperphosphatemia

Based on primary pathology and duration, therapy to reduce serum PO_4 may not be necessary. If acute hyperphosphatemia is a consequence of acute cell lysis (e.g., rhabdomyolysis) or iatrogenic (e.g., phosphate enemas), fluid therapy and diuretics are the treatment of choice. Diuretics, in addition to decreasing the deleterious effects of pigment nephropathy, also increase PO_4 excretion.

In humans and small animals, chronic renal failure is a common cause of hyperphosphatemia, although this rarely occurs in horses. Dietary PO_4 restriction must be implemented when treating chronic hyperphosphatemia. Dialysis and phosphate binders are either impractical or very expensive in horses. In addition, conditions associated with prolonged hyperphosphatemia usually carry a poor prognosis. In horses with chronic hyperphosphatemia from vitamin D toxicity glucocorticoid therapy is unlikely to be beneficial. Calcium carbonate is a cheap phosphate binder that also provides calcium supplementation. Calcium acetate is another alternative. In people and small animals, phosphate-binding polymers (e.g., sevelamer) are the most effective way to treat chronic hyperphosphatemia.

Magnesium and Disease

Ramiro E. Toribio, DVM, MS, PhD, Dipl ACVIM

MAGNESIUM

Magnesium (Mg) is an essential macroelement involved in physiologic processes such as enzymatic activation, intermediary metabolism of carbohydrates, fats, and proteins, nucleic acid metabolism, regulation of membrane function, nerve function, muscle contraction, and cell proliferation.[272,273] Over 600 enzymatic reactions require Mg as a cofactor or activator.[274] Extracellular Mg is not under tight hormonal homeostatic control like calcium (Ca) is, and plasma concentrations depend on gastrointestinal absorption, renal excretion, and bone exchange.[272,273] However, various hormonal and nonhormonal factors influence extracellular Mg concentrations.[272] Because a multitude of cellular processes are highly dependent on Mg, intracellular concentrations are tightly regulated.[274] Many transporters and channels involved with intracellular Mg homeostasis have been recently identified, and their importance is evident in genetic disorders linked to Ca and Mg disturbances.[274] Similar to total calcium and ionized calcium (Ca^{2+}), total magnesium (tMg) in biologic fluids exists in fractions bound to proteins, chelated to organic anions, and ionized/active/free Mg (Mg^{2+}). Because Mg as a solute interacts with more water molecules than other biologic cations and its binding to water is very stable, transmembrane permeability for Mg is lower than for Na^+, K^+, or Ca^{2+}.[274-277] Thus Mg movement through channels and carrier proteins is highly energy dependent.[274-277] In addition, because of its chemical and electrical characteristics, Mg is a powerful Ca antagonist.[274-278] Despite similar electrochemical properties, in biologic systems Ca can be a death trigger whereas Mg is not, and Mg can block Ca-induced cell death.[276]

Mg concentrations are higher in the intracellular compartment, where it binds to negatively charged molecules (such as ATP, DNA, and RNA), enzymes, and other proteins. Any ATP-dependent reaction also requires Mg^{2+} because ATP is associated with Mg^{2+} ($ATP{\cdot}Mg^{2+}$).[274-277] A similar dependency exists for GTP ($GTP{\cdot}Mg^{2+}$). Approximately 90% of the intracellular Mg is bound to ribosomes, polynucleotides, and to the phosphate backbone of nucleic acids to facilitate base pairing.[277] Mg^{2+} is required for genomic stability, nucleotide repair, and DNA replication fidelity.[274,279] Mitochondria function as intracellular stores for Mg^{2+}. By interacting with various proteins, Mg affects the cell cycle and stabilizes cell membranes.[274,276-279] Mg^{2+} interacts with Ca^{2+} in the intra- and extracellular compartments.[274,276-278] Depending on the physiologic system or binding molecule, Mg^{2+} and Ca^{2+} interact in the following ways: (1) Mg^{2+} and Ca^{2+} can have similar biologic effects, (2) Mg^{2+} and Ca^{2+} can bind to molecules with different affinities, resulting in synergy or antagonism, (3) they can antagonize each other, or (4) their effects can be interdependent. The pathophysiology of Mg-related disorders is often complicated by the Ca^{2+} status of the affected animal. Likewise, Ca^{2+}-related pathologies can be confounded by Mg^{2+} status. Hypomagnesemia exaggerates the effects of Ca^{2+} whereas hypermagnesemia may antagonize the actions of Ca^{2+}. Hyperexcitability from hypocalcemia can be exacerbated by low Mg^{2+} concentrations.[274]

Mg concentrations in body fluids are reported in mg/dL, mEq/L, or mmol/L. As the atomic weight of Mg is 24.3 and its valence is 2+, 1 mEq of Mg is equal to 12.15 mg (0.5 mmol) of Mg. Conversion factors are as follows: mmol/L = mg/dL × 0.41; mg/dL = mmol/L × 2.43; mg/dL = mEq/L × 1.21; mmol/L = mEq/L × 0.5. Dietary magnesium is reported as %, g/kg, or parts per million (ppm; mg/kg).

Magnesium Requirements in the Horse

Based on urinary and fecal losses of Mg, it has been estimated that adult horses must absorb 5 mg/kg BW of Mg to

replace daily obligatory losses.[46] With an average absorption of 40%, dietary Mg requirements for adult horses are around 12.5 mg/kg BW/day (7.5–12.0 g/horse/day).[46] A daily supply of 13 to 15 mg/kg BW/day should cover the requirements for most adult horses,[51] but these needs can be higher depending on the animal's physiologic status. Growing, lactating, and exercising animals have higher demands; mares in early lactation and horses undergoing intense exercise may require 15 to 30 mg/kg of Mg (10–15 g/horse/day).[46] A daily Mg intake of 5 to 6 mg/kg has been associated with hypomagnesemia.[17] Mg content is lower in grasses, particularly rapidly growing spring pastures, and grains (0.1%–0.2% DM), compared with legumes (0.2–0.3% DM). Content in meals from the vegetable oil industry (soybean, cottonseed, peanut, linseed, safflower, sunflower) and molasses varies from 0.3% to 0.8%.[46] In fish meal, Mg content varies from 0.1% to 0.3%.[46]

Distribution of Magnesium within the Body

Magnesium is the fourth most abundant cation in the body behind Ca^{2+}, Na^+, and K^+, but it is the second most abundant intracellular cation after K^+.[280] Mg distribution resembles that of K^+, and their intra- and extracellular dynamics are interrelated. The body of domestic animals contains 0.05% Mg by weight of which 60% is in the skeleton (0.5%–1% bone ash), 38% in soft tissues, and 1% to 2% in the extracellular fluid (Fig. 16.12). Approximately 30% of bone Mg is surface limited and readily available to serve as an exchangeable pool to buffer acute changes in extracellular Mg concentrations, whereas the remaining 70% has structural functions as part of the hydroxyapatite lattice, and its release depends on bone resorption. Cells with higher metabolic activity have higher Mg content. Although most Mg is in the intracellular compartment, free intracellular and extracellular Mg concentrations are similar, and the transmembrane gradient is small compared with that of Ca. Red blood cell Mg concentrations are around 3 times the serum Mg concentrations and have been used to assess the Mg status of individual animals.

In circulation, total magnesium (tMg) is bound to proteins, chelated to organic anions (carbonate, sulfate, lactate, citrate), and ionized or free (Mg^{2+}) (see Fig. 16.12). Mg^{2+} is the form of Mg that is important for most biologic processes, in particular those related to neuromuscular excitability. When possible, it is better to measure Mg^{2+} than tMg concentrations. In equids, 60% of the serum tMg is ionized, 30% is protein bound, and 10% is complexed to weak acids[6,7,12,13,40,120,281] (see Fig. 16.12). Serum tMg concentrations depend on protein concentrations (albumin), whereas Mg^{2+} concentrations depend on the acid-base status. Acidosis increases Mg^{2+}, and alkalosis does the opposite. This is clinically relevant, as conditions associated with alkalosis (nasogastric reflux, duodenitis/proximal jejunitis, exercise-associated alkalosis from hyperventilation, and Cl^- loss) can lead to low Mg^{2+} concentrations, with potential clinical signs of hypomagnesemia despite normal tMg concentrations. Feeding an acidic diet with a low dietary cation anion balance (DCAB) will increase the percentage of Mg^{2+}.[40]

Reported normal serum concentrations in horses are tMg = 1.4 to 2.2 mg/dL (0.6–0.9 mmol/L) and Mg^{2+} = 0.9 to 1.5 mg/dL (0.42–0.6 mmol/L).[4,6,7,12,13,40,120,281] In foals, normal concentrations are tMg = 1.3 to 2.4 mg/dL (0.55–1.0 mmol/L) and Mg^{2+} = 1.0 to 1.9 mg/dL (0.42–0.8 mmol/L).[13,2,282] One study found higher tMg concentrations in donkeys.[120] Serum tMg and Mg^{2+} concentrations tend to be higher in foals.[13] Additionally, seasonal effects on tMg concentrations in equids have been reported, with higher serum tMg concentrations measured in summer compared with winter months.[119]

Magnesium Absorption

Mg is absorbed as a freely diffusing ion (Mg^{2+}) by paracellular (passive, nonsaturable, concentration-dependent) and transcellular (saturable, active transport) mechanisms (Fig. 16.13; also see Fig. 16.6).[273,283] Mg absorption occurs in the distal small intestine and colon of monogastric animals,[37,273,284,285] whereas in ruminants, the rumen is the main site for Mg absorption.[273,284] Transcellular Mg absorption is mediated by

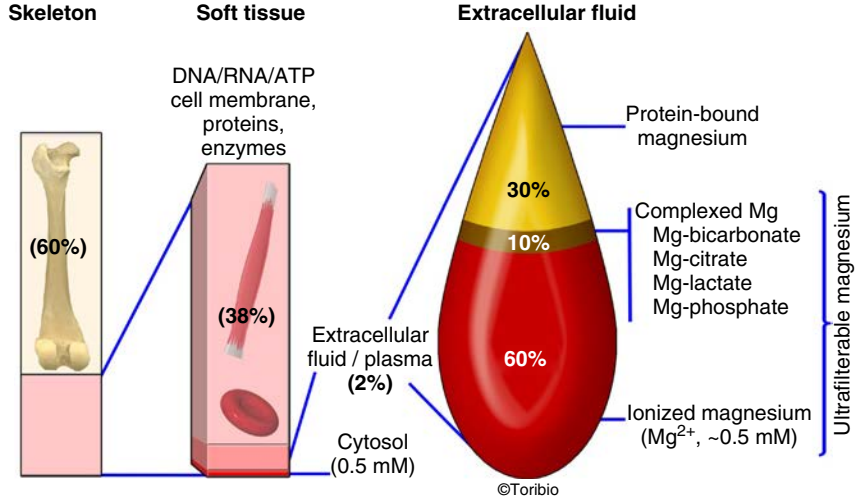


FIG. **16.12** Magnesium distribution in the body. Approximately 60% of the total body Mg is in the skeleton, 38% is in soft tissue, and 2% is in the extracellular fluid. Although most Mg is in the intracellular compartment bound to proteins and nucleotides, free cytosolic Mg concentrations are similar to extracellular Mg concentrations. In equine blood, 60% of Mg exists as a free or ionized form (Mg^{2+}), 30% is bound to proteins, and 10% is complexed to anions such as citrate, bicarbonate, phosphate, and lactate.[6,7,12,13,40,120] (Courtesy Dr. R. E. Toribio, The Ohio State University.)

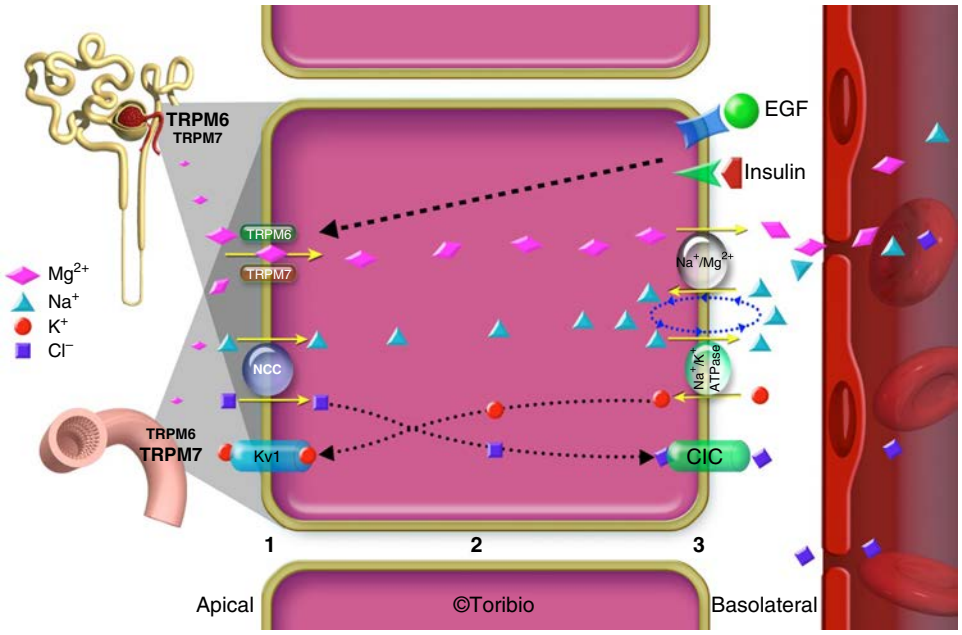


FIG. **16.13** Intestinal absorption and renal reabsorption of magnesium.[76,274,286] Apical Mg^{2+} uptake is mediated by the transient receptor potential channel melastatin member 6 (TRPM6) and member 7 (TRPM7). TRPM7 expression is ubiquitous to all segments of the intestinal tract, whereas TRPM6 is mainly present in the distal small and proximal large intestine. In the renal distal convoluted tubules, TRPM6 is the main epithelial Mg^{2+} channel. Epidermal growth factor (EGF) and insulin promote apical Mg^{2+} uptake. The Na^+/Cl^- cotransporter (NCC) enhances transcellular Mg^{2+} movement. The basolateral Na^+/K^+-ATPase creates a transcellular gradient to move Mg^{2+} across the epithelium. The voltage-gated K^+ channel (Kv1) contributes to maintaining this voltage gradient by transporting K^+ to the lumen. A putative Mg^{2+}/Na^+ exchanger moves Mg^{2+} to the basolateral side. A basolateral Cl^- channel (ClC) is responsible for Cl^- extrusion. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

the transient receptor potential channel melastatin member 6 (TRPM6) and 7 (TRPM7) (see Fig. 16.13). In other species, TRPM7 expression is ubiquitous in all segments of the intestinal tract, whereas TRPM6 is mainly present in the distal small intestine, proximal large intestine, and kidneys.[274,286] Unlike calcium, for which transcellular transport is vitamin D dependent, vitamin D has minimal effect on TRPM6 expression and Mg absorption.[287]

In horses, most Mg^{2+} absorption occurs in the small intestine, with approximately 25% being absorbed in the proximal small intestine, 30% to 35% in the distal small intestine, and 5% to 10% in the large colon.[37] Intestinal Mg absorption is proportional to dietary Mg content, but its absorptive efficiency decreases as dietary Mg content increases.[273,284] High dietary Mg leads to increased serum, erythrocyte, and tissue Mg concentrations, especially in bone.[273,288] Dietary Ca content has minimal effect on Mg absorption in monogastric animals.[273] The digestibility of Mg ranges from 30% to 50% depending on diet, activity, and age.[27,36,37,44,289,290] These values are higher in horses than other herbivores.[37] Alfalfa and alfalfa hay have the highest Mg digestibility (50%) compared with pelleted feed and cereal grains (approximately 20%–30%).[27] Digestibility of oral supplements such as MgO, $MgSO_4$, and $MgCO_3$ is similar, ranging from 50% to 70%. Magnesium digestibility is higher in foals.[36] Excessive amounts of fatty acids, oxalates, phosphates, and fiber reduce intestinal Mg^{2+} absorption. High dietary phosphate content reduces Mg absorption in horses[290]; however, phytates, calcium, and aluminum have minor influence.[37,45,289,290,291] One study found that phosphorus intake has minimal effect on Mg digestibility.[44] Malabsorption syndromes and enteropathies may decrease absorption. Prolonged use of proton pump inhibitors (e.g., omeprazole) has been linked to hypomagnesemia in people, apparently from reduced intestinal absorption.[292] This phenomenon has not been documented in veterinary medicine to date.

Magnesium Excretion

The principal routes for Mg excretion are (1) the gastrointestinal tract, (2) the kidneys, and (3) the mammary gland during lactation. In addition, Mg can be lost in sweat and to the developing fetus in pregnant animals.

The kidneys play a central role in Mg regulation, where Mg is reabsorbed in the proximal convoluted tubules (PCTs), cortical thick ascending limb (CTAL) of the loop of Henle, and the distal convoluted tubules (DCTs).[7,272,293-295] Renal Mg excretion is tightly matched to gastrointestinal Mg absorption; Mg absorbed in excess from the gastrointestinal tract is excreted by the kidneys. Tubular reabsorption is regulated by dietary availability of Mg, serum Ca^{2+} and Mg^{2+} concentrations, urinary Ca^{2+} excretion, and multiple hormones.[7,272,293,294]

Ionized and anion-bound Mg (ultrafilterable Mg) is filtered by the glomerulus, whereas protein-bound Mg is not (see Fig. 16.12). Approximately 70% of the blood Mg^{2+} is filtered by the glomeruli, with 70% to 90% being reabsorbed in different segments of the nephron.[7,294] Around 10% to 20% of the filtered Mg is reabsorbed in the PCT, 50% to 70% is reabsorbed in the CTAL, and the remaining 5% to 10% is reabsorbed in the DCT.[272] The DCT seems to be the site determining ultimate

urinary excretion of Mg.[7,283] Renal reabsorption of Mg is paracellular (PCT, CTAL) and transcellular (DCT). Paracellular reabsorption occurs in the PCT (passive, concentration-dependent) and CTAL (active, voltage-dependent), whereas transcellular (active) reabsorption takes place in the DCT (see Fig. 16.13).[7,76,286] Paracellular reabsorption of Mg in the PCT is mostly dictated by Mg concentrations in the ultrafiltrate. The mechanisms driving paracellular Mg reabsorption in the CTAL are described in detail in the calcium section (see Fig. 16.6). Claudins are intercellular proteins that determine ion permeability of epithelial tight junctions. Claudin-16 (paracellellin-1) is required for Ca^{2+} and Mg^{2+} transport at the CTAL.[76,286] Claudin-16 is more selective for Mg^{2+}, and people with inactivating mutations develop hypomagnesemia, hypercalciuria, and nephrocalcinosis.[76,286] A comparable disorder occurs with claudin-19 mutations.[76,286] Similar to Ca^{2+} transport in the CTAL, parathyroid hormone (PTH), Ca^{2+}, and Mg^{2+} at the basolateral membrane regulate paracellular reabsorption of Mg^{2+} in different species, including the horse.[7,76,274,286] A multitude of factors affect renal Mg^{2+} reabsorption (see later discussion).

In the DCT, Mg^{2+} transport is transcellular and mediated by the transient receptor potential channel melastatin member 6 (TRPM6) and 7 (TRPM7). However, TRPM6 is considered the main channel for Mg uptake.[76,274,286] The mechanisms for basolateral Mg extrusion remain unclear, but they seem to depend on Na^+ uptake and are driven by the Na^+/K^+-ATPase.[76,274,286] The Na^+/Cl^- cotransporter (NCC) enhances transcellular Mg^{2+} movement (see Fig. 16.13). Thiazide diuretics, which inhibit NCC, increase Mg^{2+} excretion, and their prolonged use in people can result in hypomagnesemia.[76,274,286] Gitelman syndrome, a human inherited condition arising from inactivating mutations of the NCC, is characterized by hypomagnesemia, hypocalciuria, hypokalemia, metabolic alkalosis, and hyperreninemic hyperaldosteronism.[286] There appears to be crosstalk between NCC and TRPM6, and a reduction in activity of one is followed by a concomitant decrease in the other.[286]

In addition to Na^+ and Cl^-, a normal K^+ status is necessary for Mg^{2+} reabsorption; the basolateral Na^+/K^+-ATPase creates ionic gradients in the CTAL and DCT. Therefore there is interdependency between Mg^{2+} and K^+; apical Mg^{2+} entry requires basolateral K^+ uptake, which also requires ATP, and therefore Mg^{2+}. Hypokalemia is often linked to hypomagnesemia in horses and foals with gastrointestinal disease and sepsis.[2,4,6,7]

PTH and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$; calcitriol) do not appear to play major roles in Mg^{2+} entry in the DCT. More important in this segment are epidermal growth factor (EGF), insulin, and extracellular concentrations of Mg^{2+}.[76,274,286] Activation of the EGF receptor enhances trafficking and activity of TRPM6, and absence of EGF causes hypomagnesemia.[76,274,286] Genetic disorders of Mg transport remain to be documented in the horse. In contrast, a number of genetic conditions affecting different Mg transporters in the CTAL and DCT occur in people.[76,274,286]

The mammary gland actively secretes Mg into milk. Mg output into milk is estimated at 3 to 6 mg/kg BW/day, and daily Mg requirements in lactating mares are 15 to 30 mg/kg BW.[46] Mg concentrations in mare's milk change from 470 mg/L at foaling (colostrum) to 90 to 120 mg/L on week 1, to 60 to 120 mg/L on week 2 to 8, and 40 to 60 mg/L by the end of lactation.[19,20,296,297] Higher values have been measured in zebras.[19,20] Mares producing larger volumes of milk are more likely to develop clinical hypomagnesemia.

Horses can also lose Mg in their sweat.[163,298] It has been proposed that the salivary glands may contribute to Mg excretion and homeostasis in the horse.[44]

Magnesium Homeostasis

There is not a precise homeostatic system for Mg^{2+}, and its extracellular concentrations depend on gastrointestinal absorption, renal excretion, and bone exchange.[272,273] However, hormonal and nonhormonal factors influence Mg absorption, excretion, and extracellular concentrations.[272,273] Hormones that *increase* renal reabsorption of Mg^{2+} include parathyroid hormone (PTH), parathyroid hormone–related protein (PTHrP), epidermal growth factor (EGF), antidiuretic hormone (vasopressin), insulin, glucagon, β-adrenergic agonists, aldosterone, and, to lesser extent, $1,25(OH)_2D$.[272,293,299-303] Activation of the calcium-sensing receptor (CaSR) in the CTAL by hypercalcemia and hypermagnesemia in various species, including the horse, increases the urinary excretion of Ca^{2+} and Mg^{2+} (see Fig. 16.6).[7,304] Impaired Mg^{2+} reabsorption occurs with osmotic diuresis (volume expansion, hyperglycemia), loop diuretics, thiazide diuretics, hypercalciuria, hypercalcemia, hypermagnesemia, hypokalemia, hypophosphatemia, renal tubular acidosis, metabolic acidosis, prostaglandins, and various toxicities (aminoglycosides, amphotericin B, cyclosporine, cantharidin).[76,274,286,293,304-307] Furosemide and hypercalcemia decrease serum tMg and Mg^{2+} concentrations in healthy horses.[7,305] Both hypercalcemia and loop diuretics act by a similar mechanism (inhibition of the $Na^+/K^+/2Cl^-$ cotransporter) in the CTAL.[7,304] PTH, on the other hand, increases the activity of the $Na^+/K^+/2Cl^-$ cotransporter and renal outer medullary K^+ channel (ROMK) in the CTAL, thereby increasing paracellular reabsorption of Ca^{2+} and Mg^{2+} (see Fig. 16.6).[1,7,76,274,286,307] PTH indirectly increases Mg^{2+} release from the skeleton during bone resorption. Hypermagnesemia decreases PTH secretion, serum Ca^{2+} concentrations, and renal Ca^{2+} reabsorption in horses by activating the CaSR in the CTAL and parathyroid chief cells.

Magnesium and the Central Nervous System

In the central nervous system (CNS), Mg^{2+} is directly or indirectly (via ATP) required for processes involving transmembrane ion traffic and neurotransmitter release. Mg^{2+} interacts with Ca^{2+} in the intracellular and extracellular compartments, and these interactions can be additive, synergistic, or antagonistic. For example, hypocalcemia can alter neuronal function, and the Mg status can worsen the severity of clinical signs. Mg ions are necessary for the activity of the Na^+/K^+-ATPase, Mg^{2+}-ATPase, H^+/K^+-ATPase pump (proton pump), plasma membrane Ca^{2+} ATPase (PMCA), Na^+/Ca^{2+} exchanger (NCX), *N*-methyl-D-aspartate (NMDA) receptor (NMDAR), Ca^{2+} channels, and other proteins involved in neuronal and glial function. The neuroprotective actions of Mg^{2+} are mainly due to its ability to compete with Ca^{2+} at a number of proteins (e.g., NCX, NMDAR, Ca^{2+} channels) that increase intracellular Ca^{2+} concentrations. Mg^{2+} also blocks Na^+ channels. In addition, Mg^{2+} may protect brain cells against free radical injury, neurotoxicity, and inflammation.[308]

The NMDAR is a cation-selective (Ca^{2+}, Na^+) channel that mediates neuronal plasticity, rhythmicity, and excitability. Upon excessive activation by glutamate, Ca^{2+} and Na^+ influx triggers excitotoxic death, a process that is blocked by cations such as Zn^{2+} and Mg^{2+}.[309,310] This protective action by Mg^{2+} may have therapeutic implications to acute brain injury,

ischemia, and hypoxia. This might be even more important in the developing neonatal brain, where the NMDAR is a predominant mediator of excitotoxicity.[309] Under this premise, Mg^{2+} has been used to treat foals with hypoxic ischemic encephalopathy and horses with brain injury[311-313]; however, controlled studies on the therapeutic benefits of Mg^{2+} in horses or foals with cerebral injury are lacking. The clinical scenario appears to be different under experimental conditions of brain injury, where several studies have shown that $MgSO_4$ improves outcome.[314,315] By blocking Ca^{2+} channels in vascular smooth muscle, Mg^{2+} is a vasodilator[316] (an effect that could be beneficial or not, depending on the condition and severity).

Mg^{2+} is also a modulator of the pain response. Similar to ketamine, Mg^{2+} reduces neuronal Ca^{2+} entry by antagonizing the NMDAR, which reduces excitability or nociception. This is likely the justification for using intravenous $MgSO_4$ in combination with chloral hydrate in earlier equine general anesthetic combinations. In addition, this knowledge has been used in humans to manage excessive nociception and pain hypersensitivity (wind-up syndrome). The analgesic benefits of Mg^{2+} for equine conditions remains to be demonstrated. Mg is also often used in show horses under the premise of its calming effects; however, this is inappropriate, given that the intent of use is to confer a competitive advantage.

Mg^{2+} reduces neuroexcitability and nerve conduction by blocking presynaptic Ca^{2+}-dependent acetylcholine release and excitation-secretion coupling.[8,284] High Ca^{2+} promotes whereas high Mg^{2+} inhibits the release of acetylcholine from the presynaptic terminal.[317] Mg depletion contributes to tetany by increasing the release of acetylcholine at the neuromuscular junction and delaying its degradation by acetylcholinesterase.[8,284]

The Ca^{2+} blocking actions of Mg^{2+} on smooth muscle are being used in human medicine to treat conditions such as hypertension and asthma.[318]

Disorders of Magnesium

Hypomagnesemic conditions are well described in the literature, whereas hypermagnesemic disorders are rarely encountered. In people, low Mg concentrations have been linked to a multitude of chronic conditions, including cardiovascular (hypertension, dysrhythmias), neurologic (migraines, attention deficits, depression, irritability, Alzheimer disease, eclampsia), respiratory (asthma), endocrine (low PTH secretion, PTH resistance, hypovitaminosis D, vitamin D resistance, osteoporosis), metabolic (insulin resistance/type 2 diabetes mellitus, metabolic syndrome), and electrolyte (hypokalemia, hypocalcemia) disorders.[318] Equine veterinary medicine has not yet solidified the importance of Mg depletion in chronic diseases of the horse.

Acute Mg deficiency may occur with gastrointestinal and renal diseases, sepsis, endotoxemia, endocrine dysfunction, alterations of acid-base status, and cell lysis. Stress hormones, such as catecholamine and insulin, can worsen the clinical signs of hypomagnesemia by stimulating intracellular Mg^{2+} shifts.[284] The severity of hypomagnesemia is the result of reduced dietary intake, decreased absorption, excessive mobilization of endogenous stores, increased urinary excretion, tissue redistribution, or a combination of these effects.

Hypomagnesemia and Equine Disease

In large animals, hypomagnesemia is most frequently diagnosed in ruminants, but in recent years, it has become a subject of clinical attention in acutely ill horses and foals.[4,40,284,319-321] Hypomagnesemia was identified as the most common electrolyte abnormality in critically ill canine and feline patients.[322] Chronic hypomagnesemia is usually linked to inadequate dietary Mg^{2+} intake, whereas acute hypomagnesemia can be the result of multiple disorders.[284,321] In the case of ruminants, primary hypomagnesemia (grass staggers, tetany) is typically observed in animals feeding exclusively on fresh rapidly growing pastures that are low in Mg content in early spring.[40,284,321]

Mg plays essential roles in inflammation, protection against free radical injury, and neurotoxicity.[323,324] However, information on the role of Mg in equine inflammatory conditions is limited.[4,36,40,325] Hypomagnesemia has been associated with increased cytokine production and systemic inflammation.[323,324] A similar process is likely to occur in critically ill horses and foals where the same proinflammatory cytokines are elevated.[4,6,326,327] Horses and foals with gastrointestinal disease often have endotoxemia and hypomagnesemia.[2,4,6,327,328] The induction of experimental endotoxemia in healthy horses decreased both tMg and Mg^{2+}, supporting a role for Mg in equine inflammatory disease.[6]

One study in horses that had colic surgery found that serum tMg and Mg^{2+} concentrations were below the reference range in 17% and 54% of animals, respectively.[155] The same study found that nonsurviving horses had significantly lower preoperative serum Mg^{2+} concentrations. Toribio and colleagues found that 78% of horses with severe gastrointestinal disease had ionized hypomagnesemia.[4] In another study in hospitalized horses, 48.7% of animals were hypomagnesemic although no association with mortality or length of hospitalization was found.[320] An association between hypomagnesemia, severity of disease, and mortality has been demonstrated in other species, and there is increasing evidence that this also applies to sick horses and foals.[2,4,6,155,320] Hurcombe and colleagues did not find an association between serum Mg^{2+} concentrations and mortality in critically ill septic foals, although 15% of foals were hypomagnesemic.[2] Another study found higher tMg concentrations in foals with evidence of perinatal asphyxia syndrome compared with healthy and septic foals.[282] The same study found higher tMg concentrations on admission in nonsurviving compared to surviving sick foals.[282]

Equine cantharidiasis (blister beetle toxicosis) is a condition characterized by acute hypomagnesemia and hypocalcemia. It is reported in the central, south, and midwestern United States and associated with the ingestion of alfalfa contaminated with blister beetles (*Epicauta* spp.), which produce cantharidin (cantharidic acid).[159,188,189] Cantharidin causes necrosis of the intestinal mucosa, renal tubules (acute tubular necrosis), and myocardium. Clinical signs include synchronous diaphragmatic flutter (SDF; thumps), muscle fasciculation, ataxia, dyspnea, laryngeal spasm, cardiac dysrhythmias, colic, diarrhea, endotoxemia, dehydration, hypotension, hematuria, stranguria, pollakiuria, and sudden death.[159,188,189]

An association between hypomagnesemia and hypocalcemia has been demonstrated in lactating mares, transported horses, and horses with intestinal strangulation, ileus, enterocolitis, endotoxemia, SDF, and blister beetle poisoning.[4,6,155,189,329] Patients with hypocalcemia and hypomagnesemia often fail to respond to calcium therapy until normomagnesemia is restored.[330] The mechanisms through which hypomagnesemia can promote hypocalcemia are numerous. Mg depletion can impair PTH synthesis and secretion and could lead to target organ resistance to PTH.[331] This translates into decreased renal reabsorption of Ca^{2+} and Mg^{2+}

(waste), reduced bone resorption, and less renal synthesis of active vitamin D (1,25[OH]$_2$D). In other words, hypocalcemia from Mg deficiency may be attributed to impaired PTH secretion (parathyroid gland dysfunction) combined with target organ (bone, kidney) insensitivity to PTH.

Contrary to other species in which there is a link between hypomagnesemia and metabolic conditions,[332] an association between obesity, insulin resistance, laminitis, and Mg deficiency has not been demonstrated in the horse.[333] Mg^{2+} is important for insulin receptor signal transduction and glucose metabolism, and insulin increases intracellular Mg^{2+} shifts.[332] Insulin is also important for renal Mg^{2+} reabsorption.[76,274] Thus, insulin resistance may lead to renal Mg^{2+} wasting from increased tubular flow (hyperglycemia) and decreased Mg^{2+} reabsorption.[332] Subsequently, Mg depletion could worsen insulin signaling, creating a vicious cycle.

Magnesium and Electrolytes

Mg is a cofactor for the Na^+/K^+-ATPase that maintains the cell membrane potential. Hypomagnesemia leads to loss of intracellular K^+, intracellular Na^+ accumulation, and lowering of the resting membrane potential, which causes hyperexcitability, seizures, arrhythmias, muscle weakness, muscle fasciculations, SDF, and cardiac arrhythmias. Information on a cause-effect relationship between hypomagnesemia and electrolyte abnormalities in horses or foals is minimal; however, hypomagnesemia is often associated with hypokalemia, in particular in foals and ponies receiving parenteral nutrition. Interestingly, hypophosphatemia is also a frequent finding in these animals. Because of their close interactions, interdependency, and homeostasis, more often than not horses with hypomagnesemia also have hypocalcemia. As previously mentioned, PTH secretion and action require Mg^{2+}, and at the same time, PTH promotes renal Mg^{2+} reabsorption.

Clinical Signs of Magnesium Deficiency

Hypomagnesemia results from reduced Mg intake (poor nutrition, low-Mg diets), reduced absorption (diarrhea, malabsorption, intestinal resection), redistribution (third space and intracellular sequestration), and increased losses (renal tubular disorders, gentamicin and/or furosemide administration, hypercalcemia, gestation, lactation, and intense exercise). Specific clinical signs of hypomagnesemia are often overlooked in horses and foals.

Mg depletion is associated with muscle weakness, tremors, seizures, hypokalemia, hypocalcemia, cardiac arrhythmias (ventricular arrhythmias, supraventricular tachycardia, atrial fibrillation), ileus, and SDF. Electrocardiographic changes associated with hypomagnesemia include prolonged P-R interval, widening of the QRS complex, depression of the ST segment, and tall T waves.[334] Hypocalcemic tetany has been reported in adult horses and ponies[329,335] and in foals fed Mg-deficient diets.[325] Affected foals exhibited nervousness, muscular tremors, ataxia, profuse sweating, hyperpnea, collapse, and convulsions.[325] On necropsy, all foals had severe mineralization of the elastic fibers of the aorta and pulmonary artery.[36,325] In one study of colic patients, nonsurviving horses had significantly lower preoperative serum Mg^{2+} concentrations.[155]

Diagnosis of Hypomagnesemia

The easiest way to assess the Mg status is by determining serum tMg and Mg^{2+} concentrations. Serum Mg^{2+} is more reliable because it is the active form of Mg and is minimally affected by protein concentrations. In animals with respiratory or metabolic alkalosis (observed after prolonged exercise, proximal enteritis), serum Ca^{2+} and Mg^{2+} concentrations may be low from increased protein binding. Methods to assess total body Mg status include 24-hour urinary Mg excretion, % Mg retention following parenteral Mg loading, and red blood cell Mg content. Of these, the 24-hour urinary excretion and the retention tests have been validated in horses.[40]

To assess renal excretion of Mg, urine is collected over 24 hours; Mg excretion is then expressed as mg/kg/day. Urinary Mg excretion is useful to assess dietary intake. Mg excretion is low when dietary intake is reduced.[40] The fractional excretion of Mg (FMg) can be determined by expressing the renal Mg excretion relative to creatinine excretion.[6,7,40] FMg in healthy horses ranges from 15% to 35%,[6,7,40] and values less than 6% indicate inadequate dietary Mg intake.[40] Muscle Mg content has been used to estimate total body stores in foals and horses.[40,336] One study evaluated Mg muscle content and intracellular Mg^{2+} concentrations in horses fed a Mg-deficient diet, with no differences found in muscle Mg content compared with control horses; however, intracellular Mg^{2+} concentrations were lower in Mg-deficient horses.[40]

The Mg retention test to assess total body status was evaluated in horses receiving Mg-deficient diets.[40] $MgSO_4$ was administered at 10 mg/kg IV of elemental Mg (100 mg/kg of $MgSO_4$). The percent retention (%Ret) was calculated as %Ret = (1 − [Mg excretion in 24 hours]/[Mg infused]) × 100). In this study, the 24-hour Mg excretion was a more sensitive indicator of reduced Mg intake than the Mg retention test, and the spot FMg reflected the 24-hour excretion of Mg.[40] Thus a spot FMg can be used as a simpler way to assess the Mg status.

The value of measuring cerebrospinal fluid and vitreous humor Mg concentrations as a postmortem tool to confirm equine hypomagnesemia remains to be evaluated. This is a well-accepted approach to diagnose hypomagnesemia in ruminants, however.

Treatment of Hypomagnesemia

Mg for oral administration is available as magnesium sulfate ($MgSO_4$; Epsom salts), magnesium oxide (MgO), magnesium carbonate ($MgCO_3$), magnesium chloride ($MgCl_2$), and magnesium hydroxide (Mg[OH]$_2$). $MgSO_4$ and $MgCl_2$ are available for intravenous administration. The type of salt and the route of administration are important when supplementing Mg. For $MgSO_4$ (9.7% Mg), a dose of 100 mg/kg will provide 9.7 mg/kg of elemental Mg whereas for $MgCl_2$ (25.5% Mg) a dose of 100 mg/kg will provide 25.5 mg/kg of elemental Mg. Attention to this detail is important, as overdosing can be fatal. Likewise, excessive oral (nasogastric) supplementation with $MgSO_4$ can be laxative and induce depression.

Recommended IV doses of $MgSO_4$ in horses vary from 25 to 150 mg/kg/day diluted in 0.9% NaCl, dextrose, or polyionic isotonic solutions. Constant rate infusions (CRI) of $MgSO_4$ at 100 to 150 mg/kg/day meet the daily requirements in foals and horses. For most horses with gastrointestinal disease and hypomagnesemia, supplementing 1 g of $MgSO_4$ per liter of crystalloid solution at 1 to 2 times the maintenance fluid rate should restore normomagnesemia or at least replace renal and intestinal losses. Horses tolerate 10 to 20 g of $MgSO_4$ IV administered over 10 to 20 minutes well. Some animals may require higher doses of $MgSO_4$, in particular those with evidence of systemic inflammation receiving intravenous calcium salts at the same time.

In foals, $MgSO_4$ can be given as a bolus (40–60 mg/kg IV) over 10 to 20 minutes, in replacement fluids, or via CRI. $MgSO_4$ CRI rates used in foals with hypoxic ischemic encephalopathy and seizures (50 mg/kg/hr loading dose, followed by 10–25 mg/kg/hr) work well in animals with severe hypomagnesemia. Alternatively, an initial dose (40–60 mg/kg IV) over 10 minutes followed by 25 to 50 mg/kg every 4 to 6 hours or a CRI of 50 to 150 mg/kg/day is safe. To put it in perspective, $MgSO_4$ doses used in children with hypocalcemia and hypomagnesemia range from 25 to 50 mg/kg IV or IM over 10 to 20 minutes every 4 to 6 hours, slowly, or via CRI at 30 to 60 mg/kg/day. Monitoring Mg^{2+} concentrations is strongly recommended to facilitate adjustment of dosage if necessary.

$MgSO_4$ is used to treat ventricular arrhythmias, in particular quinidine intoxication (torsades de pointes) and should be considered in horses with refractory ileus and SDF. Doses of $MgSO_4$ for ventricular arrhythmias vary from 2 to 6 mg/kg/min IV, up to a total of 50 mg/kg.

Oral supplementation with MgO, $MgCO_3$, or $MgSO_4$ should be considered in animals with chronic hypomagnesemia, malabsorption, renal disease, and exercise-associated hypomagnesemia. Recommended doses are 30 to 50 mg/kg/day for MgO, 60 to 80 mg/kg/day for $MgCO_3$, or 80 to 100 mg/kg/day for $MgSO_4$. These doses are safe for horses, in particular compared with the cathartic doses of $MgSO_4$ (0.5–1.0 g/kg).

Oral administration of Mg failed to improve morphometric measurements and insulin sensitivity in obese horses with evidence of insulin resistance and laminitis.[333]

A combination of enteral psyllium (1 g/kg) and $MgSO_4$ (1 g/kg) for multiple days was more effective than each constituent alone at removing sand from the large colon of horses.[337]

Hypermagnesemia

Hypermagnesemia is rarely documented in horses and in general is iatrogenic from Mg overdose, associated with renal failure, or observed in animals with extensive cellular damage (myonecrosis, cancer, hemolysis, severe sepsis). The presence of hyperkalemia and hyperphosphatemia is another indicator that suggests cellular damage. Hypermagnesemia has been reported in horses treated with Epsom salts and dioctyl sodium sulfosuccinate for large colon impaction.[338] Clinical signs in these horses included sweating, hyperexcitability, muscle tremors, recumbency, flaccid paralysis, tachycardia, tachypnea, widening of the QRS complex, and prolonged PQ interval. Very high Mg^{2+} concentrations can induce depression, bradycardia, and hypotension. Treatment of hypermagnesemia includes 0.9% NaCl, furosemide, and parenteral calcium administration. Sodium sulfate (Na_2SO_4) is considered a safer and more efficacious treatment for large colon impactions than $MgSO_4$.

Thyroid Gland

Ramiro E. Toribio

The thyroid gland has two embryologic origins: the *endoderm of the primitive larynx* that will give origin to the follicular cells that secrete thyroid hormones and the *ultimobranchial body of the fourth pharyngeal pouch* that contains neuroendocrine cells (from the neural crest) that will give rise to the C cells (parafollicular cells) that secrete calcitonin.

In the horse, the thyroid glands are two discrete, firm lobes located in the dorsolateral aspect of the third to sixth tracheal rings.[339,340] The two lobes are connected by a narrow isthmus that contains fibrous tissue. In most healthy horses the thyroid glands are not visible but can be palpated as firm and movable structures. The weight of the thyroid glands relative to body weight is largest for fetuses and foals (mean, 0.28 g/kg; range, 0.12–0.67) and decreases with age, and the ratio for adults is 0.08 g/kg (range, 0.01–0.15).[341] For average size horses, the weight of both thyroid lobes is 30 to 40 g. Total gland weight for newborn foals is around 15 g.[342] The glands are very vascular, receiving blood supply from two major arteries that arise from the external carotid and subclavian arteries. Thyroid gland blood supply is relatively greater (4–6 mL/min/g) than other highly vascular organs. One study found equine thyroid gland blood flow (1655 ± 339 mL/min/100 g) to be threefold that of the kidneys.[343]

Gland size does not parallel function, and functional tests must be used to establish an accurate diagnosis of thyroid dysfunction.

Thyroid Hormone Function

Understanding thyroid hormone (TH) biology is important in the diagnosis, treatment, and prognosis of thyroidal and nonthyroidal disorders. Thyroid hormones (THs; triiodothyroidine—T_3; thyroxine—T_4) are essential for cell growth, differentiation, and metabolism in nearly all tissues. The circulating THs include total T_4 (tT_4), free T_4 (fT_4), total T_3 (tT_3), free T_3 (fT_3), and reverse T_3 (rT_3).

The synthesis and secretion of THs are regulated by a negative feedback system that includes the hypothalamus, the pituitary gland, and the thyroid gland (hypothalamus-pituitary-thyroid axis; HPTA). Hypothalamic parvocellular neurons projecting to the median eminence release thyrotropin-releasing hormone (TRH; thyroliberin) into capillaries of the hypophyseal-portal system. Subsequently, TRH stimulates thyrotropes in the anterior pituitary (pars distalis) to secrete thyroid-stimulating hormone (TSH, thyrotropin).[344] The hypothalamus also inhibits TSH secretion through dopamine and somatostatin.[345] TSH is a glycoprotein with two subunits (α and β)—the α subunit is shared with other pituitary and placental glycoprotein hormones (LH, FSH, eCG), and the β subunit dictates its receptor affinity and specificity. Post-translational modifications (glycosylation) are essential for the function and metabolism of TSH. These modifications have limited the use of human and canine immunoassays to measure equine TSH concentrations. TSH stimulates thyroid follicular cells (thyrocytes) to release T_4 and T_3 into systemic circulation. Subsequently, T_3 decreases TSH synthesis and TSH response to TRH stimulation (pituitary gland) and TRH mRNA expression and secretion (hypothalamus).[346-348] In various species, including the horse, TRH is also a releasing factor for prolactin and adrenocorticotropin (ACTH) release.[349-353]

In the CNS and pituitary gland, T_4 is converted to T_3 by type II deiodinase. For T_4 to gain access to hypothalamic neurons it must be transported through the choroid plexus to the lateral ventricles bound to transthyretin (T_4-binding protein).

Because of their type of placentation, in humans and rodents a great proportion of fetal THs come from placental

transfer.[354,355] Thus maternal THs are important for fetal development and maturation at the end of gestation in these species. However, this does not appear to be case for animals with epitheliochorial placentation, which depend on transplacental iodine transfer and their own thyroid glands for T_4 and T_3 synthesis.[355] In the developing fetus, T_4 and T_3 concentrations are low, whereas concentrations of the inactive metabolite, reverse T_3 (rT3), are high, reflecting maturation of the HPTA and coordination with the deiodinase system.[354] Although the physiologic significance of low T_3 concentrations throughout gestation is unknown, it has been suggested to be an ontogenic adaption to avoid excessive thermogenesis and facilitate the anabolic state of the rapidly growing fetus.[354]

Thyroid Hormone Metabolism

A major function of the thyroid gland is to actively trap and conserve iodine against a large concentration gradient (30–40×).[339,356,357] Iodine is absorbed in its soluble form, iodide (I^-), via the intestinal mucosa or any moist body surface or broken skin.[339] Iodide is actively transported and concentrated into the thyroid gland by an Na^+/I^- symporter (NIS), which is coupled to an Na^+ gradient generated by the Na^+/K^+-ATPase to promote I^- accumulation.[358,359] The transport of I^- across the thyrocyte apical membrane to the cell-to-colloid interface is mediated by pendrin.[359] Once in the lumen, the trapped I^- is oxidized to iodine by thyroid peroxidase and incorporated into tyrosine residues of thyroglobulin to form the inactive precursors, monoiodotyrosine (MIT) and diiodotyrosine (DIT). Coupling of these precursors results in the synthesis of thyroxine (T_4) and triiodothyronine (T_3). The iodinated thyroglobulin containing MIT, DIT, T_3, and T_4 is stored as an extracellular polypeptide in the follicular colloid.[360] When needed, T_4 and T_3 are cleaved from thyroglobulin, picked up by the follicular cells via endocytosis, and transferred to the systemic circulation.[339,357] Most of the TH released into circulation is T_4, and serum tT_4 concentrations are 20 times higher than tT_3. Hormone synthesis and release are mainly controlled by TSH and iodine availability. The feedback regulation of TSH is closely linked to circulating concentrations of free (unbound) THs (fT_4 and fT_3).[339,357]

Conversion of T_4 (prohormone) to T_3 (active hormone) occurs through removal of an iodine from the outer ring. Iodine removal from the inner ring results in T_4 conversion to rT_3. All circulating T_4 is directly derived from the thyroid gland whereas only 10% to 20% of T_3 in circulation is directly secreted from the thyroid gland. The major pathway for the production of T_3 is the 5′-monodeiodination of T_4 by type I deiodinase (a selenoprotein) in peripheral tissues. This is referred to as "T_3 neogenesis." In the fetus, T_3 neogenesis is low due to low type I deiodinase activity, and most deiodinase activity converts T_4 to rT_3. A switch in enzymatic activity near birth, partly due to the influence of glucocorticoids, leads to high T_3 concentrations in newborns, including foals.[355,357,361-365] THs circulate bound to plasma proteins (thyroid-binding proteins; TBP). Thyroxine-binding globulin (TBG) is the major protein (70% of T_4 and T_3 are bound to TBG); transthyretin (thyroid-binding prealbumin; TBPA) and albumin bind THs to a lesser degree. In horses, the percentages of circulating T_4 bound to TBG, TBPA, and albumin were found to be 61%, 22%, and 17%, respectively.[366] T_3 is bound to TBG and albumin but not to TBPA. Transthyretin is important to transport T_4 into the CNS. Because THs are reversibly bound to transport proteins they act as TH reservoirs. T_4 has greater protein-binding affinity and is considered the prohormone. Because a large fraction of THs are bound to plasma proteins, changes in protein concentrations may change total TH concentrations. Only free, unbound THs cross the capillary endothelium to exert their biologic functions.[367] T_3 is much more potent than T_4, and it has a shorter half-life. The half-life of T_4 in horses is approximately 50 hours.[368]

The enzymes responsible for the production of T_3 and rT_3 are also responsible for their inactivation. There are three classes of 5′-deiodinases (iodothyronine deiodinases). *Type I deiodinase* is predominantly found in peripheral tissues such as thyroid gland, kidney, and liver; is responsible for the conversion of T_4 to T_3 and rT_3; and is inhibited by propylthiouracil (PTU). Type I deiodinase activity is increased by TSH and T_3 and in hyperthyroidism but reduced in hypothyroidism. *Type II deiodinase* is found in thyroid gland, brown adipose tissue (BAT), brain, reproductive tract, and pituitary gland. It is the main T_4 to T_3 converting enzyme,[360] controls intracellular availability of T_3, and is not affected by PTU.[357] Because type II deiodinase can only deiodinate the outer T_4 ring, it cannot produce rT_3. Type II deiodinase activity is increased in hypothyroidism, likely to sustain intracellular T_3 concentrations despite low peripheral T_4 concentrations, especially in the CNS. *Type III deiodinase* is mainly found in the placenta, developing brain, and skin, only deiodinates the inner ring of T_4 and T_3, and its primary function is to regulate T_3 availability during development by inactivating T_4 and T_3 (converts T_4 to rT_3; T_3 to T_2 [diiodothyronine]). It also prevents excessive access of maternal THs to the fetus. The generation of inactive rT_3 by type I and III deiodinases is essential in TH homeostasis. Metabolites are excreted via the urine, and some are conjugated and enter the enterohepatic circulation. Most iodine is returned to the thyroid gland.

The thyroid gland is also the site of production of calcitonin, secreted by the parafollicular cells (C cells). Calcitonin is involved in calcium homeostasis.[214,339,357] (See calcium section.)

Molecular Actions of Thyroid Hormones

The function of the thyroid gland is to provide THs to meet metabolic needs, based on physiologic status and pathologic conditions. The actions of the THs are mediated by TH receptors, which belong to the superfamily of nuclear receptors. There are two types of TH receptors (TR-α and TR-β). Upon binding to T_3, TH receptors form homodimers or heterodimers with the retinoid X receptor (RXR) that are transcriptionally active, binding specific DNA sequences (response elements), to increase or suppress gene expression, depending on the target organ.

Growth, differentiation, thermogenesis, and the energy metabolism are highly dependent on THs. T_3 stimulates thermogenesis, oxidative phosphorylation, oxygen consumption, protein synthesis, metabolic rate, carbohydrate absorption, insulin sensitivity, glucose metabolism, lipid metabolism, lipoprotein lipase, lipolysis, and the conversion of cholesterol to bile salts. T_3 also promotes growth, maturation, erythropoiesis, heart rate, cardiac output, and blood flow, and it is essential for cerebral and neuronal development in young animals.[339,356] THs are also crucial for the function of other endocrine organs (adrenal gland, pancreas).

Factors that Affect Thyroid Gland Function and Testing

Many endogenous and exogenous factors can influence thyroid function and test results. Being aware of these factors is relevant to the interpretation of TH measurements as well as in the diagnosis, prognosis, and therapeutic management.

Age

There are age-related differences in TH concentrations in horses and donkeys (Tables 16.12 and 16.13).[361-365,373-377] Late in gestation there is a major drive to stimulate the HPTA.[367] Serum concentrations of T_3 and T_4 in foals are very high at birth and decrease with age,[361-365,373-380] with most of the decline occurring in the first 3 to 4 months of life.[363,380,381] Concentrations of tT_4, fT_4, tT_3, and fT_3 in the umbilical cord blood of newborn foals were 14, 5, 7, and 3 times the adult concentrations, respectively.[367] Plasma rT_3 declined rapidly as tT_3 concentrations rose for the first 24 to 48 hours after birth.[367] Another study found that plasma tT_3 concentrations decreased from 7.9 ng/mL at birth, to 0.9 ng/mL at 6 months, to 0.7 ng/mL at 9 months of age.[365] Plasma tT_3 concentration in the mares was 0.5 ng/mL. In the same study, tT_4 concentrations decreased from 233 ng/mL at birth to 49 ng/mL at 14 days and 35 ng/mL at 6 months of age.[365] Thus TH concentrations in newborn foals are approximately tenfold higher than in adult horses, and TH concentrations in the range of adults may indicate a hypothyroid state.

Gender and Breed

There is not a clear association between gender and TH concentrations. Various studies have found higher TH concentrations in males,[361,382,383] lower concentrations in stallions and

TABLE **16.12** **Thyroid Hormone Concentrations in Horses and Donkeys**

Investigators	tT_4	tT_3	fT_4	fT_3	rT3	TSH
Sommardahl et al., 2005[400]		0.49 ± 0.05 ng/mL		0.5 ± 0.2 pg/mL		
Messer et al., 1995[397]	19.97 ± 3.46 mol/L	0.79 ± 0.52 nmol/L	14.35 ± 1.16 pmol/L	1.03 ± 0.53 pmol/L	0.55 ± 0.07 nmol/L	
Messer et al., 1995[422]	19.87 ± 1.74 nmol/L	1.02 ± 0.16 nmol/L	11.55 ± 0.70 pmol/L	2.05 ± 0.33 pmol/L	0.68 ± 0.06 nmol/L	
McBride et al., 1985[403]	1.79 ± 0.17 µg/dL	62 ± 4.3 ng/dL				
Thomas and Adams, 1978[387]	1.57 ± 0.62 µg/dL (0.30–3.70 µg/dL)					
Anderson et al., 1988[383]	1.56 ± 0.081 µg/dL	67.7 ± 10.2 ng/dL	5.9 ± 0.39 pg/mL	3.22 ± 0.18 pg/mL		
Duckett et al., 1989[404]	2.13 ± 0.76 µg/dL	47.38 ± 13.66 ng/dL				
Sojka et al., 1993[369]	6.2–25.1 ng/mL	0.21–0.80 ng/mL		0.07–0.47 ng/dL		
Breuhaus, 2002[451]	12.9 ± 5.6 nmol/L	0.99 ± 0.51 nmol/L	12.2 ± 3.5 pmol/L	2.07 ± 1.14 pmol/L		0.40 ± 0.29 ng/mL
Wehr et al., 2002[370]	1.9 ± 0.4 µg/dL[a]	89.3 ± 26.2 ng/dL[a]				
Marcella, 1992[371]	0.25–3.70 µg/dL[b]	10.0–127.0 ng/dL[b]				
Marcella, 1992[371]	2.57±0.71 µg/dL	77.1 ± 45.75 ng/dL				
Frank et al., 2008[438]	1.7±0.4 µg/dL	4.4 ± 1.6 ng/dL				
Rothschild et al., 2004[439]		1.16 ± 0.16 nmol/L		2.56 ± 0.35 pmol/L		
Johnson et al., 2003[450]	10.33 ± 6.92 nmol/L	0.92 ± 0.61 nmol/L				0.10 ± 0.06 ng/mL
Hilderbran et al., 2014[431]	19.0 ± 2.22 nmol/L	0.9 ± 0.11 nmol/L	22.0 ± 2.1 pmol/L	1.7 ± 0.31 pmol/L		0.35 ± 0.05 ng/mL
Mendoza et al., 2013[377]	1.64 ± 0.15 µg/dL	47.7 ± 1.79 ng/dL	0.23 ± 0.02 ng/dL	1.01 ± 0.10 pg/mL	0.41 ± 0.04 ng/mL	
Mendoza et al., 2013[377]	3.53 ± 0.25 µg/dL[c]	67.1 ± 2.93 ng/dL[c]	0.44 ± 0.02 ng/dL[c]	1.81 ± 0.10 pg/mL[c]	0.63 ± 0.03 µg/mL[c]	
Fazio et al., 2012[392]	31.01–63.67 nmol/L	1.57–2.90 nmol/L	22.47–33.69 pmol/L	3.15–15.52 pmol/L		

[a]Ponies.
[b]Miniature Horses.
[c]Donkeys.
tT_4, Total T_4; *fT_4,* free T_4; *tT_3,* total T_3; *fT_3,* free T_3; *TSH,* thyroid-stimulating hormone.

TABLE **16.13 Thyroid Hormone Concentrations in Foals**

Investigators	Age	tT_4	tT_3	fT_4	fT_3	rT3	TSH
Himler et al., 2012[378]	1–2 day	712 nmol/L (295–1012)	7.9 nmol/L (3.2–9.5)	50.2 pmol/L (27–70)	21.2 pmol/L (6.9–34.3)	6.6 pmol/L (5.2–8.1)	
Breuhaus, 2014[379]	1 day	260.5 nmol/L (191–318)	8.2 nmol/L (6.0–11.8)	97.5 pmol/L (49–144)	10.0 pmol/L (5.6–24.2)		0.24 ng/mL (0.10–0.68)
Pirone et al., 2013[375]	Birth 3 days 7 days	394.81 ± 140.8 nmol/L 178.91 ± 73.96 nmol/L 100.67 ± 79.68 nmol/L	9.63 ± 4.76 nmol/L 9.06 ± 3.50 nmol/L 7.03 ± 2.42 nmol/L				
Irvine and Evans, 1975[363]	1–10 hour 4 day	28.86 μg/dL 11.2 μg/dL	991 ng/dL 935 ng/dL	12.12 pg/mL	2.99 pg/mL		
Murray and Luba, 1993[372]	4 day 28 day	231.7 ± 61.8 nmol/L 30.6 ± 17.4 nmol/L	7.8 ± 4.2 nmol/L 3.1 ± 0.4 nmol/L	1.2 ± 0.4 ng/dL	3.4 ± 1.1 pg/mL		
Shaftoe et al., 1988[456]	1 day	4.4–25.1 μg/dL	26.0–732.7 ng/dL				
Chen and Riley, 1981[361]	1.5–4 month	4.02–0.19 μg/dL (2.9–5.25)	192.86 ± 8.54 ng/dL (135–270)				
Dudan et al., 1987[496]	5 minute 48 hour	29.3 μg/dL 20.5 μg/dL					
Malinowski et al., 1996[365]	Birth 1 7 14 1 month 6 months	233.0 μg/dL 207.0 μg/dL 92.0 μg/dL 49.0 μg/dL 26.0 μg/dL 35.0 μg/dL	7.9 ng/mL 6.7 ng/mL 4.2 ng/mL 2.4 ng/mL 1.6 ng/mL 0.9 ng/mL				
Panzani et al., 2012[376]	Birth 1 day 7 day 14 day	481.2 ± 137.4 nmol/mL 262.2 ± 11.9 nmol/mL 117.9 ± 89.5 nmol/mL 29.6 ± 0.58 mol/mL	11.69 ± 5.69 nmol/mL 11.84 ± 4.23 nmol/mL 7.54 ± 2.67 nmol/mL 2.44 ± 011 nmol/mL				

tT_4, Total T_4; *fT_4,* free T_4; *tT_3,* total T_3; *fT_3,* free T_3; *rT_3,* reverse T_3; *TSH,* thyroid-stimulating hormone.

geldings,[374,384] similar or lower in geldings than stallions,[383] and lower concentrations in mares than in stallions,[382] whereas others have found no differences.[377,381,385-387] There is no evidence that TH concentrations differ among breeds,[361,387] although one study found higher T_3 and T_4 concentrations in donkeys compared with horses.[377] This could be attributed to genetic differences as well as metabolic size.

Physiologic Status

Mares tested at all stages of the estrous cycle had no differences in TH concentrations.[386] T_4 concentrations, however, were lower following ovulation, which is the opposite pattern seen in other species. Higher TH concentrations have been reported in pregnant mares,[388] which is similar to other mammals.[357] This may be due in part to the increased TBG concentrations during gestation.[357] T_4 concentrations were statistically higher between days 49 and 55 of gestation compared with mares in advanced pregnancy.[389] A couple of studies found no effect of pregnancy on T_4 concentrations,[383,390] although tT_3 and fT_3 concentrations were higher in pregnant mares.[383] Lower T_3 concentrations were found at the end of pregnancy[390]; however, other studies showed no differences in THs between pregnant and nonpregnant mares and jennets.[368,384,391,392] Serum TH concentrations were not different between mares that did and did not become pregnant,[393] suggesting that thyroid dysfunction is an uncommon cause of infertility. This finding also questions the practice of supplementing mares with thyroid hormones to increase pregnancy rates.[393] Serum T_4 and T_3 concentrations are higher in lactating compared with nonlactating mares.[391] TH concentrations increased during lactation in donkeys.[394]

Hormones

Exogenous and endogenous glucocorticoids suppress TSH secretion, decrease the response of TSH to TRH, inhibit deiodination (except in the perinatal period where the conversion of T_4 to T_3 is enhanced), and decrease TH release.[357,395] Glucocorticoids decrease serum T_4 and T_3 concentrations, with the effect being more profound on T_3.[396] In one study, dexamethasone administration to healthy horses for 5 days did not change tT_3 and tT_4 concentrations but increased serum rT_3 and fT_3 concentrations and blunted the fT_3 response to TSH stimulation.[397] In another study, topical dexamethasone suppressed T_4 and T_3 concentrations.[398] Catecholamines enhance the rate of deiodination of T_4 to T_3 and have potentiating effects.[399] Estrogen enhances TBG production whereas androgens tend to suppress it.[357] Oral administration of levothyroxine to healthy horses resulted in weight loss; increased tT_4, fT_4, and rT_3 concentrations; and reduced the TSH and TH response to TRH stimulation.[400]

Season and Daily Rhythm

Thyroid hormones are essential for thermogenesis and regulating resting energy expenditure, in particular during cold exposure.[401] This is a process mediated by uncoupling proteins (UCP-1, UCP-2, and UCP-3) in BAT and skeletal muscle, with involvement of the sympathetic nervous system to increase ATP production and use.[401] Cold temperatures stimulate and high temperatures inhibit the HPTA.[368,402,403] The effect of season was minimal in pregnant compared with nonpregnant mares.[391] A daily rhythm in TH concentrations has been identified in horses.[380,390,404,405] In geldings, T_4 peaked around 4:00 PM with the lowest T_4 values around 4:00 AM. T_3 peaked between 8:00 AM and 4:00 PM, with the lowest T_3 values around midnight.[404] Higher T_4 concentrations between 5:00 and 8:00 PM with lowest concentrations at 8:00 AM were reported in another study.[405] No diurnal rhythmicity in TH concentrations was found in foals.[380] Diurnal variations in glucocorticoid concentrations appear to control the diurnal variations in TH by suppressing the pituitary response to TRH.[406]

Activity

Variable results on the effect of physical activity on TH concentrations have been reported.[362,407-409] One study in young Standardbreds found that serum T_4 concentrations decreased early in the training period.[410] In the same study, serum T_4 concentrations were lower in horses with maladaptation to training. Increases in T_4 within 30 minutes after exercise with minimal differences in T_3 have been reported.[385,388] Five minutes after a maximal speed race (1200 m), serum T_3 concentrations increased in Thoroughbred racehorses although T_4 concentrations did not change.[411] Significant increases in T_3 and T_4 concentrations were found 1 hour after swimming.[412] Serum T_4, T_3, and rT_3 concentrations decreased progressively in the course of a multiday ride.[413] In a study with ponies, metabolic rate, resting heart rate, and locomotor activity were positively correlated with total T_3 but negatively correlated with total T_4 concentrations.[409]

Feeding

Feeding high-energy and high-protein diets to horses stimulates TH secretion.[414,415] Soluble carbohydrates rather than protein appear to be responsible for this increase.[416] The conversion of T_4 to T_3 following a carbohydrate meal was associated with insulin release.[417] The TH response to carbohydrates is age-dependent; by the time horses are 12 to 14 months of age, feeding high-carbohydrate and protein diets has minimal effect on TH concentrations.[417] Ponies fed once daily had greater T_3 concentrations than animals fed six times a day.[418] Another study in ponies found no effect of feed restriction on T_4 concentrations.[419]

Starvation in different species, including horses, reduces T_3 and fT_3 concentrations.[420-422] Energy deprivation inhibits the deiodination of T_4 to T_3 by the type I deiodinase to decrease T_3 but increase rT_3 concentrations. This could be a biologic adjustment to reduce metabolic rate and conserve energy. In these animals, TSH concentrations are low or within the normal range despite low TH concentrations. When the effect of food deprivation on baseline TH concentrations was evaluated in euthyroid horses, it was found that serum T_3, fT_3, and fT_4 concentrations decreased whereas rT_3 concentrations increased after 1 day of food withdrawal.[422] Serum tT_3 concentrations were lowest after 2 days of deprivation, and tT_4, fT_3, and fT_4 reached their lowest concentrations on day 4 of deprivation. Three days after the horses were placed on their normal ration, serum tT_4 and fT_4 concentrations increased significantly, and rT_3 concentrations decreased.[422] Prolonged food restriction to Shetland ponies also decreased serum tT_3 and fT_3 concentrations.[423] These studies provided valuable information in the interpretation of TH concentrations in anorectic and sick horses. For example, if a decrease (greater than 50%) in serum T_3 and T_4 concentrations can occur in 1 to 2 days of food deprivation, then the validity of measuring TH in diseased horses is questionable.

The mechanisms that regulate thyroid gland function during starvation remain unclear. Leptin, the adipocyte-derived

hormone that controls hunger, appears to be key neuroendocrine signal that regulates the HPTA during fasting and disease.[424,425] Leptin concentrations decrease during starvation in order to increase appetite and conserve energy. A decrease in leptin concentrations reduces activity of hypothalamic TRH neurons, resulting in less TRH and subsequently less TSH and TH secretion. This seems to be part of a thrifty strategy by which a reduction in leptin promotes hunger and reduces HPTA activity. This process is likely to apply to horses, by which feed restriction decreases while caloric intake increases leptin concentrations,[426-428] with THs following similar leptin patterns.[420-422]

Even though information on leptin has been published in horses and foals,[426,429,430] its direct role on thyroid gland function remains unknown.

Diseases (Nonthyroidal Illness Syndrome, Euthyroid Syndrome)

Illnesses, systemic inflammation, stress, and starvation suppress the HPTA. Because there is no clinical evidence that low TH concentrations during disease are associated with hypothyroidism, this condition has been called the *low T_3 syndrome,* the *euthyroid sick syndrome,* and more recently the *nonthyroidal illness syndrome (NTIS)* because it does not make assumptions on the metabolic status of the patient.[421] In NTIS, T_3 concentrations decrease, whereas T_4 concentrations can be low, normal, or elevated. In critically ill human patients, a significant decrease in T_4 and T_3 or an increase in rT_3 concentrations is associated with increased mortality rate.[421]

Recent studies provide evidence that NTIS is highly prevalent in horses and foals.[375,378,379,431] Low tT_3, fT_3, tT_4, and fT_4 concentrations have been measured in premature, septic, and hospitalized foals.[375,378,379] Systemic diseases reduce TH concentrations in adult horses, and this effect is more profound than the effect of fasting, glucocorticoids, or NSAIDs.[431] In at least one study, tT_3 concentrations decreased before other THs.[431] Serum TH concentrations were lower in hospitalized horses that died or were euthanized.[431] TH concentrations also decreased in sick horses in proportion to disease severity.[431] Serum fT_3, fT_4, and TSH concentrations were lower in hospitalized and premature foals that died compared with survivors.[378,379] TH and TSH concentrations were negatively correlated with the sepsis score in hospitalized newborn foals.[378,379] In septic foals, rT_3 concentrations were positively associated with the sepsis score.[378]

Based on the lack of rise in TSH concentrations in sick horses with low THs, it seems that NTIS alters all levels of the HPTA.[431] It also appears that hypothalamic and pituitary dysfunction is central to the pathogenesis of NTIS,[421] as administration of TRH to patients with NTIS increases TSH, T_4, and T_3 release.[432] This further supports the central role of leptin on the HPTA. Although NTIS can be seen as a hypothyroid state, there is no evidence that target tissues are chemically hypothyroid.[421] Any acute or chronic condition is likely to reduce HPTA activity, which does not equate to hypothyroidism.

Cytokines, glucocorticoids, and leptin are key candidates in the pathogenesis of NTIS. Cytokines such as IL1-β, IL-6, and TNF-α suppress TSH, T_4, and T_3 synthesis and secretion. IL1-β impairs TH synthesis, whereas IL-6 and TNF-α decrease T_4 and T_3 concentrations and increase rT_3 concentrations. Glucocorticoids are negative regulators of thyroid function; increased cortisol concentrations are common in horses and foals under different conditions, including stress, gastrointestinal disease, pain, sepsis, obesity, insulin resistance, and pituitary pars intermedia dysfunction (PPID; Cushing disease),[378,379,431,433-435] and low TH concentrations can be measured in the same animals.[378,379,431] As previously mentioned, the role of leptin in the pathogenesis of NTIS relates to caloric intake and energy conservation.

Low TH concentrations are commonly measured in horses with insulin resistance (equine metabolic syndrome) and laminitis; however, the clinical value of these measurements is limited. Leptin concentrations tend to be high, not low, in horses with metabolic syndrome and prone to laminitis[436,437] and, similar to people with central obesity, also have low TH concentrations, supporting the concept of impaired leptin signaling at the hypothalamic level.

There is no evidence that TH replacement therapy in humans or animals with NTIS is either beneficial or harmful.[421] Some believe that replacement therapy may be harmful because exogenous TH may inhibit the HPTA. The only clear indication for exogenous supplementation of THs in horses is to induce weight loss and enhance insulin sensitivity in obese animals, particularly those with metabolic syndrome.[438]

Exogenous Compounds

Goitrogenic substances such as thiocyanate and perchlorate compete with iodide uptake. Iodide oxidation and coupling of iodotyrosines are inhibited by drugs such as sulfonamides, phenylbutazone, phenothiazines, thiouracils, thiopental, and methimazole. One study found no suppressive effect of trimethoprim-sulfadiazine on equine thyroid function.[439] Soybean meal, linseed meal, and plants of the *Brassica* family (i.e., rapeseed) contain goitrogenic substances.[339,356] Phenylbutazone administration decreases TH concentrations in horses,[405,440] and this effect can last up to 10 days after discontinuation of phenylbutazone.[440] The TSH stimulation test may be normal or exacerbated in horses receiving phenylbutazone.[405] High levels of nitrates in the water may induce thyroid gland hypertrophy in people,[441] as nitrates reduce the iodine transport into the thyroid gland.

Propylthiouracil (PTU), in addition to affecting iodine metabolism, inhibits the peripheral conversion of T_4 to T_3. Substances that compete for TH binding sites on transporting proteins could potentially lead to a hypermetabolic state by increasing their free fractions (fT_4, fT_3).[395] Human athletes receiving anabolic steroids had decreased TSH, T_4, T_3, and TBG concentrations[441a]; however, anabolic steroid administration to horses did not affect thyroid function tests.[442]

Exogenous compounds containing iodine or iodides are of particular interest. Both iodine excess and deficiency can induce thyroid dysfunction and interfere with thyroid tests.[443] Horses are exposed to iodine or iodide compounds in the form of feedstuffs, expectorants, leg paints, shampoos, injectable counterirritants, radiographic contrast media, and antiprotozoal drugs (iodochlorhydroxyquin). Animals can respond to excessive amounts of iodine by either suppressing or accelerating hormone production.

Iodine excess reduces thyroidal iodine uptake and organification (incorporation into inactive MIT and DIT) and decreases TH concentrations and the response to the TSH and TRH stimulation tests. The end result could be hypothyroidism, which is known as the "Wolff-Chaikoff effect." Iodine-containing drugs such as amiodarone have been shown to cause this effect in people and dogs[444] but not in horses. The

second way animals and people can respond to excessive iodine exposure is by elevating TH production. This is referred to as "Jod-Basedow phenomenon" that in general is seen in individuals with iodine deficiency.[357]

Newborn foals of mares grazing *Neotyphodium coenophialum*–infected fescue had lower T_3 concentrations than controls, with no differences in T_4 and rT_3 concentrations.[445] Endophyte alkaloids are D_2 dopamine receptor agonists, and dopamine is an inhibitor of TSH secretion. It remains unclear whether the low TH concentrations in these foals are a direct consequence of the alkaloids on the fetal HPTA or an indirect effect through placental and fetal development. Endophyte alkaloids do not appear to alter thyroid function in adult horses.[446]

Diagnostic Tests

Unlike human practice in which functional and direct measurements of THs are often used, in equine medicine we are far from assessing thyroid gland function in an organized manner. Our routine approach is to determine TH concentrations, compared with reference values, make a diagnosis and specific recommendations, in many instances, ignoring the underlying cause of abnormal TH concentrations. Normal TH concentrations have a wide range of values and vary among laboratories and measuring methods (see Tables 16.12 and 16.13). For consistency, the practitioner should use the same laboratory. Ratios of circulating hormones may be indicative of dysfunction.

Equilibrium dialysis has been considered the most accurate method to measure the free fractions of THs; however, immunoassays are more often used due to practicality and increasing sensitivity. There are immunoassays available to measure tT_4, fT_4, tT_3, fT_3, and rT_3 concentrations. Radioimmunoassays (RIAs) have been validated to measure equine THs.[387,447] Quantification of T_4 and T_3 with RIA is not affected by hemolysis.[448] Enzyme-linked immunosorbent assays (ELISAs) and immunoluminescence methods are becoming popular due to their simplicity, safety (no need to work with radioactive material), and detection limits. The unbound or free fractions of THs must be measured to determine available hormone to target tissues.[449]

Serum is the preferred sample to measure T_4 and T_3 concentrations, although heparin or EDTA plasma can be used. T_4 is stable in serum at 4°C for several weeks. Avoid hemolyzed and lipemic samples, and samples from horses receiving "thyroid supplements." The T_4 secretion rate is a functional test that calculates T_4 secretion after ^{131}I injection. This method is not practical.[362]

Free THs circulate in equilibrium with protein-bound THs, and changes in TBP (TBG, TBPA, albumin) can affect TH concentrations. Immunoassays to determine human TBG and transthyretin concentrations are available but not validated for horses.

In healthy horses, the tT_4 to tT_3 ratio was 23:1, and the fT_4 to fT_3 ratio was 1.83:1.[383] A more recent study found a tT_4 to tT_3 ratio of 21:1 and an fT_4 to fT_3 ratio of 13:1.[431] In healthy newborn foals, the tT_4 to tT_3 and fT_4 to fT_3 ratios were 32:1 and 10:1, respectively.[379] These ratios increased in sick horses and foals.[379,431] Serum rT_3 concentrations are often increased in hyperthyroidism and NTIS.[19]

Commercial assays to determine human and canine TSH concentrations are available, but they have failed to measure equine TSH. Increased TSH concentrations are expected in hypothyroidism and the opposite in hyperthyroidism.[352,450] Equine TSH concentrations have been determined with customized assays.[430,439,450-452] Breuhaus validated an equine-specific double-antibody TSH RIA in an equine model of hypothyroidism.[451] In that study, hypothyroidism was induced in healthy horses by administering PTU for 6 weeks. Serum tT_3 and fT_3 fell rapidly, whereas fT_4 and tT_4 did not decrease until weeks 4 and 5, respectively. TSH concentrations remained steady until week 5, increasing thereafter. The TSH response to TRH stimulation (1 and 5 mg) was exaggerated. Similar studies of PTU-induced primary hypothyroidism found a decrease in serum of T_3, T_4, and rT_3 and an increase in TSH concentrations.[352,450]

Hormone response tests are useful to differentiate primary from secondary thyroid dysfunction, and they remove the potential influence of endogenous and exogenous factors on the results.

Thyroid-Stimulating Hormone Stimulation Test

The TSH stimulation test consists of injecting 2.5 to 5.0 IU of TSH intravenously and comparing preinjection and postinjection TH concentrations.[453,454] In normal horses, T_4 peaks 3 to 4 hours after injection at a concentration 2.4 times the preinjection values. T_3 doubles within 30 minutes and peaks at 2 hours at a concentration that is five times baseline values.[455] A normal response is characterized by a T_3 rise before the T_4 increase. When 5 IU of TSH were given intramuscularly to horses, T_4 peaked at 2 times baseline value 3 to 12 hours after injection, and T_3 peaked in 1 to 3 hours.[405] No differences in T_4 concentrations were found between 2.5 and 5 IU of TSH.[453] The suggested protocol for the IV TSH response test is to measure TH concentrations at baseline and 3 to 4 hours after injection.[455] For the intramuscular protocol, measure hormone concentrations at baseline and 3 and 6 hours after injection.[405] The protocol for newborn foals is to measure T_3 concentrations at baseline and 1 and 3 hours after the IV injection of 5 IU of TSH. A normal response is a 50% increase by 3 hours. T_4 values after TSH stimulation are variable in foals.[456] An insufficient hormonal response is consistent with primary hypothyroidism. Phenylbutazone decreases TH concentrations in horses, but it does not affect the response of the TSH stimulation test.[405] Dexamethasone blunts the TH response to the TSH stimulation test.[397] This information should be taken into account in the interpretation of the TSH stimulation test.[405,455] Treatment with dopamine agonists does not appear to affect serum T_3, T_4, rT_3, or TSH concentrations in horses.[450]

Cost and availability of medical-grade TSH limit the clinical use of this test. Instead, the TRH stimulation test is much easier and cheaper to perform.

Thyrotropin-Releasing Hormone Stimulation Test

The TRH stimulation has become a widely used method to assess thyroid gland function and PPID in horses.[453,457-462] TRH is administered intravenously, 1 mg to horses and 0.5 mg to ponies and foals, with hormone measurements at baseline, 2 and 4 hours later. In a normal response both hormones should increase twofold to threefold. An inadequate TH response occurs with primary (thyroid) or secondary (pituitary) hypothyroidism. In normal animals, T_4 peaks at 4 hours[453,459] and T_3 at 2 hours after TRH administration.[459] Side effects of TRH administration include salivation, urination, defecation,

miosis, tachycardia, tachypnea, yawning, lip movements, and trembling.[458] A low response to TRH administration suggests dysfunction at either the pituitary or the thyroid gland level. Additional tests (TSH concentrations, TSH stimulation test) may be necessary to differentiate thyroid from pituitary disease. Low TH and high TSH concentrations and a low response to TSH stimulation are consistent with thyroid gland dysfunction. Low TH concentrations with low or normal TSH concentrations indicate hypothalamic or pituitary gland dysfunction. Low TH and TSH concentrations with a normal TSH and TH response to TRH indicate a hypothalamic disorder (tertiary hypothyroidism). Levothyroxine administration blunts the response to the TRH stimulation test.[400]

Thyroid Suppression Test (T_3 Suppression Test)

Autonomous TH secretion (TSH independent) can be demonstrated by the thyroid suppression test. Exogenous administration of TH should suppress TSH and TH secretion. After T_3 administration, T_4 concentrations are expected to drop in euthyroid horses, whereas minimal to no changes in T_4 concentrations are anticipated in hyperthyroid animals (primary hyperthyroidism). This test has been used to diagnose hyperthyroidism in horses with thyroid adenomas and adenocarcinomas.[463,464] For the test, 2.5 mg of T_3 (3,3′,5-triiodo-L-thyronine) diluted in 5 mL of sterile saline solution is administered intramuscularly at 8:00 AM and 6:00 PM on days 1, 2, and 3 and at 8:00 AM on day 4. Serum T_4 and T_3 concentrations are measured 5 minutes before each T_3 dose. Additional blood samples are collected at 6:00 PM on days 4 to 10.[463,464] Failure of T_4 to suppress after T_3 administration indicates autonomous secretion of THs, which is consistent with hyperparathyroidism. In euthyroid horses, T_4 concentrations decrease by day 4 and remain low until day 10, and it takes 2 to 3 weeks for T_4 concentrations to return to baseline values.[463]

Imaging

Nuclear scintigraphy (technetium 99m [^{99m}Tc]) is useful in assessing equine thyroid gland function.[465] Abnormal uptake patterns have been seen with thyroid carcinoma in horses.[466-468] Ultrasonographic evaluation of the glands can differentiate solid structures from cystic ones.[357] Ultrasonography is the most commonly used imaging modality to assess equine thyroid gland morphology.[463,469]

Biopsy

Aspirate or biopsy sampling can help differentiate cysts, neoplasia, hyperplastic goiter, colloidal goiter, and inflammation.

Hypothyroidism

There is no evidence that hypothyroidism is common in horses or foals, although it is frequently diagnosed based on low TH concentrations. Many conditions, including obesity, insulin resistance, metabolic syndrome, laminitis, anhidrosis, rhabdomyolysis, agalactia, infertility, alopecia, and hypothermia have been attributed to low TH concentrations in horses and donkeys.[350,470-475] In foals, hypothyroidism has been linked to respiratory insufficiency, gastric ulcers, and skeletal abnormalities.[476-481]

In general terms, hypothyroidism is defined as TH deficiency, inadequate thyroid follicular activity, or disruption of the HPTA. Hypothyroidism can be classified as primary (thyroid gland), secondary (pituitary gland), and tertiary (hypothalamus). Hypothyroidism occurs from diseases that affect thyroid gland function, exogenous compounds that interfere with TH synthesis, and less frequently by disorders of the hypothalamus or pituitary gland. The liver, muscle, skeleton, kidney, heart, pancreas, adrenal, gastrointestinal tract, respiratory system, and CNS are the main target organs for THs during development.[357] Hypothyroidism manifests in many ways, and the diagnosis can be challenging because a multitude of nonthyroidal factors can alter TH concentrations, leading to result misinterpretation. Primary hypothyroidism can be caused by iodine deficiency (endemic goiter) or excess (Wolff-Chaikoff effect), thyroiditis, neoplasia, biochemical defects, thyroid agenesis, or ingestion of goitrogenic compounds that block TH synthesis. Of these, iodine deficiency, iodine excess, and neoplasia have been reported as causes of equine hypothyroidism. Goiter with no evidence of hypothyroidism has been described in foals born of mares fed excess iodine during gestation.[482] Histologic lesions consistent with Hashimoto thyroiditis were recently described in horses.[483]

Horses with primary hypothyroidism have decreased T_4 and T_3 concentrations and increased TSH concentrations.[451] Central hypothyroidism occurs from pituitary or hypothalamic dysfunction. There are reports of low TH concentrations in horses with pituitary adenoma.[457,484,485] TH resistance or insensitivity (Refetoff syndrome) denotes a defect in TH signaling,[486] which remains to be documented in domestic animals.

Hypothyroidism in Foals

The consequences of inadequate circulating TH can be devastating for the developing fetus and newborn foal. Recovery is unlikely once critical developmental stages are passed. Tests to evaluate thyroid gland function may be within normal limits at the time of examination, making it difficult to confirm a previous transient or in utero TH deficiency.[367] Iodine deficiency may result in hyperplastic goiter in foals (Fig. 16.14).

Signs

Clinical signs in hypothyroid foals relate to the essential functions of THs in the development and maturation of the nervous, respiratory, and musculoskeletal systems during the prenatal and postnatal periods. Hypothyroid foals are often affected at birth. Signs include stillbirths, prematurity, weakness, and long haircoat[342,487]; weak suckle reflex[488]; respiratory insufficiency and distress[479,,489]; incoordination[363]; lethargy, depression, and rough coat[490]; cold intolerance and hypothermia[367]; physeal dysgenesis[481]; defective ossification with carpal and tarsal bone collapse[480,481]; hypoplastic carpal bones, common digital extensor tendon rupture, forelimb contracture, and prognathism[478,491]; and delayed incisor eruption, growth retardation, and death.[492] Foals can be born apparently normal but develop skeletal lesions weeks later.[490]

Cause

The major cause of hypothyroidism in foals is nutritional. Congenital thyroid enlargement (congenital goiter) with decreased thyroid function is associated with both inadequate and excessive iodine intake by the mare. There are several reports of goiter and hypothyroidism in foals associated with ingestion of excessive amounts of iodine, in particular with kelp-supplemented rations.[342,425,443,487,493-495] The dams of these foals may or may not be affected.[425,443] Feeding 40 mg or more of iodine daily can produce this syndrome.[488]



FIG. **16.14** Newborn foal presented with respiratory distress and thyroid gland enlargement (congenital goiter). Thyroid hormone concentrations were normal, but biopsy of the thyroid gland revealed follicular hyperplasia.

Diagnosis

Because reduced iodine intake is considered the main cause of neonatal hypothyroidism and goiter, pituitary gland function (TSH secretion) may be normal or increased. The lack of TH negative feedback on pituitary thyrotropes leads to excessive TSH secretion, thyroid follicular cell hyperplasia, and thyroid enlargement (goiter).

Foals with signs of hypothyroidism, with or without enlarged thyroid glands, are suspects. The nutrition of the mare as well as other horses in the premises should be examined. Thyroid gland enlargement does not imply hypothyroidism or hyperthyroidism. Other conditions should be ruled out (e.g., branchial cysts) before performing functional tests (TSH and TRH stimulation tests) to confirm the diagnosis of hypothyroidism. When assessing TH concentrations in sick foals it is crucial to compare results to age-matched healthy foals.[378,379] It is also important to remember that high TH concentrations are normal in healthy foals (see Table 16.13). Premature and septic foals may have lower TH concentrations than controls, which does not imply hypothyroidism but NTIS.[378,379] Serum rT_3 concentrations in premature and septic foals may be lower, similar, or higher than in term foals.[378,452,496] A foal with clinical evidence of hypothyroidism could have normal TH concentrations, suggesting that TH deficiency occurred during development. Hypothyroidism in foals carries a poor prognosis, even after TH replacement therapy.

Congenital Hypothyroidism and Dysmaturity Syndrome in Foals

A syndrome in newborn foals characterized by thyroid gland hyperplasia, multiple congenital musculoskeletal deformities, and high mortality rates was first recognized in western Canada and northwest of the United States in the late 1970s.[381,476,489,491,497-500] In some outbreaks 30% to 100% of the newborn foals were affected,[476] with no sex or breed predilection.[498] Gestation is often prolonged, but these foals show signs of dysmaturity including weak to stand, silky and short hair coat, floppy ears, tendon laxity, doming of the head, and incomplete closure of the abdominal wall.[476] Musculoskeletal abnormalities include prognathism, osteochondrosis, angular and flexural limb deformities, rupture of the common digital extensor tendons, and delayed ossification of carpal and tarsal bones (Fig. 16.15).[476,498-500] Even though thyroid enlargement is rare, there is thyroid gland hyperplasia.[499] This syndrome is referred to as congenital hypothyroidism and dysmaturity (CHD). The etiology remains unknown. A case-control study found that foals with CHD had a longer gestation time and that mares grazing irrigated pastures, fed greenfeed, not receiving mineral supplements, or that left their home farm were more likely to produce affected foals.[476] High nitrate intake has been proposed as the possible cause. Nitrates can cross the placenta and impair thyroid gland function.[441] Greenfeed can concentrate high levels of nitrate and nitrite. Alfalfa, ryegrass, timothy, and cereal crops such as wheat, oats, rye, and barley also accumulate nitrate. In addition, these plants have low levels of iodine. Nitrates can also be in high concentrations in the water, particularly in areas with high use of fertilizers, feedlots, and dairies.[476] TH concentrations in foals with CHD are low or within the normal range, and their response to TSH stimulation is poor.[478] Of interest, foals with musculoskeletal disease from TH deficiency have a normal adrenocortical response to ACTH,[501] suggesting that THs more than glucocorticoids play essential roles in skeletal differentiation, at least in late pregnancy.

In the past it was believed that CHD was restricted to western Canada and the northwestern United States; however, sporadic cases in eastern Canada and the midwestern United States have been reported.[500,502,503] Of interest, CHD was recently documented in Finland.[504]

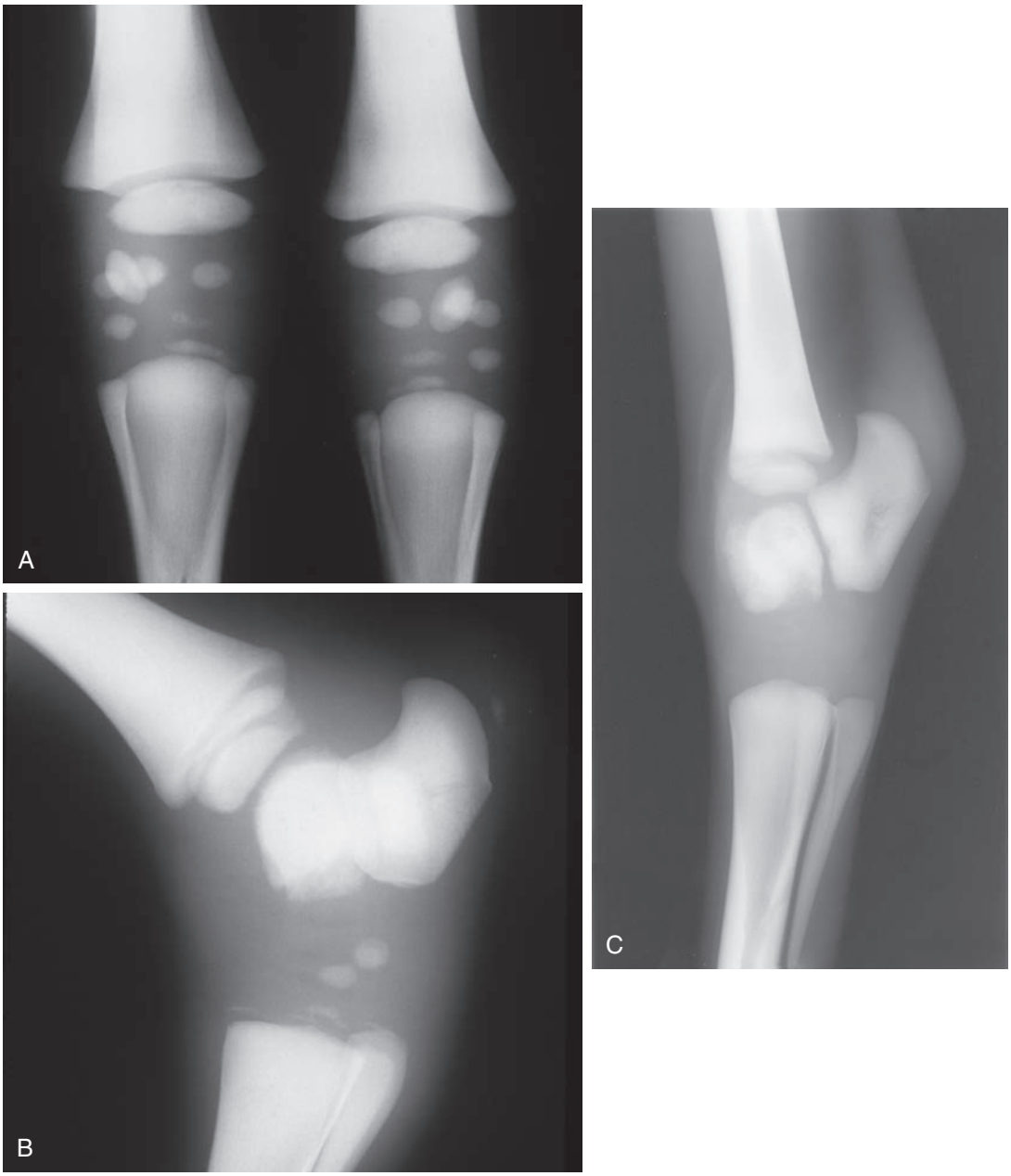


FIG. **16.15** Radiographs from newborn foals with evidence of congenital hypothyroidism and dysmaturity syndrome. Notice the (A) incomplete ossification of the cuboidal bones in the carpus and (B, C) tarsus. (A, B, Courtesy M. T. Hines. C, The Ohio State University.)

It is important for the clinician to be aware that the clinical presentation of CHD could be variable, from the foal that appears weak but otherwise mature compared with those with typical signs of the disease. This could have diagnostic, prognostic, and epidemiologic implications.

The prognosis for foals with CHD is grave, and most foals die or are euthanized within a few days after birth.[500,504] Foals that survive are likely to develop other abnormalities, particularly orthopedic disorders.

Hypothyroidism in Adult Horses

Hypothyroidism in horses is a rare condition, and unlike in foals is not life threatening. Increased cholesterol concentrations, hypothermia, bradycardia, and anemia are potential indicators of hypothyroidism; however, they are not considered reliable markers. Low-grade anemia, decreased body temperature, heart rate, respiratory rate, and cardiac output, rear- limb edema, coarse haircoat, and decreased appetite have been reported in thyroidectomized horses.[485,505,505-507] Lethargy, rearlimb edema, coarse haircoat, and decreased appetite were seen in thyroidectomized mares.[507] Reproductive problems in hypothyroid mares are irregular and absent estrus cycles.[342,505] Thyroidectomized mares could conceive and carry a foal to term.[507] Stallions exhibit decreased libido.[472,505] Thyroidectomized stallions had decreased total sperm count but normal semen characteristics and could sire foals.[492,507] Bradycardia, obesity, and lethargy were seen in mares whose thyroid function was suppressed from excessive

iodine ingestion.[342] Alopecia attributed to iodinism and low circulating TH values were documented in a horse treated with an expectorant containing iodide and topical povidone iodine.[508] One report found low T_4 concentrations in agalactic mares.[350] Poor performance in racehorses in warm seasons could be related to low TH concentrations.[407] Hypothyroidism has been implicated as a cause of anhidrosis. Low or no differences in T_3 and T_4 concentrations have been found in anhidrotic horses.[470,509,510] The TH response to the TRH stimulation test was not different between anhidrotic and matched control horses.[470]

Low TH concentrations may be present in horses with PPID and laminitis, but it is unlikely that hypothyroidism is the cause of the laminitis in these animals.[511] TH concentrations are low, whereas TRH and TSH concentrations are elevated in hypothyroid horses.[512]

Treatment of Hypothyroidism

When hypothyroidism is suspected, it is important to determine whether the animal is receiving appropriate amounts of iodine. Differentiating hypothyroidism from NTIS is critical as animals with NTIS may not require TH supplementation. Furthermore, TH therapy to euthyroid horses may be harmful, inhibiting the HPTA, and suppressing endogenous TH synthesis and secretion. The National Research Council recommends daily intakes of iodine of 1 to 5 mg per horse depending on age, physiologic status, and body weight.[342] The soil in some geographic areas (e.g., Great Lakes region) has low or marginally low iodine content. Iodinated casein and thyroprotein (5 g/day PO) have been used to reverse the effects of hypothyroidism.[473,492]

Hormone supplementation with T_4 can be effective at treating hypothyroidism unless there is a deiodination defect. A starting oral T_4 dose of 20 µg/kg/day is recommended.[385] There is a place for using levothyroxine (L-thyroxine, T_4) in horses and foals correctly diagnosed with hypothyroidism. Unfortunately, thyroid supplements are widely used for a multitude of equine conditions that not surprisingly also have low TH concentrations (obesity, laminitis, insulin resistance, infertility, poor performance, dermatopathies, anhidrosis, others).[471] According to the manufacturer, levothyroxine sodium is indicated for horses with low TH concentrations, presumably hypothyroidism, at an oral dose of 0.01 to 0.06 mg/kg. The animal should be monitored for clinical response to therapy, which can take at least 2 weeks. Periodic TH measurements are important to adjust dosing if the desired response is not achieved.

Hyperthyroidism

Hyperthyroidism is a hypermetabolic state consequence of high concentrations of free T_4 and T_3. High TH concentrations can be found in physiologic states such as pregnancy, fetuses in late pregnancy, and the first weeks of life.[361-365,367,373,374,381,389] Few documented cases of equine hyperthyroidism exist.[463,469] Tremors, excitability, tachycardia, sweating, and weight loss have been described in racehorses suspected of hyperthyroidism.[407,513] There are no reports of hyperthyroidism associated with autoimmune conditions in the horse. Hyperthyroidism from thyroid adenomas and adenocarcinomas has been documented in horses.[463,464,469] The T_3 suppression test was used to confirm the diagnosis of hyperthyroidism.[463,464]

Horses are at risk for accelerated TH production when exposed to increased quantities of iodine-containing compounds such as expectorants, counterirritants, contrast media, drugs, leg paints, and povidone-based shampoos (Jod-Basedow phenomenon).[357]

As part of the treatment of hyperthyroidism, it is important to eliminate exposure to iodine-containing compounds. Glucocorticoids may alleviate clinical signs in severe cases.[357] Hemithyroidectomy can be successful at restoring TH concentrations in horses with unilateral thyroid tumors. Horses may improve with antithyroid therapy of potassium iodide (1 g/day PO).[402,407] PTU (8 mg/kg PO q 24 h) was used successfully to treat a 19-year-old mare with a thyroid adenocarcinoma and hyperthyroidism.[464] Measuring TH in the first 2 weeks of treatment is recommended to adjust dosing.[464] Administration of PTU every other day for weeks to months appears to be safe, effective, and inexpensive. The prognosis for hyperthyroid horses is variable, depending on the cause and response to treatment. For unilateral thyroid tumors, prognosis after surgical excision is good. The use of thyroidectomy to improve behavior in unmanageable horses had variable success.[492]

Thyroid Tumors

Tumors of the thyroid gland tend to occur more frequently in lightweight breeds than in draught horses and more often in aged than in young horses.[514] There are three cell types in the equine thyroid gland: undifferentiated cells, parafollicular cells (secrete calcitonin), and follicular epithelial cells. A survey of aged horses found thyroid tumors in 30% of the animals,[515] with no tumors in horses younger than 18 years old. Masses in the throat or retropharyngeal areas could also be branchial remnant cysts, which are reported in young and mature horses.[516]

Adenoma

Adenoma is the most common neoplasia of the equine thyroid.[463,494,515,517-522] It is an age-related phenomenon, occurring mainly in horses older than 16 years. It is benign, usually unilateral, and is not associated with thyroid dysfunction. Occasionally its size may warrant surgical excision. Prognosis is good. A thyroid adenoma associated with hyperthyroidism was diagnosed in a 23-year-old gelding.[463] In this case, the diagnosis was aided by percutaneous biopsy and the use of the T_3 suppression test, in which T_4 concentration did not suppress after the IM administration of T_3. Hemithyroidectomy of the affected gland restored TH concentrations. Immunohistochemistry of equine benign thyroid tumors in old horses revealed that adenomas are often misclassified as follicular when in fact many of them originate from the C cells.[523]

Adenocarcinoma

Malignant neoplasia of the thyroid occurs less frequently. In reported cases of thyroid adenocarcinomas, horses have been euthyroid, hypothyroid, and hyperthyroid.[464,466-469,524] One case of thyroid carcinoma with systemic metastasis and concurrent pituitary adenoma was reported.[525]

Medullary Carcinoma

Medullary carcinomas (C-cell or parafollicular cell tumors) have been documented in horses.[340,526,527] In most cases, tumors are unilateral. Benign C-cell adenomas are common in old horses with no evidence of disease or endocrine dysfunction.[523,528] In one horse, a C-cell adenoma associated with multiple endocrine neoplasia was documented at necropsy.[526]

C-cell hyperplasia has been described in horses as young as 3 years of age.[518] Depending on mass size, surgical removal could be recommended to alleviate clinical signs.[340] In other cases, esthetics is the main reason for surgery. Immunohistochemistry or electron microscopy may be necessary to distinguish C-cell tumors from other thyroid neoplasias.[523]

Multiple Endocrine Neoplasia

Multiple endocrine neoplasia (MEN) is a human syndrome characterized by multiple neoplasia of glands with a neuroendocrine origin. One case of a 22-year-old Thoroughbred mare with a C-cell adenoma, a pheochromocytoma, and multicentric bilateral nodular hyperplasia of the adrenal medulla was reported.[526] A retrospective evaluation of endocrine tumors in horses that underwent necropsy suggested that hyperplasia and neoplasia of the thyroid and adrenal glands, as occur in humans with MEN, also occur in the horse.[525,526]

Treatment

In circumstances that warrant surgical excision the prognosis is best when the condition is unilateral. Potential surgical complications include infection, hemorrhage, and laryngeal hemiplegia. As the equine parathyroid glands in general are not connected to the thyroid gland, hypocalcemia is unlikely to be a complication.

Hypothalamic-Pituitary-Adrenal Axis, Steroids, and Neuroactive Steroids

Katarzyna A. Dembek and Ramiro E. Toribio

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS PHYSIOLOGY

The hypothalamic-pituitary-adrenal axis (HPAA) represents a complex regulatory system that modulates multiple homeostatic systems (metabolism, pain, hunger, thirst, blood pressure, electrolyte balance, autonomic activity, immunity, and sexual behavior), as well as the stress response to internal and external stimuli, to ensure organ function and survival. Major advances have been made in understanding HPAA physiology and pathophysiology in people and animals with various acute and chronic disorders.

Hypothalamus

The hypothalamus is located in the ventral diencephalon and below the thalamus. It is part of the limbic system (with the hippocampus, amygdala, thalamic nuclei, mammillary body, limbic cortex, and other structures) and mediates autonomic, endocrine, and behavioral functions. It is the master regulator of numerous physiologic processes.[529,530] It generates and receives humoral and neural information; coordinates different levels of autonomic communication between the CNS and central and peripheral organs; and regulates body temperature, hunger, thirst, circadian and circannual rhythmicity, sleep, reproductive drive, sexual dimorphism, growth, behavior, metabolic activity, and other essential functions.[529-531] The hypothalamus connects via neural circuits to other structures of the CNS but also sends and receives humoral information. A good example of hypothalamic humoral connection is with the pituitary gland via the portal system in the median eminence.[529,530]

Histologically, the hypothalamus is arranged in a series of nuclei with magnocellular and parvocellular neurons that secrete releasing (e.g., corticotropin-releasing hormone [CRH], thyrotropin-releasing hormone [TRH], growth hormone–releasing hormone [GHRH], gonadotropin-releasing hormone [GnRH]) and inhibitory (e.g., somatostatin, dopamine) factors into the pituitary portal system to control hormone synthesis and secretion by the adenohypophysis (e.g., adrenocorticotropic hormone [ACTH], growth hormone [GH], thyroid-stimulating hormone [TSH], prolactin).[532,533] The hypothalamus also produces peptides that are stored in the neurohypophysis (e.g., oxytocin, vasopressin), as well as factors that modulate melanotrope activity in the pituitary pars intermedia (e.g., dopamine, serotonin, TRH). The hypothalamus is responsive to peripheral hormones (leptin, ghrelin, insulin, steroids, thyroid hormones), metabolites (glucose), electrolytes (sodium), neural stimuli (light, smell, touch, vascular pressure, pheromones, temperature, input from the thorax, gastrointestinal tract, and reproductive tract), cytokines, and microbial products. The level at which hypothalamic neurons are stimulated or suppressed is dictated by set-points specific to each function (e.g., thermoregulation, thirst, osmolality, blood pressure).[532,533]

Peptides and large molecules are sensed or gain access to hypothalamic neurons through circumventricular organs, including the subfornical organ and the organum vasculosum of the lamina terminalis. These structures have a fenestrated endothelium and lack an effective blood-brain barrier allowing the hypothalamus to continuously sample systemic circulation and relay information to different parts of the CNS and are essential for feedback regulatory mechanisms that modulate neuroendocrine function.[533]

Hypothalamic-Pituitary-Adrenal Axis Dynamics

CRH, ACTH, and cortisol are secreted in a circadian fashion mediated by inputs from the pineal gland and the suprachiasmatic nucleus of the hypothalamus, with the highest concentrations observed in the morning and the lowest in the evening. Interestingly, circadian rhythm in cortisol secretion was reported in adult horses but not in neonatal foals (less than 5 days old).[534] The mean 24-hour cortisol concentration was lower in foals compared with adult horses; however, the daily endogenous cortisol production was higher in foals than in horses.[534] ACTH is released in irregular (time and amplitude) peaks that occur about 10 times per hour in the horse.[535] Seasonal variations in ACTH and cortisol secretion in fall and winter months have been documented in horses.[536-540] Therefore seasonally specific reference ranges are required to properly assess ACTH concentrations.[536-540]

Under stress or physiologic needs, the hypothalamus secretes CRH (CRF, corticoliberin, 41 amino acids [41aa]) and arginine vasopressin (AVP, antidiuretic hormone, 9aa). CRH is synthesized by parvocellular neurons located in the paraventricular nucleus (PVN).[529,532,533] In response to stress, CRH is released in the portal system of the median eminence by neurosecretory terminals of these neurons and acts on the pituitary corticotropes via the CRH-1 receptor (CRH-1R) to elicit the secretion of ACTH (39aa) and other proopiomelanocortin (POMC)-derived peptides, including β-endorphin (β-END)

and α-melanocyte–stimulating hormone (α-MSH).[529,532,533] Factors that modulate CRH secretion include brain-derived neurotropic factor (BDNF), cytokines, lipopolysaccharides (LPS), glucocorticoids, neurosteroids, nitric oxide, glutamate (via NMDAR), and γ-aminobutyric acid [GABA] (through $GABA_A$ receptors).[541-544] BDNF is a key stimulatory factor for CRH secretion.[542] AVP is synthesized by magnocellular neurons (AVP-ergic) located in the PVN and supraoptic nucleus (SON). These neurons release AVP in the portal system to stimulate the corticotropes to release ACTH, via the AVP receptor 3 (AVPR3, V1bR).[545,546] Factors that regulate AVP secretion include BDNF, LPS, cytokines, nitric oxide, angiotensin-II, and hypocortisolemia.[547-551] The regulation of AVP as it relates to the HPAA is different than AVP stored in neurosecretory terminals (Herring bodies) of the neurohypophysis where its secretion is controlled by osmolality and blood pressure.[541,546]

In humans and rats, CRH and AVP colocalize to the same parvocellular neurons[552,553]; however, data on their hypothalamic cellular distribution in the horse is minimal.[549] The relative importance of CRH and AVP to stimulate the secretion of ACTH is species dependent.[554] An in vitro study on the dynamics of ACTH secretion by equine anterior pituitary cells showed that in horses, CRH is a major pituitary ACTH secretagogue.[555] Another study in adult horses found that endotoxemia stimulates both CRH and AVP secretion and concluded that AVP appears to be more important than CRH in stimulating ACTH release during illness.[556] More recently, it has been shown that CRH and AVP are synergistic in evoking ACTH in horses, particularly during hypocortisolemia.[549] The HPAA is also regulated by neuroactive steroids via GABAergic signals.[544,557] Thus stress-derived neurosteroids and centrally generated steroids may alter the HPAA as well as other hypothalamic functions.[544,554,558,559]

The periventricular nucleus (not to be confused with paraventricular nucleus) is a diffuse group of hypothalamic neurons responsible for somatostatin, TRH, gastrin, and dopamine secretion. Periventricular TRH-ergic neurons increase and dopaminergic neurons suppress proliferation and hormone secretion by melanotropes in the pituitary pars intermedia. Dysfunction of these dopaminergic neurons in geriatric horses has been linked to the development of pituitary pars intermedia dysfunction (PPID, Cushing disease), resulting in excessive and unregulated secretion of POMC-derived peptides, including ACTH and α-MSH.[560]

Pituitary Gland (Hypophysis)

The pituitary gland is located in the sella turcica, a depression within the sphenoid bone, and connected to the hypothalamus through the infundibular stalk.[561] Histologically, the pituitary gland consist of an anterior lobe (adenohypophysis), an intermediate lobe (pars intermedia), and a posterior lobe (pars nervosa, neurohypophysis).[561,562] The anterior lobe is further divided into a pars distalis that contains most cells and a pars tuberalis, which is the proximal part of the anterior lobe surrounding the infundibulum closer to the median eminence (ventral hypothalamus). In equids, the distinction between anterior, intermediate, and posterior lobes does not reflect pituitary anatomy described for other species because the equine anterior lobe wraps the other two lobes. Therefore *pars distalis, pars intermedia,* and *pars nervosa* are better terms.[530]

Stimulatory and inhibitory factors released in the median eminence are transported to the pars distalis through the hypophyseal portal system. The proximal or primary plexus is located in the median eminence. From here, factors are transported via the hypophyseal portal vein to the distal or secondary plexus to reach target cells in the pars distalis.

Cells in the pars distalis include somatotropes, gonadotropes, lactotropes, thyrotropes, and corticotropes. Based on staining properties, these cells are chromophils and chromophobes. Chromophils include acidophils (somatotropes, lactotropes) and basophils (gonadotropes, thyrotropes, and corticotropes).[561] The somatotropes (somatotrophs) produce growth hormone (GH), lactotropes (lactotrophs, mammotrophs) produce prolactin, gonadotropes (gonadotrophs) produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thyrotropes (thyrotrophs) produce thyroid-stimulating hormone (TSH), and corticotropes (corticotrophs) produce adrenocorticotropic hormone (adrenocorticotropin, corticotropin, ACTH).[561,562]

The pars intermedia forms the junction between the pars distalis and neurohypophysis. The melanotrope (melanotroph) is the main cell in the pars intermedia, and after cleavage of POMC the main products include α-MSH, ACTH, β-lipotropin, β-endorphin (β-END), and corticotropin-like intermediate peptide (CLIP).[531,554,560,561] The pars intermedia is under the control of hypothalamic neurons mainly through dopaminergic inhibition.

The pars nervosa consists of axons from magnocellular neurons extending from the SON and PVN. These neurons store and release AVP and oxytocin.[530,561] AVP is released in response to hypotension and hyperosmolality. Oxytocin secretion into systemic circulation is affected by a number of tactile, stretch, and visual stimuli.

For the purpose of this section, we will focus on the corticotropes as they are responsible for the synthesis and secretion of ACTH. In the pars distalis, ACTH is the main POMC-derived peptide whereas in the pars intermedia α-MSH is the main POMC product. ACTH stimulates the adrenal gland cortex to secrete cortisol and to a lesser extent other adrenocortical layers to release other steroids.[563-567] In addition to AVP and CRH, the synthesis and secretion of ACTH is regulated by cytokines, LPS, TRH, glucocorticoids, and nitric oxide.[568,569]

Adrenal Gland

In horses, the adrenal glands lie retroperitoneally and embedded in the fat on the medial cranial pole of each kidney. They are red-brown, 7.0–8.0 × 3.0–3.5 cm, and weigh 15–20 g each. Blood supply to the adrenal gland is from the adrenal artery, which arises from either the aorta or the renal artery.[529-531] Innervation involves sympathetic fibers from the splanchnic nerve.[570] Accessory adrenal cortical tissue may be found in the adrenal gland capsule, periadrenal or perirenal adipose tissue, mesorchium, and in the vicinity of the equine testis.[570] The adrenal glands develop from two separate embryologic tissues: the medulla is derived from neural crest cells originating in proximity to the dorsal aorta, and the cortex develops from the intermediate mesoderm (urogenital ridge).[571]

The medulla is functionally related to the sympathetic nervous system and consists of chromaffin cells that secrete catecholamines (epinephrine, norepinephrine, and dopamine) in response to sympathetic stimulation.[529,531] The chromaffin cells are considered modified postganglionic neurons directly controlled by preganglionic fibers of the autonomic nervous system. Epinephrine (adrenaline) is the main adrenomedullary hormone.

The adrenal cortex derives from the mesoderm and secretes different types of steroids, which are synthesized from cholesterol. The adrenal gland cortex consists of three zones: the outermost *zona glomerulosa,* which secretes mineralocorticoids (aldosterone) in response to the renin-angiotensin-aldosterone system (angiotensin-II in particular), hyperkalemia, and changes in extracellular fluid osmolality (sodium, chloride); the *zona fasciculata,* which secretes glucocorticoids (cortisol) in response to ACTH stimulation; and the *zona reticularis,* which produces sex steroids, mostly androgens.[434,530,570]

Adrenocortical function (mainly glucocorticoid synthesis) is controlled by the HPAA. As previously mentioned, the hypothalamus secretes CRH and AVP to stimulate pituitary ACTH secretion, which in turn stimulates the adrenal cortex to secrete cortisol and to a lesser extent aldosterone and other steroids.[434,529] Subsequently, cortisol inhibits CRH, AVP, and ACTH secretion as part of a negative feedback control mechanism.[434,529]

The adrenal gland cortex is involved in a wide variety of body functions, including maintenance of fluid and electrolyte balance, immunity, defense, and energy metabolism. The adrenal gland also secretes steroids with neuroactive activity, as well as steroids that could be used as precursors in other organs. Removal or destruction of the adrenal glands leads to death unless exogenous adrenocortical hormones are administered.[529,570] Aldosterone increases renal sodium reabsorption and excretion of potassium. Cortisol increases gluconeogenesis, causes mobilization of amino acids from extrahepatic tissues to enter the gluconeogenesis, decreases glucose utilization, increases glucose concentrations, and inhibits insulin actions on glucose uptake and lipogenesis. Cortisol increases fat and amino acid mobilization to make them available during stress.[529] Endogenous glucocorticoids are antiinflammatory and immunosuppressors; hypercortisolemia results in eosinopenia and lymphopenia. Cortisol is also essential for organ maturation and differentiation (lung, gastrointestinal, endocrine), a critical step in the developing fetus and newborn.[529,531]

Function of the Fetal Adrenal Gland

Compelling evidence shows that rapid maturation of the HPAA in foals takes place in the last 5 days of gestation and continues into the first weeks of life, much later than described in other species.[572,573] Cortisol synthesis depends on three key enzymes: cholesterol side chain cleavage (cytochrome P450-scc; CYP11A1), 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17α-hydroxylase (P450-17; CYP17A1). Cytochrome P450-scc is expressed in the fetal adrenal cortex from as early as 150 days of gestation, and 3β-HSD that synthesizes progesterone from pregnenolone is present from about 280 days of gestation.[573] However, P450-17, which is required for cortisol production, is expressed 5 days before birth.[572,573] Therefore premature foals (gestational age less than 320 days) have lower basal concentrations of cortisol with higher endogenous ACTH than full-term foals after birth, in part due to the lack of negative feedback. Moreover, these foals show a blunted cortisol response to ACTH stimulation compared with normal foals.[574]

Interestingly, all three enzymes are present at birth and show no change in their expression in the perinatal period. An increased response to exogenous ACTH has been described in late gestation and birth with a positive correlation between the cortisol response of a neonatal foal and its gestational age at delivery. This suggests that other factors such as ACTH receptor (MC2R) density, ACTH clearance, and adrenal blood flow may be involved in the cortisol response to ACTH in newborn foals.[572,573]

Steroidogenesis

Adrenal steroids are synthesized from cholesterol through initial steps similar to other steroidogenic organs (testes, ovaries) (Fig. 16.16). Adrenocortical cells store small amounts of steroids; therefore a fast steroid response requires a highly coordinated enzymatic machinery for rapid hormone synthesis and secretion. ACTH regulates steroidogenesis by acute and chronic effects on the adrenal gland. ACTH binds to the G protein–coupled melanocortin type 2 receptor (MC2R) to stimulate steroidogenic acute regulatory protein (StAR) transcription and translation.[575,576] StAR increases cholesterol transfer from the outer to the inner mitochondrial membrane, where it is converted to pregnenolone in the first and rate-limiting steroidogenic step.[575,576] ACTH also increases cholesterol uptake from circulation. In fact, most cholesterol in the adrenal cortex comes from outside the gland, although this remains to be documented in horses, particularly because the equine adrenal cortex is rich in lipids. Chronically, ACTH promotes the transcription of genes encoding various steroidogenic enzymes, including cytochrome P450 and hydroxysteroid dehydrogenases. Moreover, ACTH stimulates the synthesis of basic fibroblast growth factor, epidermal growth factor, and insulin-like growth factor II (IGF-II), and these mediators stimulate adrenocortical hyperplasia and hypertrophy.[575,576]

Pregnenolone is transported to the endoplasmic reticulum to be converted to progesterone and 17α-hydroxyprogesterone, which in the zona glomerulosa and fasciculata become aldosterone or cortisol, respectively. Androgens such as dehydroepiandrosterone (DHEA), androstenedione, and

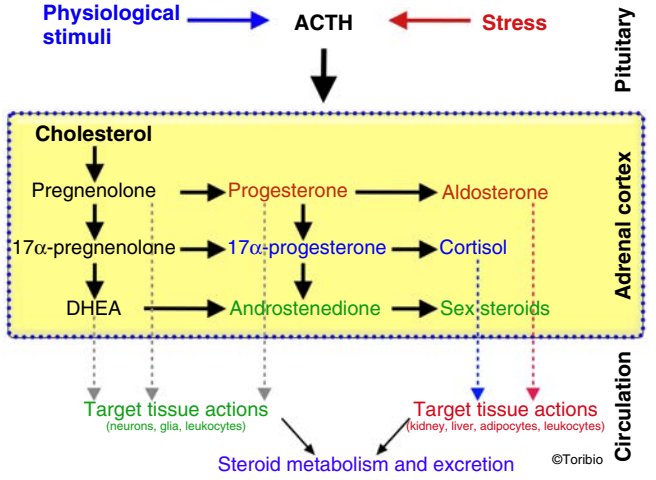


FIG. **16.16** Adrenocortical steroidogenesis. In the adrenal cortex, steroids are synthesized from cholesterol resulting in progestogens (pregnenolone, progesterone), glucocorticoids (cortisol), mineralocorticoids (aldosterone), and androgens (DHEA, androstenedione). The main products of the adrenal cortex are cortisol and aldosterone. Steroid precursors are also released into systemic circulation to exert multiple functions but are also used in other organs (e.g., nervous system) as precursors for other steroids (e.g., neurosteroids). *ACTH,* Adrenocorticotropic hormone; *DHEA,* dehydroepiandrosterone. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

epitestosterone are synthesized from 17α-hydroxyprogesterone and 17α-hydroxypregnenolone in the zona reticularis. A fraction of progestogens are released into systemic circulation to exert specific actions or are used as precursors for other steroids.

In humans, there are several genetic disorders in early steroidogenesis causing primary adrenal insufficiency such as adrenoleukodystrophy or Wolman disease (cholesterol ester storage disease).[575,576] Equivalent disorders have not been described in horses.

In the brain, progesterone, DHEA, and pregnenolone can be converted into neuroactive steroids (e.g., allopregnanolone, allotetrahydrodeoxycorticosterone [THDOC]) to promote neurogenesis, neuronal plasticity, and neuroprotection (Fig. 16.17)[577] and modulate BDNF, CRH, and AVP synthesis and secretion,[558] thus directly and indirectly influencing the HPAA. Allopregnanolone is a potent neurosteroid with a multitude of actions, including modulation of the HPAA and protection against seizures and neuronal death.[558] In humans, blood concentrations of these steroids are being used to assess adrenal gland function,[563,565-567,578,579] as well as neurologic conditions such as bipolar disorders, depression, and Alzheimer's disease. (See neurosteroid section later in this chapter.)

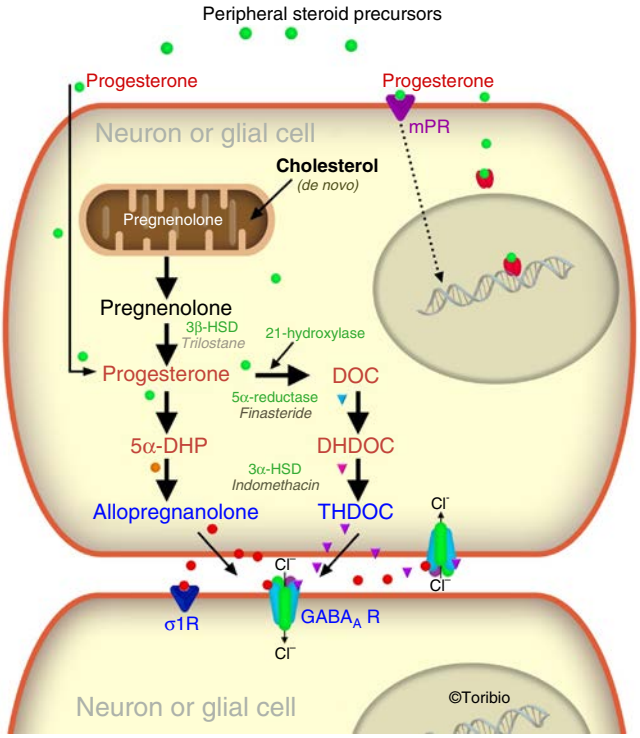


FIG. **16.17** Synthesis of neuroactive steroids by neurons and glial cells in the central nervous system. Progesterone and other steroid precursors from peripheral circulation can be taken by neurons and astrocytes to be converted into neurosteroids. These cells also have the enzymatic machinery for de novo synthesis of neuroactive steroids from cholesterol. Neuroactive steroids mainly function through the cell membrane $GABA_A$ receptor, but other receptors exist. Progesterone acts through traditional nuclear receptors as well as through cell membrane progesterone receptors (mPRs). Trilostane, finasteride, and indomethacin can inhibit specific enzymes necessary for steroidogenesis. *3α-HSD,* 3α-hydroxysteroid dehydrogenase; *3β-HSD,* 3β-hydroxysteroid dehydrogenase; *5α-DHP,* 5α-dihydroprogesterone; *DHDOC,* dehydrodeoxycorticosterone; *DOC,* deoxycorticosterone; *THDOC,* allotetrahydrodeoxycorticosterone; *$GABA_AR$,* γ-aminobutyric acid (GABA) type A receptor; *mPR,* membrane progesterone receptor; *σR,* sigma-1 receptor. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

Sepsis and the Hypothalamic-Pituitary-Adrenal Axis

Inflammation, cytokines (TNF-α, IL1-β), and LPS activate the HPAA in humans and animals.[568,580,581] In other species, inflammatory cytokines increase CRH, AVP, and ACTH mRNA expression and secretion.[582] CRH, AVP, ACTH, and cortisol concentrations are also increased in experimental models of endotoxemia.[545,556,568,580] The LPS effects on CRH mRNA expression and ACTH secretion are in part mediated by nitric oxide.[583] IL1-β and TNF-α can stimulate or suppress cortisol secretion, depending on the duration of exposure.[584] Although ACTH concentrations increase in foals with systemic inflammation,[585] the prevalence of ACTH hyposecretion in the context of HPAA dysfunction is low. Similarly, the role of hypothalamic dysregulation (decreased AVP and CRH) in the development of HPAA dysfunction is minimal compared with adrenocortical failure because most critically ill foals have high AVP[585] and CRH concentrations. Critical illness in newborn foals independent of blood pressure or osmolality can stimulate AVP secretion.[585] In humans, sepsis can lead to irreversible damage to the hypothalamus and pituitary gland.[586] Pituitary necrosis is a well-documented phenomenon in septic human patients that could lead to multiple endocrine failure (GH, ACTH, AVP, thyroid hormones).[586] A similar process may occur in foals with severe sepsis and multiorgan failure.

Adrenal Insufficiency

Adrenal insufficiency (AI) in general terms is defined as a reduced capacity of the adrenal gland to secrete adrenocortical steroids in sufficient amounts to meet physiologic needs, thus resulting in clinical and laboratory disturbances. The primary problem leading to AI could be at any level of the HPAA, but it is ultimately steroid deficiency that causes the signs of this condition that has been documented in foals and horses. It should be noted that the pathogenesis of AI in noncritically ill horses (chronic AI) is different from the AI of acute illness in horses and foals.

Types and Definitions of Adrenal Insufficiency

- *Irreversible adrenal insufficiency* or hypoadrenalism (Addison disease) results from failure of the adrenal gland to produce adrenocortical hormones (mineralocorticoids and glucocorticoids).[531]
- *Relative adrenal insufficiency (RAI)* is a transient form of AI that often occurs in critically ill people, neonatal foals, and horses. RAI is defined as an inadequate production of cortisol in relation to an increased demand during periods of severe illness and stress, and it is characterized by high ACTH/cortisol ratios.[434,531,587]
- *Critical illness–related corticosteroid insufficiency (CIRCI)* is a term promoted in recent years instead of RAI because it takes into consideration clinical findings, an exaggerated proinflammatory response from cortisol deficiency, and tissue refractoriness to corticosteroids.[531,588]

- *Iatrogenic AI,* caused by chronic glucocorticoid administration leading to suppression of the HPAA, is the most common type of AI in people and adult horses.

Adrenal insufficiency can be also classified based on the level of the HPAA that is affected.

- *Primary AI:* caused by damage or dysfunction of the adrenal gland cortex.
- *Secondary AI:* caused by impaired ACTH secretion by the pituitary gland.
- *Tertiary AI:* caused by reduced CRH and/or AVP secretion from the hypothalamus into the median eminence.

Adrenal Insufficiency in Horses

In horses, AI is also known as adrenal exhaustion, hypoadrenocorticism, turn-out, or steroid letdown syndrome, which is characterized by low cortisol concentrations and a poor response to the ACTH stimulation test. Adrenal insufficiency also occurs in critically ill horses (colic, enterocolitis, endotoxemia, sepsis) and septic foals, in part because the adrenal gland is a shock organ in equids (adrenal hemorrhage, thrombosis, and cortical necrosis are common in sick equids). Animals that recover could potentially develop adrenal atrophy and dysfunction.[531,589,590]

In adult horses, AI has been associated with the chronic administration or discontinuation of glucocorticoids or anabolic steroids.[591] The adrenal gland response to exogenous ACTH in Thoroughbred horses was not altered by training,[592] and cortisol concentrations were within normal limits in racehorses with a history of poor performance.[593] High plasma cortisol and ACTH concentrations were found in horses after maximal exercise.[594-597] Endurance horses in a 160-km ride had significantly higher midride plasma cortisol concentrations than preride concentrations.[598] A sixfold increase in cortisol secretion rate and a threefold increase in plasma cortisol concentrations was also found in exercising horses.[599] Increased total serum cortisol concentrations were reported in horses with colic[435] and lunged Royal Dutch Sport horses with altered head-and-neck position.[600] These findings suggest that the main reason for noncritically ill horses to develop AI is iatrogenic. We want to emphasize that this type of AI has a different pathogenesis from AI from critical illness.

Endogenous and exogenous glucocorticoids suppress the HPAA, and chronic exposure can induce atrophy of the zona fasciculata. The zona glomerulosa may be morphologically minimally affected, but electrolyte abnormalities are frequent. This indicates that AI in horses can involve multiple adrenocortical layers.

Horses suspected of having AI present with a history of depression, anorexia, exercise intolerance, weight loss, poor haircoat, lameness, abdominal discomfort, and seizures.[531,590,591,601-603] Therefore it is important to obtain a complete history, including performance, previous diseases, drug administration, and stressing conditions. Serum biochemical analysis may be normal, or hyponatremia, hypochloremia, hyperkalemia, and hypoglycemia may be present.[531,590,591,601-603]

Some of the clinical features of equine hypoadrenocorticism (hyponatremia and hyperkalemia without evidence of renal disease) resemble Addison disease in humans and small animals although Addison disease has not been documented in the horse. One case of an autoimmune polyendocrine syndrome with insulin-dependent diabetes mellitus and clinical signs similar to Addison disease was described in a 5-year-old Thoroughbred-cross mare.[604] Clinical abnormalities included lethargy, poor appetite, weight loss, polyuria, polydipsia, persistent hyperglycemia, hyponatremia, hypochloremia, and hyperkalemia. The mare was diagnosed postmortem with lymphocytic pancreatitis and thyroiditis, as well as AI secondary to lymphocytic adrenalitis.[604]

Adrenal insufficiency is rarely diagnosed in equine practice. However, it should be suspected in any horse with a history of anorexia, lethargy, poor body condition, poor exercise performance, history of glucocorticoid administration, electrolyte imbalances (hyponatremia, hypochloremia, hyperkalemia), and hypoglycemia.[591,604] Baseline cortisol and ACTH concentrations, as well as the ACTH stimulation test, are critical to achieve a diagnosis. Cortisol has daily fluctuations, and single measurements may not provide enough information to make a diagnosis of hypoadrenocorticism. Therefore the ACTH stimulation test is more useful in assessing adrenal gland function.[601,603,605] Horses with AI have low cortisol concentrations and do not respond or have a minimal response to the ACTH stimulation test.[601,606] In addition, measuring ACTH concentrations is important to determine other causes of hypoadrenocorticism (iatrogenic, adrenal, pituitary). It is anticipated that exogenous glucocorticoids will decrease ACTH concentrations (secondary hypoadrenocorticism), whereas adrenocortical dysfunction (primary hypoadrenocorticism) will be associated with increased ACTH concentrations from reduced glucocorticoid negative feedback. In horses with increased plasma ACTH concentrations, PPID should be ruled out.

In the ACTH stimulation test, 1 IU/kg of natural ACTH gel is administered intramuscularly.[606] For cortisol measurements, take a pre-ACTH blood sample in heparinized or plain tubes, and administer ACTH between 8:00 and 10:00 AM. Take post-ACTH blood samples 2 and 4 hours after ACTH administration. Horses with a functional adrenal gland should have twofold to threefold increases in plasma cortisol concentrations compared with baseline values.[602,606] In the synthetic ACTH ($ACTH_{1-24}$, cosyntropin, tetracosactide, tetracosactrin) stimulation test, 100 IU (1 mg) of cosyntropin (Cortrosyn®) is administered intravenously between 8:00 AM and noon.[607] Take a pre-ACTH blood sample, administer cosyntropin, and take a post-ACTH blood sample 2 hours later. Plasma cortisol concentrations should be at least twice baseline. Cosyntropin (25 IU; 250 µg) can increase cortisol concentrations twice baseline in horses. More recently, a dose of 0.1 µg/kg of cosyntropin was reported to induce maximal adrenal stimulation 30 minutes after administration in healthy horses.[605] Depending on disease duration and severity, necropsy findings in horses with hypoadrenocorticism vary from adrenocortical hemorrhages and necrosis to adrenocortical atrophy and fibrosis.

Treatment of hypoadrenocorticism involves rest, correction of serious clinical and laboratory abnormalities, and glucocorticoid replacement therapy. The duration and dose of exogenous steroids that induce adrenocortical atrophy in healthy horses are unknown. However, there is information on doses that inhibit adrenocortical secretion in horses. Dexamethasone doses as low as 4 mg suppressed the HPAA for up to 24 hours.[602] Dexamethasone (0.044–0.088 mg/kg) every 5 days for six treatments reduced cortisol concentrations for up to 4 days but did not change the response to ACTH stimulation.[608] Toutain and colleagues[609] found that dexamethasone (50 µg/kg IV or IM) suppressed adrenocortical function for up to 4 days, prednisolone sodium succinate (0.6 mg/kg IV or IM) for less than 24 hours, and prednisolone acetate

suspension (0.6 mg/kg IM) for up to 21 days. Dexamethasone (0.044 mg/kg IM) and triamcinolone (0.044 mg/kg IM) produced HPAA suppression for 7 and 14 days, respectively.[610,611] Plasma cortisol concentrations remained below baseline for 4, 10, and 15 days after intravenous, intraarticular, and intramuscular injection of triamcinolone acetonide (0.04 mg/kg), respectively.[612] A single intraarticular dose of methylprednisolone acetate (200 mg) suppressed adrenocortical function for up to 10 days.[613,614] A single intraarticular dose of betamethasone (30 mg) suppressed plasma cortisol concentrations for 3 days in Thoroughbred horses.[615] Data on the use of parenteral hydrocortisone (cortisol) in horses to treat hypoadrenocorticism are lacking; however, due to its rapid elimination in horses and foals (less than 24 hours),[534] it could be a good short-term therapeutic alternative. In fact, hydrocortisone is the recommended glucocorticoid for critically foals with AI.[616] Based on this information (half-life, adrenocortical suppression), when treating hypoadrenocorticism, hydrocortisone or prednisolone sodium succinate should be the first choice, low-dose dexamethasone the second option, and triamcinolone acetonide and prednisolone acetate should be avoided. Oral prednisolone (200–400 mg/horse) is a good option for prolonged use. Prednisone was successfully used to treat a horse with AI secondary to anabolic steroid administration and a foal with AI.[591,601] The efficacy of prednisone in horses is questionable because it is poorly absorbed in horses and the active metabolite, prednisolone, is minimally produced.[617] It is likely that animals treated with prednisone improved for reasons other than this drug.[617] This is further supported by the lack of cortisol suppression following prednisone compared with prednisolone administration.[617] In contrast, oral prednisolone has excellent bioavailability and may be more useful as a therapeutic agent in horses. As with any long-term glucocorticoid therapy, slow withdrawal is recommended.

Long-term glucocorticoid therapy is often recommended in the management of recurrent airway obstruction (RAO, equine asthma). Inhaled beclomethasone dipropionate (500 µg - 1500 µg q12h) suppressed endogenous cortisol secretion in a dose-dependent manner for 1 to 4 days after administration in horses with RAO.[618,619] Similarly, inhaled fluticasone propionate (2 mg once a day to 3 mg twice a day) was reported to reduce cortisol concentrations for 8 to 24 hours after treatment in horses with RAO.[620] Despite suppressed adrenocortical function, no clinical signs of AI or adverse systemic effects, such as laminitis or bacterial infections, occurred in treated horses.

The exaggerated proinflammatory response that occurs in foals and horses with systemic inflammatory response syndrome (SIRS) and severe sepsis can be in part attributed to adrenocortical failure and suppression of the HPAA. However, experimental and clinical data suggest that tissue resistance to glucocorticoids may be involved in HPAA dysregulation.[531,588,590] One study found that horses with SIRS that died had lower glucocorticoid binding affinity compared with survivors, suggesting that in addition to HPAA dysfunction, impaired glucocorticoid receptor activity contributes to their proinflammatory state.[590]

Adrenal Insufficiency in Foals

Our understanding of HPAA activity in healthy and sick foals has improved in recent years.[434,585,621,622] However, beyond cortisol and aldosterone, we are far from having an integrated view of the different endocrine factors released from the equine adrenal cortex, their functions, interactions, and metabolism. *Adrenal insufficiency* as a diagnosis in critically ill foals or horses is a broad term because the problem could be at any level of the HPAA. Therefore having the ability to measure key hypothalamic, pituitary, and adrenocortical factors could be valuable to narrow which component of the axis is affected. In septic foals, altered steroidogenesis seems to be the main reason for AI.[434] Another issue to consider is that single cortisol measurements may not reflect adrenal function, and comparing cortisol concentrations in proportion to ACTH concentrations may provide a better assessment of adrenocortical function. For this particular reason, the term *RAI/CIRCI* seems more appropriate because it incorporates two components of the HPAA, and the ratio between ACTH and cortisol will support a good or poor adrenocortical response. RAI/CIRCI occurs secondary to critical illness such as sepsis, severe trauma, major surgery, or acute respiratory distress syndrome (ARDS) in human patients.[587,588] Likewise, severe illness has been associated with RAI/CIRCI in equine neonates with sepsis, septic shock, and multiple organ dysfunction.[622-624]

Recent studies have shown that pituitary and adrenocortical dysfunction are frequent in critically ill foals and in many instances are linked to mortality.[434,585,622] Hart and colleagues found that 40% of septic foals had HPAA dysfunction.[622] Gold and colleagues determined the ACTH/cortisol ratio in septic foals and found that RAI was common, with nonsurviving foals having the highest ratio.[621] In a similar study, Hurcombe and colleagues assessed the AVP/ACTH and ACTH/cortisol ratios in septic foals and showed that HPAA dysfunction at the pituitary and adrenal level was frequent in these foals.[585] AVP is a major secretagogue for ACTH in the horse,[556] and a high AVP/ACTH ratio suggests a decreased ACTH response to AVP stimulation (pituitary insufficiency). Similarly, high ACTH/cortisol[585,621] and ACTH/aldosterone[434] ratios indicate decreased adrenal response to ACTH (RAI) in critically ill foals. The low cortisol concentrations observed in premature foals do not appear to be due to ACTH insufficiency as high ACTH concentrations are frequent in these foals,[625] supporting AI as a major problem in premature foals. Studies have found that both low and high cortisol concentrations were associated with nonsurvival in septic foals.[434,621,622]

Pathophysiology of Relative Adrenal Insufficiency and Critical Illness–Related Corticosteroid Insufficiency

The pathogenesis of RAI/CIRCI in horses and other species is unclear, but it is likely multifactorial and includes decreased production of CRH, ACTH, and cortisol, as well as complex interactions between the endocrine and immune systems.[531,626,627] The equine adrenal gland is a highly vascular organ; therefore it is susceptible to hemorrhages and necrosis, which are often documented in septic nonsurviving foals. Prolonged hypotension and hypoperfusion from sepsis and septic shock may lead to irreversible adrenocortical damage in critically ill foals, similar to the Waterhouse-Friderichsen syndrome described in humans.[531,588] Low cortisol concentrations in foals with AI are mainly the result of adrenocortical failure (primary AI) rather than dysfunction at the pituitary (secondary AI) or hypothalamic level (tertiary AI), which occurs less frequently. Several mechanisms can directly or indirectly lead to HPAA dysfunction, including (1) reduced CRH, AVP, and ACTH secretion; (2) decreased target tissue sensitivity to

CRH, AVP, or ACTH; (3) decreased concentrations of bioactive ACTH; (4) impaired adrenocortical steroidogenesis; (5) tissue resistance to cortisol; and (6) reduced cortisol metabolism leading to decreased ACTH secretion from the negative feedback resulting in hypocortisolemia.[531,628,629] Several drugs (e.g., ketoconazole and rifampin) are known to directly inhibit HPAA activity in critically ill humans and animals[588]; however, they are rarely used in newborn foals.

Inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α) and endotoxin (LPS) can directly stimulate the HPAA, increasing CRH, AVP, ACTH, and adrenal steroid concentrations.[568,588,626] This would be an appropriate response to the stress of systemic inflammation. However, prolonged exposure to proinflammatory cytokines and LPS can directly impair all levels of the HPAA.[568,588,626] Inappropriately low ACTH release due to increased TNF-α and IL-6 concentrations has been documented in people with severe sepsis and septic shock.[568,588] Interestingly, high ACTH concentrations were positively associated with IL-6 mRNA expression in peripheral leukocytes in septic foals.[630] High TNF-α and IL-6 concentrations were also associated with impaired glucocorticoid receptor binding in an ovine model of acute lung injury and in a rodent burn model. It is unknown, however, how a proinflammatory state affects glucocorticoid receptor expression and function in critically ill foals or horses.

Finally, hypocortisolemia may also result from substrate deficiency, as plasma high-density lipoprotein concentrations are often low in septic human patients.[631] Data on high-density lipoprotein and very-low-density lipoprotein (VLDL) levels in healthy and sick foals with RAI are lacking.

The concept of RAI was recently challenged in a study because critically ill human patients often had hypercortisolemia and reduced cortisol clearance that was attributed to decreased expression and activity of 11β-hydroxysteroid dehydrogenase type 2 (kidney) and 5β/5α-reductase (liver).[628] These patients also had lower ACTH concentrations that according to the authors were a consequence of cortisol-mediated ACTH suppression. Therefore *ACTH to cortisol dissociation (ACD)* has been introduced as a more appropriate term to describe HPAA dysfunction in people. Prolonged ACTH suppression could have adverse effects on the integrity of the HPAA that ultimately will result in AI.[628,632,633] These observations are in contrast to critically ill newborn foals with RAI that often have increased ACTH and low cortisol concentrations with increased ACTH/cortisol ratios.[434,585,621] For this reason *ACTH to cortisol imbalance (ACI)* is suggested as a more appropriate term to describe foals with RAI.[633a] This is a good example in which one must be cautious in extrapolating information from other species. Nevertheless, impaired metabolism of adrenocortical steroids might partially explain HPAA dysfunction in critically ill equine neonates.

Physiologically, about 90% of cortisol and 95% of progestogens and androgens are transported in the circulation by albumin, corticosteroid-binding globulin (CBG; transcortin), and sex hormone–binding globulin (SHBG). In neonatal foals, a significantly larger amount of circulating cortisol (30%–60%) is unbound to CBG.[6] Unbound or "free" steroids are biologically active and therefore more clinically relevant. Clinical studies in septic human patients have shown better correlation between basal and ACTH-stimulated free cortisol concentrations with disease severity and outcome than with total cortisol.[588,634] Of interest, Hart and colleagues found that baseline or ACTH-stimulated free cortisol concentrations were no better than total cortisol to diagnose CIRCI or predict disease severity and mortality in septic foals.[635]

Diagnosis

The clinical relevance of CIRCI, as well as diagnostic criteria to diagnose CIRCI, has become a controversial issue in human and veterinary critical care medicine due to conflicting data from clinical studies. Methods to diagnose RAI/CIRCI include baseline cortisol concentrations, cortisol and ACTH measurements after stimulation testing with synthetic ACTH (cosyntropin), insulin-induced hypoglycemia, and metyrapone test.[531,588] There are commercial methods to measure cortisol and ACTH concentrations in horses. Data are limited on the use of insulin-induced hypoglycemia and metyrapone test to assess HPAA activity in foals or horses.

In healthy animals and people, cortisol concentrations exhibit circadian and circannual rhythms. In addition, environmental factors, drugs, and diseases influence cortisol concentrations. Glucocorticoid concentrations during sepsis vary significantly over the time course of the illness, being highest in the early stage of sepsis, with a gradual decline in the later phases.[626] Therefore a basal cortisol concentration can sometimes be difficult to interpret and does not provide an accurate diagnosis of the HPAA dysfunction in critically ill humans and foals.[531,588] The most commonly used dynamic test for HPAA integration is the ACTH stimulation test, which permits assessment of adrenal responsiveness to exogenous synthetic ACTH (cosyntropin). Cosyntropin is traditionally used in critically ill people at high (250 μg) and low (1 μg) doses.[588] Blood samples are collected at baseline and at 30 or 60 minutes after stimulation. There are a number of definitions of CIRCI, but the most widely studied diagnostic criteria in humans are as follows: a baseline cortisol concentration less than 10 μg/dL, an increase after cosyntropin stimulation of less than 9 μg/dL (delta cortisol), or a blunted cortisol response to 1 μg of ACTH.[588,636] However, the lack of sensitivity and poor reproducibility of the ACTH stimulation test to define CIRCI limit its clinical use in critically ill people. Therefore current guidelines recommend treating pressor-resistant septic shock patients with hydrocortisone without testing the HPAA function.[587] The latest 2012 Surviving Sepsis Campaign guidelines do not recommend using the ACTH stimulation test to determine which patients should be treated with cortisol replacement therapy.[587] Interestingly, Boonen and colleagues have proposed treating critically ill patients for adrenal failure if they demonstrated clinical signs of unexplained vasoplegia, overwhelming inflammation, or coma for more than 6 days with basal plasma cortisol of less than 6 μg/dL and with an incremental cortisol response to an ACTH stimulation test (250 μg) of less than 6 μg/dL.[633] Similar criteria have not been investigated in critically ill foals.

Similar to human patients, specific criteria for CIRCI in critically ill foals are not very well established. Baseline cortisol concentrations, a cosyntropin test, and ACTH/cortisol ratio have been studied in healthy and septic foals.[585,621,622,637-639] Basal cortisol concentrations change over time in neonatal foals. Therefore age-related variation need to be considered when assessing HPAA function. Hart and colleagues reported basal cortisol concentrations in healthy foals of less than 13.5 μg/dL at birth, less than 6.7 μg/dL at 12 to 24 hours, less than 4.4 μg/dL at 36 to 48 hours, and less than 2.7 μg/dL at 5 to 7 days of age.[534] Interpretation of a single random cortisol concentration can be difficult because of individual variability and

changes during time of day and season. Therefore measurement of cortisol and other steroid concentrations in conjunction with ACTH provides more adequate assessment of the HPAA integrity. Documentation of high ACTH/cortisol,[585] as well as ACTH/aldosterone,[434] ratios in critically ill foals may suggest CIRCI; however, specific cutoff values are not very well defined because of wide ranges in these ratios. Dynamic tests such as the ACTH stimulation test are more adequate to diagnose CIRCI in critically ill people and foals.[588,622] In general, ACTH doses used to assess adrenocortical function in foals are divided into high-dose (1.0–2.0 μg/kg or higher) and low-dose (0.01–0.2 μg/kg) protocols.[622,637-640] After blood collection for baseline cortisol concentrations ACTH is administered intravenously or intramuscularly, and additional blood samples are collected 30 and 90 minutes after stimulation. High-dose ACTH produces the maximum cortisol release; therefore it is considered supraphysiologic and may be less sensitive to diagnose CIRCI. Δ-Cortisol (values at 30 minutes–baseline) concentrations are highly variable in healthy and critically ill foals, and cutoff points have not been established.[622,637-640] However, an increase from baseline cortisol concentrations of less than onefold to twofold 30 minutes after low-dose ACTH stimulation and less than threefold to fourfold 90 to 120 minutes after high-dose ACTH stimulation may be indicative of CIRCI in horses and foals.[622,637]

Insulin-induced hypoglycemia (IIH), the metyrapone test, and the CRH test assess integration of the whole HPAA axis. Metyrapone inhibits adrenocortical 11β-hydroxylase, which converts 11-deoxycortisol to cortisol. When given to individuals with normal adrenal function the reduction in cortisol concentrations stimulates ACTH and CRH release, increasing adrenocortical steroidogenesis and accumulation of 11-deoxycortisol.[531,588] IIH is considered the gold standard test for the diagnosis of HPAA dysfunction in people.[531,588] After exogenous insulin is administered, the cortisol and ACTH response to hypoglycemia is determined. The clinical use of this test in critically ill foals is limited because hypoglycemia is a frequent abnormality, ACTH and cortisol concentrations are often elevated, and insulin administration may be contraindicated. The CRH stimulation test has been described in horses using ovine CRH.[641] However, there are no data in foals on the ACTH and cortisol release in response to CRH, and most critically ill foals have elevated baseline ACTH concentrations. Therefore this test is not recommended for diagnosis of CIRCI in equine neonates.

Treatment

Currently, hydrocortisone replacement therapy (200 mg/day) is recommended in human patients with vasopressor-unresponsive septic shock. The use of the ACTH stimulation test to identify critically ill patients who should receive hydrocortisone is no longer recommended because the response to exogenous ACTH does not predict a faster resolution or better outcome.[587] Bolus administration of hydrocortisone has been associated with hyperglycemia and hypernatremia in ICU patients; therefore continuous IV infusion rather than bolus injection is preferred.[587,636] Recommendations regarding the use of hydrocortisone in septic foals are based on daily endogenous production in healthy foals.[534] The ideal diagnostic test for CIRCI in foals has not been established. Therefore a short tapering replacement therapy of hydrocortisone (1.3 mg/kg/day IV divided every 4 hours) is recommended in foals with clinical signs of CIRCI such as vasopressor-unresponsive hypotension, hypoglycemia, or persistent SIRS.[531]

Steroid Precursors and Neuroactive Steroids

Neuroactive steroids (neurosteroids) represent a multitude of steroids that directly or indirectly modify neuronal and glial cell activity, and therefore behavior, stress response, and endocrine functions. They are influenced by the reproductive cycle, pregnancy, acute and chronic stress, neurologic disorders, and injury.[642] Neurosteroids are classified as pregnane (allopregnanolone, allotetrahydrodeoxycorticosterone [THDOC]), androstane (androstanediol, etiocholanone), and sulfated (pregnenolone sulfate, dehydroepiandrosterone sulfate [DHEAS]) neurosteroids. Most research on neurosteroids has been focused on pregnenolone, progesterone, 5α-dihydroprogesterone, allopregnanolone, dehydroepiandrosterone (DHEA), and THDOC.[643,644] They are crucial for neuronal and glial plasticity, excitability, myelination, synaptic transmission, and organization of brain connectivity. DHEA is produced in the adrenal gland cortex, gonads, and neural tissue, and its main function is to serve as a precursor for the synthesis of androgens and estrogens. DHEA is the most abundant steroid in circulation, and most is in the form of DHEA sulfate, with concentrations being around 300 times higher than free DHEA.

The effects of neuroactive steroids on the CNS are mediated by classic and nonclassic pathways.[645] The classic actions are mediated by nuclear receptors leading to changes in gene transcription. This is considered the traditional mechanism by which steroids work. The nonclassic actions are rapid and involve steroid binding to cell membrane receptors including the γ-aminobutyric acid (GABA) type A receptor ($GABA_A$ receptor [$GABA_AR$]), the *N*-methyl-D-aspartate receptor (NMDAR), the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor, the 5-hydroxytryptamine type 3 (5HT3) receptor, and the sigma-1 receptor (σ1R).[645-648] There are also membrane-specific progesterone receptors (mPRs), which are G protein–coupled receptors that bind progestins. There is evidence that mPRs mediate the neuroprotective effects of progesterone and allopregnanolone by promoting cell survival and suppressing apoptosis.[649] These receptors are mainly expressed in neurons but are also present in astrocytes. $GABA_A$ receptors are widely distributed on neurons and astrocytes where they mediate the actions of neurosteroids during ischemia, brain injury, and ammonia-induced cytotoxicity. $GABA_A$ receptors are members of the ligand-gated ion channel superfamily that is coupled to the chloride channel. Opening of chloride channels in neurons causes membrane hyperpolarization and inhibition of neuronal activity, reducing neurotransmission and propagation of the action potential.

Neurosteroids modulate the acute stress response and HPAA balance by downregulating CRH and ACTH gene transcription.[643,644] However, under chronic stress, HPAA modulation by neurosteroids may be impaired due to reduced neuronal and glial 5α-reductase activity and subsequent reduction in allopregnanolone concentrations. Neurosteroid concentrations in the CNS vary dynamically depending on physiologic and pathologic conditions, including stress, anxiety, depression, and other disorders.[643] Neurosteroids may be neuroprotective during cell injury from acute hypoxia and trauma.[642,644] Both neurons and glial cells (astrocytes and oligodendrocytes) have the enzymatic machinery for de novo neurosteroid synthesis or conversion of peripheral

steroids (e.g., progesterone) into neuroactive metabolites (see Fig. 16.17).[650]

Progesterone is considered a reproductive hormone mainly produced by ovarian luteal cells and the placenta. Progesterone is also synthesized in the adrenal gland as a precursor for various adrenocortical hormones. De novo synthesis of progesterone also occurs in the CNS where it acts directly as a neurosteroid, but it can also be converted to other steroids that influence neuronal and glial activity (see Fig. 16.17).[642] Allopregnanolone is synthesized from progesterone in a two-step process that requires 5α-reductase and 3α-hydroxysteroid dehydrogenase. These enzymes are highly expressed in brain regions where GABAergic and glutamatergic neurons are present.[643,651] Peripherally, allopregnanolone is produced in the adrenal cortex and gonads. THDOC is a 21-hydroxylated derivative of deoxycorticosterone with similar properties. The major source of THDOC in the brain following stress is the adrenal cortex. Allopregnanolone and THDOC are deemed the most important neurosteroids. They have inhibitory actions including sedation, anticonvulsant effects, and anxiolytic effects and have been implicated with behavioral conditions such as bipolar disorder in humans.[652]

Sulfated neuroactive steroids such as pregnenolone sulfate and DHEAs are mainly excitatory, with anxiogenic and proconvulsant functions.[650] DHEA is a positive allosteric modulator of the NMDAR, a negative allosteric modulator of the $GABA_A$, and an agonist of σ1R. Allopregnanolone and similar steroids (THDOC) are positive allosteric modulators of the $GABA_A$ receptor but have minimal action on NMDA and AMPA receptors, which are excitatory and mediate neurotoxicity and cell death.[650] Progesterone, on the other hand, acts through nuclear and membrane receptors (NMDAR, $GABA_AR$, mPRs).[653]

Altered $GABA_A$ receptor function has been proposed as a cause of neurologic dysfunction in people (e.g., autism spectrum disorder). In immature fetal neurons, the $GABA_A$ receptor is a primary excitatory receptor.[654,655] However, just before birth, there is a transient reduction in intracellular chloride concentrations leading to an excitatory-to-inhibitory switch in the $GABA_A$ receptor. This shift in $GABA_A$ receptor signaling is in part mediated by oxytocin.[654,655] It is possible that a similar process might be involved in the pathogenesis of neonatal maladjustment syndrome in foals. Interestingly, high oxytocin, progesterone, 17α-hydroxyprogesterone, and pregnenolone concentrations on admission were associated with neurologic dysfunction in hospitalized neonatal foals.[656]

In addition to neurologic diseases, allopregnanolone and THDOC have been associated with systemic disorders such as hepatic encephalopathy (HE) and obesity.[657] The mechanisms responsible for increased neurosteroids in the brain of people with HE remain unknown, but upregulation of translocator protein TSPO (cholesterol-transporter protein) after exposure to ammonia and manganese has been proposed.[658] With regard to obesity, several neurosteroids have been associated with food intake. Allopregnanolone increased the consumption of calorie-dense food in rodents and was linked with obesity in girls.[659,660] The association of neurosteroids with HE and obesity in horses remains to be investigated.

Neuroactive Steroids and Equine Disease

Compared with other species, maturation of the HPAA in equine neonates occurs late in gestation and continues in the postnatal period.[573,661] Activity of the HPAA in the equine fetus increases 25 to 5 days before delivery.[573] As a result, the adrenal cortex develops morphologically and releases high concentrations of pregnenolone, which is converted to several progestogens by the placenta. In the last 5 days of gestation, the equine fetal adrenal cortex begins expressing 17α-hydroxylase that is required for cortisol production from progesterone.[573] Neonatal foals have high pregnane concentrations at birth that decrease gradually over the first 48 hours after foaling.[623,662] Increased pregnane and neuroactive concentrations have been associated with disease severity, neurologic abnormalities (neonatal maladjustment syndrome [NMS]), prematurity, and mortality in hospitalized foals.[623,624,656,662-665]

The mechanisms leading to pregnane elevation in critically ill foals remain unknown, although it has been proposed that high neurosteroid concentrations are a consequence of altered transition from intrauterine to extrauterine life, with subsequent neurologic dysfunction.[666] Based on their effects on $GABA_A$ receptors and pharmacologic evidence that steroid agonists can suppress neuronal function, exogenous compounds have been developed for veterinary and medical use (e.g., alphaxalone [alfaxalone]). Under this premise, one can speculate that increased steroid concentrations in sick foals can suppress neuronal activity inducing neurologic signs. To further elucidate their importance in neuronal and glial function in sick foals, neurosteroid measurements in the cerebrospinal fluid will be necessary.

Neurosteroid balance in the peripartum period could potentially explain many clinical abnormalities in these foals.[662,664,666] After all, increased neurosteroid levels at the end of gestation promote neuronal development and protect against hypoxia and ischemia.[667] It remains to be determined whether progestogen increase in critically ill foals represents a neuroprotective response to injury or a consequence of the disease. Progesterone and 17α-hydroxyprogesterone concentrations are higher in critically ill than in healthy and sick nonseptic foals.[623,656] Based on these findings and the fact that mPR-mediated actions of progestins are neuroprotective,[649] one can speculate that these steroids may have protective functions in sick foals.

Increased and decreased neuroactive steroid concentrations could be detrimental to neuronal function, depending on the condition and steroids involved. Compounds with GABAergic activity, as well as steroidogenic enzyme inhibitors (trilostane, finasteride, indomethacin), have been developed and some are under clinical evaluation for neurologic disorders (see Fig. 16.17). For example, ganaxolone, a derivative of allopregnanolone, is a selective positive allosteric modulator of the $GABA_A$ receptor under trial for the treatment of epilepsy, anxiety, and other neurologic disorders.[651] Alphaxalone is a neuroactive steroid and potent $GABA_A$ receptor agonist used as an anesthetic in dogs, cats, and horses.[668,669] Finasteride and dutasteride are 5α-reductase inhibitors used for alopecia and prostatic hypertrophy in men.[670] These compounds block the conversion of testosterone to 5α-dihydrotestosterone but also the conversion of progesterone to 5α-dihydroprogesterone, a key precursor for allopregnanolone. Under this premise we can speculate that if high concentrations of progesterone derivatives are involved in the pathogenesis of NMS, reducing their concentrations in the CNS could be beneficial. On the other hand, if progesterone derivatives are neuroprotective, blocking some of these pathways could be detrimental. This remains to be evaluated prospectively in sick foals with and without evidence of neurologic disease.

Trilostane is a competitive 3β-hydroxysteroid dehydrogenase inhibitor that blocks conversion of pregnenolone to progesterone, reducing cortisol synthesis.[671,672] Finasteride blocks terminal steps in the allopregnanolone synthesis; however, trilostane inhibits the second reaction in the steroidogenesis that may cause more side effects associated with adrenal steroid deficiency.

OTHER DISORDERS OF THE ADRENAL GLAND

Hyperadrenocorticism

Hyperadrenocorticism from pituitary pars intermedia dysfunction (PPID; equine Cushing disease) is discussed elsewhere in this chapter. Iatrogenic and adrenal hyperadrenocorticism is less common than pituitary-dependent hyperadrenocorticism (PPID) but has been described in horses.[673-675] Iatrogenic hyperadrenocorticism was induced in a horse treated for a pruritic skin condition after inject on of 12 mg of triamcinolone acetonide followed by two injections of 200 mg of the same compound, all within 6 weeks.[673] Clinical signs included depression, polyuria/polydipsia, weight loss, and laminitis. Blood and urinalysis abnormalities (neutrophilia, lymphopenia, hyperglycemia, glycosuria) were similar to those of PPID. However, this horse also had increased serum activity of γ-glutamyl transferase, aspartate transaminase, and bile acid concentrations, indicating steroid-induced hepatopathy.

The diagnosis of iatrogenic hyperadrenocorticism is based on clinical history, low baseline ACTH and cortisol concentrations, and a low cortisol response to the ACTH-stimulation test. Treatment consists of discontinuing exogenous steroids and perhaps replacement therapy with short-acting steroids.

One case of pituitary-independent (adrenal-dependent) hyperadrenocorticism has been documented.[675] In this case, a 12-year-old gelding presented with clinical signs of pituitary-dependent hyperadrenocorticism. Baseline cortisol and ACTH concentrations were within the normal range, and the cortisol response to the ACTH-stimulation test was normal. The horse was treated with bromocriptine with no improvement, and over the next 11 months the clinical signs worsened. On necropsy, an adrenocortical adenoma associated with pituitary gland and contralateral adrenal gland atrophy was present.

Adrenocortical Neoplasia

Adrenocortical tumors are rare in horses,[234,675-677] and the few cases reported in the literature were nonfunctional tumors. One case of a 12-year-old Dutch Warmblood gelding with a functional adrenocortical adenoma and clinical signs consistent with Cushing disease (hyperadrenocorticism) was recently reported.[675] Adrenocortical carcinoma characterized as a locally aggressive primary tumor with multiple metastases to abdominal and thoracic organs, lymph nodes, and proximal humerus has been describe in an 18-year-old Thoroughbred gelding and a 14-year-old Quarter Horse stallion.[234,678]

Pheochromocytoma

Pheochromocytomas are tumors that arise from the chromaffin cells of the adrenal medulla, which are part of the sympathochromaffin system (together with the sympathetic nervous system). During fetal life the chromaffin cells are associated with the sympathetic ganglia, and after birth most of them degenerate, and the few remaining cells constitute the adrenal medulla. In humans, approximately 90% of pheochromocytomas arise from the adrenal medulla and often are associated with a condition known as multiple endocrine neoplasia (MEN).[679,680] Extraadrenal pheochromocytomas have been described in humans but not in horses. In horses, these tumors have low incidence of malignancy and are usually unilateral.[681,682] Pheochromocytomas (functional and nonfunctional) are rare in horses, with a postmortem examination incidence of 0.95%.[680] Functional pheochromocytomas secrete catecholamines at a rate sufficient to cause clinical signs.[682] Thus functional pheochromocytomas are diagnosed with more frequency than nonfunctional pheochromocytomas.[680,683] It has been shown that most equine pheochromocytomas are nonfunctional and go undiagnosed.[680,684] Both epinephrine and norepinephrine have been identified as the catecholamines produced by equine pheochromocytomas.[685]

Functional pheochromocytomas in horses have no breed or gender predilection[682] and have been described in horses with a median age of 17 years (range, 13–38).[680,683,686] Because clinical signs are of acute onset and of rapid progression from intense adrenergic stimulation, they resemble those of colic, rhabdomyolysis, acute laminitis, enterocolitis, and hyperkalemic periodic paralysis. Clinical signs include abdominal pain from large hematomas or gastrointestinal distention secondary to ileus, anxiety, tachycardia, tachypnea, profuse sweating, muscle tremors, hyperthermia, dry and pale mucous membranes, increased capillary refill time, ataxia, and mydriasis.[680,682,683,686] Abortion has been documented. Clinical signs from nonfunctional pheochromocytomas in general are consistent with abdominal pain, and the median age of those horses is 27 years (range, 16–27 years).[680,682-684,686]

Hematologic abnormalities of functional pheochromocytomas include hemoconcentration, stress leukogram (mature neutrophilia with lymphopenia),[686] and leukopenia with neutropenia.[683] Hemoconcentration likely results from epinephrine-induced splenic contraction rather than dehydration.[683,684,686] Epinephrine and epinephrine-induced steroid release may be responsible for the stress leukogram. Serum biochemical abnormalities are nonspecific and include hyperlactatemia, hyperglycemia, azotemia, metabolic acidosis, hyponatremia, hyperkalemia, hypocalcemia, and hyperphosphatemia.[680,682,686] Hyperlactatemia may have been associated with catecholamine-mediated vasoconstriction or hemorrhagic shock in horses with hemoperitoneum, as well as the metabolic effects of catecholamines on gluconeogenesis. Hyperglycemia is a likely consequence of increased α- and β-adrenergic activity.[679] Hyponatremia with hyperkalemia, hypocalcemia, and hyperphosphatemia suggests acute renal failure; however, renal function in most reported cases was normal.[683,684] Hyperkalemia, hypocalcemia, and hyperphosphatemia could be from prolonged muscle activity, ischemia, and metabolic acidosis.[682-684,686] Hyperkalemia is unlikely to be from increased catecholamine concentrations as catecholamines induce hypokalemia by shifting potassium to the intracellular compartment (β-receptor mediated) and by increasing the activity of the renin-angiotensin-aldosterone system.[687] Glucosuria is also a frequent finding. Pheochromocytomas may secrete other hormones of neuroendocrine origin (calcitonin, parathyroid hormone, ACTH, CRH, somatostatin, vasoactive intestinal peptide, leu-enkephalin)[683,688-690]; however, they remain to be determined in horses.

The clinician should be aware that this condition carries a poor prognosis, and euthanasia may be a reasonable decision. Measurement of blood catecholamines or their metabolites in urine is valuable in establishing a diagnosis; however, this assessment can be a challenge and most laboratories do not measure these hormones.[682,683,686,691] Ultrasonography and measurement of blood pressure can support the diagnosis.[680]

If a diagnosis is reached, adrenalectomy can be attempted; however, the location of the adrenal glands, as well as the proximity of major blood vessels, tumor size, and arrhythmias (interaction between catecholamines and anesthetic agents),[684] make this a difficult decision.[682,684,686] Hypotensive α-adrenergic antagonists such as phentolamine, phenoxybenzamine hydrochloride, and prazosin hydrochloride are used in humans with functional pheochromocytomas before surgery.

Functional pheochromocytomas in horses do not appear to metastasize, are unilateral, and tend to bleed, and at necropsy the tumor is frequently found to be ruptured. Myocardial infarction and degeneration may be present, probably as a result of increased concentrations of catecholamines.[682-684]

Malignant pheochromocytomas have been documented in young and old horses.[526,692] A malignant pheochromocytoma was reported in a 6-month-old filly with a history of hindlimb lameness and spinal cord disease.[692] Both adrenal glands had multiple and well-circumscribed yellow masses and metastases were found in the liver, lungs, vertebral canal, left scapula, and azygous vein. A malignant pheochromocytoma was also diagnosed in a 22-year-old mare that died of massive intrauterine hemorrhage.[526] This mare also had a thyroid C-cell medullary adenoma and bilateral nodular hyperplasia of the adrenal medulla, findings that were consistent with MEN. This was the first documented case of MEN in the horse, although previous reports have been consistent with MEN.[525] A retrospective study found concurrent endocrine abnormalities including thyroid adenoma, adrenal hyperplasia, PPID, and C-cell carcinoma in 73% of horses diagnosed with pheochromocytoma postmortem. Twenty-one percent of those horses had changes consistent with MEN.[680]

The Equine Endocrine Pancreas

Teresa A. Burns and Ramiro E. Toribio

ANATOMY OF THE ENDOCRINE PANCREAS

The pancreas lies mainly on the right, in the very dorsal part of the abdomen. It has a triangular shape, composed of left and right lobes and a body. The body lies within the sigmoid flexure of the duodenum and has two regions (duodenal and intermediate). The lobes are less distinguishable than those of other species. The left lobe is longer than the right, crosses the midline, and abuts the stomach. The right lobe is close to the descending duodenum, in contact with both the right kidney and the base of the cecum. The ventral surface is attached to the right dorsal colon and base of the cecum. The dorsal surface is in direct contact with the right kidney and the liver. The portal vein perforates the pancreas at the pancreatic ring. Both the pancreatic and accessory ducts persist throughout development in equids. Exocrine pancreatic secretion is virtually constant but increases after feeding. This provides the small and large intestines with a steady supply of buffered solution, which facilitates digestion and creates a favorable environment for the microbiota. The distribution of islets of Langerhans, as well as their cellular composition, varies with location in the horse.[693,694] Islets in the left and right lobes and intermediate region contain a central mass of α-cells surrounded by β-cells, with few δ- and γ-cells.[694] Islets in the duodenal region are smaller compared with the rest of the pancreas, containing mostly β- and δ-cells but scarce α-cells. Some islets in the right lobe and duodenal region are abundant in γ-cells.[694] Regardless of location within the pancreas, α-cells are located mostly within the center of the islets, β-cells form a ring around the α-cells, and δ- and γ-cells are interspersed.[693,694] Changes in islet cell distribution with age have been documented in horses but are not well characterized otherwise (including the effect of disease).[695]

PHYSIOLOGY OF THE ENDOCRINE PANCREAS: GENERAL CONCEPTS

The regulation of energy metabolism requires coordination among different systems, including the hypothalamus, pituitary gland, gastrointestinal tract, liver, skeletal muscle, adipose tissue, and pancreas. The endocrine pancreas is the chief controller of energy homeostasis by sensing energy status and releasing hormones that facilitate energy storage during excess and promote energy release when demands increase. Pancreatic hormone secretion is influenced by endocrine and paracrine factors, metabolites (glucose, amino acids), and the autonomic nervous system. Dysfunction of the endocrine pancreas is associated with loss of glycemic control and can accompany a multitude of disorders.

The islets of Langerhans are the endocrine functional units of the pancreas. Each islet contains four primary cell types (α-, β-, δ-, and γ-cells)—α-cells secrete glucagon, β-cells secrete insulin, δ-cells secrete somatostatin, and γ-cells (PP-cells) secrete pancreatic polypeptide (PP). The β-cells also secrete urocortin-3 and C-peptide, whose functions are described later. Insulin and glucagon are considered the primary pancreatic hormones. Blood glucose and amino acid concentrations are important regulators of insulin and glucagon secretion. Insulin release is stimulated by glucose, glucagon, some amino acids, and incretin hormones (although not all to the same degree; glucose remains the primary and most profound insulin secretagogue), and it is suppressed by somatostatin. Glucagon secretion increases with hypoglycemia. Pancreatic endocrine function is also regulated by sympathetic and parasympathetic fibers through the actions of norepinephrine, epinephrine, and acetylcholine. The sympathetic system inhibits insulin and stimulates glucagon secretion, and the parasympathetic system stimulates insulin secretion during food intake.[696,697] There are CNS mechanisms that regulate insulin and glucagon secretion; however, specific pathways by which they influence the endocrine pancreas remain unclear.

Glycogenolysis is the release of glucose from stored glycogen in response to metabolic demands. Insulin suppresses glycogenolysis by activating protein phosphatase–1 and phosphodiesterase and inhibiting glycogen phosphorylase. This process is enhanced by glucagon and epinephrine. Insulin also promotes glycolysis through activation of protein phosphatase–1 and

pyruvate kinase. *Glycolysis* is a cytosolic enzymatic process in which glucose is converted into two molecules of pyruvate to generate energy (ATP) in the absence of oxygen. Through glycolysis, glucose is converted into fructose 1,6-bisphosphate (trapping), which is cleaved into phosphorylated three-carbon products that generate energy (ATP) during their oxidation to pyruvate. *Glycogenesis* is the synthesis of glycogen from glucose when energy is present in excess (in the form of glucose or ATP) to be stored in the liver and/or skeletal muscle. *Gluconeogenesis* is the synthesis of glucose from noncarbohydrate substrates such as glycogenic amino acids (e.g., alanine, glutamine, arginine, serine), triglycerides (glycerol), pyruvate, and lactate. In ruminants, as a result of the metabolic activity of the ruminal microbiota, gluconeogenesis is virtually constant using propionate as the primary substrate. The goal of gluconeogenesis is to maintain blood glucose concentration within a relatively narrow (physiologic) range during fasting or exercise. In other species, it is often linked to ketosis. It mainly occurs in the liver and kidney, with some in the brain and skeletal muscle. Insulin promotes glycogenesis and inhibits glycogenolysis and gluconeogenesis.

Insulin is the primary hormone controlling energy metabolism and storage. Its actions involve three major organic substrates (carbohydrates, proteins, and fats) and three major tissues (liver, skeletal muscle, and adipose tissue). In the liver, insulin decreases glycogenolysis, gluconeogenesis, and ketogenesis and stimulates glycogenesis, glycolysis, and fatty acid synthesis. In *adipose tissue,* insulin decreases lipolysis and stimulates fatty acid uptake, synthesis, and esterification. Hormone-sensitive lipase is an intracellular enzyme expressed in adipose and steroidogenic tissues, and its function is to mobilize stored fats through the hydrolysis of triglycerides into fatty acids. It is also important for cholesterol release from cholesteryl esters during steroidogenesis. Its activity is suppressed by insulin during energy excess and stimulated by catecholamines, adrenocorticotropic hormone (ACTH), and glucagon when energy needs increase. In *skeletal muscle,* insulin decreases proteolysis and amino acid output and increases glucose and amino acid uptake, protein synthesis, and glycogen synthesis.[698] Insulin increases the activity of adipocyte and endothelial cell lipoprotein lipase, which mediates lipoprotein (e.g., VLDL) uptake and triglyceride hydrolysis. Deficiency or mutations in lipoprotein lipase are associated with hypertriglyceridemia in other species,[699,700] although triglyceride overproduction rather than removal seems to be the main reason for hyperlipemia in ponies.[701,702] Other functions of insulin include modulation of vascular compliance, intracellular K^+ shift via Na^+/K^+ ATPase, cellular phosphate uptake, and DNA synthesis.

The main sites for insulin clearance are the liver and the kidneys. Most insulin released by the pancreas is cleared by the liver at the first passage, whereas the kidneys may be more relevant for removing insulin from systemic circulation, at least in other species.[703] Insulin removal does not necessarily imply degradation, as some receptor-bound insulin is released by the cell back into circulation.[703] Information on the kinetics (half-life, compartmental modeling) of endogenous equine insulin is limited. For humans, using the hyperinsulinemic euglycemic clamp technique, a five-compartment model has been proposed in which endogenous insulin has a mean residence time of 71 minutes: bound to hepatic receptors for 62 minutes, attached to peripheral receptors for 6 minutes, and persisting in the extracellular fluid for 3 minutes.[704] It has also been estimated that approximately 80% of total insulin is bound to hepatic receptors, suggesting that the liver is the largest reservoir for insulin.[704]

The connecting peptide (C-peptide) is the sequence within proinsulin that links the A- and B-chains of insulin. After a series of steps within the Golgi apparatus, C-peptide is removed, stored, and released with insulin in equimolar amounts. Traditionally, C-peptide has been measured as a byproduct of insulin processing as an indicator of β-cell function because unlike insulin, C-peptide is minimally metabolized by the liver. Insulin and C-peptide have different kinetics, with insulin half-life measured over the course of a few minutes, whereas C-peptide's half-life is around 30 minutes.[705] There is evidence that C-peptide is not an inert molecule but has physiologic functions, most of which remain to be elucidated.[705] It binds to a pertussis-toxin–sensitive G-protein–coupled receptor with high affinity.[705] Animal studies have shown that C-peptide ameliorates some of the complications of type 1 diabetes, including nephropathy and neuropathy.[705,706] It is believed that a major function of C-peptide is to promote normal endothelial and microvascular function. It decreases the formation of reactive oxygen species through adenosine monophosphate–activated protein kinase (AMPK), enhances nitric oxide production, reduces leukocyte adhesion, lowers proinflammatory cytokine release, and has antiapoptotic actions.[705,706] It also appears that C-peptide promotes glucose utilization and contributes to the actions of insulin at peripheral tissues. This effect does not seem to be direct but rather through facilitation of insulin-initiated signaling cascades.[705,706]

This information could be relevant to our understanding of the pathogenesis of equine disorders such as equine metabolic syndrome (EMS), pituitary pars intermedia dysfunction (PPID), hyperlipemia, and polysaccharide storage myopathy.

Glucagon has opposing effects to those of insulin on glucose metabolism. Its main targets are the liver and adipose tissue. Its key function is to increase glucose concentrations during hypoglycemia by promoting glycogenolysis and gluconeogenesis. By suppressing glycolysis, glucagon shifts intermediates to the gluconeogenic pathway. In adipose tissue, it enhances lipolysis, although this seems to be a minor effect. Glucagon secretion is stimulated by hypoglycemia, catecholamines, acetylcholine, cholecystokinin, and glucose-dependent insulinotropic polypeptide (GIP). Autonomic inputs promoting glucagon secretion are stimulated by hypoglycemia. Glucagon synthesis is suppressed by insulin, somatostatin, hyperglycemia, and neural inputs, some of which are at the hypothalamic level.[707]

Pancreatic polypeptide is secreted by the γ-cells (PP-cells), and its chief function is to reduce appetite. It also slows gastrointestinal motility, delays gastric emptying, and reduces ghrelin secretion. Vagal cholinergic activation is the main stimulus for PP secretion. Gastrin, motilin, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), ghrelin, and hypoglycemia also increase PP secretion. Somatostatin, glucocorticoids, and hyperglycemia inhibit PP secretion.

Catecholamines inhibit glucose-stimulated insulin secretion and raise glucose concentrations, both directly through hepatic effects and indirectly via modulation of glucagon secretion.

Growth hormone (GH) has a multitude of functions what will not be comprehensively discussed here. It counteracts insulin's actions on glucose and lipid metabolism and is

important in setting the basal glucose concentrations (glucostat).[698,708] There is extensive research supporting positive effects of GH on islet cells. It is involved in maintaining β-cell mass, promoting islet endocrine secretion, and contributing to normal insulin sensitivity and glucose homeostasis.[709] Most actions of GH have been attributed to insulin-like growth factor–1 (IGF-1; somatomedin-C), which is mainly synthesized by the liver (somatomedin hypothesis). Although this concept remains valid, it is also true that many GH actions are independent of IGF-1. This appears to be the case for GH's effects on the endocrine pancreas, where GH receptors are present in the β-cells whereas IGF-1 receptors are localized to endothelial and α-cells.[709] Despite these positive effects of GH on the islets of Langerhans, in peripheral tissue GH promotes insulin resistance, in part by uncoupling phosphatidylinositol 3-kinase, reducing phosphorylation of the insulin receptor and insulin receptor substrate–1 (IRS-1).[710] GH also mediates lipolysis during fasting, which seems to be a direct effect and also a consequence of interfering with insulin signaling.

Somatostatin (SST), or growth hormone–inhibiting hormone (GHIH), is a peptide produced in the hypothalamus, endocrine pancreas, proximal duodenum, and pylorus. Axons from hypothalamic SST neurons descend through the median eminence and terminate at the level of the pituitary portal venous system, from where SST will reach pituitary somatotropes to suppress GH secretion.[711] Hypothalamic SST neurons respond to high GH and IGF-1 concentrations by releasing SST as part of a feedback system. These neurons are also activated by hyperglycemia, adding another explanation for the suppressive effects of glucose on GH secretion.[712] In the pituitary gland, SST also inhibits the secretion of thyroid-stimulating hormone (TSH) and prolactin (PRL). SST may suppress ACTH secretion in other species, but information in the horse is lacking. Multiple SST agonists (octreotide, lanreotide, and pasireotide) have been developed to suppress GH, PRL, and TSH secretion in humans with acromegaly/gigantism, prolactinomas, and thyrotropinomas. In the endocrine pancreas, SST inhibits insulin, glucagon, and PP secretion. SST secretion is stimulated by urocortin-3, a peptide coreleased with insulin by the β-cells as part of the paracrine negative feedback system for glycemic control.[713] Urocortin-3 deficiency results in impaired SST secretion and exaggerated insulin secretion, creating an imbalance that has been linked to the pathophysiology of diabetes.[713] Abnormal SST has other actions within the gastrointestinal tract (reduces motility and exocrine pancreas secretion) and enteroendocrine system (inhibits most gastrointestinal hormones). Because of its multitude of actions on the pituitary gland, gastrointestinal tract, and endocrine pancreas, and considering that metabolic disorders linked to insulin dysregulation are well documented in the horse (EMS, PPID, hyperlipemia), the role of hypothalamic and pancreatic SST deserves investigation in this species.

The *enteroinsular axis* comprises enteroendocrine factors known as incretins (INtestinal seCRETion of INsulin) that regulate the secretion of pancreatic hormones. Incretins include glucagon-like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP; gastric inhibitory polypeptide), which promote insulin release and suppress glucagon secretion.[714,715] In other species, GIP is secreted by K cells in the proximal small intestine and GLP-1 by L cells in the distal small intestine.[714,715] Soluble carbohydrates, amino acids, and fatty acids promote incretin secretion. There are also neurogenic factors influencing incretin secretion. The incretin effect explains the rise in insulin concentrations before there is an increase in glycemia, which has been proposed to be a preemptive mechanism to avoid hyperglycemia and facilitate rapid peripheral glucose uptake. GLP-1 and GIP also have a multitude of nonpancreatic functions; they slow gastric emptying and acid secretion (GLP-1 in particular), promote fat deposition, enhance insulin sensitivity, modulate skeletal muscle metabolism, stimulate bone formation, increase microvascular blood flow, and reduce appetite.[714,716] Understanding the physiology of enteroendocrine cells and the gut-pancreatic-brain axis has become a major area of research, in particular to develop therapeutic strategies to modulate incretin activity in diabetes, obesity, and hypertension.[717] From all this information, it is reasonable to assume that incretin dysregulation may contribute to equine conditions related to metabolic activity, insulin secretion and sensitivity, nutrient uptake, inflammation, obesity, and satiety. There is evidence that equids have a functional enteroinsular axis.[718-721] Oral and IV soluble carbohydrates increase GIP, GLP-1, and insulin secretion in horses and ponies,[718-721] and these effects are more evident for oral than IV glucose administration.[719] Relevant to EMS and endocrinopathic laminitis, it was recently shown that insulin-resistant ponies have a hyperresponsive enteroinsular axis to oral and IV glucose.[719]

The *hypothalamic-pituitary-adrenal axis (HPAA),* specifically ACTH and cortisol, has been shown to influence β-cell function.[722] This was recently documented in foals overexposed to glucocorticoids in response to ACTH administration that had a reduced insulin response to glucose stimulation.[722] Increased free cortisol concentrations have been associated with hyperinsulinemia in horses with endocrine disease.[723] Thyroid hormones are also essential for the differentiation of other endocrine organs, including the pancreas and adrenal cortex. It is reasonable to assume that low thyroid hormone concentrations in late gestation could result in abnormal β-cell function and energy dysregulation. Insulin, through induction of hypoglycemia, also influences all levels of the equine HPAA in horses and donkeys.[724,725] Insulin administration to healthy horses increased corticotropin-releasing hormone, arginine vasopressin, ACTH, and cortisol release.[725]

Information on the role of the CNS in regulating the equine endocrine pancreas is lacking; however, it is reasonable to assume that neurologic abnormalities may contribute to disorders of insulin secretion and sensitivity (e.g., EMS, PPID). In rats, for example, before glucose enters the small intestine following consumption of a meal, insulin is secreted secondary to activity in neural inputs that appear to originate in the taste buds.[726,727] In addition, there are glucose-sensing mechanisms at the gastric, hepatic, and hypothalamic levels that are not linked to incretins.[727,728] Rodent peripheral and central ablation studies have shown that the hypothalamus senses and regulates glycemia (brain–endocrine pancreas axis) via insulin and glucagon secretion.[728,729] The parasympathetic tone to maintain β-cell proliferation and function is increased with lesions of the ventral hypothalamus, supporting the importance of vagal control.[728]

THE EQUINE ENDOCRINE PANCREAS

Relatively little is known about pancreatic physiology and pathophysiology in equids. Not only are primary pancreatic diseases reportedly rare in horses and ponies,[194] the organ itself is difficult to access for antemortem anatomic and

physiologic evaluation, sampling, or imaging. Insulin resistance (IR) and resultant hyperinsulinemia have emerged in recent years as risk factors for the development of endocrinopathic laminitis in horses and ponies based on results of several cross-sectional epizootiologic studies.[730-731] Further, experimental hyperinsulinemia has been shown to induce laminitis in normally insulin-sensitive horses and ponies.[732,733] Therefore, although the mechanistic role of insulin in the pathophysiology of laminitis remains incompletely characterized, the emerging importance of this hormone to various equine diseases is clear. It is well known that insulin resistance in equids can result in hyperinsulinemia; however, it is unknown whether this observation results from enhanced insulin secretion, decreased insulin clearance, or both. It has been proposed that hyperinsulinemia in horses with PPID results from high cortisol concentrations, whereas hyperinsulinemia in horses with EMS is a consequence of local (adipose, hepatic, lamellae) production of cortisol from inactive cortisone via 11β-hydroxysteroid dehydrogenase.[734] Administration of medications, such as glucocorticoids, has been associated with IR in horses and ponies,[735,736] likely from peripheral mechanisms rather than direct actions on the endocrine pancreas. Taking into consideration that IR is a risk factor for equine laminitis and glucocorticoids reduce insulin sensitivity, this may be a potential explanation for the development of laminitis in horses and ponies after glucocorticoid administration (not reliably reproduced experimentally, however). Functional studies will be required to clarify the importance of these mechanisms to equine IR, hyperinsulinemia, and EMS. These studies are challenging for many reasons, not the least of which are described next.

The ontogeny of equine endocrine pancreatic function has been evaluated in several studies.[737-739] The equine endocrine pancreas is functional before birth. Pancreatic β-cell maturation in the equine fetus occurs in late gestation,[739] but insulin is detectable by day 150.[738] Before day 230, glucose has no effect on fetal insulin release; however, after day 290, glucose administration evokes a rapid insulin response.[739] Fetal pancreatic insulin secretion is linked to cortisol concentrations, with a more rapid and profound insulin release observed in foals with higher cortisol concentrations near term.[739] Premature foals with low cortisol concentrations have a poor insulin response to glucose stimulation.[739,740] Variations in intrauterine growth rate have been shown to affect postnatal insulin secretion in the horse.[741] Overgrowth, not growth retardation, in utero alters equine β-cell function in the immediate neonatal period and leads to higher baseline insulin concentrations and greater insulinemic responses to glucose in affected foals.[741] Equine β-cells are responsive to glucose and arginine and release both insulin and proinsulin during the immediate postnatal period.[742] Immediately after birth, the equine β-cells are responsive to glucose administration; however, foals develop physiologic insulin resistance on the first day after birth that is likely related to high cortisol concentrations,[742] as they become very responsive days later.[740] Circumstances surrounding delivery during foaling have been shown to influence pancreatic endocrine function in foals; foals born following induction of labor have decreased insulinemic responses to glucose infusion, and this finding is correlated with a twofold to threefold increase in plasma cortisol concentration in induced foals.[743] Sex appears to play a role in the acute pancreatic response to glucose in neonatal and weanling-age foals, as filly foals were noted to have a more profound insulinemic response to glucose infusion than colt foals, even though their glucose disposal rates were similar (suggesting IR in female foals relative to their male counterparts).[744] These same authors also showed that HPAA activation may affect pancreatic insulin secretion in neonatal and weanling foals, with foals treated with ACTH daily for 5 days after foaling displaying lower insulinemic responses to glucose infusion than untreated foals.[722] This is further supported by another study in which both pregnant mares treated with dexamethasone and their foals had altered insulin dynamics following maternal treatment.[744] Another factor that could influence postnatal pancreatic β-cell function is nutrition. Insulin secretion and β-cell sensitivity to glucose were enhanced in foals born to mares that had experienced caloric restriction during midgestation.[745] Pancreatic α-cells are also functional in utero in the equine fetus, producing glucagon; however, this secretion is responsive to arginine but unresponsive to glycemia until the postnatal period.[746]

As mentioned earlier (see section on anatomy of endocrine pancreas), the pancreas is a markedly heterogeneous organ.[747] It has both exocrine and endocrine functions, with endocrine islets of Langerhans interspersed within exocrine acinar tissue. The size and distribution of islets have been shown to be quite heterogeneous across the pancreas in other species,[747] varying with both age of the animal and anatomic location of the sampled tissue (e.g., from the head, tail, or body of the pancreas); this also appears to be true in equidae.[693,694] To increase the validity of conclusions drawn from studies of islet morphology and function in horses (important for further characterization of the β-cell's role in initiation and perpetuation of equine IR), pancreatic samples of similar size should be collected from a similar anatomic location in all subjects. As the equine pancreas is often somewhat amorphous and difficult to locate within the abdomen (even during a postmortem examination), this may be a difficult task.

The endocrine tissue of the pancreas is surrounded by exocrine acinar pancreatic tissue, which is rich in proteases, lipases, amylases, and nucleases. This makes sensitive and labile analytes, such as RNA, easily degraded during extraction from pancreatic tissue homogenates, complicating gene expression studies. Additionally, autodigestion of pancreatic tissue is reported to be rapid at room temperature, requiring prompt and appropriate handling (i.e., small tissue sections to ensure rapid fixation) of pancreatic samples that are to be processed for histologic studies. Further, this propensity for autodigestion has been linked to clinical signs of pancreatic disease (such as severe abdominal pain) and the development of hypocalcemia in affected individuals associated with enzymatic saponification of regional fat. Finally, the pancreas is difficult to access surgically for antemortem sampling in the adult horse because of its location within the abdomen. Even if it were easily accessible, pancreatic biopsies are associated with a greater risk of causing iatrogenic tissue injury than other tissues as a result of the high enzymatic content and risk of pancreatitis. For these reasons, studies that involve collection of fresh pancreatic tissue in horses, although needed to advance our knowledge of equine pancreatic physiology, are practically difficult. Studies of pancreatic hormone secretion are similarly complicated; pancreatic endocrine hormones are secreted into the pancreatic veins, which coalesce to join the portal vein. A hepatic first-pass effect on pancreatic secretions complicates any assessment of these secretions that is based on sampling of peripheral venous blood. To adequately

characterize pancreatic insulin secretion—the contribution of which to equine hyperinsulinemia and IR remains incompletely described—cannulation of the portal vein and collection of postpancreatic, prehepatic blood samples would be very useful.

INSULIN SECRETION FROM THE ENDOCRINE PANCREAS

Following ingestion of a meal, the nutrient components are broken down into their respective elements by salivary, gastric, pancreatic, and intestinal enzymatic activity. Although an average grain meal (e.g., a 10% sweet feed preparation) for horses contains protein, lipid, and carbohydrate, the majority of the ration is carbohydrate (especially starch and simple sugars). Degradation of dietary starch and sugars to hexoses (glucose, galactose, and fructose) results in their absorption by simple and facilitated diffusion (cotransport with sodium) across the gastrointestinal mucosa into the portal venous blood and ultimately the systemic circulation. Although the appearance of other diet-derived substances in blood (such as certain amino acids) can induce pancreatic insulin secretion, the postprandial increase in plasma glucose concentration is a potent (and the most physiologically important) secretagogue for insulin from the β-cells of the endocrine pancreas. For the past 30 years, it has been held that transport of glucose into the β-cell (primarily through the non–insulin-dependent glucose transport proteins GLUT2 and GLUT1) during times of hyperglycemia results in increased glycolysis and ATP production. An ATP-responsive potassium channel is present on the plasma membrane of the β-cell; increased cytosolic ATP concentrations ultimately result in inhibition of this channel, reducing potassium conductance from the β-cell and resulting in relative depolarization of the cell. Increased activation of voltage-gated calcium channels on the plasma membrane and increased calcium influx activates calcium-dependent mechanisms responsible for fusion of insulin-containing cytoplasmic vesicles (also containing equimolar amounts of C-peptide) with the plasma membrane, exacting insulin release into the interstitial fluid and, ultimately, the pancreatic venous blood.

Recent work in cultured islets suggests that this mechanism is likely not the only one responsible for insulin release, however. Two phases of insulin secretion are observed in response to an enteric glycemic load: an initial, rapid phase (i.e., within the first 10 minutes) and a delayed, sustained phase (i.e., beginning approximately 15 minutes after the first phase). Pharmacologic inhibition of the ATP-sensitive potassium channels described earlier significantly attenuates the initial rapid phase of insulin secretion but has limited effect on the sustained secretion observed later. Gut-derived incretin hormones (GLP-1 and GIP) are likely responsible for this second phase of sustained insulin release and, as previously mentioned, have become attractive therapeutic targets for the treatment of relative insulin deficiency in humans with type 2 diabetes mellitus. Incretins were discovered and subsequently described following the initial observation that an oral glucose load/meal would reliably elicit a twofold to threefold greater insulinemic response than a similar glucose load given intravenously (thereby bypassing the gastrointestinal tract); the incretin response is severely impaired in humans with type 2 diabetes mellitus, an abnormality that can be reconstituted surgically (through gastric bypass procedures) or pharmacologically (through the administration of GLP-1 analogs [such as exendin-4 homologs, e.g., exenetide] or inhibitors of the enzyme that degrades incretins, dipeptidyl peptidase-4 [such as sitagliptin]).

Factors that are likely to modify this response in a horse consuming a meal of concentrate feed include, first, the composition of the meal, particularly its "glycemic index." The *glycemic index* is technically a term applied to human foodstuffs and is defined as the ability of a specific dose of the food in question to raise blood sugar compared with a similar quantity of white bread. This has been modified somewhat for use in equine nutrition, and the "relative" glycemic indices of certain cereal grains have been reported (e.g., the glycemic response of the horse to a corn meal is higher than that to one of barley). Because postprandial plasma glucose concentration is the most physiologically important secretagogue for insulin, the ability of the ingested meal to raise plasma glucose concentration would be expected to have a profound effect on the insulinemic response to that meal. This is exploited therapeutically in the dietary management of horses and ponies with EMS, as attempts are made to minimize the amount of nonstructural carbohydrate (NSC) in their diets and subsequent postprandial hyperinsulinemia.

The functional pancreatic β-cell mass, which correlates with the insulinemic response to enteral and IV glucose challenges in experimental animals, is an important variable to consider when evaluating an animal's insulinemic response to a meal. This has not been effectively characterized in equine populations, but β-cell failure (insufficient insulin response to carbohydrate challenge) appears to be uncommon in this species. Further, β-cell mass is virtually impossible to measure accurately in vivo (and presents challenges ex vivo); it can be measured experimentally, which may shed light on the mechanisms by which equine β-cell function is preserved in the setting of prolonged hyperinsulinemia (where type 2 diabetes would be expected to develop in humans).

The relative insulin sensitivity of the primary insulin-responsive tissues (skeletal muscle, liver, and adipose tissue), which are responsible for the majority of postprandial glucose disposal, has important ramifications for the insulin secretory response. Animals that are systemically insulin resistant show decreased glucose disposal from the plasma following a meal, and they display increased plasma fatty acid concentrations in both the fasting and fed states (decreased insulin-mediated inhibition of hormone-sensitive lipase in adipose tissue, decreased inhibition of hepatic synthesis and secretion of VLDL). Experimental glucotoxicity, lipotoxicity, or both have been shown to decrease insulin secretion from cultured islets in vitro over the course of several hours of glucose and lipid incubation or infusion; the mechanism for this remains to be clarified, but given that metabolic cycling of pyruvate metabolites (i.e., isocitrate-malate, citrate-malate, pyruvate-malate) is suspected to be involved in insulin secretion, the dramatic addition of metabolites to these pathways can be expected to affect this outcome (insulin secretion). Additionally, the expression and functionality of incretin receptors on β-cells have been shown to be blunted in the setting of insulin resistance; again, the mechanism for this is unclear, but this is an active area of investigation in human medicine. Finally, although the pancreas is not responsible for a large amount of insulin-dependent glucose disposal, it is insulin responsive; insulin receptor signaling is thought to be involved in

pancreatic insulin secretion, which may become dysfunctional in the setting of systemic insulin resistance (the relative importance of which is completely unknown in horses and ponies currently).

DISEASES

Diabetes Mellitus

As mentioned, diabetes mellitus is very uncommon in equids, much less common than in humans and other domestic species (such as dogs and cats). That said, the condition has been documented in horses, and it may occur more frequently than has been previously reported; whether this represents a true increase in the incidence of the disease or an increase in detection capability and/or awareness is unclear. Type 1 diabetes mellitus (insulin-dependent) was diagnosed in a 5-year-old Thoroughbred mare, for whom glycemic control was successfully achieved for 18 months following diagnosis through the administration of exogenous insulin. At necropsy following the mare's spontaneous death, lymphocytic infiltration of the pancreas (along with several other endocrine organs) was noted, suggesting an immune-mediated component to the condition.[604] Type 2 diabetes mellitus (non–insulin dependent) has been described in a Spanish Mustang.[748] Frequently sampled insulin-modified glucose tolerance testing with minimal model analysis has been used to diagnose the disease in three horses of another report, clearly documenting not only severe systemic IR but also blunted acute insulin response to glucose; the cases described in this report responded positively to medical management with diet modification, metformin, glibenclamide, and pergolide and experienced restored euglycemia.[749]

ROLE OF THE EQUINE PANCREAS IN EQUINE METABOLIC SYNDROME

Obesity, insulin resistance, and endocrinopathic laminitis have emerged as critical issues in equine medicine, causing significant morbidity, mortality, and economic loss to the equine industry.[750,751] In obese humans and experimental rodent models of nutritional obesity, systemic insulin resistance and hyperinsulinemia are followed temporally in a majority of individuals by decreased glucose tolerance, pancreatic β-cell failure, and type 2 diabetes mellitus.[752] The mechanisms by which this occurs are not completely understood; however, investigations in recent years have revealed that progressive changes in both the function and morphology (particularly increased insulin production/secretion and β-cell hyperplasia) of the endocrine pancreas occur over time in response to conditions known to increase risk for type 2 diabetes mellitus, such as genetic or nutritionally induced obesity,[64,65] high-carbohydrate feeding,[755] and high-fat feeding.[754] Failure of increased insulin secretion and β-cell hyperplasia in response to these stimuli is considered a hallmark of type 2 diabetes mellitus.[756]

In stark contrast to humans, obese horses and ponies with systemic IR chronically remain in what is termed a "prediabetic" state in human IR, characterized by hyperinsulinemic euglycemia.[757] Although increased pancreatic insulin secretion has been suggested to be responsible for the hyperinsulinemia resulting from systemic insulin resistance in equids (and evidence exists for increased pancreatic insulin secretion in response to glucose infusion in insulin-resistant equids),[758] the contribution of altered hepatic insulin clearance to increased basal insulin concentrations in these animals has been poorly characterized to date.[759] Endocrine pancreatic function (and dysfunction) would seem to be critically important to the pathophysiology of EMS, but surprisingly little information exists describing the biology of the equine endocrine pancreas in the chronically insulin-resistant equid.

Although alterations in pancreatic islet morphology are well characterized in the states of obesity and systemic insulin resistance in other species,[756] particularly in response to high-glucose diets or infusions,[755] these alterations do not appear to occur in the obese, insulin-resistant equid. Pancreatic β-cell hyperplasia has been reported to occur in the setting of the obese, chronically insulin-resistant human and rodent before the onset of secretory failure and type 2 diabetes mellitus.[747,753,760] However, this morphologic change in the pancreatic islets has also been reported to occur in response to parenteral glucose infusions over the course of as few as 6 days.[755] No association was found between body condition score or systemic insulin sensitivity and pancreatic histomorphometry in ponies in one study[761]; similarly, there was no association between pancreatic insulin content measured by ELISA and pancreatic histomorphometry, body condition score, or systemic insulin sensitivity. The results did not support morphologic changes in the equine endocrine pancreas following short-term high-carbohydrate feeding.

Given the relatively modest changes in pancreatic islet surface area that accompany marked increases in serum insulin concentrations in ponies fed a high-NSC diet, it seems important to assess both β-cell function and insulin clearance mechanisms in future studies to delineate the mechanism(s) of hyperinsulinemia in this species. Systemic IR and subsequent basal and postprandial hyperinsulinemia have been increasingly well characterized in equids over the past decade.[762-764] Hyperinsulinemia in this setting has been broadly assumed to be the result of pancreatic hypersecretion in response to hyperglycemia[731,765,766]; however, this has not been quantified well to date. Plasma insulin concentration is regulated not only by the rate of pancreatic insulin secretion (which is initially increased in the setting of systemic insulin resistance secondary to β-cell hyperplasia) but also by the rate of hepatic insulin clearance. Attempts have been made to clarify the role of hepatic insulin clearance in the setting of hyperinsulinemia in horses via assessment of plasma C-peptide concentrations following a frequently sampled IV insulin-modified glucose tolerance test.[759] However, although the results suggest that insulin clearance decreases as pancreatic secretion of insulin increases in response to dextrose infusion (supporting a role for altered clearance in hyperinsulinemia), this study was performed on a small number of horses, and the conclusions drawn should therefore be considered preliminary. Further study, including a more specific assessment of pancreatic insulin secretion (possibly through sampling of portal venous blood following administration of an insulin secretagogue), needs to be performed to further clarify this issue, which is important as it speaks directly to the pathophysiology of EMS in horses and ponies. Further, understanding the physiology of the equine endocrine pancreas in the setting of chronic systemic insulin resistance may shed light on why it is that IR in horses (analogous to "prediabetes" in humans) uncommonly progresses

to overt type 2 diabetes mellitus. Comparative, translational research on this subject may ultimately yield benefits for both veterinary and human medicine.

PANCREAS AND PITUITARY PARS INTERMEDIA DYSFUNCTION

Insulin resistance as a consequence of PPID is poorly understood. A number of horses with PPID have increased insulin concentrations,[433] but this could be reflective of excessive glucocorticoid secretion, POMC-peptides interfering with insulin signaling, or central mechanisms that influence β-cell function.

Pancreatitis

Pancreatitis, though common in other species (such as cats, dogs, and humans), appears to be an uncommonly diagnosed clinical entity in equids. This may in part be due to the fact that it is rather difficult to make this diagnosis in horses and ponies antemortem due to the lack of available definitive diagnostic tests; most published case reports of equine pancreatic disease describe a diagnosis made at necropsy.[193,194,767,768] When it does occur, however, gross and histologic features of pancreatitis in horses are reported to be similar to other species; for example, a Friesian gelding who was euthanized for chronic colic was diagnosed with chronic fibrosing pancreatitis with acinar-ductal metaplasia and ductal dysplasia, common preneoplastic changes found in humans.[767] An apparent breed, age, or sex predisposition has not been described, but conditions such as grain overload, endocrine disease (especially PPID), and parasite migration have been associated with pancreatitis in equids.[194] Additional associations have been made between pancreatitis in horses and gastrointestinal,[193,768] reproductive,[193,769] and endocrine disease.[193] Retrospective evaluation of pancreatitis cases diagnosed postmortem suggests that most cases of pancreatitis in this species are secondary or associated with another condition; however, 6 out of 43 horses in one study were diagnosed with primary pancreatitis, and therefore it seems that clinicians should at the very least be aware of this possibility.[193] The disease has also been reported in very young foals, suggesting that horses of widely variable demographics are susceptible.[192,195,770] Clinical findings reported to be most common among horses with acute pancreatitis include colic, nasogastric reflux, gastric dilation, peritonitis, and abdominal fat necrosis.[193] Evaluation of serum and peritoneal fluid lipase and amylase activities may be helpful in supporting a diagnosis, and at least one report of successful treatment of a horse with acute pancreatitis has been published.[771] More careful monitoring and diagnostic evaluation in the future may reveal pancreatitis to be more common in equidae than previously assumed.

Neoplasia

Pancreatic neoplasia, like other pancreatic disease, appears to be uncommon to rare in equidae. Exocrine pancreatic adenocarcinoma appears to be the most common tumor type, having been reported in several horses[772-775]; occasional patients have had widely disseminated disease.[774,775] Endocrine pancreatic tumors have also been reported in the horse, but again, these appear to be quite rare. Multiple glucagon-producing pancreatic neuroendocrine tumors were diagnosed in one horse,[776] and a pony with recurrent bouts of hypoglycemia was found to have multiple islet tumors and hyperinsulinism.[777]

Insulin Dysregulation and Equine Metabolic Syndrome*

Teresa A. Burns and Ramiro E. Toribio

INTRODUCTION

Obesity and insulin resistance/dysregulation (IR) are increasingly important problems in both human and veterinary medicine, and complications associated with obesity (such as type 2 diabetes mellitus, cardiovascular disease, and certain neoplasms) are diagnosed frequently in many species. Correlations have been drawn between the pathophysiology of obesity in humans to that in horses, and although similarities do exist, so do prominent species differences. Pituitary pars intermedia dysfunction (PPID; also called equine Cushing syndrome) and equine metabolic syndrome (EMS) are the two most common endocrine disorders diagnosed in horses. PPID affects older horses, whereas EMS typically develops in young and middle-aged animals. Horses with EMS often exhibit a predisposition toward obesity that becomes evident when they reach maturity, and this problem is aggravated by environmental conditions that involve high-carbohydrate feeding and/or promote weight gain. IR is a key feature of EMS and the primary reason for the condition receiving the assignation as an endocrine disorder. However, IR also occurs in a subset of patients with PPID, as well as in the setting of other systemic diseases (such as endotoxemia and sepsis); IR is therefore a clinicopathologic finding and not a disease per se. Laminitis is the most important complication of EMS, and early recognition of at-risk equids might improve detection and prevention of this painful and frequently debilitating condition.

Human Metabolic Syndrome: A Comparative View

Definition and Overview of Human Metabolic Syndrome

Obesity in humans has long been associated with adverse effects on health. More recently, a clustering of risk factors linked to obesity has been shown to predict the onset of cardiovascular disease and type 2 diabetes mellitus in affected individuals; this syndrome of risk factors, previously known as syndrome X, the cardiometabolic syndrome, or insulin resistance syndrome, is now referred to as the human metabolic syndrome (HMS).[778] The first formal definition of HMS was created by a working group of the World Health Organization in 1998.[779] The definition adopted by this group included evidence of systemic insulin resistance and at least two additional risk factors from a list of criteria including obesity, hypertension, hypertriglyceridemia, low serum high-density lipoprotein concentration, and microalbuminuria. Although seemingly inclusive, this definition has proven to be difficult to apply globally to the many diverse disease states that involve insulin resistance (such as antiretroviral therapy for HIV/AIDS, congenital lipodystrophies, polycystic ovary syndrome, Cushing syndrome, obesity, etc.), and exceptions

* With contributions to the section on equine metabolic syndrome written by Dr. Nicholas Frank derived from the third edition of this book.

to the diagnostic criteria are easily identified, particularly by individuals working in different medical specialties caring for affected patients (e.g., cardiologists, endocrinologists, gerontologists, infectious disease specialists). Consequently, the definition of metabolic syndrome in human medicine has emerged as a somewhat controversial issue.[780,781] A joint interim statement recently issued as a collaborative effort involving the International Diabetes Federation Task Force on Epidemiology and Prevention, the National Heart, Lung, and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity in 2009 suggested that the diagnosis of metabolic syndrome be made in an individual who displayed three out of the following five criteria: hypertension, hypertriglyceridemia, lowered high-density lipoprotein cholesterol, elevated fasting plasma glucose concentration, and central/truncal obesity.[782] Of these diagnostic criteria, this collaboration emphasized the importance of *body/adipose tissue morphometrics* (waist circumference, as a proxy for central/truncal obesity) as a useful screening tool for at-risk patients. The value of establishing a uniform set of diagnostic criteria to guide physicians in their management of these patients is based on both the severity of the adverse sequelae of metabolic syndrome (creating urgency to identify at-risk individuals for the purpose of prevention of these events), and the large (and increasing) number of patients affected globally; clear correlations with the management of obese equids in equine veterinary medicine can be noted here.

From an epidemiologic perspective, obesity and its associated metabolic and cardiovascular consequences have emerged over the past 10 to 15 years as critically important disease states in human medicine worldwide, creating an increasing economic burden on the health care systems of developed nations.[783] In fact, in the United States and the United Kingdom, two thirds of all adults are reported to be either overweight or obese[784-786]; accompanying this finding are increasing rates of atherothrombotic cardiovascular disease, stroke, type 2 diabetes mellitus (and all of its associated comorbidities), degenerative joint disease, and certain types of neoplastic diseases.[787,788] Perhaps more alarming is the ballooning rate of obesity among young people in industrialized countries[789,790]; in fact, the current generation of school-age children has been projected to have a life expectancy shorter than that of their parents, which is historically unprecedented.[791] Clearly, obesity is a critical health care issue in human medicine, both for the well-being of human populations and for the prioritization of the spending of increasingly limited health care funding allocations. With this economic imperative, a strong research effort has been put forth in recent years to elucidate risk factors for systemic insulin resistance and the metabolic syndrome in humans and to characterize the role that the expanded adipose tissue itself may play in the pathogenesis of the cardiometabolic sequelae of the syndrome.

Equine Metabolic Syndrome

Definition and Overview of Equine Metabolic Syndrome

Equine veterinarians have long known from clinical experience that overweight and obese horses are prone to developing laminitis. Similar to humans, obesity in horses has also been associated with peripheral insulin dysregulation/resistance.[437,766] As HMS was increasingly well described in the medical literature, correlations were drawn between this syndrome of obese humans and that noted clinically in obese horses.[792] Over time, the equine syndrome of obesity (particularly regional adiposity), peripheral insulin resistance, dyslipidemia, and an increased risk of endocrinopathic laminitis was coined the *equine metabolic syndrome* (EMS).[437,792]

The ACVIM Consensus Statement on EMS published in 2010[757] collated and synthesized most of the available research and evidence regarding this condition in horses and ponies, identifying three primary criteria for the diagnosis of EMS: increased adiposity (generalized, regional, or both, with emphasis placed on nuchal ligament and tail head adipose tissue accumulations), systemic insulin resistance (assessed in a variety of ways; see later discussion), and a predisposition to laminitis in the absence of another risk factor for the condition, such as septic disease[793] or gross enteral carbohydrate overload.[794,795] Other factors that have been reportedly associated with EMS, such as hyperleptinemia,[436,796,796] reduced adiponectin concentrations,[798] high incretin concentrations,[718,719] seasonally increased mean arterial blood pressure,[799] and altered reproductive cyclicity in mares,[800] were not included in the currently accepted diagnostic criteria. Based on recent and ongoing research findings, it is likely that these (and other) factors will be incorporated into the forthcoming definition and classification of EMS. Although the currently accepted criteria have all been subjected to reasonable objective evaluation, there remain potential flaws in their interpretation (methodology, sensitivity, etc.) that decrease their accuracy in laminitis risk prediction. Even the definition of the outcome variable, laminitis, may be ambiguous under certain circumstances.

Horses affected with EMS are typically overweight to obese, with body condition scores (BCSs) ranging from 7.0 to 9.0.[436,437,801] All breeds may be affected, although most reports indicate that breed predispositions exist, with pony breeds, Morgans, Tennessee Walking Horses, American Saddlebreds, Spanish Mustangs, Andalusians and other Spanish breeds, and Warmblood breeds overrepresented[802,803] Phenotypically and clinically, the condition is also common in Paso Fino, Criollo, and other Spanish-derived breeds in Latin America. Within at-risk breeds, affected individuals often display pronounced regional adiposity, particularly over the nuchal ligament and tailhead.[804] One report suggests that morphometric assessment of the neck of insulin-resistant horses (which incorporates assessment of the degree of nuchal ligament adiposity) can differentiate these horses from insulin-sensitive horses,[437] Interestingly, although the degree of insulin resistance has been shown to correlate reasonably well with body weight in horses,[765,766] not all obese horses are insulin resistant. Conversely, some relatively lean horses may be profoundly insulin resistant, particularly if they are members of a predisposed breed. Similar observations have been made in human medicine, and abnormalities in adipocyte function have been implicated in some of the more severe forms of insulin resistance (e.g., congenital lipodystrophy).[805] The underlying physiologic mechanisms that predispose some breeds to EMS have not yet been elucidated, but it is a tenable hypothesis that some facet of adipocyte structure and/or function is involved, at least in part. It has been suggested that components of the somatotropic axis (e.g., somatostatin) may be implicated in equine insulin dysregulation or that pancreatic cells are refractory to autoregulatory mechanisms that control insulin secretion.[806] Somatostatin is a peptide expressed by hypothalamic

neuroendocrine cells and suppresses growth hormone secretion by the pituitary gland. It is also secreted by delta cells in the pylorus/duodenum and endocrine pancreas and inhibits insulin and glucagon secretion. Under the premise that pancreatic endocrine dysregulation is involved in the pathogenesis of EMS, octreotide, a somatostatin analog, was administered to horses to assess their insulin regulation status (octreotide response test). Octreotide suppressed insulin secretion, although this effect was less evident in horses with insulin dysregulation; these findings support lack of β-cell inhibition as a potential mechanism for equine hyperinsulinemia.[806]

It is important to emphasize that the association between morphometric variables and IR is breed dependent, and although general principles apply, extrapolating from one to another type of equid may not reflect their insulin sensitivity and regulatory status.[807] It is possible (even likely) that reference ranges for interpretation of laboratory assessments of insulin dysregulation that are breed (and species) specific will be created in the future to improve the ability of clinicians to predict both disease and laminitic complications from these data.

Unlike humans with metabolic syndrome, horses with EMS do not appear to suffer from significant cardiovascular complications of their metabolic dysfunction. Although one report suggests that insulin-resistant ponies may be hypertensive,[799] no concrete evidence of cardiovascular complications related to EMS has been published. However, these horses are at increased risk for the development of laminitis,[436,808] which has been suggested (but not confirmed) to share an inflammatory pathophysiology with the more severe cardiovascular complications of obese humans, specifically, atherosclerotic coronary artery disease.[809,810] Several studies have revealed a robust correlation between markers of EMS in ponies (namely, BCS, plasma triglyceride concentration, and degree of insulin resistance) and the incidence of pasture-associated laminitis, which is perhaps the most common manifestation of endocrinopathic laminitis in horses.[436,730] In fact, the presence of more than three clinical findings of EMS in one study correctly predicted the development of laminitis in 11 of 13 ponies when these animals were exposed to a high-starch pasture.[730] Anecdotal evidence supports the contention that EMS is perceived by many equine veterinarians to be the most common cause of laminitis.

Insulin resistance in humans is often associated with increased plasma concentrations of certain markers of inflammation (notably TNF-α and IL-1β), linking abnormal carbohydrate metabolism in these people with chronic, low-grade inflammation.[811] This phenomenon has also been described in veterinary species, including dogs, mice, rats, and horses.[812,813] Obesity and insulin resistance have been loosely positively correlated with plasma concentrations of TNF-α and IL-1β in horses,[813] and several authors have suggested (based on tissue and systemic concentrations of inflammatory mediators) that EMS is associated with a systemic, low-grade inflammatory state.[813-815] The cause of laminitis in insulin-resistant equids is unknown but has been postulated to be related to a chronic proinflammatory state associated with insulin resistance[816]; this has yet to be documented conclusively in the horse, and evidence exists supporting the contention that the laminar lesions of EMS-associated laminitis are minimally inflammatory, at least acutely.[817]

Although much of the characterization for the diagnostic criteria for EMS has been performed in research animals, these criteria are meant to be applied to clinical patients for the purpose of guiding their medical care. Improving the accuracy of the diagnostic criteria will hopefully improve laminitis prevention, and improving the ease of diagnostic sample collection would potentially increase testing of general equine populations (and thus data collection, which may also be useful for research purposes). Increasing the predictive value of EMS diagnostic criteria and increasing the ease of sample collection (by validating single time point blood samples or by validating diagnostic evaluation of easily sampled tissues, such as nuchal ligament adipose tissue, skeletal muscle, or liver) would be reasonable steps to take to improve the diagnostic criteria for EMS.

The diagnosis of EMS is meant to define risk of laminitis in horses and ponies; adequate assessment of this risk requires measuring the incidence of laminitis in cohorts separated by various risk factors. Although small prospective studies on inbred populations of ponies have been performed evaluating the incidence of laminitis following exposure to pasture,[436,730] this work has not been repeated on a larger scale (or with more genetically diverse equine populations). Additionally, the same criteria defining laminitis risk in the aforementioned cohort of inbred ponies are extrapolated to light breed horses, populations on which this same type of study has not been performed. Large, prospective cohort studies (somewhat akin to the Framingham Cohort,[818] a seminal study defining risk factors for coronary artery disease and myocardial infarction in humans that has spanned several decades) would lend validation to (and perhaps reveal more accurately predictive) risk factors for endocrinopathic laminitis related to obesity in equids. If performed in conjunction with certain breed registries (breeds predisposed [gaited breeds, ponies, mules, donkeys] and breeds not predisposed [Quarter Horses, Thoroughbreds], e.g.,), data might be collected on individual animals over the lifetime of the animal (at shows, etc.). Documentation of familial tendencies and, ultimately, identification of candidate genes through linkage analyses, may lead to recognition of specific genetic determinants of abnormal carbohydrate or lipid metabolism (mitochondrial biogenesis, insulin degrading enzyme, insulin receptor [IR], anti-IR antibodies, PI3K, etc.) in horses and ponies.

Serum biomarkers that have positive correlation with metabolic risk have been fairly well characterized in human populations; although initial work incorporating serum biomarkers (such as leptin and insulin) into a "prelaminitic metabolic syndrome" has been done in ponies,[436,730] these studies have not evaluated the biomarkers that are most tightly correlated with IR in humans (namely, C-reactive protein,[811,819] plasminogen activator inhibitor–1,[820,821] serum amyloid A,[822,823] adiponectin,[824] and resistin[825,826]). Identification of additional serum biomarkers of laminitis risk, such as might be done through the long-term cohort studies mentioned earlier, might allow construction of a panel of analytes that could be easily measured in a single serum sample (adipokines, serum inflammatory proteins, proinflammatory cytokines, etc.). Again, this would require evaluation in large populations of horses (light breed horses, ponies, donkeys, and mules; predisposed breeds and nonpredisposed breeds), including evaluation of response to dietary challenge, evaluation of season, and correlation with incidence of laminitis in response to pasture exposure. Validated serum sampling protocols would increase the ease of diagnostic testing for EMS and might thereby increase the frequency of testing performed by practitioners on field cases.

Ideally, validation of serum biomarkers or a panel of such to predict IR should be performed against a gold standard.

When reviewing the equine literature on diagnostic testing of insulin and glucose dynamics, there appears to be no such gold standard (or if so, it is in name only). Insulin sensitivity is assessed by equine researchers with a diverse range of techniques[763,764]; to more cohesively move the field forward, a consensus needs to be reached on how this testing should be done. Direct comparison of the commonly used techniques (fasting insulin and glucose concentration with minimal model proxies,[731] combined glucose-insulin test [CGIT],[827] and oral glucose tolerance test [OGTT][828]) in the same cohort of horses (insulin-resistant [IR] and insulin-sensitive [IS]) to a gold standard (euglycemic-hyperinsulinemic clamp [EHC] and/or frequently sampled insulin-modified IV glucose tolerance test [FSIGTT]) should be performed to evaluate their relative benefits and shortcomings. Achievement of consensus on best laboratory testing protocol to define IR in horses (that is clinically feasible) is required to advance the field.

It has become clear in human obesity and IR that the relative roles of skeletal muscle and hepatic insulin resistance may not be equivalent. In fact, hepatic insulin resistance appears to correlate better with systemic IR and adverse disease outcomes in people than does skeletal muscle IR. EHC protocols using different insulin infusion rates (lower rate for assessing skeletal muscle IR; higher rate for assessing hepatic IR) have been described to evaluate the differential role of these tissues, and these may have some benefit in evaluation of equine IR (as the respective contributions of liver and skeletal muscle in this population remains unclear). Additionally, the role of ectopic lipid—which has been shown to correlate with systemic insulin resistance and risk outcome more tightly in humans and rodents than degree, or even location, of adipose tissue—in the pathophysiology of equine IR remains poorly described (even though a significant disease associated with equine IR, hyperlipemia, is defined by ectopic lipid accumulation). The relative ease of sampling of equine skeletal muscle and liver by minimally invasive techniques and the importance of these tissues to the pathophysiology of human metabolic syndrome makes this an attractive future possibility for diagnostic evaluation of equids with EMS.

Definitions

Insulin dysregulation/resistance refers to the failure of tissues to respond normally to insulin and/or altered insulin clearance. Skeletal muscle and adipose tissues are affected because they are two important sites for insulin-mediated glucose disposal. The liver also responds to insulin. Gluconeogenesis is inhibited and glycogen synthesis promoted as blood insulin levels rise after feeding. It should also be recognized that up to 50% of the insulin secreted by the pancreas is cleared by the liver as blood circulates through this organ before entering the systemic circulation. Hyperinsulinemia may therefore reflect increased insulin secretion, reduced insulin clearance, or a combination of these mechanisms.

Compensated insulin resistance is a term used when hyperinsulinemia is detected in an animal with reduced insulin sensitivity.[731] Insulin secretion from the pancreas increases to compensate for reduced tissue sensitivity, and euglycemia is maintained. However, recent reports suggest that resting hyperinsulinemia cannot be attributed to increased insulin secretion in all cases, which suggests that insulin clearance may be affected in some animals.[759]

Uncompensated insulin resistance refers to reduced insulin sensitivity with hyperglycemia. Insulin concentrations may be elevated or within reference range, and it is assumed that uncompensated IR develops as a result of pancreatic insufficiency in equids as in other species (although not with the same frequency, however). Compensated IR is the most common form of IR in horses and ponies, but uncompensated IR should be considered when elevated blood glucose concentrations are detected. It is appropriate to use the term *type 2 diabetes mellitus* when hyperglycemia and glucosuria are present, and this condition has been recognized in horses.[748,829,830]

As mentioned, equine metabolic syndrome is characterized by (1) obesity or regional adiposity, (2) insulin resistance, and (3) subclinical or clinical laminitis.[792] Hypertriglyceridemia has also been associated with EMS, and blood leptin concentrations are often elevated in affected animals.[437,730] Leptin is a hormone released by adipose tissue that signals the hypothalamus to suppress appetite after feed consumption. Plasma leptin concentrations generally correlate with BCS and degree of adiposity, but elevated levels are sometimes detected in leaner horses, suggesting a state of "leptin resistance" and metabolic dysregulation.[437,831] Studies have been performed to compare horses with low or high plasma leptin concentrations, and a cutoff value of 7 ng/mL has been used to define groups.[797] Elevated systemic blood pressure has also been associated with laminitis and IR in ponies kept on pasture.[799] Mean arterial blood pressure was higher in laminitis-prone ponies when measured in the summer, but values did not differ from control ponies in the winter.

Obesity is defined as an increase in body weight as a result of excessive fat accumulation within the body. When the BCS system developed by Henneke and colleagues[832] is applied, *obesity* is defined by a BCS ≥7 on a scale of 1 to 9. Obesity and IR are associated in horses and ponies.[437,833] It must be recognized, however, that body condition scoring systems are sometimes inadequate for assessing horses with endocrine disorders. Some horses with EMS and many with PPID are thin across the ribs yet have pronounced adipose tissue deposits in the neck and tailhead regions. This accumulation of adipose tissue at specific locations is referred to as *regional adiposity.*

Laminitis is the most important component of EMS because of its negative impact on the welfare of the affected horse. It is the development of laminitis that often prompts a horse owner to seek veterinary attention, and this condition presents the greatest challenge with respect to management. Subclinical laminitis takes the form of divergent growth rings (also called "founder lines") on the hooves and radiographic evidence of third phalanx rotation or distal displacement. This is often referred to as *endocrinopathic laminitis* because of its association with EMS and PPID. The term *prelaminitic metabolic syndrome* has also been used to describe ponies that are at higher risk for developing pasture-associated laminitis because of IR.[730]

Etiology

Genetic predisposition plays an important role in the development of obesity and IR in horses. Ponies tend to be more insulin resistant than horses,[833] and certain breeds and genetic lines of horses appear to be more predisposed to obesity and IR.[437,792] Obesity can develop in horses of all breeds, but this problem appears to be more common in Morgan Horses, Paso Finos, Arabians, Saddlebreds, American Quarter Horses, and Tennessee Walking Horses (among others). Horses that are predisposed to obesity are sometimes referred to as "easy keepers," as they seem to require fewer calories to maintain body weight (sometimes excessive body weight).

Most horses or ponies with EMS are obese, and this syndrome likely begins with a genetic predisposition that affects the efficiency of nutrient disposition and metabolism. Affected animals seem to require fewer calories to maintain body weight and display an enhanced appetite. Owners often report that their horse spends more time grazing when turned out on pasture. The "easy keeper" concept is relevant to the issue of genetic susceptibility. Certain breeds or genetic lines may have undergone evolutionary adaptations to survive harsher environments, and these horses or ponies may be more efficient at converting poorer quality forages into energy. Lack of exercise likely also plays a role in the development of EMS, as exercise increases both short- and long-term insulin sensitivity.[834,835]

Genetics and environment interact when affected horses are given ad libitum access to pasture, particularly when a small number of animals are grazing on a large area. Carbohydrate intake from pasture is influenced by grazing time, the area grazed, geographic location, climate, soil quality, and season.[836] In many regions of the country, pasture grass is abundant and rich in nutrients. This is accentuated when certain types of grass are sown to improve pastures for cattle or when fertilizers are used. A second point of interaction occurs when susceptible horses are fed concentrate feed. This is unnecessary for metabolically efficient animals and promotes obesity and IR. Unfortunately, many horse owners feed grain to bring their horses in from pasture or because they perceive that their horse's quality of life is adversely affected if it is kept on a hay-only diet. It is a common mistake to feed grain to horses that are able to maintain their body condition on pasture or hay alone. Horses can also be overfed if they are fed as a group or if grain is used as a reward. Some horse owners deliberately feed their horse too much because they prefer higher BCSs, and these biases are sometimes reflected in the judging at horse-shows. Other horse owners simply fail to recognize that obesity has developed in their animal.

Finally, humans interfere with an important interaction between genetics and environment by feeding horses more grain in the winter to prevent weight loss. Seasonal weight loss is a natural occurrence in wild horses and is likely to correct problems with obesity that have developed during other times of the year.

Clinical Signs and Differential Diagnoses

Obesity is detected on physical examination of the horse and determining a BCS. A horse suffering from EMS is shown in Fig. 16.18. Regional adiposity is commonly detected in IR horses, and mean neck circumference has been negatively correlated with insulin sensitivity in obese horses with IR.[436,437,807] Neck circumference can be measured with a tape measure. These measurements are made by dividing the distance along a line from the poll to the cranial aspect of the withers (x) by 4 and measuring the circumference of the neck at three equidistant points within x (0.25x, 0.50x, and 0.75x). Expansion of the adipose tissue within the neck is commonly referred to as a "cresty neck" and, with the exception of stallions, this finding is suggestive of IR in horses.[437] Recent work investigating the relationship between neck circumference, nuchal adiposity, and plasma insulin and leptin concentrations in Andalusian horses suggests that although neck circumference measurements may be heavily influenced by sex (and do not seem to correlate with hormonal measurements well), ultrasonographic measurement of the thickness of nuchal ligament adipose tissue is not similarly influenced (and correlates well with hormone measurements).[837] Validation of ultrasonographic parameters/measurements that correlate with metabolic risk in horses would likely be clinically useful.



FIG. **16.18** Photograph of a 13-year-old Appaloosa gelding with equine metabolic syndrome. (Courtesy Dr. R. E. Toribio, The Ohio State University.)

Enlarged fat deposits may also be found close to the tailhead or appear as randomly distributed subcutaneous swellings. Geldings are sometimes presented for evaluation of preputial swelling, and mares may show enlargement of the mammary glands. Both of these problems are related to adipose tissue accumulation, which can also interfere with lymphatic return in these regions and result in local edema. Affected horses with edema in these areas (especially when they are stall restricted for extended periods of time) usually experience resolution with exercise. Supraorbital fat accumulation is also a frequent finding. Equine metabolic syndrome is sometimes recognized when horses develop other medical problems, including colic, hyperlipemia, and reproductive issues. Horses with EMS may develop pedunculated lipomas at a younger age, presumably because of additional fat surrounding the abdominal viscera. Obesity has also been associated with abnormal reproductive cycling in mares.[74]

Regional adiposity is a feature of EMS, but it must be recognized that fat redistribution is also seen in older horses with PPID. It is therefore necessary to examine the patient for clinical signs of PPID, including delayed hair coat shedding, hypertrichosis, hyperhidrosis, skeletal muscle atrophy, polyuria, polydipsia, and/or recurrent pyogenic infections.[433] Affected horses can enter a *transition state* when PPID develops in an animal that has previously been diagnosed with EMS. Delayed hair coat shedding is often the first sign of this transition, and owners should be advised to watch for this change.

Horses with EMS commonly suffer from laminitis, which is recognized when lameness is detected, hoof testers are applied to the feet, or pedal radiographs are performed. Divergent growth rings indicating abnormal hoof growth can be observed in horses with or without a history of laminitis (Fig. 16.19). Some horses are not lame on physical examination but have radiographic evidence of third phalanx rotation or distal displacement. Laminitis often first develops after the horse has been grazing on pasture grass that is rich in NSCs. This happens at times of the year when grass is abundant and growing rapidly or when responding to cooler temperatures in the fall. Owners of horses with recurrent laminitis also report



FIG. **16.19** Abnormal hoof growth (divergent growth rings) from a horse with equine metabolic syndrome and a history of chronic laminitis.

that laminitic episodes occur after changes in season or with the onset of cold weather.

Clinical Pathology

Complete blood count and serum biochemical analysis results are usually unremarkable, with the exception of plasma glucose concentrations, which are discussed later. Hyperglycemia is more commonly associated with PPID and may be accompanied by mature neutrophilia and lymphopenia. Elevated serum triglyceride concentrations are sometimes detected in ponies[730] or horses[437] with obesity and IR, but this finding is inconsistent in horses with EMS. Horses with long-standing EMS have been noted to have mildly elevated γ-glutamyl transferase activity, a change that may be associated with hepatic lipidosis.

Low resting total triiodothyronine (tT_3) and total thyroxine (tT_4) concentrations are sometimes measured in horses with EMS, but the significance of this finding has been overstated. It is a common misconception that obese horses suffer from hypothyroidism because obesity is associated with hypothyroidism in dogs and humans. However, serum thyroid hormone concentrations rise appropriately when thyrotropin-releasing hormone is injected intravenously,[603] suggesting that the hypothalamic–pituitary gland–thyroid gland axis is functioning normally in these patients. It is therefore likely that low serum thyroid hormone concentrations simply reflect responses to extrathyroidal changes in metabolism[838]. Breuhaus and colleagues[839] detected lower serum total T_4 and free T_4 concentrations in horses with systemic illness. Phenylbutazone also significantly lowers serum tT_4 concentrations in horses,[440] and this drug is commonly used to treat laminitis in horses with EMS.

DIAGNOSTIC TESTING

Equine Insulin Resistance

Clinical Assessment of Insulin Resistance in Equids

Assessment of systemic insulin resistance is thought to be critically important to the definition of EMS, particularly given the recent direct association of increased plasma insulin concentration with laminitis.[732,733] IR in equids is frequently measured both clinically and in the research setting; however, very often the methodology used in these two settings is different, making comparisons between larger, clinical, population-based studies and smaller experimental studies difficult. Measurement of fasting serum insulin concentration (in conjunction with an assessment of plasma glucose concentration) is the most common field test, based on the availability of the methodology and the ease with which a single blood sample can be collected. However, dynamic aspects of insulin and glucose dynamics may not be revealed by a single sample; a single "normal" insulin concentration (which is a parameter that also currently requires additional definition in horses and ponies) often does not predict normal insulin sensitivity in response to an oral or IV glucose challenge, many of which have not been directly compared in the same cohort of horses. Preliminary work comparing the results of the basal insulin concentration, the oral sugar test, and the combined glucose and insulin test to those of the frequently sampled insulin-modified IV glucose tolerance test (FSIGTT) in the same cohort of 12 horses revealed the basal insulin concentration and oral sugar test (OST) to have inadequate sensitivity for use as screening tests in the diagnosis of IR in light breed horses; although abnormal results of these tests strongly suggest IR, normal results do not rule out disease.[840] Validation of some of the more clinically feasible tests (such as fasting serum insulin concentrations or the combined glucose and insulin test) against the gold standards in experimental studies (the euglycemic, hyperinsulinemic clamp or the frequently sampled intravenous insulin and glucose tolerance test with minimal model kinetic analysis) would be helpful in gaining acceptance of a standard methodology in measuring IR in horses and ponies for future research and clinical use.

Several laboratory tests have been evaluated and utilized in humans and horses for the diagnosis and monitoring of IR, including fasting plasma insulin and glucose concentrations,[437,833] the oral glucose tolerance test,[833] the IV glucose tolerance test,[841] the modified IV insulin and glucose tolerance test,[437] the IV insulin tolerance test,[833] the euglycemic/hyperinsulinemic clamp technique,[842] and the FSIGTT.[763] Of these methods, only the FSIGTT and the euglycemic/hyperinsulinemic clamp technique are well-established methods for accurate quantitative assessment of insulin action.[843]

Baseline Blood Tests

The baseline blood tests most commonly employed in evaluation of IR in horses include fasting glucose and insulin concentrations (Table 16.14). Other analytes, such as leptin[436,437] and high-molecular-weight adiponectin,[844] may be validated as useful diagnostic biomarkers in the future. Lipid metabolites (such as triglycerides and free fatty acids) may also provide additional information about the degree of IR and negative energy balance, and liver enzymes may serve to identify animals that are at risk for hepatic lipidosis. As part of the routine evaluation of a horse with IR and laminitis, it is also recommended to measure resting ACTH concentrations, particularly in geriatric animals.[433]

Measuring basal blood glucose and insulin concentrations has been recommended as a screening test for EMS; however, recent work suggests that although this test is quite specific, its sensitivity is likely unacceptable for a test to be used for rule-out screening.[840] Horses should not be provided feedstuffs containing greater than 10% NSC on a dry matter basis within

TABLE **16.14 Guidelines for Interpreting Baseline Glucose and Insulin Concentrations in Blood Samples from Fasted Horses**

Glucose (mg/dL)	Insulin (μIU/mL)	Interpretation[a]
<100	<20	Not consistent with insulin resistance (IR) Retest at a later date or perform the combined glucose-insulin test (CGIT) if the equine metabolic syndrome (EMS) phenotype is recognized Address obesity if present
<100	>20	Normoglycemia with hyperinsulinemia Horse displays compensated IR; there may be an increased risk of laminitis Recommend weight management plan, diet changes, and exercise
<100	>100	Normoglycemia with marked hyperinsulinemia Severe compensated IR; increased risk of laminitis Strictly control diet and consider pharmacologic intervention
>100	>20	Horse is losing its ability to regulate glucose associated with pancreatic insufficiency Transitioning from compensated to uncompensated IR; high risk of laminitis Test for pituitary pars intermedia dysfunction (PPID)
>120	<20	Glucose levels are unregulated and pancreatic insufficiency has developed Horse suffers from uncompensated IR; high risk of laminitis Check urine glucose for evidence of diabetes mellitus and test for PPID

[a]Patient is not in pain or stressed; all feed withheld for 6 hours before sampling.

6 to 8 hours of testing in order to achieve the most accurate interpretation of the results; however, there is debate about the proper dietary preparation for assessment of insulin and glucose dynamics in equids, and recommendations will likely be further clarified in the near future. A standardized method of pretest preparatory feeding has been suggested,[804] in which the horse in question is fed one flake of grass hay (less than 10% NSC) overnight (after 10:00 PM), with blood collected in the morning (ideally between 7 and 10 AM). Blood samples should be submitted for analysis to a laboratory performing a diagnostic test validated for use in equine samples. Horses are considered to have IR if they display fasting glucose concentrations greater than 110 mg/dL (6.1 mmol/L) and fasting insulin concentrations greater than 20 μIU/mL.[804]

There are two important limitations that must be considered when using this test for IR in horses. First, environmental conditions significantly affect results, so care must be taken to collect blood samples under standardized conditions. Second, insulin assays vary between laboratories, so different laboratories yield different results. This does not mean that laboratories are using inaccurate assays; it relates instead to the method used. It is therefore very important to interpret results according to the reference range provided by the laboratory. Clinicians are advised to send samples to a single laboratory and to select one that routinely handles equine serum or plasma. An insulin concentration above the reference range (for horses) provided by the laboratory defines hyperinsulinemia and therefore suggests IR. A resting insulin concentration above 20 μIU/mL defines IR if the horse has been deprived of feed for approximately 6 hours. If hay has been fed, a cutoff value of 30 μIU/mL can be used to define IR, but it is advisable to recheck the patient after feed deprivation. Note that some laboratories report insulin concentrations in pmol/L, and an approximate conversion factor of 7 is used to convert units from μIU/mL to pmol/L (i.e., 30 μIU/mL is approximately equal to 210 pmol/L). Units of μIU/mL may also be written as mIU/L.

In one cohort of Welsh and Welsh cross ponies, resting insulin concentrations greater than 32 μIU/mL had a sensitivity and specificity to predict laminitis of 100% and 78%, respectively, when the ponies were exposed to spring pasture.[436] Fundamental to the poor specificity of these basal blood tests, it is now recognized that many EMS-affected animals that exhibit abnormal hyperinsulinemia following dynamic challenge tests (enteral or parenteral) have normal basal insulin concentrations. In one report, evaluation of basal serum insulin concentration was normal (less than 20 μIU/mL in 12 of 12 horses); according to FSIGTT with minimal model analysis, 7 of these horses were actually insulin resistant.[840] Based on these results, basal insulin concentration, although specific, has insufficient sensitivity to be used as a single screening test for IR in equids. Marked interday variation in serum insulin concentrations has also been reported and represents an additional limitation to interpretation of this test.

The glucose-to-insulin ratio can be calculated from basal insulin and glucose concentration measurements by dividing the glucose concentration in mg/dL by the insulin concentration in μIU/mL (or mIU/L). The correct units must be used, so insulin concentrations reported in pmol/L must be divided by 7 to provide values in μIU/mL. Proponents of this test consider a ratio below 10 to be indicative of IR and refer to horses with ratios less than 4.5 as severely insulin resistant or decompensated. If a glucose concentration of 90 mg/dL is assumed, these cutoff values are equivalent to serum insulin concentrations of 9 and 20 μIU/mL, respectively; these values fall within most normal reference ranges for insulin in fasted horses. Glucose-to-insulin ratios below 10 are commonly detected in healthy Quarter Horse and Tennessee Walking Horse mares, and some fall below 4.5 (N. Frank, personal communication). Use of the glucose-to-insulin ratio therefore leads to the overdiagnosis of IR in horses. One other important limitation of the glucose-to-insulin ratio is that insulin results differ between laboratories, depending on the testing procedure used. However, most laboratories use the same colorimetric assay to measure glucose, so these results are more consistent. When resting insulin concentrations are interpreted independently, the reference range for the laboratory can be used to account for differences in methodology. However, this flexibility does not exist for the glucose-to-insulin ratio because set cutoff values have been established. It is therefore conceivable that insulin results from one laboratory will cause the ratio to be low,

whereas results from another laboratory will give a ratio that falls within acceptable range. It should also be noted that glucose concentrations decrease in blood samples if there is any delay between collection and centrifugation. Finally, a horse with uncompensated IR or diabetes mellitus will have a high glucose concentration with a low insulin concentration, and this may result in a glucose-to-insulin ratio that exceeds 10 and will therefore be considered normal. Care should therefore be used in interpretation of this ratio if it is used in the diagnosis and management of IR in horses.

Proxy estimates of insulin sensitivity and pancreatic β-cell function from single measurements of glucose and insulin have been used for diagnosis of IR.[730,731,804] Suggested proxies have included the glucose-to-insulin ratio, the reciprocal of the square root of insulin (RISQI; $\text{insulin}^{-0.5}$), the quantitative insulin sensitivity check index (QUICKI; 1/[log(insulin) + log(glucose)]), the homeostatic model assessment of insulin resistance (HOMA-IR; [glucose × insulin]/22.5), and the modified insulin-to-glucose ratio (MIRG; 800 − 0.3 × [insulin − 50]^2/[glucose − 30]),[731] the latter being an estimate of pancreatic β-cell responsiveness. These proxy measurements were useful for distinguishing groups of laminitis-prone and control ponies,[731] but they did not accurately predict predisposition to laminitis in individual ponies. Seasonal variation in the proxy measurements of insulin sensitivity also have been observed, confounding interpretation.[845]

Most insulin-resistant horses maintain blood glucose concentrations within the reference ranges provided by laboratories, as they are most frequently in a state of compensated IR (hyperinsulinemic euglycemia). However, glucose concentrations may tend to be toward the high end of the reference range, and affected horses are likely to have exaggerated blood glucose responses to high-NSC feeds. It is therefore important to deprive horses of feed (preferred approach) for 6 hours or to feed only hay the night before blood collection for testing. Most reference ranges for blood glucose in adult horses include values of 80 to 120 mg/dL, with values from fasted horses expected to be between 80 and 100 mg/dL, barring a significantly stressful blood collection. A blood glucose concentration above 120 mg/dL suggests hyperglycemia. Glucose concentrations reported in mmol/L must be multiplied by 18 to convert to mg/dL.

It is important to measure blood glucose concentrations, because examining only resting insulin concentrations would not allow detection of uncompensated IR or diabetes mellitus. Insulin concentrations can return to reference range in these patients as insulin secretion decreases due to pancreatic insufficiency. However, blood glucose concentrations rise as a consequence of IR and reduced insulin secretion, so patients can be identified by the presence of hyperglycemia (and sometimes glucosuria).

Serum triglyceride concentration may be a useful test for diagnosis and monitoring of EMS patients. Increased serum triglyceride concentration was associated with the EMS phenotype in a closed herd of Welsh ponies (97.2 ± 10.7 mg/dL for ponies with previous laminitis vs. 52.3 ± 3.6 mg/dL for non-laminitic ponies).[730] In another cohort of mixed-breed ponies, animals with the EMS phenotype had higher serum triglycerides in summer but not in winter.[799] A recent multicenter collaborative study of a large cohort of horses and ponies (n > 600) has also demonstrated that serum triglyceride concentrations are higher in animals with a history of laminitis and other features of EMS.[846]

Further refinement of the case definition of EMS will likely include measured plasma or serum adipokine concentrations. Increased leptin concentrations have been associated with IR and laminitis in horses and ponies.[436,437] In one cohort of ponies, leptin concentrations greater than 7.3 ng/mL had a sensitivity and specificity of 83% and 78%, respectively, to predict laminitis.[436] However, in recent studies, serum leptin was not useful for the identification laminitis-prone animals although, consistent with previous work in horses and other species, leptin concentrations were positively correlated with body condition score.[847] Immunoassays for adiponectin and high-molecular-weight adiponectin have been validated for horse serum,[844] with concentrations being higher in lean horses and lower in EMS and laminitic horses.[848] This analyte shows diagnostic potential when the assay becomes commercially available to equine practitioners and reference ranges are validated.

Dynamic Testing for Equine Insulin Resistance

When results of basal insulin concentrations are variable or when fasting insulin concentration is normal in the face of clinical or historical findings suggestive of EMS, dynamic evaluation of insulin sensitivity is recommended. These tests are also useful to assess severity of IR, to monitor disease progression, and to evaluate response to treatment. Dynamic tests include the IV glucose tolerance test (IVGTT), the combined glucose-insulin test (CGIT), the oral glucose tolerance test (OGTT)/oral sugar test (OST), and the two-step insulin response test (IRT). Methods for quantitative assessment of insulin sensitivity, such as the euglycemic hyperinsulinemic clamp and minimal model analysis of a frequently sampled insulin-modified IV glucose tolerance test (FSIGTT), provide valuable information in the research setting, but these tests are impractical for clinical use.[804]

The IVGTT has historically been one of the most frequently used methods for assessment of glucose tolerance in the horse.[804] Horses should be held off feed for 6 to 8 hours before testing, as for basal insulin concentration assessment. Dextrose (150–300 mg/kg BW; 50% solution) is administered intravenously after collection of a baseline blood sample, and subsequent blood samples are collected every 30 minutes for 180 minutes. Blood glucose should be measured with a handheld glucometer at baseline and in all samples collected after dextrose administration (this can easily be done in a point-of-care fashion). In insulin-sensitive horses, glucose concentrations should be back to baseline values by 150 minutes after dextrose administration. Horses with elevated blood glucose concentration at 180 minutes are likely to have IR. This test can be simplified for field use by collecting samples at 0, 150, and 180 minutes after dextrose administration.

The CGIT has been designed to assess insulin sensitivity by determining the time when blood glucose concentrations return to baseline values after the administration of simultaneous IV boluses of dextrose and insulin.[804] An IV catheter is placed before testing. The horse is fasted as for baseline insulin concentration assessment. After collection of a baseline blood sample, dextrose (150 mg/kg BW; 50% solution) is administered intravenously and immediately followed by IV injection of regular insulin (0.1 IU/kg BW). Blood samples are collected at 0, 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135, and 150 minutes to measure glucose concentrations. Insulin concentration can be measured in a sample collected 45 minutes

after administration. A horse is considered to have IR if blood glucose concentration is above baseline or insulin concentration is greater than 100 μIU/mL 45 minutes after dextrose administration (Fig. 16.20). This test can be performed in the field, with glucose concentrations measured stall-side with a handheld glucometer. Hypoglycemia may complicate this test, especially in insulin-sensitive individuals, and dextrose solution should be available to treat affected horses if needed. A modification of this test that may be more clinically useful for on-farm use is to collect a baseline blood sample, administer the proscribed doses of dextrose and insulin, and measure glucose and insulin concentrations 45 minutes after administration (with the results interpreted as previously outlined). Extrapolating the equine CGIT to donkeys and likely ponies can be misleading.[90] In insulin-sensitive donkeys, the glucose curve is shifted to the right, reaching nadir at 120 minutes compared with 75 minutes for horses, taking 240 minutes for glucose concentrations to return to baseline compared with 150 minutes for horses.[827,849] Moreover, 45 minutes after insulin administration, donkeys could have glucose concentrations above baseline, which under equine reference criteria would categorize them as insulin resistant.[849] This indicates that for an equivalent glucose and insulin dose, insulin-mediated glucose uptake (and perhaps pancreatic insulin release) in donkeys is delayed, supporting evolutionary differences in insulin sensitivity between equid species.

The OST has recently been recommended as a preferred method for dynamic assessment of insulin dysregulation in clinical settings. Light corn syrup is administered at a dosage of 15 mL per 100 kg BW after fasting as described earlier for other tests. Blood samples are collected 60 and 90 minutes after corn syrup administration for measurement of glucose and insulin concentrations. Animals that have insulin concentration greater than 60 μIU/mL at 60 or 90 minutes after administration are likely IR. Additionally, blood glucose concentrations greater than 125 mg/dL at 60 or 90 minutes are considered excessive and associated with IR.

An in-feed OGTT has also been described for use in the diagnosis of EMS. The horse is fed a meal of dextrose powder (1 g/kg BW) mixed with a small amount (about 1 lb or 450 g) of a low-glycemic feedstuff (hay chaff). A blood sample for measurement of insulin concentration is collected 2 hours after feeding; values greater than 85 μIU/mL are considered abnormal. For both the OST and in-feed OGTT, owners may be concerned about laminitis risk associated with the administration of sugar/glucose to insulin-resistant animals; however, this risk appears to be very small based on clinical experience. Additionally, investigators who have administered hundreds of OSTs to horses and ponies for epidemiologic and genetic studies of EMS have reported no new laminitic episodes immediately after testing (Dr. Jane Manfredi, personal communication); the risk appears to be very low.

A two-step insulin tolerance test has been designed in which the blood glucose response to an IV injection of insulin (0.1 U/kg regular human insulin) is assessed. Blood glucose concentration is measured at baseline and 30 minutes after injection of insulin. The time to reach 50% reduction of glucose from baseline is calculated from these two measurements. This test has been suggested to represent a safe, rapid, and low-cost method for diagnosis of equine IR and has recently been reported to correlate moderately to the OST in horses with EMS.[850]

Hyperinsulinemia in the Insulin-Resistant Equid

Insulin Clearance. Several methods have been described in the literature attempting to quantify insulin secretion in many species (including horses and ponies). Unfortunately, the methods that are technically simplest (such as measurement of insulin in peripheral venous blood following oral or IV glucose challenge) are heavily confounded by the effect of hepatic first-pass clearance on portal blood (which contains pancreatic insulin secretion). Plasma insulin concentration can be shown to readily increase in a dose-dependent fashion in response to a carbohydrate challenge (oral vs. IV), and this is presumed to result at least in part from pancreatic secretion. However, the relative contributions of altered insulin clearance and altered pancreatic secretion cannot be evaluated effectively with this sampling method and assay (even with analysis via minimal model kinetics).

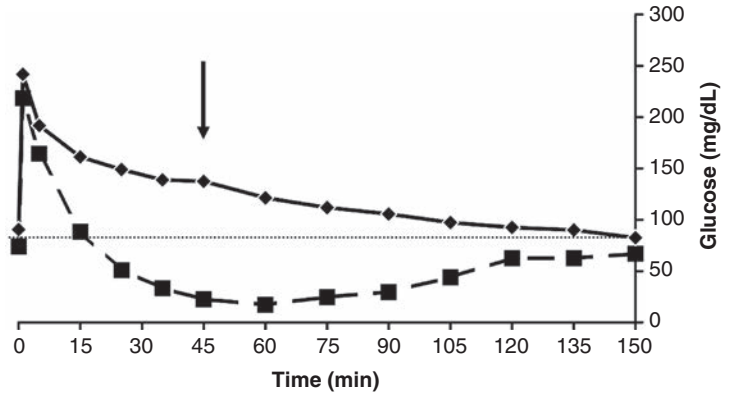


FIG. **16.20** Blood glucose concentrations during the combined glucose and insulin test (CGIT) in a healthy nonobese mixed breed horse *(dashed line)* and an obese insulin-resistant Morgan mare *(solid line).* The *horizontal line* represents the approximate baseline plasma glucose concentration, and the *arrow* indicates the time point (45 minutes after injection) selected to define insulin resistance using this test. Blood glucose concentrations remain above the baseline value for 45 minutes or longer if the horse is IR. Figure adapted from Eiler and colleagues.[827] (Courtesy Dr. N. Frank, Tufts University.)

Assessment of plasma C-peptide concentrations concurrently with determination of insulin concentrations might be more useful. C-peptide is a cleavage product of proinsulin that is secreted in equimolar amounts with insulin, but (in contrast to insulin) it is primarily excreted unchanged in the urine. Measurement of C-peptide concentrations would allow evaluation of β-cell function independent of hepatic action on pancreatic secretions and the use of this technique in the horse has been published.[759] However, C-peptide human-specific immunoassays require further validation in healthy and insulin-resistant horses and ponies. This method is also not commercially available for equine samples, limiting its use in clinical patients. Finally, single time point evaluations of plasma C-peptide concentrations (which would again be most useful for equine practitioners) have not been validated on a large scale, still requiring a glucose stimulation test to evaluate β-cell function.

Direct measurement of insulin secretion from pancreas would require sampling portal venous blood following enteral or IV carbohydrate challenge; this has been reported in experimental rodent models. This would be at best very challenging in horses and ponies, as surgical access to the portal vein is difficult and accompanied by significant risk to the animal. However, this remains the only way to truly accurately measure prehepatic insulin flux from the pancreas.[851-853]

Increased Insulin Release. Regarding the involvement of other hormones in insulin release from the pancreas, as described earlier, incretins have been shown to be critically important mediators of insulin release during increased plasma glucose concentrations (in fact, they have little effect on insulin secretion during euglycemia, increasing their attractiveness as therapeutic agents, as they are accompanied by little to no risk of hypoglycemic episodes, in contrast to insulin).[854] The enteroinsular axis is a poorly characterized system in the horse that likely contributes to equine metabolic disorders.[855] In general terms, this axis comprises factors from the gut that stimulate pancreatic islet cells but specifically refers to the incretins. Of the gastrointestinal hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide–1 (GLP-1) are considered incretins (which promote insulin release and suppress glucagon secretion). In other species, GIP is secreted by K cells (proximal small intestine) and GLP-1 by L cells (from proglucagon; distal small intestine). Nutrients within the small intestine (particularly carbohydrates) promote incretin secretion. Incretin activity explains the increase in insulin secretion that occurs before a rise in blood glucose, which is considered a preemptive mechanism to avoid hyperglycemia and facilitate peripheral tissue glucose uptake. Incretin mimetics (e.g., GLP-1 agonists such as exenatide and liraglutide) are used in humans and small animals for the treatment of type 2 diabetes mellitus. Dipeptidyl peptidase–4 (DPP-4) inactivates GIP and GLP-1. Based on this property, novel drugs (gliptins) that inhibit DPP-4 have been developed to prolong the incretin effect, promote insulin secretion, and reduce glycemia. These drugs have also been approved to be used alone or in combination with metformin to treat patients in which normalizing glycemia is difficult. In addition to their actions on the endocrine pancreas, GIP and GLP-1 have a multitude of other functions; they inhibit gastric emptying and acid secretion, promote insulin sensitivity, influence fat metabolism, and modulate skeletal muscle metabolism microvascular blood flow.[716] GIP enhances resistin secretion via MAPK, activates PI3K, and suppresses AMPK signaling.[714] Increased GIP concentrations have been linked to human obesity. Obese individuals have increased GIP levels and a higher response to a meal than lean individuals.[856] Impaired function of the enteroinsular axis has been linked to inadequate insulin secretion in people with type 2 diabetes mellitus. One can speculate from this information that incretin investigation should be considered a priority to better understand equine endocrinology and metabolism but also to elucidate the importance of the incretins in EMS. Few studies have investigated incretins in horses.[719,720,857]

The incretin effect has been shown to be present in equids,[719,857] and recent work involving administration of IV and enteral carbohydrate loads to ponies has suggested that activity of the enteroinsular axis (EIA) likely contributes to excessive postprandial hyperinsulinemia observed in these animals.[719] The EIA may ultimately be an effective therapeutic target for equids with EMS/IR, but in contrast to humans, the goal may be to suppress its activity to minimize insulin secretion in equids (rather than to promote insulin secretion, as in humans).

Another system that deserves further investigation as it relates to energy homeostasis in horses is the somatotropic axis. Consisting of growth hormone (GH) and IGF-1, the equine somatotropic axis is likely to be integrally involved with energy metabolism, insulin sensitivity, fat accumulation/distribution, and integumentary health. It is well accepted that GH has antiinsulin properties whereas IGF-1 has insulin-like actions and may be a vascular protective factor.[858,859] High and low GH concentrations have been linked to HMS, whereas low IGF-1 seems to be a consistent finding in affected individuals. A number of studies have measured GH and IGF-1 in horses and have associated their plasma concentrations with diet and BCS.[420,797] It is well documented in other species that GH can decrease insulin sensitivity by uncoupling PI3K signaling (a signaling pathway critical for insulin/growth factor receptor signaling; discussed earlier[710]). Increased GH and decreased IGF-1 concentrations have been associated with insulin resistance and the risk of developing metabolic syndrome.[860] It has also been suggested that low IGF-1 concentrations could be a useful marker to identify people at risk for IR and cardiovascular disease.[860] The role of the somatotropic axis in the pathogenesis of equine obesity and endocrinopathic laminitis has received little evaluation, although preliminary evidence suggests that serum IGF-1 concentrations are not increased in experimental animals subjected to a euglycemic hyperinsulinemic clamp (under the conditions of which lamellar gene expression of insulin receptor and IGF-1 receptor is reportedly decreased).[861] However, it is reasonable to assume that GH elevations could interfere with glucose uptake and lipid metabolism and contribute to the development of insulin resistance. Stimulation of the IGF-1 receptor may alter numerous events relative to laminitis including lamellar cell proliferation, cytoskeletal regulation, and cell adhesion processes, suggesting a connection between this signaling system and the pathogenesis of hyperinsulinemic laminitis.[861,862] This also supports potential involvement of the somatotropic axis in maintaining lamellar health.

Insulin degrading enzyme (IDE) is thought to be the primary enzyme responsible for insulin degradation following its release from the endocrine pancreas.[863] Although IDE is ubiquitously expressed (in insulin-responsive and non–insulin-responsive tissues, suggesting that it subserves alternate functions in different tissues), it is thought that hepatic IDE is

responsible for the degradation of the majority of plasma insulin (at least 60%).[863] Following binding to the insulin receptor, insulin and its receptor are internalized by endocytosis in a process that is enhanced by insulin signaling (negative feedback loop). Endosomal acidification is thought to be required for insulin release from its receptor (which can be recycled back to the plasma membrane); IDE can then degrade free insulin. IDE is present in the cytosol, cell membrane, and some organelles; it is also secreted into the extracellular space.[863] The insulin-induced IDE activity has been shown in hepatoma cell lines to be inhibited by elevated concentrations of glucose in the culture media, suggesting a mechanism by which increased alimentation (especially increased dietary NSC) could decrease liver insulin clearance and, consequently, inappropriate hyperinsulinemia.[864] Conversely, normal insulin signaling has been shown to upregulate IDE activity, most of these studies having been performed in the setting of experimental models of Alzheimer disease. (β-Amyloid is a target of IDE whose accumulation has been shown to correlate with increased risk for this condition.) Patients with Alzheimer disease show decreased IDE transcript and activity in brain tissue.[865] Further, IDE-knockout mice display hyperinsulinemia, decreased glucose tolerance, and type 2 diabetes mellitus, implicating the enzyme in some of the most characteristic aspects of the metabolic syndrome.

It is certainly possible that manipulation of insulin clearance (e.g., through pharmacologic activation of IDE or modification of the diet to increase hepatic insulin clearance) might be useful in the management of EMS. However, first it must be established that altered insulin clearance is responsible for the majority of the hyperinsulinemia observed in these patients (e.g., versus exuberant pancreatic insulin secretion in response to insulin resistance). This has not been demonstrated in equids. Experimentally, particularly if budget and time were not limiting factors, comparison of serum insulin concentrations across the liver (i.e., in portal venous blood and systemic venous blood [ideally right atrial blood]) in response to both oral and IV glucose tolerance tests with frequent sampling would identify the relative contributions of pancreatic secretion and hepatic clearance of insulin to the systemic insulin concentrations observed following the testing. Because of the technical difficulty associated with this technique, evaluation of C-peptide responses in concert with insulin responses to an oral and IV glucose tolerance test might be an easier, more efficient way to arrive at the same answer.

Insulin Signaling and Insulin Resistance. Recent research in humans and experimental rodent models has revealed the importance of aberrant insulin signaling in both the pathogenesis of the metabolic syndrome and the risk of adverse consequences in affected patients.[866,867] Not only is basal and stimulated hyperinsulinemia considered a marker/diagnostic criterion for at-risk individuals, it is also considered to play direct and indirect roles in the pathophysiology of diseases associated with metabolic syndrome.[782] Knowledge of insulin signaling in health and mechanisms by which it becomes dysfunctional in the insulin-resistant individual is imperative for the design of rational treatment and prevention programs.

Glucose, the primary carbohydrate fuel of cellular intermediary metabolism, crosses cell membranes via facilitated diffusion through various members of a family of glucose transport proteins (GLUTs), of which 14 have been described in mammalian systems to date.[868] In the postprandial state, elevation in plasma glucose concentration following its absorption from the gastrointestinal tract is a potent secretagogue for insulin from β cells in the islets of Langerhans of the endocrine pancreas. Secretion of insulin from the endocrine pancreas results in increased plasma concentration of this hormone and its subsequent delivery via the systemic circulation to its target tissues (primarily skeletal muscle, adipose tissue, and liver); here insulin binds its receptor present on the cell surface.[869]

Binding of insulin to its receptor, a heterotetrameric receptor tyrosine kinase composed of two α and two β subunits, results in autophosphorylation of critical cytoplasmic tyrosine residues on the β-chains of the receptor and recruitment of insulin receptor substrate (IRS) proteins to the cytoplasmic side of the plasma membrane.[870,871] Although several IRSs have been described (at least four), IRS-1 and IRS-2 primarily subserve the functions of signal transduction through the insulin receptor (IRS-1 is of primary importance in skeletal muscle and adipose tissue; IRS-2 is of greater importance in liver).[872] IRSs contain multiple tyrosine and serine phosphorylation sites; in general (with a few exceptions), tyrosine phosphorylation of IRSs by activated insulin receptor results in conformational changes in IRSs that activate and promote their function as docking proteins, recruiting additional SH-2 domain-containing molecules involved in insulin signal transduction through two main pathways.[872] Serine phosphorylation of IRSs is broadly inhibitory to their function in recruiting downstream effectors of signaling; although more than 50 canonical serine phosphorylation sites have been described on IRS-1, the functions of all of which have not been fully characterized to date, phosphorylation of some of these (such as Ser312/307) results in retention of IRS-1 in the cytosol in association with 14-3-3 proteins and enhanced IRS-1 ubiquitination and degradation. In this way, serine phosphorylation of IRSs serves as an important site of negative feedback of insulin action; it is also a primary mechanism responsible for acquired insulin resistance in pathologic states, such as obesity and inflammation.[867]

As mentioned, IRSs recruit effectors of two intracellular signaling cascades that are activated by insulin binding to its receptor: the mitogen-activated protein kinase (MAPK) pathway, responsible for the mitogenic and differentiation effects of insulin, does not play a significant role in glucose metabolism. Activation of the phosphatidylinositol-3-kinase (PI3K) pathway is responsible for the increased transcellular glucose conductance observed following insulin binding. Tyrosine phosphorylation of IRS-1 and/or IRS-2 results in recruitment of the p85 subunit of PI3K to the plasma membrane, where its primary substrate(s) phospholipids are located. Phosphorylation of PIP2 by the catalytic p110 subunit of PI3K and subsequent generation of PIP3 results in activation of phosphatidylinositol-dependent protein kinase (PDK1). PDK1 phosphorylates PKB/Akt, a kinase that then phosphorylates Akt substrate of 160 kDa (AS160).[752,871,873-875] Glucose transporter type 4 (GLUT4), the primary insulin-responsive glucose transporter protein in skeletal muscle, is sequestered in cytoplasmic vesicles in the absence of insulin receptor activation; AS160 is involved (likely with many other molecules) in promoting this retention. On phosphorylation and inhibition of AS160, GLUT4-containing vesicles translocate to and fuse with the plasma membrane, resulting in increased cell surface expression of GLUT4. Plasma glucose then is allowed to enter skeletal muscle cells through GLUT4-facilitated diffusion, until these transporters are resequestered following cessation of the insulin signal.[869] Other GLUTs are expressed in skeletal

muscle (e.g., GLUT1, GLUT2); however, they are not insulin responsive and their involvement in glucose transport into skeletal muscle is considered minor in comparison with that of GLUT4.[868] Intracellular glucose trapping and prevention of back-diffusion through GLUT4 are prevented via rapid phosphorylation of intracytoplasmic glucose to glucose-6-phosphate (which does not pass through GLUTs) by glucokinase/hexokinase. These are the essential steps that promote insulin-mediated glucose movement from the systemic circulation into the skeletal myocyte, which is primarily responsible for insulin-mediated plasma glucose disposal postprandially.

Insulin Resistance/Dysregulation in Obese Horses with Equine Metabolic Syndrome. Relationships between obesity and IR are complex. It should be remembered that *not all obese horses are insulin resistant and IR is not always accompanied by obesity.* However, these conditions are associated, and there is evidence that IR predisposes ponies to laminitis. Adipose tissues expand as obesity develops through an increase in adipocyte size (hypertrophy) and/or number (hyperplasia). This is manifested as generalized obesity or regional adiposity, and either can be accompanied by IR. In horses, regional adiposity increases the size of adipose tissues within the nuchal crest, and neck circumference has been negatively correlated with insulin sensitivity in obese insulin-resistant horses. Fat thickness over the rump has also been used as a measure of body fat mass in horses, and some horses develop enlarged fat pads in this area.

Horses that are evolutionarily adapted to sparse forages and harsher conditions may be predisposed to obesity because lipid accumulates more readily when excessive calories are provided in the diet. This state of overnutrition is often induced when genetically predisposed horses are turned out on pasture. The natural equine diet contains little fat, but excess glucose can be converted into fat via de novo lipogenesis. Fats are used for energy or stored as triglyceride within cells. When the storage capacity of adipose tissues is exceeded, fats are directed toward nonadipose tissues (repartitioning). Skeletal muscle, liver, and pancreatic tissues attempt to utilize fats by increasing β-oxidation, but lipid can accumulate within these tissues and alter normal cellular functions. This pathophysiologic mechanism is sometimes referred to as lipotoxicity.

Reactive oxygen species are also generated as oxidative pathways are upregulated. Oxidant damage contributes to lipotoxicity and lipid-induced programmed cell death (lipoapoptosis). Inflammation accompanies these events, resulting in the release of TNF-α and IL-6 from adipose tissues. Adipocytes release these proinflammatory cytokines that act locally or enter the blood. Hormones and cytokines released from adipose tissues are referred to as adipokines. Leptin is an adipokine that is involved in the satiety response within the brain. Obese and insulin-resistant horses often have higher plasma leptin concentrations.[436,796,876] In contrast, the release of adiponectin decreases with obesity and IR, and this may serve as a marker for EMS in the future.[798,844,877] Insulin resistance develops when insulin-sensitive adipose, liver, and skeletal muscle tissues fail to respond normally. Insulin receptors, downstream signaling pathways, and glucose transporters such as GLUT4 are potential sites of dysregulation. More insulin is released from pancreatic β-cells to compensate for reduced tissue insulin sensitivity, which results in hyperinsulinemia. Pancreatic insufficiency develops as a result of chronic IR or the effects of lipid accumulation within pancreatic tissues. Uncompensated IR occurs when the pancreas fails to sustain the higher rate of insulin secretion necessary to compensate for reduced tissue insulin sensitivity, and hyperglycemia develops as a consequence.

All of the pieces of the puzzle must be assembled before we can fully understand the relationship between IR and laminitis in horses and ponies. At present, we are limited to the knowledge that IR predisposes ponies to pasture-associated laminitis and that the disease can be experimentally induced by infusing insulin intravenously over several days. There are three broad mechanisms by which IR and obesity could predispose horses to laminitis: (1) altered blood flow or endothelial cell dysfunction within blood vessels of the foot, (2) impaired nutrient delivery to hoof tissues, or (3) a proinflammatory or prooxidative state induced by chronic obesity and IR. These processes are likely to contribute to the lower threshold for laminitis recognized in horses with EMS.

Lean Horses with Equine Metabolic Syndrome. Our current understanding of chronic IR in the lean horse is very limited, but several explanations have been proposed. One explanation is that the liver responds differently to nutrients in affected animals. In one study, ponies with IR showed exaggerated insulin responses to a meal containing fructose, which suggests that the liver responds differently to certain nutrients in horses with EMS and this may represent *hepatic insulin resistance.* Another proposed cause of IR in leaner animals is the increased production of cortisol locally within adipose tissues surrounding the abdominal organs. This is thought to result from increased 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) activity within visceral adipose tissues, which increases local cortisol production and leads to the development of IR. Johnson referred to this condition as *peripheral Cushing disease,* but this term is not recommended because it is easily confused with equine Cushing disease, which results from pituitary pars intermedia dysfunction. Further studies are required to determine the activity of 11β-HSD1 in horses with EMS.

Equine Metabolic Syndrome–Associated Laminitis (Endocrinopathic Laminitis). The equine digital laminae are a unique integumental structure in which the adhesion of the epidermal laminae (continuous with the hoof wall) to the dermal laminae (attached to the third phalanx; P3) allows for the suspension of P3 (and therefore the entire musculoskeletal system and weight of the horse) within the hoof wall. The cell type responsible for this adhesion, and which is the site of dysadhesion leading to laminar failure in laminitis, is the laminar basal epithelial cell (LBEC). The LBEC is a polarized, highly differentiated epithelial cell with critical adherence functions on its basement membrane aspect, maintaining adequate adhesion capacity to support the weight of the horse within the hoof wall. In addition to this essential function, the equine LBEC behaves similarly to keratinocytes in other locations (and species), responding to extracellular events via a multitude of signaling mechanisms related to inflammation, endocrine/paracrine factors, and epithelial stress in sepsis-related laminitis. Although there has been a great deal of effort put forth to find a common signaling pathway by which diverse systemic diseases can lead to laminitis/laminar failure, it is more likely that the reason various pathologic entities result in laminar dysfunction is that any insult to the LBEC alters their polarity, resulting in basement membrane dysadhesion and laminar separation due to the incredible stresses placed on the digital laminae. Thus the cause of laminar failure in pasture-associated laminitis (PAL) may bear little similarity to that occurring

in the other types of laminitis regarding extracellular events or even cell signaling; however, a similar laminar dysfunction results due to unique disarray of cytoskeletal and adhesion functions and subsequent dysadhesion of the LBEC from the underlying basement membrane.[794]

Although equine laminitis has been associated with many diverse causes (including gastrointestinal tract disorders, retained placenta or metritis, severe infections, and excessive weight bearing),[793] PAL is now reported to be the most common cause of laminitis, affecting primarily horses and ponies with a phenotype of obesity (especially regional adiposity) and insulin resistance when they are exposed to NSC-rich pastures.[436,730,751] Clinical observations and epidemiologic studies support that risk of PAL is highest when equids are grazing pastures with high NSC content (i.e., fructans, simple sugars, and/or starch), and theories on the pathogenesis of PAL have been extrapolated from experimental models of alimentary carbohydrate overload. However, the extent to which these severe models reflect events during development of EMS-related laminitis may be questioned. First, it is likely that the amount and rate of starch or fructan administered for induction of carbohydrate overload in these studies far exceeds the quantity of NSC ingested by grazing equids. Second, previous studies have not used animals with an EMS phenotype and thus have not addressed the interaction between diet, phenotypic, and genetic factors associated with laminitis susceptibility.

The LBEC provides the load-bearing capability of the digital laminae through its adherence to its associated basement membrane, neutralizing forces both from the weight of the animal and ground reaction. Its function is critically important, and separation/failure of the LBEC from the basement membrane results in detachment of the dermal and epidermal laminae, which often results in displacement of the pedal bone within the hoof capsule. As previously mentioned, the LBEC is a highly polarized epithelial cell whose function is critically dependent on its adherence both to its neighboring epithelial cells and to its anchoring basement membrane. It is tempting to speculate that decreased adenosine-5′-monophosphate-activated protein kinase (AMPK) activity (which has been recently observed in laminar tissue of ponies challenged with an increased dietary carbohydrate load, a stimulus that has been associated with induction of laminitis in IR equids[878]) might induce loss of cytoskeletal polarity of the laminar basal cell (such as has been observed in vitro in other epithelial cell model systems[879]) and subsequent detachment from its basement membrane. Loss of polarity of epithelial basal cells and decreased maintenance of epithelial tight junctions may lead to mechanical disruption of the laminar interface secondary to constant tension from the deep digital flexor tendon. Recent evidence of decreased AMPK phosphorylation in a model of endocrinopathic laminitis[878] suggests that laminar decreased AMPK activity may be pathologic; however, it is unclear what role it plays in normal and abnormal laminar physiology. Although the net effects of AMPK activation within the digital laminae are unclear currently, it is reasonable to assume that it plays an important role in this highly polarized cell type, as it has been shown to do in diverse epithelial cell types in other species.

Laminitis is associated with a diverse range of clinical diseases in horses, from sepsis to endocrine diseases associated with IR. IR, which has been shown to occur in obesity and in sepsis in horses, can result in hyperinsulinemia due to decreased glucose transport into insulin-responsive tissues (especially skeletal muscle). Although IR and subsequent glucose deprivation have been suggested in the past as potential mechanisms underlying laminar failure in equids with EMS, it has recently been shown that the primary glucose transporter type expressed in laminar tissue is GLUT1[880], which is neither insulin dependent nor highly insulin responsive.[128] The digital laminae may actually experience an increased glucose flux in insulin-resistant individuals, particularly after consumption of a high-NSC meal. Increased glucose uptake (passive and concentration-dependent through GLUT1) is associated with increased ATP production and a decreased intracellular AMP:ATP ratio, conditions in which AMPK phosphorylation (and activation) would be expected to be decreased.[881] If LBEC polarity (assumed to be important for their proper function) is similarly dependent on AMPK function as has been shown in other epithelial cell types in vitro,[882] this scenario may logically result in laminar failure (which is observed clinically).

Hyperinsulinemia has been repeatedly identified as an important risk factor for the development of endocrinopathic laminitis, reported by several groups independently in cross-sectional studies.[437,730,801] More recently, a direct link between insulin and laminitis has been identified through the experimental induction of laminitis via iatrogenic hyperinsulinemia in normal ponies[732] and Standardbred horses.[733] Insulin is a pleiotropic hormone whose functions have been classically limited to regulation of carbohydrate, lipid, and protein metabolism in times of energy excess (i.e., in the postprandial period).[883] However, signaling through the insulin receptor has been shown to activate not only pathways that direct the fates of nutritional metabolites through PI3K activation but also regulation of cellular replication and extracellular matrix production through activation of MAPK pathways.[871] Direct effects of insulin on laminar epidermal basal cells, with subsequent alterations of their extracellular matrix (which supports the integrity of the laminar basement membrane), may play a role in endocrinopathic laminitis. Recent studies reporting little evidence of insulin receptor expression on laminar epidermal cells make this theory less likely.[758] Insulin signaling through its receptor may have a greater effect on the perfusion of the laminae, as the insulin receptor appears to be expressed primarily within the laminar microvasculature.[758]

Incubation of endothelial cells with increased concentrations of insulin in vitro has been shown to alter their insulin responsiveness, inducing cellular insulin resistance after a relatively short exposure (24–48 hours).[884] Interestingly, it is after approximately 72 hours of experimental hyperinsulinemia that horses and ponies are observed to become laminitic.[732] Rapid downregulation of surface expression of the insulin receptor has been documented on endothelial cells in response to treatment with exogenous insulin, which may partially explain the endothelial insulin resistance observed in response to hyperinsulinemia in vivo. Signaling through the insulin receptor also tends to be self-limiting[872]; this mechanism for insulin resistance may be more important in the laminae, where endothelial expression of the insulin receptor appears to be increased in the setting of hyperinsulinemia.[758] Altered vascular responsiveness in insulin-resistant individuals is thought to result from decreased insulin-mediated elaboration of nitric oxide (NO) and its vasodilatory effects on vascular smooth muscle.[885] Attenuation of PI3K signaling results in decreased activity of endothelial NOS (eNOS) and decreased NO production. Concurrently, whereas defective PI3K signaling is observed from the insulin-bound insulin receptor in endothelial IR, signaling through the MAPK

pathway appears to be preserved. Increased production of the vasoconstrictor endothelin-1 through this pathway, along with decreased NO elaboration, is thought to result in inappropriate vasoconstriction, failure to achieve flow-dependent vasodilation, and hypertension. Local perfusion, particularly in those tissues lacking collateral circulation, may be inadequate and may activate stress kinase systems, such as MAPK and AMPK. NO also has been reported to have important antiinflammatory and anticoagulant effects, loss of which has been associated with the propagation of atherothrombotic lesions in humans and experimental animal models of metabolic syndrome.[875] In horses with IR, pathologic changes in vasomotor tone to a tissue with poor collateral circulation (the digital laminae) may promote hypoxia/ischemia, both of which have been reported to play roles in the pathogenesis of laminitis.[886] Thrombi have been noted within the laminar microvasculature in experimental and naturally occurring laminitis.[887]

Insulin may also be triggering signaling in laminar epithelial cells through the IGF-1 receptor, not the insulin receptor (which does not appear to be present at significant levels on laminar epithelial cells). Insulin has been shown to bind and activate IGF-1R at supraphysiologic concentrations in cell cultures (concentrations that have been observed in insulin-resistant equids in response to a dietary carbohydrate challenge),[888] and IGF-1R's distribution within the laminae appears to be much more extensive than that of the insulin receptor.[758] Whereas the insulin receptor appears to be limited to the laminar microvasculature, IGF-1R is present on laminar epithelial cells, endothelial cells, and fibroblasts (and likely other cell types). The importance of IGF-1R signaling in the laminar epidermis, particularly its effects on extracellular matrix and cytoskeletal components, is currently unclear but is an active area of investigation.

Necropsy Findings

Horses and ponies with EMS are obese or exhibit regional adiposity and have evidence of laminitis. Hepatic lipidosis has been noted on some pathology reports, but it is unclear whether this simply reflects increased lipid storage or provides evidence of a pathologic process.

Management

Management of IR involves three approaches: (1) reducing the sugar and starch content of the feeds provided to the horse, (2) limiting or eliminating access to pasture until IR improves, and (3) increasing exercise. The first approach involves selection of hay with a lower sugar and starch content, which can be determined by submitting a sample for analysis. Equi-Analytical Laboratories analyzes hay and provides starch, ethanol-soluble carbohydrate (ESC), and water-soluble carbohydrate (WSC) content. ESCs include simple sugars such as monosaccharides and disaccharides, whereas WSC includes fructans as well. The nonstructural carbohydrate (NSC) content of the hay should be calculated from the values provided by the laboratory. Unfortunately, researchers disagree about the appropriate method of calculating NSC content. Some think that the WSC must be included, so NSC is calculated by adding WSC and starch values together. However, others argue that only ESC is relevant because fructans are primarily digested in the large intestine and therefore do not contribute to the rise in blood glucose concentrations after a meal. At present, we recommend that NSC be calculated by adding starch and ESC together, and this level should ideally fall below 10% as-fed in the hay. It is important to recognize that the relative importance of NSC intake varies with the severity of IR. Horses with severe IR (resting insulin concentration greater than 100 μIU/mL) must be maintained on a stringent diet with an NSC content less than 10%, whereas mildly affected animals can be fed hay with higher levels. If hay has already been purchased and the NSC level is between 10% and 20%, soaking the hay in cold water for 3-6 hours before feeding should lower the sugar content to a level that is safer for EMS-affected horses.[888a]

Leaner horses with EMS are challenging to manage from a dietary standpoint because more calories must be provided without exacerbating IR. This can be accomplished by feeding one of the commercial low-sugar/low-starch (low-NSC) pelleted feeds that are now available to horse owners. These feeds vary in their NSC content, so the severity of IR must be taken into account before one is selected. It is also better to divide the daily ration into multiple small meals and to feed hay beforehand to slow gastric emptying. These strategies are employed to lower the glycemic response to the meal, which is the degree to which blood glucose concentrations rise in response to the feed. Individual horses may respond differently to the same feed, so it is advisable to recheck glucose and insulin concentrations 7 to 14 days after a new diet has been initiated. Severely insulin-resistant horses are particularly difficult to manage and may require individual diet formulation, with the help of a nutritionist. Rinsed and then soaked molasses-free beet pulp can be fed as a lower cost alternative to commercial feeds. This feed has a low glycemic response yet provides calories through hindgut fermentation and volatile fatty acid production.

Weight loss should be induced in obese horses by restricting the total number of calories consumed. In horses that are being overfed, removal of all concentrates from the diet is often sufficient to achieve the ideal body weight. Total caloric intake should initially be met by feeding hay in amounts equivalent to 1.5% to 2.0% of current BW (e.g., 18–24 lb [8–11 kg] hay per day for a 1200-lb [540 kg] horse). If the horse does not lose weight, the amount fed should be lowered over several weeks to 1.5% of ideal BW (e.g., 15 lb [6.8 kg] hay for an ideal weight of 1000 lb [450 kg]). The horse should not be restricted to less than 1.0% of its ideal body weight in forage daily at any point; this represents severe caloric restriction and, particularly when restricted acutely, may increase risk of hyperlipidemia/hyperlipemia in EMS-affected equids.

These strategies are effective for horses kept in stalls or dirt paddocks, but weight loss is more difficult to achieve when horses are grazing on pasture. Strategies for limiting grass consumption on pasture include short (less than 1 hour) turnout periods; confinement in a small paddock, round pen, or area enclosed with electric fence; or use of a grazing muzzle. Appropriate vitamin and mineral supplements should be provided to horses confined to dirt paddocks or stalls. Pasture access must remain restricted or eliminated until insulin sensitivity has improved because carbohydrates consumed on pasture can trigger gastrointestinal events that lead to laminitis in susceptible horses. Grass is also unregulated source of starch and sugar, so these carbohydrates will continue to exacerbate IR, even when other aspects of the diet have been controlled.

Laminitis must be considered when managing horses with EMS, and horses with recurrent laminitis may require permanent restriction of their pasture access. These patients can be housed in dirt paddocks so that they are able to exercise once laminitis is controlled. Mildly affected horses can be returned

to pasture if IR can be controlled, but care should be taken to restrict pasture access when the grass is going through dynamic phases, such as rapid growth in the spring or senescence in the fall. Measurement of pasture grass NSC content at different times of the day has revealed that grazing in the early morning is likely to be safer for horses with IR, except after a hard frost when grasses accumulate sugars.

Pharmacotherapy

Most horses or ponies with EMS can be effectively managed by lowering the sugar and starch content of the diet, reducing body fat mass if the animal is obese, instituting an exercise program, and limiting or eliminating access to pasture. However, it takes time for these management changes to take effect, so pharmacologic interventions can be considered to accelerate the improvement if the patient suffers from recurrent laminitis. Two options are currently available to clinicians, and each has its advantages and disadvantages. Levothyroxine sodium can be administered to accelerate weight loss and improve insulin sensitivity in obese horses but should be avoided in leaner horses because of the reduction in BW. Metformin may also be useful; this drug improves insulin sensitivity in insulin-resistant horses and ponies without affecting body weight[838]. However, the long-term effects of metformin on insulin sensitivity have not been determined, and the safety of this drug must be evaluated in horses.

Levothyroxine Sodium

Weight loss can be accelerated and insulin sensitivity improved in obese insulin-resistant horses when levothyroxine sodium is administered orally at a dosage of 48 mg (total per 500-kg horse) per day for 3 to 6 months. Smaller ponies and Miniature Horses can be given 24 mg of levothyroxine sodium per day for the same time period. Thyro L (Lloyd, Inc., Shenandoah, IA) is recommended for this purpose, and 1 teaspoon (tsp) of powder provides 12 mg of levothyroxine sodium, so 4 tsp should be administered in the feed or by mouth once daily. When levothyroxine treatment is discontinued, horses should be weaned off the drug by lowering the dosage to 24 mg (2 tsp) per day for 2 weeks and then 1 tsp (12 mg) per day for 2 weeks. The benefits of treating horses with levothyroxine at lower dosages for longer periods have not been evaluated.

Measured serum total thyroxine (tT_4) concentrations are elevated when levothyroxine is administered at a dosage of 48 mg (4 tsp) per day, but levels vary considerably within and between horses. Serum tT4 concentrations often range between 40 and 100 ng/mL in treated horses, indicating that levothyroxine sodium is being given at a supraphysiologic dosage. However, clinical signs of hyperthyroidism such as sweating or tachycardia have not been observed in treated horses. It is important for horses to be placed on a controlled diet when levothyroxine sodium is administered because it has been subjectively observed that feed intake increases in response to treatment. Weight loss is unlikely to occur if the horse is given free access to pasture, which will be perceived by owners as treatment failure.

Metformin

Energy Regulation and Insulin Resistance: Adenosine-5′-Monophosphate-Activated Protein Kinase. Adenosine-5′-monophosphate-activated protein kinase (AMPK) is a eukaryotic heterotrimeric protein complex that is highly conserved among phyla and widely distributed in most tissue types of multicellular organisms.[889,890] Activation of AMPK kinase activity results in broad changes in carbohydrate, lipid, and protein metabolism that in general serve to decrease energy consumption (anabolic pathways, such as glycogen, lipid, and protein synthesis) and increase energy production (catabolic pathways, such as glucose uptake, glycolysis, glycogenolysis, lipolysis, lipid oxidation).[891]

The role of AMPK in skeletal muscle has been shown to be responsible (at least in part) for the insulin-sensitizing and non–insulin-dependent effects on glucose transport following exercise. AMPK activation, which may occur in exercising muscle via several mechanisms (increased AMP:ATP ratio with fatiguing exercise [LKB1], increased cytoplasmic calcium concentration [CaMKK]),[892] results in phosphorylation of AS160 and translocation of GLUT4 to the cytoplasmic membrane in the absence of insulin signaling; AMPK also enhances the activity of insulin signaling at multiple levels (most importantly, IRSs), but increased glucose disposal during exercise has been shown to be non–insulin dependent (at least in part).[893]

AMPK may display significant cross-talk with insulin (and IGF-1) signaling at multiple levels.[894] For example, AMPK has been shown to phosphorylate IRS-1 at a serine site that increases its affinity for PI3K, an effect that increases insulin signaling (and thereby promotes insulin sensitivity). AMPK also promotes the oxidation of accumulated ectopic lipid in multiple tissues, lipids that likely signal through PKCè to inhibit IRS-1 and insulin signaling (again, promoting increased insulin sensitivity).[895] AMPK may also directly phosphorylate and inhibit the activity of AS160, which serves to retain GLUT4 in cytoplasmic vesicles in the absence of insulin signaling; this is likely the mechanism (or at least part of the mechanism) by which AMPK elicits non–insulin-dependent GLUT4 translocation and increased glucose disposal that is observed with muscle contraction.[894] Conversely, Akt/PKB (an important kinase effector of the PI3K pathway, signaling downstream of insulin receptor) may phosphorylate AMPK alpha subunits and inhibit their phosphorylation by LKB1. Insulin/AMPK interactions are very complex and may ultimately be determined to be highly tissue specific in the setting of metabolic syndrome.

The liver, although insulin responsive in many ways, is not dependent on insulin for many of its functions in glucose metabolism. Insulin signaling in the liver tends to inhibit glycogenolysis and gluconeogenesis, encouraging instead glycogen storage; these effects are attenuated in the setting of insulin resistance, with persistent gluconeogenesis and exacerbation of hyperglycemia (especially in patients with type 2 diabetes mellitus).[896] In skeletal muscle, however, glucose uptake occurs primarily through GLUT4, which is largely insulin responsive.[897] In muscle, insulin resistance is associated with decreased glucose disposal and the accumulation of intramyocellular lipid.[898-900] Although the role of AMPK in liver and skeletal muscle is likely complex and affected by multiple inputs, it has been suggested since the late 1990s that AMPK activation could potentially reverse many of the characteristic abnormalities of carbohydrate and lipid metabolism that are observed in insulin-resistant individuals. This has led to the development and widespread clinical use of functional AMPK agonists, such as metformin, in the treatment of humans with the metabolic syndrome and type 2 diabetes mellitus.[901]

Administration of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMPK agonist, to healthy horses

reduced blood glucose and increased insulin concentrations, increased skeletal muscle phosphorylated AMPK, with no effect on GLUT4 and GLUT1, but increased GLUT8 protein.[902] This information supports the use of AMPK agonists to promote glucose uptake in horses with clinical evidence of IR.

Metformin, a functional AMPK agonist, is an orally bioavailable biguanide compound that has been used clinically as an antihyperglycemic and insulin-sensitizing therapeutic in the treatment of human metabolic syndrome and type 2 diabetes mellitus.[901] The pharmacokinetics and pharmacodynamics of this drug have been well characterized in humans; however, although metformin has been used empirically to treat equids afflicted with EMS[903] (and some pharmacokinetic studies have been published[904,905]), peer-reviewed pharmacodynamic information regarding metformin in equids is limited.[905,906] When the effects of metformin on insulin sensitivity were assessed in horses and ponies with IR, a positive response was detected initially without hypoglycemia developing, but long-term results were variable.[905] Metformin was administered at a dosage of 15 mg/kg orally twice daily in the aforementioned study, but the pharmacokinetics of this drug have recently been examined, and results suggest that the drug should be given every 8 hours (Anna Firshman, personal communication). Further studies are required to assess the safety and efficacy of this treatment for IR.

Additionally, it is now recognized that AMPK may have profound effects on the integrity and confluence of epithelial cells in cell culture, including their ability to remodel tight junctions following application of a cellular stressor.[882] AMPK, then, may provide an attractive link between dietary carbohydrate content, insulin sensitivity, and laminar epithelial dysfunction. Investigation of the effects of AMPK activation in the digital laminae in response to dietary carbohydrate content may open new avenues of inquiry regarding the pathophysiology of endocrinopathic laminitis, ideally leading to the development of novel therapies for treating this difficult disease.

Other Treatments for Equine Insulin Resistance

Numerous other treatments have been proposed for the management of IR in horses including magnesium supplementation, chromium, clenbuterol, cinnamon, and thiazolidinediones. Each of these therapies may be of some benefit, but further studies are required to establish their efficacy; the studies that have been performed to date do not support a significant therapeutic effect in horses. For example, multiple studies have been published investigating both the pharmacokinetics[907] and pharmacodynamics[908,909] of pioglitazone (a thiazolidinedione drug and a peroxisome-proliferator activated receptor-gamma agonist); although this drug appears to be orally bioavailable in horses, it appears to have relatively little insulin-sensitizing effect in this species, at least when administered over the short term. Additional study may reveal a utility for this class of drug in insulin-resistant equids, but current knowledge does not suggest strong therapeutic benefit for patients with EMS. Regarding neutraceutical preparations, although little research exists to guide rational clinical use of these products, preliminary work suggests that at least one commercially available nutritional supplement had no detectable effect on the degree of IR in treated horses.[333]

Pituitary Pars Intermedia Dysfunction

Teresa A. Burns, Dianne McFarlane, and Ramiro E. Toribio

A clinical syndrome associated with hypertrichosis (hirsutism), skeletal muscle atrophy, weight loss, polyuria and polydipsia (PU/PD), and laminitis is well recognized in aged horses and ponies.[676,910-914] The syndrome was originally referred to as equine Cushing disease due to similarities to Cushing disease in humans and dogs, in which there is excessive and autonomous secretion of proopiomelanocortin (POMC)–derived peptides, including adrenocorticotropic hormone (ACTH, adrenocorticotropin, corticotropin), resulting in pituitary-dependent hyperadrenocorticism. However, in equids, pituitary adenomas arise from the pars intermedia (intermediate lobe) of the pituitary gland, unlike in humans and dogs, in which pituitary adenomas commonly develop from the anterior lobe (pars distalis, adenohypophysis). As a result, in the horse, hormones from the pars intermedia are excessively secreted as described later. *Pituitary pars intermedia dysfunction* (PPID) therefore seems to be a more appropriate term to describe the syndrome because, unlike humans, adrenocortical hyperplasia and hypercortisolemia are not consistent findings in affected horses.

CLINICAL SIGNS

Clinical signs of PPID in aged horses and ponies include hypertrichosis, poor haircoat, laminitis, PU/PD, muscle wasting, weight loss/repartitioning, docility, lethargy, hyperhidrosis, narcolepsy (and other signs of CNS dysfunction, including seizure), blindness, decreased responsiveness to painful stimuli, increased appetite, and recurrent infections.[606,910,911,915,916] Although there are many clinical signs associated with PPID, only a subset of the signs typically manifest in an affected individual, perhaps due to differences in the secretion profile of POMC-derived peptides or the degree of compression of adjacent neuroendocrine tissues.

Hypertrichosis (55%–80%) is considered the most frequent clinical sign in PPID horses, although these estimates may be biased due to the increased tendency to test aged horses displaying haircoat abnormalities for PPID.[676,910,911,915,917] Hypertrichotic horses have a long, thick, often curly haircoat (Fig. 16.21). Other haircoat abnormalities may include a failure to shed in a complete or timely manner; some horses shed in late spring and grow a winter coat early in the fall. Retention of long guard hairs in the jugular furrow, on the distal limbs, within the intermandibular space, or along the ventral abdomen is also a frequent finding. The pathogenesis of hypertrichosis is unknown, but one study demonstrated that horses with PPID have a greater percentage of hair follicles that remain in anagen phase than do age-matched unaffected horses.[918] This study also reported an increased proportion of telogen-phase hair follicles in PPID-affected horses following 6 months of treatment with pergolide mesylate.[918]

Chronic laminitis occurs in approximately 30% of horses with PPID.[919] The presentation of endocrinopathic laminitis is frequently more insidious than laminitis due to other causes (such as sepsis). It has been speculated that laminitis in the setting of PPID may result from increased concentrations of endogenous glucocorticoids, which are known to be associated



FIG. **16.21** Horse diagnosed with pituitary pars intermedia dysfunction based on basal ACTH concentrations (A). The same horse 6 months later after initiation of management (dental care, deworming, diet change) and treatment with pergolide mesylate (Prascend®) (B).

with systemic insulin resistance in equids.[735,736,920-923] However, more current data suggest that insulin dysregulation (ID) or insulin resistance (IR) and the associated hyperinsulinemia more likely have a role in inciting laminitis in horses with PPID, as the connection between hyperinsulinemia and equine laminitis has become increasingly robust following intense research efforts in the past decade.[732,733] The connection with glucocorticoids is weaker, though, as hypercortisolemia is an inconsistent finding in PPID-affected equids.[924-926] Laminitis-prone ponies have been reported to have increased serum insulin concentrations compared with control ponies.[799] Experimental maintenance of prolonged hyperinsulinemia in clinically normal ponies and Standardbred horses resulted in consistent induction of laminitis within 72 hours of onset of hyperinsulinemia, suggesting that increased serum insulin concentration in the absence of hyperglycemia can induce laminitis.[732,733] Additionally, one study reported that horses with PPID displayed significantly lower mean basal rates of glucose metabolism compared with healthy and non-PPID diseased control horses when tested with the euglycemic-hyperinsulinemic clamp method.[927] This may represent an age-related (expected, normal) decrease in peripheral insulin sensitivity (similar to that well described in many species), as more recent work comparing horses with PPID to age-matched control horses with this same test showed no difference between the two groups with respect to their glucose disposal rates.[928] In geriatric horses, PPID does not necessarily impair insulin sensitivity.[928] Horses with PPID do not appear to be consistently hyperglycemic when assessed via plasma fructosamine concentrations (which provide a medium-term assessment of average blood glucose concentration); the authors of one report found no difference in fructosamine concentration between horses with PPID and control animals. Horses experiencing recent bouts of laminitis, however, were reported to have elevated fructosamine concentrations, suggesting that laminitis (or more specifically, laminitic pain) was more likely to be associated with hyperglycemia than PPID.[929] Finally, in a study comparing laminitic and nonlaminitic horses with PPID, hyperinsulinemia was documented in all of the laminitic individuals (median 74.1 mIU/L, interquartile range 49.9–349.5 mIU/L) and none of the nonlaminitic individuals (less than 20 mIU/L [less than 20 μIU/mL]), again implicating hyperinsulinemia in the pathophysiology of laminitis in this setting.[919] Regardless of the underlying cause of laminitis, PPID should be included in the differential diagnosis for any aged horse presented with chronic laminitis, even if other clinical signs of PPID are not present.[433]

Polyuria and polydipsia have been reported in up to 76% of horses diagnosed with PPID.[911] Horses housed in stalls are most likely to have obvious PU/PD, as the condition is much less likely to be detected in animals housed extensively on pasture, calling into question the accuracy of assessments of the prevalence of PU/PD in horses with PPID. The pathogenesis behind the development of PU/PD is unclear. It has been speculated that compression of the pars nervosa by an expanding pars intermedia adenoma may result in decreased secretion of vasopressin (AVP, antidiuretic hormone [ADH]).[910,930] Hyperglycemia, which may be a laboratory finding in these horses (albeit inconsistently[929]), may induce osmotic diuresis. Glucocorticoids can increase glomerular filtration rate and may contribute to diuresis, but hypercortisolemia is also inconsistent in horses with PPID.[677,926] It is important to rule out other differentials for PU/PD, including neurogenic diabetes insipidus, nephrogenic diabetes insipidus, psychogenic polydipsia, and hyperglycemia of various origins (chronic pain, pancreatic disease, pheochromocytoma, insulin resistance).

Weight loss or repartitioning is reported in the majority of horses with PPID (up to 88%) and is often an early clinical sign[911]; however, some horses may be obese or in good body condition.[931] Affected horses often have a weak and pendulous abdomen, a sway back, and a poorly developed topline. The pot-bellied appearance is probably the result of lack of tone in the abdominal musculature. The muscle wasting along the dorsal midline and elsewhere results in prominence of the tuber coxae and tuber sacrale regions. It is unclear what the effect of different POMC-derived peptides is on equine energy metabolism or muscle atrophy. Weight loss in some horses may be the result of other conditions associated with aging (such as dental problems, poor nutrition, or chronic disease) or PPID (opportunist infections including parasitism secondary to immunosuppression) rather than the direct effect of an abnormally regulated hypothalamus-pituitary-adrenal axis (HPAA) on body condition. Glucocorticoids, in addition to being immunomodulatory, can have catabolic effects on skeletal muscle; however, the role of increased concentration of cortisol on weight loss in PPID is unclear. Muscle tissue from horses with PPID shows selective atrophy of type 2 muscle fibers and differential expression of m-calpain (a major proteolytic system that may play a role in the development of muscle atrophy in horses with PPID)[932,933]; however, the specific role of POMC hormones on muscle atrophy has not been investigated. In horses with normal or low cortisol concentrations, causes other than hypercortisolemia may be responsible for the weight loss/redistribution.

Increased docility in affected horses is often reported by owners and clinicians. Horses with PPID have increased plasma and cerebrospinal fluid concentrations of β-endorphin (β-END),[934] which may explain the docility and lethargy in some of these animals. Increased β-END concentrations may also explain the decreased responsiveness to painful stimuli and corneal stimulation that is observed in affected animals.[935] The decreased corneal sensation that develops both with age and PPID may also explain the frequency of chronic ulcerative keratitis in these populations.[935]

Neurologic impairment, although uncommon, has been reported in horses with PPID and includes blindness, seizures, and narcolepsy.[433] Blindness may result due to compression of the overlying optic chiasm by the enlarged pituitary gland; however, blindness can occur in horses with PPID in the absence of postmortem evidence of optic nerve damage. An increased occurrence of narcolepsy or sleep disorders has been suggested to occur in horses with PPID. The reason that some of these horses develop narcolepsy is unknown. One explanation may be that the removal of dopaminergic control may alter the sleep-wake cycle as occurs in humans. Narcolepsy can result from decreased orexin activity in the hypothalamus. Orexins (hypocretins) are peptide neurotransmitters expressed in the lateral hypothalamus (orexinergic neurons) that are important in the sleep-wake cycle; the absence of orexin function results in narcolepsy.[936] Horses with PPID have been shown to have decreased cerebrospinal fluid concentrations of hypocretin (orexin).[937]

Excessive sweating (hyperhidrosis) and, less commonly, lack of sweating (anhidrosis) are reported in horses with PPID, although the mechanisms underlying abnormal sweating remain unclear.

The expanded supraorbital adipose tissue often observed in horses with PPID results from fat redistribution.[911] This finding may be observed in horses that do not exhibit generalized obesity. Likewise, some horses at higher body condition scores may have prominent nuchal ligament ("cresty neck") and tailhead adipose tissue accumulation (regional adiposity). It is unclear if PPID causes adiposity (generalized or regional) or if it is the consequence of EMS (or insulin dysregulation) occurring as a comorbidity in horses with PPID. Limited data would suggest that EMS is present in approximately one third of horses with PPID and that certain clinical signs, such as laminitis and adiposity, may be a result of the EMS rather than PPID.[919,928]

Skin infections (such as dermatophilosis and pyoderma), sinusitis, pneumonia, and abscess formation (dental, pulmonary, subsolar, etc.) are common sequelae of PPID. The precise mechanism resulting in immune impairment is unknown, but the increased concentration of immunosuppressive hormones, including α-melanocyte-stimulating hormone (α-MSH), cortisol, insulin, and/or β-END (or their ratios) is likely a contributing factor. Abnormalities in neutrophil function have been shown in horses with PPID compared with age-matched control horses; neutrophils from PPID-affected horses display decreased oxidative burst capacity and functional adhesion, both of which may increase the animal's risk for opportunistic infections.[938] Additionally, horses with PPID have been shown to have a shorter strongyle-type egg reappearance period following anthelmintic administration than age-matched control horses, again suggesting that the hormonal milieu associated with this disease affects the host's responsiveness to various types of infections.[939] Management of these infections is an important part of treating equine PPID.

Abnormal estrus cycles and infertility have been reported in horses with PPID.[912] This may be a direct or indirect effect of PPID, or an unrelated effect of advanced age, as fertility (particularly in mares) has been determined to significantly decline with age in otherwise healthy mares.[940-942] Compression of the pars distalis or hypothalamus by expanding diseased pars intermedia could have a profound effect on reproductive hormones. In addition, a generalized decrease in dopamine output could directly affect release of reproductive hormones, as many are under dopaminergic regulation, including gonadotropin-releasing hormone and prolactin. There are no controlled studies documenting the relationship between treated and untreated horses with PPID and fertility.

EPIDEMIOLOGY

Compared with some other equine endocrine disorders, PPID appears to be a common clinical syndrome. That said, no large-scale prospective clinical studies have accurately documented the incidence of the disease to date. Additionally, estimates of the prevalence of the condition vary somewhat. In a UK study of more than 300 horses ≥15 years of age, horses were tested using plasma ACTH (assessed with seasonally adjusted reference ranges) and α-MSH concentration, and 21.2% of horses were positive for PPID.[943] Advancing age and hypertrichosis were significantly associated with PPID diagnosis in that study, which also showed that the disease is underrecognized by horse owners.[943] An equine veterinary practice from the eastern United States reported that 30% of surveyed horse owners reported haircoat changes in their horses suggestive of PPID.[944,945] Postmortem surveys also suggest that the disease is quite common in aged horses, with rates of gross or microscopic pathology of the pars intermedia reported to be from 30% to 40% of examined horses.[46,47] An incidence of 15% to 30% is markedly greater than originally reported in the 1970s (0.075–0.15% of 4000 horses of all ages seen in an ambulatory practice diagnosed by the presence of generalized hypertrichosis).[914] Hypertrichosis had 71% sensitivity, 95% specificity, 91% positive predictive value, and 84% negative predictive value for diagnosing the disease where a definitive diagnosis was based on postmortem examination of the pituitary gland.[462] At this time, it is unclear whether this change reflects a true increase in prevalence of disease, a change in equine demographics with a larger aged population, or an increased awareness and earlier recognition of disease due to change in diagnostic testing methods or frequency.

PPID has been diagnosed in horses as young as 7 years of age (range 7–40; average 19–21).[676,910,911,931,947] Although all breeds are susceptible, ponies and Morgan Horses may be predisposed.[433,676,911,915,948] Despite earlier studies suggesting PPID to be more prevalent in females[910,949-951] or males,[911] there appears to be no sex predilection.[433,676,911,931,952] The disease also occurs in donkeys, but prevalence studies are lacking.

Pars intermedia adenomas and microadenomas are often observed at postmortem examination in elderly horses with no previous history or reported clinical signs of this condition.[462,946] The significance of these clinically silent histologic findings is unknown.

PATHOGENESIS

Pars intermedia hyperplasia and hypertrophy are consistent findings in PPID,[606,953] and data suggest that loss of inhibitory

control of pars intermedia cell function is associated with triggering unregulated cell proliferation and the development of adenomas. The pars intermedia in horses and other species is inhibited by dopamine of hypothalamic origin.[954] There are three neuroendocrine dopaminergic tracts: the tuberoinfundibular, tuberohypophyseal, and periventricular dopaminergic neurons.[955] It is the periventricular neurons that appear most critical in regulating cell proliferation and hormone production and release in the pars intermedia.[956] The periventricular neurons originate in the periventricular nucleus, adjacent to the third ventricle, and project through the infundibular stalk, terminating in the pars intermedia. In the pars intermedia, the periventricular neurons directly innervate melanotropes, where they release dopamine that interacts with dopaminergic D_2-type receptors on melanotropes. In the presence of dopamine, the D_2 receptors act to inhibit POMC mRNA expression and POMC-derived intermediate lobe hormone release. In rodents, surgical disruption of the periventricular neurons results in increased α-MSH release, confirming a melanotropic inhibitory role for the periventricular neurons.[956] In contrast, prolactin release is regulated primarily by the tuberoinfundibular dopaminergic neurons. These originate in the arcuate nucleus and release dopamine at the median eminence. From there, dopamine is transported to the anterior lobe through the hypothalamo-hypophyseal portal system.[955]

Loss of dopaminergic inhibition in horses with PPID was suggested by the data of Orth and colleagues[957] (who found that the D_2-receptor agonists, bromocriptine or pergolide, decreased POMC-derived peptide concentrations in horses with PPID) and the work of Millington and colleagues[934] (who found low concentrations of dopamine and dopamine metabolites in the pars intermedia of horses with PPID). These data suggested that although dopamine was lacking in the pars intermedia of horses with PPID, responsiveness to dopamine and exogenous dopaminergic agonists was intact (including exogenous dopaminergic agonists).[957] This understanding has made it possible to recommend dopaminergic agonists (e.g., pergolide) in the symptomatic treatment of PPID. Several studies[433,917,958,959] have documented the efficacy of dopamine agonists in the treatment of PPID as described later. Studies have suggested that the loss of dopaminergic input to the pars intermedia is a consequence of neurodegeneration of the periventricular neurons.[560] A loss of dopaminergic cell bodies in the periventricular nucleus of the hypothalamus and the nerve terminals in the pars intermedia was demonstrated in horses with PPID using immunohistochemistry.[560] In addition, in horses with PPID dopaminergic nerve terminals showed evidence of oxidative stress, suggesting that free radical damage may participate in periventricular neuronal damage and cell loss. Oxidative stress has been implicated as a contributing factor in the pathogenesis of many other neurodegenerative diseases.[960] Evidence from other species suggests that additional hypothalamic factors, such as TRH, serotonin, and GABA, are important in regulating melanotrope function in the pars intermedia.[934,961] In horses, a direct positive effect of TRH on melanotropes has been shown.[962] When equine melanotropes were maintained in culture, treatment with TRH resulted in a release of α-MSH. In addition, horses administered TRH have a rapid and substantial increase in plasma α-MSH concentration.[962] In other species, serotonin is also a positive releasing factor for pars intermedia cells.[954] However, the role of serotonin on equine pars intermedia function is unclear, and Millington and colleagues[934] found no difference in serotonin concentrations in the pars intermedia of affected versus control horses. GABA has a tonic inhibitory effect on POMC synthesis in the pars intermedia of other species[961]; however, the role of GABA in the pathogenesis of equine PPID has not been investigated.

Both the anterior lobe (corticotropes) and the intermediate lobe (melanotropes) synthesize the identical hormone precursor molecule, POMC[963]; however, in the intermediate lobe, the posttranslational cleavage and processing of POMC is different from that in the anterior lobe.[934,964] In the anterior lobe, the major products of POMC are ACTH and β-lipotropin (β-LPH) (Fig. 16.22). In the intermediate lobe, ACTH is further cleaved to produce α-MSH and corticotropin-like intermediate lobe peptide (CLIP), and β-LPH is processed to β-END.[964,965] Therefore in the pars intermedia, ACTH is a minor (2%) posttranslational product of POMC.[65] It also appears that POMC production and cleavage in the pars intermedia of the horse with PPID remains intact, but postcleavage modification of the peptides is altered.[934,963] Millington and colleagues[934] found that the posttranslational processing of β-END in normal tissues was different from that in equine pituitary adenomas, with significantly more of the active (agonist) form of β-END present in the pituitary of horses with PPID.

Although progress has been made in our understanding of the pathogenesis of PPID in horses, many questions remain unanswered. For example, why do a number of horses with PPID have low cortisol concentrations despite high ACTH concentrations? Cortisol concentrations are variable in affected horses,[457,606,951,966] and adrenocortical hyperplasia is not a consistent finding.[910,951] Therefore it is difficult to explain hyperglycemia, neutrophilia, lymphopenia, and other clinical signs such as laminitis and predisposition to infections as a result of glucocorticoid excess. Cortisol may be responsible for some of the clinical signs associated with PPID; however, many studies have found that cortisol concentrations in horses with PPID may be within or below the normal range, suggesting that unidentified factors may be as important as or more important than hypercortisolemia in the pathogenesis of this condition. Furthermore, these findings are consistent with the low frequency (20%) of adrenocortical hyperplasia in horses diagnosed with PPID.[910,951] One explanation for the low cortisol concentration in some affected horses may be that glucocorticoid synthesis and secretion from the adrenal gland is downregulated from excessive and prolonged exposure to ACTH. Alternatively, ACTH secreted in horses with PPID may be immunologically reactive but biologically inert (perhaps due to posttranslational modifications). Finally, recent work has demonstrated that although horses with PPID have similar total cortisol concentrations to control animals, their free cortisol fraction is increased; decreased plasma cortisol-binding capacity could therefore contribute to the pathophysiology of PPID, even in the absence of overt hypercortisolemia.[723]

PATHOLOGIC FINDINGS

Horses with PPID typically have a grossly enlarged pituitary due to hypertrophy, hyperplasia, and adenoma formation in the pars intermedia (Fig. 16.23). Adenomas of the pars intermedia are white-to-yellow, nodular or multinodular masses that can infiltrate or compress the pars distalis and pars nervosa.[910,951] In some horses, pituitary microadenomas (smaller than 1 cm) may be evident. Microscopically, these adenomas

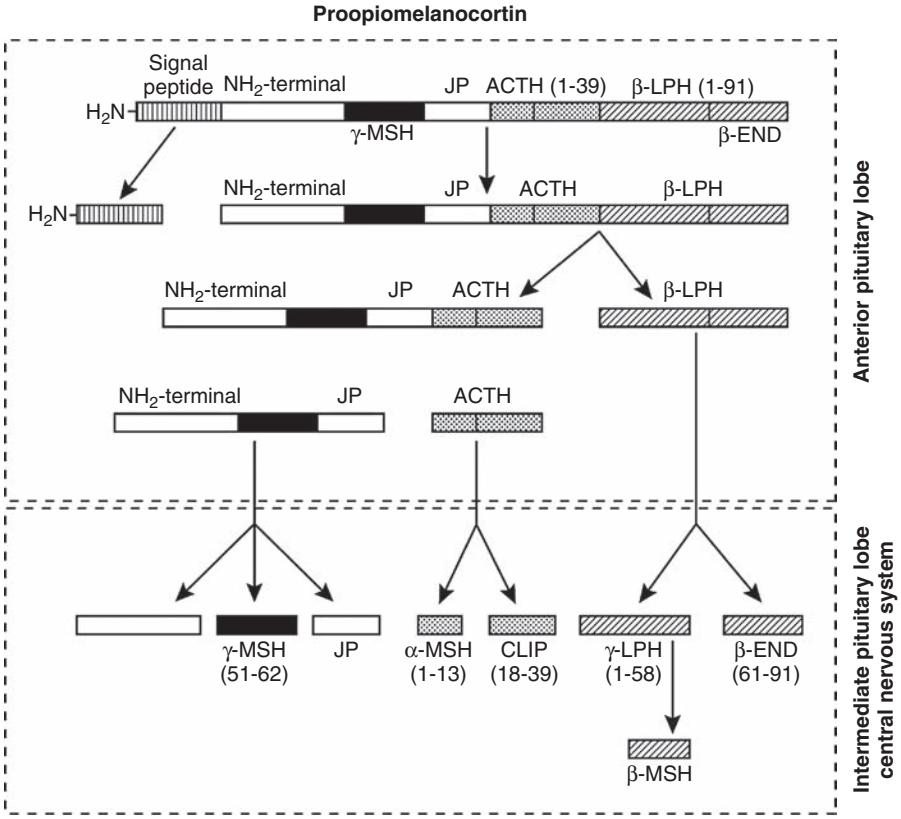


FIG. **16.22** Processing of proopiomelanocortin (POMC) in the anterior pituitary lobe, the intermediate pituitary lobe, and the central nervous system. In the anterior lobe, ACTH and β-LPH are the major products of POMC. In the intermediate lobe and in the central nervous system, ACTH is cleaved to produce α-MSH and CLIP, and β-LPH is cleaved to produce β-END. *ACTH*, adrenocorticotropic hormone; *-LPH*, β-, γ- lipotropin; *-MSH*, α-, β-, γ-melanocyte-stimulating hormone; *CLIP*, corticotropin-like intermediate lobe peptide; *β-END*, β-endorphin; *JP*, joining peptide. (Adapted from Castro and colleagues.[965])

consist of large columnar, spindle-shaped, or polyhedral cells that form palisades and pseudoacini.[910] The tumor cells are slightly acidophilic with hematoxylin and eosin staining and chromophobic with trichrome stain.[910] Pars intermedia adenomas may compress the overlying hypothalamus or optic chiasm, resulting in damage to nuclei that are important in endocrine and metabolic functions.

Adrenocortical hyperplasia is not a consistent finding; 1 of 5,[51] 4 of 19,[910] and 14 of 32[951a] horses had hyperplasia of the adrenal cortex. The lack of cortical hyperplasia in most horses with PPID supports the contention that the clinical signs in these horses are not the result of hyperadrenocorticism. The variability among affected horses to the ACTH stimulation test (measuring cortisol release) may be the result of inconsistent adrenal hyperplasia.

Skin biopsies reveal normal hair follicles in anagen phase, normal epidermis and dermal collagen, and a lack of the characteristic lesions present in dogs with Cushing disease from cortisol excess; however, a relative lack of follicles in telogen phase may characterize the skin biopsy from a patient with untreated PPID.[918] Although laminitis is a relatively common finding in horses with PPID, the histopathologic features in the laminae of these horses are not specific to this condition.[919,967]

LABORATORY TESTING

The diagnosis of PPID in many cases can be suggested by history, clinical signs, and preliminary laboratory results. Initial testing should include complete blood count, serum chemistry profile, and urinalysis. Horses with PPID may have a normal complete blood cell count and serum chemistry profile. Clinicopathologic abnormalities reported in horses with PPID include anemia, neutrophilia, lymphopenia, eosinopenia, hyperglycemia, hyperlipemia, increased liver enzymes, and glucosuria.[353,676,911,947,949-951] Persistent hyperglycemia is not uncommon in clinically affected horses; over 45% (94% in one study) of horses with PPID had hyperglycemia.[676,911,915,949,951] Many horses with PPID and hyperglycemia have insulin dysregulation (hyperglycemia and hyperinsulinemia), but neither finding should be considered diagnostic of PPID.[968] Although insulin-resistant hyperglycemia can be present, it is also possible that hyperglycemia in some horses may result from painful or stressful conditions (such as laminitis).[929] Glucosuria is a frequent finding (up to 77% in one study) in horses with PPID and hyperglycemia.[911] Hyperlipemia may be present in affected horses, but it is more frequent in ponies.[926,969]

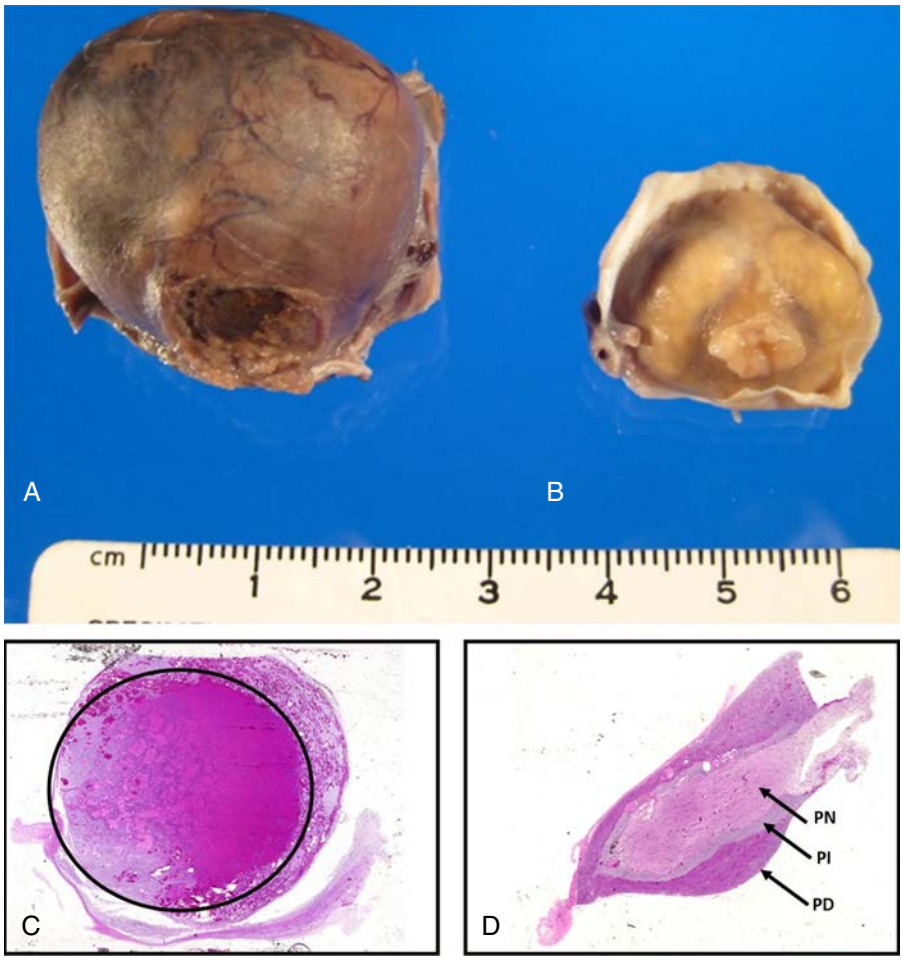


FIG. **16.23** Pituitary gland from a horse with advanced PPID showing marked enlargement and rounding (A), compared with that from a healthy horse (B). A sagittal section of the pituitary gland from A (C) shows a large adenoma *(black circle)* with compression of the adjacent pituitary structures. Three distinct lobes (pars distalis [PD], pars intermedia [PI], and pars nervosa [PN]) are apparent in the sagittal section of the pituitary gland from the healthy horse (D) from B (hematoxylin and eosin staining).

DIAGNOSTIC TESTING

Specific diagnostic testing for PPID may be indicated based on history, clinical signs, and laboratory findings. Many tests for the diagnosis of PPID have been evaluated; however, based on the limited number of horses studied and the variable results from some of these studies, it is difficult to recommend them for wholesale clinical use without additional validation. One limitation in the diagnosis of PPID is establishing a "gold standard" against which to evaluate new diagnostic tests, and currently perhaps the only well-accepted gold standard is postmortem examination interpreted in conjunction with clinical signs. In fact, even this postmortem "gold standard" (histopathologic evaluation of the pituitary gland) has been questioned as insufficiently accurate.[970] Another important consideration is the season during which the testing is performed. The secretory output of the equine pars intermedia is known to increase in the fall (Northern Hemisphere), and as a result, false-positive diagnostic tests may occur when testing is performed between August and October unless seasonally adjusted reference ranges are used for interpretation (ACTH).[537-540]

Baseline Cortisol Concentrations

Measurement of baseline plasma concentrations of cortisol has been proposed to aid in the diagnosis of PPID. However, in horses with PPID, resting plasma concentrations of total cortisol may be within the normal range,[951] mildly increased,[606] or decreased,[457,966] and therefore measuring baseline plasma cortisol concentrations to diagnose PPID is not recommended. Healthy horses have diurnal variations in plasma cortisol concentrations, with higher concentrations in the morning than in the late evening, although significant individual variation occurs.[971,972] Minimal diurnal variations in cortisol concentrations have been reported in horses with PPID.[606] However, circadian cortisol variability is commonly blunted in horses with other diseases or stress-related conditions,[973] thus precluding its usefulness as a diagnostic test.

In addition to plasma, cortisol concentrations have been determined in saliva and urine of healthy horses and horses with PPID.[925,926,972] van der Kolk and colleagues[972] measured salivary cortisol concentrations in healthy horses and found that there was a trend for higher salivary cortisol concentrations in the morning than in the evening although the

differences were not statistically significant. Those authors also found that plasma and salivary cortisol concentrations were correlated and that both increased or decreased following administration of ACTH or dexamethasone, respectively. Salivary cortisol concentration testing is not recommended for use in diagnosing PPID. Tear cortisol concentrations were increased in some horses and ponies with PPID compared with healthy controls.[974]

Although total plasma and salivary cortisol concentration have been shown to be insufficiently sensitive and specific for use in diagnosing PPID, recent work has demonstrated that the free cortisol fraction in plasma is more discriminatory for equine endocrine disease.[723] Horses with PPID, obese horses, and horses with insulin dysregulation all had higher free cortisol fractions than healthy control horses (even thought their total cortisol concentrations were similar). Although the diagnostic utility of this finding is currently unclear, free cortisol may be more relevant to the pathogenesis of equine endocrinopathies than total cortisol and warrants further study.

Urinary Corticoid:Creatinine Ratio

The urinary corticoid:creatinine (c:c) ratio has been determined in ponies and horses suffering from PPID.[925,926] van der Kolk and colleagues[925] found that horses with PPID had higher urinary concentrations of corticoids and a higher c:c ratio than healthy horses; however, in their study there was overlap in the c:c ratio between healthy and diseased horses resulting in a number of false-positive and false-negative results, thus limiting the value of this test alone for the diagnosis of PPID. In addition, horses were included in that study on the basis of the ACTH stimulation test, which itself has significant variability among horses with PPID and between healthy ponies and horses.[915] No ponies were included in the control group, and most of the affected animals were ponies. Additional studies comparing the c:c ratio to well-accepted and validated diagnostic procedures are necessary to recommend this test over other tests.

Dexamethasone Suppression Test

The dexamethasone suppression test (DST) has been a well-accepted and recommended test for the diagnosis of PPID in horses.[606,975,976] This test is based on the lack of suppression of plasma cortisol concentrations in horses with PPID at 19 to 24 hours after dexamethasone administration.[606] It has been suggested that plasma cortisol concentrations are not suppressed by dexamethasone administration in horses with PPID due to production of ACTH from melanotropes of the pars intermedia which, as described earlier, are not responsive to cortisol feedback.[606] The most commonly used protocol for dexamethasone suppression is the overnight DST. A baseline blood sample is collected for serum cortisol measurement between 4 and 6 PM, after which dexamethasone is administered (40 µg/kg IM). Blood samples are collected at 15 and 19 hours (8 AM and 12 PM) after dexamethasone administration. Many clinicians will omit the 15-hour sample and collect only a single cortisol sample at 19 hours after dexamethasone administration. Plasma cortisol concentrations should be below 1 µg/dL in horses that suppress normally; concentrations above this level suggest PPID. This test provides simplicity, is inexpensive, and is well accepted by clinicians. Although the test is useful in distinguishing horses with PPID from horses with an apparently functional HPAA, both false positives and false negatives may occur. False positives are common when horses are tested during the fall, as there are no seasonally adjusted reference ranges against which to evaluate the results of this test. Therefore this test should likely only be performed between the months of November and July (Northern Hemisphere). Donaldson and colleagues[539] reported false-positive rates of 20% and 40% in healthy ponies and horses, respectively, when tested in the fall. False-negative test results most commonly are observed in the early course of the disease. Horses with clinical indicators of PPID and normal dexamethasone suppression should be monitored and retested after 4 to 6 months; alternatively, a test that can be interpreted against seasonally adjusted reference ranges (ACTH) or a more sensitive diagnostic test (TRH stimulation test) should be performed.[977] Although many clinicians are concerned with steroid-induced laminitis as a result of the DST test, no adverse effects of this test were reported when a large number of horses with PPID were evaluated.[606] Use of this test has recently been largely supplanted by tests that are more convenient, more sensitive, less time-consuming, and easier to interpret (namely, the baseline ACTH concentration and the TRH simulation test).

Baseline ACTH Concentrations

Increased plasma concentrations of ACTH and POMC-derived peptides (such as α-MSH) have been reported in horses with PPID.[537-539,603,915,916,934,957,963,978] Couetil and colleagues[915] found that plasma ACTH concentrations for the diagnosis of PPID had a sensitivity of 90.9% in horses and 81.8% in ponies, and a specificity of 100% using dexamethasone suppression as the gold standard.[915] Other studies have reported false-positive and false-negative results.[931] Determination of resting ACTH concentrations is a good initial test for PPID, and testing in the autumn months may increase the sensitivity of this test when interpreted according to seasonally adjusted reference ranges.[537,538,952,978] Plasma ACTH concentrations are higher in the fall in normal animals, and this effect is exacerbated in animals with PPID, which enhances the sensitivity of the test (increasing the separation between normal and abnormal values). It is important to consider when determining baseline ACTH concentrations that stressful conditions may increase ACTH plasma concentrations, although the likelihood of this interfering in the interpretation of this test is considered unlikely in all but the most critically ill equine patients. Furthermore, although circadian and ultradian variation in plasma ACTH concentration has been documented in horses, this is unlikely to affect the interpretation of this test for the diagnosis of PPID.[979] Assessment of ACTH in multiple samples from the same patient has not been shown to be diagnostically superior to a single measurement.[979,980] A recent study found no advantage of paired over single ACTH measurement, with similar sensitivities and specificities.[980] Blood samples to determine ACTH concentrations should be collected in EDTA tubes (ideally plastic), kept at 4°C, centrifuged within 8 hours, and plasma shipped on ice immediately or stored frozen until analysis.[915,959] ACTH appears to be stable at room temperature longer than previously believed.[981] One study found minimal changes in ACTH concentrations over 48 hours when whole blood was stored at 4°C.[981] In the same study, concentrations did not change in whole blood over 8 hours when samples were kept at room temperature. Regardless, it is important to follow recommendations when handling samples for ACTH measurements. Pretest feeding recommendations have not been standardized for the baseline ACTH or TRH stimulation test, but fed state has recently been

shown by one group to affect the results of both of these tests (with baseline and stimulated ACTH concentrations higher in fed animals than fasted animals),[982] whereas others reported no effect of feeding conditions on either baseline or TRH-stimulated ACTH concentration.[983]

ACTH Stimulation Test

Administration of ACTH (1 IU/kg of natural ACTH gel IM; 25–100 IU [250–1000 μg] of synthetic $ACTH_{1-24}$ [cosyntropin; tetracosactide; tetracosactrin] IV) has been used in different studies with different results.[603,606,607,676,916,925] A horse with PPID would be expected to have an exaggerated (at least fourfold) cortisol response to ACTH stimulation, and when an increased response is present in a horse with elevated resting ACTH concentrations, a diagnosis of PPID can be supported.[603] Dybdal and colleagues[606] failed to detect a statistical difference in cortisol concentrations between control horses and horses with PPID after intramuscular administration of 1 IU/kg of natural ACTH gel between 8 and 10 AM, samples being collected in heparinized tubes before, and 2, 4, 8, and 12 hours after ACTH administration. van der Kolk and colleagues[916] found a significantly higher increase in cortisol concentrations in horses with PPID after IV administration of 25 IU of synthetic $ACTH_{1-24}$ at 9 AM, with samples collected in EDTA tubes just before and 2 hours after ACTH administration. Although the ACTH stimulation test is more useful in assessing adrenal gland function or exhaustion, it does not seem to be an effective test to assess HPAA function in horses suspected of having PPID. Combining the ACTH stimulation test with the DST failed to improve the tests' performance in distinguishing horses with PPID from control horses.[606]

TRH Stimulation Test

Beech and Garcia evaluated the effect of TRH administration on plasma cortisol concentrations in healthy horses and in horses with PPID.[457] They found that TRH administration (1 mg IV) to horses with PPID, in addition to increasing T_3 and T_4 concentrations, also resulted in a significant increase in plasma concentrations of cortisol within 15 minutes after TRH administration, and cortisol concentrations remained elevated for 90 minutes. No significant increases in plasma cortisol concentrations were present in healthy horses. Eiler and colleagues[966] evaluated the TRH stimulation test in two horses suspected of having PPID and three healthy horses and found that maximal cortisol concentrations overall were not statistically different between groups; however, too few horses were included to make any conclusion.[966] The sensitivity and diagnostic performance of the TRH stimulation test was subsequently shown to be improved by measuring ACTH as the output variable, rather than cortisol (see discussion of immunoreactive vs. biologically active ACTH earlier).[924] TRH has been shown to be a direct releasing factor for equine melanotropes, and TRH increases the secretion of α-MSH and ACTH in both normal horses and those with PPID. The diagnostic utility of this test lies in the fact that ACTH concentrations are higher and more prolonged following TRH administration in horses with PPID than those without.[924,984,985] This test likely has the greatest sensitivity for detection of subclinical PPID of the diagnostic tests currently available, and, as such, this stimulation test has been advocated for use in cases with vague clinical signs or equivocal results of baseline ACTH concentrations.[977] Seasonal variability in the results of this test have also been reported,[986] and although the sensitivity would be expected to be maximized during the autumn months (as with baseline ACTH concentrations), seasonally adjusted reference ranges have not yet been created for this test. Therefore testing is only recommended during the months of November–July in the Northern Hemisphere.[977]

A veterinary-labeled TRH solution is not available for equine practitioners. However, chemical-grade TRH powder can be purchased from a number of companies, diluted in saline solution, filter sterilized, and frozen in 1-mg aliquots until use. Veterinary compounding pharmacies are also offering TRH as an injectable solution. Side effects that may be observed in horses after TRH administration include yawning, lip movements, salivation, trembling, and coughing.

To administer the test, 1 mg of compounded, filter-sterilized TRH should be administered intravenously following collection of a blood sample in an EDTA tube (as for ACTH, described earlier). An additional blood sample in EDTA should be collected at 10 minutes after TRH administration, and both samples should be submitted for measurement of ACTH concentration; $[ACTH]_{10min}$ values greater than 110 pg/mL are consistent with a diagnosis of PPID.[977] A sample at 30 minutes is also acceptable using a cutoff of 65 pg/mL. The baseline sample can be omitted to improve economic feasibility.

Combined DST/TRH Test

The combination of a DST with the TRH stimulation test was evaluated as a diagnostic method for PPID in 42 horses.[462] After baseline sampling in EDTA-containing tubes, dexamethasone (40 μg/kg IV) was administered (8–10 AM). A second sample was collected 3 hours later, and then TRH (1.1 mg IV) was administered. Blood samples were collected at 3.5 (30 minutes after TRH administration) and 24 hours after dexamethasone administration. The test was considered positive if cortisol concentration was greater than 1 μg/dL at 24 hours, or the 3-hour cortisol concentration increased by ≥66% 30 minutes after TRH administration. Performance of the test was evaluated using postmortem diagnosis as the gold standard. The TRH component alone had a sensitivity of 41% and a specificity of 92%, and the DST alone had a sensitivity of 65% and a specificity of 76%. When performed as a combined test, the sensitivity (88%) and specificity (76%) improved. Disadvantages include expense and the need for multiple sample collections. This test has, like other tests, been supplanted by the baseline ACTH and TRH stimulation tests.

Resting Insulin Concentrations

An assessment of insulin regulation should be performed in all horses suspected of having PPID as hyperinsulinemia is a strong predictor of laminitis in this population. Other conditions, such as EMS and stress, are also associated with hyperinsulinemia or ID; therefore the presence of hyperinsulinemia or ID alone is not diagnostic. Although hyperinsulinemia alone is not diagnostic for PPID, it does have prognostic value, as horses or ponies with hyperinsulinemia are more likely to develop laminitis.[799,987] Horses with PPID that were laminitic were much more likely to be hyperinsulinemic than horses with PPID that were nonlaminitic in one study.[919] Therefore measurement of fasting insulin and glucose is recommended in horses suspected of having PPID. If resting insulin and glucose is not diagnostic but ID is suspected, further tests may be useful to confirm ID, such as the combined glucose-insulin test, the oral sugar test, or more involved methods (such as the

frequently sampled insulin-modified IV glucose tolerance test or the euglycemic-hyperinsulinemic clamp). Details on this testing can be found in the section on the equine metabolic syndrome.

Determination of POMC Peptides

Based on our current knowledge of equine pars intermedia dysfunction, it would be reasonable to determine plasma concentrations of POMC-derived peptides (ACTH, α-MSH, β-END, CLIP) in horses suspected of having PPID. These peptides were found to be increased in horses with PPID.[537,913,915,916,934,957,963,988] Determination of POMC-derived peptides in the clinical setting has been limited to ACTH plasma concentrations, and the use of human immunoassays to determine equine ACTH concentrations has been validated.[915]

Plasma and cerebrospinal fluid β-END concentrations were found to be increased several-fold in horses with PPID.[934] Furthermore, pars intermedia POMC peptides (α-MSH, β-END, CLIP) were disproportionally higher compared with ACTH plasma concentrations in affected horses.[957,963] The sensitivity of α-MSH, either baseline concentration or used as the output variable following TRH stimulation, has been reported to be similar to that of ACTH, but the specificity might be greater.[952,985] Although a human-based radioimmunoassay has been validated for use with horse samples,[988] this test is not currently commercially available, but it would be assumed to be clinically useful should it become so in the future.

Domperidone Response Test

Another test that has been evaluated for diagnosis of PPID is the domperidone response test.[985,989] Domperidone is a dopamine receptor antagonist. In horses with PPID, administration of domperidone is suggested to exacerbate the loss of dopaminergic inhibition and result in release of ACTH from the pars intermedia. In a study of 33 horses, plasma ACTH concentration 8 hours after domperidone administration was positively correlated with histologic PPID severity score and morphometric analysis of pars intermedia size, although not all animals considered "positive" by histology and domperidone test were exhibiting clinical signs of disease. In the absence of any clinical signs, it is difficult to determine whether the relatively mild histologic changes (diffuse pars intermedia hyperplasia) were indicative of early disease or associated with an increased function of the intermediate lobe for other reasons. In a second study, the domperidone response test was compared with the TRH stimulation test in 28 horses using histology and clinical signs as the diagnostic gold standard.[985] In this population, evaluating ACTH concentration 30 minutes after TRH administration was more accurate at diagnosing PPID than use of a doubling of ACTH following domperidone administration.[985]

Radiographic Methods

Computed tomography (CT) has been used to diagnose PPID in horses.[990-992] Although measurements of the pituitary by CT correlate reasonably well with postmortem measurements, they have marginal accuracy.[992] Ventral radiography and contrast venography were successfully used to diagnose PPID in horses and ponies.[993] Magnetic resonance imaging (MRI) is the preferred diagnostic imaging modality in human pituitary disease. MRI was useful at identifying pituitary gland and pars intermedia size in horses with PPID.[994] Pituitary microadenomas and macroadenomas could be visualized, and MRI scoring using T2-weighted images was correlated with the histologic score.[994] Although available to equine clinicians, these methods are far from being practical in the diagnosis of PPID, as they require anesthesia and expensive equipment and are not cost-effective compared with other diagnostic methods.

TREATMENT

Optimal treatment of PPID includes a combination of management and pharmaceutical interventions. PPID is observed primarily in older horses; therefore it is important to treat the dysfunctional pituitary, as well as many of the pathologic conditions associated with aging. Special attention should be given to providing optimal management including high-quality feed, dental care, deworming, and farrier care. It is also important to pay particular attention to the skin and haircoat. Many horses cannot properly shed their winter coat and as a result may develop hyperthermia. In addition, horses with PPID are prone to skin infections. The most difficult complication to manage is laminitis, which may be complicated by overfeeding of hay, grass, or grain containing excessive nonstructural carbohydrate. Therefore reducing the amount of soluble carbohydrate in the diet is advisable (even in nonlaminitic animals). Exercise is also recommended for nonlaminitic patients. Due to the insidious nature of the laminitis observed in horses with endocrinopathies, close monitoring of hoof growth and freedom of movement is desirable in all aged horses. Periodic radiographic assessment of the feet in horses at risk of PPID may be prudent.

As discussed earlier in this section, the regulation of POMC peptide synthesis and secretion is under the influence of the hypothalamic periventricular dopaminergic systems. POMC synthesis and secretion by the pars intermedia of horses with PPID typically remains responsive to similar releasing and inhibiting factors as the pars intermedia from unaffected horses and other species.[957] Based on this information, the medical treatment of PPID involves the use of dopamine agonists or serotonin antagonists.

Pergolide is the most commonly used dopamine D_2 receptor agonist for the treatment of PPID; however, others including bromocriptine and cabergoline have been investigated.[995-1001] The oral absorption of pergolide in horses is good, with a half-life similar to humans (around 20 hours).[1002] However, for an equivalent dose, plasma pergolide concentrations are higher in horses than humans, and this difference has been attributed to higher volume of distribution, lower bioavailability, and extensive first-pass metabolism in people.[1002]

Bromocriptine can be given either orally or by subcutaneous injection (0.03–0.09 mg/kg twice a day),[675,957,675] but its use in treatment of PPID is limited. Cabergoline, similar to bromocriptine, lisuride, and pergolide, is an ergot derivative with potent dopamine D_2 receptor agonist actions used in people to suppress pituitary prolactin secretion (lactotropes) in conditions associated hyperprolactinemia, as adjunctive therapy of acromegaly, and in Parkinson disease. Due to its high affinity for D_2 receptors, it has the potential to be used in the treatment of PPID. Cabergoline in a slow-release vehicle was shown to decrease PI response to stimulation in healthy mares and mares with insulin resistance; however, its efficacy in the treatment of PPID has not been explored.[1000,1001]

Oral pergolide is widely used and has been shown to be beneficial in the treatment of horses and ponies with PPID, and a

Food and Drug Administration (FDA)-approved formulation is now commercially available (Prascend™, Boehringer-Ingelheim). Lifelong treatment is recommended following diagnosis.[917,931,998] Doses of pergolide from 0.2 mg up to 5 mg/horse per day, once a day, or divided twice daily have been used in ponies and horses, with treatment durations of greater than 5 years.[958] Treatment typically starts with a total daily dose of 1.0 mg/day, and if there is no clinical evidence of improvement by 4 to 6 weeks, the dose should be increased gradually, every 3 to 4 weeks by 0.5 mg. Success of treatment should be assessed by documenting both improvement of clinical signs and normalization of diagnostic test results. Most horses show improvement within 6 to 8 weeks of treatment with doses of 1.0 to 2.0 mg of pergolide/day (see Fig. 16.21). Failure to respond to pergolide treatment may occur in very advanced disease, if a poor-quality drug is obtained from a compounding pharmacy, if the drug is not consumed completely by the patient, or if the diagnosis is incorrect. Periodic reassessments of ACTH or TRH stimulation are recommended, particularly if the patient experiences an exacerbation of clinical signs, as the dose required to control disease may change over time.[917] Long-term oral administration of pergolide is effective at reducing ACTH concentrations in aged horses, and ACTH increased to previous values by 10 days after discontinuation.[958] Of interest, 2 days after pergolide discontinuation, ACTH concentrations increased in a number of horses despite detectable plasma pergolide concentrations.[958] The pharmacokinetic properties of IV pergolide have been investigated.[1003] Although two studies found a half-life of >20 hours,[958,1002] a third study found a terminal elimination half-life of 5.6 hours,[1003] indicating that twice-daily dosing may be necessary in some horses to maintain appropriate concentrations. A number of horses treated with pergolide may develop anorexia, which typically resolves by reducing the dose.[931] Although long-term pergolide therapy has been associated with fibrotic cardiac valvular lesions in humans,[1004] similar lesions have not been reported in horses to date.

A second drug suggested to be useful in treatment of PPID is cyproheptadine, although horses treated with pergolide show a better response than horses treated with cyproheptadine.[917,931] Further, no FDA-approved formulation of cyproheptadine is currently available for horses. Cyproheptadine is a nonselective 5-hydroxytryptamine receptor blocking agent (serotonin antagonist) that reduces ACTH and β-END secretion from ACTH-producing tumors in humans.[1005] There is limited information on the serotonin functions in the hypothalamus and pituitary gland of the horse, and no difference in serotonin concentration was found between the pars intermedia of horses with PPID and control horses.[934] However, this drug has been widely used alone or in combination with pergolide to treat PPID.[917,959] Serotonin is also a histamine receptor (H_1) antagonist with weak anticholinergic activity. Serotonin has been shown to inhibit the release of prolactin from anterior pituitary cells in a non–serotonin receptor–dependent fashion by blocking calcium influx at the cell membrane.[1006] Cyproheptadine has been used for many years to treat horses with PPID with variable results. Cyproheptadine has also been proposed for the treatment of photic headshaking in horses.[1007] It is estimated that one third of horses with PPID show improvement when treated with cyproheptadine.[917,948] This drug has been recommended at 0.25 mg/kg orally once a day for 4 to 8 weeks, ideally in conjunction with pergolide treatment.[917] If there is no clinical evidence of improvement and no reduction in plasma ACTH concentrations, the dosage frequency should be increased to twice a day for 1 month, at which time ACTH concentrations should be determined or the TRH stimulation repeated.

Alternative therapeutic compounds have been suggested to treat horses with PPID. Aqueous extracts of *Vitex agnus-castus* (chasteberry, chaste tree, vitex, Evitex, and Hormonise for veterinary use) have been used to treat horses with PPID. *Vitex agnus-castus* extracts contain compounds (diterpenoids) that stimulate dopamine D_2 receptor activity and inhibit different opioid receptors.[1008] Because of its dopaminergic effects, *Vitex* has been recommended as an alternative phytotherapeutic agent in the treatment of hyperprolactinemia and premenstrual syndrome in women.[1009] In a blinded study, there was no clinical improvement or improvement in diagnostic test results in 14 PPID-affected horses treated with *Vitex agnus-castus* extract.[1010] Although there is a physiologic and pharmacologic rationale in the possible use of these extracts to increase dopaminergic activity in the equine pituitary, there is currently no evidence to recommend *Vitex* to treat horses with PPID, particularly given the other treatment options available.

Trilostane is an enzymatic inhibitor of 3-β-hydroxysteroid dehydrogenase, an important enzyme in steroid synthesis. However, there is little information on the effects of trilostane in equine steroid metabolism.[1011] Trilostane has been proposed for the treatment of PPID, as well as in the treatment of EMS.[1012] In a study of 20 horses or ponies treated over 1 to 2 years, improvement in clinical signs other than hypertrichosis was recorded; however, DST results remained abnormal, suggesting an insufficient response to the drug.[1012] Until the mid-1980s, trilostane was recommended to treat Cushing disease in humans; however, due to its lack of potency in blocking cortisol synthesis, trilostane is no longer recommended to treat this condition.[1013] In horses with PPID, only few have evidence of adrenal hyperplasia; therefore treatments directed at restoring pars intermedia function and limiting expansion of the pituitary mass are considered more appropriate than those that target cortisol synthesis inhibition.

Pituitary-Independent Hyperadrenocorticism (Adrenal Cushing Syndrome)

In pituitary- or ACTH-independent hyperadrenocorticism, the adrenal cortex secretes excessive amounts of steroids, cortisol in particular, that are not subject to negative feedback. In other species this typically occurs with adrenal tumors. Primary adrenocortical tumors are rare in horses,[234,675-677] and the few cases reported in the literature were nonfunctional tumors. A 12-year-old Dutch Warmblood gelding was presented with a history of polyphagia, polydipsia, reduced muscle mass, hyperhydrosis, lethargy, and delayed shedding of the haircoat.[675] The horse had persistent hyperglycemia and a normal adrenocortical response to ACTH stimulation. After unsuccessful treatment with bromocriptine, the horse was euthanized, and a unilateral adrenocortical adenoma was found.

Adrenocortical carcinomas have been documented in horses and are usually nonfunctional.[234,677] Of interest, one adrenocortical carcinoma in a 14-year-old Quarter Horse stallion presented for inappetance, weight loss, and abdominal pain was associated with hypercalcemia of malignancy, which is a paraneoplastic syndrome from excessive secretion of parathyroid hormone–related peptide.[234]

Anhidrosis

Ramiro E. Toribio

Equine anhidrosis (dry coat, nonsweating) is defined as the inability of horses to sweat in response to appropriate stimulation. Partial anhidrosis (hypohidrosis) is the most common presentation of this condition.

EPIDEMIOLOGY

Anhidrosis is frequent in the tropics and in areas with high temperatures and humidity, including the southern United States, Asia, Oceania, and Latin America. Earlier reports of "dry coat" were documented in tropical British colonies where Thoroughbred horses transported from England and Australia to tropical regions after arrival developed profuse sweating and subsequently transitioned to poor or insufficient sweating.[1014] It was initially proposed that anhidrosis resulted from a lack of adaptation to local conditions, in particular of horses from cold and temperate climates moving to warmer and humid areas.[1015] Some studies found no differences in the prevalence of anhidrosis between horses native to the southern United States and those from other states or countries,[510,1016,1017] whereas others showed an association between anhidrosis with the geographic origin of the horse.[1018] One study even found anhidrosis to be more frequent in horses native to Florida.[1017] These conflicting results are likely due to dissimilar sample populations, environmental conditions, inclusion criteria (geographic origin, age, breed, sex, type of activity), and data analysis.

The prevalence of anhidrosis across the United States is unknown, although it has been estimated to range from 6% to 20% in southern states.[510,1016,1017,1019] One study in Thoroughbred horses in central Florida found a prevalence of 6.1%, with horses in training and nonpregnant mares having the highest prevalence, but the disease was infrequent in young horses.[510] An epidemiologic survey of anhidrosis in Florida published in 2010 included 500 farms and 4620 horses.[1018] In that study, horses were classified as anhidrotic by the farm owner, manager, or veterinarian. Intradermal β-adrenergic receptor agonist testing was not employed because it is rarely used by field clinicians to make the diagnosis. Farms were classified as affected if they reported the condition in at least one horse. At the farm level, the prevalence of anhidrosis was 11.2% whereas at the animal level it was 1.8%. The prevalence differed from 0.08% in northern Florida to 4.3% in southern Florida. Farms located in central and southern Florida were more than twice as likely to have horses with anhidrosis than those in northern Florida. The odds of anhidrosis were higher in show and riding instruction than in ranch operations.[1018] Of interest, Thoroughbred and Warmblood horses, animals born in western or midwestern states, and those with a familial history of this condition were more likely to have anhidrosis.[1018] The disease seems to be more common in retired horses or horses used for nonracing performance than horses used for halter, pleasure, trail riding, or work.[1018] In Korea, the prevalence of anhidrosis in Thoroughbred horses, based on the intradermal epinephrine skin test, was 22%.[1020] In Pakistan, a prevalence of 12.4% was published.[1021]

Anhidrosis does not seem to be associated with exercise as previously suggested but rather with environmental conditions.[1016] There is no clear association with sex, breed, coat color, or country of origin.[510,1016,1017,1020] There seems to be a genetic component. For example, horses with a familial history of anhidrosis were 22 times more likely to be afflicted with this disorder. The influence of diet is controversial, with some clinicians claiming that anhidrosis is associated with high-protein diets and insufficient electrolyte intake.

PHYSIOLOGY

Thermal adaptation to heterogeneous environmental temperatures by endotherm animals (birds and mammals) is the result of evolutionary adjustments and selection pressure to maintain optimal biologic functions to succeed as a species. Physiologic functions are in great part dictated by the body temperature. It is clear then that there must be a thermal homeostatic system that senses and maintains body temperature within a narrow range (homeotherms) by coordinating mechanisms of heat loss, heat conservation, and heat generation. Losing the ability to thermoregulate results in hypothermia or hyperthermia, depending on surrounding temperature and humidity and results from pathologic conditions that alter temperature sensing, heat generation, or the hypothalamic temperature set-point. For heat loss, animals use radiation, conduction, convection, respiration, and evaporation. Radiation is simply the emission of infrared energy away from the body. Convection occurs when heat is transferred from the body to moving air or water. Conduction takes place when there is surface-to-surface thermal transfer to a solid structure or water. During respiration, colder air moves into the lungs, and warmer air is exhaled into the environment. In the case of evaporation (sweating), heat loss occurs through the conversion of water into gas. Specifically, vaporization removes heat from the body by converting water into vapor (evaporative cooling) through a process that requires energy.

The efficiency of each of these mechanisms depends on the species, physical activity, diseases, and environmental conditions. Conduction and convection are influenced by vasoconstriction and vasodilation. When ambient temperature is higher than body temperature, radiation, conduction, and convection become ineffective (the opposite can occur and the body may gain heat). In that case, evaporation and respiration become very important. This is evident in dogs that mostly rely on the respiratory system for heat exchange (panting). Any process that interferes with heat dissipation will lead to hyperthermia, tachypnea, stress, and, in severe cases, heat stroke, organ failure, and death.

The equine sweat glands (400–1128/cm^2) are apocrine, associated with the hair follicle (epitrichial), and consist of a secretory portion and a serpentine duct that opens into the follicular canal.[1022-1026] An important feature of the equine sweat glands compared with other species is their rich capillary network.[1023,1025] Details of equine thermoregulation are discussed elsewhere.[1027]

Sweat evaporation, followed by respiration, are the main ways for equids and humans to dissipate heat.[1028-1030] Sweating in horses is controlled by endocrine, neural, and vascular mechanisms.[1025,1031] Neural regulation mainly involves sympathetic β-adrenergic innervation whereas endocrine mechanisms consist of β-adrenergic receptor stimulation by circulating catecholamines.[1030] It appears that the neural sympathetic control of sweating in equidae is indirect by controlling regional blood flow whereas the humoral sympathetic

control is direct to the glands.[1023] This is evident in horses with Horner syndrome in which sympathetic denervation results in regional sweating that is believed to be caused by vasodilation and increased cutaneous blood flow.[1032]

The central neural pathway to the sweat gland is primarily sympathetic.[1033] Efferent fibers travel from the hypothalamus via the tegmentum to the intermediolateral column of the spinal cord to emerge from the ventral horns and synapse in the sympathetic ganglia. From here, postganglionic nonmyelinated fibers join peripheral nerves to reach the sweat glands. These sympathetic fibers control sweating, pilomotor response, cutaneous vasoconstriction, and vasodilation.[1033] Sympathetic fibers reaching sweat glands, at least in people, are cholinergic and adrenergic. In contrast to humans where neural cholinergic (muscarinic receptors) stimulation is important for sweat production,[1033] β_2-adrenergic receptor activation appears to dominate sweating in the horse.[1019,1023,1024,1026] Cholinergic innervation to the equine sweat glands has been demonstrated, and it has been proposed that it might be important in regulating regional blood flow.[1031,1034] The relative role of purinergic (ATP, adenosine) and nitrinergic (nitric oxide) stimulation remains unclear.[1023-1026,1031,1034-1038] Equine sweat glands are responsive to purinergic receptor activation.[1039] The importance of nitric oxide in equine sweat gland physiology is supported by the fact that inhibition of nitric oxide synthase attenuates sweating in healthy horses.[1036,1037] It has been proposed that the effect of nitric oxide could be through cutaneous vasodilation or by modulating the sympathetic control of sweating.[1036,1037]

PATHOPHYSIOLOGY

Even though a clear understanding on the pathogenesis of equine anhidrosis is lacking, proposed mechanisms include deficiency in β_2-adrenoreceptor agonists (deficient β_2-adrenergic stimulation), a poor sweat gland response to β_2-adrenergic stimulation (receptor refractoriness or downregulation), and inflammation.[1016] In regard to the neural control of sweating, it has been suggested that hypothalamic dysfunction from severe hyperthermia may be involved[1019]; however, there is no evidence to substantiate this hypothesis. It seems that the pathogenesis of anhidrosis results from a combination of insufficient neural and endocrine stimulation. Horses with skin denervation can sweat, although their response to thermal stimulation is impaired.[1040,1041] One study found that sweat gland innervation of horses with anhidrosis was normal,[1015] making denervation unlikely in the pathogenesis of anhidrosis.[1019,1040] A clinical example supporting that factors other than lack of autonomic innervation contribute to equine anhidrosis is equine Horner syndrome in which sympathetic denervation results in excessive sweating.

With regard to the role of the adrenal medulla in the pathogenesis of equine anhidrosis, some studies have shown that medullary dysfunction is improbable in the development of this disease.[1019,1040-1042] Similarly, vascular dysfunction to the sweat glands seems unlikely. Instead it has been suggested that abnormal dermal vascular perfusion could be relevant to the pathogenesis of equine anhidrosis.[1023,1035]

The fact that epinephrine concentrations in anhidrotic horses are normal or elevated,[1043] but sweat glands have a decreased response to adrenergic stimulation, indicates that receptor downregulation could be involved.[1015] Downregulation of β_2-adrenoreceptors has been demonstrated in anhidrotic horses.[1044] It is possible that excessive or prolonged exposure to catecholamines leads to reduced receptor signaling. The presence of sweat gland atrophy, cell flattening, and ductal obstruction on histologic examination does not imply that these changes are the cause but rather consequences of this disease.[1035,1045,1046] There is no evidence that inflammation or immune-mediated processes are central to the pathogenesis of equine anhidrosis. In humans, idiopathic sudomotor failure or idiopathic generalized anhidrosis, a condition characterized by reduced or lack of sweating, has been proposed to be caused by abnormal acetylcholine binding, reduced number of muscarinic receptors, or immune-mediated interference with cholinergic signaling.[1047] High immunoglobulin E (IgE) concentrations, the presence of pain (cholinergic urticaria), and a positive response to glucocorticoid therapy support an immune component in humans[1047] but remain unclear in horses, even though histologic abnormalities have been documented.[1023,1035,1046,1048]

It was recently shown that purinergic agonists increase intracellular calcium concentrations in sweat glands from healthy horses,[1039] and it has been suggested that purinergic P2Y receptors may be relevant to equine anhidrosis.[1049] Purinergic agonists stimulated electrolyte transport in sweat gland cells from normal horses but failed to elicit a response in cells from anhidrotic horses.[1038] Similarly, β_2-adrenergic receptor agonist stimulation failed to provoke a response in cells from anhidrotic horses.[1038] This indicates that both purinergic and adrenergic ion transport mechanisms may be involved in the pathogenesis of this condition. Assuming that abnormal β_2-adrenergic receptor signaling is central to the pathogenesis of anhidrosis, receptor polymorphisms could support the genetic predisposition of this condition. However, it is feasible that defects in other proteins, not necessarily the receptors per se, are implicated. The low expression of the water channel aquaporin-5 in anhidrotic horses may be of clinical relevance to the pathophysiology of anhidrosis, but it does not imply that is the cause.[1045]

The store-operated Ca^{2+} entry (SOCE) involves multiple proteins that increase intracellular Ca^{2+} to maintain the secretory function of exocrine cells.[1050] One of these proteins is stromal interaction molecule 1 (STIM1), which acts as a Ca^{2+} sensor at the endoplasmic reticulum membrane to modulate Ca^{2+} entry into the cell.[1050] Of interest, decreased STIM1 mRNA expression and protein activity were found in sweat glands isolated from anhidrotic horses, indicating that SOCE contributes to equine sweat production and abnormalities in this system may be involved in the pathogenesis of anhidrosis.[1051] Bestrophin-2 is an anion channel that facilitates Ca^{2+}-associated ionic transport and monovalent ion exchange into sweat via the $Na^+/K^+/2Cl^-$ cotransporter, and downregulation of these proteins has been documented in animal models of anhidrosis and suspected to occur in humans with idiopathic anhidrosis.[1052]

The role of thyroid hormones in the pathogenesis of anhidrosis is unclear. It is believed by some that anhidrosis is caused by hypothyroidism; however, there are no data to substantiate this. Some studies have found lower thyroid hormones concentrations in anhidrotic horses whereas others have not.[470,509,510,1017] Lower thyroid hormone concentrations in horses with clinical signs of anhidrosis may represent an adaptation to the disease (nonthyroidal illness syndrome). Baseline thyroid hormones and the thyroid hormone response to the TRH stimulation test were not different between anhidrotic

and matched control horses.[470] The fact that some horses may show improvement with L-thyroxine or iodinated casein supplementation indicates that thyroid hormones may enhance adrenoreceptor sensitivity, as documented in other species.[1053] It has been claimed that abnormal electrolyte concentrations are part of the pathogenesis of anhidrosis; however, in controlled studies no differences have been found between anhidrotic and healthy horses.[510]

Anhidrosis, heat stress, and exercise intolerance are documented conditions in equids with pituitary pars intermedia dysfunction, particularly in tropical climates.[1054] It has been proposed that drug-induced anhidrosis is the likely cause of hyperthermia in foals treated with macrolide antibiotics, particularly erythromycin.[1055]

CLINICAL SIGNS

Typical clinical signs include depression, anorexia, poor performance, tachypnea, hyperthermia (particularly after exercise), lack of or decreased sweating, alopecia, dry coat, and flaky skin, mainly over the face. Some areas may continue to sweat, predominantly under the saddle, mane, and inguinal and axillary regions. Initial signs are nonspecific and related to poor performance and respiratory problems. Tachypnea is the most common clinical sign in horses with anhidrosis.[1018] Early recognition of anhidrosis is important, particularly in hot climates where a delay in core temperature cooling can lead to heat stroke. In addition, anhidrosis represents a major stress to the animal, which can predispose to other diseases.

DIAGNOSIS

In general, the diagnosis of anhidrosis is based on clinical signs. The first specific method developed for the diagnosis of anhidrosis was the intradermal injection of epinephrine.[1056] Horses with anhidrosis will have a delayed or absent sweat response to this test. However, because epinephrine is not specific to β_2-receptors, α-adrenergic effects may mask the response of the sweat glands. Subsequently, tests based on β_2-receptor agonists (salbutamol, terbutaline) have been developed.[1029,1057] These methods are semiquantitative. Saline solution and serial dilutions of salbutamol or terbutaline (10^{-3}–10^{-8} w/v) are injected intradermally over the side of the neck.[1029,1057] Sweating is evident by 10 minutes, but the final response is evaluated at 20 to 30 minutes. In the terbutaline test, terbulatine sulfate (1 mg/mL [1000 mg/L]) in tenfold dilutions with 0.9% NaCl (1000, 100, 10, 1, 0.1, 0.01, 0.001, and 0 mg/L) is injected intradermally (0.1 mL) with a 25-gauge needle at 5 cm apart.[1029,1057] For the epinephrine response test, 0.1 mL of epinephrine in tenfold dilutions (1:1000, 1:10,000, 1:100,000; 1:1,000,000) is injected as previously described. Horses with anhidrosis have minimal to no response to any of the dilutions,[1019,1029,1057] whereas normal horses have a sweat response to most dilutions.

Differential diagnoses are limited to the respiratory tract as most anhidrotic and hypohidrotic horses have tachypnea and an exaggerated respiratory response (tachypnea) to exercise. In addition, body temperatures in these animals remain elevated for longer periods of time than in healthy animals. Idiopathic hyperthermia should be considered in foals of draft breeds with thermoregulatory problems. The etiology is unknown. These animals have persistent hyperthermia and tachypnea with minimal to no evidence of systemic or pulmonary disease and tend to be bright and alert. This condition resolves in 3 to 7 days with supportive management that includes cooling the environment, alcohol baths, and cooled intravenous fluids.

TREATMENT, PREVENTION, AND PROGNOSIS

There is no specific treatment for equine anhidrosis. Keeping affected horses in a cool environment and reducing physical activity is central to the palliative treatment of anhidrosis. Measures that reduce environmental temperature/increase heat loss include shades, fans, air conditioning, misting, and water cooling. Because many afflicted horses are used for various performance activities, unfortunately some management measures may not be practical or are not implemented. On hot days, it is recommended to work these horses in the coolest part of the day. In severe cases and, if feasible, moving these animals to cooler climates should be considered. Dietary modifications and mineral supplementation may help. The use of salts and electrolytes, including NaCl (table salt), KCl (light salt), $NaHCO_3$ (baking soda), and $MgSO_4$ (Epsom salt) may be beneficial, although controlled studies are lacking. A multitude of supplements containing electrolytes, amino acids, L-tyrosine (catecholamine precursor), cobalt, vitamin E (antioxidant), vitamin C (antioxidant), vitamin B_{12}, other water-soluble vitamins, and herbal remedies, to name a few, are available to treat anhidrosis, although their efficacy has not been scientifically evaluated. Methyldopa, an antihypertensive agent that decreases central sympathetic activity (sympatholytic), has also been used, but there is no research to support its efficacy in anhidrotic horses. Antihistamines have been unsuccessful. It has been reported that daily oral administration of iodinated casein (10–15 g/day) or sodium levothyroxine (0.5–3.0 mg/lb; 1–6 mg/100 kg) improved clinical signs.[1019,1035] As there is information from other species that thyroid hormones enhance β_2-adrenoreceptor sensitivity, these supplements should be considered in some horses with anhidrosis, keeping in mind potential complications (iatrogenic hyperthyroidism, weight loss, hyperthermia).

The use of β_2-receptor agonists is controversial as available information indicates that β_2-receptor downregulation is the result of excessive adrenergic stimulation. However, some clinicians report that the use of clenbuterol in horses with mild anhidrosis or hypohidrosis when environmental temperature and humidity are elevated could ameliorate the severity of the clinical signs. A recent study found no effect of prolonged clenbuterol treatment on the sweat response to intradermal epinephrine administration in Thoroughbred horses.[1058]

There is no evidence that anhidrosis is an immune-mediated process; however, glucocorticoids have been a proposed treatment. A combination of glucocorticoids with clenbuterol has been suggested under the rationale that β_2-receptor agonists may potentiate the antiinflammatory effects of corticoids. Another justification is that glucocorticoids may enhance β_2-adrenoreceptor response to agonists. Again, this approach remains unclear as excessive adrenergic stimulation seems to be part of the problem. It is also important to avoid the use of sedatives (α_2-receptor agonists) that may stimulate sweating in the horse.

Acupuncture and acupressure are nontraditional treatments claimed by horse owners, trainers, and some veterinarians to improve sweating in anhidrotic horses, particularly when other therapies have failed. In one retrospective study

that included anhidrotic horses treated with acupuncture and herbal medicine, according to the owners, clinical signs improved in treated horses.[1059] A limitation of this study was the inclusion criteria that likely introduced client bias—only anhidrotic horses with complete medical controls and the ability to contact clients for follow-up were included.[1059] A field evaluation of acupuncture combined with herbal medication in horses diagnosed with anhidrosis based on the intradermal terbutaline sweat test in Florida failed to demonstrate a clear difference between treated and placebo horses.[1060] In that study, sweating improved in treated horses but statistical significance compared with placebo horses could not be achieved.[1060] According to the author, acupuncture combined with herbal medicine may potentially improve sweating in anhidrotic horses, but the effect is short-lasting after discontinuation of treatment.[1060] One 12-oz (355 mL) can of beer every morning is recommended in some lay magazines as an effective treatment for anhidrosis. Some sources claim that dark beer is more effective.

The prognosis for horses with anhidrosis is guarded and dictated by the severity of the condition, response to therapy, geography, and implementation of palliative measures.

REFERENCES

1. Toribio RE. Disorders of calcium and phosphate metabolism in horses. *Vet Clin North Am Equine Pract*. 2011;27:129–147.
2. Hurcombe SD, Toribio RE, Slovis NM, et al. Calcium regulating hormones and serum calcium and magnesium concentrations in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2009;23:335–343.
3. Kamr AM, Dembek KA, Reed SM, et al. Vitamin D Metabolites and Their Association with Calcium, Phosphorus, and PTH Concentrations, Severity of Illness, and Mortality in Hospitalized Equine Neonates. *PLoS One*. 2015;10:e0127684.
4. Toribio RE, Kohn CW, Chew DJ, Sams RA, Rosol TJ. Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis. *Am J Vet Res*. 2001;62:938–947.
5. Toribio RE, Kohn CW, Capen CC, Rosol TJ. Parathyroid Hormone (PTH) Secretion, PTH mRNA and Calcium-Sensing Receptor mRNA Expression in Equine Parathyroid Cells, and Effects of IL-1, IL-6, and TNF-alpha on Equine Parathyroid Cell Function. *J Mol Endocrinol*. 2003.
6. Toribio RE, Kohn CW, Hardy J, Rosol TJ. Alterations in serum parathyroid hormone and electrolyte concentrations and urinary excretion of electrolytes in horses with induced endotoxemia. *J Vet Intern Med*. 2005;19:223–231.
7. Toribio RE, Kohn CW, Rourke KM, Levine AL, Rosol TJ. Effects of hypercalcemia on serum concentrations of magnesium, potassium, and phosphate and urinary excretion of electrolytes in horses. *Am J Vet Res*. 2007;68:543–554.
8. Rosol TJ, Capen CC. Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals*. San Diego: Academic Press; 1997:619–702.
9. Aguilera-Tejero E, Sanchez J, Almaden Y, Mayer-Valor R, Rodriguez M, Felsenfeld AJ. Hysteresis of the PTH-calcium curve during hypocalcemia in the dog: effect of the rate and linearity of calcium decrease and sequential episodes of hypocalcemia. *J Bone Miner Res*. 1996;11:1226–1233.
10. Hurwitz S. Homeostatic control of plasma calcium concentration. *Crit Rev Biochem Mol Biol*. 1996;31:41–100.
11. Kohn CW, Brooks CL. Failure of pH to predict ionized calcium percentage in healthy horses. *Am J Vet Res*. 1990;51:1206–1210.
12. Lopez I, Estepa JC, Mendoza FJ, Mayer-Valor R, Aguilera-Tejero E. Fractionation of calcium and magnesium in equine serum. *Am J Vet Res*. 2006;67:463–466.
13. Berlin D, Aroch I. Concentrations of ionized and total magnesium and calcium in healthy horses: effects of age, pregnancy, lactation, pH and sample type. *Vet J*. 2009;181:305–311.
14. Hilgard P. Experimental hypercalcaemia and whole blood clotting. *J Clin Pathol*. 1973;26:616–619.
15. Schryver HF, Hintz HF. Minerals. In: Robinson NE, ed. *Current Therary in Equine Medicine*. Philadelphia: W.B. Saunders; 1987:393–405.
16. National Research Council. *Nutrient Requirements of Horses*. 5th ed. Washington, D.C.: National Academic Press; 1989.
17. Meyer H, Ahlswede L. Magnesium metabolism in the horse. *Zentralbl Veterinarmed A*. 1977;24:128–139.
18. Jordan RM, Meyers VS, Yoho B, Spurrel FA. Effect of calcium and phosphorus levels on growth, reproduction, and bone development of ponies. *J Anim Sci*. 1975;40:78.
19. Schryver HF, Oftedal OT, Williams J, Soderholm LV, Hintz HF. Lactation in the horse: the mineral composition of mare milk. *J Nutr*. 1986;116:2142–2147.
20. Schryver HF, Oftedal OT, Williams J, Cymbaluk NF, Antczak D, Hintz HF. A comparison of the mineral composition of milk of domestic and captive wild equids (Equus przewalski, E. zebra, E. burchelli, E. caballus, E. assinus). *Comp Biochem Physiol A*. 1986;85:233–235.
21. Schryver HF, Craig PH, Hintz HF. Calcium metabolism in ponies fed varying levels of calcium. *J Nutr*. 1970;100:955–964.
22. Schryver HF, Hintz HF, Craig PH. Phosphorus metabolism in ponies fed varying levels of phosphorus. *J Nutr*. 1971;101:1257–1263.
23. Schryver HF, Hintz HF, Lowe JE. Calcium and phosphorus in the nutrition of the horse. *Cornell Vet*. 1974;64:493–515.
24. National Research Council. *Mineral tolerance of domestic animals*. Washington, D.C.: National Academic Press; 1980.
25. Hintz HF, Meakim DW. A Comparison of the 1978 National Research Council's Recommendations of Nutrient Requirements of Horses With Recent Studies. *Equine Vet J*. 1981;13(3):187–191.
26. Schryver HF, Foose TJ, Williams J, Hintz HF. Calcium excretion in feces of ungulates. *Comp Biochem Physiol A*. 1983;74:375–379.
27. Meyer H, Stadermann B, Schnurpel B. The influence of type of diet (roughage or concentrate) on the plasma level, renal excretion, and apparent digestibility of calcium and magnesium in resting and exercising horses. *J Equine Vet Sci*. 1992;12:233–239.
28. Schryver HF, Craig PH, Hintz HF, Hogue DE, Lowe JE. The site of calcium absorption in the horse. *J Nutr*. 1970;100:1127–1131.
29. Schryver HF, Hintz HF, Craig PH. Calcium metabolism in ponies fed a high phosphorus diet. *J Nutr*. 1971;101:259–264.
30. Schryver HF, Hintz HF, Lowe JE. Calcium and phosphorus inter-relationships in horse nutrition. *Equine Vet J*. 1971;3:102–109.
31. Swartzman JA, Hintz HF, Schryver HF. Inhibition of calcium absorption in ponies fed diets containing oxalic acid. *Am J Vet Res*. 1978;39:1621–1623.
32. McKenzie RA, Gartner RJ, Blaney BJ, Glanville RJ. Control of nutritional secondary hyperparathyroidism in grazing horses with calcium plus phosphorus supplementation. *Aust Vet J*. 1981;57:554–557.
33. McKenzie EC, Valberg SJ, Godden SM, et al. Plasma and urine electrolyte and mineral concentrations in Thoroughbred horses with recurrent exertional rhabdomyolysis after consumption of diets varying in cation-anion balance. *Am J Vet Res*. 2002;63:1053–1060.
34. Glade MJ, Krook L, Schryver HF, Hintz HF. Calcium metabolism in glucocorticoid-treated pony foals. *J Nutr*. 1982;112:77–86.

35. Glade MJ, Krook L. Glucocorticoid-induced inhibition of osteolysis and the development of osteopetrosis, osteonecrosis and osteoporosis. *Cornell Vet*. 1982;72:76–91.
36. Harrington DD. Influence of magnesium deficiency on horse foal tissue concentration of Mg, calcium and phosphorus. *Br J Nutr*. 1975;34:45–57.
37. Hintz HF, Schryver HF. Magnesium metabolism in the horse. *J Anim Sci*. 1972;35:755–759.
38. Caple IW, Doake PA, Ellis PG. Assessment of the calcium and phosphorus nutrition in horses by analysis of urine. *Aust Vet J*. 1982;58:125–131.
39. Coffman J. Percent creatinine clearance ratios. *Vet Med Small Anim Clin*. 1980;75:671–676.
40. Stewart AJ, Hardy J, Kohn CW, Toribio RE, Hinchcliff KW, Silver B. Validation of diagnostic tests for determination of magnesium status in horses with reduced magnesium intake. *Am J Vet Res*. 2004;65:422–430.
41. Endres DB, Rude RK. Mineral and Bone Metabolism. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. St. Louis, Mo: Elsevier Saunders; 2006:1891–1963.
42. Schryver HF, Hintz HF, Craig PH, Hogue DE, Lowe JE. Site of phosphorus absorption from the intestine of the horse. *J Nutr*. 1972;102:143–147.
43. Schryver HF. Intestinal absorption of calcium and phosphorus by horses. *J S Afr Vet Assoc*. 1975;46:39–45.
44. van Doorn DA, Everts H, Wouterse H, Homan S, Beynen AC. Influence of high phosphorus intake on salivary and plasma concentrations, and urinary phosphorus excretion in mature ponies. *J Anim Physiol Anim Nutr (Berl)*. 2011;95:154–160.
45. Fowler AL, Hansen TL, Strasinger LA, Harlow BE, Lawrence LM. Phosphorus digestibility and phytate degradation by yearlings and mature horses. *J Anim Sci*. 2015;93:5735–5742.
46. National Research Council (U.S.). *Committee on Nutrient Requirements of Horses. Nutrient requirements of horses*. 6th rev. ed. Washington, DC: National Academies Press; 2007.
47. van Doorn DA, Everts H, Wouterse H, Beynen AC. The apparent digestibility of phytate phosphorus and the influence of supplemental phytase in horses. *J Anim Sci*. 2004;82:1756–1763.
48. Hintz HF, Williams AJ, Rogoff J, Schryver HF. Availability of phosphorus in wheatbran when fed to ponies. *J Anim Sci*. 1973;36:522–525.
49. Matsui T, Murakami Y, Yano H, Fujikawa H, Osawa T, Asai Y. Phytate and phosphorus movements in the digestive tract of horses. *Equine Vet J Suppl*. 1999:505–507.
50. Lavin TE, Nielsen BD, Zingsheim JN, et al. Effects of phytase supplementation in mature horses fed alfalfa hay and pelleted concentrate diets. *J Anim Sci*. 2013;91:1719–1727.
51. Hintz HF, Schryver HF. Magnesium, calcium and phosphorus metabolism in ponies fed varying levels of magnesium. *J Anim Sci*. 1973;37:927–930.
52. Roose KA, Hoekstra KE, Pagan JD, Geor RJ. Effect of an aluminum supplement on nutrient digestibility and mineral metabolism in Thoroughbred horses. *16th Equine Nutr Physiol Soc Symp*. 2001:364–369.
53. Mundy GR, Guise TA. Hormonal control of calcium homeostasis. *Clin Chem*. 1999;45:1347–1352.
54. Suva LJ, Winslow GA, Wettenhall RE, et al. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. *Science*. 1987;237:893–896.
55. Wysolmerski JJ, Stewart AF. The physiology of parathyroid hormone-related protein: an emerging role as a developmental factor. *Annu Rev Physiol*. 1998;60:431–460.
56. Aguilera-Tejero E, Garfia B, Estepa JC, Lopez I, Mayer-Valor R, Rodriguez M. Effects of exercise and EDTA administration on blood ionized calcium and parathyroid hormone in horses. *Am J Vet Res*. 1998;59:1605–1607.
57. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab*. 1983;56:572–581.
58. Estepa JC, Aguilera-Tejero E, Mayer-Valor R, Almaden Y, Felsenfeld AJ, Rodriguez M. Measurement of parathyroid hormone in horses. *Equine Vet J*. 1998;30:476–481.
59. Felsenfeld AJ, Llach F. Parathyroid gland function in chronic renal failure. *Kidney Int*. 1993;43:771–789.
60. Brown EM, Gamba G, Riccardi D, et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature*. 1993;366:575–580.
61. Rourke KM, Kohn CW, Rosol TJ, Toribio RE. Equus caballus parathyroid hormone precursor (PTH) mRNA. *NIH-GenBank*. 2006. Retrieved from http://www.ncbi.nlm.nih.gov/nuccore/DQ399295.1.
62. Capen CC, Rosol TJ. Pathobiology of Parathyroid Hormone and Parathyroid Hormone-Related Protein: Introducing and Evolving Concepts. In: LiVolsi VA, DeLellis RA, eds. *Pathobiology of the Parathyroid and Thyroid Glands*. Baltimore: Williams & Wilkins; 1993:1–33.
63. Dambacher MA, Fischer JA, Hunziker WH, et al. Distribution of circulating immunoreactive components of parathyroid hormone in normal subjects and in patients with primary and secondary hyperparathyroidism: the role of the kidney and of the serum calcium concentration. *Clin Sci (Lond)*. 1979;57:435–443.
64. Kronengerg HM, Bringhurst FR, Segre GV, Potts Jr JT. Parathyroid hormone biosynthesis and metabolism. In: Bilezikian JP, Marcus R, Levine MA, eds. *The Parathyroids: Basic and Clinical Concepts*. San Diego: Academic Press; 2001:17–30.
65. Martin KJ, Hruska KA, Freitag JJ, Klahr S, Slatopolsky E. The peripheral metabolism of parathyroid hormone. *N Engl J Med*. 1979;301:1092–1098.
66. Bringhurst FR, Stern AM, Yotts M, Mizrahi N, Segre GV, Potts Jr JT. Peripheral metabolism of PTH: fate of biologically active amino terminus in vivo. *Am J Physiol*. 1988;255:E886–E893.
67. Fox J, Scott M, Nissenson RA, Heath III H. Effect of plasma calcium concentration on the metabolic clearance rate of parathyroid hormone in the dog. *J Lab Clin Med*. 1983;102:70–77.
68. Segre GV, D'Amour P, Hultman A, Potts Jr JT. Effects of hepatectomy, nephrectomy, and nephrectomy/uremia on the metabolism of parathyroid hormone in the rat. *J Clin Invest*. 1981;67:439–448.
69. Silver J, Russell J, Sherwood LM. Regulation by vitamin D metabolites of messenger ribonucleic acid for preproparathyroid hormone in isolated bovine parathyroid cells. *Proc Natl Acad Sci U S A*. 1985;82:4270–4273.
70. Moallem E, Kilav R, Silver J, Naveh-Many T. RNA-Protein binding and post-transcriptional regulation of parathyroid hormone gene expression by calcium and phosphate. *J Biol Chem*. 1998;273:5253–5259.
71. Bringhurst FR. Physiologic actions of PTH and PTHrP. II. Renal actions. In: Bilezikian JP, Marcus R, Levine MA, eds. *The Parathyroids: Basic and Clinical Concepts*. San Diego: Academic Press; 2001:223–243.
72. Bellorin-Font E, Lopez C, Diaz K, Pernalete N, Lopez M, Starosta R. Role of protein kinase C on the acute desensitization of renal cortical adenylate cyclase to parathyroid hormone. *Kidney Int*. 1995;47:38–44.
73. de Rouffignac C, Quamme G. Renal magnesium handling and its hormonal control. *Physiol Rev*. 1994;74:305–322.
74. Friedman PA. Codependence of renal calcium and sodium transport. *Annu Rev Physiol*. 1998;60:179–197.
75. Brown EM. Physiology and pathophysiology of the extracellular calcium-sensing receptor. *Am J Med*. 1999;106:238–253.
76. Houillier P. Mechanisms and regulation of renal magnesium transport. *Annu Rev Physiol*. 2014;76:411–430.

77. Simon DB, Lu Y, Choate KA, et al. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science*. 1999;285:103–106.
78. Hoenderop JG, van der Kemp AW, Hartog A, et al. Molecular identification of the apical Ca^{2+} channel in 1, 25-dihydroxyvitamin D3-responsive epithelia. *J Biol Chem*. 1999;274:8375–8378.
79. Hoenderop JG, Willems PH, Bindels RJ. Toward a comprehensive molecular model of active calcium reabsorption. *Am J Physiol Renal Physiol*. 2000;278:F352–F360.
80. Rourke KM, Coe S, Kohn CW, Rosol TJ, Mendoza FJ, Toribio RE. Cloning, comparative sequence analysis and mRNA expression of calcium-transporting genes in horses. *Gen Comp Endocrinol*. 2010;167:6–10.

80a. Wilkens MR, Marholt L, Eigendorf N, et al. Trans- and paracellular calcium transport along the small and large intestine in horses. *Comp Biochem Physiol A Mol Integr Physiol*. 2017;204:157–163.

81. Gmaj P, Murer H. Cellular mechanisms of inorganic phosphate transport in kidney. *Physiol Rev*. 1986;66:36–70.
82. Malmstrom K, Murer H. Parathyroid hormone regulates phosphate transport in OK cells via an irreversible inactivation of a membrane protein. *FEBS Lett*. 1987;216:257–260.
83. Filipovic N, Stojevic Z, Plevnik N, Masek T, Prvanovic N, Tucek Z. The influence of age on bone metabolism in mares during late pregnancy and lactation. *Res Vet Sci*. 2014;97:194–198.
84. Teitelbaum SL. Bone resorption by osteoclasts. *Science*. 2000;289:1504–1508.
85. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. 1997;89:309–319.
86. Lepage OM, Carstanjen B, Uebelhart D. Non-invasive assessment of equine bone: an update. *Vet J*. 2001;161:10–22.
87. Price JS. Biochemical markers of bone metabolism in horses: potentials and limitations? *Vet J*. 1998;156:163–165.
88. Carstanjen B, Hoyle NR, Gabriel A, Detilleux J, Amory H, Remy B. Assessment of Bone Formation- and Bone Resorption-Markers in Horses. *J Bone Miner Res*. 2002;17:S319.
89. Holick MF, Frommer JE, McNeill SC, Richtand NM, Henley JW, Potts Jr JT. Photometabolism of 7-dehydrocholesterol to previtamin D3 in skin. *Biochem Biophys Res Commun*. 1977;76:107–114.
90. Fu GK, Lin D, Zhang MY, et al. Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D–dependent rickets type 1. *Mol Endocrinol*. 1997;11:1961–1970.
91. Kuro-o M. Overview of the FGF23-Klotho axis. *Pediatr Nephrol*. 2010;25:583–590.
92. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiol Rev*. 2012;92:131–155.
93. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. *Am J Physiol*. 1999;277:F157–F175.
94. Friedman PA, Gesek FA. Cellular calcium transport in renal epithelia: measurement, mechanisms, and regulation. *Physiol Rev*. 1995;75:429–471.
95. Wasserman RH, Fullmer CS. Vitamin D and intestinal calcium transport: facts, speculations and hypotheses. *J Nutr*. 1995;125:1971S–1979S.
96. Szabo A, Merke J, Beier E, Mall G, Ritz E. 1,25(OH)2 vitamin D3 inhibits parathyroid cell proliferation in experimental uremia. *Kidney Int*. 1989;35:1049–1056.
97. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab*. 2009;94:26–34.
98. Borella E, Nesher G, Israeli E, Shoenfeld Y. Vitamin D: a new anti-infective agent? *Ann N Y Acad Sci*. 2014;1317:76–83.
99. Zittermann A, Gummert JF. Nonclassical vitamin D action. *Nutrients*. 2010;2:408–425.
100. Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol*. 2009;4:1151–1165.
101. Guo C, Gombart AF. The antibiotic effects of vitamin D. *Endocr Metab Immune Disord Drug Targets*. 2014;14:255–266.
102. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. *N Engl J Med*. 2009;360:1912–1914.
103. Nair P, Lee P, Reynolds C, et al. Significant perturbation of vitamin D-parathyroid-calcium axis and adverse clinical outcomes in critically ill patients. *Intensive Care Med*. 2013;39:267–274.
104. Jeng L, Yamshchikov AV, Judd SE, et al. Alterations in vitamin D and anti-microbial peptides in patients in the intensive care unit with sepsis. *J Transl Med*. 2009;7:28.
105. Leaf DE, Croy HE, Abrahams SJ, Raed A, Waikar SS. Cathelicidin antimicrobial protein, vitamin D, and risk of death in critically ill patients. *Crit Care*. 2015;19:80.
106. Lee P, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? *Intensive Care Med*. 2009;35:2028–2032.
107. Breidenbach A, Schlumbohm C, Harmeyer J. Peculiarities of vitamin D and of the calcium and phosphate homeostatic system in horses. *Vet Res*. 1998;29:173–186.
108. Horst RL, Littledike ET, Riley JL, Napoli JL. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals. *Anal Biochem*. 1981;116:189–203.
109. Horst RL, Littledike ET. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. *Comp Biochem Physiol B*. 1982;73:485–489.
110. Maenpaa PH, Lappetelainen R, Virkkunen J. Serum retinol, 25-hydroxyvitamin D and alpha-tocopherol of racing trotters in Finland. *Equine Vet J*. 1987;19:237–240.
111. Maenpaa PH, Koskinen T, Koskinen E. Serum profiles of vitamins A, E and D in mares and foals during different seasons. *J Anim Sci*. 1988;66:1418–1423.
112. Maenpaa PH, Pirhonen A, Koskinen E. Vitamin A, E and D nutrition in mares and foals during the winter season: effect of feeding two different vitamin-mineral concentrates. *J Anim Sci*. 1988;66:1424–1429.
113. Smith BS, Wright H. 25-Hydroxyvitamin D concentrations in equine serum. *Vet Rec*. 1984;115:579.
114. Enbergs H, Karp HP, Schonherr U. Course of blood levels of calcium, inorganic phosphate, alkaline phosphatase, parathyroid hormone and calcidiol (25-OH-D3) in one and two year old thoroughbred horses. *Dtsch Tierarztl Wochenschr*. 1996;103:491–493.
115. Pozza ME, Kaewsakhorn T, Trinarong C, Inpanbutr N, Toribio RE. Serum vitamin D, calcium, and phosphorus concentrations in ponies, horses and foals from the United States and Thailand. *Vet J*. 2014;199:451–456.
116. El Shorafa WM, Feaster JP, Ott EA, Asquith RL. Effect of vitamin D and sunlight on growth and bone development of young ponies. *J Anim Sci*. 1979;48:882–886.
117. Azarpeykan S, Dittmer KE, Gee EK, et al. Influence of blanketing and season on vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. *Domest Anim Endocrinol*. 2016;56:75–84.
118. Piccione G, Assenza A, Fazio F, Bergero D, Caola G. Daily rhythms of serum vitamin D-metabolites, calcium and phosphorus in horses. *Acta Veterinaria Brno*. 2008;77:151–157.
119. Azarpeykan S, Dittmer KE, Gee EK, et al. Circadian rhythm of calciotropic hormones, serum calcium, phosphorus and magnesium during the shortest and longest days of the year in horses in New Zealand. *J Anim Physiol Anim Nutr (Berl)*. 2016;100:1058–1066.
120. Lopez I, Estepa JC, Mendoza FJ, Rodriguez M, Aguilera-Tejero E. Serum concentrations of calcium, phosphorus, magnesium and calciotropic hormones in donkeys. *Am J Vet Res*. 2006;67:1333–1336.

121. Ceylan E, Dede S, Deger Y, Yoruk I. Investigation of the Effects of Carrying Heavy Load on Prooxidation/Antioxidant Status and Vitamin D-3 in Healthy Horses. *Asian J Anim Vet Adv*. 2009;4:41–46.
122. Toribio RE, Kohn CW, Leone GW, Capen CC, Rosol TJ. Molecular cloning and expression of equine calcitonin, calcitonin gene-related peptide-I, and calcitonin gene-related peptide-II. *Mol Cell Endocrinol*. 2002.
123. Garrett JE, Tamir H, Kifor O, et al. Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. *Endocrinology*. 1995;136:5202–5211.
124. Munson PL, Hirsch PF. Importance of calcitonin in physiology, clinical pharmacology, and medicine. *Bone Miner*. 1992;16:162–165.
125. Woodrow JP, Sharpe CJ, Fudge NJ, Hoff AO, Gagel RF, Kovacs CS. Calcitonin plays a critical role in regulating skeletal mineral metabolism during lactation. *Endocrinology*. 2006;147:4010–4021.
126. Blahser S. Immunocytochemical demonstration of calcitonin-containing C-cells in the thyroid glands of different mammals. *Cell Tissue Res*. 1978;186:551–558.
127. Gray AW, Davies ME, Jeffcott LB. Generation and activity of equine osteoclasts in vitro: effects of the bisphosphonate pamidronate (APD). *Res Vet Sci*. 2002;72:105–113.
128. Garel JM, Martin-Rosset W, Barlet JP. Plasma immunoreactive calcitonin levels in pregnant mares and newborn foals. *Horm Metab Res*. 1975;7:429–432.
129. Sandusky Jr GE, Wightman KA. Application of the peroxidase-antiperoxidase procedure to the localization of pituitary hormones and calcitonin in various domestic animals and human beings. *Am J Vet Res*. 1985;46:739–741.
130. Wysolmerski JJ. Parathyroid hormone-related protein: an update. *J Clin Endocrinol Metab*. 2012;97:2947–2956.
131. Care AD, Abbas SK, Ousey J, Johnson L. The relationship between the concentration of ionised calcium and parathyroid hormone-related protein (PTHrP[1-34]) in the milk of mares. *Equine Vet J*. 1997;29:186–189.
132. Barton MH, Sharma P, LeRoy BE, Howerth EW. Hypercalcemia and high serum parathyroid hormone-related protein concentration in a horse with multiple myeloma. *J Am Vet Med Assoc*. 2004;225:409–413, 376.
133. Karcher LF, Le Net JL, Turner BF, Reimers TJ, Tennant BC. Pseudohyperparathyroidism in a mare associated with squamous cell carcinoma of the vulva. *Cornell Vet*. 1990;80:153–162.
134. Marr CM, Love S, Pirie HM. Clinical, ultrasonographic and pathological findings in a horse with splenic lymphosarcoma and pseudohyperparathyroidism. *Equine Vet J*. 1989;21:221–226.
135. Meuten DJ, Price SM, Seiler RM, Krook L. Gastrict carcinoma with pseudohyperparathyroidism in a horse. *Cornell Vet*. 1978;68:179–195.
136. Rosol TJ, Nagode LA, Robertson JT, Leeth BD, Steinmeyer CL, Allen CM. Humoral hypercalcemia of malignancy associated with ameloblastoma in a horse. *J Am Vet Med Assoc*. 1994;204:1930–1933.
137. Berndt T, Kumar R. Phosphatonins and the regulation of phosphate homeostasis. *Annu Rev Physiol*. 2007;69:341–359.
138. Kuro-o M. Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism. *Nat Rev Nephrol*. 2013;9:650–660.
139. Lanske B, Razzaque MS. Molecular interactions of FGF23 and PTH in phosphate regulation. *Kidney Int*. 2014;86:1072–1074.
140. Gattineni J, Bates C, Twombley K, et al. FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1. *Am J Physiol Renal Physiol*. 2009;297:F282–F291.
141. Schiavi SC, Kumar R. The phosphatonin pathway: new insights in phosphate homeostasis. *Kidney Int*. 2004;65:1–14.
142. Hu MC, Kuro-o M, Moe OW. The emerging role of klotho in clinical nephrology. *Nephrol Dial Transplant*. 2012;27:2650–2657.
143. Kamr A, Dembek K, Hildreth III B, Reed SM, Slovis N, Barr B, Zaghawa A, Toribio R. The fibroblast growth factor-23/klotho axis in healthy and hospitalized foals. *ACVIM Abstracts*. 2016;E20:1504.
144. Beyer MJ, Freestone JF, Reimer JM, Bernard WV, Rueve ER. Idiopathic hypocalcemia in foals. *J Vet Intern Med*. 1997;11:356–360.
145. Aguilera-Tejero E, Estepa JC, Lopez I, Bas S, Rodriguez M. Polycystic kidneys as a cause of chronic renal failure and secondary hypoparathyroidism in a horse. *Equine Vet J*. 2000;32:167–169.
146. Couetil LL, Sojka JE, Nachreiner RF. Primary hypoparathyroidism in a horse. *J Vet Intern Med*. 1998;12:45–49.
147. Frank N, Hawkins JF, Couetil LL, Raymond JT. Primary hyperparathyroidism with osteodystrophia fibrosa of the facial bones in a pony. *J Am Vet Med Assoc*. 1998;212:84–86.
148. Tomlinson JE, Johnson AL, Ross MW, et al. Successful detection and removal of a functional parathyroid adenoma in a pony using technetium Tc 99m sestamibi scintigraphy. *J Vet Intern Med*. 2014;28:687–692.

148a. Cottle HJ, Hughes KJ, Thompson H, Johnston PEJ, Philbey AW. Primary hyperparathyroidism in a 17-year-old Arab × Welsh Cob pony mare with a functional parathyroid adenoma. *Equine Vet Educ*. 2016;28:477–485.

149. Villagran CC, Frank N, Schumacher J, Reel D. Persistent Hypercalcemia and Hyperparathyroidism in a Horse. *Case Reports Vet Medicine*. 2014;2014:1–6.
150. Ronen N, Van Heerden J, van Amstel SR. Clinical and biochemistry findings, and parathyroid hormone concentrations in three horses with secondary hyperparathyroidism. *J S Afr Vet Assoc*. 1992;63:134–136.
151. Harrington DD, Page EH. Acute vitamin D3 toxicosis in horses: case reports and experimental studies of the comparative toxicity of vitamins D2 and D3. *J Am Vet Med Assoc*. 1983;182:1358–1369.
152. Elfers RS, Bayly WM, Brobst DF, et al. Alterations in calcium, phosphorus and C-terminal parathyroid hormone levels in equine acute renal disease. *Cornell Vet*. 1986;76:317–329.
153. Aguilera-Tejero E, Estepa JC, Lopez I, Bas S, Garfia B, Rodriguez M. Plasma ionized calcium and parathyroid hormone concentrations in horses after endurance rides. *J Am Vet Med Assoc*. 2001;219:488–490.
154. Dart AJ, Snyder JR, Spier SJ, Sullivan KE. Ionized calcium concentration in horses with surgically managed gastrointestinal disease: 147 cases (1988–1990). *J Am Vet Med Assoc*. 1992;201:1244–1248.
155. Garcia-Lopez JM, Provost PJ, Rush JE, Zicker SC, Burmaster H, Freeman LM. Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. *Am J Vet Res*. 2001;62:7–12.
156. Kaneps AJ, Knight AP, Bennett DG. Synchronous diaphragmatic flutter associated with electrolyte imbalances in a mare with colic. *Equine Pract*. 1980;2:18.
157. Baird JD. Lactation tetany (eclampsia) in a Shetland pony mare. *Aust Vet J*. 1971;47:402–404.
158. Mansmann RA, Carlson GP, White NA, Milne DW. Synchronous diaphragmatic flutter in horses. *J Am Vet Med Assoc*. 1974;165:265–270.
159. Schoeb TR, Panciera RJ. Blister beetle poisoning in horses. *J Am Vet Med Assoc*. 1978;173:75–77.
160. Dyke TM, Maclean AA. Urethral obstruction in a stallion with possible synchronous diaphragmatic flutter. *Vet Rec*. 1987;121:425–426.
161. Carlson GP, Mansmann RA. Serum electrolyte and plasma protein alterations in horses used in endurance rides. *J Am Vet Med Assoc*. 1974;165:262–264.

162. Hudson NP, Church DB, Trevena J, Nielsen IL, Major D, Hodgson DR. Primary hypoparathyroidism in two horses. *Aust Vet J*. 1999;77:504–508.
163. Kerr MG, Snow DH. Composition of sweat of the horse during prolonged epinephrine (adrenaline) infusion, heat exposure, and exercise. *Am J Vet Res*. 1983;44:1571–1577.
164. Schryver HF, Hintz HF, Lowe JE. Calcium metabolism, body composition, and sweat losses of exercised horses. *Am J Vet Res*. 1978;39:245–248.
165. Holcombe SJ, Embertson RM, Kurtz KA, Roessner HA, Wismer SE, Geor RJ, Kaneene JB. Increased serum nonesterified fatty acid and low ionised calcium concentrations are associated with post partum colic in mares. *Equine Vet J*. 2016;48:39–44.
166. Vandeplassche M, Spincemaille J, Bouters R. Aetiology, pathogenesis and treatment of retained placenta in the mare. *Equine Vet J*. 1971;3:144–147.
167. Sevinga M, Barkema HW, Hesselink JW. Serum calcium and magnesium concentrations and the use of a calcium-magnesium-borogluconate solution in the treatment of Friesian mares with retained placenta. *Theriogenology*. 2002;57:941–947.
168. Delesalle C, Dewulf J, Lefebvre RA, Schuurkes JA, Van VB, Deprez P. Use of plasma ionized calcium levels and Ca^{2+} substitution response patterns as prognostic parameters for ileus and survival in colic horses. *Vet Q*. 2005;27:157–172.
169. Toribio RE, Kohn CW, Sams RA, Capen CC, Rosol TJ. Hysteresis and Calcium Set-point for the Calcium Parathyroid Hormone (PTH) Relationship in Healthy Horses. *Gen Comp Endocrinol*. 2003.
170. Carlstedt F, Eriksson M, Kiiski R, Larsson A, Lind L. Hypocalcemia during porcine endotoxemic shock: effects of calcium administration. *Crit Care Med*. 2000;28:2909–2914.
171. Zaloga GP. Ionized hypocalcemia during sepsis. *Crit Care Med*. 2000;28:266–268.
172. Carlstedt F, Lind L, Rastad J, Stjernstrom H, Wide L, Ljunghall S. Parathyroid hormone and ionized calcium levels are related to the severity of illness and survival in critically ill patients. *Eur J Clin Invest*. 1998;28:898–903.
173. van der Kolk JH, Nachreiner RF, Refsal KR, Brouillet D, Wensing T. Heparinised blood ionised calcium concentrations in horses with colic or diarrhoea compared to normal subjects. *Equine Vet J*. 2002;34:528–531.
174. King JN, Gerring EL. Detection of endotoxin in cases of equine colic. *Vet Rec*. 1988;123:269–271.
175. Meyers K, Reed S, Keck M, Clem M, Bayly W. Circulating endotoxin-like substance(s) and altered hemostasis in horses with gastrointestinal disorders: an interim report. *Am J Vet Res*. 1982;43:2233–2238.
176. Moore JN, White NA, Berg JN, Trim CM, Garner HE. Endotoxemia following experimental intestinal strangulation obstruction in ponies. *Can J Comp Med*. 1981;45:330–332.
177. Pantaleon LG, Furr MO, McKenzie HC, Donaldson L. Effects of small- and large-volume resuscitation on coagulation and electrolytes during experimental endotoxemia in anesthetized horses. *J Vet Intern Med*. 2007;21:1374–1379.
178. Zaloga GP, Malcolm D, Chernow B, Holaday J. Endotoxin-induced hypocalcemia results in defective calcium mobilization in rats. *Circ Shock*. 1988;24:143–148.
179. Nakamura T, Mimura Y, Uno K, Yamakawa M. Parathyroid hormone activity increases during endotoxemia in conscious rats. *Horm Metab Res*. 1998;30:88–92.
180. Crouser ED, Dorinsky PM. Metabolic consequences of sepsis. Correlation with altered intracellular calcium homeostasis. *Clin Chest Med*. 1996;17:249–261.
181. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet*. 1993;341:515–518.
182. Dandona P, Nix D, Wilson MF, et al. Procalcitonin increase after endotoxin injection in normal subjects. *J Clin Endocrinol Metab*. 1994;79:1605–1608.
183. Sperber SJ, Blevins DD, Francis JB. Hypercalcitoninemia, hypocalcemia, and toxic shock syndrome. *Rev Infect Dis*. 1990;12:736–739.
184. Schott HC, Marlin DJ, Geor RJ, et al. Changes in selected physiological and laboratory measurements in elite horses competing in a 160 km endurance ride. *Equine Vet J Suppl*. 2006:37–42.
185. Vervuert I, Stanik K, Coenen M. Effects of different levels of calcium and phosphorus intake on calcium homeostasis in exercising horses. *Equine Vet J Suppl*. 2006:659–663.
186. McCutcheon LJ, Geor RJ, Hare MJ, Ecker GL, Lindinger MI. Sweating rate and sweat composition during exercise and recovery in ambient heat and humidity. *Equine Vet J Suppl*. 1995:153–157.
187. Geiser DR, Andrews FM, Rohrbach BW, White SL, Maykuth PL, Green EM, Provenza MK. Blood ionized calcium concentrations in horses before and after the cross-country phase of three-day event competition. *Am J Vet Res*. 1995;56:1502–1505.
188. Gulick MA, MacAllister CG, Panciera R. Equine cantharidiasis. *Comp Cont Educ Equine Pract*. 1996;18:77–83.
189. Helman RG, Edwards WC. Clinical features of blister beetle poisoning in equids: 70 cases (1983–1996). *J Am Vet Med Assoc*. 1997;211:1018–1021.
190. Torbeck R, Pan M, DeMoll E, Levitt J. Cantharidin: a comprehensive review of the clinical literature. *Dermatol Online J*. 2014;20.
191. Lopez JR, Linares N, Cordovez G, Terzic A. Elevated myoplasmic calcium in exercise-induced equine rhabdomyolysis. *Pflugers Arch*. 1995;430:293–295.
192. Taintor J, Sartin EA, Waldridge BM, Schumacher J. Acute pancreatitis in a 3-day-old foal. *J Vet Intern Med*. 2006;20(1):210–212.
193. Yamout SZ, Nieto JE, Anderson J, De Cock HE, Vapniarsky N, Aleman M. Pathological evidence of pancreatitis in 43 horses (1986–2011). *Equine Vet J Suppl*. 2012:45–50.
194. Newman SJ. Equine pancreatic disease: A review and characterization of the lesions of four cases (2005–2014). *J Vet Diagn Invest*. 2015;27(1):92–96.
195. Ollivett TL, Divers TJ, Cushing T, et al. Acute pancreatitis in two five-day-old appaloosa foals. *Equine Vet J Suppl*. 2012;41:96–99.
196. Wong D, Sponseller B, Miles K, Butt T, Kersh K, Myers R. Failure of Technetium Tc 99m sestamibi scanning to detect abnormal parathyroid tissue in a horse and a mule with primary hyperparathyroidism. *J Vet Intern Med*. 2004;18:589–593.
197. Bienfet V, Dewaele A, Van Essch R. A primary parathyroid disorder. Osteofibrosis caused by a parathyroid adenoma in a Shetland pony. *Ann Med Vet*. 1964;108:252–256.
198. Krook L, Lowe JE. Nutritional secondary hyperparathyroidism in the horse. *Pathol Vet*. 1964;65:26–56.
199. Peauroi JR, Fisher DJ, Mohr FC, Vivrette SL. Primary hyperparathyroidism caused by a functional parathyroid adenoma in a horse. *J Am Vet Med Assoc*. 1998;212:1915–1918.
200. Roussel AJ, Thatcher CD. Primary hyperparathyroidism in a pony mare. *Comp Cont Educ Equine Pract*. 1987;9:781–783.
201. Almaden Y, Canalejo A, Hernandez A, Ballesteros E, Garcia-Navarro S, Torres A, Rodriguez M. Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *J Bone Miner Res*. 1996;11:970–976.
202. Brobst DF, Bayly WM, Reed SM. Parathyroid hormone evaluation in normal horses and horses with renal failure. *Equine Vet Sci*. 1982;2:150.
203. Varnell G. A peculiar and unusual disease of the osseous tissue in the horse; resembling in many characteristics mollitis ossium, rachitis, osteoporosis and fatty degeneration of bone. *Veterinarian*. 1860;33:493.
204. Joyce JR, Pierce KR, Romane WM, Baker JM. Clinical study of nutritional secondary hyperparathyroidism in horses. *J Am Vet Med Assoc*. 1971;158:2033–2042.

205. Clarke CJ, Roeder PL, Dixon PM. Nasal obstruction caused by nutritional osteodystrophia fibrosa in a group of Ethiopian horses. *Vet Rec*. 1996;138:568–570.
206. Jaramillo Morales C, Zapata Marín J, Agudelo Agudelo P, Sánchez Piedrahita L, García Osorio A, Aguilar Pérez LC. Hiperparatiroidismo nutricional de origen secundario en 3 yeguas de raza Criollo Colombiano en Antioquia. *J Agric Anim Sci*. 2015;4:74–82.
207. Walthall JC, McKenzie RA. Osteodystrophia fibrosa in horses at pasture in Queensland: field and laboratory observations. *Aust Vet J*. 1976;52:11–16.
208. Stewart J, Liyou O, Wilson G. Bighead in Horses—Not an Ancient Disease. *Aust Equine Vet*. 2010;29:55–62.
209. David JB, Cohen ND, Nachreiner R. Equine nutritional secondary hyperparathyroidism. *Compendium Cont Educ Vet*. 1997;19:1380–1387.
210. Ospina JC, Doncel Díaz B. Prevalence and pathologic features of fibrous osteodystrophy in horses in Colombia: 23 cases (1971–2012). *Proc Am Coll Vet Pathol*. 2013;142.
211. Herbert EW, Dittmer KE. Acute and chronic oxalate toxicity in Miniature Horses associated with soursob (Oxalis pes-caprae) ingestion. *Equine Vet Educ*. 2016. http://dx.doi.org/10.1111/eve.12605.
212. Rahman MM, Kawamura O. Oxalate Accumulation in Forage Plants: Some Agronomic, Climatic and Genetic Aspects. *Asian-Australas JAnimal Sci*. 2011;24:439–448.
213. Rahman MM, Abdullah RB, Wan Khadijah WE. A review of oxalate poisoning in domestic animals: tolerance and performance aspects. *J Anim Physiol Anim Nutr (Berl)*. 2013;97:605–614.
214. Argenzio RA, Lowe JE, Hintz HF, Schryver HF. Calcium and phosphorus homeostasis in horses. *J Nutr*. 1974;104:18–27.
215. Benders NA, Junker K, Wensing T, van den Ingh TS, van der Kolk JH. Diagnosis of secondary hyperparathyroidism in a pony using intact parathyroid hormone radioimmunoassay. *Vet Rec*. 2001;149:185–187.
216. Hintz HF, Kallfelz FA. Some nutritional problems of horses. *Equine Vet J*. 1981;13:183–186.
217. Freestone JF, Seahorn TL. Miscellaneous conditions of the equine head. *Vet Clin North Am Equine Pract*. 1993;9:235–242.
218. Kintner JH, Holt RL. Equine osteomalacia. *Philipine J Sci*. 1932;49:1–89.
219. Brook D. Osteoporosis in a six year old pony. *Equine Vet J*. 1975;7:46–48.
220. Bertone JJ. Nutritional secondary hyperparathyroidism. In: Robinson NE, ed. *Current Therary in Equine Medicine*. Philadelphia: W.B. Saunders; 1992:119–122.
221. Cymbaluk NF, Millar JD, Christensen DA. Oxalate concentration in feeds and its metabolism by ponies. *Can J Anim Sci*. 1986;66:1107–1116.
222. Boland RL. Solanum malacoxylon: a toxic plant which affects animal calcium metabolism. *Biomed Environ Sci*. 1988;1:414–423.
223. Harrington DD. Acute vitamin D2 (ergocalciferol) toxicosis in horses: case report and experimental studies. *J Am Vet Med Assoc*. 1982;180:867–873.
224. Hughes MR, McCain TA, Chang SY, Haussler MR, Villareale M, Wasserman RH. Presence of 1,25-dihydroxyvitamin D3-glycoside in the calcinogenic plant Cestrum diurnum. *Nature*. 1977;268:347–349.
225. Kasali OB, Krook L, Pond WG, Wasserman RH. Cestrum diurnum intoxication in normal and hyperparathyroid pigs. *Cornell Vet*. 1977;67:190–221.
226. Krook L, Wasserman RH, McEntee K, Brokken TD, Teigland MB. Cestrum diurnum poisoning in Florida cattle. *Cornell Vet*. 1975;65:557–575.
227. Krook L, Wasserman RH, Shively JN, Tashjian Jr AH, Brokken TD, Morton JF. Hypercalcemia and calcinosis in Florida horses: implication of the shrub, Cestrum diurnum, as the causative agent. *Cornell Vet*. 1975;65:26–56.
228. Worker NA, Carrillo BJ. "Enteque seco," calcification and wasting in grazing animals in the Argentine. *Nature*. 1967;215:72–74.
229. Muylle E, Oyaert W, de Roose P, Van den HC. Hypercalcaemia and mineralisation of non-osseous tissues in horses due to vitamin-D toxicity. *Zentralbl Veterinarmed A*. 1974;21:638–643.
230. Mello JR. Calcinosis—calcinogenic plants. *Toxicon*. 2003;41:1–12.
231. Braun U, Diener M, Hilbe M, Busch M, Bischoff M, Brosi G. [Enzootic calcinosis in 16 cows from 6 dairy farms in Unterengadin]. *Schweiz Arch Tierheilkd*. 2000;142:333–338.
232. Grabner A, Kraft W, Essich G, Hanichen T. Enzootic calcinosis in the horse. *Tierarztl Prax Suppl*. 1985;1:84–93.
233. Bockisch F, Aboling S, Coenen M, Vervuert I. Yellow oat grass intoxication in horses: Pitfalls by producing hay from extensive landscapes? A case report. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2015;43:296–304.
234. Fix AS, Miller LD. Equine adrenocortical carcinoma with hypercalcemia. *Vet Pathol*. 1987;24:190–192.
235. Mair TS, Yeo SP, Lucke VM. Hypercalcaemia and soft tissue mineralisation associated with lymphosarcoma in two horses. *Vet Rec*. 1990;126:99–101.
236. Sellers RS, Toribio RE, Blomme EA. Idiopathic systemic granulomatous disease and macrophage expression of PTHrP in a miniature pony. *J Comp Pathol*. 2001;125:214–218.
237. Anderson CA, Divers TJ. Systemic granulomatous inflammation in a horse grazing hairy vetch. *J Am Vet Med Assoc*. 1983;183:569–570.
238. Woods LW, Johnson B, Hietala SK, Galey FD, Gillen D. Systemic granulomatous disease in a horse grazing pasture containing vetch (Vicia sp.). *J Vet Diagn Invest*. 1992;4:356–360.
239. Peters M, Graf G, Pohlenz J. Idiopathic systemic granulomatous disease with encephalitis in a horse. *J Vet Med A Physiol Pathol Clin Med*. 2003;50:108–112.
240. Reijerkerk EP, Veldhuis Kroeze EJ, Sloet van Oldruitenborgh-Oosterbaan MM. Generalized sarcoidosis in two horses. *Tijdschr Diergeneeskd*. 2008;133:654–661.
241. Panciera RJ, Mosier DA, Ritchey JW. Hairy vetch (Vicia villosa Roth) poisoning in cattle: update and experimental induction of disease. *J Vet Diagn Invest*. 1992;4:318–325.
242. Tan JY, Valberg SJ, Sebastian MM, et al. Suspected systemic calcinosis and calciphylaxis in 5 horses. *Can Vet J*. 2010;51:993–999.
243. Anderson JD, Galuppo LD, Barr BC, et al. Clinical and scintigraphic findings in horses with a bone fragility disorder: 16 cases (1980–2006). *J Am Vet Med Assoc*. 2008;232:1694–1699.
244. Durham MG, Armstrong CM. Fractures and bone deformities in 18 horses with silicosis. *Proc Am Assoc Equine Pract*. 2006;52:1–7.
245. Arens AM, Puchalski SM, Whitcomb MB, Bell R, Gardner IA, Stover SM. Comparison of the use of scapular ultrasonography, physical examination, and measurement of serum biomarkers of bone turnover versus scintigraphy for detection of bone fragility syndrome in horses. *J Am Vet Med Assoc*. 2013;242:76–85.
246. Arens AM, Barr B, Puchalski SM, et al. Osteoporosis associated with pulmonary silicosis in an equine bone fragility syndrome. *Vet Pathol*. 2011;48:593–615.
247. Schwartz LW, Knight HD, Whittig LD, Malloy RL, Abraham JL, Tyler NK. Silicate pneumoconiosis and pulmonary fibrosis in horses from the Monterey-Carmel peninsula. *Chest*. 1981;80:82–85.
248. Katzman SA, Nieto JE, Arens AM, et al. Use of zoledronate for treatment of a bone fragility disorder in horses. *J Am Vet Med Assoc*. 2012;240:1323–1328.
249. DiBartola SP, Willard MD. Disorders of phosphorus: hypophosphatemia and hyperphosphatemia. In: DiBartola SP, ed. *Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice*. Philadelphia, PA: Elsevier Saunders; 2005:195–209.

250. Liamis G, Milionis HJ, Elisaf M. Medication-induced hypophosphatemia: a review. *QJM*. 2010;103:449–459.
251. Krapf R, Vetsch R, Vetsch W, Hulter HN. Chronic metabolic acidosis increases the serum concentration of 1,25-dihydroxyvitamin D in humans by stimulating its production rate. Critical role of acidosis-induced renal hypophosphatemia. *J Clin Invest*. 1992;90:2456–2463.
252. Geerse DA, Bindels AJ, Kuiper MA, Roos AN, Spronk PE, Schultz MJ. Treatment of hypophosphatemia in the intensive care unit: a review. *Crit Care*. 2010;14:R147.
253. Gaasbeek A, Meinders AE. Hypophosphatemia: an update on its etiology and treatment. *Am J Med*. 2005;118:1094–1101.
254. Amanzadeh J, Reilly Jr RF. Hypophosphatemia: an evidence-based approach to its clinical consequences and management. *Nat Clin Pract Nephrol*. 2006;2:136–148.
255. O'Brien TM, Coberly L. Severe hypophosphatemia in respiratory alkalosis. *Adv Stud Med*. 2003;3:345–348.
256. Witham CL, Stull CL. Metabolic responses of chronically starved horses to refeeding with three isoenergetic diets. *J Am Vet Med Assoc*. 1998;212:691–696.
257. Liamis G, Liberopoulos E, Barkas F, Elisaf M. Spurious electrolyte disorders: a diagnostic challenge for clinicians. *Am J Nephrol*. 2013;38:50–57.
258. Toribio RE. Disorders of calcium and phosphorus. In: Reed SM, Bayly WM, Sellon DC, eds. *Equine Internal Medicine*. St. Louis, MO: Saunders/Elsevier; 2010:1277–1291.
259. Jubb TF, Jerrett IV, Browning JW, Thomas KW. Haemoglobinuria and hypophosphataemia in postparturient dairy cows without dietary deficiency of phosphorus. *Aust Vet J*. 1990;67:86–89.
260. Willard MD, Zerbe CA, Schall WD, Johnson C, Crow SE, Jones R. Severe hypophosphatemia associated with diabetes mellitus in six dogs and one cat. *J Am Vet Med Assoc*. 1987;190:1007–1010.
261. Adams LG, Hardy RM, Weiss DJ, Bartges JW. Hypophosphatemia and hemolytic anemia associated with diabetes mellitus and hepatic lipidosis in cats. *J Vet Intern Med*. 1993;7:266–271.
262. Ogawa E, Kobayashi K, Yoshiura N, Mukai J. Hemolytic anemia and red blood cell metabolic disorder attributable to low phosphorus intake in cows. *Am J Vet Res*. 1989;50:388–392.
263. Toribio RE. Phosphorus homeostasis and derangements. In: Magdesian KG, Fielding CL, eds. *Equine Fluid Therapy*. Ames, IA: Wiley Blackwell; 2015:88–100.
264. Charron T, Bernard F, Skrobik Y, Simoneau N, Gagnon N, Leblanc M. Intravenous phosphate in the intensive care unit: more aggressive repletion regimens for moderate and severe hypophosphatemia. *Intensive Care Med*. 2003;29:1273–1278.
265. Clark CL, Sacks GS, Dickerson RN, Kudsk KA, Brown RO. Treatment of hypophosphatemia in patients receiving specialized nutrition support using a graduated dosing scheme: results from a prospective clinical trial. *Crit Care Med*. 1995;23:1504–1511.
266. Goff JP. Calcium, magnesium, and phosphorus. In: Smith BP, ed. *Large Animal Internal Medicine*. St. Louis, Mo: Mosby; 2009:1369–1377.
267. Bollaert PE, Levy B, Nace L, Laterre PF, Larcan A. Hemodynamic and metabolic effects of rapid correction of hypophosphatemia in patients with septic shock. *Chest*. 1995;107:1698–1701.
268. Larner AJ. Pseudohyperphosphatemia. *Clin Biochem*. 1995;28:391–393.
269. Barutcuoglu B, Parildar Z, Mutaf I, Habif S, Bayindir O. Spuriously elevated inorganic phosphate level in a multiple myeloma patient. *Clin Lab Haematol*. 2003;25:271–274.
270. Caudarella R, Vescini F, Buffa A, Francucci CM. Hyperphosphatemia: effects on bone metabolism and cardiovascular risk. *J Endocrinol Invest*. 2007;30:29–34.
271. Walton RJ, Russell RG, Smith R. Changes in the renal and extrarenal handling of phosphate induced by disodium etidronate (EHDP) in man. *Clin Sci Mol Med*. 1975;49:45–56.
272. Quamme GA, de RC. Epithelial magnesium transport and regulation by the kidney. *Front Biosci*. 2000;5:D694–D711.
273. Schweigel M, Martens H. Magnesium transport in the gastrointestinal tract. *Front Biosci*. 2000;5:D666–D677.
274. de Baaij JH, Hoenderop JG, Bindels RJ. Magnesium in man: implications for health and disease. *Physiol Rev*. 2015;95:1–46.
275. Wolf FI, Torsello A, Fasanella S, Cittadini A. Cell physiology of magnesium. *Mol Aspects Med*. 2003;24:11–26.
276. Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J*. 2012;5:i3–i14.
277. Wolf FI, Cittadini A. Chemistry and biochemistry of magnesium. *Mol Aspects Med*. 2003;24:3–9.
278. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A, Magnesium. An update on physiological, clinical and analytical aspects. *Clin Chim Acta*. 2000;294:1–26.
279. Hartwig A. Role of magnesium in genomic stability. *Mutat Res*. 2001;475:113–121.
280. Altura BM, Altura BT. Role of magnesium in patho-physiological processes and the clinical utility of magnesium ion selective electrodes. *Scand J Clin Lab Invest Suppl*. 1996;224:211–234.
281. Navarro M, Monreal L, Segura D, Armengou L, Anor S. A comparison of traditional and quantitative analysis of acid-base and electrolyte imbalances in horses with gastrointestinal disorders. *J Vet Intern Med*. 2005;19:871–877.
282. Mariella J, Isani G, Andreani G, Freccero F, Carpene E, Castagnetti C. Total plasma magnesium in healthy and critically ill foals. *Theriogenology*. 2016;85:180–185.
283. Hoenderop JG, Bindels RJ. Epithelial Ca^{2+} and Mg^{2+} channels in health and disease. *J Am Soc Nephrol*. 2005;16:15–26.
284. Martens H, Schweigel M. Pathophysiology of grass tetany and other hypomagnesemias. Implications for clinical management. *Vet Clin North Am Food Anim Pract*. 2000;16:339–368.
285. Schweigel M, Park HS, Etschmann B, Martens H. Characterization of the Na^{+}-dependent Mg^{2+} transport in sheep ruminal epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. 2006;290:G56–G65.
286. de Baaij JH, Hoenderop JG, Bindels RJ. Regulation of magnesium balance: lessons learned from human genetic disease. *Clin Kidney J*. 2012;5:i15–i24.
287. Groenestege WM, Hoenderop JG, van den HL, Knoers N, Bindels RJ. The epithelial Mg^{2+} channel transient receptor potential melastatin 6 is regulated by dietary Mg^{2+} content and estrogens. *J Am Soc Nephrol*. 2006;17:1035–1043.
288. Chester-Jones H, Fontenot JP, Veit HP. Physiological and pathological effects of feeding high levels of magnesium to steers. *J Anim Sci*. 1990;68:4400–4413.
289. van Doorn DA, van der Spek ME, Everts H, Wouterse H, Beynen AC. The influence of calcium intake on phosphorus digestibility in mature ponies. *J Anim Physiol Anim Nutr (Berl)*. 2004;88:412–418.
290. van Doorn DA, Schaafstra FJ, Wouterse H, et al. Repeated measurements of P retention in ponies fed rations with various Ca:P ratios. *J Anim Sci*. 2014;92:4981–4990.
291. Schryver HF, Millis DL, Soderholm LV, Williams J, Hintz HF. Metabolism of some essential minerals in ponies fed high levels of aluminum. *Cornell Vet*. 1986;76:354–360.
292. William JH, Danziger J. Proton-pump inhibitor-induced hypomagnesemia: Current research and proposed mechanisms. *World J Nephrol*. 2016;5:152–157.
293. Dai LJ, Ritchie G, Kerstan D, Kang HS, Cole DE, Quamme GA. Magnesium transport in the renal distal convoluted tubule. *Physiol Rev*. 2001;81:51–84.
294. Konrad M, Schlingmann KP, Gudermann T. Insights into the molecular nature of magnesium homeostasis. *Am J Physiol Renal Physiol*. 2004;286:F599–F605.

295. de RC, Quamme G. Renal magnesium handling and its hormonal control. *Physiol Rev*. 1994;74:305–322.
296. Ullrey DE, Struthers RD, Hendricks DG, Brent BE. Composition of mare's milk. *J Anim Sci*. 1966;25:217–222.
297. Grace ND, Pearce SG, Firth EC, Fennessy PF. Concentrations of macro- and micro-elements in the milk of pasture-fed thoroughbred mares. *Aust Vet J*. 1999;77:177–180.
298. McConaghy FF, Hodgson DR, Evans DL, Rose RJ. Equine sweat composition: effects of adrenaline infusion, exercise and training. *Equine Vet J Suppl*. 1995;158–164.
299. Dai LJ, Ritchie G, Bapty B, Quamme GA. Aldosterone potentiates hormone-stimulated Mg2+ uptake in distal convoluted tubule cells. *Am J Physiol*. 1998;274:F336–F341.
300. Dai LJ, Bapty B, Ritchie G, Quamme GA. Glucagon and arginine vasopressin stimulate Mg2+ uptake in mouse distal convoluted tubule cells. *Am J Physiol*. 1998;274:F328–F335.
301. de RC, Mandon B, Wittner M, di SA. Hormonal control of renal magnesium handling. *Miner Electrolyte Metab*. 1993;19:226–231.
302. Kang HS, Kerstan D, Dai LJ, Ritchie G, Quamme GA. Beta-adrenergic agonists stimulate Mg(2+) uptake in mouse distal convoluted tubule cells. *Am J Physiol Renal Physiol*. 2000;279:F1116–F1123.
303. Dai LJ, Ritchie G, Bapty BW, Kerstan D, Quamme GA. Insulin stimulates Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol*. 1999;277:F907–F913.
304. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. 2001;81:239–297.
305. Freestone JF, Gossett K, Carlson GP, Church G. Exercise induced alterations in the serum muscle enzymes, erythrocyte potassium and plasma constituents following feed withdrawal or furosemide and sodium bicarbonate administration in the horse. *J Vet Intern Med*. 1991;5:40–46.
306. Norris CR, Nelson RW, Christopher MM. Serum total and ionized magnesium concentrations and urinary fractional excretion of magnesium in cats with diabetes mellitus and diabetic ketoacidosis. *J Am Vet Med Assoc*. 1999;215:1455–1459.
307. van der Wijst J, Bindels RJ, Hoenderop JG. Mg^{2+} homeostasis: the balancing act of TRPM6. *Curr Opin Nephrol Hypertens*. 2014;23:361–369.
308. Weglicki WB. Hypomagnesemia and inflammation: clinical and basic aspects. *Annu Rev Nutr*. 2012;32:55–71.
309. Mishra OP, Fritz KI, ivoria-Papadopoulos M. NMDA receptor and neonatal hypoxic brain injury. *Ment Retard Dev Disabil Res Rev*. 2001;7:249–253.
310. Levenson CW. Regulation of the NMDA receptor: implications for neuropsychological development. *Nutr Rev*. 2006;64:428–432.
311. Wilkins PA. Magnesium Infusion in Hypoxic Ischemic Encephalopathy. *Proc 19th Annual ACVIM Forum*. 2001:242–244.
312. MacKay RJ. Brain injury after head trauma: pathophysiology, diagnosis, and treatment. *Vet Clin North Am Equine Pract*. 2004;20:199–216.
313. Wong DM, Wilkins PA, Bain FT, Brockus CW. Neonatal encephalopathy in foals. *Comp Cont Educ Vet*. 2011:E1–E10.
314. Siemkowicz E. Magnesium sulfate solution dramatically improves immediate recovery of rats from hypoxia. *Resuscitation*. 1997;35:53–59.
315. Hallak M, Hotra JW, Kupsky WJ. Magnesium sulfate protection of fetal rat brain from severe maternal hypoxia. *Obstet Gynecol*. 2000;96:124–128.
316. Seelig JM, Wei EP, Kontos HA, Choi SC, Becker DP. Effect of changes in magnesium ion concentration on cat cerebral arterioles. *Am J Physiol*. 1983;245:H22–H26.
317. Fawcett WJ, Haxby EJ, Male DA. Magnesium: physiology and pharmacology. *Br J Anaesth*. 1999;83:302–320.
318. Grober U, Schmidt J, Kisters K. Magnesium in Prevention and Therapy. *Nutrients*. 2015;7:8199–8226.
319. Hurcombe SDA, Toribio RE, Slovis NM, et al. Calcium regulating hormones and serum calcium and magnesium concentrations in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2009.
320. Johansson AM, Gardner SY, Jones SL, Fuquay LR, Reagan VH, Levine JF. Hypomagnesemia in hospitalized horses. *J Vet Intern Med*. 2003;17:860–867.
321. Stewart AJ. Magnesium disorders in horses. *Vet Clin North Am Equine Pract*. 2011;27:149–163.
322. Dhupa N, Proulx J. Hypocalcemia and hypomagnesemia. *Vet Clin North Am Small Anim Pract*. 1998;28:587–608.
323. Weglicki WB, Phillips TM, Freedman AM, Cassidy MM, Dickens BF. Magnesium-deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol Cell Biochem*. 1992;110:169–173.
324. Kramer JH, Misik V, Weglicki WB. Magnesium-deficiency potentiates free radical production associated with postischemic injury to rat hearts: vitamin E affords protection. *Free Radic Biol Med*. 1994;16:713–723.
325. Harrington DD. Pathological features of magnesium deficiency in young horses fed purified rations. *Am J Vet Res*. 1974;35:503–513.
326. Bueno AC, Seahorn TL, Cornick-Seahorn J, Horohov DW, Moore RM. Plasma and urine nitric oxide concentrations in horses given below a low dose of endotoxin. *Am J Vet Res*. 1999;60:969–976.
327. Seethanathan P, Bottoms GD, Schafer K. Characterization of release of tumor necrosis factor, interleukin-1, and superoxide anion from equine white blood cells in response to endotoxin. *Am J Vet Res*. 1990;51:1221–1225.
328. Barton MH, Morris DD, Norton N, Prasse KW. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. *J Vet Intern Med*. 1998;12:26–35.
329. Meijer P. [Two cases of tetany in the horse (author's transl)]. *Tijdschr Diergeneeskd*. 1982;107:329–332.
330. Fatemi S, Ryzen E, Flores J, Endres DB, Rude RK. Effect of experimental human magnesium depletion on parathyroid hormone secretion and 1,25-dihydroxyvitamin D metabolism. *J Clin Endocrinol Metab*. 1991;73:1067–1072.
331. Abbott LG, Rude RK. Clinical manifestations of magnesium deficiency. *Miner Electrolyte Metab*. 1993;19:314–322.
332. Barbagallo M, Dominguez LJ, Galioto A, et al. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med*. 2003;24:39–52.
333. Chameroy KA, Frank N, Elliott SB, Boston RC. Effects of a supplement containing chromium and magnesium on morphometric measurements, resting glucose, insulin concentrations and insulin sensitivity in laminitic obese horses. *Equine Vet J*. 2011;43(4):494–499.
334. Marr CM. Arrhythmias. *Cardiology of the horse*. London: W.B. Saunders; 1999:190–205.
335. Green HH, Allcroft WM, Montgomerie RF. Hypomagnesemia in equine transit tetany. *J Comp Pathol Therap*. 1935;48:74–79.
336. Grace ND, Pearce SG, Firth EC, Fennessy PF. Content and distribution of macro- and micro-elements in the body of pasture-fed young horses. *Aust Vet J*. 1999;77:172–176.
337. Niinisto K, Hewetson M, Kaikkonen R, Sykes BW, Raekallio M. Comparison of the effects of enteral psyllium, magnesium sulphate and their combination for removal of sand from the large colon of horses. *Vet J*. 2014;202:608–611.
338. Henninger RW, Horst J. Magnesium toxicosis in two horses. *J Am Vet Med Assoc*. 1997;211:82–85.
339. Kaneko JJ. Thyroid function. In: Kaneko JJ, ed. *Biochemistry of Domestic Animals*. San Diego: Academic Press; 1989:630.
340. Lucke VM, Lane JG. C-cell tumours of the thyroid in the horse. *Equine Vet J*. 1984;16:28–30.
341. Dimock WW, Westerfield C, Doll ER. The equine thyroid in health and disease. *J Am Vet Med Assoc*. 1944;104:313.

342. Drew B, Barber WP, Williams DG. The effect of excess dietary iodine on pregnant mares and foals. *Vet Rec*. 1975;97:93–95.
343. Manohar M, Goetz TE, Saupe B, Hutchens E, Coney E. Thyroid, renal, and splanchnic circulation in horses at rest and during short-term exercise. *Am J Vet Res*. 1995;56:1356–1361.
344. Jackson IM. Thyrotropin-releasing hormone. *N Engl J Med*. 1982;306:145–155.
345. Reichlin S. Neuroendocrinology. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. *Williams' Textbook of Endocrinology*. Philadelphia: W.B. Saunders; 1998:165–248.
346. Dahl GE, Evans NP, Thrun LA, Karsch FJ. A central negative feedback action of thyroid hormones on thyrotropin-releasing hormone secretion. *Endocrinology*. 1994;135:2392–2397.
347. Dyess EM, Segerson TP, Liposits Z, et al. Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. *Endocrinology*. 1988;123:2291–2297.
348. Segerson TP, Kauer J, Wolfe HC, et al. Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science*. 1987;238:78–80.
349. Thompson DL, Nett TM. Thyroid stimulating hormone and prolactin secretion after thyrotropin releasing hormone administration to mares—dose-response during anestrus in winter and during estrus in summer. *Domest Anim Endocrinol*. 1984;1:263–268.
350. Thompson FN, Caudle AB, Kemppainen RJ, Nett TM, Brown J, Williams DJ. Thyroidal and prolactin secretion in agalactic mares. *Theriogenology*. 1986;25:575–580.
351. Gentry LR, Thompson Jr DL, Stelzer AM. Responses of seasonally anovulatory mares to daily administration of thyrotropin-releasing hormone and (or) gonadotropin-releasing hormone analog. *J Anim Sci*. 2002;80:208–213.
352. Pruett HE, Thompson Jr DL, Cartmill JA, Williams CC, Gentry LR. Thyrotropin releasing hormone interactions with growth hormone secretion in horses. *J Anim Sci*. 2003;81:2343–2351.
353. Beech J. Evaluation of thyroid, adrenal, and pituitary function. *Vet Clin North Am Equine Pract*. 1987;3:649–660.
354. Chung HR. Adrenal and thyroid function in the fetus and preterm infant. *Korean J Pediatr*. 2014;57:425–433.
355. Forhead AJ, Fowden AL. Thyroid hormones in fetal growth and prepartum maturation. *J Endocrinol*. 2014;221:R87–R103.
356. Capen CC, Martin SL. The thyroid gland. In: McDonald LE, ed. *Veterinary Endocrinology and Reproduction*. Philadelphia: Lea and Febiger; 1989:58.
357. Ingbar SH. The thyroid gland. In: Wilson JD, Foster DW, eds. *Williams' Textbook of Endocrinology*. Philadelphia: W.B. Saunders; 1985:682.
358. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature*. 1996;379:458–460.
359. Fong P. Thyroid iodide efflux: a team effort? *J Physiol*. 2011;589:5929–5939.
360. Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiol Rev*. 2001;81:1097–1142.
361. Chen CL, Riley AM. Serum thyroxine and triiodothyronine concentrations in neonatal foals and mature horses. *Am J Vet Res*. 1981;42:1415–1417.
362. Irvine CH. Thyroxine secretion rate in the horse in various physiological states. *J Endocrinol*. 1967;39:313–320.
363. Irvine CH, Evans MJ. Postnatal changes in total and free thyroxine and triiodothyronine in foal serum. *J Reprod Fertil Suppl*. 1975:709–715.
364. Irvine CH, Evans MJ. Hypothyroidism in foals. *N Z Vet J*. 1977;25:354.
365. Malinowski K, Christensen RA, Hafs HD, Scanes CG. Age and breed differences in thyroid hormones, insulin-like growth factor (IGF)-I and IGF binding proteins in female horses. *J Anim Sci*. 1996;74:1936–1942.
366. Larsson M, Pettersson T, Carlstrom A. Thyroid hormone binding in serum of 15 vertebrate species: isolation of thyroxine-binding globulin and prealbumin analogs. *Gen Comp Endocrinol*. 1985;58:360–375.
367. Irvine CH. Hypothyroidism in the foal. *Equine Vet J*. 1984;16:302–306.
368. Katovich M, Evans JW, Sanchez O. Effects of season, pregnancy and lactation on thyroxine turnover in the mare. *J Anim Sci*. 1974;38:811–818.
369. Sojka JE, Johnson MA, Bottoms GD. Serum triiodothyronine, total thyroxine, and free thyroxine concentrations in horses. *Am J Vet Res*. 1993;54:52–55.
370. Wehr U, Englschalk B, Kienzle E, Rambeck WA. Iodine balance in relation to iodine intake in ponies. *J Nutr*. 2002;132:1767S–1768S.
371. Marcella KL. General care of miniature horses. Part 2. *Equine Pract*. 1992;14:26–28.
372. Murray MJ, Luba NK. Plasma gastrin and somatostatin, and serum thyroxine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3) and cortisol concentrations in foals from birth to 28 days of age. *Equine Vet J*. 1993;25:237–239.
373. Khan VTS. Studies on thyroidal states in equines during normal and certain disturbed conditions of reproduction. *Mysore J Agr Sci*. 1980;14:1382.
374. Motley JS. Use of radioactive triiodothyronine in the study of thyroid function in normal horses. *Vet Med Small Anim Clin*. 1972;67:1225–1228.
375. Pirrone A, Panzani S, Govoni N, Castagnetti C, Veronesi MC. Thyroid hormone concentrations in foals affected by perinatal asphyxia syndrome. *Theriogenology*. 2013;80:624–629.
376. Panzani S, Comin A, Galeati G, et al. How type of parturition and health status influence hormonal and metabolic profiles in newborn foals. *Theriogenology*. 2012;77:1167–1177.
377. Mendoza FJ, Perez-Ecija RA, Toribio RE, Estepa JC. Thyroid hormone concentrations differ between donkeys and horses. *Equine Vet J*. 2013;45:214–218.
378. Himler M, Hurcombe SD, Griffin A, et al. Presumptive nonthyroidal illness syndrome in critically ill foals. *Equine Vet J Suppl*. 2012:43–47.
379. Breuhaus BA. Thyroid function and dysfunction in term and premature equine neonates. *J Vet Intern Med*. 2014;28:1301–1309.
380. Komosa M, Flisinska-Bojanowska A, Gill J. Development of diurnal rhythm in some metabolic parameters in foals. *Comp Biochem Physiol A Comp Physiol*. 1990;95:549–552.
381. McCall CA, Potter GD, Kreider JL, Jenkins WL. Physiological-responses in foals weaned by abrupt or gradual methods. *J Equine Vet Sci*. 1987;7:368–374.
382. Reap M, Cass C, Hightower D. Thyroxine and triodothyronine levels in ten species of animals. *Southwest Vet*. 1978;31:31.
383. Anderson RR, Nixon DA, Akasha MA. Total and free thyroxine and triiodothyronine in blood serum of mammals. *Comp Biochem Physiol A*. 1988;89:401–404.
384. Irvine CH. Protein bound iodine in the horse. *Am J Vet Res*. 1967;28:1687.
385. deMartin BW. Study on the thyroid function of thoroughbred horses by means of "in vitro" ^{125}I-T3 modified and ^{125}I-T4 tests. *Rev Fac Med Vet Zootec Univ S Paulo*. 1975;12:107.
386. Kelley ST, Oehme FW, Brandt GW. Measurement of thyroid gland function during the estrous cycle of nine mares. *Am J Vet Res*. 1974;35:657–660.
387. Thomas Jr CL, Adams JC. Radioimmunoassay of equine serum for thyroxine: reference values. *Am J Vet Res*. 1978;39:1239.
388. deMartin BW. Study on the thyroid function of male and female thoroughbred horses in different times after winning races at the Hippodrome Cidade Jardim, with the use of "in vitro" ^{125}I-T3, and ^{125}I-T4 tests. *Rev Fac Med Vet Zootec Univ S Paulo*. 1977;14:199.

389. deMartin BW. Study on the thyroid function of thoroughbred females in varying stages of pregnancy using "in vitro" tests ^{125}I-T3 and ^{125}I-T4. *Rev Fac Med Vet Zootec Univ S Paulo*. 1975;12:121.
390. Flisinska-Bojanowska A, Komosa M, Gill J. Influence of pregnancy on diurnal and seasonal changes in cortisol, T_3 and T_4 levels in the mare blood serum. *Comp Biochem Physiol A Comp Physiol*. 1991;98:23–30.
391. Fazio E, Medica P, Cravana C, Bruschetta G, Ferlazzo A. Seasonal thyroid and lipid profiles in Thoroughbred pregnant and nonpregnant mares (Equus caballus). *Theriogenology*. 2016.
392. Fazio E, Medica P, Cravana C, Ferlazzo A. Total and free iodothyronines profile in the donkey (Equus asinus) over a 12-month period. *Acta Vet BRNO*. 2012;81:239–244.
393. Meredith TB, Dobrinski I. Thyroid function and pregnancy status in broodmares. *J Am Vet Med Assoc*. 2004;224:892–894.
394. Todini L, Salimei E, Malfatti A, Ferraro S, Fantuz F. Thyroid hormones in milk and blood of lactating donkeys as affected by stage of lactation and dietary supplementation with trace elements. *J Dairy Res*. 2012;79:232–237.
395. Hershman JM. Use of thyrotropin-releasing hormone in clinical medicine. *Med Clin North Am*. 1978;62:313–325.
396. Gittoes NJL, Franklyn JA, Sarne DH, Refetoff S, Sheppard MC. Thyroid function tests. In: DeGroot LJ, Jameson JL, eds. *Endocrinology*. Philadelphia: W.B. Saunders; 2001:1361–1398.
397. Messer NT, Ganjam VK, Nachreiner RF, Krause GF. Effect of dexamethasone administration on serum thyroid hormone concentrations in clinically normal horses. *J Am Vet Med Assoc*. 1995;206:63–66.
398. Abraham G, Allersmeier M, Schusser GF, Ungemach FR. Serum thyroid hormone, insulin, glucose, triglycerides and protein concentrations in normal horses: association with topical dexamethasone usage. *Vet J*. 2011;188:307–312.
399. Galton VA. Thyroid hormone-catecholamine interrelationships. *Endocrinology*. 1965;77:278–284.
400. Sommardahl CS, Frank N, Elliott SB, et al. Effects of oral administration of levothyroxine sodium on serum concentrations of thyroid gland hormones and responses to injections of thyrotropin-releasing hormone in healthy adult mares. *Am J Vet Res*. 2005;66:1025–1031.
401. Nillni EA. Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. *Front Neuroendocrinol*. 2010;31:134–156.
402. Irvine CH. Thyroid Function in the Horse. *Proc 12th Am Assoc Equine Pract Conv*. 1966:197–205.
403. McBride GE, Christopherson RJ, Sauer W. Metabolic-rate and plasma thyroid-hormone concentrations of mature horses in response to changes in ambient-temperature. *Can J Anim Sci*. 1985;65:375–382.
404. Duckett WM, Manning JP, Weston PG. Thyroid hormone periodicity in healthy adult geldings. *Equine Vet J*. 1989;21:123–125.
405. Morris DD, Garcia M. Thyroid-stimulating hormone: response test in healthy horses, and effect of phenylbutazone on equine thyroid hormones. *Am J Vet Res*. 1983;44:503–507.
406. Brabant G, Brabant A, Ranft U, et al. Circadian and pulsatile thyrotropin secretion in euthyroid man under the influence of thyroid hormone and glucocorticoid administration. *J Clin Endocrinol Metab*. 1987;65:83–88.
407. Irvine CHG. The role of hormones in exercise physiology. In: Snow DH, Persson SGB, Rose RJ, eds. *Equine Exercise Physiology*. Cambridge: Granta Editions; 1983:377.
408. Takagi S, Ito K, Shibata H. Effects of training on plasma fibrinogen concentration and thyroid hormone level in young race horses. *Exper Results Equine Health Lab*. 1974;11:94.
409. Brinkmann L, Gerken M, Hambly C, Speakman JR, Riek A. Thyroid hormones correlate with field metabolic rate in ponies, Equus ferus caballus. *J Exp Biol*. 2016;219:2559–2566.
410. Leleu C, Haentjens F. Morphological, haemato-biochemical and endocrine changes in young Standardbreds with "maladaptation" to early training. *Equine Vet J Suppl*. 2010:171–178.
411. Gonzalez O, Gonzalez E, Sanchez C, et al. Effect of exercise on erythrocyte beta-adrenergic receptors and plasma concentrations of catecholamines and thyroid hormones in Thoroughbred horses. *Equine Vet J*. 1998;30:72–78.
412. Garcia MC, Beech J. Endocrinologic, hematologic, and heart rate changes in swimming horses. *Am J Vet Res*. 1986;47:2004–2006.
413. Graves EA, Schott HC, Marteniuk JV, Refsal KR, Nachreiner RF. Thyroid hormone responses to endurance exercise. *Equine Vet J Suppl*. 2006:32–36.
414. Gupta S, Glade MJ. Hormonal responses to high and low planes of nutrition in weanling Thoroughbreds. *Equine Vet Data*. 1983;4:170.
415. Powell DM, Lawrence LM, Fitzgerald BP, et al. Effect of short-term feed restriction and calorie source on hormonal and metabolic responses in geldings receiving a small meal. *J Anim Sci*. 2000;78:3107–3113.
416. Biesik LM, Glade MJ. Changes in serum hormone concentrations in weanling horses following gastric infusion of sucrose or casein. *Nutr Rep Int*. 1986;33:651–658.
417. Glade MJ, Reimers TJ. Effects of dietary energy supply on serum thyroxine, tri-iodothyronine and insulin concentrations in young horses. *J Endocrinol*. 1985;104:93–98.
418. Youket RJ, Carnevale JM, Houpt KA, Houpt TR. Humoral, hormonal and behavioral correlates of feeding in ponies: the effects of meal frequency. *J Anim Sci*. 1985;61:1103–1110.
419. Brinkmann L, Gerken M, Riek A. Effect of long-term feed restriction on the health status and welfare of a robust horse breed, the Shetland pony (Equus ferus caballus). *Res Vet Sci*. 2013;94:826–831.
420. Christensen RA, Malinowski K, Massenzio AM, Hafs HD, Scanes CG. Acute effects of short-term feed deprivation and refeeding on circulating concentrations of metabolites, insulin-like growth factor I, insulin-like growth factor binding proteins, somatotropin, and thyroid hormones in adult geldings. *J Anim Sci*. 1997;75(5):1351–1358.
421. De Groot LJ. Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinol Metab*. 1999;84:151–164.
422. Messer NT, Johnson PJ, Refsal KR, Nachreiner RF, Ganjam VK, Krause GF. Effect of food deprivation on baseline iodothyronine and cortisol concentrations in healthy, adult horses. *Am J Vet Res*. 1995;56:116–121.
423. Suwannachot P, Verkleij CB, Kocsis S, Enzerink E, Everts ME. Prolonged food restriction and mild exercise in Shetland ponies: effects on weight gain, thyroid hormone concentrations and muscle Na(+),K(+)-ATPase. *J Endocrinol*. 2000;167:321–329.
424. Boelen A, Wiersinga WM, Fliers E. Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. *Thyroid*. 2008;18:123–129.
425. Flier JS, Harris M, Hollenberg AN. Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. *J Clin Invest*. 2000;105:859–861.
426. McManus CJ, Fitzgerald BP. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. *Domest Anim Endocrinol*. 2000;19:1–13.
427. Buff PR, Morrison CD, Ganjam VK, Keisler DH. Effects of short-term feed deprivation and melatonin implants on circadian patterns of leptin in the horse. *J Anim Sci*. 2005;83:1023–1032.
428. Steelman SM, Michael-Eller EM, Gibbs PG, Potter GD. Meal size and feeding frequency influence serum leptin concentration in yearling horses. *J Anim Sci*. 2006;84:2391–2398.

429. Fitzgerald BP, McManus CJ. Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. *Biol Reprod*. 2000;63:335–340.
430. Berg EL, McNamara DL, Keisler DH. Endocrine profiles of periparturient mares and their foals. *J Anim Sci*. 2007;85:1660–1668.
431. Hilderbran AC, Breuhaus BA, Refsal KR. Nonthyroidal illness syndrome in adult horses. *J Vet Intern Med*. 2014;28:609–617.
432. Van den BG, de Zegher F, Baxter RC, et al. Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. *J Clin Endocrinol Metab*. 1998;83:309–319.
433. McFarlane D. Equine pituitary pars intermedia dysfunction. *Vet Clin North Am Equine Pract*. 2011;27(1):93–113.
434. Dembek KA, Onasch K, Hurcombe SD, et al. Renin-angiotensin-aldosterone system and hypothalamic-pituitary-adrenal axis in hospitalized newborn foals. *J Vet Intern Med*. 2013;27:331–338.
435. Mair TS, Sherlock CE, Boden LA. Serum cortisol concentrations in horses with colic. *Vet J*. 2014;201:370–377.
436. Carter RA, Treiber KH, Geor RJ, Douglass L, Harris PA. Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. *Equine Vet J*. 2009;41(2):171–178.
437. Frank N, Elliott SB, Brandt LE, Keisler DH. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J Am Vet Med Assoc*. 2006;228(9):1383–1390.
438. Frank N, Buchanan BR, Elliott SB. Effects of long-term oral administration of levothyroxine sodium on serum thyroid hormone concentrations, clinicopathologic variables, and echocardiographic measurements in healthy adult horses. *Am J Vet Res*. 2008;69:68–75.
439. Rothschild CM, Hines MT, Breuhaus B, Gay J, Sellon DC. Effects of trimethoprim-sulfadiazine on thyroid function of horses. *J Vet Intern Med*. 2004;18:370–373.
440. Ramirez S, Wolfsheimer KJ, Moore RM, Mora F, Bueno AC, Mirza T. Duration of effects of phenylbutazone on serum total thyroxine and free thyroxine concentrations in horses. *J Vet Intern Med*. 1997;11:371–374.
441. van Maanen JM, van Dijk A, Mulder K, et al. Consumption of drinking water with high nitrate levels causes hypertrophy of the thyroid. *Toxicol Lett*. 1994;72:365–374.
441a. Alèn M, Rahkila P, Reinilä M, Vihko R. Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes. *Am J Sports Med*. 1987;15(4):357–361.
442. Morris DD, Garcia MC. Effects of phenylbutazone and anabolic steroids on adrenal and thyroid gland function tests in healthy horses. *Am J Vet Res*. 1985;46:359–364.
443. Eroksuz H, Eroksuz Y, Ozer H, Ceribasi AO, Yaman I, Ilhan N. Equine goiter associated with excess dietary iodine. *Vet Hum Toxicol*. 2004;46:147–149.
444. Laurberg P. Amiodarone inhibits T4 and T3 secretion but does not affect T_4 deiodination to T_3 in perfused dog thyroid lobes. *Thyroidology*. 1988:1–4.
445. Boosinger TR, Brendemuehl JP, Bransby DL, Wright JC, Kemppainen RJ, Kee DD. Prolonged gestation, decreased triiodothyronine concentration, and thyroid gland histomorphologic features in newborn foals of mares grazing Acremonion coenophialum-infected fescue. *Am J Vet Res*. 1995;56:66–69.
446. Breuhaus BA. Thyroid function in mature horses ingesting endophyte-infected fescue seed. *J Am Vet Med Assoc*. 2003;223:340–345.
447. Reimers TJ, Cowan RG, Davidson HP, Colby ED. Validation of radioimmunoassay for triiodothyronine, thyroxine, and hydrocortisone (cortisol) in canine, feline, and equine sera. *Am J Vet Res*. 1981;42:2016–2021.
448. Reimers TJ, Lamb SV, Bartlett SA, Matamoros RA, Cowan RG, Engle JS. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. *Am J Vet Res*. 1991;52:1075–1080.
449. Irvine CHG. Measurement of free and total T_4 and T_3 in domestic animals. In: Stockigt JR, Nagatake S, eds. *Thyroid Research VIII*. New York: Pergamon; 1980:252.
450. Johnson PJ, Messer NT, Ganjam VK, et al. Effects of propylthiouracil and bromocryptine on serum concentrations of thyrotrophin and thyroid hormones in normal female horses. *Equine Vet J*. 2003;35:296–301.
451. Breuhaus BA. Thyroid-stimulating hormone in adult euthyroid and hypothyroid horses. *J Vet Intern Med*. 2002;16:109–115.
452. Breuhaus BA, LaFevers DH. Thyroid Function in Normal, Sick and Premature Foals. *Proc 23rd Annual ACVIM Forum*. 2005.
453. Harris P, Marlin D, Gray J. Equine thyroid-function tests—a preliminary investigation. *Brit Vet J*. 1992;148:71–80.
454. Held JP, Oliver JW. A sampling protocol for the thyrotropin-stimulation test in the horse. *J Am Vet Med Assoc*. 1984;184:326–327.
455. Oliver JW, Held JP. Thyrotropin stimulation test—new perspective on value of monitoring triiodothyronine. *J Am Vet Med Assoc*. 1985;187:931–934.
456. Shaftoe S, Schick MP, Chen CL. Thyroid-stimulating hormone response tests in one-day-old foals. *J Equine Vet Sci*. 1988;8:310–312.
457. Beech J, Garcia M. Hormonal response to thyrotropin-releasing hormone in healthy horses and in horses with pituitary adenoma. *Am J Vet Res*. 1985;46:1941–1943.
458. Chen CL, Li WI. Effect of thyrotropin releasing hormone (TRH) on serum levels of thyroid hormones in thoroughbred mares. *J Equine Sci*. 1986;6:58.
459. Lothrop Jr CD, Nolan HL. Equine thyroid function assessment with the thyrotropin-releasing hormone response test. *Am J Vet Res*. 1986;47:942–944.
460. Thompson Jr DL, Godke RA, Nett TM. Effects of melatonin and thyrotropin releasing hormone on mares during the nonbreeding season. *J Anim Sci*. 1983;56:668–677.
461. Beech J, Boston R, Lindborg S, Russell GE. Adrenocorticotropin concentration following administration of thyrotropin-releasing hormone in healthy horses and those with pituitary pars intermedia dysfunction and pituitary gland hyperplasia. *J Am Vet Med Assoc*. 2007;231:417–426.
462. Frank N, Andrews FM, Sommardahl CS, Eiler H, Rohrbach BW, Donnell RL. Evaluation of the combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test for detection of pars intermedia pituitary adenomas in horses. *J Vet Intern Med*. 2006;20:987–993.
463. Alberts MK, McCann JP, Woods PR. Hemithyroidectomy in a horse with confirmed hyperthyroidism. *J Am Vet Med Assoc*. 2000;217:1051–1054, 1009.
464. Tan RH, Davies SE, Crisman MV, Coyle L, Daniel GB. Propylthiouracil for treatment of hyperthyroidism in a horse. *J Vet Intern Med*. 2008;22:1253–1258.
465. Hillidge CJ, Theodorakis MC, Duckett WM. Scintigraphic Evaluation of Equine Thyroid Function. *Proc 27th Am Assoc Equine Pract Conv*. 1981:477–478.
466. Held JP, Patton CS, Toal RL, Geiser DR. Work intolerance in a horse with thyroid carcinoma. *J Am Vet Med Assoc*. 1985;187:1044–1045.
467. Hillidge CJ, Sanecki RK, Theodorakis MC. Thyroid carcinoma in a horse. *J Am Vet Med Assoc*. 1982;181:711–714.
468. Joyce JR, Thompson RB, Kyzar JR, Hightower D. Thyroid carcinoma in a horse. *J Am Vet Med Assoc*. 1976;168:610–612.
469. Ramirez S, McClure JJ, Moore RM, Wolfsheimer KJ, Gaunt SD, Mirza MH, Taylor W. Hyperthyroidism associated with a thyroid adenocarcinoma in a 21-year-old gelding. *J Vet Intern Med*. 1998;12:475–477.

470. Breuhaus BA. Thyroid function in anhidrotic horses. *J Vet Intern Med*. 2009;23:168–173.
471. Messer NT, Johnson PJ. Evidence-based literature pertaining to thyroid dysfunction and Cushing's syndrome in the horse. *Vet Clin North Am Equine Pract*. 2007;23:329–364.
472. Stanley O, Hillidge CJ. Alopecia associated with hypothyroidism in a horse. *Equine Vet J*. 1982;14:165–167.
473. Waldron-Mease E. Hypothyroidism and myopathy in racing thoroughbreds and standardbreds. *J Equine Med Surg*. 1979;3:124.
474. Breuhaus BA. Disorders of the equine thyroid gland. *Vet Clin North Am Equine Pract*. 2011;27:115–128.
475. Stephen JO, Baptiste KE, Townsend HG. Clinical and pathologic findings in donkeys with hypothermia: 10 cases (1988–1998). *J Am Vet Med Assoc*. 2000;216:725–729.
476. Allen AL, Townsend HG, Doige CE, Fretz PB. A case-control study of the congenital hypothyroidism and dysmaturity syndrome of foals. *Can Vet J*. 1996;37:349–351.
477. Furr MO, Murray MJ, Ferguson DC. The effects of stress on gastric ulceration, T3, T4, reverse T3 and cortisol in neonatal foals. *Equine Vet J*. 1992;24:37–40.
478. McLaughlin BG, Doige CE, McLaughlin PS. Thyroid-hormone levels in foals with congenital musculoskeletal lesions. *Can Vet J*. 1986;27:264–267.
479. Murray MJ. Hypothyroidism and respiratory insufficiency in a neonatal foal. *J Am Vet Med Assoc*. 1990;197:1635–1638.
480. Shaver JR, Fretz PB, Doige CE. Skeletal manifestations of suspected hypothyroidism in two foals. *J Equine Med Surg*. 1979;3:269.
481. Vivrette SL, Reimers TJ, Krook L. Skeletal disease in a hypothyroid foal. *Cornell Vet*. 1984;74:373–386.
482. Durham AE. Congenital goitre in two colt foals born to mares fed excess iodine during pregnancy. *Equine Vet Educ*. 1995;7:239–241.
483. Perillo A, Passantino G, Passantino L, et al. First observation of an Hashimoto thyroiditis-like disease in horses from Eastern Europe: histopathological and immunological findings. *Immunopharmacol Immunotoxicol*. 2005;27:241–253.
484. Green EM, Hunt EL. Hypophyseal neoplasia in a pony. *Compend Contin Ed Practic Vet*. 1985;7:S249.
485. Lowe JE, Kallfelz FA. Thyroidectomy and the T4 Test to Assess Thyroid Dysfunction in the Horse and Pony. *Proc 16th Am Assoc Equine Pract Conv*. 1970:135–154.
486. Refetoff S, Bassett JH, Beck-Peccoz P, et al. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. *Eur Thyroid J*. 2014;3:7–9.
487. Conway DA, Cosgrove JS. Equine goiter. *Irish Vet J*. 1980;34:29–31.
488. Baker JR, Wyn-Jones G, Eley JL. Case of equine goitre. *Vet Rec*. 1983;112:407–408.
489. Doige CE, McLaughlin BG. Hyperplastic goitre in newborn foals in Western Canada. *Can Vet J*. 1981;22:42–45.
490. McLaughlin BG, Doige CE. A study of ossification of carpal and tarsal bones in normal and hypothyroid foals. *Can Vet J*. 1982;23:164–168.
491. McLaughlin BG, Doige CE. Congenital musculoskeletal lesions and hyperplastic goitre in foals. *Can Vet J*. 1981;22:130–133.
492. Lowe JE, Baldwin BH, Foote RH, Hillman RB, Kallfelz FA. Equine hypothyroidism: the long term effects of thyroidectomy on metabolism and growth in mares and stallions. *Cornell Vet*. 1974;64:276–295.
493. Baker HJ, Lindsey JR. Equine goiter due to excess dietary iodide. *J Am Vet Med Assoc*. 1968;153:1618–1630.
494. Cubillos V, Norambuena L, Espinoza E. [Cell growth and neoplasms of the thyroid gland in horses]. *Zentralbl Veterinarmed A*. 1981;28:201–208.
495. Driscoll J, Hintz HF, Schryver HF. Goiter in foals caused by excessive iodine. *J Am Vet Med Assoc*. 1978;173:858–859.
496. Dudan FE, Ferguson DC, Little TV. Circulating Serum Thyroxine (T_4), Triiodothyronine (T_3) and Reverse T_3 (RT_3) in Neonatal Term and Preterm Foals. *Proc 5th Annual ACVIM Forum*. 1987;881.
497. McLaughlin BG, Doige CE, Fretz PB, Pharr JW. Carpal bone lesions associated with angular limb deformities in foals. *J Am Vet Med Assoc*. 1981;178:224–230.
498. Allen AL, Doige CE, Fretz PB, Townsend HG. Hyperplasia of the thyroid gland and concurrent musculoskeletal deformities in western Canadian foals: reexamination of a previously described syndrome. *Can Vet J*. 1994;35:31–38.
499. Allen AL. Hyperplasia of the thyroid gland and musculoskeletal deformities in two equine abortuses. *Can Vet J*. 1995;36:234–236.
500. Allen AL. Congenital hypothyroidism in horses: looking back and looking ahead. *Equine Vet Educ*. 2014;26:190–193.
501. Card CE, Manning ST. Response of newborn foals with thyroid musculoskeletal disease to adrenocorticotrophic hormone (ACTH). *J Reprod Fertil Suppl*. 2000:709–715.
502. Lohr CV, Polster U, Kuhnert P, Karger A, Rurangirwa FR, Teifke JP. Mesenteric lymphangitis and sepsis due to RTX toxin-producing Actinobacillus spp in 2 foals with hypothyroidism-dysmaturity syndrome. *Vet Pathol*. 2012;49:592–601.
503. Gawrylash SK. Thyroid hyperplasia and musculoskeletal deformity in a standardbred filly in Ontario. *Can Vet J*. 2004;45:424–426.
504. Koikkalainen K, Knuuttila A, Karikoski N, Syrja P, Hewetson M. Congenital hypothyroidism and dysmaturity syndrome in foals: first reported cases in Europe. *Equine Vet Educ*. 2014;26:181–189.
505. Lowe JE, Baldwin BH, Foote RH, Hillman RB, Kallefelz FA. Semen characteristics in thyroidectomized stallions. *J Reprod Fertil Suppl*. 1975:81–86.
506. Vischer CM, Foreman JH, Constable PD, et al. Hemodynamic effects of thyroidectomy in sedentary horses. *Am J Vet Res*. 1999;60:14–21.
507. Lowe JE, Foote RH, Baldwin BH, Hillman RB, Kallfelz FA. Reproductive patterns in cyclic and pregnant thyroidectomized mares. *J Reprod Fertil Suppl*. 1987;35:281–288.
508. Fadok VA, Wild S. Suspected cutaneous iodism in a horse. *J Am Vet Med Assoc*. 1983;183:1104–1106.
509. Poomvises P, Gesmankit P, Tawatsin A. Studies on serum triiodothyronine and thyroxine in anhidrotic horses. *Centaur*. 1986;II:139.
510. Mayhew IG, Ferguson HO. Clinical, clinicopathologic, and epidemiologic features of anhidrosis in central Florida Thoroughbred horses. *J Vet Intern Med*. 1987;1:136–141.
511. Hood DM, Hightower D, Amoss MS. Thyroid function in horses affected with laminitis. *Southwest Vet*. 1987;38:85.
512. Alexander SL, Irvine CH, Evans MJ. Inter-relationships between the secretory dynamics of thyrotrophin-releasing hormone, thyrotrophin and prolactin in periovulatory mares: effect of hypothyroidism. *J Neuroendocrinol*. 2004;16:906–915.
513. deMartin BW. Study on the thyroid function of Thoroughbred horses using 131I-TBI. *Rev Fac Med Vet Zootec Univ S Paulo*. 1973;10:35.
514. Tateyama S, Tanimura N, Moritomo Y, et al. The ultimobranchial remnant and its hyperplasia or adenoma in equine thyroid gland. *Nippon Juigaku Zasshi*. 1988;50:714–722.
515. Dalefield RR, Palmer DN. The frequent occurrence of thyroid tumours in aged horses. *J Comp Pathol*. 1994;110:57–64.
516. Nolen-Walston RD, Parente EJ, Madigan JE, David F, Knafo SE, Engiles JB. Branchial remnant cysts of mature and juvenile horses. *Equine Vet J*. 2009;41:918–923.
517. Damodaran S, Ramachandran PV. A survey of neoplasms in equidae. *Centaur*. 1986;II:161.
518. Hopper LD, Kennedy GA, Taylor WA. Diagnosing and treating thyroid adenoma in a horse. *Vet Med*. 1987;82:1252.

519. Hovda LR, Shaftoe S, Rose ML, Clemmons LH. Mediastinal squamous cell carcinoma and thyroid carcinoma in an aged horse. *J Am Vet Med Assoc*. 1990;197:1187–1189.
520. Ralston SL, Nockels CF, Squires EL. Differences in diagnostic test results and hematologic data between aged and young horses. *Am J Vet Res*. 1988;49:1387–1392.
521. Schlotthauer CF. The incidence and types of disease of the thyroid gland of adult horses. *J Am Vet Med Assoc*. 1931;78:211.
522. Yoshikawa T, Yoshikawa H, Oyamada T, Suzuki K. A follicular adenoma with C-cell hyperplasia in the equine thyroid. *Nippon Juigaku Zasshi*. 1984;46:615–623.
523. Ueki H, Kowatari Y, Oyamada T, Oikawa M, Yoshikawa H. Non-functional C-cell adenoma in aged horses. *J Comp Pathol*. 2004;131:157–165.
524. Elce YA, Ross MW, Davidson EJ, Tulleners EP. Unilateral thyroidectomy in 6 horses. *Vet Surg*. 2003;32:187–190.
525. Chiba S, Okada K, Numakunai S, Ohshima K. A case of equine thyroid follicular carcinoma accompanied with adenohypophysial adenoma. *Nippon Juigaku Zasshi*. 1987;49:551–554.
526. De Cock HE, MacLachlan NJ. Simultaneous occurrence of multiple neoplasms and hyperplasias in the adrenal and thyroid gland of the horse resembling multiple endocrine neoplasia syndrome: case report and retrospective identification of additional cases. *Vet Pathol*. 1999;36:633–636.
527. van der Velden MA, Meulenaar H. Medullary thyroid carcinoma in a horse. *Vet Pathol*. 1986;23:622–624.
528. Kuwamura M, Shirota A, Yamate J, Kotani T, Ohashi F, Sakuma S. C-cell adenoma containing variously sized thyroid follicles in a horse. *J Vet Med Sci*. 1998;60:387–389.
529. Hurcombe SD. Hypothalamic-pituitary gland axis function and dysfunction in horses. *Vet Clin North Am Equine Pract*. 2011;27:1–17.
530. van der Kolk JH, Fouche N, Gross JJ, Gerber V, Bruckmaier RM. A comparison between the equine and bovine hypothalamus-pituitary-adrenocortical axis. *Domest Anim Endocrinol*. 2016;56(suppl):S101–S111.
531. Hart KA, Barton MH. Adrenocortical insufficiency in horses and foals. *Vet Clin North Am Equine Pract*. 2011;27:19–34.
532. Belda X, Fuentes S, Daviu N, Nadal R, Armario A. Stress-induced sensitization: the hypothalamic-pituitary-adrenal axis and beyond. *Stress*. 2015;18:269–279.
533. Keller-Wood M. Hypothalamic-pituitary-adrenal axis—feedback control. *Compr Physiol*. 2015;5:1161–1182.
534. Hart KA, Dirikolu L, Ferguson DC, Norton NA, Barton MH. Daily endogenous cortisol production and hydrocortisone pharmacokinetics in adult horses and neonatal foals. *Am J Vet Res*. 2012;73:68–75.
535. Alexander SL, Irvine CH, Donald RA. Dynamics of the regulation of the hypothalamo-pituitary-adrenal (HPA) axis determined using a nonsurgical method for collecting pituitary venous blood from horses. *Front Neuroendocrinol*. 1996;17:1–50.
536. Schreiber CM, Stewart AJ, Kwessi E, Behrend EN, Wright JC, Kemppainen RJ, Busch KA. Seasonal variation in results of diagnostic tests for pituitary pars intermedia dysfunction in older, clinically normal geldings. *J Am Vet Med Assoc*. 2012;241:241–248.
537. Beech J, Boston RC, McFarlane D, Lindborg S. Evaluation of plasma ACTH, alpha-melanocyte-stimulating hormone, and insulin concentrations during various photoperiods in clinically normal horses and ponies and those with pituitary pars intermedia dysfunction. *J Am Vet Med Assoc*. 2009;235:715–722.
538. Cordero M, Brorsen BW, McFarlane D. Circadian and circannual rhythms of cortisol, ACTH, and alpha-melanocyte-stimulating hormone in healthy horses. *Domest Anim Endocrinol*. 2012;43:317–324.
539. Donaldson MT, McDonnell SM, Schanbacher BJ, Lamb SV, McFarlane D, Beech J. Variation in plasma adrenocorticotropic hormone concentration and dexamethasone suppression test results with season, age, and sex in healthy ponies and horses. *J Vet Intern Med*. 2005;19:217–222.
540. McFarlane D, Paradis MR, Zimmel D, et al. The effect of geographic location, breed, and pituitary dysfunction on seasonal adrenocorticotropin and alpha-melanocyte-stimulating hormone plasma concentrations in horses. *J Vet Intern Med*. 2011;25:872–881.
541. Itoi K, Jiang YQ, Iwasaki Y, Watson SJ. Regulatory mechanisms of corticotropin-releasing hormone and vasopressin gene expression in the hypothalamus. *J Neuroendocrinol*. 2004;16:348–355.
542. Jeanneteau FD, Lambert WM, Ismaili N, et al. BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proc Natl Acad Sci U S A*. 2012;109:1305–1310.
543. Marini AM, Popolo M, Pan H, Blondeau N, Lipsky RH. Brain adaptation to stressful stimuli: a new perspective on potential therapeutic approaches based on BDNF and NMDA receptors. *CNS Neurol Disord Drug Targets*. 2008;7:382–390.
544. Camille ML, Maguire J. GABAergic regulation of the HPA and HPG axes and the impact of stress on reproductive function. *J Steroid Biochem Mol Biol*. 2016;160:196–203.
545. Polito A, Sonneville R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoS One*. 2011;6:e25905.
546. Volpi S, Rabadan-Diehl C, Aguilera G. Vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation. *Stress*. 2004;7:75–83.
547. Givalois L, Naert G, Rage F, Ixart G, Arancibia S, Tapia-Arancibia L. A single brain-derived neurotrophic factor injection modifies hypothalamo-pituitary-adrenocortical axis activity in adult male rats. *Mol Cell Neurosci*. 2004;27:280–295.
548. Grinevich V, Ma XM, Jirikowski G, Verbalis J, Aguilera G. Lipopolysaccharide endotoxin potentiates the effect of osmotic stimulation on vasopressin synthesis and secretion in the rat hypothalamus. *J Neuroendocrinol*. 2003;15:141–149.
549. Keenan DM, Alexander S, Irvine C, Veldhuis JD. Quantifying nonlinear interactions within the hypothalamo-pituitary-adrenal axis in the conscious horse. *Endocrinology*. 2009;150:1941–1951.
550. Naert G, Ixart G, Tapia-Arancibia L, Givalois L. Continuous i.c.v. infusion of brain-derived neurotrophic factor modifies hypothalamic-pituitary-adrenal axis activity, locomotor activity and body temperature rhythms in adult male rats. *Neuroscience*. 2006;139:779–789.
551. Xu Z, Hu F, Shi L, et al. Angiotensin-induced vasopressin release and activation of hypothalamic neuron in pre-term fetuses. *Peptides*. 2005;26:307–314.
552. Bartanusz V, Jezova D, Bertini LT, Tilders FJ, Aubry JM, Kiss JZ. Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotrophic paraventricular neurons. *Endocrinology*. 1993;132:895–902.
553. Mouri T, Itoi K, Takahashi K, et al. Colocalization of corticotropin-releasing factor and vasopressin in the paraventricular nucleus of the human hypothalamu. *Neuroendocrinology*. 1993;57:34–39.
554. Goncharova ND. Stress responsiveness of the hypothalamic-pituitary-adrenal axis: age-related features of the vasopressinergic regulation. *Front Endocrinol (Lausanne)*. 2013;4:26.
555. Evans MJ, Marshall AG, Kitson NE, Summers K, Donald RA. Factors affecting ACTH release from perifused equine anterior pituitary cells. *J Endocrinol*. 1993;137:391–401.
556. Alexander SL, Irvine CH. The effect of endotoxin administration on the secretory dynamics of oxytocin in follicular phase mares: relationship to stress axis hormones. *J Neuroendocrinol*. 2002;14:540–548.
557. Crowley SK, Girdler SS. Neurosteroid, GABAergic and hypothalamic pituitary adrenal (HPA) axis regulation: what is the current state of knowledge in humans? *Psychopharmacology (Berl)*. 2014;231:3619–3634.
558. Naert G, Maurice T, Tapia-Arancibia L, Givalois L. Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. *Psychoneuroendocrinology*. 2007;32:1062–1078.

559. Wirth MM. Beyond the HPA Axis: Progesterone-Derived Neuroactive Steroids in Human Stress and Emotion. *Front Endocrinol (Lausanne)*. 2011;2:19.
560. McFarlane D, Dybdal N, Donaldson MT, Miller L, Cribb AE. Nitration and increased alpha-synuclein expression associated with dopaminergic neurodegeneration in equine pituitary pars intermedia dysfunction. *J Neuroendocrinol*. 2005;17:73–80.
561. Amar AP, Weiss MH. Pituitary anatomy and physiology. *Neurosurg Clin North Am*. 2003;14:11–23. v.
562. Hong GK, Payne SC, Jane Jr JA. Anatomy, Physiology, and Laboratory Evaluation of the Pituitary Gland. *Otolaryngol Clin North Am*. 2016;49:21–32.
563. Al-Aridi R, Abdelmannan D, Arafah BM. Biochemical diagnosis of adrenal insufficiency: the added value of dehydroepiandrosterone sulfate measurements. *Endocr Pract*. 2011;17:261–270.
564. Contreras LN, Arregger AL, Persi GG, Gonzalez NS, Cardoso EM. A new less-invasive and more informative low-dose ACTH test: salivary steroids in response to intramuscular corticotrophin. *Clin Endocrinol (Oxf)*. 2004;61:675–682.
565. Holst JP, Soldin SJ, Tractenberg RE, et al. Use of steroid profiles in determining the cause of adrenal insufficiency. *Steroids*. 2007;72:71–84.
566. Xing Y, Edwards MA, Ahlem C, et al. The effects of ACTH on steroid metabolomic profiles in human adrenal cells. *J Endocrinol*. 2011;209:327–335.
567. Yamakita N, Murai T, Kokubo Y, Hayashi M, Akai A, Yasuda K. Dehydroepiandrosterone sulphate is increased and dehydroepiandrosterone-response to corticotrophin-releasing hormone is decreased in the hyperthyroid state compared with the euthyroid state. *Clin Endocrinol (Oxf)*. 2001;55:797–803.
568. Beishuizen A, Thijs LG. Endotoxin and the hypothalamo-pituitary-adrenal (HPA) axis. *J Endotoxin Res*. 2003;9:3–24.
569. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev*. 1999;79:1–71.
570. Vrezas I, Willenberg HS, Bornstein SR. Adrenal Cortex, Development, Anatomy, Physiology. In: De Groot LJ, Chrousos G, Dungan K, et al, eds. *Endotext [Internet]*. South Dartmouth (MA): MDText.com, Inc; 2000-2013.
571. Vinson GP. Functional Zonation of the Adult Mammalian Adrenal Cortex. *Front Neurosci*. 2016;10:238.
572. Conley AJ. Review of the reproductive endocrinology of the pregnant and parturient mare. *Theriogenology*. 2016;86:355–365.
573. Fowden AL, Forhead AJ, Ousey JC. Endocrine adaptations in the foal over the perinatal period. *Equine Vet J Suppl*. 2012:130–139.
574. Rossdale PD, Silver M, Ellis L, Frauenfelder H. Response of the adrenal cortex to tetracosactrin (ACTH1-24) in the premature and full-term foal. *J Reprod Fertil Suppl*. 1982;32:545–553.
575. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. 2011;32:81–151.
576. Miller WL. Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol*. 2013;379:62–73.
577. Mayo W, Lemaire V, Malaterre J, et al. Pregnenolone sulfate enhances neurogenesis and PSA-NCAM in young and aged hippocampus. *Neurobiol Aging*. 2005;26:103–114.
578. Jonklaas J, Holst JP, Verbalis JG, Pehlivanova M, Soldin SJ. Changes in Steroid Concentrations with the Timing of ACTH Stimulation Testing in Normal Subjects. *Endocr Pract*. 2011:1–23.
579. Mohn CE, Fernandez-Solari J, De Laurentiis A, et al. The rapid release of corticosterone from the adrenal induced by ACTH is mediated by nitric oxide acting by prostaglandin E_2. *Proc Natl Acad Sci U S A*. 2005;102:6213–6218.
580. Dadoun F, Guillaume V, Sauze N, et al. Effect of endotoxin on the hypothalamic-pituitary-adrenal axis in sheep. *Eur J Endocrinol*. 1998;138:193–197.
581. Toribio RE. Endocrine dysregulation in critically ill foals and horses. *Vet Clin North Am Equine Pract*. 2011;27:35–47.
582. Suda T, Tozawa F, Ushiyama T, Sumitomo T, Yamada M, Demura H. Interleukin-1 stimulates corticotropin-releasing factor gene expression in rat hypothalamus. *Endocrinology*. 1990;126:1223–1228.
583. Akasaka S, Nomura M, Nishii H, et al. The hypothalamo-pituitary axis responses to lipopolysaccharide-induced endotoxemia in mice lacking inducible nitric oxide synthase. *Brain Res*. 2006;1089:1–9.
584. Tkachenko IV, Jaaskelainen T, Jaaskelainen J, Palvimo JJ, Voutilainen R. Interleukins 1alpha and 1beta as regulators of steroidogenesis in human NCI-H295R adrenocortical cells. *Steroids*. 2011;76:1103–1115.
585. Hurcombe SD, Toribio RE, Slovis N, et al. Blood arginine vasopressin, adrenocorticotropin hormone, and cortisol concentrations at admission in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2008;22:639–647.
586. Prigent H, Maxime V, Annane D. Science review: mechanisms of impaired adrenal function in sepsis and molecular actions of glucocorticoids. *Crit Care*. 2004;8:243–252.
587. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med*. 2013;(41):580–637.
588. Marik PE. Critical illness-related corticosteroid insufficiency. *Chest*. 2009;135:181–193.
589. Hinchcliff KW, Rush BR, Farris JW. Evaluation of plasma catecholamine and serum cortisol concentrations in horses with colic. *J Am Vet Med Assoc*. 2005;227:276–280.
590. Hoffman CJ, McKenzie III HC, Furr MO, Desrochers A. Glucocorticoid receptor density and binding affinity in healthy horses and horses with systemic inflammatory response syndrome. *J Vet Intern Med*. 2015;29:626–635.
591. Dowling PM, Williams MA, Clark TP. Adrenal insufficiency associated with long-term anabolic steroid administration in a horse. *J Am Vet Med Assoc*. 1993;203:1166–1169.
592. Wilson DW, Kingery S, Snow DH. The effect of training on adrenocortical function in Thoroughbred racehorses. In: Persson SGB, Lindholm A, Jeffcott LB, eds. *Equine Exercise Physiology 3*. Davis: ICEEP Publications; 1991:482–489.
593. Baker HW, Baker ID, Epstein VM, Hudson B. Effect of stress on steroid hormone levels in racehorses. *Aust Vet J*. 1982;58:70–71.
594. Cayado P, Munoz-Escassi B, Dominguez C, et al. Hormone response to training and competition in athletic horses. *Equine Vet J Suppl*. 2006:274–278.
595. Church DB, Evans DL, Lewis DR, Rose RJ. The effect of exercise on plasma adrenocorticotropin, cortisol and insulin in the horse and adaptations with training. In: Gillespie JR, Robinson NE, eds. *Equine Exercise Physiology*. Davis: ICEEP Publications; 1987:506–515.
596. Linden A, Art T, Amory H, Desmecht D, Lekeux P. Comparison of the adrenocortical response to both pharmacological and physiological stresses in sport horses. *Zentralbl Veterinarmed A*. 1990;37:601–604.
597. Snow DH, Mackenzie G. Some metabolic effects of maximal exercise in the horse and adaptations with training. *Equine Vet J*. 1977;9:134–140.
598. Dybdal NO, Gribble D, Madigan JE, Stabenfelt GH. Alterations in plasma corticosteroids, insulin and selected metabolites in horses used in endurance rides. *Equine Vet J*. 1980;12:137–140.
599. Lassourd V, Gayrard V, Laroute V, et al. Cortisol disposition and production rate in horses during rest and exercise. *Am J Physiol*. 1996;271:R25–R33.
600. Smiet E, Van Dierendonck MC, Sleutjens J, et al. Effect of different head and neck positions on behaviour, heart rate variability and cortisol levels in lunged Royal Dutch Sport horses. *Vet J*. 2014;202:26–32.

601. Couetil LL, Hoffman AM. Adrenal insufficiency in a neonatal foal. *J Am Vet Med Assoc*. 1998;212:1594–1596.
602. Dybdal NO. Endocrine and metabolic diseases. In: Smith BP, ed. *Large Animal Internal Medicine*. St. Louis: Mosby; 2002:1233–1265.
603. Sojka JE, Levy M. Evaluation of endocrine function. *Vet Clin North Am Equine Pract*. 1995;11:415–435.
604. Giri JK, Magdesian KG, Gaffney PM. Insulin-dependent diabetes mellitus associated with presumed autoimmune polyendocrine syndrome in a mare. *Can Vet J*. 2011;52(5):506–512.
605. Stewart AJ, Behrend EN, Wright JC, et al. Validation of a low-dose ACTH stimulation test in healthy adult horses. *J Am Vet Med Assoc*. 2011;239:834–841.
606. Dybdal NO, Hargreaves KM, Madigan JE, Gribble DH, Kennedy PC, Stabenfeldt GH. Diagnostic testing for pituitary pars intermedia dysfunction in horses. *J Am Vet Med Assoc*. 1994;204:627–632.
607. Eiler H, Goble D, Oliver J. Adrenal gland function in the horse: effects of cosyntropin (synthetic) and corticotropin (natural) stimulation. *Am J Vet Res*. 1979;40:724–726.
608. MacHarg MA, Bottoms GD, Carter GK, Johnson MA. Effects of multiple intramuscular injections and doses of dexamethasone on plasma cortisol concentrations and adrenal responses to ACTH in horses. *Am J Vet Res*. 1985;46:2285–2287.
609. Toutain PL, Brandon RA, de Pomyers H, Alvinerie M, Baggot JD. Dexamethasone and prednisolone in the horse: pharmacokinetics and action on the adrenal gland. *Am J Vet Res*. 1984;45:1750–1756.
610. Ekstrand C, Bondesson U, Gabrielsson J, et al. Plasma concentration-dependent suppression of endogenous hydrocortisone in the horse after intramuscular administration of dexamethasone-21-isonicotinate. *J Vet Pharmacol Ther*. 2015;38:235–242.
611. Slone DE, Purohit RC, Ganjam VK, Lowe JL. Sodium retention and cortisol (hydrocortisone) suppression caused by dexamethasone and triamcinolone in equids. *Am J Vet Res*. 1983;44:280–283.
612. Soma LR, Uboh CE, You Y, Guan F, Boston RC. Pharmacokinetics of intra-articular, intravenous, and intramuscular administration of triamcinolone acetonide and its effect on endogenous plasma hydrocortisone and cortisone concentrations in horses. *Am J Vet Res*. 2011;72:1234–1242.
613. Menendez MI, Phelps MA, Hothem EA, Bertone AL. Pharmacokinetics of methylprednisolone acetate after intra-articular administration and subsequent suppression of endogenous hydrocortisone secretion in exercising horses. *Am J Vet Res*. 2012;73:1453–1461.
614. Soma LR, Uboh CE, Luo Y, Guan F, Moate PJ, Boston RC. Pharmacokinetics of methylprednisolone acetate after intra-articular administration and its effect on endogenous hydrocortisone and cortisone secretion in horses. *Am J Vet Res*. 2006;67:654–662.
615. Menendez MI, Phelps MA, Bertone AL. Pharmacokinetics of intra-articular betamethasone sodium phosphate and betamethasone acetate and endogenous hydrocortisone suppression in exercising horses. *J Vet Pharmacol Ther*. 2016;39:22–26.
616. Hart KA, Barton MH, Vandenplas ML, Hurley DJ. Effects of low-dose hydrocortisone therapy on immune function in neonatal horses. *Pediatr Res*. 2011;70:72–77.
617. Peroni DL, Stanley S, Kollias-Baker C, Robinson NE. Prednisone per os is likely to have limited efficacy in horses. *Equine Vet J*. 2002;34:283–287.
618. Rush BR, Trevino IC, Matson CJ, Hakala JE. Serum cortisol concentrations in response to incremental doses of inhaled beclomethasone dipropionate. *Equine Vet J*. 1999;31:258–261.
619. Rush BR, Raub ES, Thomsen MM, Davis EG, Matson CJ, Hakala JE. Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction. *J Am Vet Med Assoc*. 2000;217:359–364.
620. Munoz T, Leclere M, Jean D, Lavoie JP. Serum cortisol concentration in horses with heaves treated with fluticasone proprionate over a 1 year period. *Res Vet Sci*. 2015;98:112–114.
621. Gold JR, Divers TJ, Barton MH, et al. Plasma adrenocorticotropin, cortisol, and adrenocorticotropin/cortisol ratios in septic and normal-term foals. *J Vet Intern Med*. 2007;21:791–796.
622. Hart KA, Slovis NM, Barton MH. Hypothalamic-pituitary-adrenal axis dysfunction in hospitalized neonatal foals. *J Vet Intern Med*. 2009;23:901–912.
623. Dembek KA, Johnson L, Timko K, et al. Dynamics of Adrenal Steroids, Steroid Precursors and Neurosteroids in Neonatal Foals during Hospitalization. *Proc 33rd Annual ACVIM Forum*. 2015;1241.
624. Dembek KA, Minuto J, Burns T, Barr B, Slovis NM, Toribio RE. Association of Androgen and Pregnane Response to ACTH Stimulation with Adrenal Dysfunction in Hospitalized Foals. *Proc 34th Annual ACVIM Forum*. 2016;1503.
625. Silver M, Ousey JC, Dudan FE, Fowden AL, Knox J, Cash RS, Rossdale PD. Studies on equine prematurity 2: Post natal adrenocortical activity in relation to plasma adrenocorticotrophic hormone and catecholamine levels in term and premature foals. *Equine Vet J*. 1984;16:278–286.
626. Annane D. Adrenal insufficiency in sepsis. *Curr Pharm Des*. 2008;14:1882–1886.
627. Annane D. Defining critical illness-related corticosteroid insufficiency: one step forward! *Crit Care Med*. 2010;38:721–722.
628. Boonen E, Bornstein SR, Van den BG. New insights into the controversy of adrenal function during critical illness. *Lancet Diabetes Endocrinol*. 2015;3:805–815.
629. Jellyman JK, Valenzuela OA, Allen VL, Forhead AJ, Holdstock NB, Fowden AL. Neonatal glucocorticoid overexposure programs pituitary-adrenal function in ponies. *Domest Anim Endocrinol*. 2015;50:45–49.
630. Gold JR, Cohen ND, Welsh Jr TH. Association of adrenocorticotrophin and cortisol concentrations with peripheral blood leukocyte cytokine gene expression in septic and nonseptic neonatal foals. *J Vet Intern Med*. 2012;26:654–661.
631. Wu A, Hinds CJ, Thiemermann C. High-density lipoproteins in sepsis and septic shock: metabolism, actions, and therapeutic applications. *Shock*. 2004;21:210–221.
632. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med*. 2013;368:1477–1488.
633. Boonen E, Van den BG. Mechanisms in endocrinology: new concepts to further unravel adrenal insufficiency during critical illness. *Eur J Endocrinol*. 2016;175:R1–R9.
633a. Dembek KA, Timko KJ, Johnson LM, et al. Steroids, steroid precursors, and neuroactive steroids in critically ill equine neonates. *Vet J*. 2017. in press.
634. Marik PE, Zaloga GP. Adrenal insufficiency during septic shock. *Crit Care Med*. 2003;31:141–145.
635. Hart KA, Barton MH, Ferguson DC, et al. Serum free cortisol fraction in healthy and septic neonatal foals. *J Vet Intern Med*. 2011;25:345–355.
636. Marik PE, Pastores SM, Annane D, et al. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med*. 2008;36:1937–1949.
637. Hart KA, Heusner GL, Norton NA, Barton MH. Hypothalamic-pituitary-adrenal axis assessment in healthy term neonatal foals utilizing a paired low dose/high dose ACTH stimulation test. *J Vet Intern Med*. 2009;23:344–351.
638. Wong DM, Vo DT, Alcott CJ, et al. Adrenocorticotropic hormone stimulation tests in healthy foals from birth to 12 weeks of age. *Can J Vet Res*. 2009;73:65–72.

639. Wong DM, Vo DT, Alcott CJ, Peterson AD, Sponseller BA, Hsu WH. Baseline plasma cortisol and ACTH concentrations and response to low-dose ACTH stimulation testing in ill foals. *J Am Vet Med Assoc*. 2009;234:126–132.
640. Stewart AJ, Wright JC, Behrend EN, Martin LG, Kemppainen RJ, Busch KA. Validation of a low-dose adrenocorticotropic hormone stimulation test in healthy neonatal foals. *J Am Vet Med Assoc*. 2013;243:399–405.
641. Reijerkerk EP, Visser EK, van Reenen CG, van der Kolk JH. Effects of various doses of ovine CRH on plasma and saliva cortisol concentrations in horses. *Am J Vet Res*. 2009;70:361–364.
642. Guennoun R, Labombarda F, Gonzalez Deniselle MC, Liere P, De Nicola AF, Schumacher M. Progesterone and allopregnanolone in the central nervous system: response to injury and implication for neuroprotection. *J Steroid Biochem Mol Biol*. 2015;146:48–61.
643. Bali A, Jaggi AS. Multifunctional aspects of allopregnanolone in stress and related disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:64–78.
644. Weng JH, Chung BC. Nongenomic actions of neurosteroid pregnenolone and its metabolites. *Steroids*. 2016;111:54–59.
645. Faroni A, Magnaghi V. The neurosteroid allopregnanolone modulates specific functions in central and peripheral glial cells. *Front Endocrinol (Lausanne)*. 2011;2:103.
646. Maurice T. Neurosteroids and sigma1 receptors, biochemical and behavioral relevance. *Pharmacopsychiatry*. 2004;37(suppl 3):S171–S182.
647. Sedlacek M, Korinek M, Petrovic M, et al. Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission. *Physiol Res*. 2008;57(suppl 3):S49–S57.
648. Zheng P. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol*. 2009;89:134–152.
649. Thomas P, Pang Y. Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. *Neuroendocrinology*. 2012;96:162–171.
650. Rahmani B, Ghasemi R, Dargahi L, Ahmadiani A, Haeri A. Neurosteroids; potential underpinning roles in maintaining homeostasis. *Gen Comp Endocrinol*. 2016;225:242–250.
651. Reddy DS, Rogawski MA. Neurosteroids—Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, eds. *Jasper's Basic Mechanisms of the Epilepsies [Internet]*. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
652. Carta MG, Bhat KM, Preti A. GABAergic neuroactive steroids: a new frontier in bipolar disorders? *Behav Brain Funct*. 2012;8:61.
653. Borowicz KK, Piskorska B, Banach M, Czuczwar SJ. Neuroprotective actions of neurosteroids. *Front Endocrinol (Lausanne)*. 2011;2:50.
654. Tyzio R, Cossart R, Khalilov I, et al. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science*. 2006;314:1788–1792.
655. Tyzio R, Nardou R, Ferrari DC, et al. Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science*. 2014;343:675–679.
656. Dembek KA, Brown C, Mudge MM, Reed SM, David B, Toribio RE. Association of Oxytocin and Neurosteroids with Neonatal Maladjustment Syndrome (NMS) in Hospitalized Foals. *Proc 33rd Annual ACVIM Forum*. 2016;1503.
657. Ahboucha S, Butterworth RF. The neurosteroid system: an emerging therapeutic target for hepatic encephalopathy. *Metab Brain Dis*. 2007;22:291–308.
658. Butterworth RF. Neurosteroids in hepatic encephalopathy: Novel insights and new therapeutic opportunities. *J Steroid Biochem Mol Biol*. 2016;160:94–97.
659. Holmberg E, Johansson M, Backstrom T, Haage D. Allopregnanolone preferentially induces energy-rich food intake in male Wistar rats. *Physiol Rep*. 2014;2:e12190.
660. Predieri B, Luisi S, Casarosa E, et al. High basal serum allopregnanolone levels in overweight girls. *Int J Obes (Lond)*. 2007;31:543–549.
661. O'connor SJ, Gardner DS, Ousey JC, et al. Development of baroreflex and endocrine responses to hypotensive stress in newborn foals and lambs. *Pflugers Arch*. 2005;450:298–306.
662. Houghton E, Holtan D, Grainger L, Voller BE, Rossdale PD, Ousey JC. Plasma progestagen concentrations in the normal and dysmature newborn foal. *J Reprod Fertil Suppl*. 1991;44:609–617.
663. Holtan DW, Houghton E, Silver M, Fowden AL, Ousey J, Rossdale PD. Plasma progestagens in the mare, fetus and newborn foal. *J Reprod Fertil Suppl*. 1991;44:517–528.
664. Madigan JE, Haggettt EF, Pickles KJ, et al. Allopregnanolone infusion induced neurobehavioural alterations in a neonatal foal: is this a clue to the pathogenesis of neonatal maladjustment syndrome? *Equine Vet J Suppl*. 2012;44(suppl 41):109–112.
665. Rossdale PD, Ousey JC, McGladdery AJ, et al. A retrospective study of increased plasma progestagen concentrations in compromised neonatal foals. *Reprod Fertil Dev*. 1995;7:567–575.
666. Aleman M, Pickles KJ, Conley AJ, et al. Abnormal plasma neuroactive progestagen derivatives in ill, neonatal foals presented to the neonatal intensive care unit. *Equine Vet J*. 2013;45:661–665.
667. Hirst JJ, Cumberland AL, Shaw JC, et al. Loss of neurosteroid-mediated protection following stress during fetal life. *J Steroid Biochem Mol Biol*. 2016;160:181–188.
668. Chiu KW, Robson S, Devi JL, Woodward A, Whittem T. The cardiopulmonary effects and quality of anesthesia after induction with alfaxalone in 2-hydroxypropyl-beta-cyclodextrin in dogs and cats: a systematic review. *J Vet Pharmacol Ther*. 2016.
669. Wakuno A, Aoki M, Kushiro A, et al. Comparison of alfaxalone, ketamine and thiopental for anaesthetic induction and recovery in Thoroughbred horses premedicated with medetomidine and midazolam. *Equine Vet J*. 2017;49:94–98.
670. Kelly Y, Blanco A, Tosti A. Androgenetic Alopecia: An Update of Treatment Options. *Drugs*. 2016;76:1349–1364.
671. Woolcock AD, Bugbee AC, Creevy KE. Evaluation of baseline cortisol concentration to monitor efficacy of twice-daily administration of trilostane to dogs with pituitary-dependent hyperadrenocorticism: 22 cases (2008–2012). *J Am Vet Med Assoc*. 2016;248:814–821.
672. McGowan CM, Neiger R. Efficacy of trilostane for the treatment of equine Cushing's syndrome. *Equine Vet J*. 2003;35:414–418.
673. Cohen ND, Carter GK. Steroid hepatopathy in a horse with glucocorticoid-induced hyperadrenocorticism. *J Am Vet Med Assoc*. 1992;200:1682–1684.
674. Traver DS, Bottoms GD. Adrenal Dysfunction. *Proc 24th Am Assoc Equine Pract Conv*. 1981:499–514.
675. van der Kolk JH, Ijzer J, Overgaauw PA, van der Linde-Sipman JS. Pituitary-independent Cushing's syndrome in a horse. *Equine Vet J*. 2001;33:110–112.
676. van der Kolk JH, Kalsbeek HC, van Garderen E, Wensing T, Breukink HJ. Equine pituitary neoplasia: a clinical report of 21 cases (1990–1992). *Vet Rec*. 1993;133:594–597.
677. van der Kolk JH, Mars MH, van dG I. Adrenocortical carcinoma in a 12-year-old mare. *Vet Rec*. 1994;134:113–115.
678. Young AC, Hoffmann KL, Begg AP, Major DA. Acute lameness associated with osseous metastasis of a peri-renal carcinoma in a horse. *Aust Vet J*. 2010;88:346–350.
679. Cryer PE. Diseases of the sympathochromaffin system. In: Felig P, Baxter JD, Frohman LA, eds. *Endocrinology and Metabolism*. New York: McGraw-Hill, Inc; 1995:713–748.

680. Luethy D, Habecker P, Murphy B, Nolen-Walston R. Clinical and Pathological Features of Pheochromocytoma in the Horse: A Multi-Center Retrospective Study of 37 Cases (2007–2014). *J Vet Intern Med*. 2016;30:309–313.
681. Buckingham JD. Case report. Pheochromocytoma in a mare. *Can Vet J*. 1970;11:205–208.
682. Yovich JV, Horney FD, Hardee GE. Pheochromocytoma in the horse and measurement of norepinephrine levels in horses. *Can Vet J*. 1984;25:21–25.
683. Duckett WM, Snyder JR, Harkema JR, Carlson GP. Functional pheochromocytoma in a horse. *Comp Cont Educ Equine Pract*. 1987;9:1118–1121.
684. Johnson PJ, Goetz TE, Foreman JH, Zachary JF. Pheochromocytoma in two horses. *J Am Vet Med Assoc*. 1995;206:837–841.
685. Gelberg H, Cockerell GL, Minor RR. A light and electron microscopic study of a normal adrenal medulla and a pheochromocytoma from a horse. *Vet Pathol*. 1979;16:395–404.
686. Yovich JV, Ducharme NG. Ruptured pheochromocytoma in a mare with colic. *J Am Vet Med Assoc*. 1983;183:462–464.
687. Kolloch RE, Kruse HJ, Friedrich R, Ruppert M, Overlack A, Stumpe KO. Role of epinephrine-induced hypokalemia in the regulation of renin and aldosterone in humans. *J Lab Clin Med*. 1996;127:50–56.
688. Ivanova RS, Dashev GI. Neuroendocrine features of adrenal pheochromocytomas: histological and immunocytochemical evaluation. *Neoplasma*. 1990;37:219–224.
689. Moreno AM, Castilla-Guerra L, Martinez-Torres MC, Torres-Olivera F, Fernandez E, Galera-Davidson H. Expression of neuropeptides and other neuroendocrine markers in human phaeochromocytomas. *Neuropeptides*. 1999;33:159–163.
690. Wilson RB, Holscher MA, Kasselberg AG, Jones M. Leu-enkephalin and somatostatin immunoreactivities in canine and equine pheochromocytomas. *Vet Pathol*. 1986;23:96–98.
691. Hardee GE, Wang LJ, Semrad SD, Trim CM. Catecholamines in equine and bovine plasmas. *J Vet Pharmacol Ther*. 1982;5:279–284.
692. Froscher BG, Power HT. Malignant pheochromocytoma in a foal. *J Am Vet Med Assoc*. 1982;181:494–496.
693. Helmstaedter V, Feurle GE, Forssmann WG. Insulin-, glucagon-, and somatostatin-immunoreactive endocrine cells in the equine pancreas. *Cell Tissue Res*. 1976;172:447–454.
694. Furuoka H, Ito H, Hamada M, Suwa T, Satoh H, Itakura C. Immunocytochemical component of endocrine cells in pancreatic islets of horses. *Nihon Juigaku Zasshi*. 1989;51:35–43.
695. Forssmann A. The ultrastructure of the cell types in the endocrine pancreas of the horse. *Cell Tissue Res*. 1976;167:179–195.
696. Gilon P, Henquin JC. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocrine Reviews*. 2001;22:565–604.
697. McClain DA. The endocrine pancreas. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and clinical principles*. Totowa, New Jersey: Humana Press; 1997:349–360.
698. Felig P, Bergman M. The endocrine pancreas: Diabetes mellitus. In: Felig P, Baxter JD, Frohman LA, eds. *Endocrinology and Metabolism*. New York: McGraw-Hill; 1995:1107–1250.
699. Brunzell JD. Familial lipoprotein lipase deficiency. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. *GeneReviews(R)*. Seattle (WA): University of Washington, Seattle; 1993.
700. Reue K, Doolittle MH. Naturally occurring mutations in mice affecting lipid transport and metabolism. *J Lipid Res*. 1996;37:1387–1405.
701. Breidenbach A, Fuhrmann H, Deegen E, Lindholm A, Sallmann HP. Studies on equine lipid metabolism. 2. Lipolytic activities of plasma and tissue lipases in large horses and ponies. *Zentralbl Veterinarmed A*. 1999;46:39–48.
702. Watson TD, Burns L, Love S, Packard CJ, Shepherd J. Plasma lipids, lipoproteins and post-heparin lipases in ponies with hyperlipaemia. *Equine Vet J*. 1992;24:341–346.
703. Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. *Endocr Rev*. 1998;19:608–624.
704. Hovorka R, Powrie JK, Smith GD, Sonksen PH, Carson ER, Jones RH. Five-compartment model of insulin kinetics and its use to investigate action of chloroquine in NIDDM. *Am J Physiol*. 1993;265:E162–E175.
705. Yosten GL, Kolar GR. The physiology of proinsulin C-peptide: Unanswered questions and a proposed model. *Physiology (Bethesda)*. 2015;30:327–332.
706. Hills CE, Brunskill NJ. Cellular and physiological effects of C-peptide. *Clin Sci (London)*. 2009;116:565–574.
707. Rutter GA. Regulating glucagon secretion: somatostatin in the spotlight. *Diabetes*. 2009;58:299–301.
708. Dominici FP, Turyn D. Growth hormone-induced alterations in the insulin-signaling system. *Exp Biol Med*. 2002;227:149–157.
709. Huang Y, Chang Y. Regulation of pancreatic islet beta-cell mass by growth factor and hormone signaling. *Prog Mol Biol Transl Sci*. 2014;121:321–349.
710. Takano A, Haruta T, Iwata M, et al. Growth hormone induces cellular insulin resistance by uncoupling phosphatidylinositol 3-kinase and its downstream signals in 3T3-L1 adipocytes. *Diabetes*. 2001;50(8):1891–1900.
711. Eigler T, Ben-Schlomo A. Somatostatin system: molecular mechanisms regulating anterior pituitary hormones. *J Mol Endocrinol*. 2014;53:R1–R19.
712. Park C, Yang I, Woo J, et al. Acute hyperglycemia and activation of the beta-adrenergic system exhibit synergistic inhibitory actions on growth hormone (GH) releasing hormone–induced GH release. *Eur J Endocrinol*. 2003;148:635–640.
713. van der Meulen T, Donaldson CJ, Caceres E, et al. Urocortin3 mediates somatostatin-dependent negative feedback control of insulin secretion. *Nat Med*. 2015;21(7):769–776.
714. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: similarities and differences. *J Diabetes Investig*. 2010;1(1-2):8–23.
715. Seino Y, Yabe D. Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: Incretin actions beyond the pancreas. *J Diabetes Investig*. 2013;4:108–130.
716. Abdulla H, Phillips B, Smith K, Wilkinson D, Atherton PJ, Idris I. Physiological mechanisms of action of incretin and insulin in regulating skeletal muscle metabolism. *Curr Diabetes Rev*. 2014;10:327–335.
717. Ezcurra M, Reimann F, Gribble FM, Emery E. Molecular mechanisms of incretin hormone secretion. *Curr Opin Pharmacol*. 2013;13:922–927.
718. Chameroy KA, Frank N, Elliott SB, Boston RC. Comparison of plasma active glucagon-like peptide 1 concentrations in normal horses and those with equine metabolic syndrome and in horses placed on a high-grain diet. *J Equine Vet Sci*. 2016;40:16–25.
719. de Laat MA, McGree JM, Sillence MN. Equine hyperinsulinemia: investigation of the enteroinsular axis during insulin dysregulation. *Am J Physiol Endocrinol Metab*. 2016;310(1):E61–E72.
720. Bamford NJ, Baskerville CL, Harris PA, Bailey SR. Postprandial glucose, insulin, and glucagon-like peptide-1 responses of different equine breeds adapted to meals containing micronized maize. *J Anim Sci*. 2015;93(7):3377–3383.
721. Schmidt O, Deegen E, Fuhrmann H, Duhlmeier R, Sallmann HP. Effects of fat feeding and energy level on plasma metabolites and hormones in shetland ponies. *J Vet Med A Physiol Pathol Clin Med*. 2001;48(1):39–49.
722. Jellyman JK, Allen VL, Holdstock NB, Fowden AL. Glucocorticoid overexposure in neonatal life alters pancreatic beta-cell function in newborn foals. *J Anim Sci*. 2013;91(1):104–110.
723. Hart KA, Wochele DM, Norton NA, McFarlane D, Wooldridge AA, Frank N. Effect of age, season, body condition, and endocrine status on serum free cortisol fraction and insulin concentration in horses. *J Vet Intern Med*. 2016;30(2):653–663.

724. Forhead AJ, Dobson H. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. *Res Vet Sci*. 1997;62:265–269.
725. Alexander SL, Roud HK, Irvine CH. Effect of insulin-induced hypoglycaemia on secretion patterns and rates of corticotrophin-releasing hormone, arginine vasopressin and adrenocorticotrophin in horses. *J Endocrinol*. 1997;153(3):401–409.
726. Berthoud HR, Trimble ER, Bereiter DA, Jeanrenaud B. Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rats. *Am J Physiol*. 1980;238:E336–E340.
727. Berthoud HR, Powley TL. Identification of vagal preganglionics that mediate cephalic phase insulin response. *Am J Physiol*. 1990;258:R523–R530.
728. Thorens B. Brain glucose sensing and neural regulation of insulin and glucagon secretion. *Diabetes Obes Metab*. 2011;13(suppl 1):82–88.
729. Taborsky Jr GJ. The physiology of glucagon. *J Diabetes Sci Technol*. 2010;4:1338–1344.
730. Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J Am Vet Med Assoc*. 2006;228(10):1538–1545.
731. Treiber KH, Kronfeld DS, Hess TM, Boston RC, Harris PA. Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. *Am J Vet Res*. 2005;66(12):2114–2121.
732. Asplin KE, Sillence MN, Pollitt CC, McGowan CM. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet J*. 2007;174(3):530–535.
733. de Laat MA, McGowan CM, Sillence MN, Pollitt CC. Equine laminitis: induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Vet J*. 2010;42(2):129–135.
734. Johnson PJ, Ganjam VK, Slight SH, Kreeger JM, Messer NT. Tissue-specific dysregulation of cortisol metabolism in equine laminitis. *Equine Vet J*. 2004;36:41–45.
735. Bailey SR, Menzies-Gow NJ, Harris PA, et al. Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis. *J Am Vet Med Assoc*. 2007;231:1365–1373.
736. Tiley HA, Geor RJ, McCutcheon LJ. Effects of dexamethasone administration on insulin resistance and components of insulin signaling and glucose metabolism in equine skeletal muscle. *Am J Vet Res*. 2008;69(1):51–58.
737. Fowden AL, Barnes RJ, Comline RS, Silver M. Pancreatic beta-cell function in the fetal foal and mare. *J Endocrinol*. 1980;87(2):293–301.
738. Fowden AL, Silver M, Ellis L, Ousey J, Rossdale PD. Studies on equine prematurity 3: Insulin secretion in the foal during the perinatal period. *Equine Vet J*. 1984;16(4):286–291.
739. Fowden AL, Gardner DS, Ousey JC, Giussani DA, Forhead AJ. Maturation of pancreatic beta-cell function in the fetal horse during late gestation. *J Endocrinol*. 2005;186(3):467–473.
740. Fowden AL, Ellis L, Rossdale PD. Pancreatic beta cell function in the neonatal foal. *J Reprod Fertil Suppl*. 1982;32:529–535.
741. Forhead AJ, Ousey JC, Allen WR, Fowden AL. Postnatal insulin secretion and sensitivity after manipulation of fetal growth by embryo transfer in the horse. *J Endocrinol*. 2004;181(3):459–467.
742. Holdstock NB, Allen VL, Bloomfield MR, Hales CN, Fowden AL. Development of insulin and proinsulin secretion in newborn pony foals. *J Endocrinol*. 2004;181(3):469–476.
743. Holdstock NB, Allen VL, Fowden AL. Pancreatic endocrine function in newborn pony foals after induced or spontaneous delivery at term. *Equine Vet J Suppl*. 2012;(41):30–37 (41).
744. Jellyman JK, Valenzuela OA, Allen VL, Holdstock NB, Fowden AL. Sex-associated differences in pancreatic beta cell function in healthy preweaning pony foals. *Equine Vet J*. 2014;46(6):722–728.
745. Ousey JC, Fowden AL, Wilsher S, Allen WR. The effects of maternal health and body condition on the endocrine responses of neonatal foals. *Equine Vet J*. 2008;40(7):673–679.
746. Fowden AL, Forhead AJ, Bloomfield M, Taylor PM, Silver M. Pancreatic alpha cell function in the fetal foal during late gestation. *Exp Physiol*. 1999;84(4):697–705.
747. Augstein P, Salzsieder E. Morphology of pancreatic islets: a time course of pre-diabetes in zucker fatty rats. *Methods Mol Biol*. 2009;560:159–189.
748. Johnson PJ, Scotty NC, Wiedmeyer C, Messer NT, Kreeger JM. Diabetes mellitus in a domesticated Spanish Mustang. *J Am Vet Med Assoc*. 2005;226(4):584–588. 542.
749. Durham AE, Hughes KJ, Cottle HJ, Rendle DI, Boston RC. Type 2 diabetes mellitus with pancreatic beta cell dysfunction in 3 horses confirmed with minimal model analysis. *Equine Vet J*. 2009;41(9):924–929.
750. American Association of Equine Practitioners. AAEP membership equine research study report. www.aaepfoundation.org. Updated 2009. Accessed March 14, 2011.
751. USDA-NAHMS. Lameness and laminitis in US horses. *U S Department Agriculture National Anim Health Monit Syst*. 2000. April (#N318.0400).
752. Frojdo S, Vidal H, Pirola L. Alterations of insulin signaling in type 2 diabetes: A review of the current evidence from humans. *Biochim Biophys Acta*. 2009;1792(2):83–92.
753. Paulsen SJ, Jelsing J, Madsen AN, et al. Characterization of beta-cell mass and insulin resistance in diet-induced obese and diet-resistant rats. *Obesity (Silver Spring)*. 2010;18(2):266–273.
754. Mercado AB, Castells S. Pancreatic beta-cell hyperactivity in morbidly obese adolescents. *Pediatr Endocrinol Rev*. 2006;3(suppl 4):560–563.
755. Alonso LC, Yokoe T, Zhang P, et al. Glucose infusion in mice: a new model to induce beta-cell replication. *Diabetes*. 2007;56(7):1792–1801.
756. Heit JJ, Karnik SK, Kim SK. Intrinsic regulators of pancreatic beta-cell proliferation. *Annu Rev Cell Dev Biol*. 2006;22:311–338.
757. Frank N, Geor RJ, Bailey SR, Durham AE, Johnson PJ. American College of Veterinary Internal Medicine. Equine metabolic syndrome. *J Vet Intern Med*. 2010;24(3):467–475.
758. Burns TA, Watts MR, Weber PS, McCutcheon LJ, Geor RJ, Belknap JK. Distribution of insulin receptor and insulin-like growth factor-1 receptor in the digital laminae of mixed-breed ponies: an immunohistochemical study. *Equine Vet J*. 2013;45:326–332.
759. Toth F, Frank N, Martin-Jimenez T, Elliott SB, Geor RJ, Boston RC. Measurement of C-peptide concentrations and responses to somatostatin, glucose infusion, and insulin resistance in horses. *Equine Vet J*. 2010;42(2):149–155.
760. Meier JJ, Butler AE, Saisho Y, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes*. 2008;57(6):1584–1594.
761. Burns TA, Watts MR, Geor RJ, McCutcheon LJ, Belknap JK. Evaluation of the response of the endocrine pancreas to short-term high-carbohydrate feeding in mixed breed ponies. *Proc 29th Annual ACVIM Forum*. 2011:668–669.
762. Pratt SE, Geor RJ, McCutcheon LJ. Effects of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in standardbred horses. *Equine Vet J Suppl*. 2006;36(36):579–584.
763. Pratt SE, Geor RJ, McCutcheon LJ. Repeatability of 2 methods for assessment of insulin sensitivity and glucose dynamics in horses. *J Vet Intern Med*. 2005;19(6):883–888.
764. Firshman AM, Valberg SJ. Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Vet J*. 2007;39(6):567–575.
765. Carter RA, McCutcheon LJ, George LA, Smith TL, Frank N, Geor RJ. Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *Am J Vet Res*. 2009;70(10):1250–1258.

766. Hoffman RM, Boston RC, Stefanovski D, Kronfeld DS, Harris PA. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *J Anim Sci*. 2003;81(9):2333–2342.
767. Leipig M, Abenthum K, Wollanke B, et al. Chronic pancreatitis with acinar-ductal metaplasia and ductal dysplasia in a horse. *J Comp Pathol*. 2015;153(2-3):131–134.
768. Lohmann KL, Allen AL. Chronic active pancreatitis as a cause of transverse colonic obstruction and colic in a horse. *Can Vet J*. 2015;56(11):1177–1180.
769. Gomez DE, Radtke CL, Russell LA, Lopez A, Wichtel MW. Acute pancreatitis following granulosa cell tumor removal in a mare. *Can Vet J*. 2015;56(10):1049–1052.
770. Buote M. Cholangiohepatitis and pancreatitis secondary to severe gastroduodenal ulceration in a foal. *Can Ve. J*. 2003;44(9):746–748.
771. Bakos Z, Krajcsovics L, Toth J. Successful medical treatment of acute pancreatitis in a horse. *Vet Rec*. 2008;162(3):95–96.
772. Carrick JB, Morris DD, Harmon BG, Fawzi M. Hematuria and weight loss in a mare with pancreatic adenocarcinoma. *Cornell Vet*. 1992;82(1):91–97.
773. Church S, West HJ, Baker JR. Two cases of pancreatic adenocarcinoma in horses. *Equine Vet J*. 1987;19(1):77–79.
774. de Brot S, Junge H, Hilbe M. Acinar cell carcinoma of exocrine pancreas in two horses. *J Comp Pathol*. 2014;150(4): 388–392.
775. Rendle DI, Hewetson M, Barron R, Baily JE. Tachypnoea and pleural effusion in a mare with metastatic pancreatic adenocarcinoma. *Vet Rec*. 2006;159(11):356–359.
776. Herbach N, Nagel L, Zwick T, Hermanns W. Multiple glucagon-producing pancreatic neuroendocrine tumors in a horse (equus caballus). *Vet Pathol*. 2014;51(3):607–611.
777. Ross MW, Lowe JE, Cooper BJ, Reimers TJ, Froscher BA. Hypoglycemic seizures in a shetland pony. *Cornell Vet*. 1983;73(2):151–169.
778. Day C. Metabolic syndrome, or what you will: Definitions and epidemiology. *Diab Vasc Dis Res*. 2007;4(1):32–38.
779. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15(7):539–553.
780. Stolar M. Metabolic syndrome: Controversial but useful. *Cleve Clin J Med*. 2007;74(3):199–202. 205-208.
781. Saely CH, Rein P, Drexel H. The metabolic syndrome and risk of cardiovascular disease and diabetes: experiences with the new diagnostic criteria from the international diabetes federation. *Horm Metab Res*. 2007;39(9):642–650.
782. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120(16):1640–1645.
783. Polonsky KS. The past 200 years in diabetes. *N Engl J Med*. 2012;367(14):1332–1340.
784. Ljungvall A, Zimmerman FJ. Bigger bodies: Long-term trends and disparities in obesity and body-mass index among U.S. adults, 1960–2008. *Soc Sci Med*. 2012;75(1):109–119.
785. Martinson ML. Income inequality in health at all ages: a comparison of the United States and England. *Am J Public Health*. 2012;102(11):2049–2056.
786. Stevens VL, Jacobs EJ, Sun J, et al. Weight cycling and mortality in a large prospective US study. *Am J Epidemiol*. 2012;175(8):785–792.
787. Daviglus ML, Talavera GA, Aviles-Santa ML, et al. Prevalence of major cardiovascular risk factors and cardiovascular diseases among hispanic/latino individuals of diverse backgrounds in the United States. *JAMA*. 2012;308(17):1775–1784.
788. Kraschnewski JL, Sciamanna CN, Stuckey HL, et al. A silent response to the obesity epidemic: Decline in US physician weight counseling. *Med Care*. 2013;51(2):186–192.
789. Robbins JM, Mallya G, Polansky M, Schwarz DF. Prevalence, disparities, and trends in obesity and severe obesity among students in the Philadelphia, Pennsylvania, school district, 2006–2010. *Prev Chronic Dis*. 2012;9:E145.
790. Turchiano M, Sweat V, Fierman A, Convit A. Obesity, metabolic syndrome, and insulin resistance in urban high school students of minority race/ethnicity. *Arch Pediatr Adolesc Med*. 2012;166(11):1030–1036.
791. Bray GA, Bellanger T. Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. *Endocrine*. 2006;29(1):109–117.
792. Johnson PJ. The equine metabolic syndrome peripheral Cushing's syndrome. *Vet Clin North Am Equine Pract*. 2002;18(2):271–293.
793. Belknap JK, Moore JN, Crouser EC. Sepsis—from human organ failure to laminar failure. *Vet Immunol Immunopathol*. 2009;129(3-4):155–157.
794. Pollitt CC, Visser MB. Carbohydrate alimentary overload laminitis. *Vet Clin North Am Equine Pract*. 2010;26(1):65–78.
795. van Eps AW, Pollitt CC. Equine laminitis induced with oligofructose. *Equine Vet J*. 2006;38(3):203–208.
796. Kearns CF, McKeever KH, Roegner V, Brady SM, Malinowski K. Adiponectin and leptin are related to fat mass in horses. *Vet J*. 2006;172(3):460–465.
797. Cartmill JA, Thompson DL Jr , Storer WA, Gentry LR, Huff NK. Endocrine responses in mares and geldings with high body condition scores grouped by high vs. low resting leptin concentrations. *J Anim Sci*. 2003;81(9):2311–2321.
798. Wray H, Elliott J, Bailey SR, Harris PA, Menzies-Gow NJ. Plasma concentrations of inflammatory markers in previously laminitic ponies. *Equine Vet J*. 2013;45(5):546–551.
799. Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ. Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. *Am J Vet Res*. 2008;69(1):122–129.
800. Waller CA, Thompson Jr DL, Cartmill JA, Storer WA, Huff NK. Reproduction in high body condition mares with high versus low leptin concentrations. *Theriogenology*. 2006;66(4):923–928.
801. Kronfeld DS, Treiber KH, Hess TM, et al. Metabolic syndrome in healthy ponies facilitates nutritional countermeasures against pasture laminitis. *J Nutr*. 2006;136(suppl 7):2090S–2093S.
802. Johnson PJ, Messer NT, Ganjam VK. Cushing's syndromes, insulin resistance and endocrinopathic laminitis. *Equine Vet J*. 2004;36(3):194–198.
803. Morgan R, Keen J, McGowan C. Equine metabolic syndrome. *Vet. Rec*. 2015;177(7):173–179.
804. Frank N. Equine metabolic syndrome. *Vet Clin North Am Equine Pract*. 2011;27(1):73–92.
805. Wildman RP, Muntner P, Reynolds K, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: Prevalence and correlates of 2 phenotypes among the US population (NHANES 1999–2004). *Arch Intern Med*. 2008;168(15):1617–1624.
806. Frank N, Hermida P, Sanchez-Londono A, Uricchio C, Singh R. Development of an octreotide response test for detection of insulin dysregulation in horses. *Proc. 34th Annual ACVIM Forum*. 2016;1502.
807. Carter RA, Geor RJ, Burton Staniar W, Cubitt TA, Harris PA. Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *Vet J*. 2009;179(2):204–210.
808. Treiber KH, Kronfeld DS, Geor RJ. Insulin resistance in equids: Possible role in laminitis. *J Nutr*. 2006;136(suppl 7):2094S–2098S.

809. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol*. 2010;314(1):1–16.
810. Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: The role of glucose, lipids and endotoxin. *J Endocrinol*. 2013;216(1):T1–T15.
811. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. 1999;19(4):972–978.
812. Vick MM, Murphy BA, Sessions DR, et al. Effects of systemic inflammation on insulin sensitivity in horses and inflammatory cytokine expression in adipose tissue. *Am J Vet Res*. 2008;69(1):130–139.
813. Vick MM, Adams AA, Murphy BA, et al. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *J Anim Sci*. 2007;85(5):1144–1155.
814. Treiber K, Carter R, Gay L, Williams C, Geor R. Inflammatory and redox status of ponies with a history of pasture-associated laminitis. *Vet Immunol Immunopathol*. 2009;129(3–4):216–220.
815. Burns TA, Geor RJ, Mudge MC, McCutcheon LJ, Hinchcliff KW, Belknap JK. Proinflammatory cytokine and chemokine gene expression profiles in subcutaneous and visceral adipose tissue depots of insulin-resistant and insulin-sensitive light breed horses. *J Vet Intern Med*. 2010;24(4):932–939.
816. Johnson PJ, Wiedmeyer CE, LaCarrubba A, Ganjam VK, Messer NT 4th. Laminitis and the equine metabolic syndrome. *Vet Clin North Am Equine Pract*. 2010;26(2):239–255.
817. Burns TA, Watts MR, Weber PS, McCutcheon LJ, Geor RJ, Belknap JK. Laminar inflammatory events in lean and obese ponies subjected to high carbohydrate feeding: Implications for pasture-associated laminitis. *Equine Vet J*. 2015;47(4):489–493.
818. Wilson PW, Meigs JB. Cardiometabolic risk: a Framingham perspective. *Int J Obes (Lond)*. 2008;32(suppl 2):S17–S20.
819. Behre CJ, Fagerberg B, Hulten LM, Hulthe J. The reciprocal association of adipocytokines with insulin resistance and C-reactive protein in clinically healthy men. *Metabolism*. 2005;54(4):439–444.
820. Cao YL, Wang YX, Wang DF, Meng X, Zhang J. Correlation between omental TNF-alpha protein and plasma PAI-1 in obesity subjects. *Int J Cardiol*. 2008;128(3):399–405.
821. Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity. *Ann Med*. 2000;32(suppl 1):78–84.
822. Jylhava J, Haarala A, Eklund C, et al. Serum amyloid A is independently associated with metabolic risk factors but not with early atherosclerosis: The cardiovascular risk in young finns study. *J Intern Med*. 2009;266(3):286–295.
823. van Bussel BC, Ferreira I, van de Waarenburg MP, et al. Multiple inflammatory biomarker detection in a prospective cohort study: A cross-validation between well-established single-biomarker techniques and an electrochemiluminescense-based multi-array platform. *PLoS One*. 2013;8(3):e58576.
824. Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci (Lond)*. 2006;110(3):267–278.
825. Ou HC, Lee WJ, Wu CM, Chen JF, Sheu WH. Aspirin prevents resistin-induced endothelial dysfunction by modulating AMPK, ROS, and akt/eNOS signaling. *J Vasc Surg*. 2012;55(4):1104–1115.
826. Chen XD, Lei T, Xia T, Gan L, Yang ZQ. Increased expression of resistin and tumour necrosis factor-alpha in pig adipose tissue as well as effect of feeding treatment on resistin and cAMP pathway. *Diabetes Obes Metab*. 2004;6(4):271–279.
827. Eiler H, Frank N, Andrews FM, Oliver JW, Fecteau KA. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. *Am J Vet Res*. 2005;66(9):1598–1604.
828. Brojer JT, Nostell KE, Essen-Gustavsson B, Hedenstrom UO. Effect of repeated oral administration of glucose and leucine immediately after exercise on plasma insulin concentration and glycogen synthesis in horses. *Am J Vet Res*. 2012;73(6):867–874.
829. Baker JR, Ritchie HE. Diabetes mellitus in the horse: a case report and review of the literature. *Equine Vet J*. 1974;6(1):7–11.
830. Jeffrey JR. Diabetes mellitus secondary to chronic pancreatitis in a pony. *J Am Vet Med Assoc*. 1968;153(9):1168–1175.
831. Gentry LR, Thompson Jr DL, Gentry Jr GT, Davis KA, Godke RA, Cartmill JA. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J Anim Sci*. 2002;80(10):2695–2703.
832. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15(4):371–372.
833. Jeffcott LB, Field JR, McLean JG, O'Dea K. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. *Equine Vet J*. 1986;18(2):97–101.
834. Freestone JF, Beadle R, Shoemaker K, Bessin RT, Wolfsheimer KJ, Church C. Improved insulin sensitivity in hyperinsulinaemic ponies through physical conditioning and controlled feed intake. *Equine Vet J*. 1992;24(3):187–190.
835. Hawley JA. Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. *Diabetes Metab Res Rev*. 2004;20:383–393.
836. Vervuert I, Coenen M, Dahlhoff S, Sommer W. Fructan concentrations in grass, silages, and hay. *Proc Equine Sci Soc*. 2005:309–310.
837. Martin-Gimenez T, de Blas I, Aquilera-Tejero E, Diez de Castro E, Aguirre-Pascasio CN. Endocrine, morphometric, and ultrasonographic characterization of neck adiposity in andalusian horses. *Domest Anim Endocrinol*. 2016;56:57–62.
838. Vick MM, Sessions DR, Murphy BA, Kennedy EL, Reedy SE, Fitzgerald BP. Obesity is associated with altered metabolic and reproductive activity in the mare: Effects of metformin on insulin sensitivity and reproductive cyclicity. *Reprod Fertil Dev*. 2006;18(6):609–617.
839. Breuhaus BA, Refsal KR, Beyerlein SL. Measurement of free thyroxine concentration in horses by equilibrium dialysis. *J Vet Internl Med*. 2006;20:371–376.
840. Dunbar LK, Mielnicki KA, Dembek KA, Toribio RE, Burns TA. Evaluation of four diagnostic tests for insulin dysregulation in adult light-breed horses. *J Vet Intern Med*. 2016;30(3):885–891.
841. Ralston SL. Insulin and glucose regulation. *Vet Clin North Am Equine Pract*. 2002;18(2):295–304, vii.
842. Rijnen KE, van der Kolk JH. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. *Am J Vet Res*. 2003;64(10):1260–1264.
843. Radziuk J. Insulin sensitivity and its measurement: Structural commonalities among the methods. *J Clin Endocrinol Metab*. 2000;85(12):4426–4433.
844. Wooldridge AA, Edwards HG, Plaisance EP, et al. Evaluation of high-molecular weight adiponectin in horses. *Am J Vet Res*. 2012;73(8):1230–1240.
845. Borer KE, Bailey SR, Menzies-Gow NJ. Use of proxy measurements of insulin sensitivity and insulin secretory responses to distinguish between normal and previously laminitic ponies. *Equine Vet J*. 2012;44:444–448.
846. Geor RJ, McCue ME, Schultz N. Current understanding of equine metabolic syndrome. *Proc 59th Am Assoc Equine Pract Conv*. 2013:297–304.
847. Schultz N, Geor RJ, Manfredi JM. Factors associated with leptin and adiponectin concentrations in a large across breed cohort of horses and ponies. *J Vet Intern Med*. 2014;28:998.

848. Menzies-Gow NJ, Harris PA, Elliott J. Prospective cohort study evaluating risk factors for the development of pasture-associated laminitis in the united kingdom. *Equine Vet J*. 2016;Jul 1.
849. Mendoza FJ, Aguilera-Aguilera R, Gonzalez-De Cara CA, Toribio RE, Estepa JC, Perez-Ecija A. Characterization of the intravenous glucose tolerance test and the combined glucose-insulin test in donkeys. *Vet J*. 2015;206(3):371–376.
850. Bertin FR, Sojka-Kritchevsky JE. Comparison of a 2-step insulin-response test to conventional insulin-sensitivity testing in horses. *Domest Anim Endocrinol*. 2013;44:19–25.
851. Matveyenko AV, Veldhuis JD, Butler PC. Measurement of pulsatile insulin secretion in the rat: Direct sampling from the hepatic portal vein. *Am J Physiol Endocrinol Metab*. 2008;295(3). E569–E574.
852. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P. Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. *Am J Physiol*. 1995;269(3 pt 1):E478–E488.
853. Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC. Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab*. 2000;85(12):4491–4499.
854. Chrysant SG, Chrysant GS. Clinical implications of cardiovascular preventing pleiotropic effects of dipeptidyl peptidase-4 inhibitors. *Am J Cardiol*. 2012;109(11):1681–1685.
855. de Graaf-Roelfsema E. Glucose homeostasis and the enteroinsular axis in the horse: a possible role in equine metabolic syndrome. *Vet J*. 2014;199(1):11–18.
856. Vilsboll T, Krarup T, Sonne J, et al. Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2003;88(6):2706–2713.
857. Duhlmeier R, Deegen E, Fuhrmann H, Widdel A, Sallmann HP. Glucose-dependent insulinotropic polypeptide (GIP) and the enteroinsular axis in equines (equus caballus). *Comp Biochem Physiol A Mol Integr Physiol*. 2001;129(2–3): 563–575.
858. Conti E, Carrozza C, Capoluongo E, et al. Insulin-like growth factor-1 as a vascular protective factor. *Circulation*. 2004;110(15):2260–2265.
859. Yuen KC, Dunger DB. Therapeutic aspects of growth hormone and insulin-like growth factor-I treatment on visceral fat and insulin sensitivity in adults. *Diabetes Obes Metab*. 2007;9(1):11–22.
860. Sesti G, Sciacqua A, Cardellini M, et al. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. *Diabetes Care*. 2005;28(1):120–125.
861. de Laat MA, Pollitt CC, Kyaw-Tanner MT, McGowan CM, Sillence MN. A potential role for lamellar insulin-like growth factor-1 receptor in the pathogenesis of hyperinsulinaemic laminitis. *Vet J*. 2013;197(2):302–306.
862. Mezi S, Todi L, Orsi E, Angeloni A, Mancini P. Involvement of the src-cortactin pathway in migration induced by IGF-1 and EGF in human breast cancer cells. *Int J Oncol*. 2012;41(6):2128–2138.
863. Fernandez-Gamba A, Leal MC, Morelli L, Castano EM. Insulin-degrading enzyme: Structure-function relationship and its possible roles in health and disease. *Curr Pharm Des*. 2009;15(31):3644–3655.
864. Valera Mora ME, Scarfone A, Calvani M, Greco AV, Mingrone G. Insulin clearance in obesity. *J Am Coll Nutr*. 2003;22(6):487–493.
865. Messier C, Teutenberg K. The role of insulin, insulin growth factor, and insulin-degrading enzyme in brain aging and alzheimer's disease. *Neural Plast*. 2005;12(4):311–328.
866. Wang Q, Jin T. The role of insulin signaling in the development of beta-cell dysfunction and diabetes. *Islets*. 2009;1(2):95–101.
867. Tanti JF, Jager J. Cellular mechanisms of insulin resistance: Role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. *Curr Opin Pharmacol*. 2009;9(6):753–762.
868. Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. *IUBMB Life*. 2010;62(5):315–333.
869. Leto D, Saltiel AR. Regulation of glucose transport by insulin: Traffic control of GLUT4. *Nat Rev Mol Cell Biol*. 2012;13(6):383–396.
870. Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. *Trends Cell Biol*. 2002;12(2):65–71.
871. Vigneri R, Squatrito S, Sciacca L. Insulin and its analogs: Actions via insulin and IGF receptors. *Acta Diabetol*. 2010;47(4):271–278.
872. Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*. 2012;55(10):2565–2582.
873. Avogaro A, de Kreutzenberg SV, Fadini GP. Insulin signaling and life span. *Pflugers Arch*. 2010;459(2):301–314.
874. Jensen M, De Meyts P. Molecular mechanisms of differential intracellular signaling from the insulin receptor. *Vitam Horm*. 2009;80:51–75.
875. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiol Rev*. 2009;89(1):27–71.
876. Gordon ME, McKeever KH, Betros CL, Manso Filho HC. Plasma leptin, ghrelin and adiponectin concentrations in young fit racehorses versus mature unfit standardbreds. *Vet J*. 2007;173(1):91–100.
877. Menzies-Gow NJ, Wray H, Bailey SR, Harris PA, Elliott J. The effect of exercise on plasma concentrations of inflammatory markers in normal and previously laminitic ponies. *Equine Vet J*. 2014;46(3):317–321.
878. Burns T, Watts M, Geor R, McCutcheon L, Belknap J. Effect of dietary carbohydrate challenge on activation of 5′-adenosine monophosphate activated protein kinase (AMPK) in liver, skeletal muscle, and digital laminae of lean and obese ponies. *J Vet Intern Med*. 2012;26(3):735.
879. Miranda L, Carpentier S, Platek A, et al. AMP-activated protein kinase induces actin cytoskeleton reorganization in epithelial cells. *Biochem Biophys Res Commun*. 2010;396(3): 656–661.
880. Asplin KE, Curlewis JD, McGowan CM, Pollitt CC, Sillence MN. Glucose transport in the equine hoof. *Equine Vet J*. 2011;43(2):196–201.
881. Hardie DG. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes Dev*. 2011;25(18):1895–1908.
882. Zhang L, Li J, Young LH, Caplan MJ. AMP-activated protein kinase regulates the assembly of epithelial tight junctions. *Proc Natl Acad Sci U S A*. 2006;103(46):17272–17277.
883. Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2012;32(9):2052–2059.
884. Schulman IH, Zhou MS. Vascular insulin resistance: a potential link between cardiovascular and metabolic diseases. *Curr Hypertens Rep*. 2009;11(1):48–55.
885. Muniyappa R, Yavuz S. Metabolic actions of angiotensin II and insulin: a microvascular endothelial balancing act. *Mol Cell Endocrinol*. 2013;378:59–69.
886. Robertson TP, Bailey SR, Peroni JF. Equine laminitis: a journey to the dark side of venous. *Vet Immunol Immunopathol*. 2009;129(3–4):164–166.
887. Noschka E, Moore JN, Peroni JF, Lewis SJ, Morrow JD, Robertson TP. Thromboxane and isoprostanes as inflammatory and vasoactive mediators in black walnut heartwood extract induced equine laminitis. *Vet Immunol Immunopathol*. 2009;129(3–4):200–210.

888. Varewijck AJ, Janssen JA. Insulin and its analogues and their affinities for the IGF1 receptor. *Endocr Relat Cancer*. 2012;19(5):F63–F75.
888a. Longland AC, Barfoot C, Harris PA. Effects of soaking on the water-soluble carbohydrate and crude protein content of hay. *Vet Rec*. 2011;168(23):618.
889. Hardie DG. AMP-activated/SNF1 protein kinases: Conserved guardians of cellular energy. *Nat Rev Mol Cell Biol*. 2007;8(10):774–785.
890. Hardie DG. Organismal carbohydrate and lipid homeostasis. *Cold Spring Harb Perspect Biol*. 2012;4(5). 10.1101/cshperspect.a006031.
891. Hardie DG. Sensing of energy and nutrients by AMP-activated protein kinase. *Am J Clin Nutr*. 2011;93(4):891S–896S.
892. Hardie DG. Energy sensing by the AMP-activated protein kinase and its effects on muscle metabolism. *Proc Nutr Soc*. 2011;70(1):92–99.
893. Lefort N, St-Amand E, Morasse S, Cote CH, Marette A. The alpha-subunit of AMPK is essential for submaximal contraction-mediated glucose transport in skeletal muscle in vitro. *Am J Physiol Endocrinol Metab*. 2008;295(6):E1447–E1454.
894. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res*. 2007;100(3):328–341.
895. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol*. 2012;13(4):251–262.
896. Meshkani R, Adeli K. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. *Clin Biochem*. 2009;42(13–14):1331–1346.
897. Joost HG, Thorens B. The extended GLUT-family of sugar/polyol transport facilitators: Nomenclature, sequence characteristics, and potential function of its novel members (review). *Mol Membr Biol*. 2001;18(4):247–256.
898. Corcoran MP, Lamon-Fava S, Fielding RA. Skeletal muscle lipid deposition and insulin resistance: Effect of dietary fatty acids and exercise. *Am J Clin Nutr*. 2007;85(3):662–677.
899. Eckardt K, Taube A, Eckel J. Obesity-associated insulin resistance in skeletal muscle: role of lipid accumulation and physical inactivity. *Rev Endocr Metab Disord*. 2011;12(3):163–172.
900. Franklin RM, Kanaley JA. Intramyocellular lipids: Effect of age, obesity, and exercise. *Phys Sportsmed*. 2009;37(1):20–26.
901. Mehnert H. Metformin, the rebirth of a biguanide: Mechanism of action and place in the prevention and treatment of insulin resistance. *Exp Clin Endocrinol Diabetes*. 2001;109(suppl 2). S259– S264.
902. de Laat MA, Robinson MA, Gruntmeir KJ, Lui Y, Soma LR, Lacombe VA. AICAR administration affects glucose metabolism by upregulating the novel glucose transporter, GLUT8, in equine skeletal muscle. *Vet J*. 2015;205(3):381–386.
903. Durham AE, Rendle DI, Newton JE. The effect of metformin on measurements of insulin sensitivity and beta cell response in 18 horses and ponies with insulin resistance. *Equine Vet J*. 2008;40(5):493–500.
904. Hustace JL, Firshman AM, Mata JE. Pharmacokinetics and bioavailability of metformin in horses. *Am J Vet Res*. 2009;70(5):665–668.
905. Tinworth KD, Edwards S, Noble GK, Harris PA, Sillence MN, Hackett LP. Pharmacokinetics of metformin after enteral administration in insulin-resistant ponies. *Am J Vet Res*. 2010;71(10):1201–1206.
906. Rendle DI, Rutledge F, Hughes KJ, Heller J, Durham AE. Effects of metformin hydrochloride on blood glucose and insulin responses to oral dextrose in horses. *Equine Vet J*. 2013;45(6):751–754.
907. Wearn JM, Crisman MV, Davis JL, et al. Pharmacokinetics of pioglitazone after multiple oral dose administration in horses. *J Vet Pharmacol Ther*. 2011;34(3):252–258.
908. Wearn JG, Suagee JK, Crisman MV, et al. Effects of the insulin sensitizing drug, pioglitazone, and lipopolysaccharide administration on markers of systemic inflammation and clinical parameters in horses. *Vet Immunol Immunopathol*. 2012;145(1–2):42–49.
909. Suagee JK, Corl BA, Wearn JG, et al. Effects of the insulin-sensitizing drug pioglitazone and lipopolysaccharide administration on insulin sensitivity in horses. *J Vet Intern Med*. 2011;25(2):356–364.
910. Heinrichs M, Baumgartner W, Capen CC. Immunocytochemical demonstration of proopiomelanocortin-derived peptides in pituitary adenomas of the pars intermedia in horses. *Vet Pathol*. 1990;27:419–425.
911. Hillyer MH, Taylor FGR, Mair TS, Murphy D, Watson TDG, Love S. Diagnosis of hyperadrenocorticism in the horse. *Equine Vet Educ*. 1992;4:121–134.
912. Love S. Equine Cushing's disease. *Br Vet J*. 1993;149:139–153.
913. Moore JN, Steiss J, Nicholson WE, Orth DN. A case of pituitary adrenocorticotropin-dependent Cushing's syndrome in the horse. *Endocrinology*. 1979;104:576–582.
914. Evans DR. The recognition and diagnosis of a pituitary tumor in the horse. *Proc Am Assoc Equine Pract*. 1972;18:417–419.
915. Couetil L, Paradis MR, Knoll J. Plasma adrenocorticotropin concentration in healthy horses and in horses with clinical signs of hyperadrenocorticism. *J Vet Intern Med*. 1996;10:1–6.
916. van der Kolk JH, Wensing T, Kalsbeek HC, Breukink HJ. Laboratory diagnosis of equine pituitary pars intermedia adenoma. *Domest Anim Endocrinol*. 1995;12:35–39.
917. Donaldson MT, LaMonte BH, Morresey P, Smith G, Beech J. Treatment with pergolide or cyproheptadine of pituitary pars intermedia dysfunction (equine Cushing's disease). *J Vet Intern Med*. 2002;16:742–746.
918. Innera M, Petersen AD, Desjardins DR, Steficek BA, Rosser Jr EJ, Schott HC. Comparison of hair follicle histology between horses with pituitary pars intermedia dysfunction and excessive hair growth and normal aged horses. *Vet Dermatol*. 2013;24:212–217.
919. Karikoski NP, Patterson-Kane JC, Singer ER, McFarlane D, McGowan CM. Lamellar pathology in horses with pituitary pars intermedia dysfunction. *Equine Vet J*. 2016;48:472–478.
920. Cartmill JA, Thompson Jr DL, Storer WA, Crowley JC, Huff NK, Waller CA. Effect of dexamethasone, feeding time, and insulin infusion on leptin concentrations in stallions. *J Anim Sci*. 2005;83:1875–1881.
921. Firshman AM, Valberg SJ, Karges TL, Benedict LE, Annandale EJ, Seaquist ER. Serum creatine kinase response to exercise during dexamethasone-induced insulin resistance in Quarter Horses with polysaccharide storage myopathy. *Am J Vet Res*. 2005;66:1718–1723.
922. Haffner JC, Eiler H, Hoffman RM, Fecteau KA, Oliver JW. Effect of a single dose of dexamethasone on glucose homeostasis in healthy horses by using the combined intravenous glucose and insulin test. *J Anim Sci*. 2009;87:131–135.
923. Tiley HA, Geor RJ, McCutcheon LJ. Effects of dexamethasone on glucose dynamics and insulin sensitivity in healthy horses. *Am J Vet Res*. 2007;68:753–759.
924. Beech J, Boston R, Lindborg S. Comparison of cortisol and ACTH responses after administration of thyrotropin releasing hormone in normal horses and those with pituitary pars intermedia dysfunction. *J Vet Intern Med*. 2011;25:1431–1438.
925. van der Kolk JH, Kalsbeek HC, Wensing T, Breukink HJ. Urinary concentration of corticoids in normal horses and horses with hyperadrenocorticism. *Res Vet Sci*. 1994;56:126–128.
926. van der Kolk JH, Wensing T. Urinary concentration of corticoids in ponies with hyperlipoproteinaemia or hyperadrenocorticism. *Vet Q*. 2000;22:55–57.
927. Klinkhamer K, Menheere PP, van der Kolk JH. Basal glucose metabolism and peripheral insulin sensitivity in equine pituitary pars intermedia dysfunction. *Vet Q*. 2011;31:19–28.

928. Mastro LM, Adams AA, Urschel KL. Pituitary pars intermedia dysfunction does not necessarily impair insulin sensitivity in old horses. *Domest Anim Endocrinol*. 2015;50:14–25.
929. Knowles EJ, Menzies-Gow NJ, Mair TS. Plasma fructosamine concentrations in horses with pituitary pars intermedia dysfunction with and without laminitis. *Equine Vet J*. 2014;46:249–251.
930. Loeb WF, Capen CC, Johnson LE. Adenomas of the pars intermedia associated with hyperglycemia and glycosuria in two horses. *Cornell Vet*. 1966;56:623–639.
931. Schott HC, Coursen CL, Eberhart SW, et al. The Michigan Cushing's project. *Proc Am Assoc Equine Pract*. 2001;47: 22–24.
932. Aleman M, Watson JL, Williams DC, LeCouteur RA, Nieto JE, Shelton GD. Myopathy in horses with pituitary pars intermedia dysfunction (Cushing's disease). *Neuromuscul Disord*. 2006;16:737–744.
933. Aleman M, Nieto JE. Gene expression of proteolytic systems and growth regulators of skeletal muscle in horses with myopathy associated with pituitary pars intermedia dysfunction. *Am J Vet Res*. 2010;71:664–670.
934. Millington WR, Dybdal NO, Dawson Jr R, Manzini C, Mueller GP. Equine Cushing's disease: differential regulation of beta-endorphin processing in tumors of the intermediate pituitary. *Endocrinology*. 1988;123:1598–1604.
935. Miller C, Utter ML, Beech J. Evaluation of the effects of age and pituitary pars intermedia dysfunction on corneal sensitivity in horses. *Am J Vet Res*. 2013;74:1030–1035.
936. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*. 1999;98:437–451.
937. McFarlane D. Cerebrospinal fluid concentration of hypocretin-1 in horses with equine pituitary pars intermedia disease and its relationship to oxidative stress. *J Vet Intern Med*. 2007;21:602.
938. McFarlane D, Hill K, Anton J. Neutrophil function in healthy aged horses and horses with pituitary dysfunction. *Vet Immunol Immunopathol*. 2015;165:99–106.
939. McFarlane D, Hale GM, Johnson EM, Maxwell LK. Fecal egg counts after anthelmintic administration to aged horses and horses with pituitary pars intermedia dysfunction. *J Am Vet Med Assoc*. 2010;236:330–334.
940. Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. *Equine Vet J*. 2007;39:438–445.
941. Hanlon DW, Stevenson M, Evans MJ, Firth EC. Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses. *N Z Vet J*. 2012;60:329–334.
942. Hemberg E, Lundeheim N, Einarsson S. Reproductive performance of Thoroughbred mares in Sweden. *Reprod Domest Anim*. 2004;39:81–85.
943. McGowan TW, Pinchbeck GP, McGowan CM. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. *Equine Vet J*. 2013;45:74–79.
944. Brosnahan MM, Paradis MR. Demographic and clinical characteristics of geriatric horses: 467 cases (1989–1999). *J Am Vet Med Assoc*. 2003;223:93–98.
945. Brosnahan MM, Paradis MR. Assessment of clinical characteristics, management practices, and activities of geriatric horses. *J Am Vet Med Assoc*. 2003;223:99–103.
946. van der Kolk JH, Heinrichs M, van Amerongen JD, Stooker RC, in de Wal LJ, van den Ingh TS. Evaluation of pituitary gland anatomy and histopathologic findings in clinically normal horses and horses and ponies with pituitary pars intermedia adenoma. *Am J Vet Res*. 2004;65:1701–1707.
947. Field JR, Wolf C. Cushing's syndrome in a horse. *Equine Vet J*. 1988;20:301–304.
948. Schott HC. Pituitary pars intermedia dysfunction: equine Cushing's disease. *Vet Clin North Am Equine Pract*. 2002;18:237–270.
949. Beech J. Tumors of the pituitary gland. In: Robinson NE, ed. *Current Therapy in Equine Medicine 1*. Philadelphia: W.B. Saunders; 1983:164–169.
950. Beech J. Tumors of the pituitary gland (pars intermedia). In: Robinson NE, ed. *Current Therapy in Equine Medicine 2*. Philadelphia: W.B. Saunders; 1987:182–185.
951. Boujon CE, Bestetti GE, Meier HP, Straub R, Junker U, Rossi GL. Equine pituitary adenoma: a functional and morphological study. *J Comp Pathol*. 1993;109:163–178.
951a Glover CM, Miller LM, Dybdal NO, Lopez A, Duckett WM, McFarlane D. Extrapituitary and Pituitary Pathological Findings in Horses with Pituitary Pars Intermedia Dysfunction: A Retrospective Study. *J Equine Vet Sci*. 2009;29(3):146–153.
952. McGowan TW, Pinchbeck GP, McGowan CM. Evaluation of basal plasma alpha-melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. *Equine Vet J*. 2013;45:66–73.
953. Beech J. Diseases of the pituitary gland. In: Colahan PT, Merritt AM, Moore JN, Mayhew IG, eds. *Equine Medicine and Surgery*. St. Louis: Mosby, Inc; 1999:1951–1956.
954. Saland LC. The mammalian pituitary intermediate lobe: an update on innervation and regulation. *Brain Res Bull*. 2001;54:587–593.
955. DeMaria JE, Lerant AA, Freeman ME. Prolactin activates all three populations of hypothalamic neuroendocrine dopaminergic neurons in ovariectomized rats. *Brain Res*. 1999;837:236–241.
956. Goudreau JL, Lindley SE, Lookingland KJ, Moore KE. Evidence that hypothalamic periventricular dopamine neurons innervate the intermediate lobe of the rat pituitary. *Neuroendocrinology*. 1992;56:100–105.
957. Orth DN, Holscher MA, Wilson MG, Nicholson WE, Plue RE, Mount CD. Equine Cushing's disease: plasma immunoreactive proopiolipomelanocortin peptide and cortisol levels basally and in response to diagnostic tests. *Endocrinology*. 1982;110:1430–1441.
958. McFarlane D, Banse H, Knych HK, Maxwell LK. Pharmacokinetic and pharmacodynamic properties of pergolide mesylate following long-term administration to horses with pituitary pars intermedia dysfunction. *J Vet Pharmacol Ther*. 2016.
959. Perkins GA, Lamb S, Erb HN, Schanbacher B, Nydam DV, Divers TJ. Plasma adrenocorticotropin (ACTH) concentrations and clinical response in horses treated for equine Cushing's disease with cyproheptadine or pergolide. *Equine Vet J*. 2002;34:679–685.
960. McFarlane D. Advantages and limitations of the equine disease, pituitary pars intermedia dysfunction as a model of spontaneous dopaminergic neurodegenerative disease. *Ageing Res Rev*. 2007;6:54–63.
961. Garcia dY, Li S, Pelletier G. Regulation of proopiomelanocortin gene expression by endogenous ligands of the GABAA receptor complex as evaluated by in situ hybridization in the rat pars intermedia. *Brain Res*. 1997;750:277–284.
962. McFarlane D, Beech J, Cribb A. Alpha-melanocyte stimulating hormone release in response to thyrotropin releasing hormone in healthy horses, horses with pituitary pars intermedia dysfunction and equine pars intermedia explants. *Domest Anim Endocrinol*. 2006;30:276–288.
963. Wilson MG, Nicholson WE, Holscher MA, Sherrell BJ, Mount CD, Orth DN. Proopiolipomelanocortin peptides in normal pituitary, pituitary tumor, and plasma of normal and Cushing's horses. *Endocrinology*. 1982;110:941–954.
964. Orth DN, Nicholson WE. Bioactive and immunoreactive adrenocorticotropin in normal equine pituitary and in pituitary tumors of horses with Cushing's disease. *Endocrinology*. 1982;111:559–563.

965. Castro MG, Morrison E. Post-translational processing of proopiomelanocortin in the pituitary and in the brain. *Crit Rev Neurobiol*. 1997;11:35–57.
966. Eiler H, Oliver JW, Andrews FM, Fecteau KA, Green EM, McCracken M. Results of a combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test in healthy horses and horses suspected to have a pars intermedia pituitary adenoma. *J Am Vet Med Assoc*. 1997;211:79–81.
967. Karikoski NP, McGowan CM, Singer ER, Asplin KE, Tulamo RM, Patterson-Kane JC. Pathology of Natural Cases of Equine Endocrinopathic Laminitis Associated with Hyperinsulinemia. *Vet Pathol*. 2015;52:945–956.
968. Garcia MC, Beech J. Equine intravenous glucose tolerance test: glucose and insulin responses of healthy horses fed grain or hay and of horses with pituitary adenoma. *Am J Vet Res*. 1986;47:570–572.
969. van der Kolk JH, Wensing T, Kalsbeek HC, Breukink HJ. Lipid metabolism in horses with hyperadrenocorticism. *J Am Vet Med Assoc*. 1995;206:1010–1012.
970. McFarlane D, Miller LM, Craig LE, et al. Agreement in histologic assessments of the pituitary pars intermedia in aged horses. *Am J Vet Res*. 2005;66:2055–2059.
971. Bottoms GD, Roesel OF, Rausch FD, Akins EL. Circadian variation in plasma cortisol and corticosterone in pigs and mares. *Am J Vet Res*. 1972;33:785–790.
972. van der Kolk JH, Nachreiner RF, Schott HC, Refsal KR, Zanella AJ. Salivary and plasma concentration of cortisol in normal horses and horses with Cushing's disease. *Equine Vet J*. 2001;33:211–213.
973. Douglas RH. Circadian cortisol rhythmicity and equine Cushing's-like disease. *J Eq Vet Sci*. 2000;19:684–686.
974. Hart KA, Kitchings KM, Kimura S, Norton NA, Myrna KE. Measurement of cortisol concentration in the tears of horses and ponies with pituitary pars intermedia dysfunction. *Am J Vet Res*. 2016;77:1236–1244.
975. Levy M, Sojka JE, Dybdal NO. Diagnosis and treatment of equine Cushing's disease. *Compend Contin Educ Pract Vet*. 1999;21:766–769.
976. Sojka JE, Johnson MA, Bottoms GD. The effect of starting time on dexamethasone suppression test results in horses. *Domest Anim Endocrinol*. 1993;10:1–5.
977. Frank N, Andrews F, Durham A, Kritchevsky J, McFarlane D, Schott H. Recommendations for the diagnosis and treatment of pituitary pars intermedia dysfunction (PPID). *Havemeyer Equine Geriatric Workshop II and 3rd Equine Endocrine Summit*. 2014.
978. Lee ZY, Zylstra R, Haritou SJ. The use of adrenocorticotrophic hormone as a potential biomarker of pituitary pars intermedia dysfunction in horses. *Vet J*. 2010;185:58–61.
979. Rendle DI, Litchfield E, Heller J, Hughes KJ. Investigation of rhythms of secretion and repeatability of plasma adrenocorticotropic hormone concentrations in healthy horses and horses with pituitary pars intermedia dysfunction. *Equine Vet J*. 2014;46:113–117.
980. Rendle DI, Duz M, Beech J, Parkin T, Durham AE. Investigation of single and paired measurements of adrenocorticotropic hormone for the diagnosis of pituitary pars intermedia dysfunction in horses. *J Vet Intern Med*. 2015;29:355–361.
981. Prutton JS, Kass PH, Watson JL, Pusterla N. Pre-analytical stability of adrenocorticotrophic hormone from healthy horses in whole blood, plasma and frozen plasma samples. *Vet J*. 2015;204:123–124.
982. Diez de CE, Lopez I, Cortes B, Pineda C, Garfia B, guilera-Tejero E. Influence of feeding status, time of the day, and season on baseline adrenocorticotropic hormone and the response to thyrotropin releasing hormone-stimulation test in healthy horses. *Domest Anim Endocrinol*. 2014;48:77–83.
983. Restifo MM, Frank N, Hermida P, Sanchez-Londono A. Effects of withholding feed on thyrotropin-releasing hormone stimulation test results and effects of combined testing on oral sugar test and thyrotropin-releasing hormone stimulation test results in horses. *Am J Vet Res*. 2016;77:738–748.
984. Thompson JC, Ellison R, Gillet RBL. Problems in the diagnosis of pituitary adenoma (Cushing's syndrome) in horses. *N Z Vet J*. 1995;43:79–82.
985. Beech J, McFarlane D, Lindborg S, Sojka JE, Boston RC. Alpha-Melanocyte-stimulating hormone and adrenocorticotropin concentrations in response to thyrotropin-releasing hormone and comparison with adrenocorticotropin concentration after domperidone administration in healthy horses and horses with pituitary pars intermedia dysfunction. *J Am Vet Med Assoc*. 2011;238:1305–1315.
986. Funk RA, Stewart AJ, Wooldridge AA, et al. Seasonal changes in plasma adrenocorticotropic hormone and alpha-melanocyte-stimulating hormone in response to thyrotropin-releasing hormone in normal, aged horses. *J Vet Intern Med*. 2011; 25:579–585.
987. McGowan CM, Frost R, Pfeiffer DU, Neiger R. Serum insulin concentrations in horses with equine Cushing's syndrome: response to a cortisol inhibitor and prognostic value. *Equine Vet J*. 2004;36:295–298.
988. McFarlane D, Donaldson MT, McDonnell SM, Cribb AE. Effects of season and sample handling on measurement of plasma alpha-melanocyte-stimulating hormone concentrations in horses and ponies. *Am J Vet Res*. 2004;65:1463–1468.
989. Miller MA, Pardo ID, Jackson LP, Moore GE, Sojka JE. Correlation of pituitary histomorphometry with adrenocorticotrophic hormone response to domperidone administration in the diagnosis of equine pituitary pars intermedia dysfunction. *Vet Pathol*. 2008;45:26–38.
990. Allen JR, Barbee DD, Crisman MV. Diagnosis of pituitary tumors by computed tomography-part I. *Compend Contin Educ Pract Vet*. 1988;10:1103–1106.
991. McKlveen TL, Jones JC, Sponenberg DP, Scarratt K, Ward DL, Aardema Jr CH. Assessment of the accuracy of computed tomography for measurement of normal equine pituitary glands. *Am J Vet Res*. 2003;64:1387–1394.
992. Pease AP, Schott HC, Howey EB, Patterson JS. Computed tomographic findings in the pituitary gland and brain of horses with pituitary pars intermedia dysfunction. *J Vet Intern Med*. 2011;25:1144–1151.
993. Levy M, Blevins WE, Janovitz EB. Radiological diagnosis of pituitary adenoma in the horse. *Proc Third Congress World Equine Vet Assoc*. 1993;18.
994. Pease A, Patterson J, Howey E, McFarlane D, van der Kolk H, Schott II H. Comparison of magnetic resonance imaging and histological scores for assessing pituitary pars intermedia enlargement in horses with pituitary pars intermedia dysfunction. *Proc 32nd Annual ACVIM Forum*. 2014:1113–1114.
995. Beck DJ. Effective long term treatment of a suspected pituitary adenoma with bromocriptine mesylate in a pony. *Am J Vet Res*. 1985;46:1941–1943.
996. Beech J. Treatment of hypophyseal adenomas. *Compend Contin Educ Pract Vet*. 4:119–121.
997. Krieger DT, Amorosa L, Linick F. Cyproheptadine-induced remission of Cushing's disease. *N Engl J Med*. 1975;293:893–896.
998. Munoz MC, Doreste F, Ferrer O, Gonzalez J, Montoya JA. Pergolide treatment for Cushing's syndrome in a horse. *Vet Rec*. 1996;139:41–43.
999. Peters D. Low dose pergolide mesylate treatment for equine hypophyseal adenomas (Cushing's syndrome). *Proc Am Assoc Equine Pract*. 1995;41:154–155.
1000. Oberhaus EL, Thompson DL, Arana Valencia N, Gilley RM. The effects of long-term treatment with cabergoline on the vernal transition in mares. *J Equine Vet Sci*. 2014;34:104.
1001. Valencia NA, Thompson Jr DL, Oberhaus EL. Dopaminergic (cabergoline) and antidopaminergic (sulpiride) effects on indices of insulin sensitivity in horses. *J Equine Vet Sci*. 2015;35:418.
1002. Gehring R, Beard L, Wright A, Coetzee J, Havel J, Apley M. Single-dose oral pharmacokinetics of pergolide mesylate in healthy adult mares. *Vet Ther*. 2010;11:E1–E8.

1003. Rendle DI, Hughes KJ, Doran GS, Edwards SH. Pharmacokinetics of pergolide after intravenous administration to horses. *Am J Vet Res*. 2015;76:155–160.
1004. Antonini A, Poewe W. Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. *Lancet Neurol*. 2007;6:826–829.
1005. Tanakol R, Alagol F, Azizlerli H, Sandalci O, Terzioglu T, Berker F. Cyproheptadine treatment in Cushing's disease. *J Endocrinol Invest*. 1996;19:242–247.
1006. Lamberts SW, Verleun T, Oosterom R. The mechanism of action of cyproheptadine on prolactin release by cultured anterior pituitary cells. *Life Sci*. 1985;36:2257–2262.
1007. Madigan JE, Kortz G, Murphy C, Rodger L. Photic headshaking in the horse: 7 cases. *Equine Vet J*. 1995;27:306–311.
1008. Meier B, Berger D, Hoberg E, Sticher O, Schaffner W. Pharmacological activities of Vitex agnus-castus extracts in vitro. *Phytomedicine*. 2000;7:373–381.
1009. Berger D, Schaffner W, Schrader E, Meier B, Brattstrom A. Efficacy of Vitex agnus castus L. extract Ze 440 in patients with pre-menstrual syndrome (PMS). *Arch Gynecol Obstet*. 2000;264:150–153.
1010. Beech J, Donaldson MT, Lindborg S. Comparison of Vitex agnus castus extract and pergolide in treatment of equine cushing's syndrome. *Proc Am Assoc Equine Pract*. 2002;48:175–177.
1011. Schutzer WE, Kerby JL, Holtan DW. Differential effect of trilostane on the progestin milieu in the pregnant mare. *J Reprod Fertil*. 1996;107:241–248.
1012. McGowan CM, Neiger R. Efficacy of trilostane for the treatment of equine Cushing's syndrome. *Equine Vet J*. 2003;35:414–418.
1013. Dewis P, Anderson DC, Bu'lock DE, Earnshaw R, Kelly WF. Experience with trilostane in the treatment of Cushing's syndrome. *Clin Endocrinol (Oxf)*. 1983;18:533–540.
1014. Wright TWW, Tull TC. A preliminary report of an investigation of a condition known as "dry coat" in horses. *Vet J*. 1925;81:235.
1015. Evans CL, Nisbet AM, Ross KA. A histological study of the sweat glands of normal and dry-coated horses. *J Comp Pathol*. 1957;67:397–405.
1016. Warner A, Mayhew IG. Equine anhidrosis: a review of pathophysiologic mechanisms. *Vet Res Commun*. 1983;6:249–264.
1017. Warner AE, Mayhew IG. Equine anhidrosis: a survey of affected horses in Florida. *J Am Vet Med Assoc*. 1982;180:627–629.
1018. Johnson EB, MacKay RJ, Hernandez JA. An epidemiologic study of anhidrosis in horses in Florida. *J Am Vet Med Assoc*. 2010;236:1091–1097.
1019. Hubert JD, Beadle RE, Norwood G. Equine anhidrosis. *Vet Clin North Am Equine Pract*. 2002;18:355–369.
1020. Yang J, Lim YK. Prevalence of anhidrosis in Thoroughbred racehorses in Korea. *J Anim Sci Technol*. 2011;53:571–573.
1021. Rasheed SU, Khan MS, Rehman ZU, Avais M, Khan JA, Shabbir MZ. A study on prevalence and treatment of anhidrosis in horses. *J Anim Plant Sci*. 2016;20:70–72.
1022. Watanabe A, Kanemaki N, Matsuura K. Distribution densities of hair follicles in racehorses. *Jpn J Equine Sci*. 1993;4:55–60.
1023. Jenkinson DM, Elder HY, Bovell DL. Equine sweating and anhidrosis Part 1—equine sweating. *Vet Dermatol*. 2006;17:361–392.
1024. Bijman J, Quinton PM. Predominantly beta-adrenergic control of equine sweating. *Am J Physiol*. 1984;246:R349–R353.
1025. Scott CM, Marlin DJ, Schroter RC. Quantification of the response of equine apocrine sweat glands to beta2-adrenergic stimulation. *Equine Vet J*. 2001;33:605–612.
1026. Snow DH. Identification of the receptor involved in adrenaline mediated sweating in the horse. *Res Vet Sci*. 1977;23:246–247.
1027. McCutcheon LJ, Geor RJ. Thermoregulation and exercise-associated heat illness. In: Hinchcliff KW, Kaneps AJ, Geor RJ, eds. *Equine Sorts Medicine and Surgery Basic and Clinical Sciences of the Equine Athlete*. Elsevier; 2014:901–918.
1028. Kingston JK, Geor RJ, McCutcheon LJ. Rate and composition of sweat fluid losses are unaltered by hypohydration during prolonged exercise in horses. *J Appl Physiol*. 1985;1997(83):1133–1143.
1029. MacKay RJ. Quantitative intradermal terbutaline sweat test in horses. *Equine Vet J*. 2008;40:518–520.
1030. Hodgson DR, McCutcheon LJ, Byrd SK, et al. Dissipation of metabolic heat in the horse during exercise. *J Appl Physiol*. 1993;74:1161–1170.
1031. Jenkinson DM, Blackburn PS. The distribution of nerves, non-oamine oxidase and cholinesterase in the skin of the horse. *Res Vet Sci*. 1968;9:165–169.
1032. DeLahunta A, Glass E. Lower motor neuron: general visceral efferent system. In: DeLahunta A, Glass E, eds. *Veterinary Neuroanatomy and Clinical Neurology*. St. Louis, Mo: Saunders/Elsevier; 2009:168–191.
1033. Shibasaki M, Crandall CG. Mechanisms and controllers of eccrine sweating in humans. *Front Biosci (Schol Ed)*. 2010;2:685–696.
1034. Bell M, Montagna W. Innervation of sweat glands in horses and dogs. *Br J Dermatol*. 1972;86:160–163.
1035. Jenkinson DM, Elder HY, Bovell DL. Equine sweating and anhidrosis. Part 2: anhidrosis. *Vet Dermatol*. 2007;18:2–11.
1036. Mills PC, Marlin DJ, Scott CM, Smith NC. Nitric oxide and thermoregulation during exercise in the horse. *J Appl Physiol*. 1985;1997(82):1035–1039.
1037. Mills PC, Scott CM, Marlin DJ. Effects of nitric oxide inhibition on thermoregulation during exercise in the horse. *Ann N Y Acad Sci*. 1997;813:591–599.
1038. Wilson DC, Corbett AD, Steel C, Pannirselvam R, Bovell DL. A preliminary study of the short circuit current (Isc) responses of sweat gland cells from normal and anhidrotic horses to purinergic and adrenergic agonists. *Vet Dermatol*. 2007;18:152–160.
1039. Bovell DL, Riggs CM, Sidlow G, Troester S, MacLaren W, Yip W, Ko WH. Evidence of purinergic neurotransmission in isolated, intact horse sweat glands. *Vet Dermatol*. 2013;24: 398–396.
1040. Robertshaw D. Proceedings: neural and humoral control of apocrine glands. *J Invest Dermatol*. 1974;63:160–167.
1041. Robertshaw D, Taylor CR. Sweat gland function of the donkey (Equus asinus). *J Physiol*. 1969;205:79–89.
1042. Beadle RE, Norwood GL, Brencick VA. Summertime plasma catecholamine concentrations in healthy and anhidrotic horses in Louisiana. *Am J Vet Res*. 1982;43:1446–1448.
1043. Marlin DJ, Schroter RC, Scott CM, et al. Sweating and skin temperature responses of normal and anhidrotic horses to intravenous adrenaline. *Equine Vet J Suppl*. 1999;30:362–369.
1044. Rakhit S, Murdoch R, Wilson SM. Persistent desensitisation of the beta 2 adrenoceptors expressed by cultured equine sweat gland epithelial cells. *J Exp Biol*. 1998;201:259–266.
1045. Bovell DL, Lindsay SL, Corbett AD, Steel C. Immunolocalization of aquaporin-5 expression in sweat gland cells from normal and anhidrotic horses. *Vet Dermatol*. 2006;17:17–23.
1046. Jenkinson DM, Montgomery I, Elder HY, Mason DK, Collins EA, Snow DH. Ultrastructural variations in the sweat glands of anhidrotic horses. *Equine Vet J*. 1985;17:287–291.
1047. Nakazato Y, Tamura N, Ohkuma A, Yoshimaru K, Shimazu K. Idiopathic pure sudomotor failure: anhidrosis due to deficits in cholinergic transmission. *Neurology*. 2004;63:1476–1480.
1048. Jenkinson DM, Loney C, Elder HY, Montgomery I, Mason DK. Effects of season and lower ambient temperature on the structure of the sweat glands in anhidrotic horses. *Equine Vet J*. 1989;21:59–65.
1049. Moran SA, Bovell DL, Corbett AD, et al. Comparison of P2Y Receptor Subtypes in Equine Sweat Gland Epithelial Cells From Normal and Anhidrotic Animals. *Proc Physiol Soc 16. Poster Commun PC17*. 2009.
1050. Bovell DL. The human eccrine sweat gland: Structure, function and disorders. *J Local Global Health Sci*. 2015;2015:1–16.
1051. Robertson J, Bovell DL. Pharmacological blockers of STIM1 inhibit increases in intracellular calcium in horse sweat gland cells. *FASEB*. 2014;28:650.

1052. Cui CY, Childress V, Piao Y, et al. Forkhead transcription factor FoxA1 regulates sweat secretion through Bestrophin 2 anion channel and Na-K-Cl cotransporter 1. *Proc Natl Acad Sci U S A*. 2012;109:1199–1203.
1053. Bilezikian JP, Loeb JN. The influence of hyperthyroidism and hypothyroidism on alpha- and beta-adrenergic receptor systems and adrenergic responsiveness. *Endocr Rev*. 1983;4:378–388.
1054. Spelta CW, Axon JE. Case series of equine pituitary pars intermedia dysfunction in a tropical climate. *Aust Vet J*. 2012;90:451–456.
1055. Stieler AL, Sanchez LC, Mallicote MF, Martabano BB, Burrow JA, MacKay RJ. Macrolide-induced hyperthermia in foals: Role of impaired sweat responses. *Equine Vet J*. 2016;48:590–594.
1056. Evans CL. Physiological mechanisms that underlie sweating in the horse. *Br Vet J*. 1966;122:117–123.
1057. Guthrie AJ, Van den Berg JS, Killeen VM, Nichas E. Use of a semi-quantitative sweat test in thoroughbred horses. *J S Afr Vet Assoc*. 1992;63:162–165.
1058. Read JR, Boston RC, Abraham G, Bauquier SH, Soma LR, Nolen-Walston RD. Effect of prolonged administration of clenbuterol on airway reactivity and sweating in horses with inflammatory airway disease. *Am J Vet Res*. 2012;73:140–145.
1059. Atria S, Carson E, Xie H, Tangjitjaroen W. Acupuncture and Chinese herbal medicine treatment of eighteen Florida horses with anhidrosis. *Am J Tradit Chinese Vet Med*. 2010;5:30–42.
1060. Mallicote M. Anhidrosis: Can acupuncture help non-sweaters? *Vet Extension Univ Fla*. 2013. https://vetmed-extension.sites.medinfo.ufl.edu/files/2013/05/Healthy-Horses-2013-Presentations.pdf.

CHAPTER 17

Disorders of the Eye and Vision

Mary Lassaline

The equine eye is unique in its large size, lateral placement, and propensity to injury relative to other large animal species, with the latter likely associated with the horse's temperament as a flight animal that relies on running as its primary defense. Examination of the equine eye can be challenging given this temperament, particularly if the eye is painful, but having the proper setting, equipment, and knowledge of normal anatomy can facilitate this sometimes difficult task.

Ophthalmology is a visual specialty with many ophthalmic diagnoses made based on visual inspection. The ability to describe what is seen during an ophthalmic examination in complete and precise terms, whether verbally or pictorially, and distill a problem list from examination findings is critical to proceed accurately with development of differential diagnoses, as well as a diagnostic and therapeutic plan. Precision in reporting the examination findings can be optimized by translating continuous or analog information into discrete findings, such as characterizing blepharospasm numerically by the percent eyelids are open, as well as grading features such as conjunctival hyperemia, chemosis, and corneal edema as mild, moderate, or severe.

Examination

The ophthalmic examination should begin in ambient light and before sedation with a "hands off" assessment of facial, orbital, and eyelid symmetry and globe position and mobility; evaluation for the presence of ocular discharge or blepharospasm; and measurement of the menace response (which requires functional cranial nerves II and VII, as well as involving the visual cortex), dazzle reflex (which requires functional cranial nerves II and VII), and direct and indirect pupillary light reflexes (which require functional cranial nerves II and III). Assessment of the pupillary light reflexes should be repeated in a darkened examination area and is most accurately performed using a bright focal light source. Ocular discharge should be characterized by its nature (i.e., serous, mucoid, mucopurulent) and by its severity. Vision can be evaluated with behavioral tests, including ability to navigate obstacles such as stepping over cavaletti and walking around jump standards without making errors. There is not a well-established standardized routine for testing vision, but minimally, vision in each eye should be tested separately with the opposite eye masked, as well as testing binocular vision. To test vision in each eye separately, one eye can be covered with a towel tucked into the halter in a way that allows the towel to be immediately pulled off the covered eye should the horse become fractious or difficult to control. The horse's willingness to proceed and ability to walk without tripping or stumbling over obstacles can provide a rough correlate with vision. It is important to perform the vision test in both ambient light and dim light to test both photopic, or cone-driven, and scotopic, or rod-driven, vision, respectively. Some horses with mild visual impairment may only show deficits (e.g., reluctance to move forward or tripping over obstacles) when moving from light to dark or vice versa.

Following the "hands off" examination, ocular structures should be examined from front to back of the eye, with sedation as needed to facilitate examination. An auriculopalpebral (AP) nerve block, which provides akinesia of the superior eyelid and therefore can facilitate forcibly opening the eyelids, is often not required if the patient is compliant and the examiner goes slowly but is indicated if the structural integrity of the globe is threatened by a deep or perforating corneal defect. The AP nerve can be palpated as it courses over the zygomatic arch in the area of the temporofrontal suture. To block the AP nerve, 1.5 mL of 2% lidocaine or mepivacaine should be injected using a 25-gauge, ⅝-inch needle over the zygomatic arch in this area. If ocular pain makes examination difficult, anesthesia should be provided, as well as akinesia, using a topical anesthetic for corneal pain and a local nerve block for eyelid pain.

Before instillation of any solution such as a topical anesthetic onto the cornea, samples for bacterial or fungal culture and measurement of aqueous tear production should be obtained. Aqueous tear production can be measured with the Schirmer tear test (STT) using commercially available test strips. Normal wetting has been considered to be 20 mm or greater in 30 seconds, with most horses wetting the entire 35-mm strip in less than 1 minute. However, there appears to be wide variability in STT values in normal horses and ponies.[1]

Intraocular pressure (IOP) measurement should be included in a complete ophthalmic examination. Two types of tonometers, applanation and rebound, are most commonly used to estimate IOP in the equine eye. Applanation tonometers measure the force required to flatten, or applanate, a precise area of the corneal surface and, making assumptions about physical features of the cornea such as thickness and curvature, convert this force to an estimate of IOP. Rebound tonometers use characteristics of the motion created when a probe is electromagnetically

propelled to come in contact with and then rebounds from the corneal surface. In one study evaluating the use of rebound tonometers to measure IOP in normal horses, values obtained with the rebound tonometer were an average of 1 mm Hg higher in horses compared with values obtained with the applanation tonometer.[2] It is important to understand the estimates calculated by these types of tonometers. The applanation tonometer displays each individual reading followed by a measure of central tendency and a measure of agreement among the readings. The rebound tonometer, in contrast, internally averages each new reading with any prior readings in a series of six consecutive readings and displays, following each of the first five readings, the running average value rather than the individual value. After the sixth reading, the lowest and highest readings are discarded, and the average of the remaining four values is displayed, or, if there is excessive deviation among measurements, a problem with probe motion, or misalignment with the central portion of the cornea, the display shows an error message. Applanation tonometry is best performed following instillation of a topical anesthetic and allows IOP measurement with the patient in any position. Rebound tonometry, in contrast, does not require the use of a topical anesthetic but does require that the tonometer probe be parallel to the horizon.

Head position can introduce artifact into IOP tonometry measurements, with higher readings obtained when the head is positioned below the heart, as with sedation, and thus care should be taken to ensure that the head is elevated above the level of the heart when IOP is being determined.[3] Sedation can lower IOP estimates, with xylazine administration associated with a decrease in tonometry readings of over 20%,[4] and detomidine administration, similarly, can be associated with a decrease in IOP readings of 15% to 20%.[5] Topical atropine administration can be associated with changes in IOP. In one study of atropine's effect on IOP in normal horses, twice-daily administration was associated with an 11% average decrease in IOP, but one horse in that study had a significant IOP increase following atropine administration.[6]

TOPICAL ANESTHETICS

Topical anesthetics typically used to desensitize the cornea before applanation tonometry, as well as before collecting samples for corneal cytology, include proparacaine and tetracaine. Proparacaine provided corneal anesthesia for about 25 minutes in horses in one study, with maximal anesthetic effect in less than 5 minutes; however, a single dose may not provide complete corneal desensitization.[7] Tetracaine decreased corneal sensitivity for 30 to 60 minutes, depending on the concentration and volume of drug administered, with a single drop of 0.5% tetracaine lasting 30 minutes, two drops of 0.5% tetracaine lasting 60 minutes, and a single drop of 1.0% tetracaine lasting 50 minutes. A maximal anesthetic effect was achieved for about 5 minutes with a single drop of 0.5% tetracaine and for over 15 minutes with two drops of 0.5% tetracaine and a single drop of 1.0% tetracaine. Taken together, these results suggest that the use of a second drop or a stronger concentration of topical tetracaine increased both the degree and duration of the maximal anesthetic effect on the equine cornea.[8] In a study evaluating the effect and duration of proparacaine and tetracaine, maximal effect occurred at 10 minutes, and duration of a viscous tetracaine solution was longer at 30 minutes than that of aqueous tetracaine and proparacaine at 20 minutes.[9]

The anterior segment of the eye, including the lens and structures anterior to it, is best examined with a bright focal light source such as a Finoff transilluminator, although a handheld slit-lamp biomicroscope can help identify and localize lesions in the anterior segment by providing magnification and depth information. The posterior segment of the eye, including the vitreous, optic nerve, retinal blood vessels, and tapetal and nontapetal fundus, should be evaluated with both indirect and direct ophthalmoscopy. Indirect ophthalmoscopy using a 15D or 20D lens and a Finoff transilluminator provides a panoramic view of the fundus, although it is viewed as if rotated 180 degrees, whereas direct ophthalmoscopy provides a view magnified 10× relative to that seen with the 20D lens, and therefore requires looking in more locations to see a similar area of the fundus. Both should be performed.

Mydriasis is recommended for a better view of structures posterior to the iris but should not be performed if glaucoma is suspected or has been diagnosed or if lens instability or luxation is suspected or has been diagnosed. Owner consent should be obtained before instillation of a mydriatic agent to ensure that the owner gives permission to dilate pupils, as well as understands the anticipated duration of mydriasis and potential altered depth perception and visual impairment associated with mydriasis. If the owner declines to allow the pupils to be dilated, it should be made clear that lens and posterior segment examination will be limited. A short-acting mydriatic such as 1% tropicamide should be used instead of a more potent drug such as 1% atropine, which can result in mydriasis for at least 2 weeks in normal horses.[10]

Corneal aesthesiometers such as the Cochet-Bonnet can be used to estimate corneal sensitivity by measuring the threshold pressure required to stimulate corneal sensory nerve endings and thereby induce the corneal reflex, which typically results in a blink, but may also result in retraction of the globe and elevation of the third eyelid. Corneal sensitivity in horses decreases with age and in horses with pituitary pars intermedia dysfunction (PPID). Because decreased corneal sensitivity is associated with impaired wound healing, increasing age and PPID may increase the risk for nonhealing or recurrent corneal ulcers in horses.[11] One study that established reference ranges for corneal touch threshold in the horse found that the axial cornea is the most sensitive and found a negative correlation between age and corneal sensitivity in the axial, inferior, and temporal cornea.[12] Corneal sensitivity is also decreased in sick neonatal foals relative to healthy foals and adults.[13]

IMAGING

Ocular ultrasound is indicated for evaluation of intraocular contents when one or more of the ocular transmitting media are opaque, including opacification of the cornea, aqueous and vitreous humors, and the lens. The most common indications for ocular ultrasound are in eyes with a cataract to evaluate for a retinal detachment, after traumatic hyphema to assess posterior segment damage, or in eyes with severe corneal opacification. In addition, ocular ultrasound can be used to evaluate the orbit in instances of exophthalmos or orbital trauma. Ultrasound biomicroscopy can be used to determine depth of a corneal lesion such as a squamous cell carcinoma or to examine an anterior uveal mass for extent of involvement. Ocular ultrasound should not be performed if the structural integrity of the globe is suspect, because of the risk of iatrogenic globe

rupture and expulsion of intraocular contents caused by pressure on a fragile or perforated globe.

DISORDERS OF THE EQUINE EYELIDS

Normal Anatomy

Sensory innervation of the equine eyelids is provided by the supraorbital (frontal), infratrochlear, lacrimal, and zygomatic branches of the trigeminal nerve (cranial nerve V).

Entropion

The most common eyelid disorder in foals is entropion, which is inward rolling of the eyelid margin. If entropion is severe enough that facial hairs contact the cornea, corneal pain and ulceration can result. Entropion may be the result of a primary conformational abnormality or occur secondary to prematurity or dehydration in foals with enophthalmos or ocular pain resulting in spastic entropion. Manual reduction and frequent topical ocular lubrication with artificial tear ointment to prevent corneal ulceration may be the only treatment required in some foals, particularly if entropion is secondary to dehydration, and will self-correct when the systemic condition resolves. Severe or persistent entropion may be temporarily corrected by placement of three vertical mattress imbricating sutures to position the eyelid correctly. Sedation and local block is typically sufficient for suture placement. Overcorrection should be avoided to prevent the inability to close the eyelids during blinking. Primary (anatomic) entropion requiring permanent surgical correction involving skin excision is rare.

Ectopic Cilia

An ectopic cilium, an aberrant eyelash that grows through the conjunctiva directly toward the cornea, should be considered as a differential diagnosis for horses with epiphora, blepharospasm, and keratitis and in whom no foreign object can be found. In a series of seven horses with ectopic cilium, a single translucent cilium was detected in the upper eyelid palpebral conjunctiva, emerging from within a small focal region of conjunctival pallor approximately 5 mm from the eyelid margin. Corneal ulceration was not observed in any of these horses, and most responded well to transconjunctival surgical excision.[14]

Distichiasis

Distichiasis, single or multiple cilia emerging from meibomian gland openings along the eyelid margin, should be considered as a differential diagnosis for horses with chronic or recurrent ulcerative or nonulcerative keratitis. Distichia may be soft and of little clinical significance or stiff, causing corneal pain and ulceration. Treatment of distichiasis has been well described and includes epilation, cryoepilation, and tarsal plate excision. There is a breed predisposition for distichiasis in the Friesian horse, and therefore careful examination of the eyelid margins should be performed in Friesians with keratitis.[15,16]

Laceration

Eyelid lacerations should be repaired immediately, with primary closure if possible. The vascular supply to the eyelid is extensive, and many apparently avascular segments of eyelid recover after repair. Amputating pedicles of eyelid tissue should be avoided to preserve the eyelid margin and prevent subsequent corneal vascularization and ulceration associated with eyelid margin loss. Eyelid lacerations should be repaired using two-layer closure, ensuring accurate anatomic apposition of the wound edges and eyelid margin. Minor surgical repairs can be performed using sedation and local nerve blocks, but general anesthesia is warranted if the injury is severe. Postoperative therapy should include a systemic nonsteroidal antiinflammatory drug (NSAID) to reduce pain and inflammation, a systemic antibiotic to prevent infection, and tetanus toxoid. Topical medication is not required for eyelid injuries unless corneal or anterior segment damage accompanies the injury, and administration of topical ophthalmic medications may actually disrupt the laceration repair. A modified Kuhnt-Szymanowski procedure has been used to correct cicatricial ectropion resulting from removal of eyelid tissue during an initial eyelid laceration repair.[17]



FIG. **17.1** Squamous cell carcinoma (SCC) of the nictitans (third eyelid). This raised rough mass with a cauliflower-like appearance is typical for the appearance of SCC of the nictitans.

Squamous Cell Carcinoma

The most common neoplasm of the equine eyelid is squamous cell carcinoma (SCC) (Fig. 17.1). In addition, sarcoid, melanoma, mast cell tumor, lymphosarcoma, basal cell carcinoma, and papilloma can affect the eyelids. The differential diagnosis for eyelid neoplasms includes parasitic diseases such as ocular habronemiasis and other causes of granulomatous skin disease. Treatment of eyelid neoplasia depends on the location, size, tumor type, age and purpose of the horse, cost, surgical skill, and equipment available. Treatment modalities include surgical excision, radiation therapy, intralesional chemotherapy, hyperthermia, immunotherapy, cryosurgery, or a combination of these. The aim of therapy is to eliminate or halt progression of the tumor while maintaining eyelid function and preserving the eye and vision. Complete excision with primary closure is the optimal treatment but often cannot be achieved because of limitations on availability of tissue for reconstruction and the extensive and aggressive nature of many eyelid neoplasms. If eyelid margins cannot be apposed after tumor excision, more involved blepharoplastic techniques, such as advancement skin flaps, may be indicated. SCC involving the eyelid is usually erosive and ulcerative, and invasion of adjacent soft and bony orbital tissue is possible if the disease is untreated.

Cisplatin is a platinum-based chemotherapy drug that binds to and causes crosslinking of deoxyribonucleic acid (DNA), which triggers apoptosis of tumor cells. It is most commonly used intralesionally, in conjunction with surgical excision for larger tumors. A large retrospective study of 573 equids with 630 cutaneous tumors over a 10-year period confirmed the value of intratumoral chemotherapy with cisplatin with local control at 4 years in 88% of 151 tumors histologically confirmed as SCC.[18] In that study, large tumors, tumors with gross residual disease following surgical excision and debulking, and tumors that had been treated previously with another treatment modality had the lowest treatment efficacy. In treated patients, local reactions were most likely after the third and fourth treatments. The therapeutic protocol used in the Theon study[18] involved a series of four percutaneous intratumoral injections of a viscous slow-release 3.3-mg/mL cisplatin solution suspended in sesame oil to provide slow drug release, given at 2-week intervals, with 1 mg of cisplatin injected per cubic centimeter of tumor tissue. Cisplatin-containing biodegradable beads have been used to treat cutaneous SCC,[19] but these can cause adverse effects on the cornea associated with high local concentrations of the drug. The beads contain approximately 1.6 mg of cisplatin per bead, and placement of beads with 2-cm spacing between beads theoretically results in cisplatin concentrations comparable to injection at 1 mg/mL tissue.

Photodynamic therapy (PDT) involves the use of photochemical reactions mediated through the interaction of photosensitizing agents, light, and oxygen. Tumor tissue is injected with verteporfin, and laser radiation is applied. This induces oxidative damage to the microvasculature, contributing to ischemic tumor death. In one study of 10 horses with periocular SCC treated with excision and PDT, no horses demonstrated tumor recurrence with a minimum of 25 months of follow-up.[20]

Sarcoid

Equine sarcoid is the most common skin tumor in horses and can provide a significant therapeutic challenge, whether surgical or medical management is used. Periorbital sarcoids can be particularly difficult to treat given the importance of preserving eyelid function for maintaining corneal health and given the thin elastic nature of eyelid skin. Six types of sarcoid, have been described: occult, verrucose, nodular, fibroblastic, mixed, and malignant. In a review of 445 cases of sarcoid over the 25-year period from 1974 to 1999, treatment with radiation resulted in the best outcome, with almost 100% resolution in 66 cases, although treatment with bacillus Calmette-Guérin (BCG) immunomodulation for fibroblastic and nodular sarcoids was associated with good outcomes in 69% of 300 cases. Of the 42 cases that received no treatment, 27 had lesions too extensive to treat at initial referral and were euthanized. Recurrence rates for other therapeutic modalities are as follows, in order of increasing recurrence rates: gamma radiation brachytherapy, 53 cases, 8% recurrence; topical 5-fluorouracil cream application, 9 cases, 33% recurrence; AW4 topical treatment (unlicensed material containing 10% 5-fluorouracil and oil of rosemary), 146 cases, 65% recurrence; cisplatin injection, 18 cases, 66% recurrence; surgical excision alone, 28 cases, 82% recurrence; cryosurgery, 23 cases, 91% recurrence.[21] However, in a more recent retrospective study evaluating the long-term outcome of intratumoral chemotherapy with cisplatin for cutaneous tumors in horses, cure rate was 96% with a minimum follow-up of 2 years after one course of treatment with four injections for 409 sarcoids.[18]

DISORDERS OF THE EQUINE CONJUNCTIVA AND THIRD EYELID

Normal Anatomy

The conjunctiva is divided into bulbar and palpebral surfaces. The conjunctiva merges with the cornea at the limbus, with the eyelid at the eyelid margin, and the bulbar and palpebral conjunctiva join to form the fornix. In general, isolates of gram-positive organisms are more common than gram-negative organisms from normal equine conjunctiva, but fungi are commonly isolated as well.

Dermoid

Conjunctival dermoid is one of the most common congenital ocular abnormalities in horses but congenital conjunctival diseases are rare in horses. A dermoid is a congenital mass of skin and associated glands, hair, and hair follicles that can affect the cornea and adjacent conjunctiva. A dermoid that is irritating the globe or adnexa should be surgically removed by superficial keratectomy or conjunctivectomy.

Foreign Body

Conjunctival foreign bodies should be considered in any horse with a recurrent corneal erosion, ocular pain, or conjunctivitis and can be detected by examination of the superior and inferior fornices and the bulbar and palpebral surface of the nictitans after topical administration of 0.5% proparacaine. Magnification may be necessary for foreign body detection. In addition, irrigation of the nasolacrimal system may yield foreign material associated with chronic conjunctivitis or dacryocystitis.

Parasitic Disease

Parasites of the equine conjunctiva include *Thelazia lacrymalis, Habronema* spp., and *Onchocerca cervicalis.* Although parasitic infection of the conjunctiva is not typically associated with clinical signs, chronic conjunctivitis, seromucoid discharge, and conjunctival nodules can occur. Treatment includes removal of the adult worms, irrigation of the nasolacrimal system, topical corticosteroids to reduce inflammation, and fly control. Ocular manifestations of equine cutaneous habronemiasis such as intense pruritus, epiphora, and yellow caseous "sulfur granules" result when the house or stable fly deposits the larvae of the *Habronema* spp. on or around the eye. Ocular lesions of habronemiasis are most common at the medial canthus and may involve the skin, nasolacrimal duct, third eyelid, or conjunctiva. Treatment may involve excision of sulfur granules, local and systemic corticosteroid therapy, and systemic ivermectin. The aim of therapy is to kill the larvae while controlling the resulting inflammation. Fly control is an essential part of an overall management program.

The blood-sucking midge *Culicoides nubeculosus* transmits *O. cervicalis* microfilariae. After transmission the immature microfilariae migrate through lymphatics and can travel to ocular and adnexal tissues. The prevalence of equine onchocerciasis increases with age, with an overall prevalence of 50% to 60% in horses in the southeastern and midwestern United States,[22] and with a prevalence on histologic examination of the lateral bulbar conjunctiva of 10.8% in a study of

368 equine eyes.[23] The pathogenesis is thought to involve the death of the microfilariae, release of antigens, and development of hypersensitivity in susceptible horses. Treatment is indicated in those horses with active conjunctivitis, keratitis, or uveitis attributable to onchocerciasis and includes antiinflammatory agents such as topical corticosteroids and systemic flunixin meglumine and ivermectin.

Squamous Cell Carcinoma

SCC is the predominant tumor type of the third eyelid (Fig. 17.2). Other neoplasms affecting the conjunctiva and third eyelid include lymphosarcoma, melanocytoma, mastocytoma, hemangioma, and angiosarcoma. Complete surgical excision of the third eyelid can be curative for tumors that have not extended beyond the third eyelid. Surgical excision can typically be performed using sedation and local nerve blocks, without suturing the bulbar and palpebral conjunctiva closed. A rare complication of third eyelid removal without suturing the surgical incision is orbital fat prolapse, which is easily repaired by excising the prolapsed fat, creating a fresh surgical conjunctival margin with sharp dissection, and suturing the conjunctival margin closed. In one case series including 50 horses with third eyelid neoplasia, there was recurrence in 20%, occurring at a median of 1.5 years after excision, suggesting that long-term monitoring is important for all patients in which third eyelid excision is performed.[24]



FIG. **17.2** Third eyelid SCC that is more extensive. This mass was better visualized by retropulsing the globe to elevate the third eyelid.

DISORDERS OF THE EQUINE NASOLACRIMAL SYSTEM

The nasolacrimal system consists of the superior and inferior puncta and canaliculi, nasolacrimal sac, nasolacrimal duct, and nasal punctum located on the floor of the nasal vestibule. The nasolacrimal system carries tears from the medial canthus of the eye to the nasal vestibule. Abnormalities of the nasolacrimal system result in epiphora, an overflow of tears, or ocular discharge that can be serous, mucoid, or mucopurulent and must be differentiated from reflex lacrimation resulting from ocular irritation or inflammation. The Jones test evaluates nasolacrimal duct patency by instillation of fluorescein stain in the conjunctival cul-de-sac and observation of its appearance at the nasal opening. Nasolacrimal duct irrigation can be performed in an anterograde manner, from the eyelid to the nasal punctum, or retrograde, from the nasal punctum. Topical anesthetic should be used before nasolacrimal duct irrigation. Radiographic imaging of the nasolacrimal duct, dacryocystorhinography, can be performed using contrast material injected into the duct from the superior eyelid punctum. Lateral and oblique radiographic views provide the best visualization of the nasolacrimal duct. Indications for dacryocystorhinography include chronic epiphora, inability to irrigate the nasolacrimal duct, suspicion of a nasolacrimal foreign body, and evaluation of the nasolacrimal duct for congenital or acquired abnormalities. The nasolacrimal duct can also be imaged using computed tomography (CT).

Abnormalities of the nasolacrimal system can be congenital or acquired. In the horse the most common congenital abnormality is atresia of the distal portion of the nasolacrimal duct and the nasal punctum, which results in mucoid epiphora at 3 to 4 months of age. Additional congenital abnormalities include atresia of an eyelid punctum, abnormal placement of an eyelid punctum, and multiple nasal openings. Whether these conditions are inherited is unknown. Acquired conditions of the nasolacrimal system include dacryocystitis, foreign body obstruction, trauma, and involvement secondarily in neoplastic or inflammatory conditions of the medial canthus or nasal passages. Dacryocystitis appears as a mucopurulent ocular discharge without associated ocular inflammation. Dacryocystitis and foreign body obstructions are treated by nasolacrimal irrigation and bacterial and fungal culture with or without the aid of radiographic or CT evaluation. Topical broad-spectrum ophthalmic antibiotic solution should be administered after irrigation to prevent infection, and topical corticosteroids or systemic NSAIDs may reduce swelling and inflammation.

Surgical repair of the nasolacrimal duct involves surgical trephination (conjunctivorhinotomy) to create a new outflow pathway for the tears. After a new duct is created, a stent connecting the eyelid and nasal puncta is kept in place for several weeks postoperatively to keep the repaired nasolacrimal duct patent until the nasal mucosa completely lines it. These stents can become dislodged, resulting in obstruction of the repaired duct. Canaliculosinostomy to divert lacrimal secretions into the sinuses, which can be performed in the standing sedated horse, can improve epiphora caused by nasolacrimal duct obstruction and can provide a good functional and cosmetic outcome.[25]

DISORDERS OF THE EQUINE CORNEA

Normal Anatomy

The cornea consists of the epithelium, stroma, Descemet's membrane, and endothelium. The epithelium is 7 to 15 cells thick and is replaced every 7 to 10 days. The corneal endothelium is a monolayer of cells with little to no regenerative capacity, and therefore damage to the endothelium is of great significance, because complete repair is often not possible and results in permanent corneal edema. The normal cornea is transparent and avascular. Sensory innervation comes from the ophthalmic branch of the trigeminal nerve. The epithelium and anterior stroma are innervated richly with sensory

nerves, whereas the middle and inner cornea is less well supplied. Nutrition and waste removal are carried out by the tear film, aqueous humor, and diffusion to and from the scleral and conjunctival blood vessels. The cornea is maintained in a state of relative deturgescence by the mechanical barrier of the epithelium and the endothelial pump mechanism.

Changes in Corneal Clarity

The normally clear cornea can become opaque and take on a color that correlates with the mechanism underlying the corneal opacity, including white with fibrosis, yellow with a cellular infiltrate, brown with pigment, red with corneal vascularization, sparkling with lipid or mineralization, or blue with corneal edema. Corneal edema results when either the epithelial barrier or endothelial pump function is disrupted. Chronic irritation of the cornea results in superficial vascularization, whereas inflammation of the anterior uvea results in deep corneal vascularization. Corneal pigmentation often follows vascularization, as well as deep corneal infection, although this is not as common in horses as in dogs and cats. Cellular infiltration of the cornea occurs in neoplastic, infectious, and inflammatory disease. All of these processes can occur alone or in combination, resulting in an alteration of corneal transparency. In addition, scar formation or deposits of mineral or phospholipid can alter corneal transparency Establishing and eliminating causes of these changes are essential.

Corneal Ulcer

A corneal ulcer is a break in the corneal epithelium. Clinically, ulceration results in lacrimation, blepharospasm, photophobia, conjunctival hyperemia, corneal edema, and possibly miosis and aqueous flare. A corneal ulcer is diagnosed by positive fluorescein staining of the cornea, although corneal ulcers that involve a complete loss of the corneal epithelium and stroma, called *descemetoceles*, do not take up fluorescein stain (Fig. 17.3). Fluorescein stain is absorbed by the water-soluble corneal stroma but not the corneal epithelium or Descemet's membrane, giving a descemetocele its characteristic "green donut" appearance, with a ring of stain-positive stroma surrounding a dark center. Corneal ulcers should be characterized by size, depth, the presence or absence of corneal cellular infiltration, and signs of reflex anterior uveitis such as aqueous flare and miosis.

Uncomplicated Corneal Ulcers

Treatment of an uncomplicated corneal ulcer involves controlling pain and inflammation, eliminating or preventing infection, and preventing secondary complications. Healing occurs by migration and mitosis of the adjacent epithelial cells and, depending on the size of the ulcer, should be complete in 2 to 6 days. Common treatment for an uncomplicated corneal ulcer includes a topical broad-spectrum antibiotic, topical atropine to control reflex uveitis, and a systemic NSAID.

Nonhealing Ulcers

Corneal ulcers that have not healed within 7 to 10 days are generally classified as nonhealing. Causes of persistent ulceration, including infection and continued mechanical trauma, need to be ruled out before classification as nonhealing. Histologically, characteristics of nonhealing corneal ulcers in horses are similar to those in other species and include epithelial nonadherence and dysmaturity, as well as mild to moderate stromal inflammation; however, the anterior stromal acellular hyaline zone commonly found in canine spontaneous chronic corneal epithelial defects (SCCEDs) is not consistently found in horses with nonhealing ulcers.[26] The term *indolent ulcer* is usually reserved for SCCEDs in humans and dogs.



FIG. **17.3** Descemetocele with diffuse corneal edema, corneal vascularization, and hypopyon.



FIG. **17.4** Oblique corneal laceration resulting in a flap of cornea.

Medical therapy for uncomplicated ulcers is unlikely to lead to resolution of nonhealing ulcers without some additional intervention. In one case series of 23 horses with superficial nonhealing ulcer, complete reepithelialization of the cornea following a procedural intervention such as debridement, grid keratotomy, or superficial keratectomy in addition to medical therapy occurred in approximately 2 to 3 weeks.[27] Another case series of 60 horses with nonhealing ulcers of approximately 1 month's duration suggested that diamond burr debridement may be an effective treatment for nonhealing ulcers, with 92% achieving complete reepithelialization within an average of 16 days.[28]

Complicated Ulcers

Complicated corneal ulcers are those that fail to heal in an appropriate time, are secondarily infected, have an ongoing source of irritation or ulceration, have a collagenase component, are associated with corneal vascularization, or are worsening despite appropriate treatment (Figs. 17.4–17.12).

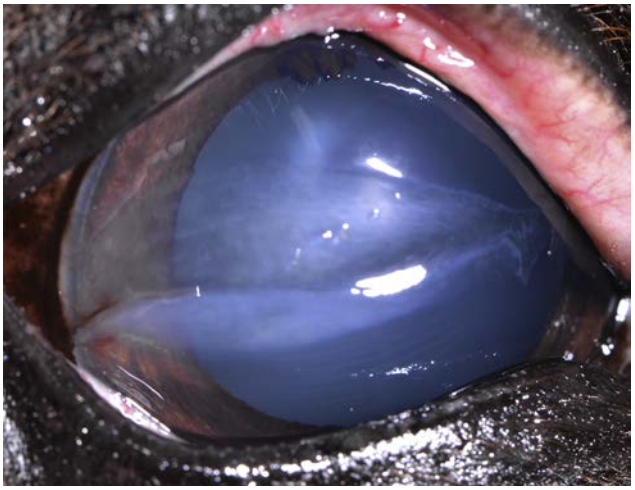


FIG. **17.5** Horizontal corneal laceration associated with corneal edema.

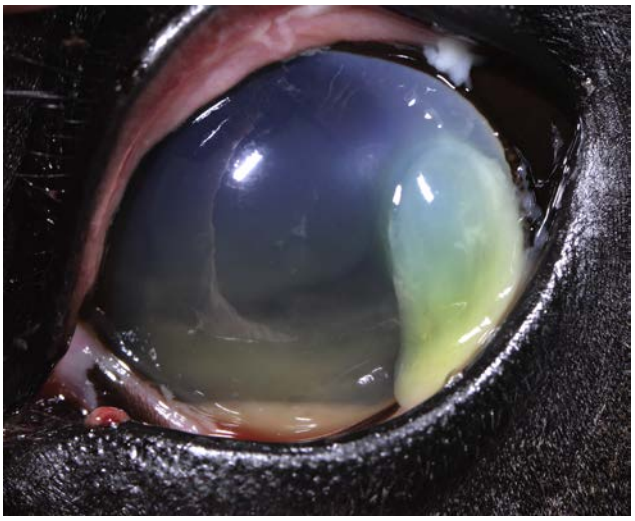


FIG. **17.6** "Melting" corneal ulcer with severe keratomalacia. This corneal ulcer should be viewed as an emergency and treated empirically until diagnostic results are available.



FIG. **17.7** Melting corneal ulcer in a foal. These often go undetected because foals with melting ulcers do not always show signs of ocular discomfort until quite late in the disease course, when the ulcer may have become full thickness.

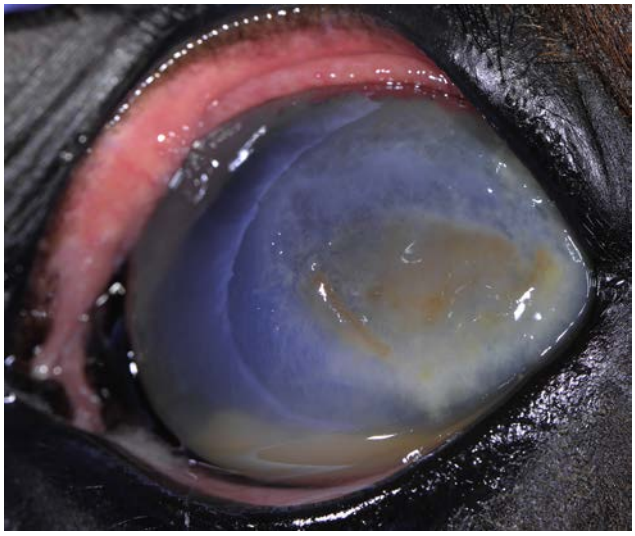


FIG. **17.8** Fungal keratitis from which a dematiaceous fungal organism was cultured. These fungal organisms have pigmented hyphae and appear pigmented clinically. Dematiaceous fungi isolated from the equine cornea include *Curvularia* and *Cladosporium*.

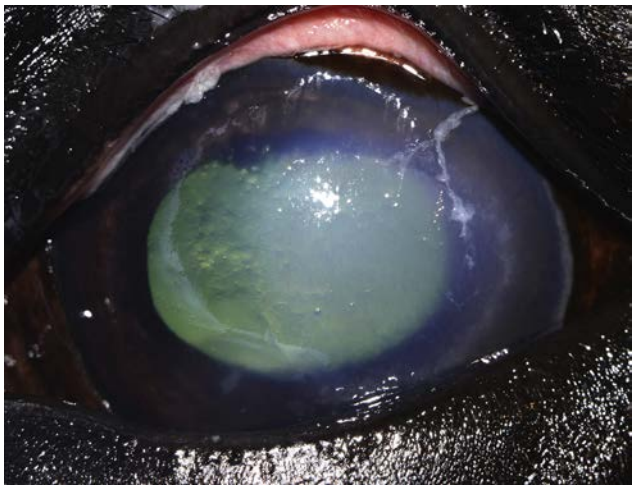


FIG. **17.9** Early fungal keratitis, characterized by only mild corneal edema but often accompanied by severe ocular pain and a rough gritty appearance to the cornea.

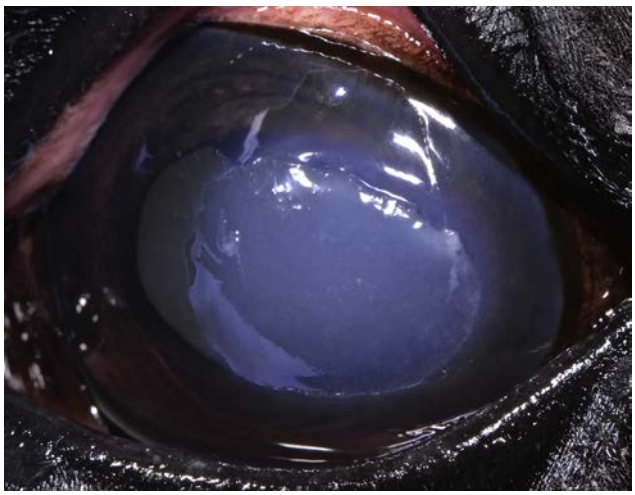


FIG. **17.10** Progression of fungal keratitis from Fig. 17.9, in which a plaque has formed over the ulcerated area. Such plaques are characteristic of fungal keratitis.

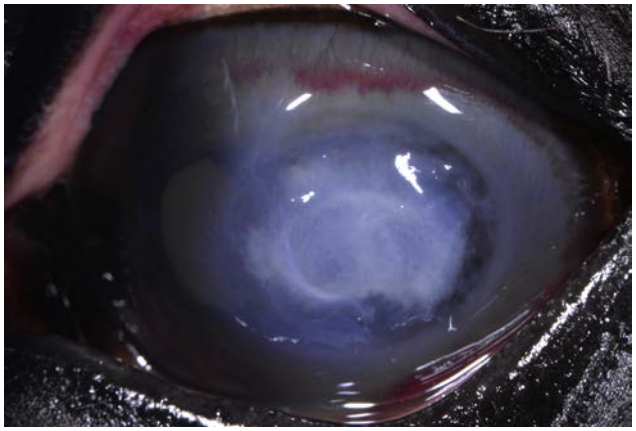


FIG. **17.11** Progression of fungal keratitis from Figs. 17.9 and 17.10, in which a deep groove has developed in the temporal periphery of the corneal ulcer.



FIG. **17.12** Progression of fungal keratitis from Figs. 17.9–17.11, in which a full-thickness perforation has developed. Ultimately, this patient underwent a corneal transplant and conjunctival graft that restored the structural integrity of the globe, and some vision was retained.

Secondary infection of a corneal ulcer is suggested by increasing pain, corneal edema, interstitial keratitis associated with an increase in stromal inflammatory cells, corneal vascularization, purulent discharge, severe anterior uveitis, and stromal necrosis and liquefaction. Diagnostics, including corneal bacterial and possibly fungal culture and corneal cytology, should guide medical therapy. In many instances complicated corneal ulcers require frequent and prolonged therapy, and placement of a subpalpebral lavage system may be indicated to ensure adequate treatment. Many complicated corneal ulcers require a combination of surgical and medical therapy to improve the likelihood of a successful outcome. In vivo confocal microscopy may provide a diagnosis that is not available using more routine diagnostics such as corneal culture and cytology.[29] Medical therapy of a complicated ulcer typically involves treatment with a topical antimicrobial, antifungal, anticollagenase, and atropine, as well as a systemic NSAID.

Antibiotics

Initial choice of antimicrobial therapy can be guided by cytology if bacterial rods or cocci are observed. If no bacteria or bacterial rods are seen, a second-generation fluoroquinolone such as ofloxacin is typically a good choice to treat for a possible *Pseudomonas* infection. A cephalosporin such as cefazolin can be added to treat for possible infection with a beta-hemolytic *Streptococcus* spp. if cocci are observed. Evaluating the transcorneal penetration of a drug can help estimate a drug's potential to reach its target corneal tissue. Many ophthalmic antibiotics have poor penetration through an intact corneal epithelium, but this is not an issue when treating an infected corneal ulcer. Second-generation fluoroquinolones such as ciprofloxacin and ofloxacin generally have good in vitro activity against common equine corneal pathogens. Ofloxacin and the fourth-generation fluoroquinolones such as moxifloxacin tend to have better in vitro activity against gram-positive pathogens. Moxifloxacin has better corneal penetration than ciprofloxacin.[30] In a retrospective study of bacterial ulcerative keratitis in 65 horses, *Pseudomonas aeruginosa* was the most common isolate (22%), followed by *Streptococcus equi* subspecies *zooepidemicus* (20%), and there was increased resistance of *Pseudomonas* spp. to both gentamicin and tobramycin over the study period.[31]

Antifungals

Natamycin is the only commercially available antifungal approved by the U.S. Food and Drug Administration for ophthalmic use. All other antifungal drugs must be compounded for ophthalmic use. In horses, voriconazole has established absorption into the aqueous humor and plasma[32] and has been shown to be well tolerated when administered both subconjunctivally[33] and intrastromally,[34] as well as topically. In one study of antifungal susceptibility of commonly isolated equine corneal fungal pathogens using banked isolates from horses with ulcerative fungal keratitis in Florida, Missouri, Tennessee, and Georgia, 14 fungal isolates, 8 *Aspergillus* and 4 *Fusarium* spp., were collectively significantly more susceptible to voriconazole than to four other antifungal agents (natamycin, itraconazole, fluconazole, and ketoconazole). In that study, *Aspergillus* spp. were most susceptible to voriconazole, miconazole, and itraconazole. *Fusarium* spp. were most susceptible to natamycin and voriconazole.[35] Silver sulfadiazine proved inhibitory and fungicidal for 17 equine corneal fungal isolates in another study.[36]

Anticollagenases

The corneal stroma can degrade rapidly, or "melt," in horses with corneal ulcers. This stromal collagenolysis is caused by proteolytic enzymes produced by microorganisms, inflammatory cells, corneal epithelial cells, and fibroblasts. There are two main enzyme families involved in corneal stromal degradation: matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9, and serine proteases such as neutrophil elastase. MMP-2, synthesized by corneal keratocytes, is involved in routine repair of damaged collagen molecules, whereas MMP-9, produced by corneal epithelial cells and polymorphonuclear neutrophils, is involved in repair following corneal wounding. Protease inhibitors such as serum, which inhibits both matrix metalloproteinases and neutrophil elastase, can reduce the progression of corneal stromal ulcers, speed healing, and minimize scarring.

Several studies provide empiric support for the use of anticollagenases in the treatment of complicated corneal ulcers in horses. First, in an in vitro study of the effect of anticollagenases on MMP activity, a significant reduction in the in vitro

activity of equine MMPs was observed following treatment with several anticollagenases, including EDTA, doxycycline, *N*-acetylcysteine, and equine serum.[37] Second, in a study of the utility of oral treatment of horses with melting corneal ulcers with the antibiotic doxycycline, which has anticollagenase activity in addition to its antibiotic activity, oral doxycycline was found to be present in the preocular tear film of eight normal broodmares given 20 mg/kg of doxycycline orally once daily. This dose of doxycycline was well tolerated by horses in the study, suggesting that it may be a useful treatment for horses with melting corneal ulcers.[38]

Systemic NSAIDs

Ocular pain associated with corneal ulcers may be alleviated by systemic administration of NSAIDs that also help control ocular inflammation, but systemic NSAID administration can be associated with undesirable effects in horses, including renal and gastrointestinal tract toxicoses. To address concerns regarding the development of NSAID-associated renal toxicity, serum creatinine concentration should be measured in horses treated with systemic NSAIDs to obtain a baseline value and then monitored for the development of azotemia. It is also important to monitor horses for the development of right dorsal colitis associated with systemic NSAID use. A drop in total protein will often occur before the development of any clinical signs such as loose manure, inappetance, or weight loss in horses with right dorsal colitis, and therefore monitoring hematocrit and total protein can enable early detection of protein loss. If azotemia or hypoproteinemia is attributed to NSAID toxicity (i.e., based on additional diagnostics including urinalysis for renal toxicity and abdominal ultrasound for right dorsal colitis), systemic NSAID therapy should be discontinued to avoid permanent damage to the kidneys or gastrointestinal tract.

Topical administration of NSAIDs may reduce pain and inflammation associated with keratitis; however, toxic effects on corneal epithelial cells and prolonged healing times and even corneal malacia can result when used in the face of corneal ulceration. Topical treatment with opioid agonists-antagonists such as morphine and nalbuphine has been suggested to reduce corneal pain without interfering with corneal wound healing and to avoid the complications associated with systemic NSAID administration, but the efficacy of these drugs at reducing corneal pain in horses has not been evaluated. One study evaluating nalbuphine used topically on normal equine corneas found that it had no effect on corneal sensitivity, and thus although it appeared to cause no harm it is unlikely to provide any pain relief. Therefore its use is not recommended.[39]

Subpalpebral Lavage Catheters

A subpalpebral lavage (SPL) catheter is a convenient method of delivering medications in solution form to the equine ocular surface that is most useful when the horse is difficult to treat, treatment will be long term, the lids or cornea are very fragile, or the frequency of treatment is high. If placed incorrectly, however, corneal ulcers can result. A common reason for referral for horses with corneal ulcers is inability to administer topical medication. If there is any question that topical medications can be successfully administered without an SPL, one should be placed.

Commercial SPL kits are available through MILA International, Inc. These come equipped with a 12-gauge needle and tubing that is either 36 or 60 inches long. The shorter tubing may be insufficient to reach the withers in a larger horse, and it can be difficult to reach the injection port to administer medications when the shorter tubing is used in a patient that elevates the head and neck in anticipation of treatment. The SPL may be placed either in the superior or inferior lid.[40] Temperament of the horse may contribute to a decision to place the SPL in the inferior lid, in patients for whom it would prove challenging to replace any of the butterfly sutures holding the SPL tubing in place on the face, because of difficulty handling or restraining such a patient. An example might be a foal that has not been frequently handled, is not halter-broken, and might be unduly stressed by resuturing the tubing to the face. If the butterfly suture breaks in an inferior-lid SPL, there is little risk of the SPL footplate slipping and causing an SPL ulcer. If a suture breaks in a superior lid SPL, however, footplate migration onto the corneal surface can easily cause an ulcer that can be difficult to resolve. One study summarizing data from 135 horses treated with SPLs suggested that complications may be more frequent when SPLs are placed in the lower rather than the upper lid, although complication rates reported in this study were high overall.[41] If properly placed and monitored, complications are unlikely to occur. Adequate sedation is mandatory to place an SPL. It is typical for a higher dose of sedative than is expected to be required to achieve adequate sedation because of most patients' intense dislike of eyelid manipulation and of finger insertion between an eyelid and a painful cornea.

To place an SPL, an auriculopalpebral block should be performed both for an inferior SPL as well as a superior one, to paralyze the orbicularis muscle and thereby prevent strong blepharospasm from compromising access to the conjunctival fornix. For a superior SPL, a supraorbital block of the frontal nerve will provide anesthesia of the medial upper lid and may completely desensitize the intended SPL trochar site; however a local block may be necessary as well. For an inferior SPL, a local block at the intended SPL trochar site using 1 to 1.5 mL of lidocaine is required. Before inserting the SPL trochar through the eyelid, the cornea and conjunctiva should be desensitized with a topical anesthetic; the lid skin, cornea, and conjunctival surface prepared with dilute Betadine solution; and the adequacy of sedation and local anesthesia tested by inserting a finger under the lid through which the SPL will be placed and pushing down on that finger, through the lid, with a finger on the opposite hand. If the patient tolerates this, insertion of the SPL trochar will most likely be easily tolerated as well. Wearing sterile gloves, the SPL trochar should be held in the dominant hand and the opposite hand used to open the eyelid as needed to allow insertion of the SPL trochar, guided by the index finger, to the conjunctival fornix. It is mandatory that the trochar pass through the eyelid at the depth of the conjunctival fornix, no closer to the lid margin, to prevent footplate movement over the cornea and subsequent corneal ulceration. Inserting the trochar through the skin typically requires a strong effort on the part of the veterinarian. After the tubing is drawn the remainder of the way through the eyelid, one butterfly should be placed about 25 mm from the wound where the SPL tubing exits the skin and a single interrupted suture using 2-0 nonabsorbable suture placed through the butterfly tape on either side of the tubing. The butterfly must be well adhered to the tubing, so that the tubing does not slip toward the lid, dislodging the footplate. A second butterfly should be placed about 25 mm down the tubing from the first, as a backup in case the first breaks. The SPL tubing should

then be woven through a braid in the forelock to prevent slippage over the ear, and through several braids along the mane to secure it to the neck. A 20-gauge catheter should be inserted into the distal end of the SPL tubing by backing the stylet out of the catheter just a small distance, to prevent lacerating the SPL tubing with the stylet, and inserting the catheter into the tubing up to the catheter hub and then removing the stylet. The catheter provides stability for the end of the tubing and a place to affix an injection port to allow injection of ophthalmic solutions into the system. The patency of the SPL should be tested by injecting sterile saline or eyewash slowly through the injection port and tubing until it appears through the palpebral fissure and runs down the face. The SPL should be checked every time medication is injected through it to ensure that the tubing has not slid through the butterfly, allowing the footplate to rub on the cornea, and to ensure that medication is directed through the tubing onto the cornea rather than being injected subconjunctivally.

The volume of medication injected into the catheter is 0.2 mL of medication per dose. There are different methods of administering medications through the SPL, each with advantages and disadvantages. First, each dose of medication may be followed with 1 mL of air, to clear the tubing and deliver undiluted medication to the ocular surface. The advantage of this pattern of medication administration is that it gives the eye time to absorb the first medication before it receives any additional medication, and medications do not sit in the tube where they could become inactivated by mixing with other medications, heat, or light. Alternatively, each dose of medication may be delivered without flushing the tubing with air. The advantage of this method is that air is not blown on the corneal surface. There is some evidence that mixing medications, and that leaving medications in the tubing rather than flushing them out, may not change their efficacy.[42,43] Regardless of the method chosen, at least 5 minutes should elapse between the administration of each medication if medications are injected individually. Continuous rate infusion is another alternative.[44]

With a sensible patient, it is reasonable to allow small paddock turnout even with the SPL in place, rather than restricting activity to stall rest with hand walking as is typical for more rambunctious patients, but only with willingness to accept the risk that the SPL will be damaged or dislodged and may need to be replaced (e.g., if the patient rolls and breaks the tubing or catches it on a fence or foot). Placing a hard plastic cup mask on the horse to prevent self-trauma to the lavage system is preferred by some veterinarians, and these masks can be invaluable for protecting the SPL. However, hard cup masks can also discourage visual inspection of the eye and the SPL; provide a damp, dark, warm environment that may not be optimal for healing; and encourage development of a moist dermatitis where the hard cup contacts the skin. Additional options for protecting the SPL system include Lycra racing blinkers and Lycra hoods designed to protect the mane and keep the SPL sutures and tubing protected. Horses with corneal ulcers are unlikely to rub the eye unless there is an additional discomfort such as a poorly fitted hard cup mask or SPL footplate that has slipped onto the cornea.

Friesian Stromal Loss

A syndrome of corneal stromal loss has been described in nine Friesian horses (Fig. 17.13). This syndrome is unusual in its lack of accompanying reflex uveitis, as well as in its bilaterally symmetric location, which is typically inferiotemporal.



FIG. **17.13** Bilateral corneal stromal loss in a Friesian gelding.

Although disease is bilaterally symmetric in location, onset in asymmetric in its development in each eye. This bilateral corneal stromal loss appears to be progressive and can result in perforation but has responded well to surgical repair. This syndrome, which may be a variant of a form of corneal degeneration called pellucid margination degeneration, has been reported more frequently in males. It may have a genetic component in Friesian horses.[45]

Iris Prolapse

Full-thickness corneal perforation is often called iris prolapse (Fig. 17.14). Iris prolapse may be ulcerative or traumatic and is considered a surgical emergency in either case. In one retrospective study of 32 horses with iris prolapse repaired with a conjunctival graft, 73% of perforated globes were retained, and 37% of eyes had vision. Risk factors associated with a good prognosis for retention of the globe following surgical repair of iris prolapse include being traumatic rather than ulcerative, and risk factors associated with a good prognosis for a visual outcome include duration less than 15 days, lacerations less than 15 mm, and lacerations involving cornea alone rather than extending to, along, or beyond the limbus.[46] A more recent retrospective study including 37 horses with iris prolapse caused by corneal ulcers with keratomalacia showed that penetrating keratoplasty alone or in combination with an overlying graft of conjunctiva or amniotic membrane can achieve a successful visual outcome, with 65% of horses had vision postoperatively and 89% of affected globes retained rather than enucleated, which is an increase in globe survival and visual outcome relative to the previous study in which conjunctival graft alone was used as the surgical repair.[47]

Stromal Abscess

Stromal abscesses, which often appear clinically as yellow, tan, or white circular to multifocal stromal lesions associated with intense ocular pain, blepharospasm, tearing, variable corneal vascularization, and secondary uveitis, occur more frequently in horses than any other species (Fig. 17.15). The hallmark of a corneal stromal abscess is ocular pain that is disproportionate to the apparent severity of the lesion. Stromal abscesses occur beneath intact epithelium and are thus fluorescein negative.

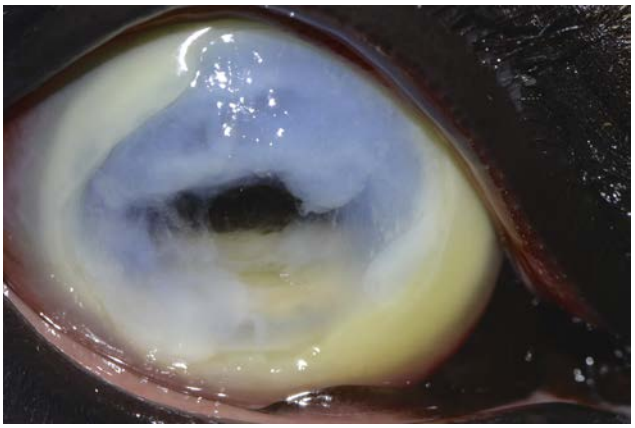


FIG. **17.14** Iris prolapse characterized by a dark brown opacity in the axial cornea surrounded by malacic corneal stroma and a severe stromal infiltrate of white blood cells.

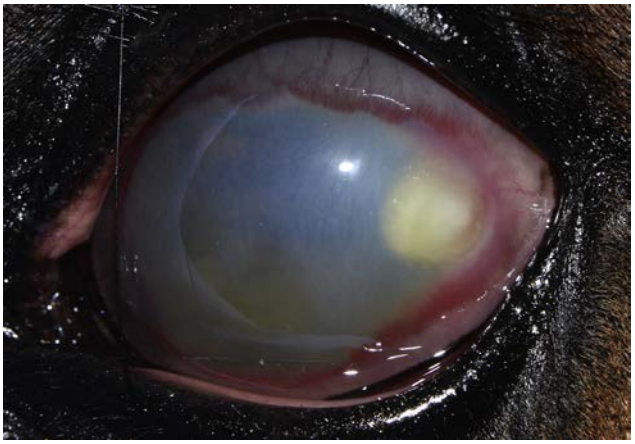


FIG. **17.15** Stromal abscess deep in the temporal cornea characterized by a yellow opacity, severe diffuse corneal edema, corneal vascularization, and a miotic pupil.

When uveitis is severe, corneal edema and vascularization may preclude view of the abscess. Extreme caution must be exercised when treating a corneal opacity that is fluorescein negative (i.e., there is no corneal ulcer present) with a topical steroid, as steroids can exacerbate an abscess. Stromal abscesses may be bacterial or fungal in etiology; however, without an epithelial defect, corneal culture and cytology are not viable diagnostics, and therefore treatment should be directed at both bacterial and fungal agents. Medical therapy is as for an infected ulcer although drug penetration may be poor through the intact epithelium. Debridement of the overlying epithelium to improve drug penetration is not recommended to avoid creation of an open wound that is susceptible to infection. Medical therapy is prolonged in many cases, from several weeks to several months, and typically involves topical antibiotics and antifungals. Stromal abscesses can be associated with severe secondary uveitis, which should be treated with topical atropine and systemic NSAIDs. Stromal abscesses that vascularize will often heal with medical therapy alone; however, lack of vascularization, or persistence or progression of the uveitis in the face of aggressive or appropriate medical therapy, may warrant surgical intervention. Surgical therapy includes excision of the abscessed stroma with supportive



FIG. **17.16** Eosinophilic keratitis, which is a characterized by a caseous pinkish white plaque often located at the limbus.

grafting procedures to return the cornea to normal tectonic strength. Surgical procedures most often involve partial-thickness grafts such as the posterior lamellar keratoplasty (PLK) or deep lamellar endothelial keratoplasty (DLEK).[48] Surgical therapy combined with medical therapy is typically associated with a shorter duration of therapy and faster recovery than treatment with medical therapy alone, although scar formation nearly always occurs.[49]

Corneal Grafting Biomaterials

A number of biomaterials have been used to reconstruct the diseased equine cornea, with the choice of biomaterial determined by the goal of reconstruction: restoration or preservation of tectonic integrity of the globe, replacement of missing or excised disease tissue, preservation or restoration of vision, or improved cosmesis. Conjunctival grafts have traditionally been used to provide a vascular supply but can cause significant scarring. Corneal tissue from a donor can replace missing tissue but can also result in significant scarring. Amniotic membrane can be used to reconstruct the ocular surface, and its use can optimize the visual outcome by reducing scarring because it is avascular, strong, antiangiogenic, and antiinflammatory.[50] Porcine urinary bladder extracellular matrix grafts, which have the advantages over other biomaterials of commercial availability and shelf storage, may be a viable alternative to other biomaterials for corneal grafting in horses, based on a 94% vision and globe retention rate in one retrospective study including 17 cases.[51]

Eosinophilic Keratitis

Eosinophilic keratitis is an inflammatory condition of the cornea characterized initially by severe pain and limbal corneal ulcers, often accompanied by a caseous discharge and severe chemosis and conjunctival hyperemia (Fig. 17.16). In early stages of the disease, clinical signs are often limited to the eyelids and conjunctiva, with severe blepharospasm, blepharitis, and conjunctival hyperemia. If treated at this stage with systemic corticosteroids, corneal disease can sometimes be prevented. The disease has a seasonal occurrence in the summer, likely associated with an environmental stimulus or insect

vector. The presence of eosinophils on cytology is diagnostic. Treatment with topical steroids is often associated with secondary fungal infection. The use of a systemic steroid such as prednisolone or dexamethasone and an antihistamine such as cetirizine may reduce disease severity. Treatment is typically prolonged. Recurrence from year to year in the same patient is reported. In one study of 27 horses diagnosed with eosinophilic keratitis, time to disease resolution averaged 3.7 months, with a significantly shorter time to resolution in horses treated with systemic dexamethasone (2.2 months) than in horses not so treated (4.2 months).[52] In that same study, only 8% of horses treated with cetirizine experienced a recurrence of clinical signs of eosinophilic keratitis in subsequent years; however, 57% of horses not treated with cetirizine experience recurrence.

Idiopathic Nonulcerative Keratitis

Immune-mediated keratitis has been described as a chronic (i.e., greater than 3 months in duration), nonulcerative corneal opacity that is not believed to be caused by an infectious agent and that typically responds to topical immunosuppressive drugs such as prednisolone acetate, dexamethasone, cyclosporine, or tacrolimus. Three types have been described based on the depth of the stromal infiltrated: superficial, characterized by a stromal infiltrate, diffuse vascularization, and response to medical therapy or keratectomy; midstromal, characterized by a stromal infiltrate, mild focal edema, vascularization, and response to cyclosporine; and endothelial, characterized by an infiltrate, diffuse edema, and the poorest prognosis. Immune-mediated keratitis is not typically associated with uveitis or with pain. Medical therapy tends to be constant, and in some cases surgery can be curative.[53]

An investigation of the histopathologic changes associated with immune-mediated keratitis identified nonspecific abnormalities including corneal cellular infiltrate, edema, neovascularization, and secondary degenerative changes. Immunohistochemistry suggested a predominantly T-cell driven process with CD3+ staining. Immunoglobulins detected in diseased cornea suggested localhumoral involvement, but corneal-binding immunoglobulins were not detected in serum or aqueous humor from horses with immune-mediated keratitis, suggesting that systemic humoral immunity is not a component of immune-mediated keratitis. Medical therapy of included corneas may have affected the results of this study.[54]

Episcleral silicone matrix cyclosporine implants have been developed to deliver immunosuppressive therapy locally in horses with immune-mediated keratitis. These implants have been shown to be well tolerated and to control keratitis (i.e., reduce severity of keratitis such that no or only one medication is required) for approximately 6 months when they are placed at an average of 4 months' disease duration for superficial immune-mediated keratitis and an average of 10 months' disease duration for endothelial immune-mediated keratitis. In the same retrospective study, keratitis was not controlled by episcleral silicone matrix cyclosporine implants when placed at an average of 16 months' disease duration for midstromal immune-mediated keratitis, with an average of 5 months of follow-up after implant placement.[55]

Squamous Cell Carcinoma

SCC may occur on the cornea, typically appearing as a vascular, raised pink lesion at the limbus (Figs. 17.17 and 17.18). Ultraviolet light has been implicated in the development of SCC by causing mutations in a tumor suppressor gene, p53. Additionally, cyclooxygenase-derived prostaglandins, specifically COX-2, may be upregulated in association with p53 mutations, stimulating tumor growth, metastasis, and angiogenesis.

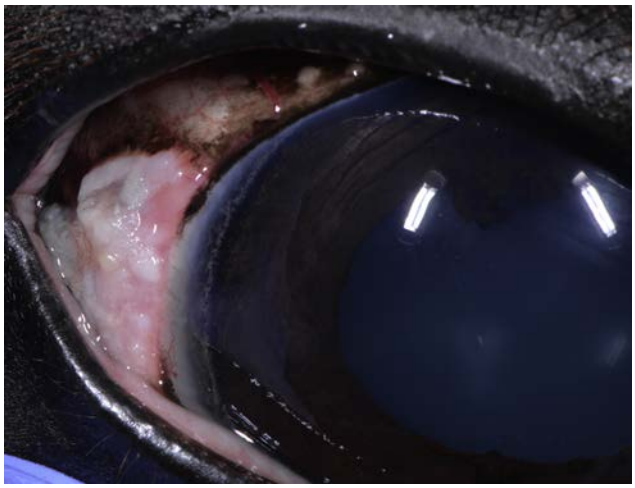


FIG. **17.17** Limbal SCC temporally that appears confined to the conjunctiva that is not covered by the upper or lower eyelid.

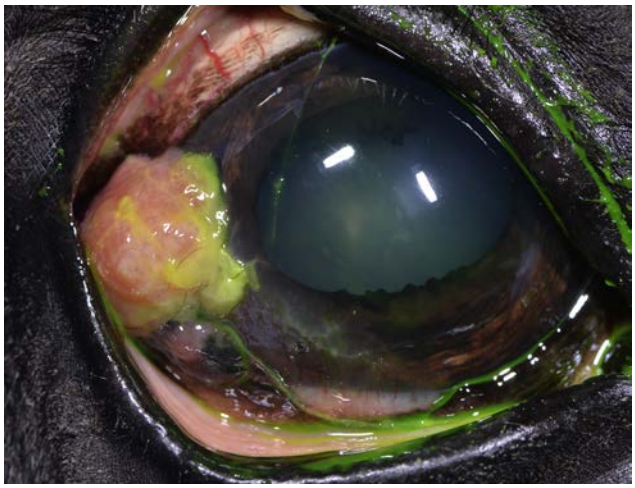


FIG. **17.18** Limbal SCC with a classic "cauliflower" appearance.

Numerous therapies exist for SCC, as with any disease for which there is no perfect treatment. The choice of therapy may not be as critical as early intervention. Excision is often combined with an adjunctive therapy. Excision of SCC involving the nictitans can typically be performed standing, if the tumor is focal and complete excision can be accomplished. Cryoablation is typically used for lesions less than 2 mm in thickness. Optimal cryonecrosis is achieved between −20°C and −40°C, using a double freeze-thaw technique. A fast freeze and slow thaw facilitates cryodestruction. Cryotherapy has the advantages of being accessible and portable. Adverse effects are uncommon, although excessive granulation tissue can be associated with cryotherapy for limbal tumors. Cryotherapy can be used for lid, nictitans, and limbal SCC.[56] 5-Fluorouracil is a pyrimidine analog that acts as an antimetabolite by noncompetitive inhibition of thymidylate synthase. It can be used topically for limbal SCC

and intralesionally for lid SCC. Mitomycin-C is an aziridine-containing natural product isolated from *Streptomyces* species. It acts as a potent DNA crosslinker. Its use topically is based on a retrospective study of 10 human patients with extensive recurrent limbal SCC.[57] Topical use in horses has been shown to be effective.[58,59]

Radiation has been used as primary and adjunctive therapy to treat SCC. Strontium 90 (Sr90) penetrates to a depth of approximately 3 mm, with 80% of the radiation dose absorbed in the first 2 mm, and thus may be effective for limbal SCC.[60] Iridium 129 (Ir129) is delivered via implants for eyelid SCC. Cobalt 60 (Co60) is associated with deeper penetration and thus does not have periocular application because of risks of collateral damage.

Haflingers may be overrepresented among horses with limbal SCC and may be diagnosed at a younger age than other breeds, with an average age at diagnosis of less than 9 years in one study. Affected Haflingers appear closely related, suggesting a possible heritable basis for limbal SCC.[61]

DISORDERS OF THE EQUINE UVEA

Normal Anatomy

The uvea, the vascular tunic of the eye, comprises the iris and ciliary body anteriorly and the choroid posteriorly. Histologically, the uvea contains blood vessels, pigment cells, smooth muscle, and, in the horse, a noncellular fibrous tapetum in the choroid. The ciliary body is the source of aqueous humor, which supplies nutrition to the cornea and lens. The choroid supplies nutrition to the retina and also serves as a heat sink to protect the photoreceptors from the heat generated by light striking the retina. The tapetum, contained within the superior choroid, reflects light back across the retina, thereby maximizing the use of available light. The uvea is the primary site of the blood-ocular barrier, an immunologic barrier to the internal components of the eye. Uveitis, or inflammation of the uveal tract, disrupts this barrier. Spasm of the smooth muscles in the iris and ciliary body occurs with uveitis and results in intraocular pain, photophobia, and constriction of the pupil, or miosis.

The equine pupil is horizontally elliptic, with the dorsal and ventral margins having pigmented prominences termed *corpora nigra*. The pupillary light reflex in the horse is similar to that of other species, with a direct and a consensual response. The afferent fibers of the pupillary light reflex are carried in cranial nerve II, and the efferent parasympathetic fibers are carried in cranial nerve III. Abnormalities in pupil response to a light stimulus result from afferent lesions involving the retina or optic nerve. Afferent abnormalities are associated with loss of vision in the affected eye. Efferent lesions are rare but include damage to cranial nerve III with an associated ventrolateral strabismus and abnormalities of the iris itself such as synechiae. Glaucoma, which is an optic neuropathy characterized by high intraocular pressure, can cause mydriasis secondary to afferent optic nerve damage and/or because of an efferent inability of the pupil to constrict in the face of high intraocular pressure. It is important to differentiate excited horses with sympathetic override, weak light source, and improper examination technique from an abnormal pupillary light response.



FIG. **17.19** Corpora nigra cyst at the inferior pupil margin. These structures originate from the posterior pigmented epithelium of the iris.

Sympathetic nerve fibers supply the dilator muscles of the iris. These fibers travel in the spinal cord, emerge in the ventral nerve roots at the first and second thoracic nerves, course through the thorax, run in association with the internal carotid artery, synapse at the cranial cervical sympathetic ganglion, and travel to the eye with the ocular arteries. Damage to the sympathetic fibers results in Horner's syndrome. Clinical signs include ptosis, miosis, enophthalmos, protrusion of the third eyelid, and sweating on the affected side of the face and neck. Treatment is directed toward the primary disease, if possible. In some horses the clinical signs of Horner's syndrome resolve without treatment.

Iris Stromal Hypoplasia

An abnormally thin iris stroma, particularly in horses with a blue iris, can protrude anteriorly as aqueous humor, formed by the ciliary body in the posterior chamber, accumulates behind the iris. This iris stromal hypoplasia can be mistaken for a solid iris mass; however, transillumination can differentiate between the two.

Corpora Nigra Cyst

Cysts of the anterior uvea are formed by the posterior pigmented epithelium of the iris (Fig. 17.19). Treatment of these cysts is not required unless they are numerous or impair sight. In a retrospective study of the use of semiconductor diode laser to deflate and coagulate anterior uveal cysts in dogs, cats, and horses, in nine horses with corpora nigra cysts at the pupillary margin, no recurrences were reported in any treated horses, and undesirable behavior such as spooking or shying that had been observed before diode laser treatment improved or resolved in all cases.[62]

Uveitis

Uveitis is an inflammation of the iris and ciliary body (anterior) and choroid (posterior). Uveitis is a common manifestation of many ocular and systemic infectious and noninfectious diseases, and as such, an attempt to ascertain the cause is essential. Differentiating uveitis from other ophthalmic diseases resulting in a red and painful eye, such as glaucoma, corneal ulceration, and conjunctivitis, is also essential.

Acute inflammation of the anterior uvea results in spasm of the iris and ciliary muscles and a breakdown in the blood-ocular barrier, leading to miosis and photophobia. Eyelids and conjunctiva may be swollen and hyperemic in the acute phase of anterior uveitis. Protein and cells leak into the anterior chamber, resulting in aqueous flare, hypopyon, and keratitic precipitates. The cornea may show diffuse edema, and the IOP will be low in acute uveitis. Chronic inflammatory changes in the aqueous humor can result in secondary corneal endothelial and lens changes such as corneal edema and cataract, as well as synechiae, which are adhesions between the iris and adjacent cornea (anterior synechiae) or lens (posterior synechiae). The lens may stain a yellow color as a result of chronic serum leakage. The corpora nigra will atrophy with chronic uveitis, appearing abnormally small and smooth. Inflammation of the choroid, because of its close association with the retina, usually also involves the retina and is termed *chorioretinitis.* Chorioretinitis is diagnosed by direct or indirect ophthalmoscopy and results in retinal and subretinal transudate and exudate, retinal vascular changes, hemorrhage, retinal detachment, and retinal degeneration. The optic nerve may show hyperemia and later atrophy. Vitreal changes may be observed in association with posterior uveitis. This may include vitreous debris, cells, liquefaction, and yellow discoloration of the vitreous. Choroidal depigmentation and areas of repigmentation or pigment clumping may be present. Other chronic changes resulting from intraocular inflammation include secondary glaucoma, retinal detachment, blindness, and phthisis bulbi (Fig. 17.20). The development of intraocular changes following uveitis is related directly to the duration and severity of the acute episode and is exacerbated by recurrent episodes of inflammation.

Uveitis may be primary or secondary to other ophthalmic or systemic disease. Ocular causes of secondary uveitis include blunt or penetrating ocular trauma, intraocular neoplasia, corneal ulceration, lens rupture, cataract, and parasitic infiltration. Corneal ulceration results in secondary anterior uveitis through a reflex pathway involving the ophthalmic branch of cranial nerve V. Fluorescein staining of the cornea therefore is indicated in eyes with anterior uveitis. The lens is considered to be an immune-privileged site and as such is capable of stimulating an inflammatory reaction. Lens protein is exposed by traumatic rupture of the lens capsule or during the degenerative process of a hypermature cataract undergoing liquefaction and leakage. Either of these processes can lead to anterior uveitis, with rupture of the lens resulting in the more severe inflammation and frequently secondary glaucoma. Uveitis resulting from direct ocular trauma may be associated with rupture of the fibrous tunic of the eye, hyphema, lens luxation, corneal endothelial damage, retinal detachment, or proptosis.



FIG. **17.20** Phthisis bulbi in an Appaloosa with chronic equine recurrent uveitis (ERU).

Systemic diseases resulting in anterior and posterior uveitis are numerous and include bacteremia, septicemia, immune-complex disease, viremia, disseminated mycoses, and the syndrome of equine recurrent uveitis. Common infectious causes of uveitis include *Streptococcus equi* subsp. *equi* (strangles) and *Leptospira interrogans* serogroup *pomona,* as well as gram-negative sepsis.

Foals with neonatal septicemia are especially prone to anterior and posterior uveitis. The uveitis in these foals is usually sterile. Systemic antimicrobial therapy, topical and systemic antiinflammatory therapy, and topical atropine 1% therapy are the treatments of choice. Copious fibrin production may result from anterior uveitis, especially in young foals, and can result in posterior synechia and opacification of the lens capsule. Intracameral tissue plasminogen activator, 25 to 75 µg administered in the first few days after fibrin formation, can result in complete lysis and resolution.

Equine Recurrent Uveitis

Equine recurrent uveitis (ERU) has been described as the leading cause of blindness in horses. Alternate names include moon blindness and periodic ophthalmia. A review of records of 224 horses with ERU correlated the high frequency of blindness, globe loss, and loss of horses' function, with change of ownership and even euthanasia being common sequelae to ERU.[63] Uveitis starts as blood-ocular barrier compromise, such that blood vessels of the iris, ciliary body, and choroid become "leaky," allowing cells and inflammatory cytokines to enter the eye. Infectious agents may initiate the initial flare-up of uveitis, but immune response shifts between different autoantigens may be responsible for recurrent episodes. Corneal, lens, and retinal autoantigens have been identified in the equine eye that are believed to be targeted by the immune system in horses with ERU; these self-antigens perpetuate the disease. ERU is mediated by a Th1 response directed against several retinal proteins, including interphotoreceptor-retinoid binding protein and cellular retinaldehyde-binding protein. Retinal Mueller glial cells may play a fatal role in uveitic disease progression by directly triggering inflammation processes through the expression and secretion of interferon-gamma.[64]

Although leptospirosis has been implicated in the initiation of ERU, the continued presence of *Leptospira* spp. organisms in the aqueous or vitreous humors does not appear to play a direct role in the pathogenesis of ERU, based on evaluation of aqueous humor, vitreous humor, and serum samples of 24 clinically normal horses, 52 horses with ERU, and 17 horses with ocular inflammation not associated with ERU for the presence of bacterial DNA and intraocular production of antibodies against *Leptospira* spp.[65] However, in another study evaluating aqueous and vitreous samples from 31 horses with ERU and 21 control horses by *Leptospira* culture, polymerase chain reaction (PCR), and microagglutination test (MAT), horses with ERU had a high prevalence of *Leptospira* infection based on PCR and MAT but not *Leptospira* culture, suggesting

that the diagnosis of intraocular *Leptospira* infection requires invasive testing.[66] Given its potential role in the pathogenesis and persistence of ERU, the complicated diagnostic picture, and the low risk of treating for *Leptospira* infection relative to the consequences associated with persistent ocular pain, inflammation, and possible blindness, treating for leptospirosis in acute uveitis cases with no other apparent underlying cause (e.g., blunt trauma, corneal ulceration, cataract) is probably reasonable.

Appaloosa horses are predisposed to ERU, suggesting a genetic link. Evaluation of the association between genetic markers on equine chromosomes 1 (ECA1) and 20 (ECA20) in 53 Appaloosas with ERU and 43 healthy Appaloosa controls showed strong associations between ERU disease status and genetic markers in the TRPM1 gene on ECA1 and the equine major histocompatibility complex (ELA) on ECA20.[67]

Cyclosporine A (CsA) implants have been developed to decrease the frequency and severity of flare-ups of ERU. Most horses with CsA implants have less inflammation and fewer attacks postoperatively.[68] Standard treatment of ERU flare-ups remains topical corticosteroids and NSAIDs, topical atropine, and systemic antiinflammatories (both corticosteroids and NSAIDs). There are risks and complications associated with long-term use of any of these drugs.

DISORDERS OF THE EQUINE LENS

Normal Anatomy

The lens originates from surface ectoderm and is a clear, avascular, biconvex structure suspended posterior to the iris by lenticular zonules arising from the ciliary body. The lens depends on the aqueous and vitreous humors to supply nutrition and remove waste products.

Luxation

Lens luxation and subluxation can be congenital or acquired. Acquired lens luxation results from glaucoma and buphthalmos, trauma, or chronic uveitis. Once luxated, the lens can be displaced anteriorly or posteriorly. A posteriorly luxated lens results in liquefaction of the vitreous from lens movement. The lens settles inferiorly, may or may not attach to the retina, and generally does not result in significant problems or require therapy. Anterior lens luxation can cause anterior uveitis and trauma to the corneal endothelium, resulting in diffuse corneal edema, obstruction of aqueous humor circulation, and secondary glaucoma (Fig. 17.21). Treatment of anterior lens luxation in a potentially visual eye involves surgical removal of the luxated lens, although the success of this procedure in the horse has not been well documented. In a blind, painful eye, enucleation is warranted. It is possible for an anteriorly luxated lens to be repositioned into the posterior segment with digital pressure; however, complications such as retinal detachment and vitreal prolapse resulting in secondary glaucoma can occur.

Cataract

A cataract is any opacity of the lens or its capsule (Figs. 17.22–17.24). Cataracts can be unilateral or bilateral and should be classified according to age of onset, location, and degree of lens affected to aid in determining the cause and likelihood of progression. In general, anterior, cortical, and equatorial cataracts are more likely to progress than are nuclear and posterior cortical



FIG. **17.22** Early immature cataract in the right eye of a young adult horse.



FIG. **17.21** Chronic anterior luxation of a lens with a brunescent cataract (a very advanced nuclear cataract that has become brown and opaque).

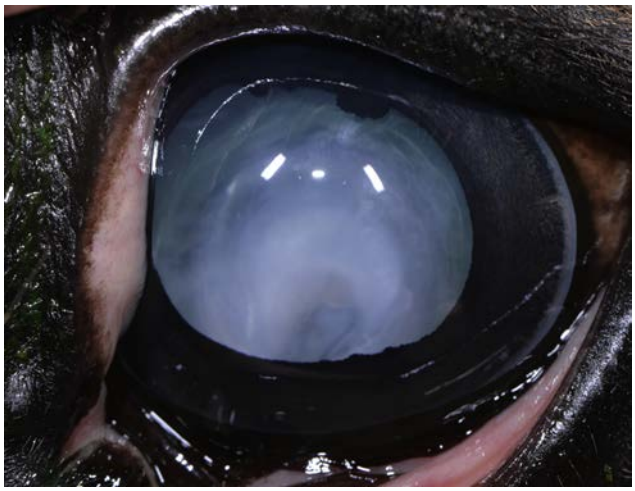


FIG. **17.23** Late immature cataract in the left eye of the horse pictured in Fig. 17.22.



FIG. **17.24** Immature cataract in a foal.

cataracts. Classification by age of onset includes congenital, juvenile, adult, and senile. Causes of cataract formation include inherited, inflammatory, traumatic, metabolic, toxic, and nutritional abnormalities, with the most common cause of cataracts in horses being secondary to ERU. Suspected inherited cataracts have been reported in several breeds of horses and are thought to occur in other breeds, but definitive support is difficult because of the small number of offspring produced. If the cause of a cataract cannot be determined and intraocular inflammation is not evident, breeding of a horse with a cataract should be discouraged. The only treatment for cataracts is surgical. Cataracts that are unilateral or not sufficiently severe to interfere significantly with vision may not warrant treatment. Many horses develop age-related cataracts that have variable effects on functional vision. A decision to treat cataracts should always be considered in the context of the degree of functional visual impairment, the presence of active inflammation, and the intended use of the horse. Before cataract surgery, retinal anatomy and function should be evaluated using ocular ultrasound and electroretinography.

Success rates of equine cataract surgery have been reported in two recent retrospective studies. In one review of records of 95 horses undergoing cataract surgery, including 44 foals and 24 horses with cataracts secondary to ERU, 95% regained vision postoperatively but the percent of horses with eye sight decreased rapidly from 87% at less than 1 month, 49% at 6 months, 35% at 1 year, and only 26% had vision at greater than 2 years. However, horses not available for follow-up were not included as 'had vision' but not removed from the denominator so were effectively counted as 'had no vision', potentially underestimating long-term success.[69] In another retrospective study of 41 horses undergoing cataract surgery, 26% with chronic uveitis, 54% had vision at an average last follow-up of 35 months. In that study, visual outcome was not associated with patient age or placement of an artificial lens, but fewer horses with uveitis (25%) had vision at last follow-up compared with horses without uveitis (65%).[70] Intraocular lenses have been developed to correct vision in the equine eye following cataract surgery.[71]

DISORDERS OF THE EQUINE RETINA

Normal Anatomy

The horse has a paurangiotic (partially vascularized) retina with 30 to 60 small retinal vessels radiating from the margin of the optic disk that are visible for a distance of one to two disk diameters. Beyond this distance, the remainder of the equine retina is avascular, with nutrition supplied to the retina from the underlying choroidal blood vessels. The equine fundus is divided into dorsal tapetal and ventral nontapetal regions, with the oval, salmon-colored optic disk located in the nontapetal fundus. Variations in tapetal color are related to coat color and include green, yellow, orange, and blue-green. The tapetum of the horse is fibrous and penetrated by small choroidal vessels that appear as dark dots in the tapetum, termed the *stars of Winslow.* The ventral, nontapetal fundus is generally dark brown or black but can appear lighter or nonpigmented depending on coat color. Retinal function can be tested by performing an electroretinogram (ERG). ERGs can be performed in standing sedated horses.[72]

Retinal Detachment

Retinal detachment can be congenital or acquired, partial or complete. Because the equine retina depends almost totally on the underlying choroid for its blood supply, detachment results in rapid severe retinal degeneration and irreversible blindness. Common causes of retinal detachment in the horse include an inherited abnormality associated with retinal dysplasia, ERU, and trauma. Treatment is limited to controlling the inciting disease and use of systemic antiinflammatory drugs, but restoration of vision is not possible. Surgical reattachment of the retina has not been successfully performed in the horse.

Chorioretinitis

Inflammation of the retina and choroid in the horse most often results from ERU and may be associated with concomitant anterior uveitis and optic neuritis. In addition, the vascular nature of the choroid results in susceptibility to blood-borne disease, as with bacteremia, septicemia, and viremia. Treatment of chorioretinitis includes systemic therapy for the primary infectious disease, if present, and flunixin meglumine to decrease inflammation. Topical treatment is indicated only with anterior uveal involvement. The sequelae of chorioretinitis include retinal degeneration, retinal detachment, and optic nerve atrophy.

Although pinpoint chorioretinal scars ("bullet hole" lesions) have been associated with chronic chorioretinitis, outer retinal function as measured by ERG does not appear to be compromised in horses with extensive bullet hole lesions.[73]

DISORDERS OF THE EQUINE OPTIC NERVE

Normal Anatomy

The equine optic nerve is oval, salmon-pink, and located in the nontapetal region of the fundus. Arterioles and venules extend a short distance from the optic nerve into the surrounding peripapillary retina. On direct ophthalmoscopic examination the margin of the optic nerve is sharp and well defined, as are the retinal blood vessels. The nerve fiber layer of the retina may be visible as linear white streaks radiating outward from the optic nerve.

Glaucoma

Glaucoma is a disease characterized by elevation of IOP that is incompatible with health of the optic nerve. In horses, glaucoma is usually secondary to uveitis. Clinical signs include

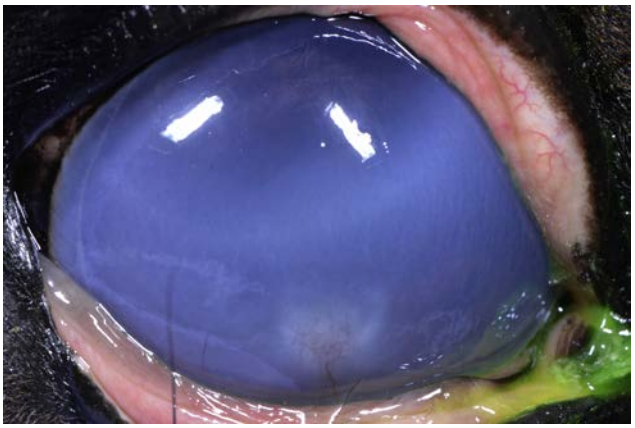


FIG. **17.25** Glaucoma characterized by severe diffuse corneal edema.

mydriasis, corneal edema, scleral injection, ciliary flush, and, in chronic cases, buphthalmos (Fig. 17.25). Haab's striae, which represent breaks in Descemet's membrane, may be associated with glaucoma but can also be seen with no apparent cause and may represent inflammatory lesions. Glaucoma is diagnosed based on clinical signs and measurement of IOP. Several different types of tonometers are commercially available and lend themselves well to field use. Glaucoma can be classified as primary, resulting from anatomic abnormalities associated with the iridocorneal angle, or secondary to other ocular disease such as uveitis, lens luxation, or intraocular neoplasia. Primary glaucoma is not well characterized in the horse.

Therapy for glaucoma can be divided into two general categories: drugs that decrease aqueous production, such as β-blockers and carbonic anhydrase inhibitors, and drugs that increase aqueous outflow, such as prostaglandin analogs. Another way of categorizing glaucoma medications would be based on their function, as rescue versus maintenance drugs. Some drugs, such as the prostaglandin analogs (e.g., latanoprost), serve both rescue and maintenance functions. Unfortunately, the prostaglandin analogs, which have proven to control IOP well in other species, do not decrease IOP in horses. The two drugs most helpful in decreasing IOP in horses with glaucoma are timolol and dorzolamide, which are available as a combination product called Cosopt. These drugs have been shown to be associated with a small but significant decrease in IOP in normal horses,[74] but no controlled studies of their efficacy in horses with glaucoma have been performed. Brinzolamide, a topical ophthalmic carbonic anhydrase inhibitor with a pH closer to physiologic and that is therefore less likely to be associated with ocular irritation, has been shown to decrease IOP in normal horses with both once- and twice-daily dosing.[75]

As with medical therapy, surgical treatments for glaucoma can be classified based on their mechanism of action as either decreasing aqueous production (e.g., cyclodestructive procedures, which damage the ciliary body) or increasing aqueous outflow (e.g., anterior chamber shunts). Diode laser transcleral cyclophotocoagulation (TSCP) can provide long-term control of IOP in horses with glaucoma but typically does not eliminate the need for long-term topical treatment.[76] TSCP can often be performed under standing sedation rather than general anesthesia. Horses undergoing TSCP typically have a 3-week period in which IOP spikes in association with laser-induced uveitis but then settles down to a stable postoperative level.

Endocyclophotocoagulation and gonioimplant placement have become increasingly commonly performed by veterinary ophthalmologists treating canine glaucoma patients, but neither of these surgical therapies has yet achieved consistent success with equine glaucoma patients. In one experimental study in which Ahmed gonioimplants were placed in seven normal eyes of four horses, IOP was significantly decreased for the entire 4-week study period, suggesting that gonioimplants may be a viable therapeutic option for horses with glaucoma.[77] A case report describing the placement of a Baerveldt gonioimplant in a horse with glaucoma documented IOP control for 402 days, suggesting that long-term IOP control may be achieved with gonioimplants.[78]

Traumatic Optic Neuropathy

Trauma to the head of the horse has been associated with acute unilateral or bilateral blindness resulting from optic nerve damage. The pupil in the affected eye is dilated, but the remainder of the ophthalmic examination may be normal initially. Occasionally, retinal hemorrhage and papilledema are present. Fundic examination 3 to 4 weeks after the traumatic episode reveals optic nerve pallor and absence of retinal blood vessels, indicating atrophy. The cause of this lesion is hypothesized to be stretching of the optic nerve with subsequent rupture of the optic nerve axons or trauma from bony fractures adjacent to the optic nerve, although anatomic studies have not been performed to confirm this. Systemic antiinflammatory therapy may be beneficial in the acute phase; however, the prognosis for return of vision is guarded. Optic nerve atrophy can also be caused by ERU and chronic glaucoma.

DISORDERS OF THE EQUINE ORBIT

Normal Anatomy

The complete bony orbital rim that protects the equine globe is formed by the frontal bone dorsally, the lacrimal bone medially, the zygomatic bone ventrally, and the temporal bone laterally.

Orbital Fracture

The zygomatic process of the frontal bone and the zygomatic arch, which form the dorsal orbital rim, are prone to fracture and resultant opening into the frontal and caudal maxillary sinuses. Epistaxis can occur when the turbinate bones are fractured, and nasolacrimal duct obstruction can result from a lacrimal bone fracture. In a retrospective study of 18 horses diagnosed with orbital fractures that were confirmed by skull radiographs or CT, 56% had orbital rim fractures, 72% had open fractures, and 83% of the fractures were displaced. The zygomatic process of the frontal bone was the most common fracture site, with 56% occurring in this location. Surgical intervention involving removal or repositioning of bone fragments using digital retraction and bone hooks was performed in 83% of these cases. The globe was retained in 89% of cases and was visual in 78%, suggesting that horses diagnosed with orbital fractures have a favorable prognosis for vision.[79]

Neoplasia

The most common orbital tumors are neuroendocrine tumors and extra-adrenal paragangliomas, which grow slowly and rarely metastasize.[80] Clinical signs of retrobulbar neoplasia include exophthalmos, strabismus, elevation of the third

eyelid, periorbital swelling, decreased retropulsion of the globe, chemosis, mydriasis, and vision loss. Ocular ultrasound may identify a mass lesion with or without indentation of the globe, but this can be difficult to interpret. CT can identify the location and extent of an orbital tumor.[80] Although the equine orbit and sinuses can provide room for orbital tumors to progress to an advanced stage before diagnosis and therefore the prognosis for globe survival is poor, exenteration can be associated with increased survival times by preventing extension beyond the orbit.

Enucleation

Enucleation may be warranted for globes that are blind and intractably painful when medical therapy or other surgical alternatives are not viable. Two standard approaches to enucleation are transpalpebral and subconjunctival. In a transpalpebral enucleation, the eyelids are closed and the eyelid margin is removed in a single bloc of tissue with the conjunctiva, third eyelid, and globe, which can minimize orbital contamination in cases of infectious or neoplastic ocular disease. In a subconjunctival enucleation, the globe is removed before removal of the eyelid margin, which can be associated with decreased surgical time and hemorrhage.

A transpalpebral enucleation in a standing sedated horse can safely be performed in most horses and eliminates the risks associated with general anesthesia.[81] In one retrospective report of 40 horses enucleated under standing sedation, acceptable local anesthesia and lid akinesia was provided using a combination of an auriculopalpebral motor block, a supraorbital block of the frontal nerve, a subcutaneous ring block around the palpebrae to desensitize the infratrochlear, lacrimal, and zygomatic nerves, and a retrobulbar four-point block using a 20-gauge bent spinal needle inserted through the eyelid skin.[82] Surgical drapes are typically not used to avoid exaggerated head movement, although a small sterile drape can be placed over the nose and cheek band of the halter after the globe is excised to cover the region ventral to the eye and thereby prevent contamination of the suture during wound closure.

The effect of unilateral enucleation on vision and potential loss of performance in horses is not well understood. In one study of 34 horses who had undergone unilateral enucleation, 74% were reported to be acutely blind at the time of enucleation, and 85% returned to their presurgical discipline. The duration of vision loss before enucleation did not appear to affect whether horses returned to their previous discipline.[83] In the Pollock study, 30 of 32 horses for whom follow-up information was available returned to their previous use, with two retired to breeding or pasture.[82]

REFERENCES

1. Beech J, Zappala R, Smith G, Lindborg S. Schirmer tear test results in normal horses and ponies: effect of age, season, environment, sex, time of day and placement of strips. *Vet Ophthalmol*. 2003;6(3):251–254.
2. Knollinger AM, La Croix NC, Barrett PM, Miller PE. Evaluation of a rebound tonometer for measuring intraocular pressure in dogs and horses. *J Am Vet Med Assoc*. 2005;227(2):244–248.
3. Komáromy AM, Garg CD, Ying G-S, Liu C. Effect of head position on intraocular pressure in horses. *Am J Vet Res*. 2006;67(7):1232–1235.
4. van der Woerdt A, Gilger BC, Wilkie DA, Strauch SM. Effect of auriculopalpebral nerve block and intravenous administration of xylazine on intraocular pressure and corneal thickness in horses. *Am J Vet Res*. 1995;56(2):155–158.
5. Holve DL. Effect of sedation with detomidine on intraocular pressure with and without topical anesthesia in clinically normal horses. *J Am Vet Med Assoc*. 2012;240(3):308–311.
6. Herring IP, Pickett JP, Champagne ES, Troy GC, Marini M. Effect of topical 1% atropine sulfate on intraocular pressure in normal horses. *Vet Ophthalmol*. 2000;3(2-3):139–143.
7. Kalf KL, Utter ME, Wotman KL. Evaluation of duration of corneal anesthesia induced with ophthalmic 0.5% proparacaine hydrochloride by use of a Cochet-Bonnet aesthesiometer in clinically normal horses. *Am J Vet Res*. 2008;69(12):1655–1658. http://dx.doi.org/10.2460/ajvr.69.12.1655.
8. Monclin S, Farnir F, Grauwels M. Duration of corneal anaesthesia following multiple doses and two concentrations of tetracaine hydrochloride eyedrops on the normal equine cornea. *Equine Vet J*. 2011;43(1):69–73.
9. Sharrow-Reabe KL, Townsend WM. Effects of action of proparacaine and tetracaine topical ophthalmic formulations on corneal sensitivity in horses. *J Am Vet Med Assoc*. 2012;241(12):1645–1649.
10. Davis JL, Stewart T, Brazik E, Gilger BC. The effect of topical administration of atropine sulfate on the normal equine pupil: influence of age, breed and gender. *Vet Ophthalmol*. 2003;6(4):329–332.
11. Miller C, Utter ML, Beech J. Evaluation of the effects of age and pituitary pars intermedia dysfunction on corneal sensitivity in horses. *Am J Vet Res*. 2013;74(7):1030–1035. http://dx.doi.org/10.2460/ajvr.74.7.1030.
12. Kaps S, Richter M, Spiess BM. Corneal esthesiometry in the healthy horse. *Vet Ophthalmol*. 2003;6(2):151–155.
13. Brooks DE, Clark CK, Lester GD. Cochet-Bonnet aesthesiometer-determined corneal sensitivity in neonatal foals and adult horses. *Vet Ophthalmol*. 2000;3(2-3):133–137.
14. Hurn S, Turner A, Cowan CM. Ectopic cilium in seven horses. *Vet Ophthalmol*. 2005;8(3):199–202.
15. Hermans H, Ensink J. Treatment and long-term follow-up of distichiasis, with special reference to the Friesian horse: a case series. *Equine Vet J*. 2014;46(4):458–462.
16. Utter M, Wotman K. Distichiasis causing recurrent corneal ulceration in two Friesian horses. *Equine Vet Educ*. 2012;24(11):556–560.
17. Henriksen M. d. L, Plummer CE, Brooks DE. Modified Kuhnt-Szymanowski surgical procedure for secondary cicatricial ectropion in a horse. *Vet Ophthalmol*. 2013;16(4):276–281.
18. Theon AP, Wilson WD, Magdesian KG, Pusterla N, Snyder JR, Galuppo LD. Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995–2004). *J Am Vet Med Assoc*. 2007;230(10):1506–1513. http://dx.doi.org/10.2460/javma.230.10.1506.
19. Hewes CA, Sullins KE. Use of cisplatin-containing biodegradable beads for treatment of cutaneous neoplasia in equidae: 59 cases (2000–2004). *J Am Vet Med Assoc*. 2006;229(10):1617–1622.
20. Giuliano EA, Johnson PJ, Delgado C, Pearce JW, Moore CP. Local photodynamic therapy delays recurrence of equine periocular squamous cell carcinoma compared to cryotherapy. *Vet Ophthalmol*. 2014;17(s1):37–45.
21. Knottenbelt DC, Kelly DF. The diagnosis and treatment of periorbital sarcoid in the horse: 445 cases from 1974 to 1999. *Vet Ophthalmol*. 2000;3(2-3):169–191.
22. Cummings E, James ER. Prevalence of equine onchocerciasis in southeastern and midwestern United States. *J Am Vet Med Assoc*. 1985;186(11):1202–1203.
23. Schmidt GM, Krehbiel JD, Coley SC, Leid RW. Equine ocular onchocerciasis: histopathologic study. *Am J Vet Res*. 1982;43(8):1371–1375.
24. Scherrer NM, Lassaline-Utter M, McKenna BC. Characterization and outcome following excision of masses in the nictitating membranes of horses: 50 cases (1998–2012). *J Am Vet Med Assoc*. 2014;245(7):812–815. http://dx.doi.org/10.2460/javma.245.7.812.

25. Brink P, Schumacher J. Canaliculosinostomy as a long-term treatment of seven horses for permanent obstruction of the nasolacrimal duct. *Vet Surg*. 2015.
26. Hempstead JE, Clode AB, Borst LB, Gilger BC. Histopathological features of equine superficial, nonhealing, corneal ulcers. *Vet Ophthalmol*. 2014;17(suppl 1):46–52.
27. Michau T, Schwabenton B, Davidson M, Gilger B. Superficial, nonhealing corneal ulcers in horses: 23 cases (1989–2003). *Vet Ophthalmol*. 2003;6(4):291–297.
28. Lassaline-Utter M, Cutler TJ, Michau TM, Nunnery CM. Treatment of nonhealing corneal ulcers in 60 horses with diamond burr debridement (2010–2013). *Vet Ophthalmol*. 2014;17(suppl 1):76–81. http://dx.doi.org/10.1111/vop.12148.
29. Ledbetter EC, Irby NL, Schaefer DMW. In vivo confocal microscopy of corneal microscopic foreign bodies in horses. *Vet Ophthalmol*. 2014.
30. Clode AB, Davis JL, Salmon J, LaFevers H, Gilger BC. Aqueous humor and plasma concentrations of ciprofloxacin and moxifloxacin following topical ocular administration in ophthalmologically normal horses. *Am J Vet Res*. 2010;71(5):564–569.
31. Sauer P, Andrew S, Lassaline M, Gelatt K, Denis H. Changes in antibiotic resistance in equine bacterial ulcerative keratitis (1991–2000): 65 horses. *Vet Ophthalmol*. 2003;6(4):309–313.
32. Clode AB, Davis JL, Salmon J, Michau TM, Gilger BC. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. *Am J Vet Res*. 2006;67(2):296–301.
33. Gilmour M. Subconjunctival voriconazole for the treatment of mycotic keratitis in a horse. *Equine Vet Educ*. 2012;24(10):489–492.
34. Smith KM, Pucket JD, Gilmour MA. Treatment of six cases of equine corneal stromal abscessation with intracorneal injection of 5% voriconazole solution. *Vet Ophthalmol*. 2014;17(s1):179–185.
35. Pearce JW, Giuliano EA, Moore CP. In vitro susceptibility patterns of *Aspergillus* and *Fusarium* species isolated from equine ulcerative keratomycosis cases in the midwestern and southern United States with inclusion of the new antifungal agent voriconazole. *Vet Ophthalmol*. 2009;12(5):318–324.
36. Betbeze CM, Wu CC, Krohne SG, Stiles J. In vitro fungistatic and fungicidal activities of silver sulfadiazine and natamycin on pathogenic fungi isolated from horses with keratomycosis. *Am J Vet Res*. 2006;67(10):1788–1793.
37. Ollivier FJ, Brooks DE, Kallberg ME, et al. Evaluation of various compounds to inhibit activity of matrix metalloproteinases in the tear film of horses with ulcerative keratitis. *Am J Vet Res*. 2003;64(9):1081–1087.
38. Baker A, Plummer CE, Szabo NJ, Barrie KP, Brooks DE. Doxycycline levels in preocular tear film of horses following oral administration. *Vet Ophthalmol*. 2008;11(6):381–385.
39. Wotman KL, Utter ME. Effect of treatment with a topical ophthalmic preparation of 1% nalbuphine solution on corneal sensitivity in clinically normal horses. *Am J Vet Res*. 2010;71(2):223–228. http://dx.doi.org/10.2460/ajvr.71.2.223.
40. Giuliano EA, Maggs DJ, Moore CP, Boland LA, Champagne ES, Galle LE. Inferomedial placement of a single-entry subpalpebral lavage tube for treatment of equine eye disease. *Vet Ophthalmol*. 2000;3(2-3):153–156.
41. Cornelissen S, Finding E, Bowen I, Bullard C, Hallowell G. Factors affecting complication rates with subpalpebral lavage catheter use in horses. *Equine Vet J*. 2015;47(S48). 21–21.
42. Scotty NC, Brooks DE, Schuman Rose CD. In vitro efficacy of an ophthalmic drug combination against corneal pathogens of horses. *Am J Vet Res*. 2008;69(1):101–107. http://dx.doi.org/10.2460/ajvr.69.1.101.
43. Johns IC, Beech J, Benson CE, Parente LL. In vitro evaluation of the antibiotic activity of combinations of ophthalmic drugs against common equine ocular pathogens. *J Equine Vet Sci*. 2010;30(5):231–236.
44. Myrna KE, Herring IP. Constant rate infusion for topical ocular delivery in horses: a pilot study. *Vet Ophthalmol*. 2006;9(1):1–5.
45. Lassaline-Utter M, Gemensky-Metzler AJ, Scherrer NM, et al. Corneal dystrophy in Friesian horses may represent a variant of pellucid marginal degeneration. *Vet Ophthalmol*. 2014;17(suppl 1):186–194. http://dx.doi.org/10.1111/vop.12152.
46. Chmielewski NT, Brooks DE, Smith PJ, Hendrix DVH, Whittaker C, Gelatt KN. Visual outcome and ocular survival following iris prolapse in the horse: a review of 32 cases. *Equine Vet J*. 1997;29(1):31–39.
47. Linde Henriksen M, Plummer CE, Mangan B, et al. Visual outcome after corneal transplantation for corneal perforation and iris prolapse in 37 horses: 1998–2010. *Equine Vet J*. 2012;44(S43):115–119.
48. Brooks DE, Plummer C, Kallberg M, et al. Corneal transplantation for inflammatory keratopathies in the horse: visual outcome in 206 cases (1993–2007). *Vet Ophthalmol*. 2008;11(2):123–133.
49. de Linde Henriksen M, Andersen PH, Thomsen PD, et al. Equine deep stromal abscesses (51 cases—2004–2009)—Part 1: the clinical aspects with attention to the duration of the corneal disease, treatment history, clinical appearance, and microbiology results. *Vet Ophthalmol*. 2014;17(suppl 1):6–13. http://dx.doi.org/10.1111/vop.12103.
50. Plummer CE, Ollivier F, Kallberg M, et al. The use of amniotic membrane transplantation for ocular surface reconstruction: a review and series of 58 equine clinical cases (2002–2008). *Vet Ophthalmol*. 2009;12(suppl 1):17–24. http://dx.doi.org/10.1111/j.1463-5224.2009.00741.x.
51. Mancuso LA, Lassaline M, Scherrer NM. Porcine urinary bladder extracellular matrix grafts (ACell Vet® Corneal Discs) for keratomalacia in 17 equids (2012–2013). *Vet Ophthalmol*. 2014.
52. Lassaline-Utter M, Miller C, Wotman KL. Eosinophilic keratitis in 46 eyes of 27 horses in the mid-Atlantic United States (2008–2012). *Vet Ophthalmol*. 2014;17(5):311–320. http://dx.doi.org/10.1111/vop.12076.
53. Gilger BC, Michau TM, Salmon JH. Immune-mediated keratitis in horses: 19 cases (1998–2004). *Vet Ophthalmol*. 2005;8(4):233–239. http://dx.doi.org/10.1111/j.1463-5224.2005.00393.x.
54. Pate DO, Clode AB, Olivry T, Cullen JM, Salmon JH, Gilger BC. Immunohistochemical and immunopathologic characterization of superficial stromal immune-mediated keratitis in horses. *Am J Vet Res*. 2012;73(7):1067–1073. http://dx.doi.org/10.2460/ajvr.73.7.1067.
55. Gilger BC, Stoppini R, Wilkie DA, et al. Treatment of immune-mediated keratitis in horses with episcleral silicone matrix cyclosporine delivery devices. *Vet Ophthalmol*. 2014;17(suppl 1):23–30. http://dx.doi.org/10.1111/vop.12087.
56. Bosch G, Klein WR. Superficial keratectomy and cryosurgery as therapy for limbal neoplasms in 13 horses. *Vet Ophthalmol*. 2005;8(4):241–246.
57. Shields CL, Naseripour M, Shields JA. Topical mitomycin C for extensive, recurrent conjunctival-corneal squamous cell carcinoma. *Amer J Ophthalmol*. 2002;133:601–606.
58. Clode AB, Miller C, McMullen Jr RJ, Gilger BC. A retrospective comparison of surgical removal and subsequent CO_2 laser ablation versus topical administration of mitomycin C as therapy for equine corneolimbal squamous cell carcinoma. *Vet Ophthalmol*. 2012;15(4):254–262. http://dx.doi.org/10.1111/j.1463-5224.2011.00982.x.
59. Rayner S, Zyl NV. The use of mitomycin C as an adjunctive treatment for equine ocular squamous cell carcinoma. *Aust Vet J*. 2006;84(1-2):43–46.
60. Plummer CE, Smith S, Andrew SE, et al. Combined keratectomy, strontium-90 irradiation and permanent bulbar conjunctival grafts for corneolimbal squamous cell carcinomas in horses (1990–2002): 38 horses. *Vet Ophthalmol*. 2007;10(1):37–42. http://dx.doi.org/10.1111/j.1463-5224.2007.00489.x.

61. Lassaline M, Cranford TL, Latimer CA, Bellone RR. Limbal squamous cell carcinoma in Haflinger horses. *Vet Ophthalmol*. 2014. http://dx.doi.org/10.1111/vop.12229.
62. Gemensky-Metzler AJ, Wilkie DA, Cook CS. The use of semiconductor diode laser for deflation and coagulation of anterior uveal cysts in dogs, cats and horses: a report of 20 cases. *Vet Ophthalmol*. 2004;7(5):360–368.
63. Gerding JC, Gilger BC. Prognosis and impact of equine recurrent uveitis. *Equine Vet J*. 2015. http://dx.doi.org/10.1111/evj.12451.
64. Deeg CA. Ocular immunology in equine recurrent uveitis. *Vet Ophthalmol*. 2008;11(s1):61–65.
65. Gilger BC, Salmon JH, Na YY, et al. Role of bacteria in the pathogenesis of recurrent uveitis in horses from the southeastern United States. *Am J Vet Res*. 2008;69(10):1329–1335.
66. Polle F, Storey E, Eades S, et al. Role of intraocular *Leptospira* infections in the pathogenesis of equine recurrent uveitis in the southern United States. *J Equine Vet Sci*. 2014;34(11):1300–1306.
67. Fritz K, Kaese H, Valberg S, et al. Genetic risk factors for insidious equine recurrent uveitis in Appaloosa horses. *Animal Genetics*. 2014;45(3):392–399.
68. Gilger BC, Wilkie DA, Clode AB, et al. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. *Vet Ophthalmol*. 2010;13(5):294–300. http://dx.doi.org/10.1111/j.1463-5224.2010.00807.x.
69. Brooks DE, Plummer CE, Carastro SM, Utter ME. Visual outcomes of phacoemulsification cataract surgery in horses: 1990–2013. *Vet Ophthalmol*. 2014;17(suppl 1):117–128. http://dx.doi.org/10.1111/vop.12168.
70. Edelmann ML, McMullen R, Stoppini R, Clode A, Gilger BC. Retrospective evaluation of phacoemulsification and aspiration in 41 horses (46 eyes): visual outcomes vs. age, intraocular lens, and uveitis status. *Vet Ophthalmol*. 2014;17(suppl 1):160–167.
71. McMullen RJ, Davidson MG, Campbell NB, Salmon JH, Gilger BC. Evaluation of 30- and 25-diopter intraocular lens implants in equine eyes after surgical extraction of the lens. *Am J Vet Res*. 2010;71(7):809–816. http://dx.doi.org/10.2460/ajvr.71.7.809.
72. Komáromy AM, Andrew SE, Sapp HL, Brooks DE, Dawson WW. Flash electroretinography in standing horses using the DTL™ microfiber electrode. *Vet Ophthalmol*. 2003;6(1):27–33.
73. Allbaugh RA, Ben-Shlomo G, Whitley RD. Electroretinogram evaluation of equine eyes with extensive "bullet-hole" fundic lesions. *Vet Ophthalmol*. 2014;17(s1):129–133.
74. Willis AM, Robbin TE, Hoshaw-Woodard S, Wilkie DA, Schmall ML. Effect of topical administration of 2% dorzolamide hydrochloride or 2% dorzolamide hydrochloride-0.5% timolol maleate on intraocular pressure in clinically normal horses. *Am J Vet Res*. 2001;62(5):709–713.
75. Germann S, Matheis F, Rampazzo A, Burger D, Roos M, Spiess B. Effects of topical administration of 1% brinzolamide on intraocular pressure in clinically normal horses. *Equine Vet J*. 2008;40(7):662–665.
76. Annear MJ, Wilkie DA, Gemensky-Metzler AJ. Semiconductor diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in horses: a retrospective study of 42 eyes. *Vet Ophthalmol*. 2010;13(3):204–209.
77. Townsend W, Langohr I, Mouney M, Moore G. Feasibility of aqueous shunts for reduction of intraocular pressure in horses. *Equine Vet J*. 2014;46(2):239–243.
78. Wilson R, Dees D, Wagner L, Monheit B, O'Gan D. Use of a Baerveldt gonioimplant for secondary glaucoma in a horse. *Equine Vet Educ*. 2015;27:346–351.
79. Gerding JC, Clode A, Gilger BC, Montgomery KW. Equine orbital fractures: a review of 18 cases (2006–2013). *Vet Ophthalmol*. 2014;17(suppl 1):97–106. http://dx.doi.org/10.1111/vop.12162.
80. Montgomery KW. Equine ocular neoplasia: a review. *Equine Vet Educ*. 2014.
81. Hewes CA, Keoughan GC, Gutierrez-Nibeyro S. Standing enucleation in the horse: a report of 5 cases. *Can Vet J*. 2007;48(5):512.
82. Pollock PJ, Russell T, Hughes TK, Archer MR, Perkins JD. Transpalpebral eye enucleation in 40 standing horses. *Veterinary Surgery*. 2008;37(3):306–309.
83. Utter ME, Wotman KL, Covert KR. Return to work following unilateral enucleation in 34 horses (2000–2008). *Equine Vet J*. 2010;42(2):156–160. http://dx.doi.org/10.2746/042516409X479577.

CHAPTER 18

Disorders of the Skin

Ann M. Rashmir-Raven*

Disorders of equine skin are common and can be both debilitating to the horse and a significant source of frustration for the owner. Horses suffer a broad range of dermatitides including infectious, allergic, heritable, and idiopathic disease; immune-mediated disorders; and cancers. Evaluation of the skin in the equine patient is an important part of the physical examination. Lesions can be identified and treated while they are still small, before they have a negative impact on the horse's welfare. Some skin conditions can make a horse so uncomfortable that they interfere with riding and training, and the occasional disorder can actually be life-threatening.

In addition to providing critical vital functions such as thermoregulation, sensation, and immunosurveillance, the skin on the horse can dictate a significant portion of their value with respect to coat color and pattern and the length and thickness of the mane, tail, and feathers. Because many skin disorders share the same physical characteristics, it can be difficult for the equine veterinarian to diagnose and treat problems without detailed knowledge and a few specific skills and techniques.

STRUCTURE OF THE SKIN AND ADNEXA

The Skin

Skin thickness varies between 1 and 5 mm depending on location. Thickness is greatest on the dorsum and proximal extremities and becomes thinner on the ventrum and distal portion of the extremities. Skin is thickest on the forehead, dorsal neck, dorsal thorax, and base of the tail, and it is thinnest on the ears and in the axillary, inguinal, and perianal areas.[1,2]

The skin consists of two main layers: the epidermis and dermis. The epidermis consists of the basal, spinous, granular, clear, and horny layers. Each layer has different types of cells and differs in function. The basal layer consists of a single column of cells that are attached to the basement membrane. Typical basal keratinocytes have an oval nucleus, prominent nucleolus or nucleoli, and little heterochromatin. The basal keratinocytes contain melanosomes; various metabolic and synthesizing organelles such as mitochondria, lysosomes, rough endoplasmic reticula, and Golgi complexes; and cytoskeletal structures such as keratin intermediate filaments, microfilaments, and microtubules. Structures such as desmosomes and hemidesmosomes that help form the attachment and help to shape the basal layer are also present.[3]

The spinous layer of the epidermis comprises two to four layers. The cells become progressively differentiated as they move outward toward the skin surface. The spinous layer cells first appear polyhedral and then become more flattened as they develop and move outward. The spinous cells contain cellular organelles and keratin filaments. The cells of the uppermost one to two layers of the granular layer contain small, oval, membrane-bound organelles (300 nm) with alternating lamellae called *lamellar granules* (membrane-coating granules, keratinosomes, and Odland bodies) formed in the Golgi region. They contain polar lipids such as phospholipids, glycosphingolipids, free sterols, and other probarrier lipids and hydrolytic enzymes that convert the probarrier lipids into stacks of neutral lipid-rich lamellae that assemble in the intercellular space, coat the surfaces of the cornified cells, and provide a barrier to the permeation of the skin. The spinous cells have abundant desmosomes that consist of adhesion plaques and submembranous plaques involved in cell cohesion.[4,5]

The granular layer consists of highly differentiated cells that are flattened polyhedrons with keratohyalin granules. In this layer of the skin, dephosphorylation and proteinolysis activate filaggrin, which cross-links and bundles with keratin filaments into large macrofilaments. Filaggrin is broken down in the stratum corneum to free amino acids important for the proper hydration of the epidermis. Lamellar granules present in the granular layer aggregate beneath the plasma membrane and fuse with it, opening a channel for the release of granule contents such as polysaccharides, glycoproteins, acid hydrolases, acid lipases, and probarrier lipids into the intercellular spaces. After release, polar lipids are remodeled into hydrophobic, neutral, lipid-rich lamellae that form an effective barrier. The lipid synthesis required for barrier function occurs within all nucleated cell layers of the epidermis. These lipids are stored within epidermal lamellar bodies, which are visible at the level of the spinous layer and continue throughout the granular layer. In the outermost granular layer, the contents of lamellar bodies are secreted into the intercellular domains at the stratum granulosum–stratum corneum interface. Lamellar bodies contain cholesterol, phospholipids, and glucosylceramides, as well as hydrolytic enzymes that convert the newly secreted phospholipids, glucosylceramides, and sphingomyelins into

*The editors and authors acknowledge and appreciate the contributions of Christine A. Rees as a previous contributor to this chapter. Some of her original work has been incorporated into this edition.

free fatty acids and ceramides. Sphingomyelins also act as bioactive regulators and messengers in cutaneous homeostasis and immune-mediated responses. Ceramide is an important metabolite of sphingomyelin and is one of the main lipid components in the skin barrier.[6] Ceramides are critical for barrier function and required for repair. Alterations in epidermal lipids have been linked to atopic dermatitis in humans, and sphingomyelin seems to be a potential player in insect bite allergy of the horse because alterations in its concentrations are associated with changes in clinical signs.[7]

A dense layer of highly cross-linked proteins is deposited inside the inner leaflet of the membrane. Together with the membrane, this boundary is called the *cornified cell envelope*, which is a rigid structure that resists degradation and is rich in glutamyl-lysine isopeptide cross-links. At the uppermost aspect of this layer, substances such as keratins, filaggrin, and the cornified cell envelope are passed on to the cornified cell layer.[4,5]

The cornified layer contains the largest and most numerous cells of any zone in the epidermis. Cells are large, flattened, and polyhedral; overlap with the margins; and associate through interlocking ridges and modified desmosomes. The contents of the cell are limited primarily to disulfide-bonded keratin filaments, highly cross-linked bundles of macrofilaments, and filaggrin. The compact organization of the proteins becomes looser toward the outer epidermal surface. The breakdown of filaggrin to its constituent amino acids in the outer cell layers accounts for the greater water-holding capacity of the cells and the more diffuse organization of the filaments. Urocanic acid is degraded from filaggrin with histidase and absorbs ultraviolet light. Therefore urocanic acid is important in ultraviolet light protection. The pyrrolidone carboxylic acid derived from glutamine is hygroscopic and helps to keep the skin hydrated, even in a dry environment. The preserved trilaminar plasma membrane outside the cornified cell envelope becomes discontinuous and desquamates toward the upper portion of the cornified layer so that the envelope serves as the real cell membrane.[4,5]

The dermis contains cellular elements including fibroblasts, macrophages, histiocytes, eosinophils, and mast cells in the fibrous and nonfibrous matrix. The fibrous matrix consists of collagen and elastic fibers. The nonfibrous matrix consists of glycosaminoglycans and proteoglycans. Blood vessels, nerves, lymphatics, hair, and sebaceous and apocrine glands are found within the dermis. The sebaceous and apocrine glands in the horse are larger and more numerous compared with those in other large-animal species.[4,5]

The area that separates the epidermis from the dermis is known as the *basement membrane zone*. This layer of the skin functions as a scaffold for organization (important for growth and differentiation of keratinocytes) and repair (speeds up reepithelization), acts to regulate selective permeability by only allowing the passage of molecules of the correct charge and molecular size, provides a physical intercellular barrier (tumor containment), and links epithelia to their underlying matrices. The basement membrane consists of structures such as tonofilaments, hemidesmosomes, laminin, and fibronectin; proteins such as collagen; laminin-like molecules such as entactin and nidogen; anionic glycosaminoglycans such as heparin sulfate, anchoring fibrils, oxytalan filaments, elaunin fibers, and microthread-like filamentous threadwork; and large glycoproteins such as fibrillin.[5]

Hair

The hair growth cycle begins in the growing or anagen phase and progresses through an intermediate or catagen phase into a resting or telogen phase (Fig. 18.1). The time period that hairs are in the catagen phase is short, and finding hairs in this stage of the cycle is often difficult using a trichogram (plucking hairs and looking at them under the microscope). A variety of conditions including temperature, photoperiod, genetics, and hormones can affect the hair cycle.[1,2]

Hair follicles are located within the dermis and are associated with sebaceous and apocrine glands. Hair is important in thermoregulation and in the protection of the skin from external factors. Equine skin contains simple primary hairs that do not shed all at once; rather, their hair tends to shed in patches or in a mosaic pattern.[1,2]

Ambient temperature can affect the texture of the hair. In warm temperatures the coat is comprised of thick, medullated hairs, and piloerection aids in cooling the horse. In colder temperatures the coat is comprised of longer, finer, and poorly medullated fibers that provide more insulation from the cold. The hairs of the fetlock, mane, and tail do not generally shed like body hair.[2]

Additional Skin-Related Structures

The horse and other Equidae have skin structures unique to these species, including the ergot, chestnut, and hoof. The ergot is a small mass of cornified tissue located in a tuft of hair on the flexor surface of the fetlock. It is a vestige of the second and fourth digits of extinct Equidae. The chestnut is a mass of horny tissue on the medial surface of the radius and is believed to be a vestige of the first digit. The hoof is the horny covering over the distal end of the third digit and includes the hoof wall, heel bulbs and solar surface (frog, sulci, sole, white line, hoof wall, and bars).

APPROACH TO SKIN DISORDERS

History

A detailed history of the patient can provide the clinician with useful clues to help determine the underlying cause of a dermatologic problem. Fig. 18.2 is an example of an equine dermatologic history form. Obtaining the answer to simple questions, such as, "How long as the horse had the lesion(s)?", can help distinguish a case of equine sarcoid from dermatophytosis.

Similarly, knowing if grooming aids (brushes, curry combs, etc.) are shared may help rule in or rule out contagious or infectious diseases. Current bathing practice is an important consideration; bathing with detergent-based products that are commonly used to deep clean horses or whiten the socks, stockings, or entire body of the horse may strip the skin of its protective fats and oils and contribute to both infectious and allergic processes.

Response to previous therapy is another useful historical clue. The practitioner should determine the dose and frequency of medication administration. If the appropriate drug or drug dose is not used, the patient is unlikely to respond.

Other factors to consider include feed, stabling environment, and supplements or other medications (e.g., wormers, sprays, shampoos). If any of these factors changed before the

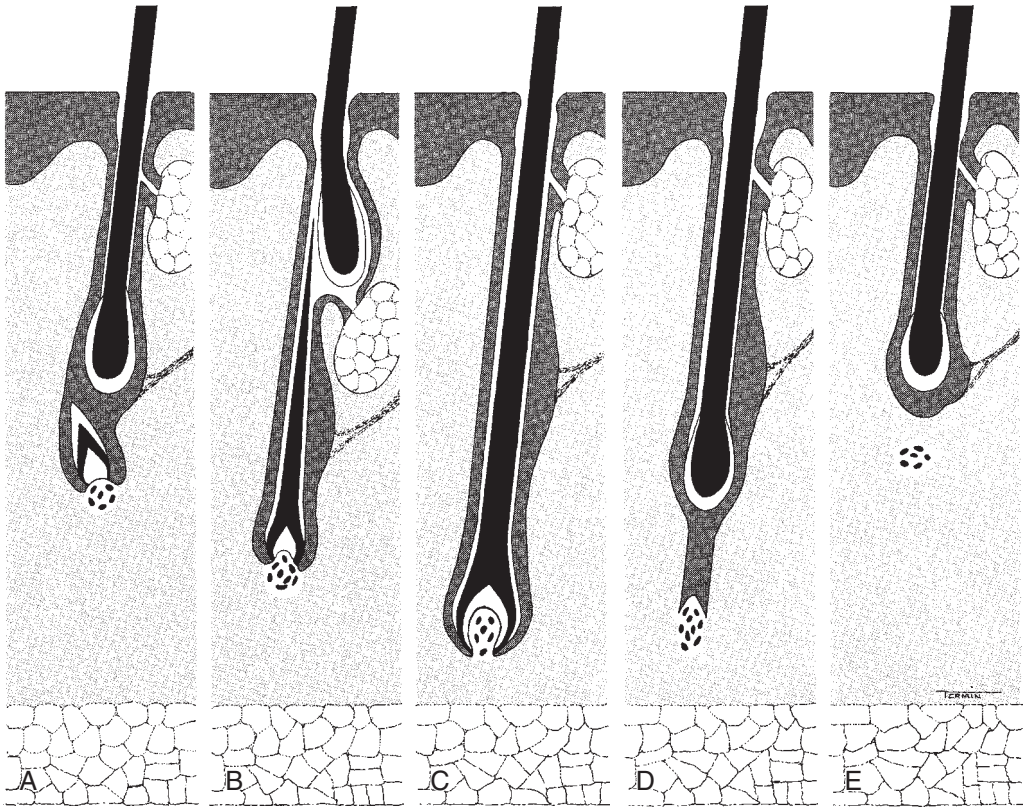


FIG. **18.1** Phases in the life cycle of a hair. (A) Anagen begins with the renewal of the intimate relationship between the papilla and the undifferentiated cells that partially enclose it. (B) As anagen proceeds, matrix cells generate a new hair that pushes upward toward the surface and in the process dislodges the old club hair. (C) Mature anagen hair follicle consists of infundibular, isthmus, and inferior segments. (D) During catagen, the entire inferior segment of the follicle shrivels upward as a thin cord of epithelial cells and is followed upward by the papilla. (E) During telogen, the club hair rests in its cornified sac at the level of the hair erector muscle. (From Moschella SL, Herley HJ. *Dermatology.* 3rd ed. Philadelphia, PA: WB Saunders; 1992.)

development of the dermatologic lesions they may be related to the problem.

Physical Examination

The examiner should consider both the character of the skin lesions and their distribution (Fig. 18.3) when performing a physical examination. Pustules, vesicles, papules, wheals or hives, erythema, and nodules are considered primary lesions. Pustules and vesicles are most common in bacterial and autoimmune skin diseases but can be so fragile that they are not easy to find. Secondary lesions include scales, crusts, excoriations, fistulae, ulcers, necrosis, hyperpigmentation, hypopigmentation, lichenification, hyperhidrosis, and scars.

Lesion location is important because many dermatologic conditions occur with a higher frequency on specific areas of the body. Parasite-related dermatologic problems are a good example of this.[1,2] For instance, pinworms can cause intense pruritus of the tail and perineum, and black flies have a predilection for the head, ears, and ventral abdomen. More detailed information concerning insect feeding preferences is presented later. In addition, special attention is given to mucous membranes, mucocutaneous junctions, the inguinal region, and feathered limbs if they are present; unless these areas are specifically examined, disease processes can be overlooked.

Patient evaluation will often include one or more of the following: grooming for mites and lice; hair sampling for dermatophytes and dermatophilus; skin scraping or a tape prep for mites, bacteria, yeast, and pinworm eggs; swabs for culture; and fine-needle aspirate or biopsy (Boxes 18.1, 18.2, and 18.3).

The clinician should also perform a complete physical examination, determining the temperature, heart rate, and respiratory rate, presence or absence of borborygmi, and quality of digital pulses. If abnormalities exist, the horse may have a systemic disease or more than one medical problem (Table 18.1). For the animal's long-term health and well-being, exploring all problems is important.

DISORDERS OF PIGMENTATION

Skin and hair color depend on melanin production in the skin. Melanocytes produce pigment at the dermal–epidermal junction and in the outer root sheath of the hair follicle. The ratio is one melanocyte for every 10 to 20 keratinocytes.

EQUINE DERMATOLOGY HISTORY FORM

Date _____

Age when purchased _____

What is this horse's use? _____

What is your complaint about the horse's skin? _____

Age of horse? _____ Age when skin problem started? _____

Where on the body did the problem start? _____

What did the skin problem look like initially? _____

_____

How has it spread or changed? _____

Is the problem continual or intermittent? _____

What season did the problem start? _____

Is the problem seasonal or year-round? _____

If seasonal, what seasons is the disease present? _____

_____

Does the horse itch? _____ If so, where? _____

_____

Do any horses in contact with the affected horse have skin problems? _____

If so, are they similar or different from this horse's problem? _____

Do any people in contact with the horse have skin problems? _____

Do you use insect control? _____ If so, describe. _____

_____

Do any relatives of this horse have skin problems? _____ If yes, explain. _____

_____

Please list any injectable, oral, or topical medications that have been used to treat the problem (veterinary or "home remedies"): _____

_____

Did any help the condition? _____ If yes, which ones? _____

Did any aggravate the condition? _____ If yes, which ones? _____

Describe the environment where the horse is kept: Indoors _____

_____ Outdoors _____

What is the horse fed? _____

What feed additives do you use? _____

What is your deworming schedule? _____

Did the horse receive ivermectin? _____ If so, when? _____

List any other major medical problems or drugs that the horse received: _____

_____

List any additional information you feel is relevant to the skin disease: _____

_____

FIG. **18.2** Sample equine history form.

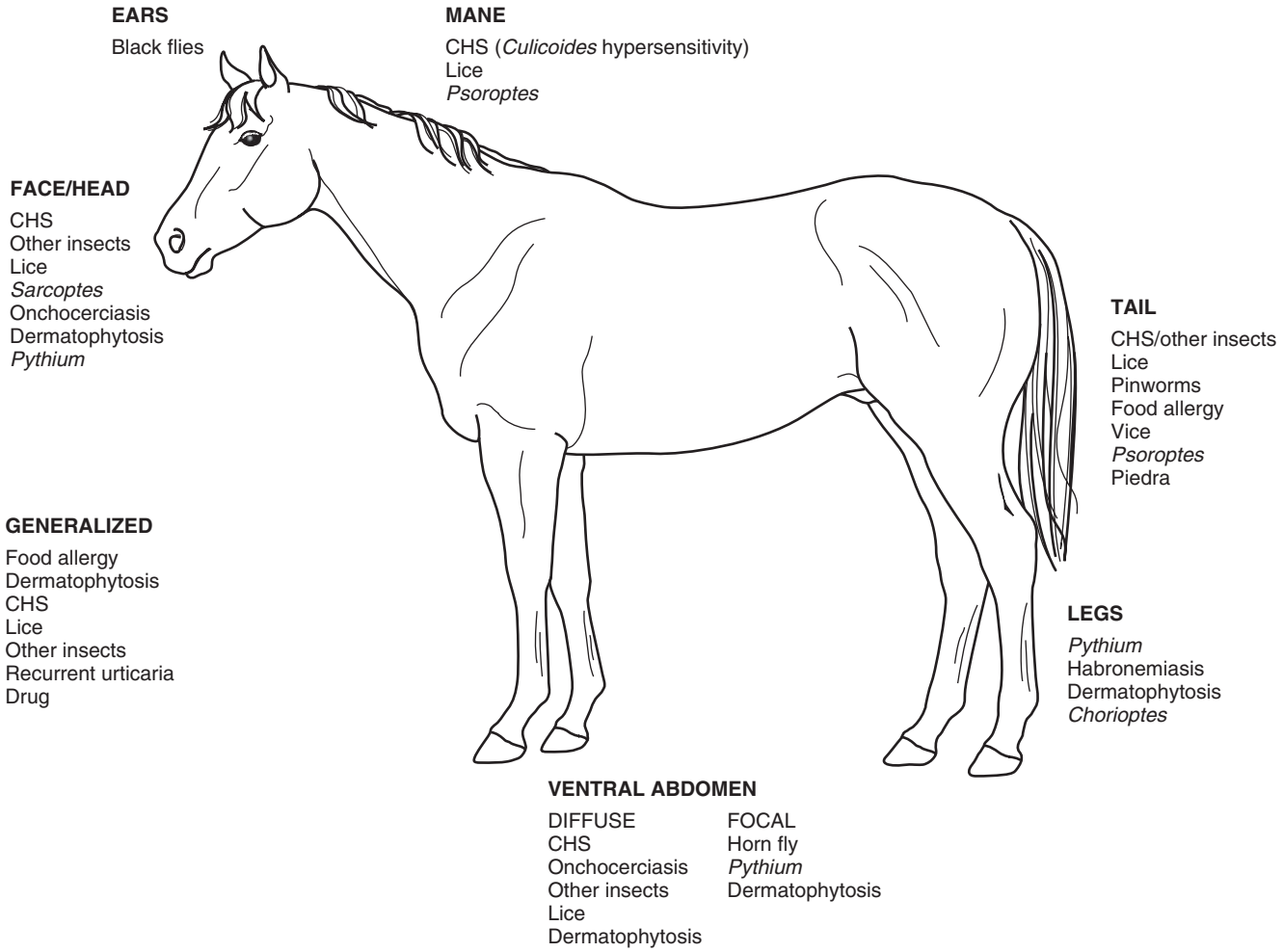


FIG. **18.3** Regional pruritus: differential diagnosis of pruritic skin diseases.

BOX 18.1 Collecting Samples for Fungal and Bacteriologic Culture

BACKGROUND CONSIDERATIONS

- **Indications:** Horses can develop a wide range of bacteriologic and fungal dermatoses. They also maintain a large population of resident bacteria and fungi that can make culture results frustrating if proper sampling technique is not used.
- **Equipment/supplies:** These include dermatophyte test medium (DTM) available from Remel (Lenexa, Kansas) for fungal cultures if planning to culture fungal lesions (see the following sections for culturing fungus from pulled hair samples), sterile needles of any size, and culture swabs with transport medium.
- **Special considerations**
  - Discontinue the use of topical and systemic antibiotics 36 hours prior to sampling whenever possible. Some dermatophytes require a more specialized medium.
  - Swabs taken from the surface of lesions generally have a low diagnostic value.
  - Viral culture or detection requires specialized transport media from the laboratory performing the procedure. Scabs and infected tissue packed in ice are often diagnostically useful for viral based disease.

BASIC PROCEDURE

- Do not cleanse with antiseptic prior to sampling.
- Locate an intact pustule or a recently infected area.
- Use a sterile needle to open the pustule prior to swabbing the lesion.
- Swab and immediately place the swab into transport medium.
- Alternatively, you may swab from the deeper layers of a biopsy specimen or from a fine-needle aspirate.
- If in-house culture is preferred, use the swab to directly plate onto culture medium.
- For deep lesions, a two-punch biopsy technique is the preferred method to obtain deep cultures.

PROCEDURE FOR FUNGAL CULTURES

Fungal cultures should be obtained from the rim of an active lesion. Hairs and scales scraped with a scalpel blade can be placed in a sterile container, such as a red top tube, and sent to the laboratory for culture. Alternatively, the samples can be cultured in-house by placing the plucked hairs directly on fungal culture medium (DTM). Push plucked hair firmly onto the two separate DTM bottles, but do not bury them. Either send the samples to an appropriate laboratory or incubate

Continued

BOX 18.1 Collecting Samples for Fungal and Bacteriologic Culture—cont'd

one sample at room temperature and one sample at 37°C. The samples should be monitored for 14 days, checking daily for color change and growth of fungal colonies. Pathogenic dermatophytes generally cause a red color change in 3 to 5 days and generate powdery or fluffy white or off-white colonies shortly thereafter. Color changes that occur 14 or more days after culture and brown, black, or green colonies are considered negative for pathogenic dermatophytes.

PROCEDURE FOR ANAEROBIC CULTURES

Anaerobic cultures must be inoculated into specialized culture bottles or vials to avoid rapid sample deterioration. Check with your specific laboratory prior to culture.

PROCEDURE FOR DIAGNOSIS OF DERMATOPHILOSIS

It is generally simpler to use a Gram stain on fresh lesions than to culture *Dermatophilus congolensis.* The scab is removed from a fresh lesion, and the underside of the scab is swabbed or pressed to a glass slide for staining. *Dermatophilus* has a characteristic railroad track appearance on Gram stain (2–6 parallel rows of gram-positive cocci); Diff-Quik, Giemsa, and other stains are also suitable choices. Alternatively, *D. congolensis* can be diagnosed on scab histopathology or lesion biopsy. Culture requires a laboratory with the capability to provide a 20% CO_2 environment. Scabs should be collected and moistened with distilled water or sterile saline.

BOX 18.2 Collecting Samples by Fine Needle Aspirate

INDICATIONS

Fine-needle aspirate is useful for diagnosing both solid tumors and fluid-filled cavities such as bullae and abscesses. Leaving the needle in place postaspiration will also serve as a guide for establishing drainage of deep abscesses.

SPECIAL CONSIDERATIONS

The use of a 5-MHz ultrasound transducer will help delineate the appropriate site or sites to aspirate abscesses. Thick-walled abscesses and those surrounded by cellulitis or edema (a common occurrence for abscesses that result subsequent to vaccination or other injections) particularly benefit from ultrasonography to avoid random exploration.

EQUIPMENT/SUPPLIES

Supplies include a 1-inch" or 1½"-inch sterile 19-gauge or 20-gauge hypodermic needle, 20-cc syringe or syringe of similar size, multiple clean glass microscope slides with or without cover slips, and blood culture or transport media if bacterial or viral culture. An ultrasonography will be useful if available in select cases with fluid-filled lesions.

PROCEDURE

- Introduce the needle with the syringe attached to the center of the mass.
- Apply suction for several seconds. Unless fluid is encountered redirect the needle and repeat the aspiration process for three to four attempts. Several passes may be necessary in solid lesions.
- Blood contamination is a common occurrence and should not discourage the veterinarian from submitting the samples. Withdraw the needle and syringe from the mass and then disconnect the needle from the syringe. Draw several cubic centimeters of air into the syringe. Replace the needle on the syringe and force the air from the syringe through the needle to expel needle contents onto clean glass slides.
- Repeat the last two steps as needed to dispel the entire aspirate. It is wise to smear the slides if the preparations are too thick to be read, although this may deform the cells. If significant fluid is encountered, it is introduced into blood culture vials or transport media for bacteriologic culture and sensitivity testing.

ADDITIONAL CONSIDERATIONS

Slide preparations that appear black in color to the naked eye are likely melanomas. If significant amounts of purulent material are encountered the needle may be left in place to direct abscess drainage; an uncontaminated culture may be obtained from the syringe contents.

BOX 18.3 Obtaining a Skin Biopsy

INDICATIONS

Skin biopsy is one of the most useful procedures available to the equine veterinarian confronted with skin disease. Skin biopsies can establish a specific diagnosis, eliminate erroneous diagnoses, and confirm complete tumor excision. A well-performed skin biopsy that is read by a trained dermatopathologist is diagnostic in more than 90% of cases. Even when not diagnostic, skin biopsy can help direct therapy or establish a group of diseases to consider.

EQUIPMENT/SUPPLIES

Supplies include a range of 6- to 9-mm biopsy instruments, standing sedation, ring block materials, sterile gloves, Brown-Adson or rat-tooth forceps, 4 × 4 gauze sponges, vial(s) of 10% neutral buffered formalin, small tissue forceps, needle holders, and suture material (preferably 2-0 nylon on a cutting needle) or 35 mm staples if the biopsy site is to be closed.

BOX 18.3 Obtaining a Skin Biopsy—cont'd

SPECIAL CONSIDERATIONS

- A full history of the horse should always be included with the sample so that the pathologist can appropriately adjust the investigation for maximum benefit.
- Antiinflammatory agents should be discontinued 2 to 3 weeks prior to skin biopsy.
- It is important to perform biopsies on areas that represent primary disease and not those with secondary bacterial infections. Whenever possible, secondary bacterial infections should be eliminated with systemic antibiotics prior to skin biopsy unless noninfected areas are available for biopsy. Multiple samples of lesions in various stages will provide the maximum amount of information. Do not vigorously prepare lesions, particularly if they have a crusting or scaling component; lesions to be used for culture should be rinsed in sterile saline without an antimicrobial scrub. A 10 or 20 scalpel blade with a handle may be necessary for lesions requiring larger biopsy samples, en bloc resection, bullae, pustules and vesicles, and deep sampling.
- Full-thickness biopsies of the coronary band can leave permanent hoof wall defects; therefore, the author recommends shaving coronary bend lesions as a first attempt to get a diagnosis. If shaving the lesion fails, then biopsy is recommended. Alternatively, some horses with immune-mediated disease also have lesions on their ergots or chestnuts. Biopsy of lesions in these areas are often diagnostic and recommended because they will not result in hoof wall issues associated with coronary band biopsy.

PROCEDURE

- Center the biopsy punch over the lesion to be biopsied.
- Rotate the punch in one direction only to avoid shearing artifacts.
- Once a full-thickness sample has been achieved, gently pull the biopsy punch away from the skin.
- Cut any remaining tissue strands with your scalpel blade being careful to obtain a full-thickness biopsy.
- Gently lift the sample off the punch with a 25-gauge needle. Forceps are not recommended because they may crush or otherwise distort the sample.
- Immediately place the harvested sample into formalin or other appropriate fixative. Samples being harvested for culture should be placed directly into blood culture or transport medium. In addition to routine histology, immunohistochemistry; bacterial, fungal, and viral culture; and electron microscopy can be obtained from biopsy specimens placed in the appropriate medium.
- If normal skin is not present on the harvested sample, a specimen of normal, adjacent skin may be used for histopathologic comparison.
- To obtain a *deep* sample for culture or histology the double-punch method can be used. Briefly, an 8-mm punch biopsy is used to access the deep tissue. Once the 8-mm sample is removed, a 6-mm biopsy punch is inserted in the same hole, and the tissue removed from this deeper biopsy is submitted for culture and/or histology.
- A simple interrupted or cruciate suture may be placed to close the punch biopsy site if indicated.
- If the sample is not amenable to punch biopsy, then a wedge biopsy is performed by making an elliptical incision centered over the lesion. Incisions should be placed in a vertical position along the planes of loose skin to avoid tissue compromise whenever possible. Large areas over such regions as the middorsum will have significant tension when closed and may be best left to heal by second intention.
- Extreme care should be exercised to minimize grasping of the sample with tissue forceps or otherwise crushing or damaging the sample.
- Samples larger than 1 cm should be partially transected at 1-cm increments to facilitate fixation.
- Large samples should be pressed subcutis side down onto a wooden tongue depressor prior to immersion to minimize artifacts of fixation. Immerse the tongue depressor and sample together to maintain orientation and minimize folding.
- Wound closure can be achieved with simple interrupted or cruciate sutures or skin staples.
- Ensure adequate tetanus prophylaxis.
- Fly repellents placed around the wound but not in the wound may be appropriate.
- Staples or sutures should be removed in 10 to 14 days.

TABLE **18.1 Skin Diseases with Systemic Manifestations**

Disease	Cutaneous Signs	Systemic Signs
ENVIRONMENTAL		
Gangrene	Wet: moist swelling, discoloration, malodorous, tissue decomposition Dry: dry, discolored, leathery skin	Depends on underlying cause; fever
Burns	Superficial: erythema, edema, pain, vesicles Deep: necrosis, ulceration, anesthesia, scarring	Shock, respiratory compromise
Selenosis	Painful coronary band, sloughing and necrosis of hoof, rough hair coat, progressive loss of long hairs of mane and tail	Lameness, weight loss
Arsenic poisoning	Severe seborrhea, ulcer, nonhealing wounds, hypertrichosis	Gastroenteritis, emaciation, variable appetite
Mercury poisoning	Progressive alopecia	Gastroenteritis, lameness, emaciation

Continued

TABLE **18.1 Skin Diseases with Systemic Manifestations—cont'd**

Disease	Cutaneous Signs	Systemic Signs
Iodism	Severe dry seborrhea with or without hair loss	Cough, variable appetite, joint pain, seromucoid nasal discharge, lacrimation
Hepatogenous photosensitization	Erythema, edema, pruritus, pain, in white or light-skinned area; vesicles and bullae may progress to oozing, necrosis, and sloughing	Acute: hepatic encephalopathy, icterus, depression, decreased appetite Chronic: weight loss, depression, neurologic signs
Ergotism	Swelling of coronary band; necrosis of feet; sloughing of ears, tail, feet	Lameness of hindlegs, fever, weight loss, poor appetite
Leucinosis	Hoof dystrophies, shedding of hair	Laminitis, lameness
Hairy vetch toxicosis	Cutaneous plaques and papules that ooze yellow pus; pruritus; hair loss	Conjunctivitis, anorexia, pyrexia, weight loss
BACTERIAL DISEASES		
Strangles	Limb edema; edema of lips, eyelids; petechial hemorrhages of mucous membranes and sclera	History of acute contagious upper respiratory infection; pyrexia, mucopurulent nasal discharge; abscesses in the mandibular or retropharyngeal lymph nodes
Corynebacterium pseudotuberculosis abscesses	Single or multiple deep abscesses that develop slowly or rapidly; 50% occurring in the pectoral or ventral abdominal area; ventral midline edema	Pitting edema, depression, fever, lameness, internal abscess, prolonged fever, abortion
Dermatophilosis	Exudative crusted lesions on dorsal trunk, face, pastern, or coronets	Depression, fever, lethargy, poor appetite, weight loss, lymphadenopathy; lesions on legs may cause edema, pain, and lameness
Actinobacillosis	Thick-walled abscess of soft tissue	In newborn foals, disease is a highly fatal septicemic disease, and skin lesions are rare
Clostridial infections	Malignant edema: swelling at site of infection, pitting edema, local erythema; skin may become hot to touch, painful, or slough; crepitus Blackleg: hot, painful, swelling that progresses to a cold painful swelling with edema and subcutaneous emphysema	High fever, anorexia, muscle tremors, acute death possible within 24–48 hours
Glanders (farcy) *Burkholderia mallei* (not in United States)	Subcutaneous nodules begin most often on the medial aspect of the hock; lesions rapidly ulcerate and drain a honey-colored material; lymphadenopathy and cording of lymphatics is common	Respiratory infection that rapidly leads to death
FUNGAL DISEASE		
Histoplasma farciminosa (epizootic lymphangitis)	Unilateral nodules on face, head, neck and occasionally trunk; nodules initially are firm but rupture and exude light-green, blood-tinged exudate; large ulcer may form and lesions may spread bilaterally	Lacrimation, conjunctivitis, and respiratory signs may occur
PARASITES		
Lice	Pruritus, scaling, alopecia	Anemia in severe infestations with sucking lice
Black flies	Painful papules and wheals that may become vesicular, hemorrhagic, and necrotic; lesion may be localized to ears or intermandibular areas	Toxin in bite can cause increased capillary permeability; depression, weakness, staggers, tachypnea, tachycardia, weak pulse, shock, and possible death
IMMUNE-MEDIATED DISEASES		
Atopy	Chronic pruritic urticaria, excoriations, alopecia, lichenification	Respiratory difficulty, especially on expiration
Pemphigus foliaceus	Crusts, scales, oozing, annular eruption, matting of the coat; lesions may be limited to the coronary band	Depression, weight loss, poor appetite, pyrexia
Bullous pemphigoid	Vesicles and bullae in mouth, groin, and axilla; crusts, ulcers	Anorexia, depression, fever
Systemic lupus erythematosus	Lymphedema, panniculitis, alopecia, leukoderma; scaling of face, neck, and trunk	Polyarthritis, thrombocytopenia, proteinuria, fever, depression, weight loss

TABLE **18.1 Skin Diseases with Systemic Manifestations—cont'd**

Disease	Cutaneous Signs	Systemic Signs
Transfusion reactions and graft-versus-host disease (may occur in horse after unmatched blood transfusion)	Exfoliative to ulcerative dermatitis, ulcerative stomatitis	Diarrhea, increased heart and respiratory rates, lacrimation, muscle fasciculation
Erythema multiforme	Symmetric maculopapular lesions, urticaria that results in annular arciform or polycyclic shapes; wheals that do not disappear	Occurs after pregnancy, drugs, neoplasia, connective tissue disease, and infections or may be idiopathic
Vasculitis	Purpura, edema, erythema, necrosis, crusts; purpura hemorrhagica causes edema and hemorrhagic swelling in tissue, mucosa, and viscera	May occur after strangles or influenza; depression, fever, reluctance to move, colic, diarrhea
Equine exfoliative eosinophilic dermatitis and stomatitis	Scaling and crusting that progress to generalized exfoliation, alopecia, ulceration, and exudation; variable pruritus	Severe, progressive weight loss; no diarrhea, ravenous appetite
Equine cutaneous amyloidosis	Papules, nodules, and plaques over the head and neck that develop rapidly	Diffuse nodules in upper respiratory tract may cause severe dyspnea
ENDOCRINE DISEASES		
Hypothyroidism	Dull rough hair coat, delayed shedding of coat, edema of face and limb	Anecdotal reports of laminitis, infertility, anhidrosis, anemia, myopathy; weight gain and decreased food intake; skeletal limb disorders have been reported in foals
Pituitary pars intermedia dysfunction	Long shaggy hair that fails to shed; mane and tail are unaffected	Polydipsia, polyuria, muscle wasting, weight loss, lethargy, swayback appearance, pendulous abdomen, blindness, chronic infections, neurologic disorders or signs
SWEAT GLAND DISORDERS		
Anhidrosis	Acute episode: none Chronic: dry hair coat, excessive scaling, partial alopecia, pruritus	Acute: labored breathing, fever, flared nostrils, lack of sweating, collapse, death Chronic: polydipsia, polyuria, poor appetite, loss of body condition
MISCELLANEOUS DISEASES		
Panniculitis	Firm to fluctuant nodules most commonly found on trunk in subcutaneous tissue that ruptures and drains an oily yellow-brown to bloody discharge	Anorexia, depression, lethargy, pyrexia
NEOPLASTIC DISEASES		
Hemangioma and hemangiosarcoma	Two types: well-circumscribed nodules that are blue-black in appearance; dark hyperkeratotic and verrucous lesions that bleed easily	Anemia
Lymphosarcoma	Single or multiple dermal-to-subcutaneous nodules, especially on trunk	Internal organ involvement; usually fatal

Melanin pigments have a wide range of colors, including black-brown eumelanins, yellow-red pheomelanins, and a range of intermediate pigments. Special tyrosinase-rich organelles called *melanosomes,* which are found in the cytoplasm of melanocytes, produce melanin pigment. Tyrosine is converted to dopa, which is oxidized to dopaquinone. The copper-containing enzyme tyrosinase catalyzes both reactions. Subsequent intermediate products polymerize, eventually forming melanin. Melanocytes secrete or inject melanosomes into adjacent keratinocytes. Production of melanin is controlled by genetics, hormones, local keratinocytes, and Langerhans cells. Ultraviolet light, inflammation, byproducts of the arachidonic acid cycle, androgens, estrogen, glucocorticoids, and thyroid hormone also may influence pigmentation.

The number, size, type, and distribution of melanosomes determine skin and hair color. Melanosomes are responsible for coat color, photoprotection, free radical scavenging, and heat conservation.

Hypopigmentation is a decrease in normal melanin pigmentation. Depigmentation specifically refers to a loss of preexisting melanin. Leukoderma and leukotrichia are clinical terms used to describe the loss of color in skin and hair, respectively. Amelanosis is a total lack of melanin.

Hyperpigmentation is almost always an acquired change. The skin and hair may become hyperpigmented. The usual cause of localized or patchy hyperpigmentation is chronic inflammation or irritation. Macular patches of noninflammatory hyperpigmentation, called *lentigo,* can occur in horses. It occurs most frequently at mucocutaneous junctions. The most important differential diagnosis for lentigo is cutaneous melanoma. A skin biopsy of the affected area is the most useful diagnostic test. Generalized hyperpigmentation of the coat or skin has not been reported; however, if observed, the condition may suggest an underlying hormonal disorder.

Overo Lethal White Syndrome

Overo lethal white syndrome (OLWS) is a leucism-associated disorder caused by a mutation in endothelin receptor B.[8] Despite what the name implies, the syndrome can be produced by mating solid-colored horses, although the incidence of producing an abnormal foal is much lower.[9] Color patterns with highest incidence (>94%) of heterozygotes are frame overo, highly white calico overo, and frame blend overo. White-patterned bloodlines with the lowest incidence of heterozygotes (<21%) are tobiano, sabino, minimally white calico overo, splashed white overo, nonframe blend overo, and breeding-stock solid.[9] Although a small number of white foals produced by these matings have normal gastrointestinal function, most of these foals are affected by myenteric aganglionosis, which results in fatal functional intestinal obstruction. DNA testing is recommended before breeding at-risk horses. Albinism, which can be mistaken for OLWS, is rare in horses and transmitted by an autosomal dominant gene. Affected animals have white skin and hair and hypopigmented irides and are photophobic.

Leukoderma

Leukoderma develops in areas of previous trauma or inflammation and may be temporary or permanent. Affected areas appear normal but depigmented. Leukoderma occurs commonly in horses with onchocerciasis, lupus erythematosus, pressure sores, ventral midline dermatitis, viral skin diseases, freezing, burns, or sun damage. Leukoderma can result from the contact of skin with chemicals that inhibit or interfere with melanogenesis, such as phenol and rubber products containing monobenzyl ether or hydroquinone.

The diagnosis is usually made by clinical signs, but elliptic biopsy of the junction of the pigmented and nonpigmented area confirms the diagnosis. Leukoderma causes no discomfort to the horse, but it may be a source of great agitation to the owner. No treatment is known.

Vitiligo of Arabian Horses

The term *vitiligo* refers to an idiopathic acquired depigmentation. Leukoderma develops in areas with no known history of previous trauma. Vitiligo can occur in any age, gender, or breed of horse but is most common in the Arabian for which it has been termed *Arabian fading syndrome* or *pinky syndrome* (Fig. 18.4). Vitiligo appears to be more common in pregnant or postpartum mares, suggesting a hormonal or stress influence. The condition may be hereditary.

With vitiligo, annular areas of depigmentation develop symmetrically on the muzzle, face, lips, and periocular areas. Depigmentation also can occur at the mucocutaneous junctions of the genital and anal area. Areas of depigmentation



FIG. **18.4** Horse with Arabian fading syndrome. Depigmented areas on the face are notable.



FIG. **18.5** Reticulated leukotrichia in a Quarter Horse.

vary between 1 mm to 2 to 3 cm in size, and spots may become confluent at the mucocutaneous junctions. Depigmentation may wax and wane, and rarely the skin repigments completely within 1 to 2 years.

History, physical examination, and skin biopsy are diagnostic. Skin biopsy shows complete absence of epidermal melanin and lack of an inflammatory infiltrate. There is limited to no success in treatment with topical glucocorticoids or vitamin-mineral supplementation (i.e., copper), and the condition is often considered heritable.

Leukotrichia

Leukotrichia is an acquired loss of pigment in hairs that occurs most commonly in areas of previous trauma or inflammation. Leukotrichia may also occur at the site of previous injections with a variety of compounds including dexamethasone and lidocaine with epinephrine.

Reticulated leukotrichia may be an inherited disorder in Quarter Horses, Thoroughbreds, and Standardbreds but can also occur in other breeds (Fig. 18.5).[10] There is no gender predilection. Affected horses begin to show clinical signs as yearlings. Linear crusts in a netlike to crosshatched design develop over the dorsum from the withers to the tail head region. Crusts are shed, and after the temporary alopecia resolves, new hair growth is white. The underlying skin is normal, and leukotrichia is permanent. This type of leukotrichia generally



FIG. **18.6** Spotted leukotrichia ("bird catcher" spots) on a 12-year-old Morgan gelding.

causes no discomfort to the horse or interference with training or use of the animal.

Spotted leukotrichia is a similar condition observed most commonly in Arabian horses, Quarter Horses, and Warmbloods. Permanent spots of leukotrichia develop over the hindquarters and trunk. They can vary in size and occur either as a single area or multiple large areas of white skin that can increase as the animal ages or can occur as small areas of leukotrichia that gradually increase in numbers as the animal ages. This type of presentation is often known as *bird catcher spots* (Fig. 18.6). On rare occasion, lesions resolve spontaneously. No treatment is known.

Hyperesthetic leukotrichia is a rare and painful form of leukotrichia. Single or multiple crusts that are intensely painful characterize this disorder. Lesions develop on the dorsal midline from the withers to the tail head. Within a few weeks, white hairs develop in the areas of crusting. The intense pain and crusting may last weeks to months. Eventually, lesions subside, but leukotrichia is permanent. The most common histologic features are the presence of large stellate cells, edema in the superficial dermis, perivascular to diffuse lymphocytic inflammation, pigmentary incontinence, apoptotic keratinocytes, and vesicle formation. The condition primarily affects Arabian and American Paint horses, occurs mainly in summer, and may spontaneously recur. No treatment is known, and large doses of glucocorticoids have not been of value.

DISORDERS OF HAIR

Horses have simple hair follicles, which are accompanied by a sebaceous and sweat gland and an arrector pili muscle. Photoperiods and ambient temperature predominantly govern the hair cycle. Nutrition, hormones, genetics, and general well-being also may influence hair growth. Hair replacement in the horse occurs in a random pattern (mosaic) with the exception of the coarse permanent hair of the mane, tail, and fetlock.

Hair Follicle Abnormalities

Main and Tail Dystrophy

Mane and tail dystrophy may be congenital or acquired.[10] Whether the condition is hereditary is unknown. Affected horses (juvenile or adult) have short, brittle, dull hairs on the mane and tail. No treatment is known, but the condition does not affect the health or function of the horse.

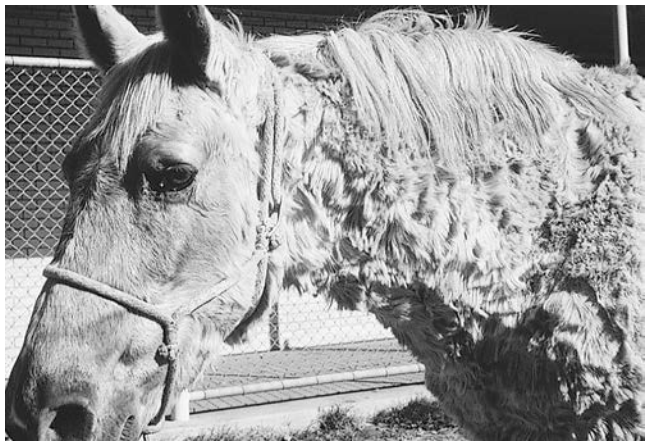


FIG. **18.7** Horse with pituitary pars intermedia dysfunction. The long hair coat did not shed in the spring.

Black or White Follicle Dystrophy

Affected horses (juvenile and adult) have abnormal hairs growing in patches of white-haired or black-haired areas. These hairs are short, brittle, and dull. Affected areas may be hypotrichotic. No treatment is available.

Curly Coat

Curly coat is a hereditary condition of Percherons, Missouri Fox Trotters, and Bashkir Horse breeds that is inherited as an autosomal recessive trait. Clinically affected horses have unusually curly coats.

Trichorexis Nodosa

Trichorrhexis nodosa is an acquired hair shaft disorder of horses caused by physical or chemical trauma. Overzealous grooming, shampoos, pesticides, alcohol, or solvents are the most common causes. The lesions are visible without magnification and appear as small white to gray nodules on the hair shaft. The hair shaft fractures and breaks easily at these sites. Microscopically, affected hairs have the appearance of two brooms shoved together. Therapy consists of identifying and eliminating the trauma.

Alopecia Areata

Alopecia areata is a rare idiopathic skin disease of horses characterized by focal areas of alopecia.[11] Lesions may be single or multiple, and the underlying skin is otherwise normal. If and when hair regrows, it may be a different color. The diagnosis is made by biopsy. Classically, in early lesions, skin biopsy shows an accumulation of lymphoid cells around the proximal end of the anagen hair follicles. This change is diagnostic but may require numerous biopsies before being observed. The prognosis is uncertain, but most horses recover within a year.

Hirsutism

Hirsutism, an excessive growth of hair with failure to shed appropriately, most commonly occurs secondary to equine pituitary pars intermedia dysfunction (PPID) as discussed in detail in Chapter 16. Abnormal pituitary function results in abnormal secretion of numerous hormones. Clinical signs are most common in older horses (12–15 years old or older), but the condition is reported occasionally in horses 7 to 10 years of age. The first clinical sign noted by an owner may be a failure to shed the coat (Fig. 18.7); however, only 10% of horses

with PPID actually demonstrate hirsutism. The coat becomes shaggy, and body hairs can reach lengths of 10 to 12 cm. Mane, tail, and fetlock hairs are unaffected. Secondary seborrhea may develop. Horses may also demonstrate abnormalities in their ability to sweat. Affected horses may be predisposed to dermatophytosis, dermatophilosis, or secondary bacterial pyoderma. Nondermatologic signs include polydipsia, polyuria, muscle wasting, lethargy, weight loss, pendulous abdomen, laminitis, and flaccid muscles. Neurologic disorders and blindness may develop.

The diagnosis is based on clinical signs and laboratory test results. Complete blood counts can reveal neutrophilia, lymphopenia, and eosinopenia. Urinalysis may reveal a low specific gravity and occasionally glucosuria. A serum chemistry panel may reveal any combination of hyperglycemia, hypercholesterolemia, and lipemia. The definitive diagnosis and treatment of PPID in horses are discussed elsewhere in Chapter 16.

Anagen Defluxion and Telogen Defluxion

Anagen defluxion refers to a condition in which a disease or drug interferes with anagen hair growth resulting in abnormal hairs or hair shaft abnormalities or both.[12] Hair loss occurs within days of the insult or drug. The condition occurs most commonly in horses with high fevers, systemic illnesses, or malnutrition (Fig. 18.8). Typically the mane and tail are not affected.

Telogen defluxion refers to a condition in which a stressful situation causes the sudden cessation of anagen hair growth and the sudden synchrony of many hair follicles in the telogen hair cycle stage.[13] Two to 3 months later a sudden shedding and a wave of new hair growth occur.

These conditions cannot be distinguished clinically. The diagnosis is based on examining the hair shaft of easily epilated hairs. Anagen defluxion is characterized by dysplastic hairs; the hair shaft may be weak or narrow, and the root end contains a root sheath. Telogen defluxion is characterized by uniform hairs with no shaft abnormalities and a nonpigmented root end lacking a root sheath. Both conditions resolve spontaneously when the animal recovers from the predisposing condition.



FIG. **18.8** Foal with telogen defluxion after illness.

Other Abnormalities of Shedding

Abnormal shedding in horses may result in large areas of alopecia that develop on the face, shoulders or over the entire body during an inappropriate time of the year (Fig. 18.9). The pathogenesis is unknown. Skin biopsy is recommended to eliminate infectious or parasitic causes of hair loss. Skin biopsy is typically normal and shows normal hair follicles in various stages of development. Horses may spontaneously recover but abnormally shed the following year. Treatment with melatonin (40 mg per horse per day PO) and thyroid hormone may be beneficial. Thyroid hormone has a hair growth stimulating effect even in the presence of normal thyroid function.

DISORDERS OF KERATINIZATION

Seborrhea

Seborrhea is a descriptive term for excessive scaling and crusting with or without greasiness, and the diagnosis is based on these characteristics. Primary seborrhea is a rare disorder of keratinization with a possible genetic predisposition in which epidermal cell turnover time and basal cell proliferation increase. Secondary seborrhea may be precipitated by any of a variety of underlying conditions such as dermatophytosis, dermatophilosis, or any inflammatory, infectious, or parasitic diseases.

Idiopathic or primary seborrhea in horses may be generalized or localized to the tail and mane. No age, gender, or breed predilection exists. Generalized seborrhea appears clinically as diffuse matting of the coat (Fig. 18.10). The texture of the coat may be oily and thick; adherent crusts are easily removed. Oily or dry scales are present at the base of the hairs. The animal may be odoriferous if affected with the oily form of the disease.



FIG. **18.9** Alopecia in a 14-year-old pony. The pony was trace-clipped and did not regrow hair for more than a year. There is also alopecia present on the point of her shoulder due to blanket rubbing.

Primary seborrhea of the mane and tail is more common and characterized by crusts or scales attached to the bases of hairs. Pain and inflammation are generally absent.

Primary seborrhea is a diagnosis of exclusion. Severe generalized seborrhea must be differentiated from pemphigus foliaceus (PF). Skin scrapings, fungal cultures, dermatophilosis preparations, and skin biopsies are indicated for all horses with suspected seborrhea. Skin biopsies may help in determining whether primary or secondary seborrhea is present. In early cases of primary generalized equine seborrhea involving little to no confounding inflammation from an opportunistic skin invader, skin biopsy findings strongly suggest a primary keratinization disorder. A significant orthokeratotic hyperkeratosis is attached to an epidermis of normal thickness. The superficial dermis shows only a noncornified epidermis, suggesting a keratinization defect. In horses with primary seborrhea that develop a secondary source of inflammation or in which the seborrheic condition is more long-standing, biopsy findings are more difficult to interpret. The cornified epidermis is acanthotic with significant orthokeratosis or parakeratotic hyperkeratosis. Superficial perivascular inflammation is often present in the superficial dermis. Fortunately, generalized primary seborrhea is rare. It tends to be symmetric, spare the appendages, and be nonpruritic unless an opportunistic bacterial or yeast infection is present. Both dry and oily forms of primary seborrhea have been described.[1,2]

Primary seborrhea is usually incurable, but satisfactory management can often be achieved with antiseborrheic shampoos. The owner should be instructed to wash the horse with a cleansing shampoo first to remove excess dirt and scale both to improve efficacy of the medicated shampoo and to decrease the amount of medicated shampoo required, thus minimizing potential for a contact reaction. Several types of shampoos may need to be explored before the most suitable shampoo is identified for an individual patient. Often, dry seborrhea responds best to sulfur-based shampoos, whereas oily seborrhea is frequently best treated with a degreasing agent such as tar-based, benzoyl peroxide, or selenium sulfide shampoos. In horses with mildly dry and flaky skin moisturizing shampoos and rinses may be adequate. Wherever possible veterinary formulations of these shampoos are recommended. Most products recommend a contact time of 10 to 15 minutes before rinsing. Thoroughly rinsing the horse is always important with any shampoo, especially in the axillary and inguinal regions, because shampoo residues commonly irritate the skin. The horse may benefit from daily shampooing for the first 1 to 2 weeks. Once the coat is normal, the owner may decrease shampooing to twice a week for the life of the horse. If shampooing dries the coat and exacerbates the seborrhea, a moisturizing spray or lotion should be used.[1,2]



FIG. **18.10** Horse with primary seborrhea.

Secondary seborrhea is much more common than primary seborrhea, and treatment requires elimination of the predisposing cause. The secondary seborrhea will then generally resolve within 1 to 2 months.

Cannon Keratosis

Cannon keratosis ("stud crud") is an idiopathic disorder characterized by the presence of plaques and patches of hyperkeratosis on the dorsal skin of the cannon bones. The rear limbs are involved much more often than the front limbs. There is no recognized age, breed, or gender predilection. Clinically, well-circumscribed plaques of tightly adherent crusts and scales with or without alopecia develop. The lesions are not pruritic.

The diagnosis is usually made by clinical examination, and skin biopsy is usually not required. Dermatophilosis and dermatophytosis should be ruled out. Treatment consists of antiseborrheic shampoos. Topical glucocorticoids may be useful. Topical vitamin A cream (tretinoin [Retin-A cream 0.1%; Ortho Pharmaceutical Corporation, Raritan, NJ]) may be beneficial but should be used judiciously.

Linear Keratosis

Linear keratosis is a fairly uncommon idiopathic disorder of keratinization. The disorder affects all ages and types of horses but Quarter Horses and Thoroughbreds may be overrepresented. Lesions develop between 1 and 5 years of age. One or more painless, nonpruritic unilateral, vertically oriented bands of alopecia and hyperkeratosis develop on the neck, forelimb, or lateral thorax. Lesions vary in size, ranging between 0.25 and 3.5 cm in width and 5 and 70 cm in length.

Diagnosis usually is based on clinical signs alone. Skin biopsy is sometimes recommended to rule out equine sarcoid, which can also occur in multiple sites in the same horse. Biopsy reveals regular, irregular, or papillated epidermal hyperplasia with significant compact orthokeratotic hyperkeratosis. Lymphocytic mural folliculitis may or may not be present.[14] Linear keratosis does not usually interfere with the function of the horse, and no treatment is necessary; surgical excision of lesions is generally not recommended because the disease will continue to progress. Topical vitamin A cream or salicylic cream may be useful keratolytics to decrease the height, width, or thickness of the keratosis, but they should be used judiciously.

DISORDERS OF COLLAGEN FORMATION

Hereditary Equine Dermal Asthenia

Hereditary equine dermal asthenia (HERDA; hyperelastosis cutis, dermatosparaxia, Ehlers-Danlos syndrome) is a specific form of Ehlers-Danlos syndrome that occurs in Quarter Horses and horses of Quarter Horse lineage including American Paint Horses and Appaloosas. HERDA is caused by a missense mutation of the peptidyl-prolyl cis-trans isomerase B gene,[15] which encodes cyclophilin B and is important in the

formation of collagen's triple helical molecule. The mutation has an autosomal recessive mode of inheritance.[16,17] Similar conditions have occurred sporadically in other breeds such as Thoroughbreds, Hanoverians, and Haflingers, but they do not share the same mutation as the Quarter Horse. Not all Quarter Horses with Ehlers-Danlos share the HERDA mutation. HERDA has become a significant concern in the Quarter Horse industry because of the high frequency of heterozygote carriers. Although HERDA occurs most frequently in cutting horse bloodlines with a carrier frequency of 28.3%,[17] horses competing in other disciplines, including reining and western pleasure, have also produced a significant number of affected individuals (9.3–12.8% carrier frequency).[17] Analysis of pedigree information from the cutting horse industry indicates that 30% of the 10 leading lifetime sires (leading lifetime cutting horse sires, all ages, all divisions) are confirmed carriers. The performance traits of these select carriers' bloodlines are highly desired, likely increasing the prevalence of HERDA.[18]

Affected homozygous horses appear normal at birth; within the first 2 years of life, however, they usually acquire loose, hyperextensible skin and wounds that result in disfiguring scars either spontaneously or from minor trauma (Fig. 18.11). Some severely affected horses also develop hematomas and seromas (Fig. 18.12). The negative effect of sunlight on the abnormal collagen has been reported as the mechanism by which most of the lesions in HERDA horses are dorsal in distribution. In addition to skin lesions, select horses affected with HERDA can be hypermobile. Most affected horses studied also suffer from abnormalities of the cornea, heart valves, tendons, ligaments, bones, and great vessels. Consequently, most affected horses are euthanized at an early age. No treatment options other than palliative therapy, which includes sunlight restriction, currently exist. The American Quarter Horse Association currently requires DNA testing for HERDA on all breeding stallions. There are currently no restrictions on the registration of horses that are heterozygous or homozygous for the HERDA mutation. Because of the autosomal recessive nature of the disease, Quarter Horse mares and horses of all breeds from HERDA-associated bloodlines should also be tested.



FIG. **18.11** Horse with severe hyperelastosis cutis.



FIG. **18.12** Horse with mild hyperelastosis cutis.

The diagnosis is based on clinical signs, physical examination, and DNA testing. When a skin biopsy is suggestive of the disease the horse should be DNA tested to confirm the diagnosis. Early lesions of HERDA show mild thinning of collagen bundles (hyperextensible areas) in the middermis.[19] As lesions progress, increased space between collagen bundles, loss of bundle compactness, and collagen bundle fraying are observed. In more advanced lesions (depending on the stage of resolution), dense inflammation or collagenous connective tissue appears to expand easily, filling a defect in the intermediate layer of the dermis. In lesions of chronically detached skin, collagen bundles become frayed with wear between the superficial and deep layers.[19] Significantly, in the absence of infection or perforation of the skin, HERDA lesions have a paucity of inflammatory infiltrate and show little evidence of physiologic repair. Biopsies taken from areas in which the skin is ulcerated often show extensive inflammation and fibrovascular proliferation, which can obscure the diagnosis. Zonal dermal separation is a distinctive histopathologic lesion associated with HERDA but will not be present in all biopsy specimens.[20] Treatment is palliative and includes sunlight restriction, dietary modification, and protection from trauma. Although most affected horses are euthanized because their fragile skin impairs their usefulness, there are a number of documented HERDA-affected horses that are able to compete successfully despite being homozygous for the condition. The prevalence and economic value of Quarter Horses that carry the genetic defect responsible for HERDA ensure that this disease will be observed for many more generations. The effect of the cyclophilin B genetic mutation on the heterozygote remains to be determined. Managed breeding strategies based on DNA testing of horses or embryos is the only method currently available to minimize the production of affected horses.

Other Collagen Disorders

Other disorders of collagen in horses include Warmblood fragile foal syndrome (WFFS) type 1[21] and junctional epidermolysis bullosa. WFFS is inherited as an autosomal recessive disorder that is caused by a point mutation in the equine procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (lysyl hydroxylase 1) gene. Affected foals are born with thin, friable skin that is characterized by tearing and ulceration from minor trauma. Small skin lesions can occur anywhere on the body but are most noted on pressure points. Lesions may also be found on the mucous membranes. Joint laxity may be so severe that it prevents foals from standing normally. In one case report the foal was born with an open abdomen. Affected foals are humanely euthanized. Histologic examination demonstrates abnormally thin dermis and markedly reduced amounts of dermal collagen

In junctional epidermolysis bullosa type 1 (JEB-1), which affects French and Belgian Draft horses,[22-25] affected foals develop blisters, erosions, and ulceration of the skin and mucous membranes shortly after birth. These lesions quickly grow larger, creating patchy skin loss over the foal's entire body. Dental abnormalities are commonly present and include early eruption of teeth and enamel pitting. Affected foals have been known to slough their hooves. Junctional epidermolysis bullosa results in secondary sepsis, which is universally fatal. The disease is caused by an insertion in LAMC2 and has autosomal recessive mode of inheritance.

Junctional epidermolysis bullosa type 2 (JEB-2) is similar to JEB-1 and affects American Saddlebreds. It is caused by a partial deletion in the *LAMA3* gene.[24] Other skin fragility syndromes in foals include vasculitis and sepsis, which are often treatable.[26]

INFECTIOUS DISEASES OF SKIN

Bacterial Diseases

Dermatophilosis

Dermatophilosis (rain scald or rain rot) is one of the most common bacterial infections of equine skin worldwide. Infections are an acute or chronic superficial pyoderma caused by the gram-positive, facultative anaerobic actinomycete *Dermatophilus congolensis.*[27] Clinical signs of dermatophilosis include regional or generalized tufted papules and crusts that can resemble small paintbrushes. Exudate may be present under the crusts in early lesions. Lesions commonly occur on the dorsum, face, neck, and distal extremities (Fig. 18.13). Severely affected horses and those with secondary bacterial infections may be febrile, depressed, lethargic, anorectic, and have a regional lymphadenopathy.

Carrier animals are the primary source of infection. Factors that affect the ability of the host's immune system to respond to infection, such as concurrent disease and/or stress, may enable the organism to proliferate and produce clinical disease. Lesions develop when the skin is exposed to chronic moisture and sustains damage.[1,2] Moisture is required for release of the infective, motile, flagellated zoospores. The zoospores are attracted to low concentrations of carbon dioxide and migrate into the skin in which they can remain viable in crusts at ambient temperatures of 28°C to 31°C for up to 42 months.[28]

Dermatophilosis is diagnosed by demonstration of the organism in direct smears of the exudate, histopathology, or a preparation made from dried or fresh crusts. Dried crusts are finely minced in a few drops of sterile saline, the preparation is allowed to dry for 45 minutes, and the preparation is stained. A fast Giemsa, Diff-Quik, or Gram stain can be used for direct smears or dried mince preparations. The organism is visualized best under 1000× oil immersion and appears as fine-branching multiseptate hyphae with transverse and longitudinally arranged cocci (railroad track appearance; Fig. 18.14). When a skin biopsy is collected from a horse with suspected dermatophilosis, submitting a specimen with the crust attached to the skin or hairs is important. Key histologic findings include folliculitis, intraepidermal pustules, intradermal edema, and alternating layers of parakeratotic (epidermal cells with retained nuclei) and orthokeratotic (keratinized epidermal cells without nuclei) hyperkeratosis with leukocyte debris.[2] The organism is often found only in crusts, and, if needed, the crust can be submitted instead of a full biopsy specimen

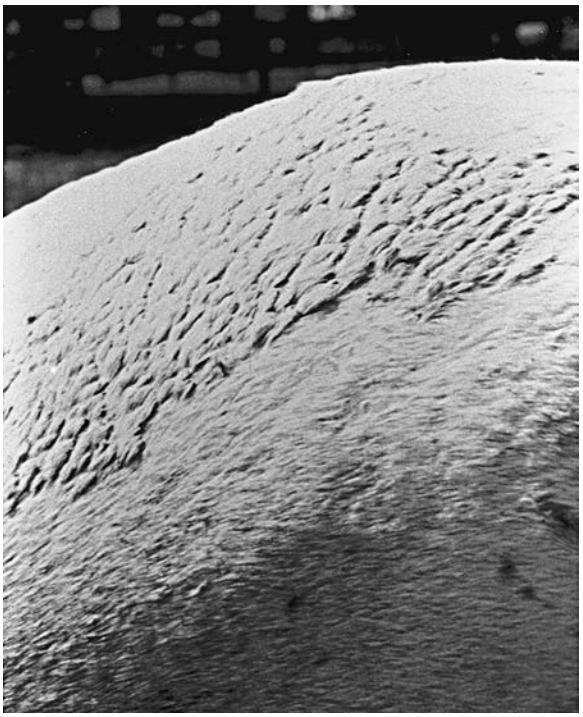


FIG. **18.13** Horse with matting of coat caused by dermatophilosis.

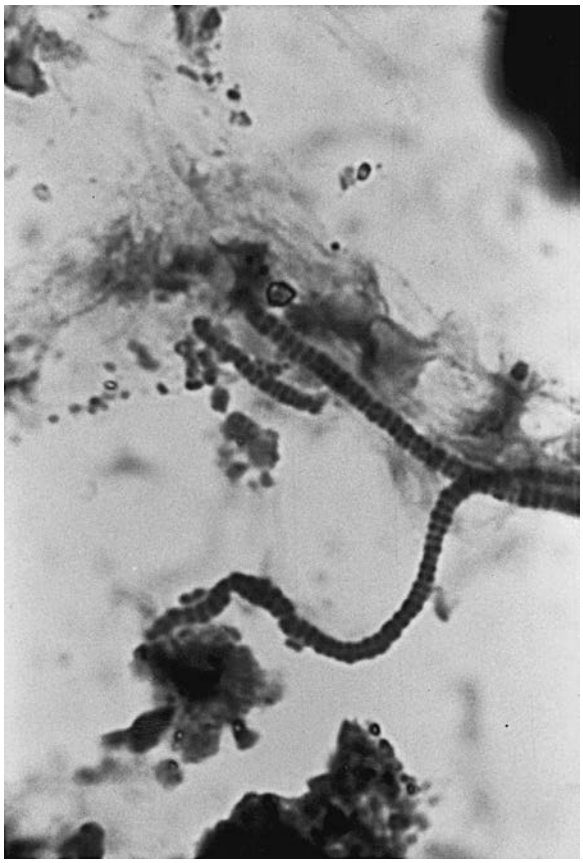


FIG. **18.14** Microscopic appearance of *Dermatophilus congolensis.*

if the history, signalment, and physical examination support a diagnosis of dermatophilosis.

Horses with mild infections can be treated by restricting their contact with moisture and providing topical antimicrobial therapy. The crusts are gently soaked and removed while bathing with a mild antibacterial shampoo such as chlorhexidine. For some horses this is quite painful and may require sedation. The horse should be dried with towels and a topical antibacterial product applied. Severely affected animals require systemic antibiotics such as procaine penicillin at 22,000 IU/kg IM twice daily for 5 to 7 days. Trimethoprim sulfa (TMS) at 10 to 15 mg/kg orally twice daily for 5 to 7 days also may be effective. In vitro sensitivity profiles suggest that doxycycline is a reasonable option for treatment of refractory cases of dermatophilosis. Regardless of all other treatment, exposure to excessive moisture must be controlled. In farm outbreaks of dermatophilosis, inadequate nutrition is a common finding, and the correction of dietary inadequacies is often enough to resolve ongoing issues.

Staphylococcal Pyoderma

Coagulase-positive *Staphylococcus* spp. are another common cause of superficial pyoderma in horses. *Streptococcus* spp. and *Corynebacterium pseudotuberculosis* can cause superficial pyodermas albeit less frequently. *Corynebacterium* infections are more commonly associated with deep pyodermas. Infections most frequently occur in the summer when heat, moisture, increased insect populations, and increased use of the horse act in concert as predisposing factors.

The pathogenesis of bacterial folliculitis in the horse is similar to that in other animals. The causative agents are normally found on the host; when the natural protective barrier of the skin is compromised, bacteria invade and multiply within hair follicles. Infection results in inflammation, destruction of the hair follicle, and shedding of the hair. In addition to the resulting alopecia, staphylococcal pyodermas often present with crusts, which can make the infection difficult to distinguish from dermatophytosis and result in staphylococcal pyodermas being underdiagnosed. Bacterial lesions tend to be more likely painful and pruritic. Infection can occur in the saddle and tack area but may be limited to the pastern on which it is a common component of the pastern dermatitis complex. Some horses with superficial (and deep) pyodermas may be in sufficient pain to present for evaluation of musculoskeletal lameness.

The definitive diagnosis of staphylococcal pyoderma is made by bacterial culture, but cytologic examination and skin biopsy will also lead to the diagnosis, sometimes more efficiently. Skin biopsy reveals varying degrees of folliculitis, intraepidermal pustules, and perivascular inflammation. Neutrophils are the primary inflammatory cell. Keratinocytes often show intracellular edema; parakeratosis and orthokeratosis are common in the crust. The bacteria can sometimes be seen within the crusts and hair follicles.

Treatment depends on severity of the clinical signs. Mild infections may be self-limiting, but moderate infections will benefit from topical therapy. Severe cases require both topical treatment and systemic antibiotics. Ideally, systemic antibiotic therapy should be based on the results of culture and sensitivity testing. Because methicillin-resistant *Staphylococcus aureus* has been cultured from horses the veterinarian should exercise caution when evaluating these horses and obtaining samples for culture. Empirically, TMS is often used as a first-line antibiotic, in part because of its spectrum of activity but also because of its ease of administration, with 15 to 30 mg/kg administered orally every 12 hours. If resistance is encountered, 2.2 mg/kg ceftiofur sodium (IM q 12 h) is often a good choice.

Corynebacterial Infections

C. pseudotuberculosis is a gram-positive, facultative, intracellular, pleomorphic bacterium with a worldwide distribution associated with many clinical forms in horses: the most common are external abscesses in the pectoral region (pigeon fever) or ventral abdominal area, internal abscess formation, and an ulcerative infection of the limbs (ulcerative lymphangitis).

Infections with *C. pseudotuberculosis* are on the rise, with infected horses being identified throughout the United States and parts of Canada. Originally reported as seasonal infections of the Western United States that occurred most often during dry, dusty conditions in the summer and fall concurrent with peak fly season, this disorder is now reported all months of the year and from horses of all ages. The organism is believed to be transmitted by flies, particularly horn flies *(Haematobia irritans),* stable flies *(Stomoxys calcitrans),* and houseflies *(Musca domestica).* The bacteria is soil borne and able to survive and persist in a wide range of environmental conditions.

Lesions develop slowly and may be single or multiple. These abscesses drain a creamy to caseous material that may be whitish to greenish. Pitting edema, ventral midline dermatitis, depression, fever, and lameness are common. The course of the disease may be chronic, and recurrent internal abscesses are frequent. Pectoral abscesses in particular are frequently quite deep (5–8 inches), and drainage can be a challenge.

In horses with ulcerative lymphangitis, lesions usually develop on the hindlimbs, especially at the fetlocks. Hard to fluctuant chains of nodules that abscess, ulcerate, and drain are most common. Old lesions heal within 1 to 2 weeks, but new lesions arise. The affected limb may be swollen and painful, and the horse may be depressed. Regional lymphatics appear corded. In chronic cases, permanent thickening of the tissue surrounding the regional lymphatics is common and can result in gait stiffness and lameness.

The diagnosis of ulcerative lymphangitis and deep abscesses is based on the appearance of the abscess and exudate. Because identifying the organism on cytologic examination of the exudate may be difficult, a culture is strongly recommended. The disease presentation seems to have a geographic component, with internal abscesses more commonly reported from California and ulcerative lymphangitis almost exclusively reported in Texas in the United States, leading to the proposal that there may be a difference in vectors in the two sites, which accounts for the difference in presentations.[29]

Treatment is protracted and is complicated by the limited response of the organism to systemic antibiotics. The veterinarian should allow the abscess to mature and then surgically incise and drain it. Administration of systemic antibiotics before abscess rupture may prolong the course of the disease with recurrence of the abscess on discontinuation of the drug. Large dosages of procaine penicillin (20,000 IU/kg IM every 12 hours) for up to 6 months have been recommended for treatment, but penicillin's lack of lipid solubility and low volume of distribution may make it a poor choice for treatment of the well-encapsulated abscesses typical of *C. pseudotuberculosis.* Based on the minimum inhibitory concentration values for a large number of isolates, doxycycline, fluoroquinolones, and potentiated sulfonamides may be better choices for first-line treatment; the final antibiotic choice should be made on the basis of culture and sensitivity.

The practitioner must initiate treatment rapidly to prevent permanent debilitation and disfigurement. In early cases treated with hydrotherapy, exercise, and surgical drainage, antibiotics should be administered for at least 30 days after clinical normalcy. Nonsteroidal antiinflammatory drugs (NSAIDs) may be beneficial. If the lesions of ulcerative lymphangitis become chronic and fibrotic, joint motion is restricted, and the prognosis is poor. Any large abscess within a body cavity or other significant structure would potentially have a poor prognosis. A vaccine against *C. pseudotuberculosis* was briefly available in 2016 but was quickly removed from the market because of adverse effects. A second generation of the vaccine may be released in the near future.

Botryomycosis

Botryomycosis, also known as *bacterial granuloma* or *staphylococcal pseudomycetoma,* is a rare problem and is most commonly seen secondary to a wound, including surgical wounds such as castration.[1] It can occur anywhere on the body, including the udder. The most commonly isolated organism found in botryomycosis is *S. aureus,* but other bacteria such as *Pseudomonas aeruginosa, Proteus* spp., *Escherichia coli,* or other nonfilamentous aerobic or anaerobic bacteria can cause the granulomatous suppuration.

Bacterial granulomas are believed to begin as traumatic injuries to the skin during which an infectious organism is inoculated into the dermis. A granulomatous reaction develops when the horse is able to contain the infection but not eradicate it. Poorly circumscribed, firm lesions that may or may not be painful are typical (Fig. 18.15). The center of the lesion may be ulcerated or drain. In some cases, variable numbers of white-to-yellow pieces of particulate matter resembling grains of sand (tissue grains) may be present in the exudate.



FIG. **18.15** Perianal bacterial granuloma. Surgical resection was curative.

Confirmation of the diagnosis is made by skin biopsy, and identification of the causative organism is confirmed by bacterial or fungal culture (if a fungus is cultured the lesion is termed a *mycetoma*). Histologic examination of the lesions typically reveals nodular to pyogranulomatous inflammation with or without tissue grains. The causative bacteria may be observed encapsulated in granules that are thought to protect it from the effects of antibiotics and phagocytosis, which leads to the chronicity of the infection. This reaction is known as the Splendore-Hoeppli phenomenon and is characterized by in vivo formation of intensely eosinophilic material around microorganisms or biologically inert material.[30]

Surgical excision or radical debulking followed by long-term antibiotic therapy based on culture and sensitivity has been the most successful treatment for botryomycosis. Organisms may be difficult to isolate from biopsy tissue samples, and the submission of large wedges of tissue obtained at the time of surgery is recommended. If possible, tissue should be obtained to submit for aerobic, anaerobic, and fungal culture from the deepest portion of the lesion using the previously described double-punch technique to increase the likelihood of isolating an infectious agent.

Viral Diseases

Viral Papillomatosis

Equine viral papillomatosis (warts) is caused by *Equus caballus* papillomavirus, a host-specific double-stranded DNA virus that belongs to the Papovavirus family. Two distinct lesions are associated with this virus: warts and aural plaques. Both lesions are characterized as benign, proliferative, epithelial, cutaneous neoplasms that are common in horses worldwide. No gender or breed predilection has been identified for either form of the disease. However, warts tend to affect young horses from 6 months to 4 years, and aural plaque is most common in horses more than 1 year of age.

Lesions originate in the basal cell layer of the epithelium following local infection by the virus. Trauma to the skin is probably required for natural infection to occur. There may be predisposing trauma caused by grazing fibrous pastures, plant thorns, insect bites, wire fence trauma, actinic dermatitis, grooming brushes, or surgical instruments. Subsequent contact of the damaged skin with contaminated combs, brushes, surgical instruments, or feeding insects can lead to infection. Black flies (*Simulium* spp.) have been strongly implicated in the transmission of aural plaques. These flies can be found feeding on the inner surface of horses' ears, producing droplets of dried blood. The papillomavirus can be transmitted through direct contact with an infected horse or indirectly through contact with contaminated fomites. Sexual contact between horses has been shown to spread the disease. The incubation period following natural infection is approximately 60 days. Warts reach maturity after a growth period of 4 to 8 weeks and tend to spontaneously regress in 3 to 4 months with the development of immunity. In contrast, aural plaques do not generally spontaneously regress.

Warts appear as multiple gray-to-pink or cream-colored cauliflower-like growths varying from 5 to 20 mm in size (Fig. 18.16). Lesions most commonly affect the muzzle, nose, and commissure of the mouth. Other sites that may be affected include other areas of the head (periocular regions),



FIG. **18.16** Warts on the muzzle of a yearling.



FIG. **18.17** Ulcerations on the tongue of a horse due to infection with vesicular stomatitis virus.

neck, distal limbs, and the external genitalia of both sexes. Warts often become necrotic before regression occurs. Most lesions are self-resolving and regress in 3 to 4 months, but some lesions may persist for up to 18 months. An inappropriate immune response should be investigated if a young horse exhibits lesions for 2 years or longer, if the horse has a generalized case of warts, or if the warts spread to atypical regions of the body. Lesions on older horses commonly persist and may remain in a static state for years.

Diagnosis of viral papillomatosis is based on the clinical appearance of the lesions. Most of the growths are unmistakable, but those on the lower limbs, anus, penis, or vulva may resemble verrucous-type sarcoids. A biopsy specimen may be obtained and submitted for histologic confirmation of the diagnosis. Histologic characteristics of papillomatosis include orthokeratotic hyperkeratosis, papillated epidermal hyperplasia with ballooning degeneration of keratinocytes, and basophilic inclusion bodies of different sizes. Aural plaques generally appear amelanotic and have a broad-based attachment.

Aural plaques are most commonly caused by a mixed infection with equine papillomavirus types (EcPV) 1 through 7.[31] The disease manifests as single or multiple, smooth or raised, gray or white lesions involving the inner surface of the pinna. Lesions tend to initially appear as small papules and enlarge progressively, eventually coalescing to become prominent hyperkeratotic plaques. Aural plaque-type lesions are less commonly found near the anus, penis, and vulva. The location of these plaques supports the belief that transmission is accomplished through insect bites and during breeding. Lesions are generally asymptomatic unless there is irritation by biting flies.

Treatment of papillomatosis lesions is not always pursued because neither warts nor aural plaques typically cause significant clinical problems. Surgical removal should be considered if lesions interfere with function. Anecdotal reports that suggest surgical removal or crushing the lesions induces remission of the lesion are unproven, and controlled studies suggest that such intervention might actually increase lesion duration. Treatment is indicated when the occasional horse with aural plaques appears to be in pain, exhibits headshaking, or develops other behavioral issues such as becoming difficult to bridle. It also may be indicated for horses with lesions that present a problem for show or sales preparation or that become irritated by trauma, insects, or secondary bacterial infections. Surgical excision, cryonecrosis, and radiofrequency hyperthermia have been advocated for the management of warts, but if large numbers of warts are present, substantial secondary trauma to normal skin can occur and result in a poor cosmetic outcome. Viral antigens released during late-phase tissue necrosis during cryosurgery treatment may enhance the body's immune response and improve the success rate of partial wart removal; spread of infection, however, is possible during this time, and the occurrence of white hairs in the region may negatively affect cosmesis. Intralesional or intravenous (IV) immunomodulatory substances and various topical caustic agents have also been used in the treatment of papillomatosis lesions. Imiquimod has demonstrated success in the treatment of aural plaques, but care must be taken to avoid overuse and excessive irritation or horses will become head shy, with some becoming difficult to bridle. Topical treatment of equine aural plaques with nitric acid, potassium nitrate, and zinc nitrate solution (Oxalic; N-vet, Uppsala, Sweden) resulted in long-term control of the lesions within 4 weeks without producing painful side effects; the lesions resolved in 91% of the horses after two treatments, with 9% of horses requiring three treatments.[32] Control of the biting insect populations will improve prognosis with any form of treatment and prevent the spread of disease. Fly masks with ear protection or application of petroleum products to the inner pinna are easy, economical means of control.

Vesicular Stomatitis

Vesicular stomatitis is a reportable viral disease that affects horses, cattle, swine sheep, goats, llamas, alpacas, and occasionally humans, in which it causes an acute influenza-like illness. The southwestern and western United States have experienced a number of vesicular stomatitis outbreaks in recent years affecting multiple states and a large number of farms. Excessive salivation is often the first sign observed. The incubation period is short (24–72 hours). Infected horses rapidly develop vesicles up to 2 cm in diameter in the mouth and on the lips. These lesions rupture, leaving large painful erosions and ulcers (Fig. 18.17). Fever and anorexia are common, and horses may refuse to drink. Rarely, lesions develop on the hooves, prepuce, and teats. Examination of the mouth initially reveals blister-like lesions along the gums, tongue, and lips with crusting scabs on the muzzle, lips, or ventral abdomen.

The disease has a seasonal occurrence in summer and fall and is believed to be transmitted to horses by insect bites. Within a group of horses, however, the disease will be transmitted via direct contact. Affected animals typically recover in approximately 2 weeks. There is no specific treatment.

Vesicular stomatitis is recognized internationally as a reportable disease with serious economic and regulatory repercussions. It is significant because of its similarity to foot-and-mouth disease and swine vesicular disease, both of which have been eradicated from the United States. Veterinarians who suspect an animal may have vesicular stomatitis or any other vesicular disease should immediately contact state or federal animal health authorities. Diagnosis is based on antibodies to fluid samples from the animal or by isolation of the virus from swabs of lesions, blister fluid, and tissue flaps. Confirmation of the diagnosis is obtained by serologic examination. Histologic findings are nonspecific and include a hyperplastic epidermis, intercellular and intracellular edema of the epidermis, reticular degeneration, spongiotic microvesicles, and focal necrosis. Superficial and deep perivascular dermatitis is present.

Owners should feed affected horses a soft gruel until oral lesions heal, usually within a few days. Permanent depigmentation may occur in areas of former ulceration. Infection results in immunity for up to 6 months.

Equine Coital Exanthema

Coital exanthema is a contagious venereal disease of horses caused by infection with equine herpesvirus type 3 (EHV-3), which is an alphaherpesvirus that is distinct from the other EHVs and endemic in most horse-breeding populations. The disease is transmitted via coitus, insects, fomites, and inhalation. Coital exanthema is characterized by the formation of painful papules, vesicles, pustules, and ulcers on the external genitalia of mares or stallions. Vesicles and bullae may be present in the mouth or nostril or on the lips. Lesions can be pruritic. Depigmentation often occurs in areas where lesions have developed and healed. Stress may precipitate recurrences.

Diagnosis is based on the appearance of lesions on the vulva, perineum, penis, or prepuce; skin biopsy; and virus isolation. Histologic findings include hyperplastic superficial and deep perivascular dermatitis with ballooning degeneration and eosinophilic intranuclear inclusion bodies.

Treatment is symptomatic, but corticosteroids are contraindicated. The negative impact of coital exanthema on equine breeding enterprises includes the forced, temporary disruption of mating activities of mares and stallions; the additional care and supportive treatment that is required for affected horses; and the risk of virus spread by either fresh or frozen semen, artificial insemination, and embryo transfer. Because coital exanthema is not a reportable disease, its true prevalence and economic impact are difficult to assess and are probably underestimated.[33] Affected horses should be isolated and removed from the breeding program for a minimum of 4 weeks. The disease has no known effect on fertility.

Horsepox

Horsepox is a relatively benign viral disease that has become nearly extinct.[34] The virus is transmitted by direct contact with an infected host or with contaminated grooming tools or tack.

Pyrexia and anorexia may be observed early in the course of the disease, and horses of all ages may be affected. The initial skin lesion is an erythematous maculopapular eruption followed by the development of vesicles. This stage is transient and may not be observed. Vesicles develop into umbilicated pustules with a depressed center and a raised erythematous border. When the pustule ruptures a crust develops and the lesion heals, often with scarring. Three clinical presentations (oral, leg, and vulvar) have been described. Oral horsepox (buccal horsepox, contagious pustular stomatitis) is characterized by the development of lesions on the inner lips and buccal mucosa. In severe forms, pox lesions may develop in the pharynx, larynx, and nostrils. Leg pox usually develops on the pasterns and fetlocks and often is confused with "scratches" or "greasy heel." Pain and lameness are common. Vulvar pox (genital horsepox) is uncommon and occurs predominantly in severely affected animals.

Diagnosis is based on history and clinical signs and confirmed by histologic examination. Intracytoplasmic inclusion bodies demonstrated by routine histopathologic examination of tissue or electron microscopy are diagnostic. Other findings include ballooning degeneration of the epidermis (stratum spinosum), reticular degeneration, acantholysis (loss of cohesion of cell in the granular area), intraepidermal microvesicles, a superficial and deep perivascular dermatitis, and intraepidermal microabscesses and pustules.

Treatment is symptomatic. Most horses recover in 2 to 4 weeks. Occasionally the disease is fatal in severely affected young horses. Recovery produces lifelong immunity. This disease also affects humans and cattle.

Fungal Diseases

Dermatophytosis (Ringworm)

Dermatophytosis (ringworm) is the most common fungal disease in the horse. *Trichophyton equinum, T. mentagrophytes, Microsporum gypseum,* and *M. canis* are the most frequent isolates.[1,2] Dermatophyte infections are self-limiting but can become a significant source of frustration, particularly in large stables.

Dermatophytes are transmitted by direct contact with an infected host or by indirect contact with contaminated fomites or the environment. Illness, poor nutrition, overcrowding, age (young or old immunosuppressed individuals), and stress predispose horses to infection. Chronic moisture from sweating or excessive bathing with detergent shampoos damages the protective barrier of the skin and enhances the opportunity for infection. The incubation period is usually several weeks. During this time, fungi invade the epidermal keratin, hair follicles, and the hair shaft itself with the aid of enzymes that are allergenic to the host. Hair shaft integrity is compromised, and hairs fall out or are fractured easily, resulting in areas of alopecia with variable crusting and scaling that are characteristic of the disease (Figs. 18.18 and 18.19). Although rare, pruritus can be severe. Secondary bacterial infections can complicate the clinical picture and diagnosis. Infection is eliminated from a particular hair on shedding or as it enters the telogen stage, or the dermatophyte elicits an inflammatory response.

Lesions are most common in areas in which tack contacts the skin but may be limited to the distal limbs; posterior pasterns; and rarely, coronary bands.

The diagnosis is confirmed by demonstration and identification of the organism. Wood's lamp examinations are generally not useful because the most commonly implicated species do not fluoresce. Commonly used diagnostic tools include direct examination of hair shafts for microconidia and hyphae, skin biopsy, and fungal culture. More recently, polymerase chain

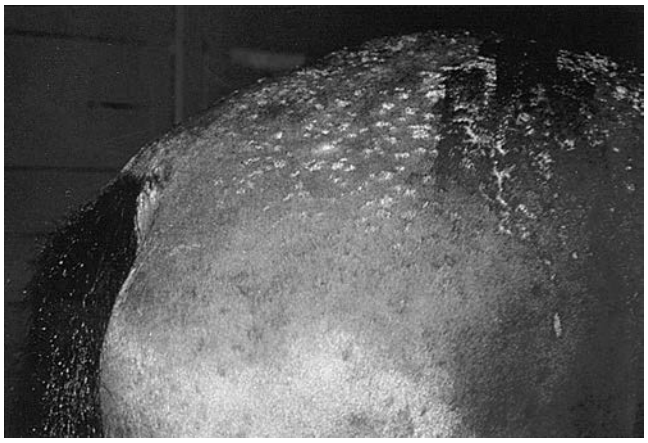


FIG. **18.18** Dermatophytosis. The papular eruption on the advancing edge of the lesions is notable.



FIG. **18.19** Classic signs of dermatophytosis (ringworm).

reaction (PCR) has become available at some laboratories. Hairs are collected for both PCR and culture from the periphery of a newly developing lesion, avoiding areas that have been previously treated. For fungal culture, the area is gently wiped with an alcohol swab to minimize growth of contaminants before collecting hair samples. Hairs should be plucked in the direction of growth and transported to a laboratory in which culturing of equine dermatophytes is routinely performed. Culturing equine dermatophytes can require medium enhancement in some instances. If biopsying, early lesions with crusts are ideal. Care should be taken to protect the crusts during skin preparation, and gently rinsing without wiping with sterile saline is sometimes recommended for this and other crusting lesions. Histologic findings include folliculitis, perifolliculitis, and furunculosis; superficial perivascular dermatitis with orthokeratosis or parakeratosis; and intraepidermal vesicular or pustular dermatitis. Septate fungal hyphae and oval spores may be present in the superficial keratin or hair follicle.[2]

Although most cases of dermatophytosis heal spontaneously within 1 to 6 months, treatment is advised to minimize contagion to other animals and humans. Weekly bathing with antifungal veterinary shampoos (2% miconazole or 1% ketoconazole) or accelerated hydrogen peroxide shampoo and application of 0.2% enilconazole as a leave-on rinse at a 1:50 dilution applied weekly for 6 to 8 weeks (Imaverol; Elanco, Basel, Switzerland) are good choices that often have better client compliance than lime sulfur or dilute bleach (1:10 with water) application; this is likely because they do not produce offensive odor or coat color alterations.

Systemic antifungal drugs, such as griseofulvin (50 mg/kg orally once daily or 100 mg/kg once daily for 7–10 days), itraconazole (5–10 mg/kg orally once daily), and 20% sodium iodine (250 mL per 500-kg horse every 7 days), have been used, but there are no controlled studies demonstrating efficacy, and use of these drugs is contraindicated in pregnant mares.

Elimination of dermatophytosis from a barn requires elimination of the organism from infected hosts and decontamination of the environment including fomites such as brushes, tack, blankets, leg wraps, and riding apparel, particularly the rider's boots. Commercial antifungal vaccines for equine dermatophytosis are not available in the United States.

Piedra

Piedra is a rare superficial fungal disease of horses that causes nodules on the hair shaft. Black or white filamentous nodules consisting of tightly packed hyphae are most commonly visible on the mane and tail. Affected hairs break at the site of infection. A definitive diagnosis is made by fungal culture and microscopic identification of the agent *Piedraia* sp. or *Trichosporon beigelii.* Treatment of affected horses includes clipping the hair and applying topical fungicides.

Sporotrichosis

Sporotrichosis is a zoonotic mycosis caused by *Sporothrix schenckii,* a dimorphic aerobic fungus. The organism lives in soil and decaying vegetation and enters the horse's skin via a traumatic wound. Early lesions begin as papules that may exude a seropurulent material. Nodules are most common on the thigh or proximal foreleg and chest (Figs. 18.20 and 18.21). If the immune system does not eliminate the organism, hard subcutaneous nodules develop along the lymphatics draining the area. The lymphatics become corded and drain thick, brown-to-red exudate. The disease rarely becomes systemic.

The definitive diagnosis is determined by demonstration of the organism via cytologic examination of exudate, culture of tissue or exudate, or skin biopsy. The organism appears as a cigar-shaped yeast in macrophages and neutrophils on Giemsa-stained smears. Histologic examination of tissue reveals nodular to diffuse, suppurative to granulomatous dermatitis. Intraepidermal microabscesses may be visible. The yeast is rarely seen in tissue sections. The number of organisms present in the tissue may vary, and repeat cultures are often necessary.

Ethylene diamine dihydroiodide (EDDI), a feed additive and an organic iodide powder, is the drug of choice because of its cost and ease of administration. It is administered at 1 to 2 mg/kg q 12 h for 7 to 10 days as a loading dose followed by 0.5 to 1.0 mg/kg q 12 h for 1 month past the resolution of clinical signs. The dose of EDDI is based on the active ingredient in the feed additive, which is usually 3.65% or 3.65 mg/g of product. Sodium iodide (20% solution) has also been successful for treating sporotrichosis in horses. A loading dose of 20 to 40 mg/kg is administered IV for 2 to 5 days, followed by once daily oral therapy (20–40 mg/kg) for 1 month after lesions disappear. Both EDDI and sodium iodide may be given orally via syringe or mixed in sweet feed. Iodism may develop and may require temporary discontinuation of therapy. Iodine cause abortion and should not be used in pregnant mares.



FIG. **18.20** Typical subcutaneous nodules with ulceration and cording of lymphatics in shoulder of 16-year-old Paint Horse mare with sporotrichosis. Sellon, Debra C., and Maureen T. Long. Equine infectious diseases. St. Louis, MO: Saunders/Elsevier, 2014.



FIG. **18.21** Sporotrichosis on distal limb of horse. Sellon, Debra C., and Maureen T. Long. Equine infectious diseases. St. Louis, MO: Saunders/Elsevier, 2014.

Mycetomata

Mycetomata are chronic pyogranulomatous infections of the skin and subcutaneous tissues caused by actinomycetes (actinomycetoma) or fungi (eumycotic mycetoma).[35,36] Mycetomata in horses have been reported in North America, South Africa, and Australia.[37] The most common agents of eumycotic mycetoma in horses belong to the *Scedosporium*/*Pseudallescheria* complex (SPC) and *Madurella* genera (i.e., *M. mycetomatis*); however, *Curvularia verruculosa, Phialophora oxyspora,* and *Aspergillus* spp. have also been occasionally detected.[36,37]

Lesions are characterized by swelling, draining tracts, and tissue granules. Regardless of the causative agent, the organism usually gains entrance to the body through traumatic inoculation. As with other granulomatous reactions the body is able to recognize the intruder and isolate it but not to eliminate it.

Lesions may vary in shape, size, texture, and color, depending on the etiologic agent. Single or multiple nodules of varying size can occur almost anywhere on the body of the horse. The nodules often are pigmented and ulcerated and may discharge tissue grains (small sandlike particles). The tissue grains are masses of hyphae from the causative organism, with dark-brown granules from the *Madurella* genera and white grains generally from SPC.[35,36] Definitive diagnosis can be made with fine-needle aspirate or excisional or wedge biopsy of a lesion. Histologic examination shows diffuse to nodular pyogranulomatous to granulomatous inflammation surrounding septate branching hyphae. Tissue grains are often visible microscopically. The hyphae contained in tissue grains or tissue may be pigmented or nonpigmented. Fungi are cultured best from tissue grains and, occasionally, exudate. Complete surgical excision is curative; if this is not possible, then additional local therapy may be beneficial.[38]

Phaeohyphomycosis

Phaeohyphomycosis (Table 18.2), sometimes known as chromomycosis, is a chronic cutaneous, subcutaneous, mucosal, and occasionally systemic infection caused by pigmented opportunistic fungi. The most commonly implicated organisms are *Alternaria* spp., *Drechslera spicifera,* and *Curvularia* spp., but *Hormodendrum, Phialophora,* and *Cladosporium* species have also been documented. Many of the organisms that cause fungal mycetomata can cause phaeohyphomycosis, but the distinguishing difference is the lack of tissue grains. Pigmented fungi (dematiaceous fungi) causing this disease are soil and vegetative saprophytes that gain entrance into the body via a wound or abrasion. The body is not able to eliminate the organism, which proliferates in tissues. Immunosuppression may be a predisposing factor.

Lesions can appear anywhere on the body but are most often found on the head and neck and may be single or multiple ulcerated cutaneous or subcutaneous nodules.[39] Some lesions are pigmented deeply on gross examination, or pigmentation is only a microscopic feature. Nodules vary in size and are cool, nonpainful, and nonpruritic. Regional granulomatous dermatitis may be present.

Definitive diagnosis is made by histologic examination and fungal culture of an excised nodule. Skin biopsy reveals suppurative to granulomatous dermatitis with pigmented septate hyphae. Observation of pigmented septate hyphae in the skin indicates opportunistic invasion of the tissue.

Complete surgical excision, and the administration of fluconazole when complete excision is not possible, for 10 days (a loading dose of 14 mg/kg given once followed by 5 mg/kg PO daily) and potassium iodide (30 mg/kg PO daily for 30 days) are the current recommended treatments.[38]

TABLE **18.2** **Fungal Diseases of Horses**

Disease	Common Causative Agents	Clinical Signs
SUPERFICIAL INFECTIONS		
Dermatophytosis	*Microsporum* and *Trichophyton* spp.	Circular patches or hair loss, crusting and scaling, urticaria, papules
Piedra	*Piedraia* spp. or *Trichosporon beigelii*	Black or white filamentous nodules on hair shaft
INTERMEDIATE INFECTIONS		
Sporotrichosis	*Sporothrix schenckii*	Papules and nodules occur along lymphatics; nodules may ulcerate, become thickened, and drain a thick brown to red-colored exudate
Phaeohyphomycosis (chronic, subcutaneous fungal infection caused by pigmented opportunistic fungi)	*Hormodendrum, Drechslera, Phialophora, Curvularia, Cladosporium*	Single or multiple nodules; lesions may be grossly or microscopically pigmented, nonpruritic, nonpainful, and cool to the touch
Mycetoma (chronic subcutaneous infection)	Filamentous bacterial organisms and opportunistic fungi	Single or multiple nodular lesions are present; ulcerated, draining tracts are common; mycetomata discharge tissue grains in contrast to phaeohyphomycosis, which does not

Pythiosis

Pythiosis (previously referred to as phycomycosis) is one of the few dermatologic emergencies in equine practice. It is caused by an aquatic fungus called *Pythium insidiosum*.[40] Originally more common in warm wet climates such as the southeastern United States and portions of South America and Australia, the organism has made its way to Michigan, Indiana, Washington State, and other similarly cooler climates. *Pythium* is a plant parasite that normally lives on aquatic vegetation and organic debris. It can live in the soil and grass for prolonged periods of time and becomes infective during periods of extended rain. Infective zoospores gain entrance into the horse via small wounds or through prolonged contact with contaminated water.

The most commonly affected areas include the distal limbs, ventrum, perioral and nasal area, and chest (Fig. 18.22). Early signs of fungal invasion include single or multiple minute foci of necrosis that progress rapidly into circular, ulcerative, and granulation-tissue–like masses with serosanguineous discharges. These masses are intensely pruritic, which is a hallmark of this disease. Lameness, regional lymph node involvement, anemia, and hypoproteinemia are common findings. If left untreated the disease will become systemic, with most horses succumbing to the disease within 6 months. The lesions of pythiosis can be confused with habronemiasis, exuberant granulation tissue, bacterial granuloma, and invasive squamous cell carcinoma (SCC), which may delay appropriate treatment. Lesions are often hemorrhagic from self-trauma and frequently weep thick, serosanguineous material thought to resemble leeches (giving it the colloquial name *Florida horse leeches*). Tracts within the lesions will contain "kunkers" (another hallmark of this disease), which are hard, gritty, and white to yellow masses that can be expressed with moderate pressure. The kunkers can be seen to branch with the naked eye, which can be used to help distinguish them from granules observed in several other skin diseases. Kunkers are composed of fungal hyphae, host exudate, and protein. The diagnosis of *Pythium* infection can be made quickly by kunker evaluation if sent to a laboratory specializing in its diagnosis. A definitive diagnosis can also be made through serology, biopsy, culture, and cytologic examination of exudate. Biopsy of early lesions of equine pythiosis reveals abundant microabscesses with eosinophils and a few neutrophils, lymphocytes, and macrophages. In chronic cases, an eosinophilic granuloma with giant cells is observed with microabscesses and kunkers at the center. With periodic acid-Schiff (PAS) and silver stains *P. insidiosum* appears as sparsely septate hyphae 6 to 10 mm in diameter. An initial diagnosis can be made grossly on lesion appearance, location, and/or the presence of kunkers. Because of the aggressiveness and severity of the disease, treatment should not be delayed while awaiting the results of additional



FIG. **18.22** Horse with bilateral pythiosis on the legs.



FIG. **18.23** Pastern dermatitis.

diagnostic tests. If properly treated early after onset of clinical signs, the outcome is favorable. The fatality rate is 100% if the animal is left untreated. The likelihood of successful resolution of lesions declines with chronicity of disease.

Lesions in horses are best treated with a combination of therapies including radical surgical excision of the lesion, three or more doses of *Pythium* immunotherapy (www.pavlab.com), and topical application of antifungal solutions. Affected horses may also benefit from regional limb perfusion with 50 mg amphotericin B[41] or oral and/or IV iodides. Prognosis for horses affected with cutaneous pythiosis is good if recognized early and immediately and aggressively treated. The disease is not a known zoonosis, but protective latex gloves should be worn when examining patients with suspected lesions.

Stachybotryotoxicosis

Stachybotryotoxicosis is a mycotoxicosis caused by the toxins of the fungus *Stachybotrys atra*.[2] This fungus grows on hay and straw and produces toxins referred to as *macrocytic trichothecenes*. These toxins cause bone marrow suppression, profound neutropenia, thrombocytopenia, and necrotic-ulcerative lesions of the skin and mucous membranes.

Lesions commonly begin at the mucocutaneous junction as focal areas of necrosis and ulceration. Petechiae, ulcers, large areas of necrosis, catarrhal rhinitis, suppurative rhinopharyngitis, and laryngitis follow. Skin lesions occur as early as 24 hours after ingestion of the toxin. Systemic signs include lethargy, anorexia, weight loss, hyperactivity, proprioceptive deficits, colic, muscular stiffness, and second-degree atrioventricular block. Affected animals develop hemorrhagic diathesis, hemorrhagic enteritis, and septicemia and die.

The diagnosis is based on history, clinical signs, and finding the toxin in the feed. If one recognizes toxicosis early in the course of the disease, withdrawal of affected feed has resulted in resolution of signs. Animals with extensive lesions and chronic exposure have a poor prognosis.

Pastern Dermatitis

Pastern dermatitis (scratches, greasy heel, and mud fever) is a descriptive term used for lesions of varied etiologies that appear on the back of the pasterns and/or heel bulbs (Fig. 18.23) and sometimes spread up the legs. Bacterial infection is usually a significant component; *D. congolensis* is frequently a copathogen with two or more other bacterial species. The lesions can be associated with other conditions including photosensitivity in horses with socks or stockings, chorioptic mange in draft horses, or simple staphylococcal bacterial folliculitis. Lesions may be scaly, crusty, oozing, or crusting and can be quite sensitive to the touch. Lameness may be present. Mild cases may resolve with clipping of excess hair from the pasterns to allow them to dry more quickly, gentle cleaning with chlorhexidine followed by thorough drying, and the topical application of water-soluble antibacterial ointments. The horse must be moved to a dry environment until lesions are completely healed. Severe cases of pastern dermatitis require systemic antibiotics, and affected horses frequently benefit from biopsy with culture and sensitivity. Silver stain of the histology specimen to examine for the presence of spirochetes is worthwhile in horses that do not respond to traditional therapy. Severely affected horses may benefit from regional limb perfusion with an antibiotic appropriate to the bacteria isolated.

Parasitic Diseases

Ectoparasites are a common cause of dermatitis in horses and can have a serious impact on the animal's welfare. They are also important vectors in the transmission of a wide variety of infectious agents.

Lice (Pediculosis)

Lice typically cause severe pruritus, which leads to self-traumatic excoriation and patchy alopecia; when left untreated the coat can become dull and scaly. Affected horses can become agitated and have a poor appetite. Foals may develop muscle fasciculations without other symptoms. Heavy infestations of sucking lice may cause anemia or severe debilitation. Infestation has no age, breed, or gender predilection.

Lice are highly host-specific, obligate parasites that spend their entire life cycle (20–40 days) on the host. In ideal environments, lice can live for 2 to 3 weeks off of the host, but less than 7 days is more typical.[42] Lice infestations are transmitted by direct or indirect contact. Grooming instruments and tack serve as fomites for transmission, and adult horses may act as asymptomatic reservoirs that are identified only when foals or other susceptible animals become infested.

Female lice attach their nits to the horse's hairs via a clear adhesive secretion. Two types of lice feed on horses. Biting lice *(Damalinia equi)* feed on epidermal debris and prefer the dorsolateral trunk. Sucking lice *(Haematopinus asini)* feed on blood and tissue fluid, and most commonly infest the mane, tail, and fetlock region.

Lice infestations can occur year round but are more common in the winter in northern climates when the longer hair coat and cooler skin and hair temperatures are more favorable to reproduction.

The diagnosis of lice infestations is made by visualizing adults or eggs or both on the horse's hair. In bright sunlight or in animals with light-colored hair the veterinarian may need to use a fine-toothed comb or brush the coat onto a darker surface to find the lice. Skin biopsies may reveal a nonspecific superficial perivascular eosinophilic dermatitis with or without intraepidermal microabscesses.[2]

When managing a lice infestation, the goal is to treat all animals on the premises. Ivermectin at 200 µg/kg orally every 2 weeks for three treatments is generally effective for treating sucking lice. Controversy exists as to whether biting lice respond as well to oral ivermectin treatment as sucking lice, but the use of topical eprinomectin solution (Eprinex Pour-on; Merial, Manukau City, New Zealand) 500 µg/kg body weight weekly once for four administrations appears to be curative. Both sucking and biting lice will respond to

insecticidal or 1% selenium sulfide shampoos, which kill the adult lice, but the hatching nits will require a second shampooing in 2 weeks. Two applications of insecticidal sprays or powders containing pyrethrins and pyrethroids at 14-day intervals are also efficacious. Older pour-on permethrin products have caused local paresthesia and local hair loss in some horses. A newer permethrin product that also contains pyriproxyfen and dinotefuran (Vecta 3D; Ceva, Lenexa, KS) does not seem to produce these untoward effects (see the section Treatment of Allergic Skin Disease), and one treatment is generally adequate for both adults and nits. Applications of imidacloprid, phoxim, and 0.25% fipronil are also effective.[43,44] Lime sulfur and pyrethrin dips have fewer associated adverse reactions in horses than do other dip treatments. Because these products do not affect lice eggs, two to three repeat applications of the dip are recommended at 2-week intervals. For optimal efficacy, one should treat the whole body of the horse when applying the dips. All products should be used according to label directions. The presence of lice in the nearby environment should be addressed by cleaning and decontaminating all tack and grooming supplies and decontaminating the stabling area using a commercial flea premise spray. Horse trailers are frequently overlooked during treatment but can be the source of infestation. Treating animals returning from shows, breeding farms, or training facilities prophylactically should be considered in high-risk facilities.

Mites (Mange)

A variety of mites may infest horses. *Sarcoptes scabiei* var. *equi* (scabies, head mange), *Chorioptes equi* (leg mange), *Psoroptes equi* (body mange), *Pyemotes tritici* (straw itch mite), and *Trombicula* and *Eutrombicula* species (chiggers) are associated with pruritic equine skin diseases.[42] The pruritus accompanying a mite infestation results from a combination of mechanical irritation and hypersensitivity to the mite and byproducts of the mite (i.e., feces). The primary skin lesion from a mite infestation is a maculopapular eruption. With chronic infestations the skin is markedly inflamed and chronically thickened, and secondary bacterial infections are often present. As of 2006, mite infestations are no longer federally reportable conditions in horses in the United States, although they may remain reportable to some state veterinary agencies. Regardless of the type of mite present on the horse, skin scrapings should be part of the diagnostic investigation. With the exception of lice and trombiculid larvae that can be seen with the naked eye, mite infestations are best confirmed by microscopic examination of skin scraping samples.

Sarcoptes scabiei. *S. scabiei* is highly contagious in horses and transiently infests the humans in contact with them. *S. scabiei* is capable of surviving off the host for up to 3 weeks, and transmission by fomites or environmental sources is possible. Scabies mites burrow in the superficial epidermis in which they lay their eggs. In early infestations, mites are found in highest concentration around the head and neck and seem to prefer the ears on horses. As disease progresses, mites spread over the entire body. Scabies infestation in the horse results in intense pruritus and generalized scaling and crusting with excoriations and lichenification. Secondary bacterial infection may occur. The scabies mite is circular with short legs, a terminal anus, and long unjointed pedicles. Unfortunately, as in other species, the scabies mites can be difficult to find on skin scrapings. Therefore a negative skin scraping does not ensure that the horse does not have scabies.

Ivermectin at a dose of 200 µg/kg orally is effective for treating horses with scabies. As in other species, the practitioner should repeat ivermectin treatment at 2-week intervals for a total of two to three treatments because the eggs hatch and new adult mites are present at that time. Alternatively, topical treatments such as lime sulfur dip, lindane, coumaphos, diazinon, malathion, or toxaphene also have been used. Affected horses and those in contact with it should be dipped every 7 to 10 days for three to six treatments. Fomites should be decontaminated (e.g., tack, brushes) as well as the environment.

Chorioptes equi. *C. equi* mites are host specific and do not parasitize humans (Fig. 18.24). They spend their entire 3-week life cycle on the host but can live off the horse for 70 days. The mite tends to be more common in the winter months. *Chorioptes* feed on epidermal debris at preferred sites, which include the distal limbs and perineum. Infested horses exhibit intense pruritus with chewing, licking, stomping of the feet, and rubbing of the tail and perineal area. In some horses only the hindlimbs are affected. Skin lesions include papules, alopecia, erythema, scabs and crusting of the pasterns, fetlocks, or perineum. Diagnosis can sometimes be made by deep skin scrapings often onto clear packing tape or a drop of oil placed on a glass slide so that the mites do not escape. If the clinical picture fits *C. equi* infestation but no mites are recovered, then the response to treatment may be used for diagnosis. Although infested horses can have a large number of easily recoverable mites, there appears to be a population of horses that has few to no recoverable mites; clinical signs on these horses may be caused by hypersensitivity. Draft-breed hors-



FIG. **18.24** *Chorioptes* mite.

es or other horses with feathered fetlocks are predisposed to *C. equi* where they may contribute to the lesions of chronic progressive lymphedema (CPL; Fig. 18.25).

C. equi should be included in the list of differential diagnoses for horses with pastern dermatitis or greasy heel. When a *C. equi* infestation is diagnosed, all horses on the property should be treated. Because *C. equi* does not cause severe clinical signs in every infested horse, chorioptic mange is often overlooked as a diagnosis when one horse in a group of horses is affected.

Treatment with topical eprinomectin solution (Eprinex Pour-on; Merial, Manukau City, New Zealand) at the manufacturer's recommended therapeutic dose for cattle of 500 µg/kg body weight weekly once for four administrations is recommended.[45] The appropriate dose volume for each horse is administered along the back line, from the withers to the proximal tail. Topical fipronil (Frontline; Merial, Iselin, NJ) is also useful, although some horses seem to become agitated when the fipronil is applied, potentially from increased activity of the mites. Both ivermectin and moxidectin have been advocated for treatment of *C. equi* in horses; however, resistance to the products may be developing. Topical dip therapy may be indicated in some horses. If a topical medication is used, clipping the hair around the fetlocks and pasterns to ensure optimal contact of the dip with the skin and mites is advisable. The premises should be decontaminated with a commercial premise spray for fleas.

Psoroptes equi. *P. equi* mites are highly contagious to other horses but do not infest humans. They are biting mites that live on the skin surface and feed on serum and cellular components. Transmission of *P. equi* mites is by direct contact and exposure to fomites. Mite infestations tend to begin on the forelock, mane, and tail and spread to the trunk. These mites have been known to infest the ear canal and cause otitis externa. Affected horses typically show headshaking or rubbing. As with many other mites, pruritus results in alopecia, papules, oozing crusts, excoriations, and ulcers on the skin. The intensity of pruritus varies. History and clinical signs suggest the diagnosis of *P. equi* mange. Skin scrapings are required to confirm a diagnosis. *P. equi* has an oval body with segmented pedicles. Treatment for *P. equi* infection is similar to that described for all mites.

Trombiculiasis. Trombiculiasis (chiggers, red bugs, and harvest mites) is caused by an infestation with larvae of free-living adult mites (genus *Eutrombicula* or *Neotrombicula*). Larvae are most prevalent in grasses, forests, or swamps in late summer and fall. Small rodents are the natural host. Pathognomonic skin lesions are papules or wheals with a small orange or red dot (trombiculid larvae) in the center. Lesions occur most commonly on the face, muzzle, distal limbs, ventral thorax, and abdomen. Trombiculiasis is seasonal, but the seasonality varies with genus and species. *E. alfreddugesi* is active in the late spring and peaks midsummer, whereas *Neotrombicula autumnalis* is most active from the late summer to midautumn.[46] Larvae feed on the host in the late afternoon and early evening. Diagnosis is based on observation of the distinctive lesion with the larvae approximately 0.2 to 0.4 mm in size, oval in shape with six legs, in the center of the skin lesion. Trombiculiasis is a self-limiting disease. Some horses are uncomfortable with this mite infestation and should be treated. Recommended treatments include lime sulfur dip, permethrin, pyrethrin, cypermethrin, and phoxim sprays or dips as a one-time treatment in association with prednisolone at a dosage of 0.5 mg/kg orally for 3 to 5 days.



FIG. **18.25** A 10-year-old draft horse with chronic progressive lymphedema. Before (A) and after (B) clipping the feathers on a horse with chronic *Chorioptes equi* and a secondary mixed bacterial infection demonstrating that feathers can hide serious skin lesions. The horse had previously been treated with ivermectin multiple times with no resolution of the pruritus or skin lesions. Eprinomectin solution applied to the horse's topline and topical wound care with systemic antibiotics were able to control disease progression.

Pyemotes tritici. *P. tritici* (straw itch mite) normally parasitizes the larvae of grain insects. Occasionally this mite parasitizes humans and horses.[15] Infestation in horses occurs from contaminated hay fed in overhead racks. The mite produces a maculopapular crusted eruption on the head, neck, and trunk that is only occasionally pruritic. The diagnosis is based on history and clinical examination. This disease is self-limiting, and the owner should remove contaminated forage or feed the horse from the ground until all contaminated forage has been consumed. Infestations from fomites or hay fed on the ground have not been reported.

Dermanyssus gallinae. The nymphs and adults of the poultry mite, *Dermanyssus gallinae,* will occasionally parasitize the horse. This mite feeds at night. The adults are oval and 0.6 to 1 mm long with eight long legs. The color of the mite is white, gray, or black. After poultry mites consume a blood meal they take on a red appearance.

This mite causes pruritic papules and crusts of the head and legs of horses. Skin scrapings and tape preparations at night yield the best chance of obtaining the mite from the horse. Mites may be more easily identified on poultry from the horse's environment. Cleaning the environment and routine mite treatments as described previously are beneficial for treatment of this mite. If it is not possible to eliminate the horse's contact with poultry, recurrence of dermatitis can be prevented by regularly applying permethrin to the horse and biannual decontamination of the horse's stable.[47]

Demodicosis. Demodectic mites are normal skin residents in horses; therefore, the rare animal that manifests clinical signs of demodicosis is likely to be immunocompromised. Two species of *Demodex* can infest horses, *D. equi* and *D. caballi,* and each affects different areas of the body. *D. caballi* prefers the eyelids and muzzle, whereas the lesions of *D. equi* are more generalized. Clinically these horses have one or more alopecic or scaling areas. The most common areas of the body affected in horses with *Demodex* are the face, neck, and sometimes shoulders. The mite is easily identified on skin scrapings of affected areas. Because the infestation is usually asymptomatic and will likely regress once the underlying cause for immunosuppression is addressed, most horses are not treated. Treatment with 2% trichlorfon topically every other day or ivermectin orally for 15 days has shown some success. Amitraz is contraindicated because it can cause colic in horses. Regardless, the veterinarian should identify and treat the underlying cause for the demodicosis, which is most commonly equine Cushing's disease (PPID).[1,2,46]

Ticks

Ticks not only harm their host locally through bite injuries, but they can also play a significant role in transmission of a variety of viral, protozoal, rickettsial, and bacterial infections. In addition, horses suffer tick paralysis similar to dogs. Tick infestations are most common in the spring and summer.

Soft (argasid) ticks lay eggs in protected areas in the environment, and newly hatched ticks suck blood and lymph from the horse and then drop off to develop into adults (one-host ticks). Soft ticks infest barns, sheds, and other areas in which animals are housed. In horses, the soft tick *Otobius megnini* (spinose ear tick) tends to infest the ears and ear canal. Clinical signs of infestation include otitis externa, head tilt, headshaking, ear rubbing, and occasionally aural hematomas. Hard (ixodid) ticks such as *Dermacentor, Ixodes,* and *Amblyomma* species are the most common ticks found on horses. They also lay their eggs in sheltered outdoor areas, but hard ticks are three-host ticks, which means they must feed on three separate hosts to complete their life cycle.

The severity of clinical signs from tick feeding depends on the density of the infestation and whether the horse develops a hypersensitivity reaction to the bites. Infestations occur most commonly on the ears, face, neck, groin, distal limbs, and tail. Early lesions consist of papular to pustular eruptions that rapidly develop into crusts, erosions, ulcers, and hair loss. Hypersensitivity reactions may be local or general. Local responses appear as nodules that develop at the site of the tick bite. Systemic reactions are characterized by whole-body urticaria or multifocal urticarial plaques. In Australia, a hypersensitivity reaction to *Boophilus microplus* has been observed in sensitized horses. Intense pruritus, papules, and wheals develop as soon as 30 minutes after ticks begin to feed.

A definitive diagnosis is made by observation of ticks attached to the horse or in the ear canal. Treatment aims at killing ticks on the horse, and knowledge of the parasiticidal resistance of the local tick population will greatly help direct treatment. Pyrethrin or pyrethroid sponge-on dips can be applied to the body of the horse, taking extra care to soak skinfold areas. Soft ticks will generally require two to three treatments. Treatment for hard ticks must be repeated throughout the tick season because of their three-host life cycle. Infestations of *O. megnini* require mechanical removal of as many ticks as possible. One part rotenone and three parts mineral oil applied twice weekly is an effective otic parasiticide for horses as are commercially available pyrethrin otic preparations (Otomite Plus; Virbac, Fort Worth, TX). Ivermectin and fipronil have also shown efficacy. A drop or spray of fipronil can be applied to the ear pinnae on a monthly basis for tick prevention.

Helminths

Onchocerciasis. Lesions of *Onchocerca cervicalis* infection begin as a simple thinning of the hair coat, often around the eyes, muzzle, neck, chest and ventral abdomen.[1,2] As the disease progresses, lesions vary from focal to generalized areas of alopecia, scales, crusts, and plaques (Figs. 18.26 and 18.27) and may become severely excoriated, ulcerated, oozing, and lichenified. Leukoderma can develop at the site of the lesion but is generally reversible. Ocular diseases associated with *O. cervicalis* include equine recurrent uveitis (which is the most common cause of blindness in horses worldwide), sclerosing keratitis, vitiligo of the bulbar conjunctiva, and conjunctival nodules.[48]

The nematode lives in the ligamentum nuchae and produces microfilariae that migrate through the skin and are ingested by the intermediate host, the *Culicoides* species. Microfilarial populations vary seasonally and are highest in the spring and summer, which are also peak season for the *Culicoides* vector. Clinical signs of onchocerciasis are believed to be caused by an idiosyncratic hypersensitivity reaction to one or more microfilarial antigens because many horses that have circulating microfilariae do not have lesions.[49]

Onchocerciasis is an important disease of horses worldwide, has no breed or gender predilection, and usually affects horses 4 years of age and older. Diagnosis of onchocerciasis is most reliably made with a mince preparation or via histologic examination of skin from a biopsy specimen. Mince preparations require a 4-mm or 6-mm punch specimen of tissue. The tissue specimen is placed in a Petri dish with a small amount of physiologic saline, minced with a scalpel blade or razor,



FIG. **18.26** Ventral midline dermatitis caused by onchocerciasis.

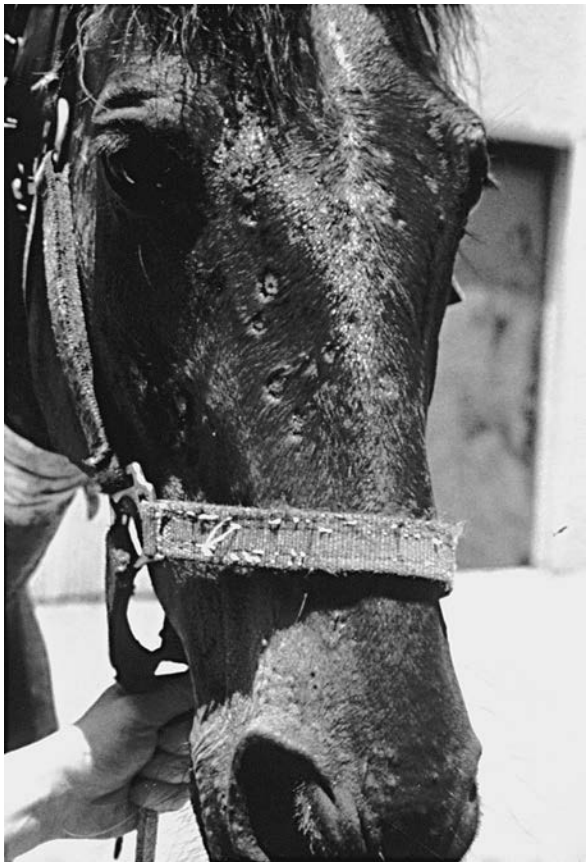


FIG. **18.27** Equine onchocercosis.

and incubated at room temperature for 30 to 60 minutes. The specimen is then examined microscopically for evidence of the rapid motion of the microfilariae. Skin biopsies reveal a superficial perivascular eosinophilic dermatitis. Often, microfilariae are visible in the superficial dermis.

Treatment with either ivermectin or moxidectin is effective for the microfilaria at the standard dosage. Some horses require two to three monthly treatments before clinical signs resolve. Approximately 25% of horses with severe disease have an adverse reaction, such as ventral midline edema or pruritus, which occurs 1 to 10 days after treatment. This reaction is believed to be caused by dying and dead microfilariae. In rare cases, severe umbilical and eyelid edema and fever may occur. Anecdotal reports suggest treatment may also precipitate an episode of uveitis.



FIG. **18.28** Recurrent *Habronema* infection on the penis of a Lusitano gelding.

Some practitioners pretreat horses with known cases of equine recurrent uveitis with nonsteroidal antiinflammatory agents. Alternatively, prednisolone at a dose of 0.5 mg/kg orally may be given, but this is not routinely recommended. None of the currently available anthelmintics kills adult parasites in the ligamentum nuchae, and affected individuals require periodic retreatment with ivermectin when clinical signs recur. The prevalence of cutaneous onchocerciasis has decreased significantly over the years with routine deworming protocols using ivermectin. The more recent use of deworming by fecal egg counts appears to have increased the incidence of *Onchocerca*-related dermatitis.

Habronemiasis. Cutaneous habronemiasis is a common nodular skin disease caused by three species of nematodes: *Habronema muscae, H. majus (H. microstoma),* and *Draschia megastoma (H. megastoma).* The housefly is an intermediate host for *H. muscae* and *D. megastoma,* whereas the stable fly is the intermediate host for *H. microstoma.* Adult nematodes live in the stomach and produce larvae, which are passed in the feces and ingested by maggots of the previously mentioned intermediate hosts. The intermediate host deposits infective larvae near the horse's mouth, which the horse then swallows to complete the cycle. Cutaneous habronemiasis occurs when the intermediate host deposits infective larvae on skin, open wounds, or chronically moist areas.

Horses with cutaneous habronemiasis have ulcerative nodules in the spring and summer that partially or completely regress in the winter. Arabians, gray horses, and horses with color dilutions (i.e., palomino, buckskin, dun) may be predisposed to developing cutaneous habronemiasis. Thoroughbred horses appear to be underrepresented. No breed, gender, or age predilection exists. Individual horses may be predisposed to cutaneous habronemiasis, exhibiting clinical signs each year, whereas other horses on the same premises never develop this condition.

Lesions commonly occur on the limbs, urethral process of the penis, prepuce, or any area with trauma to the skin (Fig. 18.28). Common periocular sites include the medial canthus, conjunctival sac, lacrimal duct, and third eyelid. Single or multiple nodules may be present. In most cases, pruritus is present, presumably caused by a hypersensitivity reaction to the parasite. The intensity of pruritus may vary from mild to severe. In severe cases in which ocular lesions are present the horse can suffer from photophobia, epiphora,

and chemosis. Dysuria can occur when lesions affect the urethral process.

Lesions often are ulcerated and appear similar to exuberant granulation tissue. Yellow granules ("sulfur granules") approximately 1 mm in diameter may be present. Microscopic examination of these granules does not reveal branching hyphae such as one frequently observes with granules from pythiosis or zygomycosis lesions.

Differential diagnoses for cutaneous habronemiasis include bacterial granuloma, fungal granuloma, pythiosis, exuberant granulation tissue, eosinophilic keratitis, SCC, and equine sarcoid. *Habronema* spp. are often present concurrently with other dermatologic conditions, and biopsy is important for a complete and definitive diagnosis.

The diagnosis is based on history, physical examination, cytologic examination, and biopsy of lesions. Occasionally the parasite itself may be directly seen and cytologic examination of exudate sometimes reveals the presence of nematode larvae. These larvae are large (3 mm × 60 μm), with a spiny tail, and are usually motile. Skin biopsies reveal a nodular to diffuse granulomatous dermatitis with large numbers of mast cells and eosinophils, and foci of coagulation necrosis are characteristic. Sometimes this area of necrosis contains cross-sections of larvae.[1,2]

Treatment of cutaneous habronemiasis should include control of the associated hypersensitivity reaction and elimination of the parasite. The routine use of macrocyclic lactones (ivermectin or moxidectin) should remove the adults from the stomach and minimize continued transmission. For horses that have ongoing lesions, repetitive ivermectin treatments spaced 2 weeks apart in addition to topical wound care is recommended. Topical wound care often includes a mixture of glucocorticoids and dimethyl sulfoxide (DMSO) to which ivermectin or moxidectin and fenthion, thiabendazole, ronnel, or trichlorfon is added. Systemic corticosteroids may be required in some horses; a common dosage of prednisolone is 0.05 to 1 mg/kg once daily for 10 to 14 days with a gradual decrease over an additional 2-week period. The addition of diethylcarbamazine (3 mg/kg PO q 24 hours) to kill the microfilaria is useful in some horses; these horses may require concurrent corticosteroid treatment to manage pruritus from dying microfilaria.

Severely affected horses will require surgical excision of lesions with follow-up topical and systemic treatment. Although cryosurgery has been recommended for cutaneous habronemiasis, the delay in healing compared with surgical removal makes the horse susceptible to reinfection for a longer period of time. In all cases of cutaneous habronemiasis, protecting the lesion from fly contact is necessary to break the reinfestation cycle.

Conjunctival habronemiasis is treatable with topical echothiophate drops instilled three times a day to kill larvae in combination with an ophthalmic ointment that contains dexamethasone and an antibiotic. Other ocular treatments include neomycin, polymyxin B, and dexamethasone applied topically three to four times daily until healed. If corneal ulcers are present, ophthalmic antibiotics without steroids are used until the ulcer is healed. After the corneal ulcer is healed, an ophthalmic dexamethasone ointment is added. Most ocular lesions heal within 5 to 18 days, with a mean of 8.2 days.[50]

Fly control is an essential part of treatment with different recommended control methods (Box 18.4).

Prognosis for horses with habronemiasis is good with appropriate and timely treatment. The veterinarian should warn the owner that habronemiasis may recur in subsequent years. Fly control is essential to preventing recurrence. Regular collection and composting of manure away from where the horse lives will also help with preventing recurrence. Placing a humidifier in the barn as a fly attractant during outbreaks of habronemiasis in drought conditions may decrease the incidence of equine infections.

BOX 18.4 Primary Management Strategies for Common Equine Pests

Stabling
- Tabanids: Diurnal and crepuscular periods
- Black flies: Diurnal and crepuscular periods
- Biting midges: Under fans; nocturnal and crepuscular periods

Exclusion devices
- Black flies: Ear nets
- Houseflies: Face masks
- Face flies: Face masks

Hay and manure management
- Stable flies: Particularly hay in pastures
- Houseflies: General sanitation

Cattle management
- Horn flies: Control pest on natural host
- Face flies: Undisturbed cattle manure requisite for larval development

Water management
- Mosquitoes: Only certain species
- Biting midges: Only certain species

Source identification and removal
- Straw itch: Normally infested hay
- Blister beetle: Normally products containing alfalfa

Restricted grazing or movement
- Chiggers: Erratic distribution in spring or fall
- Ticks: Mowing and understory control also helpful
- Tabanids: Allow horses to escape from wooded areas
- Poultry pests (sticktight fleas and lice): Separate horses from poultry

From Robinson NE, ed. *Current therapy in equine medicine.* 6th ed. St. Louis, MO: Saunders; 2009.

Pinworms. Pinworms *(Oxyuris equi)* are a common cause of tail rubbing in horses and are small white worms with a pointed tail that resembles a pin. Adult pinworms are found in the colon; the female worm migrates down the gastrointestinal tract and deposits eggs around the anus. These eggs are cemented to the skin with a thick, sticky, yellow-gray substance that can cause intense pruritus. In severe infestations, horses may also develop vague abdominal discomfort.

Eggs develop to the infective stage within a 4- to 5-day period. At this time the cement substance cracks and dries and then detaches from the skin as flakes. These flakes contain a large number of eggs that adhere to walls, buckets, and other objects in the environment.

Because ova of *O. equi* are not often observed on routine fecal flotation, a diagnosis of pinworms is based on clinical signs and identification of pinworm eggs on cellophane tape preparations. A piece of clear adhesive tape is pressed to the skin around the anus. The tape is removed and placed on a slide for microscopic examination for oval ova with an operculum or plug at one end.

Treatment is accomplished through routine deworming with ivermectin, moxidectin, pyrantel pamoate, or benzimidazoles. There are anecdotal reports of occasional resistance to ivermectin and pyrantel dewormers, but resistance must be differentiated from rapid reinfestation. If true resistance exists, fenbendazole is the treatment of choice. To avoid reinfestation the perineal area should be cleaned well at the time of deworming. Application of a light coating of oil for a few minutes followed by scrubbing with mild soap should be adequate. Infestation of pasture areas can be minimized by harrowing in midsummer. Pinworm eggs are unable to survive exposure to high temperatures, but in ideal conditions they can survive in the environment for up to 1 month.

Warbles

Warbles occasionally occur in horses and are caused by a larval stage of *Hypoderma bovis* and *H. lineatum*. Horses that develop infestation are usually pastured with cattle. Adult flies lay eggs on the hairs, and larvae migrate toward the skin surface and eventually penetrate the skin. Once in the body of the host, the larvae migrate to reach the subcutaneous tissues of the neck and trunk. A swelling develops at the site of the larvae, which eventually becomes perforated as a breathing pore is formed. Nodules are visible most often over the withers, and almost all develop a breathing pore. The nodules are often painful. Spontaneous rupture may cause anaphylaxis. Occasionally, aberrant migration causes neurologic signs.

The presence of dorsal swelling or a nodule with a breathing pore is diagnostic. The major differential consideration is collagenolytic granuloma; however, these nodules lack a breathing pore. Hypodermiasis is treated by gently enlarging the breathing pore surgically and removing the grub, removing the entire nodule surgically, or allowing the grub to fall out spontaneously. In areas of high occurrence, one may use pour-on insecticides as a preventative. Horses should be treated at the same time of year as affected cattle are treated in the area.

NUTRITIONAL AND TOXIC SKIN DISORDERS

Protein Malnutrition

Protein malnutrition in horses usually occurs from inadequate intake associated with poor quality hay or forage. Horses with long-standing systemic illness, poor dentition, significant parasitism, dysphagia, burns, proteinuria, liver disease, gastrointestinal diseases, or any other disease in which metabolic demand increases and availability of protein decreases may also suffer protein malnutrition. These horses can develop dry, dull, and brittle hair coats (Fig. 18.29). Although poor coat density has been reported in some animals, a longer hair coat with prolonged shedding may also be observed.

Iodine Toxicity

Iodine toxicity or iodism is uncommon in the horse and is generally iatrogenic in nature. Iodine is used in the treatment of infectious agents such as *P. insidiosum, S. schenckii,* and conidiobolomycosis. Even when administered at appropriate doses the prolonged treatment sometimes required can result in the classic signs of iodine toxicity, including severe dry seborrhea of the mane and tail. Some horses exhibit signs throughout their hair coat. Noncutaneous signs include cough, excessive lacrimation, salivation, nasal discharge, and joint pain. No specific therapy is indicated because horses recover spontaneously on removal of the source of excess iodine.



FIG. **18.29** Long, dry, dull hair coat in Quarter Horse and Paint yearlings with chronic protein malnutrition and parasitism. Horses also failed to shed appropriately. Dietary changes and deworming improved hair coat and overall growth. (Photograph courtesy of Dr. Judy Marteniuk.)

Selenium Toxicity

Selenium toxicosis is most common when horses eat selenium-concentrated plants that grow in soils with high selenium concentration, such as those in the Great Plains and Rocky Mountain areas of the United States. At toxic levels selenium is substituted for sulfur in sulfur-containing amino acids and alters keratinization of the hoof and hair. Toxicity occurs in any age, breed, or gender. The hair coat becomes rough, and there is a dramatic loss of mane and tail hairs. Generalized alopecia may also be visible. Lameness can be severe as the coronary band and hoof develop cracks and separate, and some hooves eventually slough (Fig. 18.30A–B).

A definitive diagnosis is made by history, clinical signs, and tissue analysis for selenium levels. Selenium in excess of 1 to 4 parts per million (ppm) from blood, 11 to 45 ppm for hair, and 8 to 20 ppm for the hoof indicates chronic toxicity. Horses should be moved from the selenium source as quickly as possible, but recovery is often prolonged and the prognosis for severely affected animals is poor. A high-protein diet rich in sulfa-containing amino acids and 2 to 3 mg orally per day of DL-methionine may be beneficial. The administration of inorganic arsenic and copper has been suggested as treatment.

Arsenic Toxicity

Clinical signs of chronic arsenic toxicity include a long hair coat, thinning of the mane and tail, and severe dry seborrhea. Horses also develop poor body condition and weight loss. At one time small doses of arsenic were commonly used in equine practice to improve hair coat and body condition, and this therapy may still be used by some veterinarians for this purpose.

Diagnosis is based on history of ingestion or treatment with arsenic or an arsenic-containing compound with the presence of compatible clinical signs. Definitive diagnosis is by measurement of arsenic levels in the kidney or liver (>10 ppm).[2] Reported treatments for chronic arsenic toxicity include sodium thiosulfate and dimercaprol.[2]



FIG. **18.30** Selenium toxicosis in a mare. Note the abnormal growth at the coronary band (A) and dramatic loss of main and tail hairs (B). (Photographs courtesy of Skip Aaroe.)

Mercury Toxicity

Mercury toxicity is rare in horses but can occur when the horse ingests feed contaminated with fungicide or ingests mercury from counterirritants. Mercury counterirritants can cause significant inflammation to the skin at the site of application and ulceration of the tongue and lips if the horse attempts to lick the substance. Systemically affected horses may have gastrointestinal disorders, depression, anorexia, and weight loss followed by generalized alopecia with subsequent loss of mane and tail hair. The diagnosis is confirmed by assaying kidney tissue in which mercury is concentrated. In addition to treatment with sodium thiosulfate and dimercaprol, orally administered potassium iodide at 4 g/day for 14 days may be beneficial.

Cutaneous Drug Reactions

Cutaneous drug reactions in horses are rare and represent an adverse reaction of the skin to any chemical compound that gains access to the skin by ingestion, injection, inhalation, or topical absorption. These reactions can occur on the first exposure to the drug, but the reaction itself may be delayed for weeks or months. It is most common, however, that the onset of the reaction occurs within 24 to 48 hours of drug administration. Drugs most likely to be involved in these eruptions include antibacterial agents (especially penicillin and sulfonamides), phenothiazine-based tranquilizers, nonsteroidal antiinflammatory agents, diuretics, and local anesthetic agents. Drug reactions are believed to involve type I, II, III, or IV hypersensitivity reactions. Urticaria is the most commonly observed sign and can be accompanied by intense pruritus, angioedema, diffuse erythema, bilateral symmetry of lesions, papular eruptions, sharply demarcated erosions and ulcerations, vesicular and bullous eruptions, photosensitization, and noninflammatory acquired alopecia. Once the drug's use is discontinued, signs may persist for varying time intervals ranging from several hours to more than 6 months. Differential diagnoses to consider include other types of hypersensitivities, systemic lupus erythematosus, and PF. Exposure to the causative drug should be carefully avoided in the future.

The diagnosis of a drug reaction depends on an accurate medication history. Skin biopsies may be helpful in supporting a clinical diagnosis. The most commonly reported patterns of inflammation are perivascular dermatitis and intraepidermal or subepidermal vesicular dermatitis.[1,2] The diagnosis may be confirmed by provocative challenge, but, as this may lead to anaphylaxis or death, it is not recommended.

Treatment requires removal of the offending drug, symptomatic therapy, and avoiding related compounds. Drug reactions usually subsides within 2 to 3 weeks but may persist for months.

Irritant Contact Dermatitis

Irritant contact dermatitis is fairly common in horses and, unlike allergic contact dermatitis, the offending substance invariably causes a reaction if left in direct contact with the skin; prior sensitization is not needed. The mechanism of irritant contact reaction depends on the causative chemical, but moisture and tissue maceration are important predisposing factors. Common irritating substances include feces, urine, wound secretions, caustic substances, crude oil, diesel oil, turpentine, blisters, leg sweats, improperly used insecticides, fly wipes, concentrated disinfectants, irritating plants, and soiled bedding.

No age, gender, or breed predilection exists for irritant contact dermatitis. Acute lesions are often erythematous, vesiculated, erosive or ulcerated, and painful. As the condition continues, necrosis, crusting, scaling, and hair loss occur. Leukoderma and leukotrichia are common and may be permanent. Pruritus and pain vary.

The diagnosis is determined from the history, clinical signs, and inspection of the horse's environment. Provocative testing is useful in identifying and confirming the causative agent, as long as the risk is minimal to the horse. Histologic examination of skin reveals necrosis, spongiotic vesicles, and ulceration.

Treatment requires identification and removal of the offending substance. One should wash affected areas daily with copious amounts of water and mild shampoo. Secondary bacterial infections should be addressed. Healing is usually rapid once the irritant is removed.

Irritant and Toxic Causes of Mucocutaneous Vesicles

Irritant reactions, toxic chemicals, or allergic reaction to drugs may result in oral mucocutaneous vesicles. One should

suspect these causes when lesions develop acutely in the absence of signs of systemic illness, in single animals within a herd, or in horses receiving medications or counterirritants. Some formulations of ivermectin dewormers, orally administered fluoroquinolone antibiotics, and NSAIDs are among the most common veterinary products to cause mucocutaneous vesicles. Mercurial compounds (commonly used in counterirritants) can also cause generalized hair loss, lameness, and emaciation in chronically intoxicated animals. Creosol and cantharidin beetles are common causes of oral ulcers. Foreign bodies such as grass awns may cause mucocutaneous ulceration and vesicles.

Envenomation

Many species of snakes, spiders, and insects have venomous bites and stings. Except for snakebites, these animals lack sufficient quantity of venom to cause more than transient pain or local inflammation. Venoms contain a variety of substances, including enzymes, peptides, polypeptides, amines, and glycosides, which act locally by causing tissue necrosis and vascular thrombosis and hemorrhage or systemically by causing widespread hemolysis and neurotoxicity.

Snake Bites

Of the poisonous snakes in the United States, bites from rattlesnakes, water moccasins, and copperheads are most common in horses.[51,52] The venom from snakes can be hemotoxic and proteolytic and produces extreme local swelling with significant tissue and red blood cell destruction.

Most bites occur in the spring and summer. Horses are most often bitten on the nose, head, neck, and legs. Snakebites may or may not involve envenomation. Bites in which no venom is injected swell minimally and are only slightly painful (dry bite). When envenomation occurs (wet bite), rapid swelling, pain, and local hemorrhage develop, usually within 60 minutes of the bite. Fang marks are often difficult to find because of tissue swelling. Edema, erythema, and tissue necrosis develop over days, and the skin may slough (Fig. 18.31). Bites occurring on the face or head are serious because of the risk of respiratory and nasal edema. If respiratory distress is severe, the horse will need a tracheotomy.

Snakebites should be treated symptomatically. The wound should be cleaned and hydrotherapy with cold water provided to minimize swelling. If swelling is already present, warm hydrotherapy may stimulate circulation and removal of tissue edema. Whether hydrotherapy increases the absorption of venom in the early stages of the bite is unknown. Broad-spectrum antibiotics are indicated to prevent secondary infection. The use of glucocorticoids is controversial but may be beneficial in decreasing inflammation and pain. Nonsteroidal antiinflammatory agents also may be beneficial. Surgical debridement and wound closure may be necessary if severe necrosis and sloughing occur. Although specific antivenoms are available, they are of limited use. Maximal benefit is attained only when they are administered within hours of the bite. The volume of antivenom necessary to treat snakebites in horses may be cost prohibitive.

Fire Ant Bites

Fire ants, *Solenopsis* spp., are common in the southern United States. Single or small numbers of stings are acutely painful, and pustules or crusts develop rapidly at the site. Horses are bitten most commonly on the legs, nose, and ventrum, but massive exposure can occur if the horse is bitten by hundreds or thousands of ants. Fortunately, horses that live in the southern regions quickly learn to avoid fire ant hills, and massive exposure is uncommon. Complications of massive exposure include infection, anaphylactic shock, bronchospasms, and sloughing of the epidermis. Single or multiple small numbers of solitary stings usually require little or no treatment. Applying an antibiotic ointment to minimize the chance of myiasis is prudent; otherwise, wounds heal without complication. If massive exposure has occurred, one may need to use systemic antibiotics and NSAIDs to decrease pain and swelling.



FIG. **18.31** Face of a horse after being bitten by an Eastern diamondback rattlesnake.

Spider Bites

Black, brown, and red widow spiders (*Latrodectus* spp.) and the brown recluse *(Loxosceles reclusa)* spider are common in America, whereas in Australia it is the black house spider *(Ixeuticus)* that causes painful, inflamed bite lesions. Spider bites are characterized by hot, edematous, painful swellings in the area of the bite. The brown recluse spider has a dermal necrotoxin that can cause significant dermal necrosis.

Treatment is symptomatic. Cold ice packs applied to the area, and systemic glucocorticoids or antihistamines, or both, may be helpful. Spraying the horse's environment with a parasiticide is important. Owners should clean the premises and remove materials that create areas for venomous spiders to hide.

IMMUNE MEDIATED SKIN DISORDERS

Hypersensitivity Reactions

Allergic dermatitis is common in equine practice and most often presents as urticaria and/or pruritus. Horses are frequently sensitive to multiple allergens, each of which contributes to the total antigen load and pruritic threshold, making it necessary to identify and correct as many factors as possible to obtain long-term control.

Urticaria (Hives)

Urticarial lesions (Fig. 18.32) are caused by dermal mast cell degranulation and release of active compounds, including histamine, platelet-activating factor, and prostaglandins. Relaxation of vascular smooth muscle cells and retraction of endothelial cells occurs and allows plasma to extravasate and form wheals. Nonimmunologic (physical) urticaria can be caused by heat, cold, pressure, and exercise. The surface of the lesions can appear normal, but with severe dermal edema serum will ooze to the skin surface, causing focal crusting and matting of the hair coat with focal hair loss. Lesions may manifest as singular eruptions, intermittent eruptions, or chronic recurrent urticaria. Wheal formations vary from 2 to 3 mm up to 20 to 40 cm. Papular wheals (3–6 mm) are often linked to biting insects. Giant wheal formation or polycyclic urticaria is often related to drug reactions. Annular wheals have a ring of edema surrounding a central, nonedematous depression. Angioedema is diffuse edema of the dermis and subcutis and is a significantly rarer manifestation of allergy than urticaria. Urticarial and angioedema lesions frequently resolve within 24 to 48 hours but may recur.

Insect bites are the most common cause of urticaria in the horse. Salivary antigens provoke an IgE-mediated inflammatory response that results in pruritus. Degranulation of mast cells and basophils causes the release of histamines, interleukins (ILs), prostaglandins, and kinins.

Treatment of acute urticaria usually includes glucocorticoid therapy. Specific treatment recommendations for acute and chronic urticaria are described in detail in the next section.



FIG. **18.32** Urticaria in a horse following the administration of penicillin.

Insect Bite Hypersensitivity

Insect bite hypersensitivity (IBH) is the most common allergic skin disease of horses[53] and ordinarily manifests as a chronic relapsing seasonal allergic dermatitis. In contrast to what the name implies, IBH is not simply a response to salivary antigens in the actual bite; rather, transcutaneous absorption and inhalation of desiccated insect parts can also incite the allergic response. Lesions are most common on the dorsal and ventral body. Affected horses may have crusted papules and evidence of self-trauma including ulcers and erosions, hair loss, lichenification, and pigmentary alterations. Chronic pruritic or nonpruritic recurrent urticaria may also be present. Some horses are so severely affected that they completely denude their entire mane and/or tail. Secondary bacterial infections and behavioral changes are common, and the disease can be a significant animal welfare issue in severely affected equids. Traditionally, IBH has been associated with insects of the genus *Culicoides;* biting midges, also known as no-see-ums; midgies; punkies; and sandflies, which belong to the Ceratopogonidae family. *Culicoides* are closely related to Simuliidae (black flies or buffalo gnats), which have also been implicated in IBH. *Haematobia* (horn flies), *Stomoxys calcitrans* (stable flies), mosquitos (Culicidae) and less commonly *Chrysops* (deer flies), *Musca* (houseflies), *Tabanus* (horse flies), bees, and wasps may also trigger IBH. Some horses have hypersensitivities to multiple biting insects.[54]

Insect hypersensitivity reactions are seasonal in colder climates but can be nonseasonal in warmer climates in which insects persist year around. Most horses with IBH begin to show clinical signs between 3 and 4 years of age; the hypersensitivity commonly exacerbates as the animal ages. There is no gender, breed, or color predisposition. Certain breeds appear to be at increased risk including Icelandic, Friesian, Arabian, and Quarter Horses. There is a demonstrated heritable component to IBH as well as an immunotolerance component, which has been demonstrated in Icelandic horses imported from Iceland in which *Culicoides* spp. are absent. Age at time of importation is positively correlated with the likelihood of development of IBH later in life. Of horses that were imported before they were 10 months old, only 6% developed IBH, whereas of those imported at 12 years of age 80% developed the disease.[55]

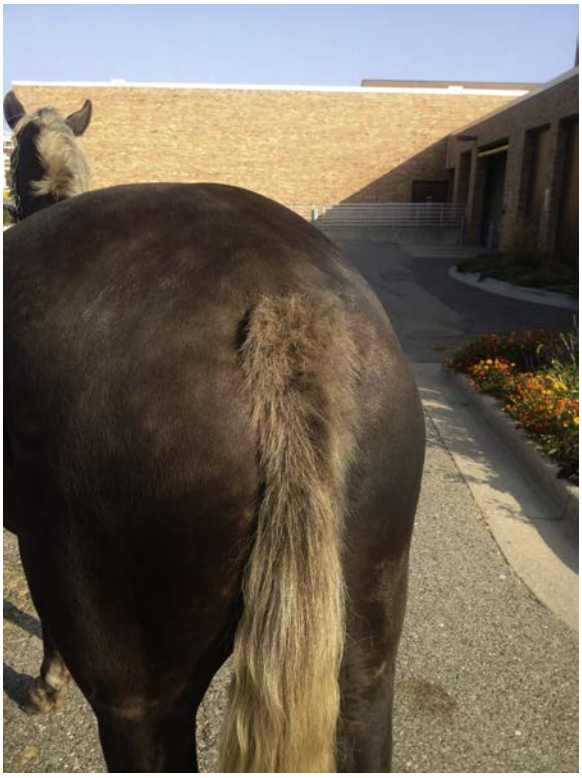


FIG. **18.33** A horse with *Culicoides* hypersensitivity. This horse has evidence of mane and tail rubbing and ventral midline dermatitis typical of this disease. Intradermal skin testing confirmed hypersensitivity to a number of additional antigens.

The distribution of IBH skin lesions varies and depends on the feeding preferences of the insect involved. Some *Culicoides* spp. commonly feed on the mane, tail, dorsum (Fig. 18.33), and ventral midline of the horse, but the face, ears, croup,



FIG. **18.34** Alopecia with some crusting around the umbilicus on a horse with horn fly infestation.

distal limbs, ventral trunk, and neck can be frequent feeding spots for other *Culicoides* spp. Black flies tend to feed on the head, ears, and ventral abdomen and may produce a generalized pattern of ventral midline edema. Horn flies typically feed around the umbilicus (Fig. 18.34). Mosquitoes prefer feeding on the lateral aspects of the body, but lesions associated with their bites may be more of a generalized papular urticaria or true hives. Stable flies prefer feeding on the lower legs but have also been known to feed on the ventral abdomen, chest, and back. The lesions in any given IBH horse may change over weeks to months as the specific population of *Culicoides* spp. or other insects present in the environment changes.

Some insects require a specific environment to propagate. Knowledge of these requirements may be helpful in establishing insect control programs. *Culicoides* spp. and mosquitoes prefer freestanding stagnant water for propagation, whereas black flies prefer moving water such as a stream, brook, or river. Horn flies are obligate parasites of cattle that require fresh cattle feces for reproduction. Several species of insects, including stable flies and houseflies, prefer decaying vegetation or manure for reproduction. They tend to be a bigger problem when sanitation practices at the stabling facility are problematic.

The time of day that an insect prefers to feed also differs among species. *Culicoides* spp. are often more active at night, and black flies are more active in the morning and late afternoon. Mosquitoes are most active at dusk and the first 2 hours after sunset. Horseflies, stable flies, black flies, and horn flies are usually active during the daylight hours. If the time of feeding is known for the potential offending insect, then the horse may be stalled during peak feeding hours to limit exposure.

The diagnosis of insect hypersensitivity is based on a compatible history, physical findings, and elimination of other causes of pruritus in the horse. Skin biopsies may reveal the presence of a superficial or deep perivascular eosinophilic dermatitis with epidermal spongiosis (intercellular edema of the epidermis), necrosis, and collagen degeneration. Although intradermal skin testing has been used to diagnose hypersensitivity, it is best used to confirm the allergens necessary for hyposensitization (see the section Treatment). The most important aspect of treatment for IBH is insect control. Fortunately, control measures are similar for most biting and flying insects (see Boxes 18.4 and 18.5). Additional treatment strategies are described in the next section.

Atopic Dermatitis

Atopy is the second most common form of skin allergy in the horse. It is an inherited hyperreactivity to environmental allergens that produce skin disease and, less commonly, respiratory disease. Atopy may also be associated with headshaking. Horses with atopy form sensitizing antibodies against a variety of antigens including grasses, pollens, dust mites, molds, trees, and fabrics. One or more of these allergens may be involved in any individual horse.[37]

A preliminary study on the skin barrier of atopic horses indicates that it is different from that of normal horses.[56] Similar abnormalities in the skin barrier in atopic dogs and humans are known to allow absorption of allergenic proteins, which are then carried to the lymph nodes. Naive T lymphocytes are activated, and IgE production is induced.[54] A variety of cytokines, including IL-4, IL-5, IL-6, IL-13, and IL-31, are released, and some stimulate itch.[54] Allergen-specific IgE binds to mast cells, basophils, Langerhans cells, and other cells within the skin and mucous membranes, and further allergens are captured. Subsequent exposure to the allergen results in amplification of the allergic response.[54]

Intradermal skin testing can be used to confirm that a horse has atopy and identify the allergens needed for hyposensitization treatment. Although serum allergy testing has also been recommended for this purpose, the sensitivity and reliability of the current tests are poor.[57] Additional strategies for treatment of atopy are provided later in this chapter.

Food Hypersensitivity

Food hypersensitivity is an immune-mediated adverse reaction to a feedstuff unrelated to any physiologic effect of the feed. Food can cause hypersensitivity by contact or ingestion. Food allergy might be suspected when a horse is presented with nonseasonal pruritic disease or urticarial signs. Pruritus and urticaria may be observed in areas less likely to be affected by an insect hypersensitivity; generalized or multifocal pruritus and urticaria are common ("grain bumps"). Pruritus limited to the base of the tail and anus would also increase the consideration of an adverse food reaction. Flaking, lichenification, and gastrointestinal signs may occur concomitantly.

There is no accurate in vitro or in vivo diagnostic test for food allergies in horses,[58] and allergen-specific immunotherapy (ASIT) for diagnosis of food allergy remains controversial. Lesions observed in skin biopsy specimens from affected horses are nonspecific (perivascular dermatitis with eosinophilia) and identical to those seen in specimens from horses with any other allergic skin disease.

Food allergies are classically considered to result from ingestion of specific feed types. In horses, however, allergic reactions to food may also occur through inhalation, or transdermal exposure. Dusty foodstuffs should be removed from the diet in case there is an inhalant allergen present. Hay and other feed storage should be investigated. Housing of the affected horse should be modified to avoid overhead storage of feed or exposure to the diets of other horses.

BOX 18.5 Parasite Control Strategies for Insect Bite Hypersensitivity

Medical treatments of insect bite hypersensitivity with corticosteroids, antihistamines, and topical shampoos or sprays is only palliative and must be coupled with practices aimed at reducing a horse's exposure to biting insects. These practices include but are not limited to the following management strategies.

USE OF INSECTICIDES AND PHARMACOLOGIC AGENTS

- Apply long-acting insect repellents twice daily, especially in the evening and early morning hours. Modern pyrethroids such as cypermethrin in combination with piperonyl butoxide to enhance potency are among the best choices. Long-acting fly repellents can also be applied to fly sheets.
- Long-acting "spot treatments" such as Vectra 3D (Ceva Animal Health, LLC, Lenexa, Kansas), which contains dinotefuran, pyriproxyfen (an insect growth regulator), and permethrin can be used. Three tubes of the "dog over 95 lb" size are used: one applied to the mane and face, one to the back and rump, and one to the ventral abdomen.
- Treatment with concentrated (44%–64%) permethrin products may result in hair loss and/or paresthesia at the site of application in the horse. Application of vitamin E oil to the application site prior to product use may mitigate this effect (Freedom 45 Spot-On [Star Horse Products NV, Carson City, Nevada].
- Insecticidal impregnated fly sheets are available and may be washed multiple times without a decrease in efficacy (Amigo Bug Blaster Fly Sheet).
- Place permethrin-impregnated tags in the mane, tail, and forelock.
- For those clients interested in natural fly repellents, Eco-Vet (Snohomish, Washington), a fatty acid-based fly repellent, is recommended.

OTHER MANAGEMENT STRATEGIES

- Use a fly sheet, fly mask with ear protection, and fly leg guards when the horse is turned out.
- Keep the horse stabled at times of day when biting insects are abundant.
- Use a good quality fan (industrial, appropriate for barn use) on the horse when the horse is stalled (many of the biting insects are poor flyers).
- Use automated fly sprayers in the affected horse's stall or throughout the stabling area.
- Use a Mosquito Magnet (Woodstream Corporation, Lititz, Pennsylvania) or similar system, which attracts and kills mosquitoes, black flies, *Culicoides,* sand flies, and other biting insects with carbon dioxide. An area the size of one acre can be controlled with the mosquito population collapsing in 6 to 8 weeks as egg-laying females are destroyed.
- Avoid turnout near insect havens such as bodies of water, manure piles, or cattle.
- Manage wet areas around the farm and routinely clean or treat water troughs.
- Use larvicidal feed supplements (SimpliFly, Farnam Corporation, Phoenix, Arizona).
- Place fly predator wasps (Spalding Laboratories, Reno, Nevada) in areas where manure accumulates.

The diagnosis of food allergy is most accurately made by eliminating the causative feed from the diet of affected horses through a food elimination trial. A complete dietary history is essential, and all components of the diet (concentrate, forage, and supplements) should be considered. A practical approach to a food elimination trial is to begin by eliminating any supplements and concentrates from the diet for 4 to 6 weeks. Major changes in diet (hay and grain) should be made gradually to prevent colic. The hay should be switched from grass hay to alfalfa or vice versa. After clinical signs disappear, feedstuffs are reintroduced to confirm that the pruritus is caused by a food allergy and that improvement was not coincidental. The offending substance is identified by reintroducing one dietary item at a time each week and observing the horse for recurrence of clinical signs, usually within 24 to 72 hours. The hypoallergenic diet is reinstituted until clinical signs subside.

Management of horses with food allergy consists of permanent dietary and pasture restrictions along with the administration of glucocorticoids and antihistamines on an as-needed basis. Omega fatty acid supplementation may be beneficial. Colloidal oatmeal shampoos can be used to minimize transdermal allergen exposure to feedstuffs. Additional information regarding treatment strategies is provided in the following section.

Contact Hypersensitivity

True contact allergies are rare in horses, perhaps in part because the hair coat acts as a protective barrier but may result from exposure to pasture plants, bedding, tack, and topical agents. Parasiticides and bath oils applied as insect repellents are some of the most commonly identified causes of contact allergy. Contact allergies, when they occur, are type IV hypersensitivity reactions. Potential allergens are usually small molecules that penetrate the skin, bind to dermal collagen or carrier proteins, and are taken up by antigen-presenting cells (Langerhans cells) in the dermis. On reexposure, an inflammatory response occurs that results in skin disease. Development of allergic contact reactions can takes months to years; a horse may suddenly develop clinical signs of a hypersensitivity reaction to a substance to which it has been exposed for years without prior incident.

Clinical signs of contact allergy vary and depend on the duration of the allergic reaction. Erythema, swelling, vesicles, papules, oozing, pain, or pruritus develops within 2 to 3 days of application of the offending substance. If untreated, lesions often progress over weeks or months to alopecia, lichenification, and crusting. The distribution of the lesions may suggest the cause: legs (plants and bedding); face and muzzle (plants and bedding); face and trunk (tack); and face, ear, neck, and trunk (insect repellents).

A definitive diagnosis of contact allergy is based on provocative exposure or patch testing. Provocative exposure requires avoiding the suspect substance for 10 days or until lesions resolve, reexposing the horse to the substance, and noting recurrence of lesions or clinical signs over the next 7 to 10 days. Provocative testing does not distinguish between an

irritant reaction and an allergic reaction but does identify the offending substance.

Patch testing requires the application of the suspect substance to an area of the skin for 48 to 72 hours. Plant material, bedding, or other particulate matter does not adhere well to the skin and is tested best under an occlusive dressing (closed patch test). For each test substance, a 3-cm^2 area of hair on the thorax or dorsum is clipped, a small amount of the suspect substance is applied to the skin, and the site is covered with a gauze sponge bandage for 48 to 72 hours. A control site should also be bandaged. On removing the bandages, the area is assessed for erythema, swelling, induration, pain, and exudation. Biopsies of the control should be obtained and the site or sites tested for histologic confirmation of the diagnosis. Liquid substances may be applied to the skin daily without using an occlusive bandage.

The suspected material should be removed from the horse's environment for a minimum of 10 days. After clinical signs regress the substance can be reintroduced. Signs of recurrence within 1 to 3 days provide a definitive diagnosis. If the causative agent cannot be identified, oral or topical glucocorticoids, mild cleansing shampoos, and omega fatty acid supplementation are used to reduce inflammation as described in the following section.

Treatment of Allergic Skin Disease

Elimination or reduction of allergen exposure is the optimum treatment for equine allergies, but unfortunately it can be impractical for many horses. Most affected horses are treated with some combination of management changes, ASIT, pharmacologic therapy, dietary supplementation, and medicated shampoos.

Pharmacologic Therapy. Corticosteroids have long been the preferred drug for pharmacologic management of allergy treatment despite their association with a variety of adverse effects. Laminitis is the most concerning potential adverse effect of corticosteroid therapy in horses, but polyuria, polydipsia, increased susceptibility to infection, mood changes, and increases in liver enzymes may also occur. The most commonly used corticosteroids for the short-term control of equine allergic reactions are prednisolone and dexamethasone. Precise treatment recommendations should be individualized for each patient and adjusted according to patient response. A suggested regimen for prednisolone is 0.5 to 1.5 mg/kg PO daily as an induction dose for 7 to 14 days and then tapered to 0.2 to 0.5 mg/kg every 48 hours for 2 to 5 weeks for maintenance. Administration of prednisolone in the morning before 9 a.m. may lessen the likelihood of the development of laminitis, possibly because it is consistent with the natural diurnal rhythm of cortisol production in the horse.[59] A recommended loading dosage for dexamethasone is 0.05 to 0.1 mg/kg, PO, IM, or IV, every 24 hours for 4 to 7 days, decreasing to a maintenance dose of 0.01 to 0.02 mg/kg every 48 to 72 hours. The injectable dexamethasone formulation can also be administered orally, but the bioavailability is only 60% to 70%. The decrease in bioavailability observed with oral dosing should be considered when developing a tapering dosage regimen for a patient.

Long-term glucocorticoid use is not recommended if it can be avoided. Antihistamines and tricyclic antidepressants may be used to reduce the dosage and length of glucocorticoid treatment. Commonly used agents include cetirizine at 0.2 to 0.4 mg/kg PO every 12 hours and hydroxyzine hydrochloride or pamoate 0.5 to 1 mg/kg, PO, every 8 to 12 hours. Alternatives include doxepin (0.5–0.75 mg/kg, PO, every 12 hours), chlorpheniramine (0.25–0.5 mg/kg, PO, every 12 hours), diphenhydramine (1–2 mg/kg, PO, every 8–12 hours), and pyrilamine maleate (0.8–1.32 mg/kg IV, IM, or subcutaneously; may be repeated in 6–12 hours if needed). Generally, oral dosing of antihistamines is preferred because of the potential for adverse reactions with IV administration. Because there can be significant variation in the response to these agents, it may be necessary to try drugs from different classes at 2-week intervals to find an effective treatment. Adverse effects associated with antihistamines include sedation and behavior changes.

Pentoxifylline (8–10 mg/kg, PO, every 8–12 hours) is a phosphodiesterase inhibitor with steroid-sparing, immunomodulating effects that can further improve the clinical signs in allergic horses. It alters T- and B-cell activation and proliferation, decreases leukocyte adhesion and aggregation, and lessens the response to inflammatory cytokines. Pentoxifylline also has rheologic effects that may minimize laminitis risk with corticosteroid therapy.

Allergen Specific Immunotherapy. The goal of ASIT or hyposensitization is to redirect the immune response to an allergen to reduce the hypersensitivity reaction. The precise mechanism(s) of action for ASIT is unknown, but several theories have been proposed. These include the production of blocking antibodies by shifting the immune response from one dominated by IgE to one dominated by IgG, altering the proportion of type 1 and type 2 T-helper cells, and induction of regulatory T cells. The percentage of horses that respond to ASIT varies between 60% and 85% and may be related to antigen preparation. ASIT may take 1 to 24 months (average 3–6 months) before effects are recognized. Because ASIT therapy is not prohibited for competition horses, it is a potentially useful therapy for this population. Unlike other treatments, ASIT may result in remission of the allergy. ASIT has been recommended for long-term control of insect hypersensitivity, recurrent urticaria, and atopy. Other therapies such as corticosteroids, antihistamines, and fatty acids may be used concurrently with ASIT to treat allergic skin disease.

Dietary Supplementation. Omega fatty acids can act synergistically with steroids and antihistamines to decrease inflammation. Fresh ground brown flax by itself has an antiinflammatory effect in the skin of allergic horses.[60] Whole flaxseed is chemically stable but will pass through the equine gastrointestinal tract intact, and ground flaxseed can become rancid at room temperature within 7 to 10 days. Whole flaxseed can be ground to an appropriate form using a small coffee grinder and fed to horses at a rate of 0.5 to 1 lb daily for a 500-kg horse. A large variety of fatty acid supplements are commercially available in capsule, powder, pellet, or oil formulations.

Medicated Shampoos. The major route of allergen delivery in humans and dogs is transcutaneous. It is unknown whether this is true for horses, but topical therapy with appropriate shampoos does appear to significantly increase comfort level and decrease the need for systemic medications presumably by removing surface irritants, bacteria, allergens, and the pruritic load. Rehydration of the skin improves the integrity of the epidermal barrier.

Horses should be bathed in cool water, which will constrict surface vessels and minimize the release of histamine and delivery of other inflammatory mediators to the skin. Surface cleaning of the skin with a nonirritating cleanser such as diluted Ivory dish soap before the use of

the medicated shampoo may be necessary for the extremely dirty horse; however, shampoos with a strong detergent component may strip the skin of ceramides and other fats important in maintaining the skin's natural barrier to allergens. Contact time (generally 10–15 minutes) is an important consideration in medicated shampoo use. Moisturizing hypoallergenic shampoos and/or colloidal oatmeal shampoos are available from a variety of manufacturers and suppliers. These shampoos help raise the pruritic threshold by cooling and moisturizing dry skin. Products that contain local anesthetics, such as pramoxine, can provide relief at least for short periods. Shampoo, sprays, and mousses containing chlorhexidine, ketoconazole, and benzoyl peroxide can help reduce secondary infections of the skin. Antibacterial shampoos can be used to reduce secondary infections and do not require the lengthy contact time of the moisturizing and colloidal oatmeal shampoos; leaving antibacterial shampoos on the horse for long periods of time has resulted in skin irritation in some horses.

Other Therapies. Anecdotal reports suggest possible benefits from a wide variety of other therapies for allergic horses. Methylsulfonylmethane (MSM), a DMSO derivative with antioxidant properties, may be effective as an aid to relieve pruritus associated with equine insect hypersensitivity. MSM is administered at 10 to 12 g/500 kg PO q 12 h initially and then tapered to q 24 h. Preliminary studies in tissue culture of equine fibroblasts indicate that avocado-soybean unsaponifiables are effective antiinflammatory agents for skin when administered at the high end of label directions.

Anecdotal reports also suggest that oclacitinib (Apoquel; Zoetis, Parsippany, NJ), a Janus kinase inhibitor that has been successful in the control of allergic dermatitis in dogs, has a positive effect in horses when administered at 0.25 mg/kg PO q 12 h for 14 days.

Pemphigus Foliaceus

PF is an uncommon but potentially life-threatening autoimmune disease of the skin. Affected horses produce autoantibodies directed against the surface proteins of keratinocytes that mediate intercellular adherence. Autoantibodies against desmoglein-1 and desmoglein-3 (DSG-1 and DSG-3) destroy the bonds that maintain cell-to-cell adhesion.[59] The cells separate from one another, leaving a space that fills with fluid, resulting in a blister or pustule close to the horse's skin surface. Although these early lesions are the preferred biopsy sites, they are quite fragile and rupture quickly after being formed. This leads to oozing, crusting, scaling, and erosions of the horse's skin (Fig. 18.35), which are the most common signs of PF.[61] Some horses may develop acute, recurrent, or chronic hives before progressing to fulminant PF. A number of horses with PF develop significant alopecia. Lesions commonly begin on the face, legs, or abdomen and spread quickly over the next 1 to 3 months. Occasionally horses have lesions that localize to the face or coronary band for prolonged periods. A number of horses with PF have considerable swelling of their distal limbs and ventral abdomen with or without pain or pruritus and in the absence of hypoproteinemia. Many horses are in so much pain they are reluctant to move and can exhibit systemic signs such as fever, depression, lethargy, anorexia, and weight loss.

Although Appaloosas, Quarter Horses, and Thoroughbreds have been implicated as being predisposed, any breed can develop PF, and there is no reported age or gender predisposition.[62] Some studies indicate a seasonality to PF (between September and February),[63] which suggests that

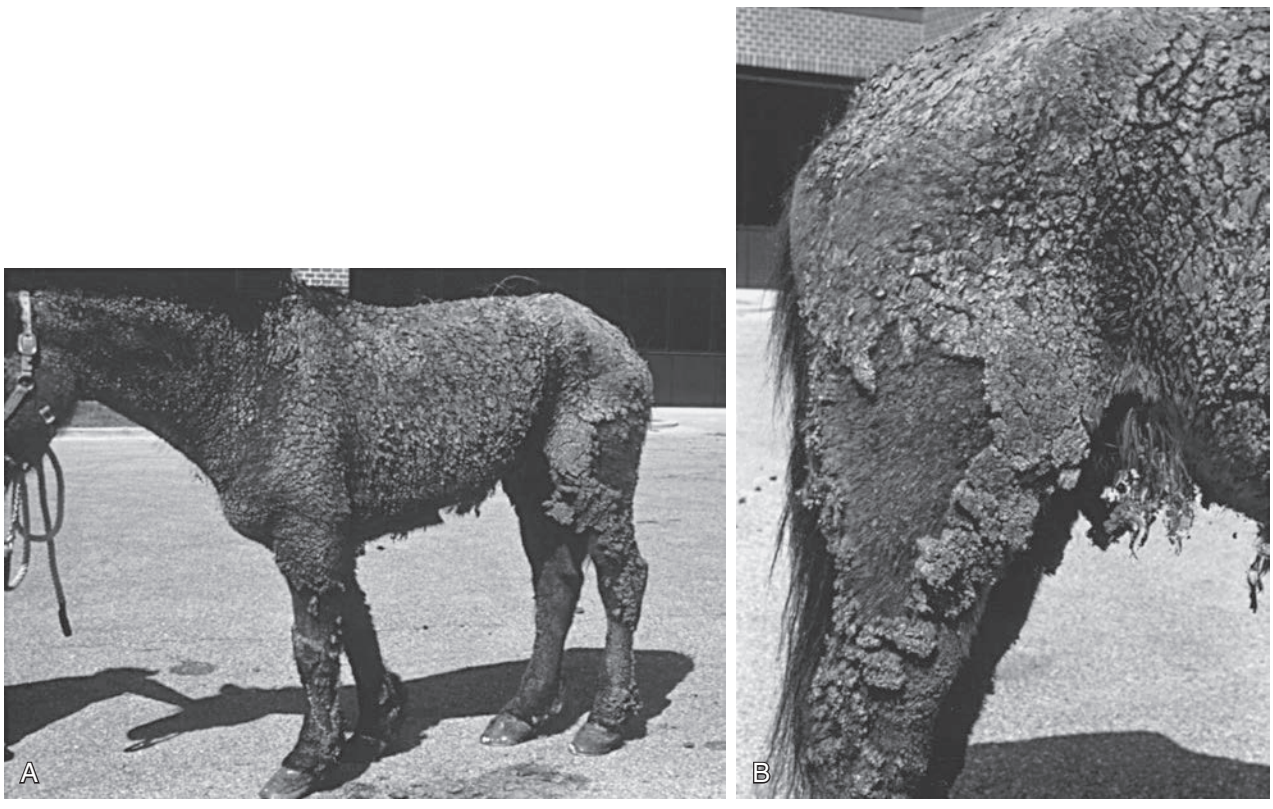


FIG. **18.35** (A) Pemphigus foliaceus in a horse. (B) Skin with significant crusting.

insect hypersensitivity, seasonal allergens such as pollens, and ultraviolet light may be predisposing or initiating factors in some horses. Drugs often used during this time frame such as antibiotics, dewormers, vaccines, and some supplements may also be involved.

It can be challenging to confirm a diagnosis of PF in horses. Early stages may be confused with allergic reactions such as drug or insect allergies, dermatophytosis, dermatophilosis, lymphoma, and defects in keratinization such as cannon keratosis. Other diseases should be ruled out with appropriate tests (special stains, fungal cultures, etc.). A tentative diagnosis of PF may be made based on impression smears of the content of intact pustules or the underside of exudative crusts (Fig. 18.36). Rafts of deeply basophilic acantholytic cells and nondegenerate neutrophils are highly suggestive of this disease.

Definitive diagnosis is made by routine histologic examination of a skin biopsy specimen. Submitting a skin biopsy with intact crusts attached to the underlying skin or hairs is critical. Histologically, PF is characterized by intragranular to subcorneal acantholysis. Layered rafts of acantholytic cells within crusts interspersed among neutrophils may be observed. A minority of horses with PF will have a predominance of eosinophils in the inflammatory infiltrate.[64] Direct immunofluorescence is unreliable for diagnosis but, if positive, reveals diffuse intercellular fluorescence.

The mainstay of treatment is high doses of glucocorticoids such as prednisolone at 1.0 to 2.5 mg/kg orally in the morning every 12 hours until no new lesions develop, which usually occurs within 7 to 10 days, at which time tapering over several weeks is indicated.[59] A maintenance dose of 0.5 mg/kg to 1 mg/kg every 48 hours is administered indefinitely. Alternatively, an immunosuppressive dose of dexamethasone can be administered at 0.02 to 0.1 mg/kg per day for 7 to 10 days and subsequently tapered to 0.01 to 0.02 q 48–72 h.

Chrysotherapy (gold salts) has also been used successfully in horses with PF. Unfortunately, aurothioglucose (Solganal; Schering Corporation, Kenilworth, NJ) is no longer available, but aurothiomalate (Myochrysine; Merck and Co., White House Station, NJ) can be used. Test doses of 20 and 50 mg are given at weekly intervals to evaluate for potential adverse effects (eosinophilia and cutaneous reactions). If no adverse reactions occur, an induction dose is given at 1 mg/kg IM weekly with a long lag phase of 6 to 12 weeks before a positive response is noted. Monitoring for stomatitis, urticaria, skin sloughing, blood dyscrasias, and proteinuria is recommended. If no adverse effects are observed, weekly therapy is continued at 1 mg/kg IM until control is achieved (6–12 weeks). Maintenance therapy is tailored to the individual animal, which may involve biweekly or monthly therapy. Complete remission of clinical signs may not be possible to achieve even with combination therapy. Weekly monitoring of a complete blood count and urinalysis is recommended for the first month and then monthly if no abnormalities are observed. Azathioprine has been administered as a steroid-sparing drug to treat equine PF. The recommended dosage for azathioprine is 1 mg/kg per day orally for 14 days then every other day or 2.2 mg/kg every other day orally. The use of omega fatty acid supplements such as fresh ground flax and bathing horses with PF in cool water may make them more comfortable.

Spontaneous regression has been reported in rare cases of young horses with PF, but most horses will require long-term if not lifelong treatment. Generally, younger horses are more likely to achieve remission than older horses and tend to have less severe disease and respond better to treatment. Unfortunately, regardless of age approximately 50% of horses that initially respond to treatment relapse and become progressively less responsive to treatment with each relapse. Horses are frequently euthanized because of the disease or because they develop laminitis possibly related to glucocorticoid treatment. Some horses die acutely when treatments fail.

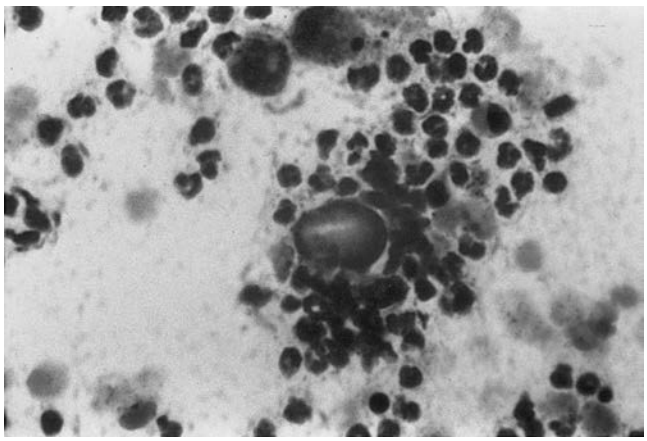


FIG. **18.36** Cytologic appearance of a touch preparation from a pemphigus lesion. Rounded cells (acanthocytes) are notable.

Pemphigus Vulgaris

Pemphigus vulgaris (PV) and bullous pemphigoid are less common than PF in horses and have a poorer prognosis.[61] Lesions of PV occur in the mouth and at other mucocutaneous junctions where vesicles, bullae, and painful ulcers are common. The immune reaction occurs at the basement membrane in which immunofluorescence has identified circulating anti-DSG-3 IgG in affected horses. Similar to PF, lesions can also occur in the mane and tail and on the coronary bands. Despite aggressive therapy the prognosis remains grave. On necropsy, horses with PV may have ulcerations in the esophagus and stomach.

Bullous Pemphigoid

Of the autoimmune diseases that make up the pemphigus complex, bullous pemphigoid is the rarest and has the poorest prognosis.[61] Similar to pemphigus vulgaris, crusts and ulcers are common, and ulcers can extend throughout the gastrointestinal tract.[61] Skin lesions occur in the axilla and groin. Vesicles and bullae rupture easily and are often crusted; secondary bacterial infections may occur. Pain, pruritus, anorexia, fever, and salivation are common.

The immune reaction in bullous pemphigoid occurs when antibody binds to the basement membrane zone and complement fixation occurs, resulting in production of inflammatory mediators chemotactic for neutrophils and eosinophils. These cells release proteolytic enzymes that disrupt the dermal-epidermal cohesion, resulting in subepidermal cleft and vesicle formation. Acantholysis is absent in this disease. A definitive diagnosis is made by eliminating other causes of vesicular lesions and by skin biopsy. Biopsy of a vesicle or intact bullae is essential; biopsy of crusted or ulcerative lesions will be nondiagnostic. Bullous pemphigoid is characterized by subepidermal vacuolar alterations and subepidermal clefts and vesicles.[1] Neutrophilic and eosinophilic

infiltration of the superficial epidermis is common. Immunohistochemistry and immunoserology are becoming increasingly helpful in the diagnosis of autoimmune blistering diseases. There are few reports of the treatment of bullous pemphigoid in horses.[1,2] Treatment is the same as that for PF but is generally less successful.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease that is rare in horses.[61] Clinical signs of SLE include weight loss, bilaterally symmetric alopecia, seborrhea, oral ulceration, and lymphadenopathy. Necropsy findings may demonstrate membranous glomerulonephritis and fibrous synovium. Some horses have concurrent Coombs test-positive hemolytic anemia and positive antinuclear antibody test results. Histologic evaluation reveals interface dermatitis with linear deposition of IgG at the basement membrane zones of the epidermis and hair follicles. In some horses with systemic lupus, profound lymphedema of the lower limbs may be the only clinical sign. The disease is treated with immunosuppressive doses of glucocorticoids similar to those of PF. The prognosis for horses with SLE is poor.

Discoid Lupus Erythematosus

Discoid lupus erythematosus is a rare skin disease in horses that is believed to be a variant of SLE. Affected horses develop areas of patchy erythema, crusting alopecia, and scaling on the face, ears, and neck. The disease can be limited to the coronary bands with or without involvement of the chestnuts and ergots in some horses. The affected coronary bands can become crumbly and exudative. Pain and lameness may occur; however, some horses with significant coronary band lesions remain surprisingly sound. Leukoderma and leukotrichia may be present.

The diagnostic test of choice is a skin biopsy. Because full-thickness biopsy of the coronary band will result in permanent abnormality of the hoof wall, biopsy of the chestnut is preferred when lesions also occur at that site. The skin biopsy reveals an interface dermatitis (hydropic or lichenoid or both). Focal hydropic degeneration of basal epidermal cells, pigmentary incontinence (pigment granules in the dermis or in dermal macrophages), and focal thickening of the basement membrane are important histologic features. Direct immunofluorescence testing may show a linear deposition of immunoglobulins at the basement membrane zone. This test is not reliable and is not recommended as a substitute for a routine histopathologic study.

Discoid lupus erythematosus is treated on an individual basis. Ideally the owner should stable the horse during daylight because sunlight aggravates the disease. Topical sunscreens and topical glucocorticoids, for example, 0.1% betamethasone 17-valerate, may be beneficial. Severe or refractory cases of discoid lupus erythematosus may require immunosuppressive doses of oral prednisolone.

Immune-Mediated Vasculitis

Vasculitis is an inflammatory reaction occurring in the wall of the blood vessel (Fig. 18.37). Vasculitides are classified by inflammatory cell type. Neutrophilic vasculitides are subdivided further into leukocytoclastic (neutrophil nuclei undergo karyorrhexis) or nonleukocytoclastic.

The immunologic mechanisms involved are typically type I and type II hypersensitivity reactions. Equine viral arteritis, equine influenza, *C. pseudotuberculosis,* and *Streptococcus* spp. (especially *S. zooepidemicus* subsp. *equi*) infections have been postulated to be precipitating factors for immune-mediated vasculitis.[65] Vasculitis also may occur following *Rhodococcus equi* pneumonia, cholangiohepatitis, and some antibiotic treatments. Often the underlying cause is not identified.

Idiopathic vasculitis has no known breed, gender, or age predilection. The most common locations for lesions are the distal limbs, ears, lips, and periocular areas.[2] Oral ulcers and bullae may be present. Skin lesions consist of purpura, edema, and erythema. Systemic signs may include pyrexia, depression, anorexia, weight loss, and lameness.

Purpura hemorrhagica is an acute noncontagious disease of horses characterized by extensive edema and hemorrhage of the subcutaneous tissue. Hemorrhage in the mucosae and viscera are common. Clinical signs usually develop within 2 to 4 weeks of a respiratory tract infection, frequently strangles or influenza. Urticaria followed by pitting edema of the distal limbs, head, and ventral abdomen is common. Severe edema of the head may compromise breathing. Tissue exudation and sloughing may occur. Pain and pruritus are rare. Affected horses usually are depressed, reluctant to move, and often anorectic.

Diagnosis of vasculitis is by skin biopsy. Obtaining skin biopsy specimens from lesions 8 to 24 hours of age is important because these lesions tend to have the most diagnostic changes. Lesions more than 24 hours old may be nondiagnostic because of intense secondary cellular infiltrates or necrosis. Skin biopsy reveals neutrophilic, eosinophilic, lymphocytic, or mixed cellular infiltrates in the vessel wall. Fibrinoid degeneration and hemorrhage are common. Direct immunofluorescence testing is occasionally useful as an adjunct diagnostic aid.



FIG. **18.37** Horse with vasculitis resulting from strangles.

Prognosis for horses with vasculitis is unpredictable. Therapy for vasculitis is discussed in Chapter 15.

NECROTIZING SKIN DISORDERS

Necrotizing skin diseases develop from a wide range of causes. The unifying characteristic is that each of these diseases may cause local or widespread full-thickness death of the skin, sloughing of tissue, ulceration, and exudation. A definitive diagnosis of the underlying cause may be difficult in some cases.

Decubital Ulcers

Decubital ulcers (pressure sores, saddle galls, and saddle sores) are caused by prolonged pressure to the skin, usually over a bony prominence. Thin and emaciated animals that spend a significant time in recumbency are at greatest risk for development of decubital ulcers. Decubital ulcers may develop from poorly fitting tack on the back, neck, or girth of the horse; from recumbency during surgery; improperly applied or maintained casts; leg wraps; or elastic bandages. The amount of pressure required to create a decubital ulcer is not great if the pressure occurs for an extended period of time. Tack and saddles may cause lesions over bony prominences or other areas subjected to prolonged pressure. Capillary circulation is stopped or severely limited, resulting in tissue anoxia and retention of metabolic wastes with tissue damage and death. Most lesions begin as small areas of tissue necrosis and progress to areas of ulceration. Secondary infections are common.[2]

Clinically, early lesions begin with hair loss, swelling, and erythema. A red-purple area of tissue discoloration may be visible. Within a few days, oozing, necrosis, and ulceration become visible. In severe cases the skin loses its elasticity, subcutaneous tissues slough, and the skin may harden. Strands of living tissue may be attached to necrotic tissue. Lesions are often malodorous. Healed lesions tend to scar, and leukotrichia or leukoderma are common sequelae.

The diagnosis is based on history and clinical examination. Areas of skin that have lost sensation or have a parchment-like feel should be suspect.

Treatment can be difficult if the horse remains recumbent. Because of the significant capillary and venous congestion, systemic antibiotics are often not effective. Wounds are managed best topically. Wounds should be cleaned daily with copious amounts of water to remove tissue debris and stimulate circulation, and topical antibiotic creams or ointments (i.e., iodine and chlorhexidine) should be applied to prevent secondary infection and to prevent the wound from becoming desiccated. Reepithelialization and wound healing greatly improve with the use of surgical dressings, but bandaging some lesions may be a challenge, such as those that occur over the wing of the ilium. In these instances, using super glue to build a frame around the lesion, which can be used to anchor elastic tape covering a donut type of padding, may be useful. Surgical debridement and skin grafting may be indicated in some cases.

Gangrene

Gangrene is a clinical term used to describe wet or dry tissue necrosis. Gangrene may result from external pressure, severe edema, burns, frostbite, snakebites, vasculitis, ergotism, fescue toxicosis, bacterial sepsis, or viral infections.[2] The characteristic lesion results from occlusion of the venous or arterial blood supply.

Dry gangrene occurs when the arterial blood supply to an area is occluded but the venous or lymphatic drainage is intact. Wet or moist gangrene is caused by occlusion or impairment of lymphatic and venous drainage plus putrefaction caused by a bacterial infection. Lesions of dry gangrene are dry, leathery, discolored, sunken, and cold to the touch. The skin may take a long time to slough. Lesions of wet gangrene are swollen, discolored, and malodorous.

The diagnosis is most often made by clinical examination and confirmed by biopsy. The primary cause of gangrene must be identified and treated.

Thermal Injuries

Burns

Excessive heat (burns) can occur from barn fires, brush fires, hot solutions, lightning, electrocution, rope burns, counterirritants, or radiation. Barn fires are the most common cause of severe burns in horses.

Large thermal injuries in horses are difficult to manage.[66] The large surface area of the burn dramatically increases the potential for fluid, electrolyte, and caloric losses. Depending on the depth of the burn, burns that cover 50% or more of the body are usually fatal.[66] Wound contamination is difficult to prevent because of the lack of a sterile environment. Horses may require long-term restraint to prevent continued trauma; wounds are often pruritic, and self-mutilation is common. Badly burned horses are frequently disfigured and have poor quality scar tissue preventing them from returning to full function.[66]

Burns are classified by the depth of the injury.[66] First-degree burns involve only the most superficial layers of the epidermis. These burns are characterized by erythema, edema, desquamation of superficial layers of the skin, and pain. The germinal layer of the epidermis is spared, and these burns heal without complication. Second-degree burns involve the entire epidermis and may be superficial or deep. Superficial second-degree burns involve the stratum corneum, stratum granulosum, and a few cells of the basal layer. Deep second-degree burns involve all layers of the epidermis. Clinically, these burns are characterized by erythema, edema at the epidermal-dermal junction, necrosis of the epidermis, accumulation of white blood cells at the basal layer of the burn, eschar (slough produced by a thermal burn) formation, and minimal pain. The only germinal cells spared are those within the ducts of sweat glands and hair follicles. Second-degree burns generally heal well with appropriate wound care. Third-degree burns are characterized by loss of epidermal and dermal components. Fluid loss occurs, along with significant cellular response at the margins and deeper tissue, eschar formation, lack of pain, shock, wound infection, and possible bacteremia and septicemia. Healing is by contraction and epithelization and occurs only from the wound margins. Infection and problems with wound healing frequently complicate these burns. Fourth-degree burns involve all the skin and underlying muscle, bone, and ligaments.

Burns cause local and systemic effects.[66] Local tissue damage results from massive protein coagulation and cellular death. In the immediate area of the burn, arteries and venules constrict and capillary beds dilate. Capillary wall permeability increases in response to vasoactive amines released as a result of tissue damage and inflammation.[66] These vascular responses result in fluid, protein, and

inflammatory cells accumulating in the wound. Vascular sludging, thrombosis, and dermal ischemia occur, resulting in further tissue damage. The tissue ischemia continues for 24 to 48 hours after the injury and is believed to be caused by the local release of thromboxane A_2. Lipid layers in the skin are destroyed, and a fourfold increase in the loss of fluid occurs. Fluid losses result in increased heat loss from evaporation and an increased metabolic rate. The full extent and depth of the burn may not be evident for several days. Neutrophil function and chemotaxis greatly decrease, predisposing the wound to local infection, bacteremia, and septicemia. The following microorganisms frequently colonize burns: *P. aeruginosa, Staphylococcus, E. coli, Klebsiella* spp., nonhemolytic *Streptococcus* spp., *Proteus* spp., *Clostridium* spp., and *Candida* spp.[13,67,68] Systemic effects are life-threatening and include hypovolemia, fluid and electrolyte losses, protein loss, pulmonary edema, anemia, increased basal metabolic rate, increased caloric needs, and depressed cell-mediated and humoral immune responses. Hypovolemia exacerbates a decrease in cardiac output after a circulating myocardial-depressant factor.

First-degree burns and superficial second-degree burns should be treated immediately with cold water to prevent further tissue necrosis. A water-soluble antibacterial cream should be applied to the wound to prevent infection. Nonsteroidal analgesics should be administered to alleviate pain and to help reduce dermal ischemia. Aspirin at 10 to 20 mg/kg orally once or twice daily decreases thromboxane production, may halt further dermal ischemia, and may be the initial drug of choice if pain is otherwise controlled.

Full-thickness burns (third and fourth degree) are treated by occlusive dressing (closed technique), eschar production (exposure technique), continuous wet dressings (semiopen technique), or excision and grafting techniques. The most practical therapy for large burns in horses is the semiopen method, leaving the eschar intact with continuous application of moist bandages and antibacterial agents. The moist dressings help prevent heat and moisture loss from the eschar, provide protection of the eschar, and help prevent bacterial invasion in the wound.

Systemic antibiotics should be used with caution, and in the absence of sepsis systemic antibiotics are contraindicated. Topical medications should be a broad-spectrum, nonirritating water-based cream such as silver sulfadiazine, which is effective against gram-negative bacteria, causes no discomfort to the horse, penetrates eschar, and has a 24-hour duration of action. Aloe vera is reported to relieve pain, decrease inflammation, stimulate cell growth, and kill bacteria and fungi. Aloe vera is most useful in the early treatment of mild burns. Horses with severe burns often succumb to respiratory disease secondary to smoke inhalation. Damage to the corneas is also common. Several excellent references for further information on long-term medical and surgical management of horses with large thermal burns are available.[66]

Frostbite

Frostbite occurs when tissue is exposed to extreme cold. Sick, debilitated, and neonatal animals are at increased risk. Cold temperatures inhibit cell metabolism and cause tissue dehydration, cell disruption by ice crystals, ischemia, and vascular damage. The most commonly affected areas are the glans penis, ear tips, coronary bands, and heels.[1,2] The initial lesion of frostbite is paleness of the skin. Erythema, scaling, and hair loss follow. Pigment loss may occur. In severe cases, necrosis and dry gangrene occur.

Mild cases of frostbite do not require treatment. More severe cases require rewarming and the application of antibiotic ointments without steroids to the area. In severe cases with necrosis and sloughing, topical wet soaks and symptomatic therapy with antibiotics may be needed to prevent sepsis. Surgical debridement should not be attempted until an obvious demarcation is present between viable and nonviable tissue. Previously frostbitten areas may be more susceptible to cold injury in the future.

Photosensitization

Photosensitization occurs when skin is exposed to ultraviolet light after being sensitized by photodynamic agents. It is an uncommon cause of dermatitis but a potentially serious problem in horses. The spectrum of disease associated with photosensitization ranges from being a simple nuisance to a life-threatening crisis.[69]

Pathogenesis

Photodynamic agents responsible for photosensitization can be phototoxic or photoallergic. Phototoxic agents produce a photosensitivity reaction in almost all animals under suitable conditions. Photoallergic agents require the animal to be sensitized to the compound. Both types of photodynamic agents reach the skin via the bloodstream or by direct contact in which they absorb wavelengths of ultraviolet or visible light that exceeds the UV-B range and become activated, ultimately damaging the epidermis and superficial blood vessels.

Four types of photosensitization are recognized in animals: primary photosensitivity (type 1 photosensitivity), hepatogenous photosensitivity (type 2 photosensitivity), photosensitivity from aberrant pigment synthesis (porphyria), and photosensitivity of uncertain etiology.

Primary photosensitization results when the photodynamic agent is ingested, systemically administered (certain antibiotics), or absorbed through the skin. Ingestion is typical of plants like St. John's wort *(Hypericum perforatum),* buckwheat (*Fagopyrum* spp.), spring parsley *(Cymopterus watsonii),* and *Ammi* spp. St. John's wort contains a red fluorescent pigment called *hypericin*. It is present in the plant's leaves as clear small dots. Clinical signs appear within 21 days. Primary photosensitization from other plants is uncommon in the horse.[69]

Contact photosensitization is associated with pastures that contain legumes like clovers. Horses with signs of contact photosensitization should be evaluated for liver disease, because some clovers can cause both contact and hepatic photosensitization. Many drugs and chemicals cause primary photosensitization through various mechanisms (see Table 18.3). Fly sprays, antimicrobial soaps, and tetracycline antibiotics are common sources.

Hepatogenous photosensitivity develops secondary to hepatic injury. When liver function is compromised, the liver cannot excrete phylloerythrin (a degradation product of chlorophyll), and it accumulates. Phylloerythrin is a photodynamic agent, and high levels render the animal photosensitive. Clinical signs develop when phylloerythrin concentrations are greater than 8.0 μg/dL. Hepatogenous photosensitization may develop secondary to liver damage and cholestasis, but it is often caused by ingestion of toxic plants and mycotoxins (see Table 18.4).

TABLE **18.3 Causes of Primary Photosensitization**

Substance	Photodynamic Agent
PLANTS	Hypericin (ingested)
Hypericum perforatum (St. John's wort, goat weed, Klamath weed), *H. pseudomaculatum* (large St. John's wort), *H. punctatum* (spotted St. John's wort)	
Fagopyrum esculentum, F. sagittatum, F. tatoricum (buckwheat)	Fagopyrin, photofagopyrin, pseudohypericin (ingested)
Cymopterus watsonii (spring parsley)	Furocoumarins
Ammi majus (Bishop's weed)	Furocoumarins
Thamnosma texana (Dutchman's breeches)	Furocoumarins (ingested)
Lolium perenne (perennial rye grass)	Perloline (ingested)
Froelichia humboldtiana	Naphthodianthrone derivative
Medicago denticulate (burr trefoil)	Aphids
Trifolium hybridum (alsike clover), *T. pretense*	Ingested and possibly contact with unknown photo and hepatotoxin
Medicago spp. (alfalfa)	Unidentified
Sphenociadium capitellatum (whiteheads, ranger's buttons)	Ingested—unidentified agent
Heracleum mantegazzlanum (giant hogweed)	Furanocoumarin (contact)
Cooperia pedunculata (rain lily)	Unidentified agent
Avena sativa (oats)	Unidentified agent
Brassica napus (rape)	Unidentified agent
Brassica rapa (mustard)	Unidentified agent
Vicia spp. (vetches)	Unidentified agent
MYCOTOXINS	
Fungi on celery and parsnips	Phytoalexins (xanthotoxin, tripsoralen)
DRUGS AND CHEMICALS	
Phenothiazine	Phenothiazine sulfoxide
Thiazides	Unidentified agent
Retinoids, therapeutic photoagents	Tazarotene (surface application)
Promazines	Unidentified agent
Acriflavines	Unidentified agent
Rose bengal	Unidentified agent
Methylene blue	Unidentified agent
Sulfonamides	Unidentified agent
Tetracyclines	Unidentified agent
Chlorpromazine	Unidentified agent
Quinidine	Unidentified agent
Coal tar derivatives	Unidentified agent
Furosemide	Unidentified agent
Some antimicrobial soaps	Unidentified agent
ENDOGENOUS METABOLITES	
Porphyrins	Genetic abnormality (porphyrin)
Phylloerythrin	Hepatic failure (ingested)
Bilirubin	Liver, blood disorders (endogenous)

Adapted from Scott DW, Miller WH, eds. *Equine dermatology*. 2nd ed. Maryland Heights, MO: Elsevier Saunders, 2011.

Pyrrolizidine alkaloids, one of the most common toxic causes of hepatogenous photosensitization, are found in plants throughout the world. Pyrrolizidine alkaloids are transported to the liver and metabolized to pyrroles. Metabolites have an antimitotic effect that prevents cells from dividing. Eventually, fibrosis and liver failure ensue. In addition to photosensitization, significant neurologic disease can develop (hepatic encephalopathy). Horses will reject plants containing pyrrolizidine alkaloids unless there is nothing else to graze. Animals more readily eat the plants when they are processed in hay or silage. Pyrrolizidine alkaloid toxicosis develops in late winter and early spring and manifests as chronic hepatitis in early summer when ultraviolet light levels increase.

Alsike clover *(Trifolium hybridum)* is unique because it causes both primary and secondary photosensitivity.[70,71]

TABLE **18.4 Causes of Hepatogenous Photosensitization**

Substance	Hepatotoxin
Diseases	
Common bile duct occlusion; inflammation, cholelithiasis, parasite migration	
Theiler's disease, serum hepatitis immunologic disease	
Chronic active hepatitis	
Cholangiohepatitis	
Ascending bacterial infection	
PLANTS	
Senecio jacobaea (tansy ragwort), *S. riddellii* (Riddell's groundsel), *S. douglasii* (woolly groundsel), *S. vulgaris* (common groundsel)	Pyrrolizidine alkaloids (retrorsine)
Amsinckia spp. (tarweed, fiddleneck)	Pyrrolizidine alkaloids
Crotalaria spp. (rattleweed)	Pyrrolizidine alkaloids
Echium plantagineum (Salvation Jane, Patterson curse)	Pyrrolizidine alkaloids
Heliotropicum europeaum (common heliotrope)	Pyrrolizidine alkaloids (lasiocarpine, heliotrine)
Cynoglossum officinale (hound tongue)	Pyrrolizidine alkaloids
Kochia scoparia (fireweed, burning bush)	Unidentified agent
Myoporum laetum (ngaio tree)	Ngaione
Lantana camara (lantana)	Lantadenes
Tribulus terrestris (goathead, puncture vine)	Unidentified agent
Nolina texana (bunchgrass, sacahuiste)	Unidentified agent
Narthecium ossifragum (bog asphodel)	Unidentified agent
Tetradymia canescens (horsebrush, rabbitbrush), *T. glabrata* (spineless horsebrush, coal-oil brush)	Unidentified agent
Trifolium hybridum (alsike clover)	Unidentified agent
Medicago spp. (Bermuda grass)	Unidentified agent
Holocalyx glaziovii (Alecrim)	Unidentified agent
Lippia spp. (whitebrush)	Unidentified agent
Panicum antidotale (blue panicum), *P. coloratum* (klein grass), *P. dichotomiflorum* (smooth witchgrass), *P. maximum* (guinea grass), *P. miliaceam* (millet), *P. virgathum* (switch grass)	Saponins (diosgenin, tamagenin, epismilagenin)
Agave lecheguilla (lecheguilla)	Saponins
Brachiaria brizantha (Australia grass), *B. decumbens* (signalgrass), *B. humidicola, Brassica rapa* (cabbage, kale)	Saponins
MYCOTOXINS	
Microcystis spp. (blue-green algae in water)	Cyclic peptide
Phomopsis leptostromiformis (on lupins)	Phomopsins
Pithomyces chartarum (facial eczema, perennial ryegrass staggers, sporodesmin)	Unidentified agent
Lupinus spp. (lupinosis, lupine)	Unidentified agent
Medicago sativa (moldy alfalfa)	Unidentified agent
Fusarium spp. (moldy corn)	T-2 toxins
Aspergillus spp.	Aflatoxin
TOXINS AND CHEMICALS	
Carbon tetrachloride	
Carbon disulfide	
Phenanthridium	
Copper	
Phosphorus	
Iron	
MISCELLANEOUS	
Serum, antiserum	Immune complex disease
Liver diseases (abscess, neoplasia, parasite migration, cholelithiasis, inflammation)	Chemical mediators

Adapted from Scott DW, Miller WH, eds. *Equine dermatology*. 2nd ed. Maryland Heights, MO: Elsevier Saunders, 2011.

Primary photosensitization occurs as an acute reaction and is not linked to liver disease. Chronic alsike clover poisoning is associated with hepatic necrosis, fibrosis, and cirrhosis. It leads to clinical signs similar to those of pyrrolizidine alkaloid toxicosis. Pastures with less than 25% alsike clover density are believed to be safe for equine grazing.

Other causes of hepatic photosensitization (Table 18.4) include crystalline hepatopathy, pharmaceutical-related hepatopathy, and hepatic disease associated with the administration of equine-origin biologics or iron-containing supplements.

Porphyria is a rare congenital form of photosensitivity arising from aberrant pigment synthesis and has not been reported in horses. In cattle, swine, and cats it is caused by a metabolic defect in uroporphyrinogen III cosynthetase, which is an enzyme required for hemoglobin synthesis. Affected cattle have discoloration of their teeth, bones, and urine that vary from shades of pink to reddish-brown. Dermal lesions occur in unpigmented areas when affected animals are subjected to sunlight.

Diagnosis

Clinical signs of photosensitization traditionally occur in poorly haired, white, or unpigmented areas, and such signs provide a presumptive diagnosis of the disorder (Fig. 18.38). Initially, erythema, edema, and pruritus may occur. Serum transudation, erosion, or ulceration of the skin may result in a secondary bacterial invasion. Jaundice is often seen with hepatogenous photosensitization caused by alterations in the liver's ability to process bilirubin. Generalized lesions suggest liver involvement and liver function should be assessed, whereas localized lesions affecting the lips and distal limbs suggest primary photosensitivity. Biopsy reveals superficial dermal blood vessel degeneration and thrombosis with perivascular inflammation. Chronic lesions may show a lymphocytic perivascular dermatitis, epidermal hyperplasia, and hyperkeratosis, with serous crust formation. Apoptotic keratinocytes, or "sunburn cells," may be present.



FIG. **18.38** Photosensitization on the muzzle of a horse. Note the distribution limited to white areas of skin that were in contact with the offending agent. (Photograph courtesy of Dr. Judy Marteniuk.)

Treatment

The goal of treatment is to prevent further damage. Treatment focuses on stall confinement away from sunlight and removing photodynamic agents. Ointments, astringents, or antiseptics can be applied. Corticosteroids may reduce inflammation and pruritus. Antibiotics may treat secondary bacterial infections. Liver disease should be treated immediately.

Photosensitivity secondary to hepatopathy produces vague, nonspecific signs including poor appetite, weight loss, icterus, fever, mild colic, and affected skin. Neurologic abnormalities are associated with advanced liver dysfunction. Other signs of liver disease may include colitis, polydipsia, and cranial nerve abnormalities such as dysphagia and inspiratory stridor.

Differential diagnoses include sunburn, contact dermatitis, dermatophilosis, photoactivated vasculitis, PF, and bacterial and fungal infections.[69]

Treatment of contact photosensitization includes removing sensitizing agents and stall confinement. Nighttime pasture access is permitted. Fly sheets, masks, boots, and the application of 30 to 55 SPF sunscreens may be sufficient in mild cases. Systemic glucocorticoids or nonsteroidal antiinflammatory agents, topical corticosteroids, pramoxine, and control of myiasis may be beneficial.

Horses with hepatogenous photosensitization may respond to appropriate medical treatment and management (Chapter 13). Any delay in diagnosis and treatment increases complications and mortality rates. The horse should be restricted from sunlight, and offending agents should be removed. Mild to moderate dermatitis is best treated with topical, water-soluble antimicrobial agents like silver sulfadiazine. Corticosteroids may calm inflammation. Cold water hydrotherapy rids the skin surface of debris, decreases inflammation, and prevents myiasis. Bandage swollen, exudative limbs with clean, nonadhesive materials and sheet cotton. Severe dermatitis and cellulitis is associated with swelling, heat, pain, lameness, and low-grade fevers (102°F to 104°F). Horses with severe infections become inappetent and show signs of discomfort. Systemic antimicrobial treatment is indicated with cellulitis. β-Lactam antimicrobials are indicated for clostridial diseases and other anaerobic bacterial infections. Standard dosing of ceftiofur sodium (2.2 mg/kg, IV or IM, q 6–8 h), procaine penicillin G (22,000 IU/kg, IM), or penicillin G potassium (22,000 IU/kg, IV, q 6 h), combined with an aminoglycoside and oral metronidazole (20–25 mg/kg, PO or per rectum, q 6–8 h) offers coverage for most bacterial organisms. Antimicrobial treatment for cellulitis should continue for at least 10 to 14 days.

Leukocytoclastic Vasculitis

Leukocytoclastic vasculitis or photoaggravated vasculitis is relatively common in horses with a significant amount of white hair and pink skin on their lower limbs. Immune deposits (IgG or complement component 3, or both) around the vessel walls have been detected in early skin lesions using direct immunofluorescence. The fact that unpigmented skin appears to be affected suggests a role of ultraviolet light in the development of this dermatologic problem. Drug reactions and



FIG. **18.39** Horse with leukocytoclastic vasculitis.

Staphylococcus spp. bacterial infection may serve as underlying causes. This dermatologic condition generally affects mature horses. Lesions are confined to the lower limb (Fig. 18.39). A lack of pigment in the affected area is believed to be the major risk factor, but pigmented skin has also been reportedly diagnosed with this condition. Lesions are usually multiple and well marked. Initially, erythema and exudation occur, which progresses to crusting and open sores or ulcerated areas. The affected area may ooze, and swelling is present. Chronic cases may develop a rough-appearing surface. This condition is more painful than pruritic. The diagnosis is confirmed with histopathologic findings of leukocytoclastic skin lesions with perivascular inflammation, blood vessel degeneration, and clots involving the small vessels in the superficial dermis. Treatment consists of systemic corticosteroids at relatively high doses for 1 week and reduced doses for another 4 to 6 weeks. A reduction in ultraviolet light exposure by bandaging the affected leg or stabling the horse indoors during daylight hours is recommended. Application of sun block (minimal SPF of 20) may be beneficial. Secondary bacterial infections should be treated.

NEOPLASTIC SKIN DISORDERS

Sarcoid

Sarcoids are the most common neoplasm in horses worldwide, with reported prevalence rates ranging from 12.9% to 67% of all equine tumors. Sarcoids consist of cutaneous accumulations of transformed fibroblasts, which are locally invasive, nonmetastatic, and rarely regress spontaneously. They can occur on any part of the body either singly or in clusters; the ventral abdomen, limbs, and head, especially around the eyes, pinnae, and commissures of the lips are the most commonly affected sites. Lesions frequently occur in areas that have experienced trauma. Early detection and responsible treatment provide the best possible outcome for affected horses.

Sarcoids have been causally associated with bovine papillomaviruses (BPV) types 1 and 2[72-74] and potentially type BR-UEL-4. Recent studies provide further support that latent BPV infections result in neoplastic transformation in genetically susceptible individuals.[75,76] This may explain the high incidence of tumor recurrence after excision alone; surgical trauma may induce proliferation and expression of latent virus, resulting in regrowth of the tumor. Viral burden has been associated with sarcoid severity, with higher viral burdens present in more aggressive tumors. Biting flies may be the route of transmission. In addition to evidence for genetic susceptibility to sarcoids, familial tendency has been identified.[77]

Sarcoid tumors commonly present with linear or focal dermal thickening. The epidermis varies from thick, rough, and hyperkeratotic to ulcerated. Tumors can also occur in the subcutaneous tissue as firm, moveable masses with an intact covering of skin. There are several forms of sarcoids that are recognized with varying treatment recommendations and prognosis. Regardless of the treatment modality used, small sarcoids are more likely to resolve than large sarcoids. Relatively small, benign sarcoid lesions may transition to more aggressive types of sarcoids if treated inappropriately. Tumor location is also an important factor when considering treatment modality. For example, periorbital tumors will require a treatment plan that will not threaten future vision. Generally, tumor duration is inversely correlated to resolution irrespective of tumor size.

Occult sarcoids are most common in the skin around the mouth, eyes, neck, and other relatively hairless areas of the body including the medial aspects of the forearm and thigh. These lesions manifest as a slightly thickened area of skin with a mildly roughened surface that is devoid of hair. They are commonly mistaken for ringworm infection or simple trauma. Occult sarcoids can stay dormant for extended periods of time before spontaneously enlarging or converting to more aggressive types (Fig. 18.40). Any sarcoid undergoing a more aggressive transformation should be treated immediately.

Verrucous (warty) sarcoids show a predilection for the face, body, groin, and sheath areas. Lesions are generally small, rarely exceeding 6 cm in diameter, with a dry, horny surface and a cauliflower-like appearance. These tumors can grow slowly over years and occasionally become quite large, sometimes covering an 8-inch-diameter or larger area. Like large occult sarcoids, large verrucous sarcoids can undergo malignant transformation.

Nodular sarcoids consist of firm and generally round masses that can vary widely in size. Nodular sarcoids are classified based on tissue involvement.[1] In type A1 the masses have no cutaneous involvement, are located subcutaneously. and are easily moveable. Type A2 masses also have no cutaneous involvement but are locally invasive with a "bound down" character; these types are very common around the eye. In type B nodules, the overlying skin cannot be moved over the nodule and is frequently abnormally thin, hyperkeratotic, or ulcerated. Type B nodules tend to be more aggressive than



FIG. **18.40** Large occult sarcoid beginning to show characteristics of a verrucous sarcoid.



FIG. **18.41** Fibroblastic (proliferative) sarcoid.

type A nodules and can be particularly problematic around the eyes and mouth. Type B1 nodules lack deep tissue invasion, whereas type B2 nodules are attached to deeper and surrounding structures. Nodular sarcoids can be easily confused with other nodular masses such as melanomas, lymphosarcomas, round cell tumors, and dermoid cysts. General predilection sites for nodular sarcoids include the groin, sheath, or eyelid areas. As with occult and verrucous sarcoids, nodular sarcoids can quickly progress to more aggressive forms, especially if the area is traumatized.

Fibroblastic sarcoids are variable in appearance and usually found on the groin, eyelid, lower limbs, previous wound sites, and sites of other sarcoid types subjected to insult. Some are well-circumscribed fibrous nodules in the dermis covered by intact epidermis (Fig. 18.41), but most have ulcerated surfaces that hemorrhage easily and may be covered by necrotic debris. These lesions often have an aggressive nature and will spread locally in the dermis. The fibroblastic sarcoid will often resemble exuberant granulation tissue. Type 1 fibroblastic sarcoids are pedunculated, with or without a discernible root, whereas type 2 have a broad base (sessile). Type 2 tumors frequently extend beneath the visible lesion a significant distance. Because fibroblastic sarcoids can be so similar in appearance to granulation tissue it is often difficult to differentiate between sarcoid regrowth and normal healing after fibroblastic sarcoids are debulked and treated. Prolonged healing and/or excessive granulation tissue postoperatively at the site of any sarcoid removal are cause for concern. Until the wound is totally healed with normal epidermal covering, sarcoid regrowth should be considered. Any wound that fails to heal may contain sarcoid tissue mixed with granulation tissue. Fibroblastic sarcoids can also be confused with pythiosis lesions. Unlike pythiosis, sarcoids are generally neither painful nor pruritic and grow more slowly.

Mixed sarcoids are composed of a variety of sarcoid types. The term *mixed* is used when no one type predominates. This type likely represents the progression of occult or verrucous types to more aggressive forms of sarcoid. Mixed sarcoids are a common consequence of biopsy or trauma (Fig. 18.42).

Malignant or malevolent sarcoids have also been described. Malignant sarcoids are "locally malignant" but, like all other forms of sarcoids, do not metastasize to other areas. A history of repeated surgeries and failed treatments of another form of sarcoid is common. Malignant sarcoids may track along or infiltrate lymphatic or other vessels and invade underlying structures (Fig. 18.43).

Differential diagnoses that must be considered when any form of sarcoid is suspected vary widely depending on the physical appearance of the lesion and may include excessive granulation tissue (proud flesh), dermatophytosis, linear hyperkeratosis, blisters, burns, rub marks, papillomatosis, hyperkeratosis, SCC, fibromas, neurofibromas, equine eosinophilic granulomas, melanoma, pythiosis, fibrosarcoma, and lymphosarcoma.

Although biopsy of occult, nodular, and small verrucous tumors is often not recommended to avoid altering the morphology and behavior of the lesion, definitive diagnosis is based on histologic examination of the affected tissue. When possible an en bloc resection of the entire tumor should be considered. If closure of the biopsy site is to be attempted, changing all surgical instrumentation and gloves is imperative to avoid accidental recontamination of the area. When either



FIG. **18.42** Originally a verrucous sarcoid, this lesion has developed a more aggressive appearance several months after biopsy.



FIG. **18.43** A large malevolent sarcoid in a Quarter Horse mare. The tumor tracks both proximally and distally from the large, ulcerated, fibroblastic mass. Several ulcerated areas are present.

an en bloc resection or punch biopsy is performed it should be followed by some additional form of treatment. Biopsy alone may cause a sarcoid to become more aggressive. When treatment of small sarcoids is considered, it may be prudent to simply treat the lesion as a tumor without a biopsy.

Histopathologically, equine sarcoids appear as a fibroblastic proliferation of cells that form whorls or interlacing bundles.[1,2] The usual overall orientation of this proliferation is perpendicular to the basement membrane, but exceptions exist. The epidermis, when present, is hyperplastic with characteristic elongated rete ridges, but normal or even atrophic epidermis with significant hyperkeratosis can occur (flat sarcoids). The best time to biopsy a sarcoid is before initial treatment; subsequent biopsies can be more difficult for the dermatopathologist to interpret. Sarcoid tumors can be difficult to differentiate from schwannomas based on histopathology alone.[78]

The presence of sarcoid lesions at prepurchase examination should be noted and the purchaser warned. Equine sarcoids can have a noticeable impact on equine health and welfare and become a significant financial burden for potential owners.

There is a wide range of treatments available for equine sarcoids. The treatment modality selected is determined by location, size, and aggressiveness of the tumor as well as by clinical experience, client commitment, and the availability of services, equipment, and facilities.

Conventional surgical excision of equine sarcoid is simple, but the recurrence rate varies between 15% and 82%, with most tumors recurring, often more aggressively, within 6 months. Surgical excision is better used to reduce tumor volume and improve therapeutic efficiency of other treatment modalities. Any time surgical excision is used in the treatment of sarcoid, a 0.5- to 1-cm margin of normal tissue is recommended. Adequate margins are not always possible, however, when the tumor is located near the orbit or similarly important structures. Primary closure is preferred when margins can be achieved. Presuturing and/or releasing incisions can be helpful to achieve primary closure. When closing en bloc sarcoid resections, particularly if releasing incisions are performed, new scalpel blades, instruments, and gloves should be used to minimize the chances of tumor spread. Adjunctive therapy is recommended any time a sarcoid is surgically removed.

Carbon dioxide laser excision of equine sarcoid may, in select cases, provide some additional benefit over conventional surgical excision. Because laser energy is absorbed by surrounding tissues, tumor cells are killed up to 0.2 mm beyond the wound margins. In addition, the CO_2 laser can be used to debulk tumors by vaporization. Reported recurrence rates for laser excision range from 17% to 33%.[79,80] Laser excision may be combined with other treatment modalities to further avoid tumor regrowth.

Cryotherapy with liquid nitrogen is cost-effective in the treatment of equine sarcoid, but recurrence rates vary widely for this procedure; case selection and method of freezing are likely to play a role in the observed variability in outcome. When using cryotherapy on large tumors they must be debulked before the standard three freeze/thaw cycles. The tumor should be rechecked at 2- to 4-week intervals and another set of freeze/thaw cycles implemented if the sarcoid appears to be recurring. Healing is by second intention or delayed closure, which will result in scarring and hair depigmentation. Because of the significant tissue destruction associated with cryotherapy, this treatment modality may be inappropriate for tumors near the eye, nostrils, or similarly significant structures. In all cases, the

client should be warned about posttreatment swelling, hyperemia, hemorrhage, necrosis, and local edema and permanent leukotrichia that will occur after treatment.

Intratumoral hyperthermia has been successful in treating equine sarcoids and can readily be combined with intralesional chemotherapy. Hyperthermia units have recently become more readily available, and their use appears to be on the rise. When used in conjunction with intralesional chemotherapy the tumor is first injected and then heated. Heating tumors to temperatures between 38°C and 43°C boosts the delivery of oxygen to the lesion, improves the distribution of chemotherapy inside the tumor, and significantly increases the uptake of the chemotherapeutic agent.

The intralesional use of platinum-based antineoplastic agents, cisplatin (injectable suspension or absorbable beads) and carboplatin (injectable suspension) consistently provides long-term control of equine sarcoids and most often offers a cosmetic end result. In one of the largest studies of sarcoid treatments ever performed, therapy with cisplatin suspension had a 96.4% resolution rate (3.7% recurrence rate) after 4 years in 573 horses.[81] Cisplatin is purchased as a compounded emulsion in sterilized sesame oil to affect a slow release allowing an elevated tumor-to-plasma drug concentration ratio. Tumors are injected a minimum of three to five times at 2-week intervals with 1 mg of cisplatin for each cubic centimeter of tumor bed and margin of normal tissue. When using carboplatin, the dosage is increased to a minimum of 1.3 mg/cm^3. Tumor volume should be accurately estimated for appropriate dosing, and this can be accomplished in a variety of ways (http://www.calculator.net/volume-calculator.html).

Small sarcoids and those with softer textures are injected with 22- to 25-gauge 1- to 1.5-inch needles. With denser sarcoid tumors, injecting cisplatin solution into the tumor may be difficult and may require a larger needle (19- to 21-gauge 1.5-inch), which is inserted through the tumor, and the chemotherapeutic agent is injected as the needle is slowly withdrawn. Injecting the tumor at a number of injection sites is usually required; the exact number of sites depends on the size and configuration of the tumor. The space between each line of injection should be 6 to 8 mm to ensure adequate diffusion of the chemotherapeutic agent. The process is repeated until the tumor is fully injected in one plane. Then the injections are rotated 90 degrees and the tumor is fully reinjected along this plane, forming a cross-hatched pattern. Because this may take some time, a three-way stopcock should be available to ensure that the oil and water emersion do not separate from one another. The tumor is reinjected every 2 weeks until resolution. Large tumors should be debulked and treated immediately regardless of whether primary closure has been achieved. Appropriate human precautions should be taken when handling any hazardous drug including these chemotherapy agents. Precautions include using a Luer-Lok syringe to help minimize potential human exposure and wearing personal protective equipment including nitrile gloves, face shield, and protective clothing. All contaminated or potentially contaminated material must be discarded into a biohazard container after use.

Bioresorbable cisplatin beads are easily implanted, are less likely to result in human contamination with the agent, and have demonstrated similarly excellent tumor resolution (9% recurrence rate). The 1.6-mg cisplatin beads release cisplatin over 30 days. Tumors should be debulked if they are >1.5 cm in size. Beads are then placed 1.5 cm apart through subcutaneous stab incisions throughout the tumor and margin. Repeating the bead implantation is recommended in 1 month if tumor remains. The beads are available from specialized equine compounding pharmacies.

Recent descriptions of treatment of equine sarcoids with cisplatin electrochemotherapy indicate a 99.5% success rate in 194 tumors[82] and 92.3% in a study of 52 tumors with one recurrence 60 months after treatment.[83] When surgical resection was combined with electrochemotherapy the success rate was 100% in 18 tumors with one recurrence 14 months later.[83] Electrochemotherapy works via a combination of a physical effect, cell membrane poration, and cytotoxic drug administration. Cell membrane poration is achieved by applying short intense electric field pulses. Pore formation on the cell membrane allows the cisplatin to enter the cell and significantly increases its local toxicity. Horses require repeat anesthesia and treatment every 4 weeks, which significantly adds to the cost of treatment and potential for adverse events. The number of recommended treatments ranges from one to five sessions.

Mitomycin-C, a DNA cross-linking agent similar to carboplatin and cisplatin, has also shown promise in the treatment of equine sarcoids. Sarcoids are injected with a 0.04% solution of mitomycin-C, at 0.5 to 1.0 mL/cm^3 of tumor every 8 weeks until resolution is achieved. In one study of 59 sarcoids, 9 of 9 periocular sarcoids resolved with an average of 1.9 injections.[84] Of the other 50 sarcoids, 48 (96%) resolved after an average of 2.4 injections. Adverse effects included the development of leukotrichia and infrequent swelling and discharge from the necrosing tumor. Recurrence rates at 6 to 30 months were zero for periocular sarcoids and 3 of 48 (6%) for other types. Additional injections were effective in horses with tumor recurrence.[84]

Electrosurgical excision of equine sarcoids had a reported treatment success rate of 86.8% in 319 tumors. Tumors were excised under general anesthesia. Direct tumor contact was avoided during the skin preparation protocol to avoid tumor spread. Margins of a ≥12-mm tumor-free zone were obtained, and the electrosurgical instrument was used with a nontouching technique to avoid iatrogenic spread of tumor cells. All wounds were closed with a new set of instruments and gloves. Unfortunately, the size of the sarcoids being treated was not described, making a comparison with other forms of treatment more difficult.[85]

A topical ointment, AW-3-LUDES, has shown success in the treatment of severe sarcoids. It contains a variety of heavy metals and the antimitotic compounds 5-fluorouracil and thiouracil. The ointment is administered on successive or alternate days for three to five treatments. A response should be evident in the following 5 to 10 weeks and will be noticed as preferential necrosis and sloughing of the sarcoid tissues.

The successful treatment of equine sarcoids by topical acyclovir application has also been reported[86] and can be a cost-effective and safe alternative or adjunct to cisplatin treatment and surgical therapy. All 47 (100%) of the sarcoids responded to acyclovir application by shrinking, with complete tumor regression observed in 68% of the lesions. Incomplete resolution was observed in 32% of the lesions, which is likely caused by acyclovir's inability to penetrate thicker areas. Acyclovir is proposed to be routinely used for the treatment of flat and mild-type sarcoids or as a relatively economical way to shrink tumors before the use of cisplatin. The drug is applied daily for a minimum of 2 months for thinner tumors, and thicker tumors will take longer to resolve and may require debulking

before treatment. The advantage of acyclovir is that it does not cause an inflammatory reaction like many other topical agents.

Imiquimod 5% (Aldara; 3M Pharmaceuticals, St. Paul, MN), an immunomodulator that stimulates macrophages, Langerhans cells, natural killer cells, and B lymphocytes, has shown efficacy in the treatment of equine sarcoid with an overall 60% resolution of nondebulked tumors.[87] In 80% of treated tumors there was a significant reduction in tumor size (75% reduction). Debulking tumors before the use of imiquimod appears to provide even better success, which is likely caused by better penetration of the product. Imiquimod is applied to the sarcoid three times a week on alternating days for 32 weeks or until the lesion resolves. Treatment can result in local inflammation, exudation, depigmentation, alopecia, and behavioral issues (if the horse does not appreciate being touched in the area of application). The use of tazarotene cream in conjunction with imiquimod has improved the overall resolution rate of sarcoids but also appears to increase pain and inflammation associated with treatment.[88]

Other forms of immunotherapy are relatively common in the treatment of equine sarcoid with bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis,* historically being used most often. Mycobacterial antigens are thought to stimulate host lymphocytes and also may stimulate an increase in natural killer cells. Success rates for treatment of periocular sarcoids with BCG range from 83% to 100%; sarcoids elsewhere on the body have much lower reported success rates (48%). Severe inflammatory reactions can occur with the use of this drug and can result in fever, necrosis, and extensive swelling. Anaphylaxis has also been reported, and pretreatment with flunixin meglumine or corticosteroids is recommended. The tumor is injected with BCG vaccine every 2 to 3 weeks for four treatments. A commercial BCG product for horses is not currently available in the United States.

Nonviable *Propionibacterium acnes* in ethanol (EqStim; Neogen Corporation, Lexington, KY), has shown variable results for sarcoid treatment. Protocols differ widely and include intralesional and/or IV injections given once weekly for 6 to 8 weeks. Susceptible lesions generally show improvement after two to three treatments and eventually necrose and slough.

Autogenous vaccines have been described to have some success in a limited number of horses.[89] This technique involves the horse-side removal, freezing, and reimplantation of a sarcoid into the same horse. The transfer of sarcoid tissue, tumor homogenates, and cell-free tumor extracts from one horse to another has been proposed to stimulate production of new tumors or regression of existing tumors. Because other less invasive treatments frequently work well and tumor transplantation bears risks, such as tumor production and transmission of other diseases, this procedure should only be attempted in the most refractory of cases and with individuals known to be negative for infectious diseases such as equine infectious anemia.

Several products containing bloodroot extracts *(Sanguinaria canadensis)* and zinc chloride have been used in the treatment of various types of skin lesions including sarcoids. The bloodroot is made into an escharotic salve, which penetrates the lesion, killing the affected cells. These preparations do not always spare the surrounding healthy tissue and are thought to work by changing the antigenicity of the sarcoids so that the body recognizes these cells as foreign and produces antibodies against them. Alternatively, the concentrated zinc chloride in these products may be adequately caustic to result in the treatment outcomes. The bloodroot extract is applied while wearing gloves to the sarcoid lesion, and a bandage is placed over the area for 4 days. If the sarcoid has not sloughed by 4 days, the treatment is repeated. A rapid response to the salve is usually noticed, with the lesion sloughing in 7 to 10 days. Repetition of the treatment is sometimes required. In one study of 125 sarcoids in 49 horses complete resolution was obtained for 65.6% of tumors, partial resolution for 23.2%, no response for 5.6%, and 5.6% worsened.[90] Size of tumor in this study was highly significant; tumors that were less than 2 cm had a 14% tumor recurrence rate. Bloodroot is a reasonable and inexpensive treatment option for small sarcoids that can be easily bandaged and for larger sarcoids in horses where significant financial constraints are present.

Various radioisotopes have been used for interstitial brachytherapy of equine sarcoids. Permanently implanted seeds of radon-222 or gold-198 and removable needles of radium-226, cobalt-60, or iridium-192 have been used. Although expensive, for recurrent, aggressive, and surgically inaccessible sarcoids, this modality should be strongly considered.

Squamous Cell Carcinoma

SCC is the second most common tumor diagnosed in horses, accounting for 20% of equine neoplasms. It is the most commonly diagnosed neoplasm of the eye, conjunctiva, ocular adnexal structures, and external genitalia but can also affect the stomach, esophagus, nasal passages, paranasal sinuses, hard palate, pharynx, larynx, perianal tissue, ear canal, tongue, hoof, and guttural pouches. Older horses are at higher risk for the development of SCC as are breeds of horses with unpigmented skin or hair surrounding their mucocutaneous junctions such as Appaloosas, American Paint horses, and Pintos.

Halflingers are also at increased risk for the development of limbal and/or third eyelid SCC despite their pigmented skin and hair.[91] A DNA marker identifies Haflingers at higher risk to develop this type of SCC, and a genetic test is commercially available. Halflingers homozygous for the risk factor (R/R) are 5.5 times more likely to develop ocular SCC than those with one copy (R/N) or no copies (N/N) of the risk factor. The increased incidence of SCC in draft breeds is also thought to be genetic.

The pathogenesis of SCC is related to ultraviolet light exposure coupled with the lack of pigmentation in sparsely haired areas. EcPV2 has been implicated as a positive etiologic agent in equine genital, ocular, and nasal SCC.[92,93] The presence of smegma, persistent phimosis, or repeated trauma may predispose stallions and geldings to developing lesions of the external genitalia.

Early clinical signs of SCC include thickening and mild exfoliation and ulceration of the skin. Mature lesions can be erosive or productive in nature. Reported tumor metastasis rate varies among SCCs, and they are generally slow to metastasize, with metastasis rates reported to vary between 2% and 18.6% of cases.[94] Poorly differentiated tumors are more likely to metastasize. Local lymph nodes are most commonly affected by the metastasis, but in rare instances, SCC may also spread to the lungs. Differential diagnoses include sarcoids, melanoma, exuberant granulation tissue, and pythiosis.

In SCC of the male genitalia, tumors usually arise from the glans penis or the inner lining of the penile sheath and spread

to the corpus cavernosum (Fig. 18.44). Early clinical signs include preputial edema and an odoriferous discharge. Intermittent hemorrhage, dysuria, pollakiuria, weight loss, and urinary incontinence may also develop. The regional lymph nodes should be assessed for metastasis by inguinal and rectal palpation. Regular examination and washing of the prepuce and penis will aid in early detection of SCC and may assist in preventing its development. SCC affecting female genitalia typically originates at the clitoris and progresses toward the vaginal vestibule and mammary glands. Hypertrophy of the clitoris and surrounding tissue and roughening of the ventral commissure may be noticed early in the disease. Ulcerative lesions of the lateral labia are also relatively common; when these are treated early they generally carry an excellent prognosis.

Lesions affecting the eyelid frequently begin as white or pink raised plaques at the edge of the eyelid and proceed to extensive ulceration and destruction of the eyelid, rapidly invading the periorbital tissues (Fig. 18.45). The earliest stages may present with bloodstaining of the tears, which progresses rapidly to a granulomatous and ulcerated lesion. Further information regarding ophthalmic SCC is available elsewhere in this text (Chapter 17).

Diagnosis of SCC can be accomplished by cytologic or histologic examination. Depending on the site and size of the lesion, excisional, wedge, punch, or elliptical biopsy can be performed. Other diagnostic methods include fine-needle aspiration of nodular lesions, cytologic examination of superficial scrapings from ulcerated lesions using a dull blade, and impression smears.



FIG. **18.44** Early squamous cell carcinoma on the penis of a 23-year-old Morgan gelding. This tumor would likely respond well to several applications of topical 5-fluorouracil.

Treatment of SCC is most successful when the lesion is recognized and therapy is initiated early in the course of the disease. Even so, recurrence of SCC is not uncommon. Debulking the tumor may be accompanied by cryosurgery, injection or implantation with cisplatin or mitomycin-C, or radiation therapy as previously described to treat sarcoids. Similarly, bloodroot extract and other topical medications can be useful.

Compared with equine sarcoid, wide surgical excision alone for treatment of early cases of SCC typically carries a more favorable prognosis; however, recurrence is always possible and the lesion characteristics may be altered with recurrence, making the tumor less likely to be recognized. When the third eyelid is removed in its entirety, care should be taken to check for extension of the tumor into nearby conjunctiva, and, for added insurance, mitomycin-C or 5-fluorouracil ophthalmic drops should be added to the treatment regimen. When using surgical excision alone in the treatment of SCC, a 0.5- to 1-cm-diameter margin of normal tissue has been recommended. This can be difficult to achieve with periocular SCC. In a study of nine horses with ocular SCC, 80% experienced tumor resolution when debulked and treated with mitomycin-C.[95] Ophthalmic 5-fluorouracil drops may provide similar or better control. The ophthalmic drops of either mitomycin-C or 5-fluorouracil are administered in a pulsed fashion. For mitomycin-C, 0.2 mL of a 0.04% solution of mitomycin-C is instilled into the conjunctival sac of the affected eye every 6 hours for 7 days. The treatment is then discontinued for 7 days, and the cycle is repeated until the tumor has resolved. For 5-fluorouracil treatment, ophthalmic drops or ointment is instilled in the affected eye q 6 to 8 h for 3 to 4 days, and then treatment is discontinued until the following month. The cycle is repeated monthly until resolution occurs. With the more recent evidence of EcPV2 playing a role in the etiology of SCC, it may be time to rethink treatment by surgical excision alone.



FIG. **18.45** Squamous cell carcinoma of the lower palpebrum in an aged Paint Horse.

There are many topical treatments available for the treatment of genital SCC. Tumors that are thick or nodular should be debulked before topical therapy. Topical 5% 5-fluorouracil cream is reported to have a 90% success rate for treatment of superficial SCC of the penis and prepuce.[96] The penis and prepuce should be cleansed and dried before rubbing a small amount (the size of a pea is adequate for most SCC if caught early) of cream into all affected tissue. The process is repeated every 2 weeks until resolution because 5-fluorouracil is retained in the prepuce for 10 to 14 days. A 40-g tube is usually sufficient for treatment of all but the most extensive disease. Application to the urethra is appropriate if necessary. The most common errors are applying too much (more inflammation than needed) and not sticking with the 2-week interval for an adequate length of time. The penis should be reevaluated every 3 months for the first year after resolution of the tumor and then every 6 months. For tumors on the external sheath, perineum, or vaginal wall, application is performed daily until the area is inflamed. The area is allowed to recover, and any residual tumor is retreated in the same fashion.

Beta irradiation (strontium-90) has been used successfully to treat SCC and is particularly good for ocular lesions because the orbit and adnexa can be preserved. Course-fractionated cobalt-60 radiotherapy has provided good to excellent long-term results in several cases with severe involvement of the paranasal sinuses.[97]

Piroxicam (80 mg/1000 lb PO once daily), a nonspecific cyclooxygenase-2 (COX-2) inhibitor, has been reported to be an effective adjunctive therapy for some horses with SCC.[98] Piroxicam is proposed to induce apoptosis and angiogenesis and stimulate the immune system through inhibition of PGE_2 production in epithelial tumors. There are conflicting reports on the production of COX-2 by SCCs in the horse[99-101] and no reported controlled studies of its use in the treatment of epithelial tumors in horses.

Melanoma

Melanomas are common tumors that arise from the malignant transformation of melanocytes. They can occur in horses and mules of any coat color, but incidence is much greater in gray and white horses. Arabians, Thoroughbreds, and Percherons may be at increased risk for developing this disease. In one study of 264 gray-skinned Camargue-type horses, the prevalence of melanomas in the overall population was 31.4%.[102] The incidence, size, and number of melanomas was significantly correlated with age, giving a prevalence of 67% at ages >15 years. Other sources indicate that approximately 80% of all gray horses will develop one or more melanomas at some location by the age of 15.[103] There appears to be no gender predilection for these tumors. The most common areas of the skin affected are underneath the tail and external genitalia (Figs. 18.46 and 18.47). Other regions less commonly affected include the ear, eyelid, neck, parotid salivary gland, guttural pouches, lips, and limbs. When melanomas do occur in horses of other colors, they may be at greater risk for becoming malignant.

The etiology of melanomas in the horse has not been clearly defined but is believed to be secondary to genetic mutations in the melanin metabolism molecular pathway. Controversy exists as to whether or not exposure to increased levels of ultraviolet radiation may also play a role in the pathogenesis. Ninety-five percent of melanomas are of little



FIG. **18.46** Multiple melanomas on the penis of a 12-year-old grey pony gelding.

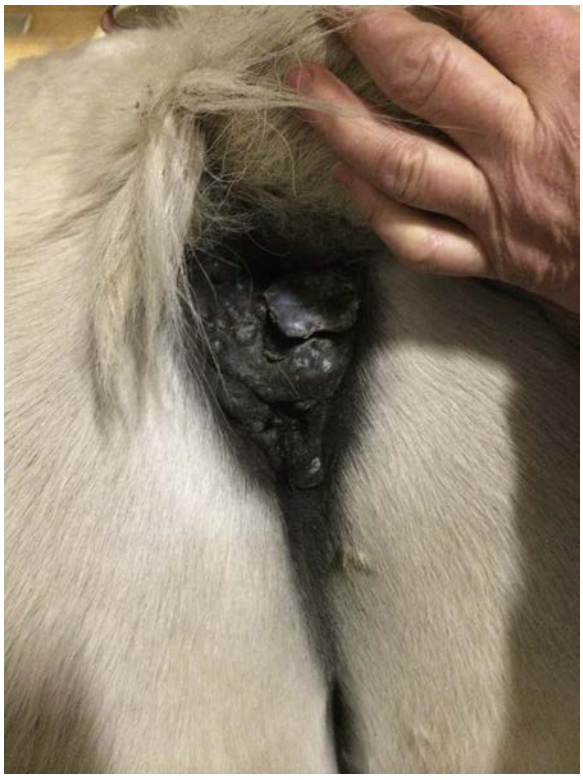


FIG. **18.47** Dermal melanomatosis in an aged pony gelding.

clinical significance except when they inhibit the use of riding tack or interfere with urination, defecation, and coitus. As horses age, however, these tumors can progress and demonstrate overt malignant behavior including local invasion and metastasis. Metastasis may occur by either hematogenous or lymphatic spread to any region in the body including lymph nodes, spleen, liver, and lungs. In rare instances melanomas exhibit rapid growth and malignant properties from the onset. Diagnosis of equine melanomas is generally based on their black gross appearance but can be confirmed using histology (Fig. 18.48).

The term *equine melanoma* or *melanocytic tumor* encompasses all histologic and clinical variants from the benign to the most anaplastic malignant variants, and in gray horses there appears to be a continuum between the two. Four distinctive manifestations of equine melanocytic tumors have been defined based on clinical presentation, histologic examination, tendency to become malignant, and response to surgical excision. *Benign melanocytic nevi (benign melanocytoma)* are characterized by their origination in the superficial dermis or at the dermoepidermal junction. Involvement of the epithelium is common. Histologically, the tumor appears as nests of large pleomorphic epithelioid cells, which contain variable amounts of pigment mixed with melanophages. Mitotic cells, binucleate cells, and euchromatic nuclei are occasionally seen. Surgical excision is largely curative. *Dermal melanomas* are located in the deep dermis and appear histologically as small, homogeneous, indistinct round or dendritic tumor cells containing condensed chromatin and dense cytoplasmic pigmentation. They demonstrate minimal malignant criteria. *Dermal melanomatosis* refers to many dermal melanomas that have coalesced. *Malignant melanomas* are characterized by pleomorphic epithelioid cells with vascular, lymphoid, and/or dermal invasion; variable pigmentation; and a moderate to high number of mitotic figures. Differential diagnosis includes habronemiasis, cutaneous collagen necrosis, mast cell tumors, and sarcoid.

Melanomas commonly appear as black or gray, solitary, discrete, firm, spherical, or flat nodules in the skin or subcutis and may have a pedicle. Tumors frequently coalesce, and hundreds of variable-sized nodules may be present without affecting the well-being of the horse. Many small tumors may be present, producing a cobblestone appearance. The overlying skin may be intact or slightly alopecic, with some tumors developing ulcers at the surface.



FIG. **18.48** Cut surface of a melanocytic tumor demonstrating dark pigment and well-encapsulated margin.

Treatment is often not necessary for small melanocytic tumors located in uncompromising locations. Large masses or masses located in areas in which they can be a nuisance are treated with surgical excision. When possible a wide margin should be attained with surgical excision. Cryosurgery can be used in conjunction with surgical excision. However, tumor regrowth is possible.

Intralesional injection or implantation of cisplatin has been very effective in the treatment of tumors less than 3 cm in diameter. The use of microwave energy (Thermofield System; Parmenides Inc., Franklin, TN) has been reported and allows for treatment of larger tumors. It is even more effective when combined with intratumoral cisplatin. Similarly, the use of cisplatin and hyperthermia improves the effect of cisplatin intratumoral therapy even in more advanced disease.[104] The few reports of electrochemotherapy in the treatment of large melanomas has been less promising.

Studies evaluating the use of cimetidine in the treatment of melanomas have shown varying results[105,106]; regression of tumors should be evident within 3 months of initiating treatment, or treatment should be discontinued. Cimetidine is administered at 2.5 mg/kg q 8 h and continued for 2 to 3 weeks following resolution of tumor growth. Some horses may require treatment their entire life. Liver enzymes should be monitored semiannually in horses receiving this medication. Similar to the treatment of SCCs, adding piroxicam (80 mg/1000 lb PO once daily) to melanoma therapy may be beneficial, but there are currently no controlled studies.

Techniques to prepare monoclonal antibodies and DNA vectors encoding eqIL12 and eqIL18 to promote immunologic recognition and tumor rejection of melanomas have been developed but are not currently available for clinical use.[107]

A canine melanoma vaccine (Oncept; Merial) is currently undergoing equine clinical trials. It contains the human DNA sequence that encodes a specific protein (tyrosinase) that is only found within melanocytes. Tyrosinase is an enzyme that is crucial to the melanocyte's ability to produce melanin and also to the survival of the melanocyte itself. The human tyrosinase protein appears similar to that of the horse. Ideally the vaccine will result in the destruction of the tyrosinase in the cancerous melanoma cells, and ultimately, the inability of the tumor cells to survive. Early results appear promising, with most horses demonstrating tumor shrinkage following vaccination and some horses demonstrating dramatic tumor shrinkage. The vaccine is given four times in 2-week intervals and then every 6 months for the remainder of the horse's life.

Mast Cell Tumor

Mast cell tumors are uncommon in horses and appear to occur more often in Arabian Horses than any other breed.[1,29] Mast cell tumors are benign and most commonly occur as a single cutaneous nodule, often located on the head (Fig. 18.49). Nodules range in size from 2 to 20 cm in diameter. The surface of the nodules may be normal, hairless, or ulcerated. Alternatively, mast cell tumors may cause diffuse swelling on a lower extremity, usually below the carpus or hock. The swelling is firm, and the overlying skin is normal in appearance. Radiographs of the limb commonly show multifocal areas of soft tissue mineralization.[64]

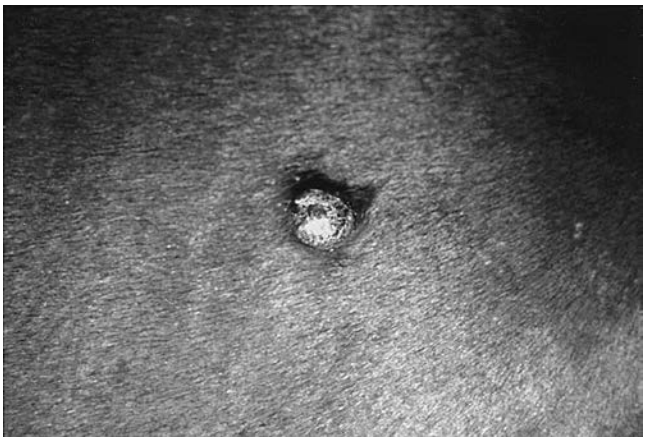


FIG. **18.49** Hyperplastic mast cell tumor in a horse.

The diagnosis of a mast cell tumor is determined by biopsy. Diffuse to nodular proliferations of mast cells occur in the dermis. Tumor cells may be well differentiated or more atypical. Tissue eosinophilia, collagen degeneration, and dystrophic mineralization are common.

Surgical excision is curative, but the tumors also respond well to sublesional triamcinolone acetonide (5–10 mg per lesion), cryosurgery, and carboplatin. Spontaneous remission may occur after incomplete excision or in young horses. Metastasis has not been reported.[29]

Cutaneous Lymphoma

Systemic lymphoma is discussed in detail in Chapter 15. Cutaneous lymphoma is rare in horses. The breed most often affected is the Quarter Horse followed by Thoroughbreds and Standardbreds. Equine cutaneous lymphomas can be classified as one of two types.[108] Cutaneous T-cell lymphoma is an epitheliotropic scaling disease widely disseminated throughout the skin. T-cell–rich, large B-cell cutaneous lymphoma or nonepitheliotropic lymphoma is a nodular disease. A definitive diagnosis is made by histologic examination of a skin biopsy with several representative tissue samples for examination. Epitheliotropic lymphoma carries a poor prognosis. Treatment strategies include prednisolone at 1 mg/kg orally once daily for 7 days and then every other day. PEG-l-asparaginase at 10,000 IU/m^2 once weekly may also be beneficial. Low-dose cyclophosphamide and immunization with *vaccinia* virus–infected autologous tumor cells were successful in inducing a 19-month remission in a 13-year-old horse.[109] Nonepitheliotropic lymphoma carries a much better prognosis. Affected horses can have one or many nodules, with some horses having a single nodule for years without showing any other effects of the tumor. The presence of underlying diseases including *Borrelia* infection and EHV-5 should be ruled out in horses with nonepitheliotropic lymphoma. When the nodules are removed in horses with one or a few nodules, they tend to recur, but many horses can live near normal life expectancies. In addition to surgical resection, the use of corticosteroids and cisplatin intralesional injections has proven to be useful in some horses.

Pseudolymphoma

Pseudolymphomata are common in horses compared with lymphomata.[2] Differentiating the two diseases is important because the prognosis for pseudolymphoma is excellent.



FIG. **18.50** Hemangioma on the dorsal surface of the hock in a yearling. (Photo courtesy of Karl Frees.)

Pseudolymphomata are nodular to papular lesions that develop in the skin as a result of chronic antigenic stimulation. The most common antigenic stimuli are believed to be insect bites and drug reactions. Lesions are most common in late summer and fall and are usually solitary but may be multiple. Pseudolymphomata usually occur on the head and trunk. Individual lesions are firm, raised, and haired.

A definitive diagnosis is made by a skin biopsy. Histologic examination of tissue shows dense dermal and subcutaneous infiltration of lymphocytes, histiocytes, plasma cells, and eosinophils. Lymphoid nodules and eosinophils are present and are an important aid in the differentiation of pseudolymphoma from lymphoma.

Pseudolymphomata often spontaneously regress but may be excised surgically or injected sublesionally with triamcinolone acetonide at 3 to 5 mg per lesion, not to exceed a total dose of 15 to 20 mg.

Hemangioma

Hemangiomas (vascular hamartoma and verrucous hemangioma) are benign tumors of the endothelial cells of blood vessels. Lesions are most common in horses less than 1 year of age, and some horses are born with them. Hemangiomas tend to be solitary tumors occurring on the distal limbs (Fig. 18.50). The clinical appearance varies and may be circumscribed, nodular, firm to fluctuant, blue to black in color, dermal or subcutaneous, hyperkeratotic, or verrucous. Ulceration and bleeding are common.

Confirmation of the diagnosis is made by skin biopsy, which reveals proliferation of blood-filled vascular spaces lined by single layers of well-differentiated endothelial cells. Equine hemangiomata are characterized by a multinodular

capillary hemangioma with hyperplasia and hyperkeratosis of the overlying epidermis.

Bloodroot extract has been used to treat these tumors as has cryosurgery, surgical excision alone, and cisplatin electrochemotherapy. Complete removal is generally curative, and there are reports of spontaneous regression. Recurrence rates are unknown.

Dermoid Cysts

A dermoid cyst is a tumor of developmental origin consisting of a wall of fibrous tissue lined with stratified epithelium containing hair follicles, sweat glands, sebaceous glands, nerves, or any combination of these. Dermoid cysts may be congenital or hereditary, have been reported in horses 6 months to 9 years of age, and are more common in Thoroughbreds. They occur frequently on the dorsal midline between the rump and withers. The cysts appear as soft, fluctuant swellings with normal overlying skin. The diagnosis is made by histologic examination after excision.

Atheroma

An atheroma is a cyst in the false nostril. These lesions are believed to develop from hair follicle retention cysts or from displaced germ material. Atheromata are present at birth, are usually unilateral, and enlarge with time. They are not usually noticeable until they enlarge to greater than 2 cm. The tumor is firm on palpation but is rarely painful.

Diagnosis is based on clinical signs. Treatment is not necessary unless the atheroma negatively impacts airflow or the owner is disturbed by the lesion. Surgical excision is the treatment of choice, but one must take care to remove the entire cyst, or recurrence or chronic drainage occur.

MISCELLANEOUS AND IDIOPATHIC SKIN DISORDERS

Anhydrosis

Anhidrosis is characterized by the inability to sweat appropriately in response to an adequate stimulus.[110] The result is exercise intolerance and dermatologic problems, and in extreme cases, collapse and death may occur. This disease is primarily a problem in hot, humid climates, such as that found in the Gulf Coast states. Symptoms are exhibited during the hottest months of the year when daytime highs can exceed 100°F, and the lows remain in the 70°F range. During the cooler months of the year, the condition tends to resolve. Cases are uncommon in temperate climates. Anhidrosis can affect up to 20% of horses in some geographic areas. The disease does not have a breed, gender, age, or coat color predisposition. Horses that were born and raised in the northern United States have been known to develop anhidrosis shortly after arriving in the southern region of the country. Horses that were born and raised in the southern regions have been known to develop the condition at age 12 or later. Native and imported horses tend to be equally affected.

The pathogenesis of anhidrosis is not clearly understood. It is believed that the condition results from a conditioned insensitivity of sweat glands to epinephrine. Studies of the sweat glands of anhidrotic horses revealed that there was a reduced number of cytoplasmic vesicles, compacted intercellular spaces, lack of evidence of myoepithelial cell contraction, and evidence of contracted ductal lumina that appeared occluded by cellular debris. These findings suggest that a failure or impairment of sweat production had occurred. The cause of these findings is not known, but hypothyroidism, hypochloremia, and downregulation and exhaustion of β-adrenergic receptors in apocrine sweat glands have been proposed.

Horses affected with anhidrosis may be presented for evaluation of respiratory disease, skin disease, exercise intolerance, and failure to sweat. These horses may exhibit exercise intolerance, tachypnea, flaring of the nostrils at rest, elevated rectal temperature, and partial or complete lack of sweating. Any sweating that is noted will most likely be restricted to the mane, axilla, and groin regions. In long-term cases, patchy alopecia of the head and neck along with seborrhea may be present, which are associated with reduced hair follicle function. Other clinical signs that may be evident include pruritus, polydipsia, polyuria, a poor appetite, and a loss of body condition.

Diagnosis of anhidrosis is accomplished by observing a lack of localized sweating in response to intradermal injections of epinephrine or terbutaline. The epinephrine or terbutaline is injected intradermally at a dose of 0.5 mL at concentrations of 1:1000, 1:10,000, 1:100,000, and 1:1,000,000. In normal horses, sweating will be evident over the injection sites within 30 minutes, at all dilutions. Anhidrotic horses will only respond to the 1:1000 dilution, and even this response may take 5 hours or more. Differential diagnoses include PF, sarcoidosis, and dermatophilosis.

The most successful remedy for anhidrosis is to move the horse to a more temperate climate. If this is not possible, the horse should be kept in a stall that is supplied with a flow of cooling air. The horse should also be trained during the coolest part of the day, and the training regimen should be modified in intensity. A variety of other treatments including electrolytes, nutritional supplements, clenbuterol, vitamin E, and iodinated casein have been reported anecdotally to help some affected horses. For some horses, reduced stress and rest for an extended period may help decrease the severity of clinical signs.

Chronic Progressive Lymphedema

CPL of draft horses is characterized by progressive swelling, hyperkeratosis, and fibrosis of the distal limbs.[111,112] The disease is seen in Shires, Clydesdales, Belgian Draft horses, and Gypsy Vanners. Clinical signs appear at an early age and progress throughout the life of the horse. Initial clinical signs mimic those of common pastern dermatitis but progress to skin thickening and folding with crusting lesions in the pastern area. Because of the heavy feathering present in affected breeds, the lesions may go unnoticed until they are extensive. Chorioptic mange and/or secondary bacterial infections are not uncommon. Chronic inflammation results in lymphedema, and lower leg enlargement becomes permanent. Treatment includes clipping the feathers, addressing the underlying skin infections, exercise, bandaging, and cold water hydrotherapy. Euthanasia is common in severely affected horses.

Dermal Panniculitis and Fat Necrosis

Panniculitis refers to inflammation of the subcutaneous fat. This condition is rare in horses and results from widespread death of lipocytes.[113] Fat cells are vulnerable to trauma, ischemia, and neighboring inflammation. When lipocytes are damaged, lipid is released and undergoes hydrolysis into glycerol

and fatty acids. Fatty acids are potent inflammatory agents that elicit further inflammatory reactions.

Dermal panniculitis is rare and may be precipitated by a wide range of causes including trauma, infections, autoimmune disease, pancreatic disease, glucocorticoid therapy, vasculitis, vitamin E deficiency, and idiopathic causes. In the horse, few cases have been reported, and their causes were obscure. Although vitamin E deficiency was suspected in a few horses, treatment with vitamin E has failed to resolve the disease in multiple cases.

Clinically, horses with panniculitis show deep-seated nodules and plaques. Lesions may be single or multiple and vary in size. They may be hard and well defined or soft and ill defined. Initially, lesions are not fixed to the overlying skin, but as the disease progresses, nodules become cystic and rupture onto the skin surface. The ulcerating nodules drain a yellow to brown to bloody oily material. Pain varies. Healed lesions may leave depressed scars. Affected animals may be febrile, depressed, lethargic, and anorectic.

A definitive diagnosis is made by skin biopsy. Samples should be obtained for histopathologic evaluation by deep excisional biopsy using a scalpel blade because skin biopsy punches do not obtain sufficiently deep samples to be diagnostic. One should request special stains (i.e., PAS, Gomori's methenamine silver, Brown and Brenn) for causative agent. Skin biopsy reveals lobular to diffuse pyogranulomatous inflammation in the panniculitis.

The practitioner should identify and treat underlying causes appropriately. Idiopathic panniculitis may respond to prednisolone at 1 to 2 mg/kg orally once daily for 7 to 14 days or to a single treatment of dexamethasone at 20 to 30 mg IM. Clinical improvement may occur within 7 to 14 days. Relapses occur, and the horse may require lifelong therapy.

Equine Axillary Nodular Necrosis

Equine axillary nodular necrosis is similar to equine eosinophilic granuloma with collagen degeneration, except that the lesions are localized to the axillary region.

The condition has no known age, gender, or breed predilection. Clinically, single or multiple nodules develop unilaterally in the axillae. The nodules are painless, nonpruritic, haired, well-circumscribed firm masses that vary in size from 0.5 to 4.0 cm or greater in diameter. Multiple lesions tend to be arranged in a row.

Skin biopsy is diagnostic. Biopsy findings include pyogranulomatous, eosinophilic dermatitis with foci of coagulation necrosis. Collagen degeneration is not a common finding. Treatment is the same as that for equine eosinophilic granuloma.

Eosinophilic Granuloma

Eosinophilic granulomas (nodular necrobiosis) are probably the most common nodular skin disease of the horse. Lesions appear as firm dermal nodules that are associated with degenerative collagen. No breed, gender, or age predisposition has been identified for this disease. Nodules usually arise in the warmer months of the year. Sites most commonly affected are the withers, back, and sides of the neck. Other areas of the body that can potentially be affected include the girth area, mane, rump, and face. Most commonly horses are brought for examination of eosinophilic granuloma because the owner is concerned that the lesion is neoplastic or the lesion occurs in an area in which it is irritated by tack.

The etiology of eosinophilic granulomas is not known. It has been suggested that a hypersensitivity reaction might play a role in the pathogenesis. Insect bites, especially those of mosquitoes, have been implicated as the cause of this disease, although the evidence for this is weak. Because lesions are common in the saddle region, it is possible that trauma is the factor in lesion development. Atopic horses may be predisposed to developing the nodules. Nodules occur as single or multiple lesions ranging from 0.5 to 10 cm in diameter, with small lesions being significantly more common (Fig. 18.51). The lesions are typically rounded, well circumscribed, and firm, with no alopecia or ulceration present. There is usually no pain or pruritus associated with the nodules. In areas in which collagenolysis is extensive and involves the superficial dermis, transepidermal elimination may occur, resulting in ulcerated lesions containing a central grayish white caseous core. The skin and coat overlying the nodules are generally normal unless traumatized. In some cases, hundreds of pea-sized nodules have been seen.

Diagnosis of eosinophilic granulomas is usually based on history and physical appearance but cannot be definitively made without a biopsy of a nodule. Culture results for bacteria and fungi will be negative. Histologic observation of a biopsy specimen shows several foci of collagenolysis, with the collagen fibers exhibiting a hyaline or granular appearance. Free hair shafts are sometimes observed within the lesions. In these horses there is usually a history of body clipping before the development of the nodules, which may suggest an association. Older nodules tend to exhibit marked dystrophic mineralization and may be mistaken as calcinosis circumscripta lesions.



FIG. **18.51** Horse with multiple eosinophilic granuloma.

Differential diagnosis includes axillary nodular necrosis, hypodermiasis, amyloidosis, and cutaneous lymphosarcoma.

Treatment of eosinophilic granuloma can be accomplished by surgical excision, intralesional corticosteroids, or systemic corticosteroids. The method of therapy used depends on the number and size of nodules. A single small lesion can be left untreated because it is asymptomatic, or if it is undesirable to the owner it can be surgically removed. If just a small number of lesions are present, intralesional corticosteroids may be the best method of treatment. Triamcinolone acetonide (3–5 mg per site, total dose not to exceed 20 mg/1000-lb horse) or methylprednisolone acetate (20 mg per site) can be injected into and under the nodule using a Luer-Lok syringe and a ⅜-inch, 23-gauge needle. Injections can be repeated at 2-week intervals if necessary. Any lesions remaining after multiple injections will probably not be susceptible to the steroids and can be surgically removed. Because of the risk of inducing laminitis, no more than 20 mg of triamcinolone should be administered at one time. If multiple lesions are present, the administration of prednisolone orally at 1 mg/kg once daily for 2 to 3 weeks is recommended. After lesions have resolved, the dosage of prednisolone should be tapered and discontinued over a 5- to 10-day period. Horses with seasonal recurrences in which insect hypersensitivity is involved and documented via intradermal skin testing might benefit from ASIT to insects. Older, mineralized lesions may be unresponsive to glucocorticoid therapy and can be surgically excised. Nodules often respond well to corticosteroid treatments, but relapses are common. Retreatment is often successful in these cases.

Multisystemic Eosinophilic Epitheliotropic Disease

Multisystemic eosinophilic epitheliotropic disease is a rare idiopathic disease of horses characterized by nodules or masses in numerous organs, ulcerative stomatitis, severe wasting, significant exfoliation, and eosinophil infiltration of the skin.[1,2,114] No gender or breed predilection exists, but lesions tend to be more common in younger horses. Early lesions begin as scaling, crusting, exudation, and fissuring at the coronary band. Oral ulcers are usually present at this stage. Over several weeks a generalized exfoliative dermatosis develops. Hairs are epilated easily, and alopecia with multifocal areas of ulceration and exudation develops. The horse may be pruritic. Affected horses without ulcerative stomatitis have a good appetite but exhibit rapid, severe weight loss. Some horses have concurrent protein-losing enteropathy or malabsorption syndrome or respiratory signs including nasal and ocular discharge, swollen submandibular lymph nodes, chronic cough, and respiratory distress.

The definitive diagnosis is based on the history, physical examination, rectal examination, and skin biopsy. Ruling out PF and bullous pemphigoid is important. The chronic wasting nature of the disease and multisystemic signs are key to making a diagnosis. Laboratory features include hypoalbuminemia, hypoproteinemia, impaired small intestinal carbohydrate absorption, and increased activities of γ-glutamyl transferase, serum alkaline phosphatase, and bile duct isoenzymes. A superficial and deep eosinophilic and lymphoplasmacytic dermatitis with irregular epidermal hyperplasia is present. Exocytosis of eosinophils and lymphocytes and necrosis of keratinocytes occurs. Perivascular collagen degeneration, lymphoid nodules, and a lichenoid inflammatory infiltrate may be present. Eosinophilic infiltrates of the pancreas, salivary glands, oral cavity, and gastrointestinal tract are common. Peripheral eosinophilia is usually absent. Abdominal ultrasound may show an enlarged liver, thickened intestine, and enlarged mesenteric and intestinal lymph nodes. In some cases thoracic radiographs may be abnormal with the presence of an interstitial pattern and nodules.

Treatment is symptomatic and includes anthelmintics, antibiotics, azathioprine, and corticosteroids. Two horses have responded to dexamethasone at 0.2 mg/kg IM for the first 5 days, followed by prednisolone at 0.5 mg/kg orally every 12 hours for 7 days and then 1.0 mg/kg every 24 hours for a week, followed by alternate-day therapy. Most horses, however, respond poorly to systemic glucocorticoids, and the prognosis is grave. A single horse with this disease showed a partial response to hydroxyurea and dexamethasone therapy.[115]

Sarcoidosis

Equine sarcoidosis (ES), also known as equine idiopathic granulomatous disease, can occur as a localized, partially generalized, or generalized condition. Although all forms are rare, it appears that they are becoming more frequently encountered in equine practice. The localized form of the disease tends to remain focused within the skin, most commonly in the lower limbs. The partially generalized and generalized forms are characterized by extensive exfoliation, severe wasting, and granulomatous inflammation of multiple organ systems.

Multiple breeds have been affected, with the most affected horses being greater than 3 years old. Mares appear to suffer a greater prevalence for this condition.[116] The skin disease begins as scaling, crusting, and alopecia on the face, limbs, and/or trunk (Fig. 18.52). Clinical signs may progress rapidly or slowly. Peripheral lymphadenopathy may develop concurrently with weight loss, muscle wasting, anorexia, exercise intolerance, and fever.

The definitive diagnosis is made by skin biopsy where multifocal nodular to diffuse lymphogranulomatous dermatitis with multinucleated giant cells are present with or without vasculitis. Granulomatous infiltrates also occur in other organs, including the lymph nodes, lung, gastrointestinal organs, liver, and spleen. A complete blood count reveals leukocytosis, increased fibrinogen, and hyperglobulinemia. Anemia caused by chronic infection may be present.



FIG. **18.52** Horse with generalized granulomatous dermatitis. (Courtesy A. A. Stannard, University of California at Davis.)

The recommended treatment includes a large loading dose of prednisolone (1–2 mg/kg orally q 24 h, given in the morning) followed by 0.2 to 1.0 mg/kg orally q 24 h for several weeks or longer. Alternatively, dexamethasone can be used for the loading dose at 0.04 to 0.08 mg/kg IM q 24 h for 7 to 14 days followed by the lower prednisolone dose. Pentoxifylline at 10 mg/kg PO q 12 h and omega fatty acids may be useful adjunctive therapies. The prognosis for both generalized and partially generalized ES is poor. The prognosis for localized ES is good for survival, but the majority of horses will need low-dose prednisolone for life for lesion control, although spontaneous remission may occur.[116,117]

REFERENCES

1. Knottenbelt DC. *Pascoe's principles and practice of equine dermatology*. London: Saunders Elsevier; 2009.
2. Scott DW, Miller WH. *Equine dermatology*. 2nd ed. St. Louis, MO: Saunders Elsevier; 2011.
3. Talukdar AH, Calhoun ML, Stinson AW. Microscopic anatomy of the skin of the horse. *Am J Vet Res*. 1972;33:2365–2390.
4. Fitzpatrick TB, Eisen AZ, Wolff K, et al. *Dermatology in general medicine*. 3rd ed. New York: McGraw-Hill; 1986.
5. Muller EJ, Galichet A, Wiener D, et al. Keratinocyte biology and pathology. *Vet Dermatol*. 2014;25:236–238.
6. Proksch E, Jensen JM, Elias PM. Skin lipids and epidermal differentiation in atopic dermatitis. *Clin Dermatol*. 2003;21:134–144.
7. Hallamaa R, Batchu K. Phospholipid analysis in sera of horses with allergic dermatitis and in matched healthy controls. *Lipids Health Dis*. 2016;15:45.
8. Yang GC, Croaker D, Zhang AL, et al. A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS); a horse variant of Hirschsprung disease. *Hum Mol Genet*. 1998;7:1047–1052.
9. Santschi EM, Vrotsos PD, Purdy AK, et al. Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white-patterned horses. *Am J Vet Res*. 2001;62:97–103.
10. Fadok VA. Update on four unusual equine dermatoses. *Vet Clin North Am Equine Pract*. 1995;11:105–110.
11. Hoolahan DE, White SD, Outerbridge CA, et al. Equine alopecia areata: a retrospective clinical descriptive study at the University of California, Davis (1980-2011). *Vet Dermatol*. 2013;24:282–e264.
12. Rosychuk RA. Noninflammatory, nonpruritic alopecia of horses. *Vet Clin North Am Equine Pract*. 2013;29:629–641.
13. Robinson NE, Sprayberry KA. *Current therapy in equine medicine*. 6 ed. St. Louis, MO: Saunders; 2009.
14. Pilsworth RC, Knottenbelt DC. Linear keratosis. *Equine Vet Educ*. 2006;18:212–213.
15. Ishikawa Y, Vranka JA, Boudko SP, et al. Mutation in cyclophilin B that causes hyperelastosis cutis in American Quarter Horse does not affect peptidylprolyl cis-trans isomerase activity but shows altered cyclophilin B-protein interactions and affects collagen folding. *J Biol Chem*. 2012;287:22253–22265.
16. Rashmir-Raven AM, W NJ, Read RW, et al. Equine hyperelastosis cutis update. *Proc Am Assoc Eq Pract*. 2004;50:47–50.
17. Tryon RC, Penedo MC, McCue ME, et al. Evaluation of allele frequencies of inherited disease genes in subgroups of American Quarter Horses. *J Am Vet Med Assoc*. 2009;234:120–125.
18. Rashmir-Raven A. Heritable equine regional dermal asthenia. *Vet Clin North Am Equine Pract*. 2013;29:689–702.
19. White SD, Affolter VK, Bannasch DL, et al. Hereditary equine regional dermal asthenia ("hyperelastosis cutis") in 50 horses: clinical, histological, immunohistological and ultrastructural findings. *Vet Dermatol*. 2004;15:207–217.
20. Brounts SH, Rashmir-Raven AM, Black SS. Zonal dermal separation: a distinctive histopathological lesion associated with hyperelastosis cutis in a Quarter Horse. *Vet Dermatol*. 2001;12:219–224.
21. Monthoux C, de Brot S, Jackson M, et al. Skin malformations in a neonatal foal tested homozygous positive for Warmblood Fragile Foal syndrome. *BMC Vet Res*. 2015;11:12.
22. Frame SR, Harrington DD, Fessler J, et al. Hereditary junctional mechanobullous disease in a foal. *J Am Vet Med Assoc*. 1988;193:1420–1424.
23. Kohn CW, Johnson GC, Garry F, et al. Mechanobullous disease in two Belgian foals. *Equine Vet J*. 1989;21:297–301.
24. Lieto LD, Cothran EG. The epitheliogenesis imperfecta locus maps to equine chromosome 8 in American Saddlebred horses. *Cytogenet Genome Res*. 2003;102:207–210.
25. Lieto LD, Swerczek TW, Cothran EG. Equine epitheliogenesis imperfecta in two American saddlebred foals is a lamina lucida defect. *Vet Pathol*. 2002;39:576–580.
26. Perkins GA, Miller WH, Divers TJ, et al. Ulcerative dermatitis, thrombocytopenia, and neutropenia in neonatal foals. *J Vet Intern Med*. 2005;19:211–216.
27. Weese JS, Yu AA. Infectious folliculitis and dermatophytosis. *Vet Clin North Am Equine Pract*. 2013;29:559–575.
28. Lloyd DH, Sellers KC. *Dermatophilus infection in domestic animals and man*. New York: Academic Press; 1976.
29. Kilcoyne I, Spier SJ, Carter CN, et al. Frequency of *Corynebacterium pseudotuberculosis* infection in horses across the United States during a 10-year period. *J Am Vet Med Assoc*. 2014;245:309–314.
30. Hussein MR. Mucocutaneous Splendore-Hoeppli phenomenon. *J Cutan Pathol*. 2008;35:979–988.
31. Mira J, Herman M, Olivo G, et al. Papillomavirus co-infections in equine aural plaques. *Vet Dermatol*. 2016;27(S1):59–60.
32. Borglund A, Bergvall K. Topical treatment of equine aural plaques with nitric acid, potassium nitrate and zinc nitrate solution (Oxalic). *Vet Dermatol*. 2016;27(S1):61–62.
33. Barrandeguy M, Thiry E. Equine coital exanthema and its potential economic implications for the equine industry. *Vet J*. 2012;191:35–40.
34. Esparza J. Has horsepox become extinct? *Vet Rec*. 2013;173:272–273.
35. Ahmed AO, van Leeuwen W, Fahal A, et al. Mycetoma caused by *Madurella mycetomatis*: a neglected infectious burden. *Lancet Infect Dis*. 2004;4:566–574.
36. Elad D. Infections caused by fungi of the *Scedosporium/Pseudallescheria* complex in veterinary species. *Vet J*. 2011;187:33–41.
37. Elad D, Blum S, Kol A, et al. Eumycetoma caused by *Madurella mycetomatis* in a mare. *Med Mycol*. 2010;48:639–642.
38. Cafarchia C, Figueredo LA, Otranto D. Fungal diseases of horses. *Vet Microbiol*. 2013;167:215–234.
39. Valentine BA, Taylor GH, Stone JK, et al. Equine cutaneous fungal granuloma: a study of 44 lesions from 34 horses. *Vet Dermatol*. 2006;17:266–272.
40. Gaastra W, Lipman LJ, De Cock AW, et al. Pythium insidiosum: an overview. *Vet Microbiol*. 2010;146:1–16.
41. Doria RG, Freitas SH, Linardi RL, et al. Treatment of pythiosis in equine limbs using intravenous regional perfusion of amphotericin B. *Vet Surg*. 2012;41:759–765.
42. Marsella R. Ectoparasites: diagnosis and management. *8th World Conference of Veterinary Dermatology.* 2016;379–387.
43. Mencke N, Larsen KS, Eydal M, et al. Natural infestation of the chewing lice *(Werneckiella equi)* on horses and treatment with imidacloprid and phoxim. *Parasitol Res*. 2004;94:367–370.
44. Mencke N, Larsen KS, Eydal M, et al. Dermatological and parasitological evaluation of infestations with chewing lice *(Werneckiella equi)* on horses and treatment using imidacloprid. *Parasitol Res*. 2005;97:7–12.
45. Ural K, Ulutas B, Kar S. Eprinomectin treatment of psoroptic mange in hunter/jumper and dressage horses: a prospective, randomized, double-blinded, placebo-controlled clinical trial. *Vet Parasitol*. 2008;156:353–357.
46. Bergvall K. Advances in acquisition, identification and treatment of equine parasites. *Clin Tech Equine Pract*. 2005;4:296–301.

47. Mignon B, Losson B. Dermatitis in a horse associated with the poultry mite *(Dermanyssus gallinae)*. *Vet Dermatol*. 2008;19:38–43.
48. Gilger B. *Equine ophthalmology*. 2nd ed. St. Louis, MO: Saunders Elsevier; 2011.
49. Stannard AA, Cello RM. *Onchocerca cervicalis* infection in horses from the western United States. *Am J Vet Res*. 1975;36:1029–1031.
50. Pusterla N, Watson JL, Wilson WD, et al. Cutaneous and ocular habronemiasis in horses: 63 cases (1988-2002). *J Am Vet Med Assoc*. 2003;222:978–982.
51. Fielding CL, Pusterla N, Magdesian KG, et al. Rattlesnake envenomation in horses: 58 cases (1992–2009). *J Am Vet Med Assoc*. 2011;238:631–635.
52. Gilliam LL, Holbrook TC, Ownby CL, et al. Cardiotoxicity, inflammation, and immune response after rattlesnake envenomation in the horse. *J Vet Intern Med*. 2012;26:1457–1463.
53. Pilsworth RC, Knottenbelt DC. Equine insect hypersensitivity. *Equine Vet Educ*. 2004;16:324–325.
54. Fadok VA. Update on equine allergies. *Vet Clin North Am Equine Pract*. 2013;29:541–550.
55. Sommer-Locher B, Endriss V, Fromm E. Various circumstances regarding initial allergen exposure and their influence on development of insect bite hypersensitivity in horses. *J Equine Vet Sci*. 2012;32:158–163.
56. Marsella R, Samuelson D, Johnson C, et al. Pilot investigation on skin barrier in equine atopic dermatitis: observations on electron microscopy and measurements of transepidermal water loss. *Vet Dermatol*. 2012;23:77.
57. Lorch G, Hillier A, Kwochka KW, et al. Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy. *J Am Vet Med Assoc*. 2001;218:1314–1322.
58. Rosenkrantz W, White S. Diagnostic workup of equine atopic disease. In: Noli C, Foster A, Rosenkrantz W, eds. *Veterinary allergy*. Oxford: John Wiley & Sons; 2013.
59. Sloet van Oldruitenborgh-Oosterbaan MM, Grinwis GC. Equine pemphigus and its differential diagnoses. 8th World Congress of Veterinary Dermatology 2016;335–340.
60. O'Neill W, McKee S, Clarke AF. Flaxseed *(Linum usitatissimum)* supplementation associated with reduced skin test lesional area in horses with *Culicoides* hypersensitivity. *Can J Vet Res*. 2002;66:272–277.
61. Rosenkrantz W. Immune-mediated dermatoses. *Vet Clin North Am Equine Pract*. 2013;29:607–613.
62. Vandenabeele SI, White SD, Affolter VK, et al. Pemphigus foliaceus in the horse: a retrospective study of 20 cases. *Vet Dermatol*. 2004;15:381–388.
63. Zabel S, Mueller RS, Fieseler KV, et al. Review of 15 cases of pemphigus foliaceus in horses and a survey of the literature. *Vet Rec*. 2005;157:505–509.
64. Scott DW, Walton DK, Slater MR, et al. Immune-mediated dermatoses in domestic animals: ten years after. *Compend Contin Educ Vet*. 1987;9:424–435.
65. Pusterla N, Watson JL, Affolter VK, et al. Purpura haemorrhagica in 53 horses. *Vet Rec*. 2003;153:118–121.
66. Hansen R, Musterman A. Treatment of burn injuries and dog bite wounds. In: Theoret C, Schumacher J, eds. *Equine wound management*. 3rd ed. Ames, IA: John Wiley & Sons; 2016.
67. Geiser DR, Walker RD. Management of thermal injuries in large animals. *Vet Clin North Am Large Anim Pract*. 1984;6:91–105.
68. Gillespie JH, Timoney JF. *Hagen and Bruner's infectious diseases of domestic animals*. Ithaca, NY: Cornell University Press; 1981.
69. Rashmir-Raven AM, McConnico RM. Photosensitivity. In: Sprayberry KA, Robinson NE, eds. *Robinson's current therapy in equine medicine*. St. Louis, MO: Saunders Elsevier; 2014:536–542.
70. Nation PN. Hepatic disease in Alberta horses: a retrospective study of "alsike clover poisoning" (1973–1988). *Can Vet J*. 1991;32:602–607.
71. Nation PN. Alsike clover poisoning: a review. *Can Vet J*. 1989;30:410–415.
72. Angelos JA, Marti E, Lazary S, et al. Characterization of BPV-like DNA in equine sarcoids. *Arch Virol*. 1991;119:95–109.
73. Chambers G, Ellsmore VA, O'Brien PM, et al. Association of bovine papillomavirus with the equine sarcoid. *J Gen Virol*. 2003;84:1055–1062.
74. Gaynor AM, Zhu KW, Dela Cruz Jr FN, et al. Localization of bovine papillomavirus nucleic acid in equine sarcoids. *Vet Pathol*. 2016;53:567–573.
75. Bugno-Poniewierska M, Staron B, Potocki L, et al. Identification of unbalanced aberrations in the genome of equine sarcoid cells using CGH technique. *Annals Anim Sci*. 2016;16:79–85.
76. Martano M, Corteggio A, Restucci B, et al. Extracellular matrix remodeling in equine sarcoid: an immunohistochemical and molecular study. *BMC Vet Res*. 2016;12:24.
77. Staiger EA, Tseng CT, Miller D, et al. Host genetic influence on papillomavirus-induced tumors in the horse. *Int J Cancer*. 2016;139:784–792.
78. Bogaert L, Heerden MV, Cock HE, et al. Molecular and immunohistochemical distinction of equine sarcoid from schwannoma. *Vet Pathol*. 2011;48:737–741.
79. Compston PC, Turner T, Wylie CE, et al. Laser surgery as a treatment for histologically confirmed sarcoids in the horse. *Equine Vet J*. 2016;48:451–456.
80. McCauley CT, Hawkins JF, Adams SB, et al. Use of a carbon dioxide laser for surgical management of cutaneous masses in horses: 32 cases (1993–2000). *J Am Vet Med Assoc*. 2002;220:1192–1197.
81. Theon AP, Wilson WD, Magdesian KG, et al. Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995–2004). *J Am Vet Med Assoc*. 2007;230:1506–1513.
82. Tamzali Y, Borde L, Rols MP, et al. Successful treatment of equine sarcoids with cisplatin electrochemotherapy: a retrospective study of 48 cases. *Equine Vet J*. 2012;44:214–220.
83. Tozon N, Kramaric P, Kos Kadunc V, et al. Electrochemotherapy as a single or adjuvant treatment to surgery of cutaneous sarcoid tumours in horses: a 31-case retrospective study. *Vet Rec*. 2016;179:627.
84. McKane SA, Coomer RP. A practical protocol for the clinical use of mitomycin-C in the treatment of sarcoids in horses. *6th Congress of the European College of Equine Internal Medicine*. 2013;704.
85. Haspeslagh M, Vlaminck LE, Martens AM. Treatment of sarcoids in equids: 230 cases (2008-2013). *J Am Vet Med Assoc*. 2016;249:311–318.
86. Stadler S, Kainzbauer C, Haralambus R, et al. Successful treatment of equine sarcoids by topical aciclovir application. *Vet Rec*. 2011;168:187.
87. Nogueira SA, Torres SM, Malone ED, et al. Efficacy of imiquimod 5% cream in the treatment of equine sarcoids: a pilot study. *Vet Dermatol*. 2006;17:259–265.
88. Tamzali Y. Use of combination of tazarotene cream and imiquimod 5% cream in the treatment of equine sarcoids: a 20 case retrospective study. *6th Congress of the European College of Equine Internal Medicine*. 2013;709.
89. Espy BM. How to treat equine sarcoids by autologous implantation. *Proc Amer Assoc Eq Pract*. 2008;54:61–72.
90. Wilford S, Woodward E, Dunkel B. Efficacy of bloodroot ointment for the treatment of equine sarcoids. *6th Congress of the European College of Equine Internal Medicine*. 2013;704.
91. Lassaline M, Cranford TL, Latimer CA, et al. Limbal squamous cell carcinoma in Haflinger horses. *Vet Ophthalmol*. 2015;18:404–408.
92. Lange CE, Tobler K, Lehner A, et al. EcPV2 DNA in equine papillomas and in situ and invasive squamous cell carcinomas supports papillomavirus etiology. *Vet Pathol*. 2013;50:686–692.

93. Schellenbacher C, Shafti-Keramat S, Huber B, et al. Establishment of an in vitro equine papillomavirus type 2 (EcPV2) neutralization assay and a VLP-based vaccine for protection of equids against EcPV2-associated genital tumors. *Virology*. 2015;486:284–290.
94. Gelatt KN, Myers Jr VS, Perman V, et al. Conjunctival squamous cell carcinoma in the horse. *J Am Vet Med Assoc*. 1974;165:617–620.
95. Malalana F, Knottenbelt D, McKane S, Mitomycin C. with or without surgery, for the treatment of ocular squamous cell carcinoma in horses. *Vet Rec*. 2010;167:373–376.
96. Fortier LA, Mac Harg MA. Topical use of 5-fluorouracil for treatment of squamous cell carcinoma of the external genitalia of horses: 11 cases (1988–1992). *J Am Vet Med Assoc*. 1994;205:1183–1185.
97. Walker MA, Schumacher J, Schmitz DG, et al. Cobalt 60 radiotherapy for treatment of squamous cell carcinoma of the nasal cavity and paranasal sinuses in three horses. *J Am Vet Med Assoc*. 1998;212:848–851.
98. Moore AS, Beam SL, Rassnick KM, et al. Long-term control of mucocutaneous squamous cell carcinoma and metastases in a horse using piroxicam. *Equine Vet J*. 2003;35:715–718.
99. Dore M. Cyclooxygenase-2 expression in animal cancers. *Vet Pathol*. 2011;48:254–265.
100. Thamm DH, Ehrhart 3rd EJ, Charles JB, et al. Cyclooxygenase-2 expression in equine tumors. *Vet Pathol*. 2008;45:825–828.
101. van den Top JG, Harkema L, Ensink JM, et al. Expression of cyclo-oxygenases-1 and -2, and microsomal prostaglandin E synthase-1 in penile and preputial papillomas and squamous cell carcinomas in the horse. *Equine Vet J*. 2014;46:618–624.
102. Fleury C, Berard F, Balme B, et al. The study of cutaneous melanomas in Camargue-type gray-skinned horses (1): clinical-pathological characterization. *Pigment Cell Res*. 2000;13:39–46.
103. Seltenhammer MH, Heere-Ress E, Brandt S, et al. Comparative histopathology of grey-horse-melanoma and human malignant melanoma. *Pigment Cell Res*. 2004;17:674–681.
104. Phillips JC, Lembcke LM. Equine melanocytic tumors. *Vet Clin North Am Equine Pract*. 2013;29:673–687.
105. Goetz TE, Ogilvie GK, Keegan KG, et al. Cimetidine for treatment of melanomas in three horses. *J Am Vet Med Assoc*. 1990;196:449–452.
106. MacGillivray KC, Sweeney RW, Del Piero F. Metastatic melanoma in horses. *J Vet Intern Med*. 2002;16:452–456.
107. Mahlmann K, Feige K, Juhls C, et al. Local and systemic effect of transfection-reagent formulated DNA vectors on equine melanoma. *BMC Vet Res*. 2015;11:132.
108. Miller CA, Durham AC, Schaffer PA, et al. Classification and clinical features in 88 cases of equine cutaneous lymphoma. *J Vet Diagn Invest*. 2015;27:86–91.
109. Gollagher RD, Ziola B, Chelack BJ, et al. Immunotherapy of equine cutaneous lymphosarcoma using low dose cyclophosphamide and autologous tumor cells infected with *Vaccinia* virus. *Can Vet J*. 1993;34:371–373.
110. Hubert JD, Beadle RE, Norwood G. Equine anhidrosis. *Vet Clin North Am Equine Pract*. 2002;18:355–369.
111. Affolter VK. Chronic progressive lymphedema in draft horses. *Vet Clin North Am Equine Pract*. 2013;29:589–605.
112. de Keyser K, Janssens S, Buys N. Chronic progressive lymphoedema in draught horses. *Equine Vet J*. 2015;47:260–266.
113. Karcher LF, Scott DW, Paradis M, et al. Sterile nodular panniculitis in five horses. *J Am Vet Med Assoc*. 1990;196:1823–1826.
114. Nimmo Wilkie JS, Yager JA, Nation PN, et al. Chronic eosinophilic dermatitis: a manifestation of a multisystemic, eosinophilic, epitheliotropic disease in five horses. *Vet Pathol*. 1985;22:297–305.
115. Hillyer MH, Mair TS. Multisystemic eosinophilic epitheliotropic disease in a horse: attempted treatment with hydroxyurea and dexamethasone. *Vet Rec*. 1992;130:392–395.
116. Sloet van Oldruitenborgh-Oosterbaan MM, Grinwis GC. Equine sarcoidosis. *Vet Clin North Am Equine Pract*. 2013;29:615–627.
117. Sloet van Oldruitenborgh-Oosterbaan MM, Grinwis GC. Equine sarcoidosis: clinical signs, diagnosis, treatment and outcome of 22 cases. *Vet Dermatol*. 2013;24:218–224.

CHAPTER 19

Disorders of the Reproductive Tract

Maria R. Schnobrich*

This chapter will review the normal anatomy and physiology of the mare and stallion with a discussion of pathology and common clinical presentation of reproductive disorders. There will also be a discussion of breeding management and advanced reproductive techniques for fertility enhancement and diagnosis of reproductive dysfunction.

Reproductive Anatomy and Physiology of the Nonpregnant Mare

Maria R. Schnobrich

INTRODUCTION

Equine reproductive anatomy and physiology deviate from those of other domestic animals in several notable ways, requiring an in-depth understanding for appropriate reproductive management and treatment. This section will serve as a review of reproductive anatomy and physiology of the nonpregnant mare, focusing on the clinically important features and highlighting where the mare differs from other species.

REPRODUCTIVE ANATOMY

The reproductive anatomy of the mare includes structures intrinsic to the reproductive tract (tubular tract and ovaries) and structures that are remote from the reproductive tract that play a role in regulating reproductive function (hypothalamus, anterior pituitary, retina, and the pineal gland) or are linked to reproductive function (mammary gland). This section will review the embryonic origin and key features of the relevant anatomic structures.

Internal Reproductive Tract

The mare's reproductive tract includes the ovaries (gonads), the tubular tract (oviducts, uterus, cervix, vagina, and vestibule), and the external genitalia (vulva and clitoris).

*The editors and authors acknowledge and appreciate the contributions of Daniel C. Sharp, Michael B. Porter, Nigel R. Perkins, John B. Chopin, Carlos R. F. Pinto, Dale L. Paccamonti, Elizabeth Metcalf, Grant S. Frazer, Michelle M. LeBlanc, Elaine M. Carnevale, Marco A. Coutinho da Silva, and Juan C. Samper as previous contributors to this chapter. Some of their original work has been incorporated into this edition.

Ovaries

The paired ovaries are composed of tissue derived from mesoderm (ovarian parenchyma and vasculature) and the primordial germ cells (oocytes).[1] The ovaries are shaped like kidney beans and are approximately 2 to 4 cm in height and 8 to 10 cm in length.[2] They are usually located at the most cranial aspect of the reproductive tract, approximately 15 cm caudal to the corresponding kidney, suspended in the mesovarium.[3,4] The ovaries are generally positioned with the long axis of the ovary parallel to the mare's spine, and the ovulation fossa (the concave portion of the ovary) directed ventrally.[4]

Resting on the abdominal viscera, the position of the ovaries is somewhat variable within the sublumbar region between the 10 and 2 o'clock position, ventral to the fourth or fifth lumbar vertebrae, approximately 4 to 8 cm craniolateral from the tip of the uterine horn.[5] Ovarian size varies with hormonal environment and follicular activity. The ovaries are usually small and inactive during anestrus (3–4 cm in length) or can be quite large (10–12 cm in length) when multiple follicles are present in estrus, in vernal or autumnal transition, or under the influence of gestational hormones. The preovulatory follicle of the mare is usually larger (≥35 mm) than that in most other domestic species and can be appreciated on transrectal palpation as a toned, fluid-filled vesicle that has a characteristic difference in consistency compared with the surrounding ovarian tissue.[3-6]

The mare's ovary is unique compared with other domestic species in two ways: (1) the cortex (containing the follicles and oocytes) is internal to the ovarian medulla (ovarian vasculature), which lies on the exterior of the ovary, and (2) ovulation occurs internally, releasing the oocyte through the ovulation fossa, whereas ovulation occurs over the entire exterior surface of the ovary in other species.[4,6] This unique anatomy of the mare's ovary makes transrectal palpation and identification of corpora lutea not practical or accurate.[4,6] In addition, pathology of the ovulation fossa can result in infertility, because the ovulated oocytes may be prevented from reaching the oviduct for fertilization.

Oviduct

The oviducts (uterine tubes) originate from the most anterior segment of the paramesonephric or müllerian ducts.[1] The concentric tissue layers of the oviduct from the external surface to the oviductal lumen consist of serosal surface, muscularis (longitudinal and circular muscular layers), submucosa, and mucosa. In the 500-kg mare they are approximately 20 to 30 cm in length and travel a tortuous path in the mesosalpinx from the ovary to the tip of the uterine horn.[4]

The oviduct is divided anatomically into three sections: the infundibulum, the ampulla, and the isthmus.[1-4] The infundibulum is a funnel-shaped dilation covering the ovulation fossa that transports the oocyte from the ovary to the ampullary region of the oviduct where fertilization occurs.[1] Following fertilization, the conceptus passes through the isthmus (the narrowest portion of the oviduct that enters the uterus at the uterotubal junction) to the uterine lumen. The uterotubal junction has a muscular sphincter that selectively allows embryos (which produce prostaglandin E_2 [PGE_2]), but not unfertilized oocytes, to pass into the uterus at approximately 5.6 to 6 days post ovulation (Fig. 19.1).[7,8] In addition, the unique anatomy and physiology of the uterotubal junction make salpingitis rare in the mare compared with other species such as cattle and swine.[6,9]

Uterus

The mare's uterus consists of two uterine horns and a uterine body that are derived from the partial fusion of the paramesonephric ducts.[1,3,5] The mare's uterine tissue layers are as follows from external to internal lumen: serosa (perimetrium), the myometrium (outer longitudinal smooth muscle fibers and inner circular fibers separated by a vascular layer), and the endometrium. The endometrium is divided histologically into three layers from deep to superficial lumen: (1) the stratum spongiosum (loose connective tissue, submucosa, and glands), (2) the stratum compactum (1-mm region beneath luminal epithelium with a high density of stellate stromal cells), and (3) the luminal epithelium (consisting of simple columnar epithelium).[4,5] The uterus, ovaries, and oviducts are suspended in the abdomen by the broad ligament in a T-shape or Y-shape when viewed from above.[3,4] The broad ligament originates from the sublumbar region, and the fibers converge over the uterine body and cervix, causing the mare's uterus to have a less distinct tubular feel when palpated transrectally compared with cattle.[6] The endometrium of the mare's uterus has 5 to 10 longitudinal folds that extend from the tips of the uterine horns through the uterine body and are continued through the cervix where they become the cervical folds seen on vaginoscopy (Fig. 19.2).[4,10] The function of the endometrial folds has been theorized to aid in sperm transport to the oviduct and to provide channels for removal of fluid and debris following breeding.[4]

The uterus is the site of semen deposition during breeding and where the developing fetus is nurtured during pregnancy. It is a dynamic and changing environment influenced by circulating and local hormones that dramatically alter uterine size, contractility, tone, secretory activity of glands, immune competence, and overall character and function.



FIG. **19.1** Oviductal papilla or uterotubal junction as viewed from hysteroscopic evaluation of the uterine lumen.

Cervix

The mare has a muscular cervix (approximately 6–7 cm in length) derived from ectodermal tissue and the fusion of the paramesonephric ducts.[1,4] The cervix of the mare differs from some other farm species in that it lacks prominent cervical rings and can easily be dilated by digital manipulation.[6] The luminal epithelium is a secretory, stratified squamous epithelium, surrounded by an internal circular and longitudinal outer muscular layer. The longitudinal folds of the uterus are continuous with the cervical folds and give the scalloped appearance of the external cervical os during estrus.[4] The external cervical os is located in the vaginal fornix (anterior vagina) and has two bands of tissue that extend from the dorsal and ventral margins to the vaginal wall (dorsal and ventral frenulum) that are often confused with adhesions (Fig. 19.3).[2,4,9]

The cervix can be appreciated with transrectal palpation as a tubular structure with variable tone, located in the anterior pelvic inlet in the region of the bladder. The cervix of the mare differs from the cow in that it is less prominent and tubular on palpation due to a more dorsal insertion of the broad ligaments and the lack of fibrocartilaginous rings. The mare's cervix cannot be grasped and used to retract the reproductive tract as in a cow.[6] The cervical length of most mares is approximately 5 to 7 cm, and the width increases during estrus to approximately 2 to 4 cm. Cervical size, tone, and character will vary with breed, age, prior trauma, and reproductive status.[2,4] The cervix is a versatile structure that in estrus provides lubrication and accommodates the stallion's penis during intromission. It then maintains tone and serves as a protective barrier to the uterine environment in diestrus and pregnancy, and finally during parturition it dilates to allow expulsion of the fetus. These dynamic changes are under the control of reproductive hormones, allowing veterinarians to use the cervix clinically as a barometer of the mare's hormonal milieu (Fig. 19.4).

Vagina

The vagina originates from the fusion of the paramesonephric ducts cranially (mesodermal origin) and the urogenital sinus



FIG. **19.2** Excised uterus postmortem with the uterine lumen exposed to show endometrial folds.

caudally (ectodermal origin).[1] It consists of a collapsed potential space extending from the cranial vaginal fornix caudally to the transverse fold.[1,11] Some authors consider the vestibule as part of the vagina, and others define the caudal border at the transverse fold.[4,11,12] The vaginal epithelium is aglandular, stratified squamous tissue and is surrounded by a thin wall of fibroelastic tissue and scant smooth muscle.[11] The cranial portion of the vagina lies in the peritoneal cavity and is covered by serosa, without a distinguishable muscular layer. The caudal vagina lies in the retroperitoneal space and is not covered with serosa. This has clinical implications, because cranial vaginal lacerations can result in peritonitis. The lumen of the vagina is normally collapsed, and the vestibulovaginal fold (or hymen) in normal mares prevents aspiration of air and fecal material into the vagina. The mucosa of the vagina should be pale pink, and fluid should only be present at certain times (lochia post foaling or small amounts of translucent fluid during estrus). Otherwise, pathology should be suspected.

The vestibulovaginal fold (transverse fold or "hymen") is a fold of tissue derived from the fusion of the paramesonephric ducts cranially and the urogenital sinus caudally.[1] This fold of tissue provides a barrier against external contamination of the cranial vagina and uterus. It is easily visualized as a sheet of pink tissue at the cranial aspect of the vestibule when one parts the vulvar lips (Fig. 19.5).[13] When a vaginal or speculum examination is performed on a maiden mare this tissue may need to be broken down manually (causing minimal discomfort). It is usually thin, occasionally fenestrated, and easily torn. The function of this tissue as a barrier can be assessed by parting the labia, and if air is aspirated into the vagina (as diagnosed by an audible noise or on visual evidence of pneumovagina on transrectal ultrasound) then it is determined that the vestibulovaginal seal is incompetent.[13] Clinically, failure of this seal may be less of a concern if the mare has an adequate vulvar seal.



FIG. **19.3** Cranial vaginal fornix demonstrating the dorsal and ventral frenulum of the cervix.

Vestibule

The vestibule, originating from the caudal urogenital sinus (ectoderm), is the region of the reproductive tract that extends from the vestibulovaginal fold to the vulvar lips.[1] The vestibule of the mare is lined by stratified squamous epithelium, and a series of branched vestibular glands are present with openings visible in the ventral and lateral vestibular walls.[11] The lateral walls of the vestibule contain erectile tissue that form the left and right vestibular bulbs, which associate abaxially with the constrictor vestibuli muscle.[4] The external urethral orifice lies beneath the vestibulovaginal fold, and the position of the urethral opening relative to the pelvis and this fold may predispose the mare to urovagina or urometra (Figs. 19.6 and 19.7).[4]

Vulva

The paired vulvar labia arise from the ectodermal tissue of the urogenital sinus and provide the first and arguably the most important external barrier to the internal reproductive tract.[1,4] The vulvar labia of normal mares appose completely, with no mucosal surface visible. The ventral commissure is rounded and houses a prominent clitoris (approximately 2 cm in diameter), lying in the clitoral fossa, which can be seen rhythmically everted during estrus ("winking"). The dorsal commissure of the vulva is pointed, and its location and degree of cranial inclination are often critically evaluated during a reproductive examination, because poor vulvar conformation has been associated with contamination and pathology (Fig. 19.8).[12,13] Mares with an increased cranial angle to the vulva, sunken anus, or poor labial apposition are predisposed to fecal contamination of the reproductive tract and may require corrective surgery (Caslick or episioplasty and Gadd or perineal body reconstruction).[5,13]

Clitoris

The mare's clitoris arises from ectodermal tissue and consists of a body and glans clitoris.[1,12] The clitoral body (5 cm in length) is formed from the crura of the ischium.[12] The mare's glans clitoris is more prominent than in most domestic species (2–3 cm in diameter), and, similar to the male homolog, the glans penis contains erectile tissue.[1,12] The clitoris lies within the clitoral

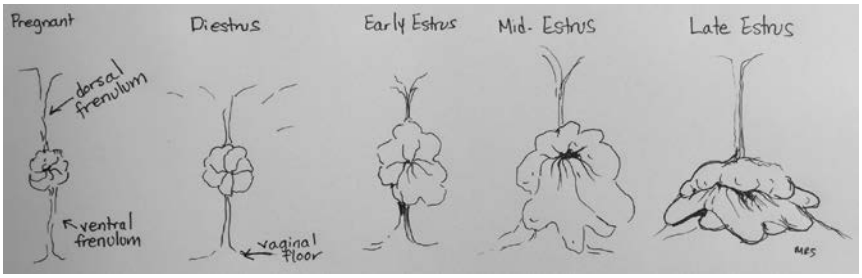


FIG. **19.4** Change in appearance of the external cervical os as viewed through vaginal speculum examination, during different hormonal environments in the mare.



FIG. **19.5** Appearance of vestibulovaginal fold (hymen) in a mare following urethral extension surgery. (Courtesy Dr. Rolf Embertson.)



FIG. **19.6** Opening of the dilated urethral sphincter under the vestibulovaginal fold. (Courtesy Dr. Rolf Embertson).

fossa (fossa clitoridis) and has three sinuses present in the skin overlying the clitoris (Fig. 19.9).[2,3] There are two smaller lateral sinuses and a larger median sinus that can accumulate debris and provide an environment that promotes growth of certain pathogenic bacteria *(Taylorella equigenitalis)*.[4,12,14]

Nonreproductive Tract Anatomy

Mammary Gland

The mammary glands are derived from ectodermal tissue and lie in the inguinal region, often inconspicuous even during lactation.[1] The mare's udder consists of left and right laterally compressed halves with its respective teat.[2] Each teat has two (sometimes three) separate lactiferous duct systems that empty into a single ostium.[2] There may be two or three ostia per teat, with each opening representing a separate duct system. During lactation the glands are soft and should not be firm and distended if the foal is nursing adequately. When not lactating, the glands involute and become soft and flaccid.

Hypothalamus and Pituitary Gland

The hypothalamus (part of the diencephalon) is located below the thalamus in the rostral aspect of the brainstem.[2,3] The hypothalamic nerve cell bodies (hypothalamic nuclei) that influence reproductive function are grouped into "tonic" and "surge" centers.[1] Neurons in these regions have axons that either terminate into a capillary system that perfuses the anterior pituitary or that terminate in the posterior pituitary to release hormones into systemic circulation.[1,2] The hypothalamohypophyseal portal system carries gonadotropin-releasing hormone (GnRH) from hypothalamic nerve endings in undiluted small concentrations to target cells in the anterior pituitary.[1,2,4]

The pituitary is divided into the posterior lobe (neurohypophysis) and the anterior lobe (adenohypophysis), which is further divided into anterior and intermediate lobes.[3,12] The anterior pituitary cells under stimulation from GnRH release their hormones into the systemic circulation. The primary hormones released from the anterior pituitary's gonadotroph cells include follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL). Neurons from hypothalamic nuclei such as the paraventricular nuclei (PVN) in the hypothalamus extend directly into the posterior pituitary, where upon stimulation of the cell body, hormones (oxytocin and vasopressin) are directly released into circulation.[1,2,4]

REPRODUCTIVE PHYSIOLOGY OF THE NONPREGNANT MARE

Reproductive Hormones

Environmental factors (daylight length, pheromones, and nutrition) transmit information through neural signaling and the pineal gland to the hypothalamus to regulate the release of GnRH.[1,4-6] The pulse frequency of release is regulated by positive and negative feedback from other reproductive hormones (inhibin, estrogens, and progestogens) secreted by the reproductive tract. The reproductive tract undergoes changes as a result of this hormonal milieu, which is dictated by the interplay of the hypothalamic-pituitary-gonadal (HPG) axis. Table 19.1 summarizes the major reproductive hormones and their primary targets and effects. Fig. 19.10 is a simplified summary of this hormonal axis and the reproductive tract illustrating the hormones, their targets, and effects.

Reproductive Phases of the Nonpregnant Mare

The mare is a seasonally polyestrous, long-day breeder with regular estrous cycles repeated when daylight is long and resources for the dam and foal are optimal.[4-6] The mare's annual reproductive rhythm (in the Northern Hemisphere) can be divided into four phases: (1) winter anestrus, (2) vernal transition, (3) ovulatory/breeding season, and (4) autumnal transition. The transition into these different phases is mediated through environmental clues, most notably length of exposure to light or photoperiod.[4,5,15]

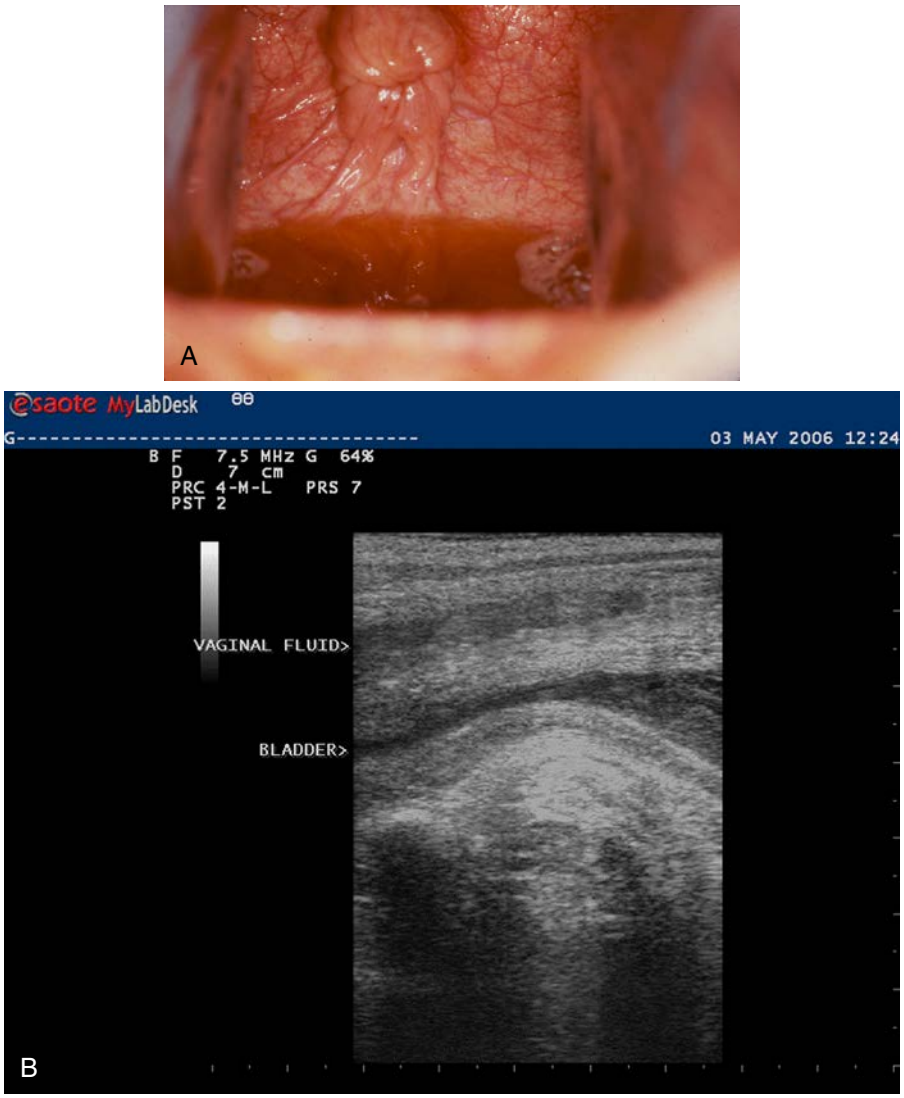


FIG. **19.7** (A) Urovagina as viewed on vaginoscopy with hyperemic vaginal mucosa and (B) as viewed on transrectal ultrasound with hypoechoic fluid present in the vagina. (Courtesy Dr. Patricia Sertich).

It is believed that this change in photoperiod is conveyed by the retina through a multineuronal pathway that modulates melatonin secretion, which then regulates hypothalamic secretion of GnRH.[4,5] Under increasing daylight, melatonin secretion is reduced (the majority of melatonin is secreted during night/dark), and melatonin's inhibitory influence on GnRH synthesis and secretion is removed, resulting in increased GnRH secretion. Increasing concentrations of GnRH stimulate follicular development and the start of the vernal transition.[4]

Winter Anestrous/Anovulatory Season

Generally the incidence of mares undergoing a period of reproductive quiescence or anestrus varies in relation to increasing distance from the equator. The incidence of anestrus at latitudes between 30° and 50° is approximately 70%, whereas at latitudes between 10° and 30°, less than 50% of mares experienced anestrus.[15-18] During anestrus the mare's production of GnRH is reduced, resulting in a decrease in pituitary secretion of FSH and LH. Consequently there is little follicular development and no corpora lutea formation, and thus circulating concentrations of estradiol and progesterone are low.

Palpation and ultrasonography of the reproductive tract during anestrous reveal small inactive ovaries (largest follicle <10 mm), a flaccid uterus, and a cervix that may have tone but will relax and give way to digital pressure or dilation. No uterine edema or intraluminal uterine fluid should be present, and the cervix is often pale pink and appears closed on vaginoscopy. Reproductive behavior is considered ambivalent, with neither strong aversion to the stallion nor demonstrative teasing. Occasionally breeding will be permitted by the anestrous mare and can be mistaken as a sign of true fertile estrus and follicular development. This is confusing because the term *anestrus* means lack of estrus or receptivity to the stallion, and as previously mentioned mares in this period have been known to allow breeding and even demonstrate receptive sexual behavior. This highlights another difference in mare reproductive physiology compared with most domestic species.



FIG. **19.8** Poor perineal conformation in a mare with episioplasty (Caslick type) in place. Note the sunken anus and cranial tilt of the dorsal vulva. (Courtesy Dr. Rolf Embertson).

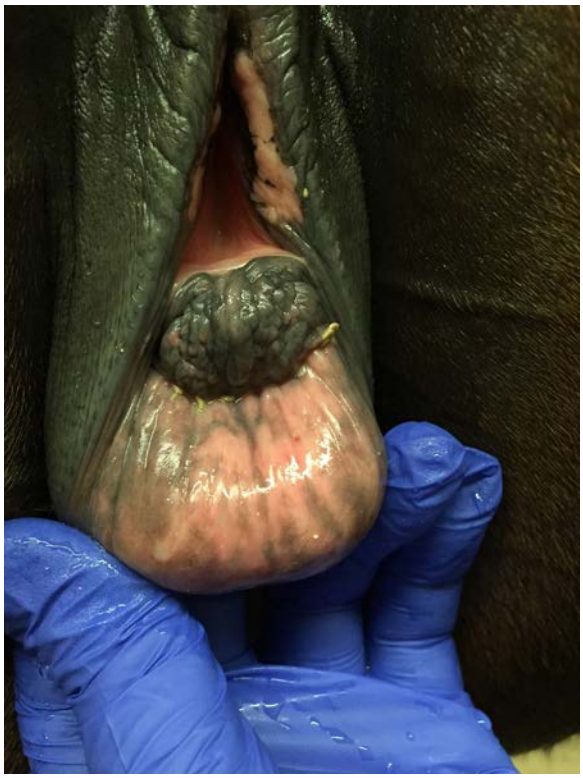


FIG. **19.9** Clitoris with smegma in the clitoral fossa.

Vernal Transition/Anovulatory Receptivity

As the length of daylight increases, hypothalamic GnRH production and secretion increases, resulting in an increase in circulating FSH and eventually LH concentrations.[4,19] Several waves (average of 3.7) of follicular growth and regression occur without ovulation before the first ovulation of the season.[20,21] The first ovulation marks the end of vernal transition and the beginning of regular interovulatory intervals or the "breeding season." FSH levels are variable during transition, whereas LH concentrations remain low, resulting in prolonged periods of estrus for approximately 1 to 2 months before ovulation.[22] Early follicular waves have decreased steroidogenesis, so estradiol elevates only in the later follicular waves that result in ovulation.[23] These transient infertile estrus periods may cause the inexperienced owner or practitioner to waste time inappropriately breeding a mare that will not ovulate, generating unnecessary costs and possibly contaminating the mare. It should be noted that not all mares have a defined transitional phase, and some remain in deep anestrus and only develop one follicular wave prior to ovulation.[24]

Reproductive evaluation of the mare during this time usually reveals large ovaries with multiple medium (>20 mm) to large follicles and no corpora lutea. Endometrial edema may vary from nonexistent to prominent, and there is a palpable increase in uterine thickness and tone. Small amounts of intrauterine fluid may be present coinciding with increased uterine edema. The mare's cervix relaxes under an environment of increasing estradiol and minimal to no progesterone. The mare's behavior is often characterized as persistent, intermittent, or irregular intervals of estrus in which the mare is receptive to mating but because of lack of ovulation will not conceive.[5,6,15] This can be a very frustrating time for owners with horses in training because they often complain of persistent estrus, which some feel interferes with performance.

Ovulatory Receptivity/Breeding Season

Following the first ovulation of transition, the mare begins to have regular interovulatory intervals (estrous cycles). The time the first ovulation occurs may vary based on individual, latitude, management, and breed. In the Northern Hemisphere, the first ovulation of the year usually occurs naturally around late April to May.[4] The average estrous cycle duration in the mare is 21 days (range 18–24), with estrus (follicular growth with sexual receptivity) lasting 4 to 7 days on average, and diestrus (active corpus luteum [CL] and nonreceptivity) lasting 14 to 15 days.[4-6]

Estrus. Estrus is defined as the behavior of sexual receptivity to the stallion but also refers to the phase in the estrous cycle when the mare is approaching ovulation and is sexually receptive.[21,22,25] Estrus in the mare, as stated previously, is typically around 7 days, lasting longer at the beginning of the breeding season and growing shorter toward the end.[4,5] During early estrus, follicles are recruited to grow under increasing FSH and LH secretion, and progesterone is low (<1 ng/mL).[1,4,5] As the cohort of follicles grow (3–5 mm/day) they secrete estradiol, which has a positive feedback on the anterior pituitary, inducing an increase in LH secretion and thus follicular maturation.[1,4,22] Inhibin, produced from the growing dominant follicles, inhibits FSH secretion, and one or two dominant follicles continue to develop (>30 mm) under stimulation from LH alone. This single follicle or occasionally two or more dominant follicles continue to grow, and when preovulatory estradiol levels are reached, an increased LH (an

TABLE **19.1 Summary of Reproductive Hormones***

Name of Hormone	Biochemical Classification	Source	Target Tissue	Main Action
GnRH	Neuropeptide (decapeptide)	Hypothalamic surge and tonic centers	Anterior lobe of the pituitary (gonadotroph cells)	Release of FSH and LH from the anterior pituitary
FSH	Glycoprotein	Anterior lobe of the pituitary (gonadotroph cells)	Ovary (granulosa cells)	Stimulates follicular development
LH	Glycoprotein	Anterior lobe of the pituitary (gonadotroph cells)	Ovary (cells of theca interna and luteal cells)	Induction of ovulation, follicular maturation Luteal tissue support
Prolactin	Protein	Anterior lobe of the pituitary (lactotroph cells)	Mammary cells	Induces maternal behavior, induction of lactation
Oxytocin	Neuropeptide (octapeptide)	Synthesized in the hypothalamus, stored and released from the posterior pituitary Evidence of production in the endometrium	Myometrium and endometrium, myoepithelial cells in the mammary gland	Induces uterine contractions and milk ejection, induces prostaglandin release and promotes maternal behavior
E_2	Steroid	Granulosa cells of the follicle, placenta	Hypothalamus, entire reproductive tract	Facilitates receptive sexual behavior, regulates GnRH secretion, enhances secretory activity of the reproductive tract, and enhances uterine motility
P4	Steroid	Corpus luteum and placenta	Uterine endometrium, myometrium, mammary gland, and hypothalamus	Inhibits sexual receptivity, alters endometrial gland secretion, promotes maintenance of pregnancy, and inhibits GnRH release
Testosterone	Steroid	Cells of the theca interna of the follicle	Brain, skeletal muscle, and granulosa cells	Substrate for E_2 synthesis, abnormal masculinization
Inhibin	Glycoprotein	Granulosa cells of the follicle	Gonadotrophs of the anterior pituitary	Inhibits FSH secretion
Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)	Prostaglandin (C-20 chain fatty acid)	Endometrium and follicle	Corpus luteum, uterine myometrium, and ovulatory follicles	Luteolysis, promotes uterine contraction, role in ovulation induction
Melatonin	Neuropeptide	Pineal gland	Hypothalamus	Inhibits GnRH secretion

E_2, Estradiol; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *P4,* progesterone; *$PGF_{2\alpha}$,* prostaglandin $F_{2\alpha}$. Adapted from Blanchard TL, Varner DD, Schumacher J, et al. *Manual of Equine Reproduction*. 2nd ed. St. Louis, MO: Mosby; 2003, and information from other sources.

LH surge) is induced, causing ovulation.[24,26,27] The process of ovulation is thought to take approximately 2 to 7 minutes and results in the oocyte being released from the follicle through the ovulation fossa. Ovulation generally occurs 1 to 2 days after the initial rise in LH, with LH concentrations reaching peak levels usually 1 day after ovulation has occurred.[1,4,26] Remarkable tissue remodeling occurs so that the oocyte can be released, and it is suggested that this process is mediated by prostaglandins (PGE_2 and $PGF_{2\alpha}$) and tissue-remodeling enzymes (MMP-1 and MMP-2).[1,23,28]

During estrus, the reproductive tract prepares for mating. There is increased lubrication of the tract as uterine and cervical glands increase mucus and glandular secretion. Ultrasonography of the ovaries reveals multiple follicles (>20 mm) that grow 3 to 5 mm/day, often with one or two dominant preovulatory follicles reaching preovulatory sizes of 35 to 50 mm.[4,5] Immediately before ovulation the follicular wall may thicken, the follicle softens, and often the mare is sensitive to palpation in the periovulatory window.[4,5,25] Endometrial edema is maximal 3 to 4 days before ovulation and then decreases approximately 1 to 2 days before ovulation.[3,26] The mare's uterus becomes more edematous and "doughy" on transrectal palpation, with the cervix relaxing significantly as ovulation approaches.[1,5,26] During estrus, scant hypoechoic intraluminal fluid can often be visualized on transrectal ultrasound in the normal mare, but it is widely believed that greater than 2 cm of fluid—or echogenic fluid at any time—is associated with decreased pregnancy rates and possible pathogy.[30,31] The cervix relaxes as ovulation nears, becoming edematous, pliable, and well lubricated. These changes serve as a tool for the clinician to evaluate the mare's hormonal environment and thus accurately time breeding (see Fig. 19.4). The behavioral signs observed during estrus include elevation of the tail in the presence of the stallion; eversion of the vulvar lips to expose the clitoris or "winking"; squatting with rotation of the pelvis, urination, and leaning toward the stallion; turning the head back toward the stallion; and moving toward the stallion.[32] Monitoring sexual

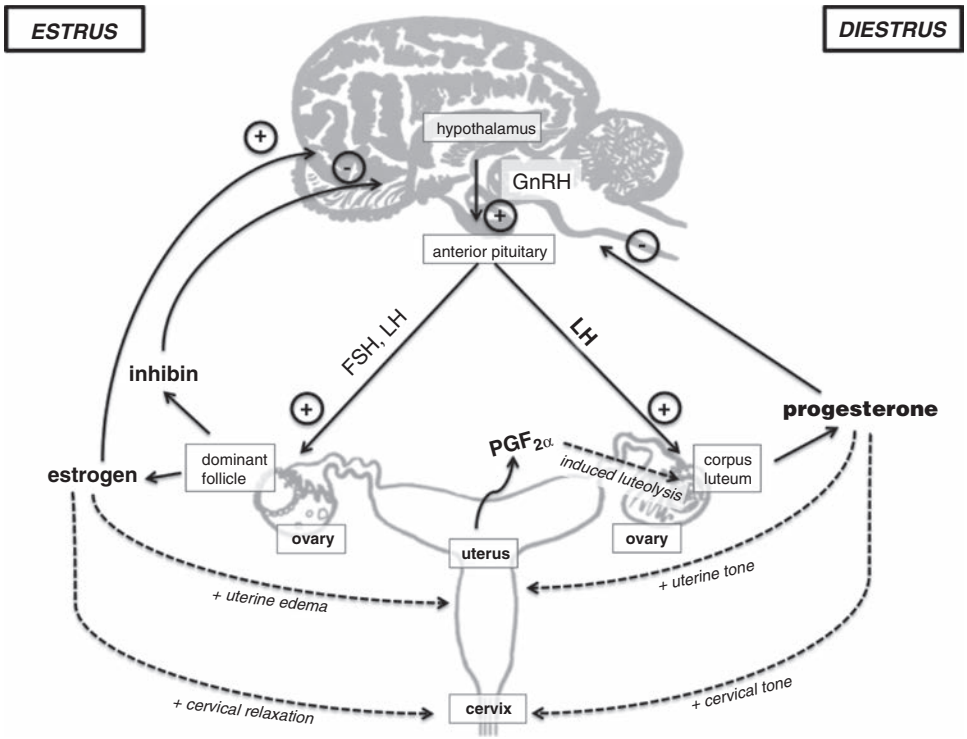


FIG. **19.10** Illustration of major reproductive hormones, their site of production, and the main targets in the nonpregnant mare. Illustration courtesy of Stephanie Gayer.

behavior of the mare is an essential part of good broodmare management because it helps to identify the current stage of the cycle and to determine the optimal time to breed or when a mare is returning to estrus.

Diestrus. Diestrus is characterized by the elevation in circulating progesterone from the CL that rises shortly after ovulation.[1,4] Progesterone levels in the blood rise from <1 ng/mL during estrus to peak levels (8–16 ng/mL) at approximately 6 days post ovulation, and progesterone levels above 4 ng/mL persist until luteolysis occurs.[4,5] Clinically this is useful if one is unsure of the mare's current stage of the estrous cycle, because a serum progesterone can determine whether there is active luteal tissue present (>1 ng/mL). FSH levels rise and fall during diestrus, inducing follicular development and estradiol secretion, but because of the inhibitory action of progesterone on LH secretion, these diestrus follicles rarely ovulate.[33] Luteolysis occurs when episodic bursts of $PGF_{2\alpha}$ from the mare's endometrium are released at 13 to 16 days post ovulation in the absence of maternal recognition of pregnancy. Luteolysis is rapid, and progesterone concentrations plummet to <1 ng/mL within 2 days of initial prostaglandin release.[4]

During diestrus, the reproductive tract functions to support an early pregnancy. The uterus becomes toned, and uterine contractions change to facilitate establishment of pregnancy.[34,35] The cervix elongates and closes, protecting the uterine environment from caudal reproductive tract contamination.[4,5] The signature feature of ovarian morphology in diestrus is the presence of an active CL, and it is important to note that follicles can be any size during diestrus (from 10–50 mm in normal mares). Because of the inside-out structure of the ovary and the restriction of ovulation to the ovulation fossa, the CL remains almost entirely within the ovary, making identification by transrectal palpation difficult. The advent of transrectal ultrasound imaging has improved diagnostic capabilities by enabling practitioners to visualize the corpora lutea, and with increasing resolution, often aged inactive corpora lutea can be visualized as well. During mid to late diestrus there is normally no uterine edema or intraluminal fluid present. The mare is categorically nonreceptive to the stallion and may kick out, strike, and demonstrate avoidance to precopulatory interactions of a stallion.[32]

Autumnal Transition. During the "autumnal transition" the mare progresses from regular interovulatory intervals to ceasing ovulation and finally into anestrus. Often changes associated with this transition begin to occur shortly after the summer solstice in the Northern Hemisphere. The beginning of this period is marked as following the last ovulation of the season.[36] The transition is characterized by irregular estrous intervals, failure of ovulation, and a prolonged or shortened luteal period. Photoperiod and environmental stressors (nutrition, temperature, and stress) are all believed to have some role in the decrease in gonadotropins during this period.[4,36] During this approximately 60-day window, GnRH, FSH, and LH production and secretion decrease, and consequently, systemic estradiol and progesterone concentrations eventually wane to basal levels.[4,36]

Reproductive tract examination at this time often reveals multiple large anovulatory follicles in early transition to minimal ovarian activity late in transition, with occasionally the last CL still visible with transrectal ultrasound. The uterus under the decreasing estradiol and progesterone becomes increasingly flaccid with minimal to no edema. The cervix often remains toned and closed because of the dwindling progesterone from the last ovulation. Mares often demonstrate erratic estrus duration and frequency, similar to the vernal transition, again making their behavior a source of frustration for owners.

Postpartum Period

Immediately after expulsion of the fetus, the equine uterus begins the rapid process of involution. The mare's placentation (diffuse and epitheliochorial) requires less endometrial repair following parturition compared with other domestic species (bovine, caprine, and ovine), enabling a mare to conceive and carry another pregnancy again shortly after foaling.[1,4,5] Histologic return of the endometrium to the pregravid size is reported to occur by 10 to 14 days, and the uterine size is reported to return to the pregravid state over a period of 23 to 32 days.[4,37,38] "Foal heat" is defined as the first period of sexual receptivity and ability to conceive after parturition and usually begins 7 to 9 days after foaling.[4] The first ovulation post foaling occurs on average at 9 days post parturition.[4] Depending on the efficiency of uterine involution, the fertility of this foal heat varies. There are many different opinions on the fertility of "foal heat" breeding, and it is likely that breed and management may have an influence. If significant fluid (>3) cm is present within the uterus at foal heat, then breeding the mare is not recommended.[38] In addition, studies regarding the effect of season on first postpartum ovulation suggest that mares that foal in January and February tend to have longer intervals from foaling to the first fertile ovulation than mares that foal in late spring. After the foal heat most mares have a second 30-day heat that occurs approximately 14 days after the end of the foal heat and may represent a more reasonable time to breed. It has been suggested based on several studies that increased pregnancy rates and decreased embryonic loss occurs when mares are bred at least 10 to 20 days postpartum. Some authors suggest an even longer period is needed following parturition to return to prepartum per cycle pregnancy rates.[38-40]

During the postpartum window in hospitalized patients, it is important to monitor daily rectal temperatures, uterine fluid accumulation, and the attitude of the mare, because significant endometritis can develop into metritis in mares not given the opportunity to ambulate and expel lochia. Although fluid within the uterine lumen by ultrasound is not abnormal, lavage and ecbolic agents may be helpful to assist with involution. In healthy mares the uterine fluid (lochia) and debris usually dissipate 7 to 10 days after parturition.[4,30,31]

Lactational Anestrus

Lactational anestrus, or inhibition of follicular development because of nursing, is not as well defined in the mare as it is in other species.[6] Because of the prevalence of foal heat and normal cyclicity in the majority of mares nursing foals, most believe it does not exist at all but is a consequence of stress from the puerperal period and perhaps increased metabolic demands. Limited studies have been performed in which foals were removed from mares at varying time points after parturition. One study indicated that removal of the foal hastens the onset of estrus.[41] Foal removal has been associated with elevated LH concentrations and increased ovarian follicle diameter in some studies and conflicting results in others.[40,41]

Mare Breeding Soundness Examination

Maria R. Schnobrich

This section will describe the reproductive evaluation of the mare (also known as a breeding soundness examination [BSE]), the procedures involved, and the common pathologies identified. A BSE of the mare may be performed for a variety of reasons including prepurchase or prebreeding examination to confirm normal reproductive tract findings, the evaluation of subfertile mares, or identification and treatment of reproductive tract pathology. Procedures that should be performed in all BSEs include proper identification of the individual, review of signalment and pertinent medical and reproductive history, general physical examination, perineal examination, transrectal palpation and ultrasonography of the reproductive tract, vaginoscopy, and digital cervical and vaginal examination. Additional procedures that may be considered depending on the purpose of the BSE and preliminary findings include uterine culture and cytology, uterine biopsy, and hysteroscopy. Next is a stepwise description of the procedures performed when conducting a thorough BSE.

TABLE **19.2 Details of Information to Be Gathered During History Taking**

History	Comments
General health	Vaccination, deworming, foot care, dental care, nutritional history, systemic diseases, and management
Athletic	Level of activity, age at which athletic career ended, any known drug usage
Cycling	Length of estrus, expression of estrus, interovulatory interval
Breeding	Number of cycles bred, breeds per cycle, natural or artificial insemination, fertility of stallion, age at which the mare was last bred
Pregnancies	Number of diagnosed pregnancies and outcomes
Foaling	Any history of dystocia, type of management
Prior reproductive examinations	Diagnoses, treatment, management
Prior reproductive surgeries	Nature of surgery, date performed, and outcome
Drug use during breeding season	Ovulation induction, estrous cycle manipulation

IDENTIFICATION

Detailed identification of the mare is of utmost importance for legal and ethical reasons. The recording of acquired brands, tattoos, and detection of a microchip should all be considered to supplement the physical description or photographs. In the worst-case scenario, improper identification can lead to erroneous conclusions and recommendations being made or to procedures being performed on the incorrect mare, which could compromise a pregnancy.

SIGNALMENT AND HISTORY

History and signalment may suggest possible problems and guide the diagnostic approach (Table 19.2). The administration of certain medications (anabolic steroids, progestogens, and GnRH vaccine) during an athletic career can affect the horse's future fertility, and the possibility of previous administration should be investigated. The use of anabolic steroids can cause masculine behavior and changes to the external

genitalia. There might also be a downregulating effect on the HPG axis, causing reproductive senescence that has to reverse over time. The use of an anti-GnRH vaccine (Equity; Pfizer Animal Health, West Ryde, Australia) is used as a reportedly effective way to inhibit estrus in fillies. Unfortunately, a number of fillies have had prolonged ovarian inactivity in their breeding career caused by this vaccination.[42-44] Several cases have been described of cytogenetically normal mares that have prolonged (four seasons or more) ovarian inactivity after the use of the GnRH vaccine. The age when the vaccine was administered may also have an effect because peripubertal fillies receiving the vaccine appear to have more prolonged periods of ovarian inactivity.

Breeding history should include detailed information from the immediate past, including information on the standard of current or previous breeding management and facilities. It is also valuable, although more difficult, to obtain historical information on the mare's entire breeding career. Particular attention should be paid to the specific details of the time and cause of any pregnancy loss; any dystocia and follow-up reproductive function; or known trauma, hemorrhage, or pathology associated with breeding, pregnancy, or foaling, because these may highlight particular regions that should be evaluated more critically during examination. Often a detailed breeding history of an infertile mare will help direct the evaluation and diagnostics used, because a mare that has never had an early pregnancy diagnosed has a different differential list compared with a mare with repeated early pregnancy loss.

In addition to breeding history, the mare's medical history and current management should be evaluated to identify areas (nutrition, hoof care, pain management, deworming and vaccination) that may need to be addressed to optimize the chances of conceiving and carrying a pregnancy to term. Any previous or current medical issue should be evaluated to determine whether it is still having any adverse effects on the mare's systemic health, and appropriate management and treatment should be initiated to optimize the overall health of the animal.

BEHAVIOR

The season of the year should be considered when assessing reproductive behavior in the mare, because transitional anestrous mares and mares in estrus can demonstrate similar behavior despite being at different reproductive phases.[45] The behavior expected for each phase of the reproductive cycle is described in the previous section. Assessing the mare's response to a stallion while in estrus is important and can help time appropriate breeding and determine whether additional steps must be taken to safely achieve live cover. If a foal is present with the mare, then estrus may not be demonstrated as readily to the stallion until the foal is taken away. In addition, shy or aggressive behavior toward the teaser by the mare while in true physiologic estrus might require additional training and management in a live-cover breeding situation. Overly aggressive, stallion-like behavior when presented with a teaser may also help confirm the presence of a hormone imbalance or reproductive pathology.

GENERAL PHYSICAL EXAMINATION

General observation of the mare while loose and being led to the stocks will allow assessment of behavior and preliminary assessment of comfort, condition, and conformation and may highlight musculoskeletal issues. A brief general physical examination should be performed to ensure that the mare has no detectable condition that could interfere with the ability to conceive or carry a foal to term. The practitioner should inspect the mammary glands visually and with palpation for evidence of abnormalities such as spurious lactation, mastitis, fibrosis, and neoplasia. Excessive body condition and abnormal fat deposits (cresty neck) or hair coat may suggest endocrine disorders such as metabolic syndrome or pituitary pars intermedia dysfunction (PPID), which may have negative effects on the mare's fertility.[45-48] Poor body condition has been associated with decreased fertility, and an underlying cause should be identified and addressed. The mare's hair coat should also be evaluated, as a poor hair coat may suggest systemic health concerns.

RESTRAINT

The mare must be adequately restrained before further examination. What constitutes adequate restraint will depend on the mare's disposition, the skill and experience of the handler and veterinarian, the procedures being performed, and the facilities available. Particular care must be exercised when performing a BSE on mares with little prior exposure to transrectal palpation or vaginal speculum placement. Stocks are generally recommended for a BSE, although adequate restraint (chemical and/or twitch or lip chain) with the mare backed out of a stall door will suffice for practitioners comfortable working under these conditions. If hysteroscopy is to be performed, then it is recommended that the mare be sedated and restrained in stocks. The stocks minimize the mare's movement during the examination and protect the veterinarian and equipment.

Chemical restraint facilitates a safe and efficient examination for certain BSE procedures that are less well tolerated or more prolonged. Chemical restraint may cause some degree of relaxation of the perineum, and therefore it is recommended to assess perineal conformation prior to sedation. In addition α_2-agonists cause increased uterine tone. The dose of sedation is based on the behavior and excitability of the mare. In general detomidine HCl (0.01–0.02 mg/kg) and butorphanol tartrate (0.01–0.02 mg/kg) administered intravenously (IV) often provide adequate sedation for most procedures. Xylazine (0.2–1.0 mg/kg) or detomidine HCl (0.006–0.01 mg/kg) is administered again if sedation is not adequate or if the procedure length dictates additional redosing.

EXAMINATION OF EXTERNAL GENITALIA

The perineal region is inspected for indications of vulvar discharge and gross abnormalities of the anogenital region. Vulvar discharge or urine scalding of the inner thighs may suggest infection, urine pooling, or urinary incontinence. Fluid and dried secretions in the tail may give evidence of vulvar discharge as well. Clitoral enlargement or abnormal anogenital distance may indicate prior exposure to androgens or progestogens or an intersex condition (Fig. 19.11). Normal vulvar lips are vertical, have good tone, and are in close apposition.

Vulvar integrity is important in forming an effective seal against contamination of the genital tract with air, urine, feces, and potential pathogens.[13,49] Vulvar conformation is assessed by digital palpation adjacent to the vulvar lips to locate the

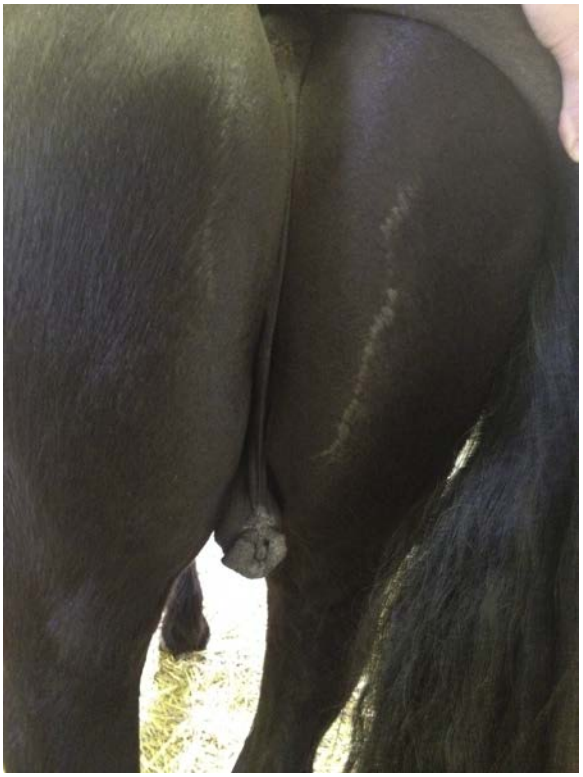


FIG. **19.11** Abnormal anogenital distance in a Friesian male pseudohermaphrodite.

position of the caudal bony pelvic brim (tuber ischii) relative to the dorsal commissure of the vulva. Pascoe's description of Caslick index has been modified to produce a simple assessment technique for vulvar conformation.[49] Generally the dorsal commissure should be level to less than 4 cm above the brim of the pelvis with well-apposed labia and minimal cranial tilt of the vulva, and the anus should be in the same vertical plane as the vulvar lips. Sunken anus, thin vulvar lips, poor labial apposition, and cranial tilt to the vulva all predispose to fecal contamination and have been associated with decreased pregnancy rates.[13,49] To determine the competency of the vulvar seal and the vestibulovaginal fold, transrectal ultrasound can be performed, and the vagina and vestibule can be evaluated for air contamination. Traditionally this evaluation has been performed by gently parting the vulvar lips to determine whether air is readily aspirated into the vagina.[13] If there is poor labial apposition or aspiration of air, then the mare may be prone to pneumovagina and at increased risk of ascending vaginitis and subsequently endometritis.[13]

- Vulvar conformation (VC) good: No Caslick is required. Dorsal commissure of the vulva is less than 4 cm above the bony pelvic brim, and vulvar lips are within 10 degrees of vertical and form an effective seal.
- VC marginal: Caslick may be required. Dorsal commissure of the vulva is 4 to 7 cm above the bony pelvic brim, vulvar lips are within 10 to 20 degrees of vertical, and vulvar lips still form an effective seal.
- VC poor: Caslick or some other corrective procedure is definitely required. Dorsal commissure of the vulva is 5 to 9 cm or more above the bony pelvic brim, vulvar lips are 30 degrees or more in front of vertical, and the vulvar seal is not effective.
- It should be noted that in certain breeds (Thoroughbreds) the Caslick's is recommended in almost every mare, as even those mares that appear to have normal vulvar conformation are predisposed to contamination, and ascending placentitis is a more common occurrence.

TRANSRECTAL PALPATION AND ULTRASOUND EXAMINATION OF THE REPRODUCTIVE TRACT

It is essential that the nonpregnant status of the mare is confirmed before any procedure is performed that could compromise a pregnancy. Transrectal palpation of the genital tract is generally considered safe and appropriate but does carry some risk of rectal mucosal trauma and potentially laceration. The risk increases if excessive force is used, if palpation continues in the presence of pneumorectum, and if the mare strains excessively. Mild sedation to minimize anxiety and reactivity may be accomplished with an α_2-agonist as described previously, with or without an opioid (e.g., butorphanol). Administration of *N*-butylscopolamine (0.3 mg/kg) by IV injection several minutes before the examination may also help prevent straining and relax the rectum, but it may interfere with accurate assessment of uterine tone.[50] As noted previously, α_2-agonists increase uterine tone, and *N*-butylscopolamine will decrease uterine tone.

Transrectal palpation of the reproductive tract should proceed with an organized, methodical approach. For example, one author, after removing all fecal material, palpates the left ovary, left uterine horn, right uterine horn, right ovary, right uterine horn, uterine body, and finally the cervix. It is important to note the ovarian position, follicular activity, size, texture, and presence of the ovulation fossa. The loss of a palpable ovulation fossa can be an early indicator of distortion of the normal ovarian shape secondary to neoplasia or other pathology.[51,52] The position of the uterus relative to the brim of the pelvis; the size, tone, sacculations, or differences in texture of the horns; and the presence and character of endometrial folds should be assessed. The general tone of the uterus should be evaluated and compared with the stage of the cycle; for example, one would expect a toned uterus in diestrus, and conflicting findings may suggest pathology. The cervical size (approximate length and width), tone, and consistency are assessed by pressing the cervix down against the pelvic floor. Cervical relaxation can most effectively be measured on a sliding scale that spans from the closed toned cervix, 0% relaxed (i.e., tightly closed, tubular cervix) to 100% relaxed (i.e., indistinguishable from adjacent uterus), and should be critically evaluated if appropriate tone is present for the stage of estrous cycle.

It is important to purposefully assess anatomic structures other than the reproductive tract as part of the palpation examination, including the pelvic inlet, caudal aorta, broad ligaments, bladder, intestines, and lymph nodes. Note the presence of soft tissue or bony changes that may narrow the diameter of the pelvic inlet, suggest pathology (aortic aneurysm, neoplasia), or potentially interfere with foaling. For example, firm swellings in the broad ligaments may suggest previous hemorrhage, neoplasia, or an abscess. Structures that may narrow the pelvic inlet and cause a potential problem for foaling include abscesses, lacerations, hematomas, neoplasia, vascular aneurysms, bladder stones, and pelvic fracture.

TABLE **19.3 Application of Transrectal Palpation and Ultrasound of the Genital Tract for Assessing Various Parameters**

Organ	Structure	Detail of Assessment	Technique
Ovary	Ovary	Size, shape, presence of ovulation fossa	P, US
		Echotexture of contents	US
		Consistency of tissue	P
	Corpus hemorrhagicum	Presence	P, US
	Corpus luteum	Presence or absence, size, shape, echotexture	US
	Follicles	Size	P, US
		Shape, wall thickness and echotexture, echotexture of contents	US
		Turgidity	P
Uterus		Diameter, shape, tone, wall thickness, endometrial folds, luminal fluid (larger amounts)	P, US
		Wall echotexture, endometrial folds, luminal fluid volume and echotexture	US
Cervix		Length, width, depth	P, US
		Echotexture	US
		Relaxation	P
Vagina and vestibule		Fluid accumulation, gas (air) accumulation	P, US

P, Transrectal palpation; *US,* ultrasound.

Transrectal ultrasound examination should be considered a routine part of a reproductive tract examination. It allows the use of electronic calipers for measuring structures and facilitates the assessment of tissue and fluid characteristics as well as subtle pathology that may not be detected by palpation. Table 19.3 presents comparative information on the application of palpation and ultrasound for assessing various genital tract structures.

The basic approach as described previously for rectal examination is used for transrectal ultrasound examination. Most practitioners use a 5- to 10-MHz linear rectal probe, and focal distance is set at a depth of 5 to 10 cm for initial evaluation. While holding a linear transducer, the practitioner extends the fingers beyond the end of the probe to assist in locating genital tract structures and prevent the probe from penetrating the rectal wall. The uterine body is initially identified as a landmark by sweeping the probe from side to side in the anterior pelvis. The uterine body is then followed cranially to the bifurcation, and the probe is swept out toward one uterine horn and ovary. Structures are examined in the same order as for palpation alone: left ovary, left horn, right horn, right ovary, uterine body, cervix, bladder, and finally vagina and vestibule. The orientation of the probe is adjusted to attempt to get a clear cross-sectional image of the uterine horns, and in the longitudinal plane the uterine body, cervix, and vagina are visualized by moving side to side while moving the transducer caudally (Figs. 19.12 and 19.13).

A simple recording system should be used with defined abbreviations. This ensures that records can be understood at a later date and facilitates an efficient communication between multiple attending veterinarians. Ovarian size, follicles, and the presence of any corpora lutea should be recorded along with any suspected cysts or abnormal structures. Uterine horn diameter should be measured about one third of the distance from the bifurcation, and the presence of endometrial edema and depth and character of any intraluminal fluid (in the dorsoventral plane) should be recorded. Uterine and cervical tone as assessed by palpation should be recorded. All follicles larger than 15 mm in diameter are recorded individually, and smaller follicles are recorded as multiple small follicles or may be measured and recorded in some cases. The tone of the follicles present should also be recorded, because often there is a loss of turgidity in preovulatory follicles.

DIAGNOSIS OF OVARIAN PATHOLOGY

Abnormal ovarian enlargement should be suspected if an ovary is greater than 10 cm in diameter and the increased size cannot be attributed to recent ovulation or a follicle and persists for more than a month.[51-53] Large ovaries may be associated with physiologic causes (e.g., persistent follicles, hematoma, multiple corpora lutea in midgestation) or pathologic processes (e.g., neoplasia, hematoma, abscess).

Neoplasia more commonly affects one ovary, although it can affect both, and typically results in an alteration in ovarian size, shape, and consistency. There appears to be no consistent pattern in the change affecting a neoplastic ovary with the exception of teratomas in which the diagnosis may be based on the detection of germ tissues, including cartilage, hair, and bone. Other neoplasias may present as single large cystic or multicystic structures or even solid tissue, and in rare cases no ultrasonographic evidence of ovarian abnormality is present. In many cases the diagnosis of ovarian neoplasia is based on persistent unilateral ovarian enlargement with obliteration of the ovulation fossa and an inactive or normally functioning contralateral ovary.[54,55] There might be a history of aberrant ovarian activity before the development of the tumor.[54] A granulosa cell tumor (GCT), the most common ovarian neoplasia, may produce varying levels of testosterone, estrogen, inhibin, and anti-müllerian hormone (AMH) but rarely produce progesterone.[56-58]

Functional ovarian tumors, such as the granulosa-theca cell tumor (GTCT) or granulosa cell tumor (GCT), may manifest as masculinization of appearance. Increased plasma

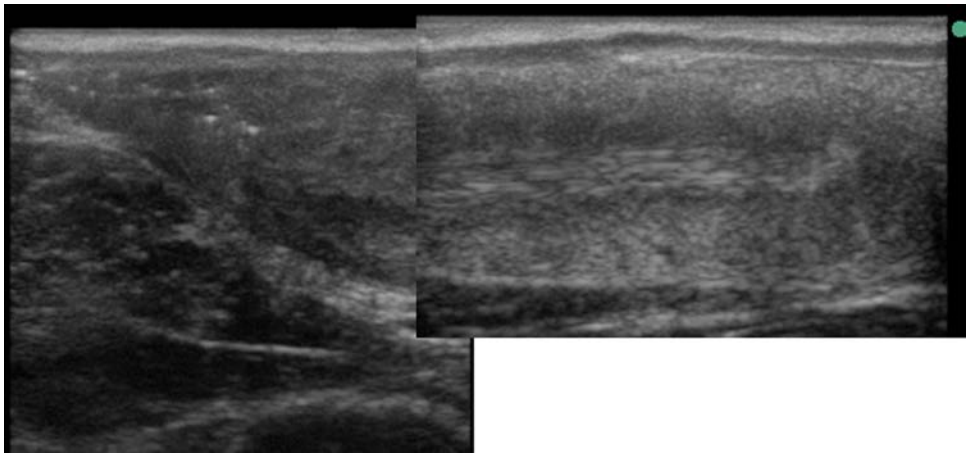


FIG. **19.12** Longitudinal image of the cervix with air in the vagina. Orientation in this image is cranial to the right and caudal to the left. This image was made by combining two images taken as the probe was positioned caudally.

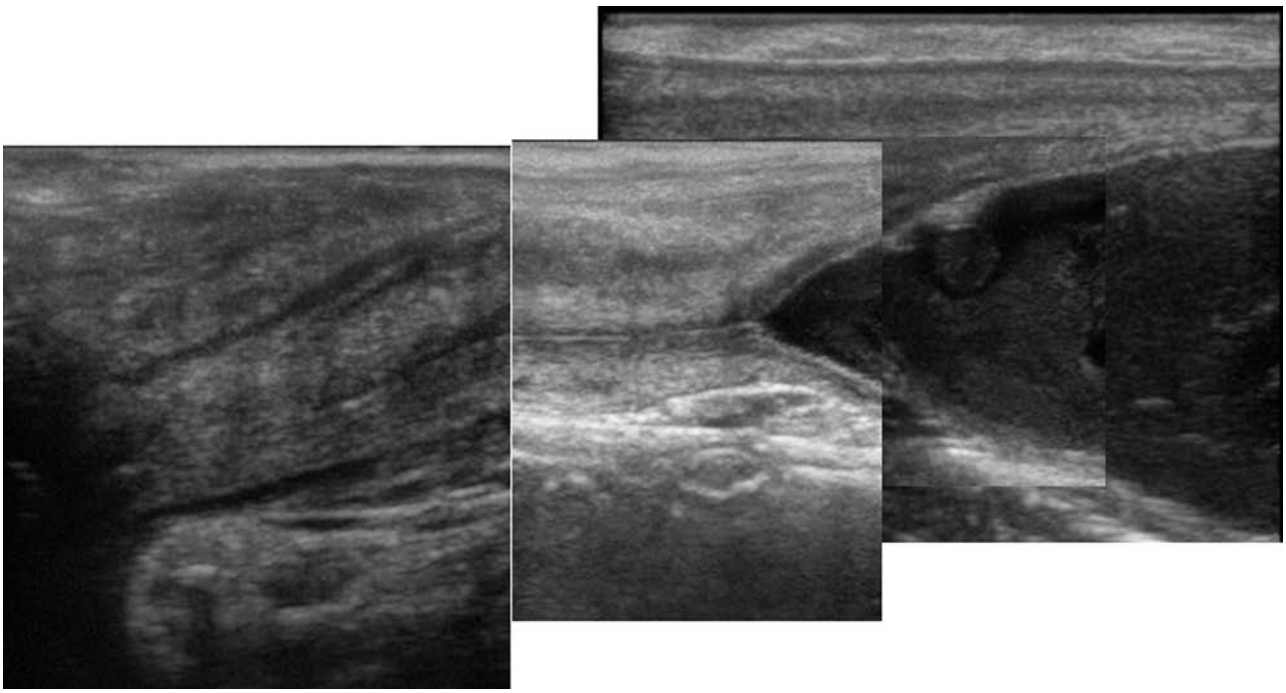


FIG. **19.13** Longitudinal image of the bladder, pelvic urethra, and opening of the ureter in the dorsal bladder wall. This image was made by combining serial images of the tract obtained with a standard linear rectal probe.

concentration of AMH, inhibin, or testosterone in conjunction with clinical signs (abnormal ovarian parenchyma or abnormal behavior) is considered diagnostic of GTCT.[57,59] The ultrasonographic appearance of most GTCT is characteristic, varying from uniformly dense multiloculated to cystic (Fig. 19.14), although it should be noted that some GTCT tumors present with no detectable abnormalities on transrectal palpation or ultrasound and are confirmed at the time of histologic tissue analysis with the only presenting complaint being abnormal behavior.[59,60]

Anovulatory follicles often grow to an unusually large size (50 mm or greater) and fill with blood, organizing into a structure with the ultrasonographic appearance of a hematoma that gradually recedes over time. Some of these structures appear to develop a thick rim of tissue resembling luteal tissue, with the mare entering a diestrus state, with progesterone concentrations greater than 4 ng/mL. Other mares do not develop a thick rim of tissue, and the structure may appear to be inert. There is a lack of consensus regarding terminology, with some authors distinguishing anovulatory hemorrhagic follicles (AHFs; no luteinization) from luteinized unruptured follicles (development of luteal rim). Others suggest that the two structures are variations of the same condition.[61,62] The condition appears to be more common in mares approaching winter anestrus and in pregnant mares under the influence of equine chorionic gonadotropin (eCG), and there is some

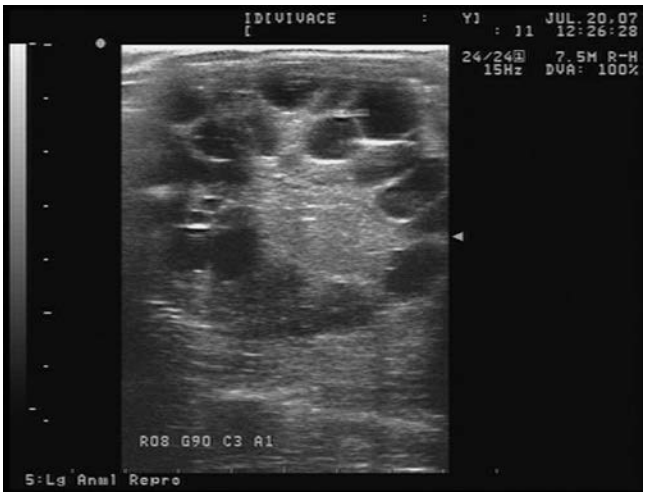


FIG. **19.14** Transrectal image of a granulosa-theca cell tumor with multiloculated "honeycomb" appearance.

evidence to suggest an increased incidence in mares administered a luteolytic agent.[61] Anovulatory follicles occur in 8% of all estrous cycles[63] but in 5% of estrous cycles in the early ovulatory season and 20% of estrous cycles in the late ovulatory season.[63] The ultrasonographic appearances of ovulatory and anovulatory follicles are largely the same. Rarely is the ovulation process normal in these structures, so mares that develop these processes may have a history of infertility and irregular estrous cycles.

Failure of the normal process of luteolysis may result in prolonged luteal maintenance. Diagnosis depends on confirmation of nonpregnant status and the presence of a CL for a period longer than the normal diestrus length (approximately 14 days)[64]. This condition, termed *pseudopregnancy,* must be differentiated from cases in which a mare is ovulating between examinations and exhibiting an apparently persistent CL. Luteolysis depends on $PGF_{2\alpha}$ release from the uterus,[65-67] which relies on the timely (approximately 12-14 days after ovulation) mechanism for endometrial $PGF_{2\alpha}$ release to be functional. An ovulation during diestrus will prolong diestrus, and the resultant CL can last for more than 2 months.[65,67] Other reasons for failure of luteolysis include the release of $PGF_{2\alpha}$ when the CL is still immature and unable to optimally respond to $PGF_{2\alpha}$ (traditionally thought to be 5 days post ovulation), the failure of $PGF_{2\alpha}$ to be released at the proper time, and low-level chronic release $PGF_{2\alpha}$ from the endometrium (which has been described in mares with endometritis).[68]

Small cystic structures may be palpated or visualized with transrectal ultrasound adjacent to the ovary. These structures often have an ovoid or serpentine shape, contain anechoic fluid, are outside the ovarian parenchyma, and are usually less than 2 cm in diameter. The structures (paraovarian cyst or epoöphoron) are typically incidental findings assumed to be cystic remnants of the mesonephric tubules and ducts.[1] It is possible that these structures could interfere with gamete transport if they obstruct normal function of the ovarian bursa and infundibulum but are almost always functionally insignificant.

Very small and inactive ovaries may be secondary to seasonal anestrus, reproductive senescence in old mares, pituitary gland dysfunction, severe malnutrition, administration of anabolic steroids, administration of anti-GnRH vaccine, or congenital infertility resulting from sex chromosome abnormalities.[45,69-71] Sex chromosome abnormalities are often associated with hypoplasia or segmental aplasia of the tubular tract. Karyotyping may be used to further investigate such mares. Chromosomal abnormalities have also been reported from subfertile mares that have foaled previously and have no detectable abnormalities of the genital tract. In light of this fact, karyotyping may be considered in mares in which routine diagnostic procedures fail to explain subfertility.[72-77]

Individuals with 63,X0 karyotype tend to be small in stature and phenotypically female, with a small uterus and ovaries.[71,74,78] These mares demonstrate irregular estrus or ambivalence in the presence of a stallion, and the uterus palpably and histologically can be within normal limits.[71,74] Plasma estrogen levels are low, and plasma LH levels are normal to high.[71] Sex reversal is a disorder that occurs in horses and most commonly results in an infertile animal.[72,76,77] The two most common forms of sex reversals are a female phenotype with male karyotype 64,XY, and a male phenotype with female karyotype 64,XX. There can be a wide phenotype variation with both conditions.[72,79]

The X trisomy is relatively rare in the horse. The XXX mare is infertile, often demonstrates erratic estrus, has normal external genitalia, and is phenotypically female with a small, thin-walled uterus and small hypoplastic ovaries. Some of these mares have been known to develop follicles and undergo what appears to be ovulation.[79-82]

Mosaics and chimeras occur when the individual has more than one cell line with different chromosomal makeups. 63,X/64,XX mosaics are phenotypically female; the uterus varies in development, but ovaries may be small and nonfunctional. These horses may show estrus and have an enlarged clitoris. Endometrial biopsy often reveals glandular hypoplasia.[70,72,73]

DIAGNOSIS OF UTERINE PATHOLOGY

Endometritis

Endometritis refers to infectious or noninfectious inflammation of the endometrium, which is a major cause of infertility in the mare. It is critical to differentiate this from metritis, in which inflammation extends to the myometrium and can cause life-threatening sequelae such as sepsis or laminitis. While endometritis compromises a mare's fertility, the mare with endometritis is generally not systemically compromised from the condition and can live a normal life if untreated. A later section in this chapter discusses diagnosis and treatment of endometritis in detail because of its importance in successful mare breeding management. This section of the text will review diagnosis of endometritis for the purpose of the BSE.

Transrectal ultrasonography is very useful for evaluating presence, quantity, and echotexture of uterine luminal fluid. Increased echogenicity of uterine fluid is positively correlated with the amount of debris or white blood cell infiltration into the fluid and is more common in older mares.[83,84] Table 19.4 presents a grading system for recording uterine fluid echotexture.[85] The presence of grade I, II, or III fluid during any stage of the cycle likely indicates endometritis. The presence of fluid in diestrus has been associated with a lower pregnancy rate and a higher embryo loss rate,[86] presumably caused by high levels of $PGF_2\alpha$ in the fluid from the neutrophils.[87] The

TABLE **19.4 Classification System for Recording Uterine Fluid Echotexture**

Grade	Ultrasound Appearance	Gross Appearance
I	White (hyperechoic)	Thick and creamy
II	Light gray	Milky
III	Black with white specks	Obvious sediment in fluid
IV	Black (anechoic)	Clear fluid

TABLE **19.5 Grading System for Recording Volume of Intrauterine Fluid**

Classification	Maximum Fluid Depth (mm)	Gross Description
VS	1–2	Barely detectable
S	1–5	Often focal
M	5–20	Obvious fluid
L	>20	Immediately apparent

L, Large; *M,* moderate; *S,* small; *VS,* very small.

presence of more than 2 cm of intraluminal fluid in the dorsoventral plane during estrus, the presence of fluid during diestrus, and fluid present 72 hours after insemination are strong indicators of mares' increased susceptibility to endometritis.[31] Table 19.5 presents a grading system for recording the volume of intrauterine fluid.

Complete absence of detectable intraluminal fluid during estrus is associated with the absence of cytologic inflammation in most cases.[31,88] The significance of anechoic (grade IV) luminal fluid is less clear. Small or very small volumes of clear fluid during estrus are likely to be normal, particularly if present during early estrus before complete cervical relaxation and maximal uterine drainage. Large volumes of clear fluid during estrus, fluid that persists into late estrus, or smaller volumes during early diestrus suggest an increased predisposition to endometritis and reduced pregnancy rates compared with mares without fluid.[88-90] Luminal fluid visible more than 12 to 20 hours after breeding indicates mating-induced endometritis.[31,90,91] This condition has been reported in 10% to 15% of mares being bred by live cover on a commercial Thoroughbred stud farm.[91] A more thorough discussion of endometritis and the significance and character of intraluminal fluid is included later in this chapter.

Discrete hyperechoic reflections present in the uterine lumen on ultrasonographic examination may indicate air, urine crystals, mucoid debris, and foreign material. Pneumouterus may be seen in normal mares soon after uterine culture, intrauterine treatment, artificial insemination (AI), or natural breeding but should not be seen more than 24 hours after breeding or treatment. The presence of pneumouterus at times other than after breeding or uterine therapy indicates loss of integrity of physical barriers to contamination (vulva, vestibulovaginal sphincter, and cervix), and the cause of this should be identified.

Endometrial Cysts

Circumscribed, discrete collections of fluid within the uterine lumen may indicate endometrial cysts, intraluminal fluid accumulation, or a conceptus. Endometrial cysts are usually classified as lymphatic or glandular. Lymphatic cysts are generally larger, anechoic, intraluminal cysts visualized by transrectal ultrasonography, whereas the glandular cysts remain small and surrounded by fibrocytes and are not easily identified with transrectal ultrasound. Cysts may protrude into the uterine lumen or remain intramural. Cysts may be single, discrete structures closely resembling a conceptus or complex, compartmentalized structures with irregular borders. Cysts that protrude into or occlude the uterine lumen may reduce fertility by interfering with embryo mobility and maternal recognition of pregnancy.[92,93] Cysts within the endometrium may interfere with gland function, and an increased number of cysts is likely an indication of a compromised endometrium.[94]

Properties of an early conceptus that can be used to distinguish it from an endometrial cyst include movement through the uterine lumen between days 10 and 16 post ovulation; spherical, symmetric appearance with specular reflection visible on ultrasound; rate of growth (cysts are assumed to grow little if at all); and appearance of the embryo proper after about day 20, or presence of heartbeat after day 25. In addition, there are palpable changes in the tract that are consistent with early pregnancy (e.g., increased uterine tone and closed, elongated cervix persisting after day 14 post ovulation), and general failure to return to estrus that would not be expected in the nonpregnant mare. The association between cysts and reduced fertility is debated, and the incidence of endometrial cysts increases with age and parity.[94,95] Large cysts or large numbers of cysts may interfere with uterine clearance, embryonic movement, maternal recognition of pregnancy, and early placentation. Extensive glandular cystic change may also adversely affect uterine gland function and compromise establishment or normal development of pregnancy. To determine whether a cyst is intraluminal and assess its location better, the uterus can be distended with sterile saline or lactated Ringer's solution (LRS), and the outline of the cyst relative to the lumen can be evaluated with transrectal ultrasound. Hysteroscopy is also helpful for identifying the degree of interference a cyst has with the uterine lumen (Fig. 19.15). Biopsy of cysts and removal by laser or manual rupture have been described. To minimize inflammation and prevent recurrence, cyst removal with a diode laser has been reported to be very effective and safe and to have improved the fertility of previously barren mares.[95]

Pyometra

Pyometra is a chronic condition with accumulation of mucoid or mucopurulent material within the uterine lumen with or without cervical occlusion. The condition may occur in the presence or absence of a CL and usually does not result in systemic signs of illness. Pyometra may be mistaken for pregnancy on palpation as a result of fluid distention of the uterus but can be diagnosed effectively with transrectal ultrasound.[96] Treatment of pyometra usually involves dilation of the cervix to allow drainage and uterine lavage with or without antibiotic use.

Adhesions

Adhesions and scarring of the endometrium may result from infusion of irritating solutions into the uterus, cesarean section, and trauma associated with foaling or dystocia. Occasionally, redundant tissue from failure of normal uterine development or the absence of one uterine horn can be seen.[97] Adhesions involving the cervix have similar initiating causes and can impair cervical function. The role of chronic endometritis or

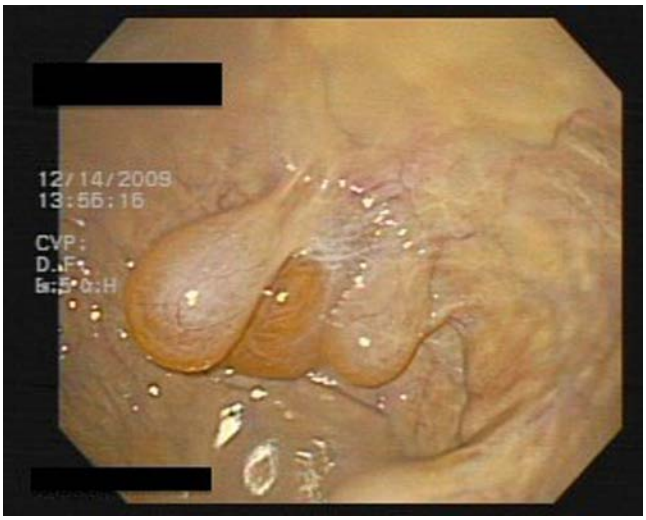


FIG. **19.15** Hysteroscopic image of a cluster of intraluminal lymphatic cysts in the uterine horn.

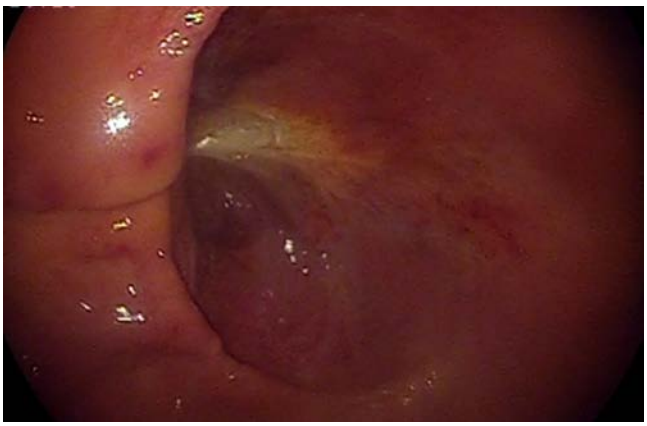


FIG. **19.16** Luminal adhesions diagnosed on hysteroscopic evaluation of a mare with chronic endometritis.

iatrogenic causes such as mechanical curettage in uterine or cervical adhesions is less clear. Adhesions may reduce fertility by interfering with function of the uterotubal junction, uterine clearance, embryo movement, maternal recognition of pregnancy, and normal placentation.[98,99]

Occlusive adhesions may result in the accumulation of fluid in the proximal portions of the tubular tract, and this may be detected on palpation or ultrasound examination of the tract. More commonly, adhesions are detected during hysteroscopy (Fig. 19.16). Occasionally these adhesions can cause a pyometra that can progress to systemic illness if infection is established without drainage.

Focal uterine lesions may respond well to surgical removal using a biopsy instrument or laser surgical equipment guided by hysteroscopy.[98] If the lesions are extensive or affecting the deeper layers of the endometrium, then the prognosis may be poor and treatment not warranted. Cervical adhesions are commonly managed by manual disruption and then repeated application of topical ointment containing an antimicrobial and steroid in an attempt to prevent recurrence. In many horses, cervical adhesions tend to recur once treatment stops.

Ventral Uterine Sacculation

Ventral sacculation is associated with a loss of normal muscle contractility in the uterine wall, with the uterine sacculations occurring most often at the ventral base of one or both uterine horns. The condition is more common in older, multiparous mares and may result in an increased risk of endometritis due to loss of the normal contractile mechanisms responsible for expelling uterine luminal fluid. This may also be linked to mares that have a more pendulous uterus. When the uterocervical angle is more ventral than horizontal, the mare is more prone to fluid accumulation because of a reduced mechanical ability to expel fluid.[99]

Vaginal and Cervical Examination

Good hygienic practice is essential in performing any vaginal procedure, particularly those that involve penetration of the cervix, to avoid inoculating the uterus. Cleanliness is perhaps the simplest and most fundamental principle of good reproductive practice. The practitioner should wrap the proximal portion of the tail at a minimum, or enclose the tail in a plastic sleeve and tie it to the mare's neck with a quick-release knot. Alternatively, an assistant may hold it out of the way. The perineal region should then be washed with water and mild detergent or povidone–iodine scrub and rinsed thoroughly. The practitioner should then wipe just inside the vulvar lips with damp cotton and clean smegma from the clitoral fossa and sinuses. This is particularly important for mares with sloping vulvar lips or if a rectal examination has just been performed. The presence of fecal matter in the vestibule without a history of any invasive procedure indicates a failure of the vulvovestibular seal.

Use of sterile, plastic sleeves for vaginal procedures is ideal. A practical alternative used by one author is to keep in a dust-free area a zipper storage bag (e.g., Ziploc) containing clean plastic sleeves to be used for vaginal procedures only. A new sleeve is taken out carefully by grasping it only at the open end, or each sleeve is turned inside out immediately before putting it on to minimize the risk of contamination. Tearing off the fingers from a plastic sleeve and putting on a sterile, surgical glove can increase sensitivity and also facilitate cleanliness. Sterile lubricant and equipment should be used. The practitioner should be meticulous to prevent contamination of either equipment or sleeves and gloves during any procedure.

Vaginal Speculum Examination

A sterile, lubricated speculum is inserted into the vestibule in a slightly dorsal direction to avoid the urethral opening and pushed through the vulvovestibular sphincter, with sphincter integrity assessed as the speculum is inserted. Because they reduce the risk of iatrogenic infection, single-use, sterile, disposable specula are preferred over metal Caslick specula for routine examinations. In some cases, a self-retaining metal Caslick speculum may provide better visualization of the vagina and vestibule.

Vaginoscopy is useful for the detection of vaginal hyperemia, fecal contamination, suppurative exudate, persistent hymen, urine pooling, varicose veins, vaginal trauma, rectovaginal defects, and cervical defects. Although examination with a vaginal speculum may be performed at any stage of the cycle, evaluation of mares suspected of urovagina is best performed during estrus, because estrogenic relaxation of the genital tract and perineal region is maximal at this time.[100] Vaginal speculum examination is an important component of

the BSE and often highlights abnormalities not identified by transrectal palpation and ultrasonography.

The timing of the vaginal examination in relation to the rectal examination is also important. If the rectal examination is performed first, then fecal contamination of the perineum and vulva is often increased. This slight disadvantage is offset by the advantage of doing the rectal ultrasound examination first to assess pneumovagina and pneumouterus. The presence of air in the vagina and uterus is difficult to interpret after a vaginal examination. However, if air is present before a vaginal examination, then it may indicate dysfunction of the vulvovestibular fold. Repeated vaginoscopy may be associated with an increased risk of iatrogenic vaginitis and persistent infection. As a result, one author prefers to perform routine assessment of cervical relaxation by transrectal palpation.

The vestibule and vestibular mucosa are evaluated by parting the vulvar lips and by continuing to look in the speculum as it is withdrawn following vaginoscopy. Varicosities of the vestibulovaginal fold are best recognized and evaluated in this manner.

Manual Examination of the Vagina and Cervix

Examination of the genital tract is incomplete without manual exploration of the vagina and cervix. The major benefit in this procedure is the detection of cervical defects. Once the hand is inserted into the anterior vagina, the forefinger is placed into the cervical lumen and the thumb in the vaginal fornix. The hand is then rotated and the entire circumference of the cervix is palpated between finger and thumb to feel for disruption in the cervical musculature. Abnormalities in the musculature or damage to submucosal layers might interfere with the ability of the cervix to close effectively. The cervical canal should be evaluated for any defects, tortuosity, or diverticula in which debris can accumulate. Cervical abnormalities (adhesions, muscular defects, and diverticula) are considered significant if they prevent the cervix from relaxing during estrus to facilitate uterine clearance, disrupt the ability to form an effective seal during diestrus and pregnancy, or serve as a nidus of infection. The examiner should also consider the length and diameter of the cervical canal in relation to the estrous stage of the mare. A partial tear involving the external os of the cervix may not require treatment if the cervix can still maintain an effective seal during diestrus. For this reason, assessment of the severity of cervical disruption should be performed when the mare is in diestrus. Alternatively, the diestrus tone can be replicated by the administration of progesterone for a few days to close the cervix.

Care should be taken while performing a manual examination of the vagina and cervix to take time to palpate the vaginal surface for pathology such as rectovaginal fistulas, vaginal lacerations and scar tissue, varicosities, urine crystals, perivaginal hematoma or abscesses, and any abnormal mass or structure that may suggest pathology. In cases in which the mare is suspected of urovagina, the integrity of the urethral sphincter should also be assessed.

Endometrial Culture and Cytology

Endometrial culture and cytology are performed as diagnostic tools for identification of endometritis, assessment of its severity, and determination of etiology. A variety of instruments and techniques have been described for sampling the mare's endometrium for cytologic analysis, microbial culture, and DNA isolation, and there is much debate regarding the methodology and interpretation of these diagnostics. At this time the gold standard based on sensitivity and specificity of correctly identifying a bacterial or fungal infection is believed to be culture of a biopsy sample obtained with a guarded technique and histologic evaluation of the sample. This is not always practical, so at a minimum a culture (double-guarded swab or guarded small-volume lavage) and cytology (double-guarded swab, swab cap, small-volume lavage, or guarded brush technique) should be performed.[101-104] Table 19.6 summarizes the sensitivity and specificity of each method for diagnosing endometritis. Generally, it is believed that a negative aerobic culture with no inflammation on cytology is reflective of no or subclinical endometritis; a negative culture with inflammation on cytology (>2 neutrophils per 400–1000× field) is a false-negative and the offending agent has likely not been identified; and a positive culture with inflammatory cytology is considered positive for endometritis.[105,106] There is debate regarding what constitutes inflammation as well as what is truly a uterine pathogen, but the reader is referred to the section on endometritis later in this chapter for a more in-depth discussion of this topic.

TABLE **19.6 Clinicopathologic Techniques Available for Diagnosing Endometritis in Broodmares**

Methodology	Test	Relative Sensitivities (Number of Disease-Positive Animals That Test Positive)	Relative Specificities (Number of Disease-Negative Animals That Test Negative)
Kalayjian swab	Culture	Low	High
	Cytology	Moderate	High
Double-guarded swab	Culture	Low	High
	Cytology	Not performed	Not performed
Low-volume lavage	Culture	Moderate	High
	Cytology	High	Moderate
Biopsy	Culture	Moderate	High
	Cytology	Moderate	High
Cytology brush	Culture	Low	High
	Cytology	High	High

Adapted from LeBlanc MM, Causey RC. Clinical and subclinical endometritis: both threats to fertility. *Reprod Domest Anim*. 2009;44:10; Nielsen JM. Endometritis in the mare: a diagnostic study comparing cultures from swab and biopsy. *Theriogenology*. 2005;64:510; and Overbeck W, Witte TS, Heuwieser W. Comparison of three diagnostic methods to identify subclinical endometritis in mares. *Theriogenology*. 2011;75:1311.

If endometritis persists despite appropriate treatment, cultures are often obtained from multiple sites in the genital tract, including endometrium, cervix, vagina, clitoral fossa, and clitoral sinuses. For the majority of mares a double-guarded endometrial swab or fluid sample is often the only sample submitted for aerobic culture, although anaerobic and fungal cultures may be useful in some cases. There is an increasing body of evidence to suggest the presence of commensal bacterial in the mare's vaginal and caudal reproductive tract, as well as possibly dormant bacteria in the endometrium, which may confuse the results of culture.[107-109] Endometrial culture should be performed before any other invasive procedure in a BSE to reduce the risk of inadvertent contamination of the uterine lumen before culture samples are obtained.

A guarded culture rod or small-volume lavage set should be used to minimize the risk of contamination with material from sites other than the uterine lumen. There is a lack of consensus on the optimal time of the cycle for obtaining endometrial culture and cytology samples. Some experts recommend performing endometrial cultures on the first or second day of estrus, when uterine secretions are increasing and the flushing action of the uterus is just beginning.[110-112] Mares with endometritis have been reported to accumulate free luminal fluid in late diestrus, and this represents an alternative time for endometrial culture, although it can result in contamination.[86] Often culture and cytology samples are obtained regardless of where the mare is in her cycle, because there is a limited period available for evaluation. If the mare's endometrium is cultured, biopsied, or the cervical barrier breached in diestrus, then it is recommended to induce luteolysis ($PGF_{2\alpha}$ administration) after the evaluation to help the mare resolve any contamination or iatrogenic inflammation.

To perform a double-guarded swab culture of the endometrium, the practitioner should carry the guarded culture rod into the vagina and through the cervix which can be accomplished through a speculum or manually. The capped rod is then passed through the cervix and the culture tip pushed through the guard and rolled against the endometrial surface for 30 to 60 seconds. The culture tip is withdrawn into the rod, and the outer sheath is rotated three times to collect endometrial cells in the cap for cytologic analysis. The guard and swab are removed gently, avoiding contamination of the cap with vaginal or vestibular secretions. The rod is then extracted without contacting the sheath and placed into a transport culture medium (Amies medium is an example). The cap's contents are tapped onto a glass slide for cytologic evaluation.[106]

The sample should ideally be transferred onto the final growing medium (e.g., trypticase soy agar (TSA), 5% sheep blood and eosin methylene blue (EMB), and a thioglycolate broth) as quickly as possible after the sample is obtained to allow quantification of colony numbers as an indication of severity of infection. When possible, quantification of colony growth should be performed, because heavy growth after 24 to 48 hours' incubation is more meaningful than a few scattered colonies. If immediate plating is not possible, then transfer the culture tip in a sterile manner into appropriate transport medium. Use of transport medium allows proliferation of some species and possibly reduced growth of others, so colony counts are not interpretable.

A wide range of organisms have been isolated from horses with acute endometritis. A combination of positive culture and evidence of inflammation on endometrial cytology is sufficient to diagnose endometritis and identify major pathogens. A positive bacterial culture in the absence of any cytologic evidence of inflammation or clinical signs is considered likely to be caused by contamination during the culture procedure and does not indicate endometritis, although *Escherichia coli* has been an agent associated with minimal inflammation and should considered if heavy growth is identified.[105,106]

In the absence of cytologic evidence, it is particularly difficult to interpret culture results. Recovery of relatively pure or heavy cultures of the following organisms may be considered indicative of endometritis: β-hemolytic streptococci, *E. coli, Pseudomonas* spp., *Klebsiella* spp., and *Candida* spp.[113-116] Recovery of other organisms without concurrent cytologic information should be viewed with more suspicion.

Endometrial cytology is used by some practitioners as a rapid screening test for detection of endometrial inflammation in mares at the beginning of the breeding season or before they are bred. When it was first described in the mare, the detection of neutrophils was correlated with positive bacteriologic findings. When no or low numbers of neutrophils were detected, bacteriologic findings were more often negative.[117] Mares with cytologic evidence of inflammation had lower 28-day pregnancy rates than mares with normal cytologic results, irrespective of culture results. Day 28 pregnancy rates were also lower in mares that had bacteria isolated from their uterus, even if cytologic results were normal.[118]

Numerous techniques have been described for collecting cytologic samples.[117-119] The approach used by the author is simple, rapid, and feasible in busy clinical practice. As previously described, a guarded culture rod (Kalayjian Industries Inc., Long Beach, CA) can be used to obtain both endometrial culture and cytology samples. The swab tip is withdrawn back into the sheath and the rod rolled against the endometrium to collect fluid and cells in the cap. After the rod is withdrawn from the mare, the cap can be cut off and tapped against a slide to make the smear from the small drop of fluid. The smear is dried and stained using any commercial Romanowsky-type staining kit (e.g., Diff-Quik; American Scientific Products, McGaw Park, IL). If no endometrial cells are seen on an initial inspection of the slide, then the sample may not have been collected from the uterus, and another sample should be taken. The presence of polymorphonucleocytes (PMNs) is generally believed to indicate infectious endometritis, but the most appropriate methodology for quantifying PMNs remains subject to debate.[120- 122] Suggestions for criteria to diagnose endometrial inflammation include observation of more than two PMNs in five high-power microscope fields (400×–1000×) or more than one PMN per 10 endometrial cells in more than one area of the slide.[121,122] In most mares with clinical endometritis, there are large numbers of PMNs evident on cytologic evaluation, and occasionally intracellular phagocytized bacteria may be seen.

Endometrial Biopsy

Preparation for endometrial biopsy is the same as for culture or cytology. There is debate as to the best time in the cycle to obtain an endometrial biopsy. Diestral samples have been recommended over estral samples because physiologic changes in the endometrium during estrus make the slides more difficult to interpret.[119,122,123] However, samples may be taken at any stage of the cycle as long as the information on the stage of cycle is provided and the person reading the slide has experience in evaluating equine endometrial biopsies. In the absence of clinically detectable pathology involving the



FIG. **19.17** Rigid endometrial biopsy rod with alligator forceps.

uterus, a single endometrial biopsy sample is representative of the entire endometrium.[124,125] However, another study found that even in clinically normal mares endometrial biopsy scores varied with the site selected; thus it may be prudent to collect more than one sample.[124,126-128]

The sterile biopsy instrument is used to recover a sample containing at least 2.5 cm of luminal epithelium. The most commonly used instrument is a rigid alligator forceps (Fig. 19.17). The hand covers the basket as the instrument is guided (with jaws closed) through the cervix and into the uterine lumen. The tip of the biopsy forceps is guided manually through the cervix. Once the instrument's tip has been passed into the uterine lumen, the guiding hand is withdrawn and inserted into the rectum to guide the closed tip to the site desired for sampling (most commonly the base of a uterine horn). The instrument is then turned on its side and the jaws gently opened. A portion of endometrium is pressed between the jaws using the index finger within the rectum, and the jaws are closed and the instrument retracted in a quick tug to remove the tissue sample.

A simpler vaginal technique is useful for collecting biopsy samples when there is no need to sample from a specific site. The instrument is introduced into the uterine lumen, and the hand is left in the vagina with the index finger in the cervical lumen. The tip of the instrument is advanced about 2 to 3 cm into the uterine lumen with the jaws closed. The jaws are opened and the instrument advanced an additional 1 to 2 cm. The instrument can then be deviated slightly to one side and the jaws closed to collect a sample. The sample is taken from the cranial uterine body close to the bifurcation. This approach has the advantage of being quick and simple. It allows the operator to return for a second sample immediately if the first sample is too small, whereas the rectovaginal technique often results in gross contamination of the instrument and vulva during the procedure, making a second sample impossible unless the mare is cleaned again and a second sterile instrument is available.

The specimen is removed from the biopsy basket using a small-gauge needle and transferred to fixative solution, preferably Bouin fixative or 10% buffered formalin. Samples placed into Bouin fixative should be transferred to 10% formalin or 80% ethanol after 12 to 24 hours for optimal retention of cell detail and tissue integrity.[119,123]

There is a strong case to be made for clinicians to read slides from their own cases or to involve a reproductive specialist familiar with biopsy interpretation. Although expert assessment by a trained pathologist may better reflect histologic tissue changes, it is vital to interpret histologic change in light of clinical and historical information about the mare. Training and practice will allow the clinician to detect most of the common pathologic changes seen in biopsy samples. An endometrial biopsy report should include the histologic evaluation and an epicrisis, which provides a biopsy grade (I, IIA, IIB, or III); prognosis for carrying a foal to term and treatment/management recommendations based on the histologic character of the endometrium; and the results of reproductive examination, endometrial culture and cytology results, and patient history.

The method of biopsy evaluation differs but should be a repeatable sequence that allows one to determine the pathology present in the endometrium. The clinician must first ensure that cellular characteristics are consistent with the stage of estrous cycle. Pathologic changes include inflammation, fibrosis, cystic glandular distention, and lymphatic stasis.[10,119,122,123,127] Generally, inflammation is evaluated by characterizing the cell type frequency, distribution, and character. The uterine luminal contents, the epithelium, the superficial endometrium (stratum compactum), and the deeper endometrium (stratus spongiosum) are all described based on the cellular infiltrate present (neutrophils, eosinophils, lymphocytes, plasma cells, and hemosiderophages), the frequency (mild, moderate, or severe), and for the endometrium, the distribution (diffuse, multifocal, perivascular, or periglandular). Acute inflammation is associated with PMNs and an increased likelihood of positive endometrial culture.[119] Increasing numbers of PMNs and their presence in deeper layers of the lamina propria indicate more severe inflammation. Chronic inflammatory change includes lymphocytes, plasma cells, eosinophils, and mast cells. Eosinophils have been associated with urometra, pneumouterus or pneumovagina, and yeast. Hemosiderophages are often associated with parturition or abortion.[119,122-126,128]

Endometrial fibrosis is believed to be a permanent degenerative change seen as increased fibrocytes surrounding the endometrial glands; when this is severe it causes the clustering of glands (fibrotic nests) or gland branches. Fibrosis can also be seen adjacent to the basement membrane of the luminal epithelium. Fibrosis often forms in concentric layers around gland branches or glands, and the number of layers of fibrosis is related to the severity of the degenerative change.[127,128] Fibrosis may be localized or diffuse, and the more widespread or severe the change, the more adverse the effect on gland function and fertility. These degenerative changes appear to be closely associated with age rather than parity.[10,127,129-132] Severe degenerative endometrial fibrosis can occur in the older maiden mare that has not had the challenges of pregnancy and exposure to semen.[127,131,132]

Cystic glandular distention may be seen in normal mares during anestrus. When diagnosed in mares during the breeding season, it is considered a pathologic change and is associated with reduced fertility. Glandular fibrosis may also be associated with cystic distention of the affected glands. Extensive fibrosis interferes with uterine gland function and may result in early embryonic death.[10,128]

Persistent endometrial atrophy is associated with an extremely poor breeding prognosis.[10,123,126,128] Large empty spaces on the biopsy slide must be interpreted with caution, because they may be artifacts associated with sample processing. If the spaces appear to be lined with an endothelial cell layer, then it indicates lymphatic stasis. Widespread lymphatic stasis may be associated with reduced contractile capability within the uterus, a doughy feel to the uterus on transrectal palpation, and reduced fertility.[119]

Assessment of severity and distribution of pathologic changes allows the sample to be classified into one of four diagnostic and prognostic levels based on a modified version of the original three-level system proposed by Kenney.[10,119,128]

Category I: No significant changes in the endometrium and no treatment required; predicted foaling rate following conception is 80% to 90%.

Category IIA: Slight to moderate inflammatory change. Scattered fibrotic change may consist of either fibrosis around individual gland branches or fewer than two fibrotic nests per high-power field. There is moderate cystic glandular distention or extensive lymphatic stasis. If two or more of these changes are present in the same sample, then the sample is classified as IIB. Expected foaling rate following conception ranges from 50% to 80%.

Category IIB: Inflammatory change is widespread and moderately severe. There is widespread fibrotic change or up to four fibrotic nests per high-power microscope field. Widespread, severe cystic glandular distention or lymphatic stasis is evident. If two or more of these changes are present, then the sample is classified as III. Expected foaling rates following conception are 10% to 50%.

Category III: The mare exhibits widespread severe changes that may include fibrosis or severe inflammation or both. The expected foaling rate following conception is less than 10%.

Use of paired biopsy samples taken at the initial diagnostic workup and again 4 weeks after completion of treatment appears to improve the usefulness of the biopsy as a prognostic indicator of a mare's fertility.[129] Mares that were classified as grade III pretreatment and that improved to grade II after treatment achieved a foaling rate of 40%, whereas mares that were still grade III after treatment had a 0% foaling rate. This is likely a result of improvement in reversible pathologic changes.[129,130,132] This approach allows effective use of the grade III categorization on the follow-up biopsy to justify recommending that such mares be culled from the breeding program or consider other alternatives (embryo transfer, oocyte transfer, or intracytoplasmic sperm injection [ICSI]).

The epicrisis of the biopsy report summarizes the histologic findings and interprets the significance of these changes in light of culture and cytology results, reproductive examination findings, and patient history. Ideally the epicrisis identifies the significant pathology and severity and guides appropriate treatment and future reproductive management.

Uterine Endoscopic Examination: Hysteroscopy

Hysteroscopy allows direct visual inspection of the uterine lumen through a flexible fiberoptic endoscope. It is indicated when other diagnostic procedures do not detect a cause for subfertility, a mare has persistent endometritis despite appropriate treatment, or to further examine a mare with suspected uterine pathology.[4-6]

Hysteroscopy is most commonly performed when the mare is in diestrus, or the cervix can remain closed enough to allow insufflations/dilation of the uterus for evaluation.[133-135] Sedation is usually required because the procedure is associated with mild discomfort. The perineum is aseptically prepared as for other vaginal or uterine procedures, and a sterilized endoscope is inserted in the vagina and guided digitally through the cervix and into the uterus. The cervical lumen is then occluded, either by insertion of one or two fingers into the cervix alongside the endoscope or by gently holding the external os of the cervix around the endoscope. The uterine lumen is distended with saline or water infused through a flexible catheter held adjacent to the endoscope or with air delivered through the endoscope. Endometrial folds should be assessed while the uterus is being distended, because they flatten out and become difficult to distinguish in the fully distended uterus. The endoscope is then manipulated through the uterine lumen, allowing visualization of the entire endometrial surface, including the tips of the uterine horns and the two oviductal papillae. Hysteroscopy allows direct visualization of a variety of abnormal changes, including inflammation, polyps or neoplasia, cysts, retained endometrial cups, bacterial or fungal plaques, adhesions, and severe scarring or fibrotic change. It can also aid in various procedures, including guided retrieval of foreign bodies, laser surgical management of conditions such as endometrial cysts or adhesions, biopsy of pathologic sections of endometrium, or hysteroscopic hydrotubation of the oviducts.

Excessive distention (>100 mm Hg) of the uterus may cause discomfort and an elevated heart rate and should be avoided.[133] Mares subjected to hysteroscopy appear to be at risk of developing subsequent endometritis caused by the inflammation associated with the procedure. It is often helpful to administer an antiinflammatory agent before the procedure (flunixin meglumine 1.1 mg/kg IV or dexamethasone 0.04 mg/kg IV) and perform a uterine lavage (1–5 L sterile saline or LRS) with oxytocin (10–20 IU intramuscularly [IM]) following the procedure. If hysteroscopy was performed when the mare was in diestrus, then administering a luteolytic agent is recommended to return the mare to estrus and facilitate recovery from the procedure. In addition, evaluating and culturing the mare's endometrium on the subsequent estrus is recommended to address any residual inflammation or infection associated with the procedure.

HORMONAL ASSAYS

In the nonpregnant mare, serum or plasma samples may be collected for measurement of a variety of reproductive hormone concentrations, including progesterone, estrogens, testosterone, inhibin, AMH, and gonadotropins. Other systemic conditions that may have effects on cyclicity and fertility include equine metabolic syndrome, PPID, and general systemic illness. The reader is referred to Chapter 16 for a discussion on appropriate testing for endocrine disorders.

Progesterone is produced by ovarian luteal tissue. Serum progesterone concentrations are low during estrus and begin to rise 12 to 24 hours after ovulation, peaking between days 5 and 10 after ovulation.[4,5,136,137] Progesterone assays in nonpregnant mares can be used to assist in determining the stage of the cycle and to confirm that ovulation has occurred. They are also used as an indirect method of pregnancy diagnosis, but false-positive results are common.[1,6]

Progesterone is necessary for early pregnancy maintenance, and primary luteal insufficiency has been linked to pregnancy loss.[138,139] Low levels of progesterone are associated with increased early pregnancy loss, and higher levels of progesterone are associated with double ovulations.[140-142] Older mares appear to require a higher level of progesterone to maintain pregnancy.[138,139] Progesterone levels on day 7 were significantly lower in mares with periovulatory intrauterine fluid

accumulation and significantly lower in mares that underwent embryonic loss.[88,139] The presence of luminal fluid in diestrus was associated with a lower progesterone level and increased embryonic loss, indicating endometritis as a cause for lower progesterone levels.[31,86,138,139]

Some researchers recommend that progesterone concentration in the peripheral blood of pregnant mares should be above 2.5 to 4 ng/mL for normal pregnancy maintenance; this will be discussed in more detail later.[142] Single samples of peripheral blood concentrations are difficult to interpret because repeated sampling of normal pregnant mares has shown that blood levels vary widely over short periods.[137,138] It is clear that in the mare the serum progesterone taken at a single time point must be critically compared with normal values for stage of cycle and pregnancy, because these change significantly as a part of normal physiology.

Thyroid gland dysfunction has been discussed as a cause of subfertility in mares with recommendations to measure total serum thyroxine (T4) or thyroxine response to administration of thyroid-stimulating hormone (TSH). Endogenous thyroxine is significantly affected by season and reproductive status.[16,143] Thyroxine is seasonally regulated when mares are kept on a constant energy balance out of the breeding season.[143,144] Although thyroidectomy of pony mares did not have any adverse effect on reproductive performance,[144] mares that continued to cycle out of season had higher levels of thyroxine than anestrous mares, which has led some clinicians to supplement subfertile mares with thyroxine.[16,143,145,146] Mares with low thyroxine levels were more likely to enter anestrus after parturition.[16] Research has demonstrated that the horse is rarely truly hypothyroid as is seen with other domestic species, and a study evaluating the effect of thyroxine supplementation of mares with low T4 (<16 µg/dL) found no benefit of supplementation on early pregnancy rates.[146] Several other studies have shown there was no association between thyroxine level and pregnancy rate 15 to 16 days after ovulation.[144,146]

Ovarian neoplasia, particularly GCTs, may be associated with increased production of testosterone, inhibin, and AMH by the affected ovary. Serum inhibin concentration is elevated in approximately 80% of mares with a GCT, and testosterone is elevated in about 50% to 60% of cases.[147-149] Recent work suggests that an increase in AMH, a hormone expressed in granulosa cells of preantral and small antral follicles, is elevated in mares with GCT tumors. It is believed that an elevated concentration of serum AMH is the most sensitive test for GCT at this time, with a sensitivity of 98%.[148-150] A study in 2013 demonstrated that the sensitivity of elevated blood AMH (95%) for the detection of histologically confirmed GCTs was significantly greater than that of either an elevation in inhibin (85%) or testosterone (55%) alone or the combination of inhibin and testosterone (89%).[149,150] Generally normal concentrations in nonpregnant mares for inhibin, testosterone, and AMH are less than 0.7 ng/mL, less than 45 pg/mL, and less than 4 ng/mL, respectively, but these values should be compared with the laboratory normal in which samples are being processed.[149,150]

Horses with PPID often exhibit variable degrees of hirsutism with a range of other systemic signs. The direct effect on the reproductive tract and fertility is currently a subject of much debate. Anecdotally, mares with PPID can have reproductive changes such as increased incidence of anovulatory follicles, loss of uterine tone, increased fluid accumulation, decreased cervical relaxation, and decreased resistance to bacterial or fungal endometritis. Diagnosis and treatment of this disorder are discussed in the later section Endometritis and Uterine Therapy.

The measurement of estrogen concentration in blood, urine, feces, milk, and saliva has been used for monitoring the estrous cycle, determining pregnancy status,[92-94] and monitoring fetal-placental viability. Peripheral blood estrogens may be either unconjugated or conjugated (bound to sulfates), and different forms of estrogens are found in the mare, particularly during pregnancy. Estrogen production in the pregnant mare will be reviewed more thoroughly later, but generally conjugated estrogens exist in the circulation at much higher concentrations than the unconjugated forms, and measuring either estrone sulfate or total conjugated estrogens has historically been used to assess pregnancy status and viability. Often a dramatic increase in peripheral blood estrone sulfate concentrations occurs in pregnant mares after day 60 of pregnancy, whereas in fecal assays this increase may take up to 150 days to occur.[153,156,157] A rapid decline of estrone sulfate concentration in a pregnant mare may indicate loss of fetal viability.[155-158] One of the difficulties of measuring specific estrogens is the cross-reactivity on most commercially available immunoassays and interpreting findings relative to normal changes in concentration.

Equine chorionic gonadotropin (eCG, formerly known as pregnant mare serum gonadotropin [PMSG]) is a hormone produced from the trophoblast cells derived from the chorionic girdle that invade the endometrium, forming "endometrial cups" at around day 35 of gestation.[1,4,6,158,159] This hormone has both FSH and LH effects on the ovary, inducing the formation of accessory CLs to help maintain pregnancy until the placenta develops and produces hormones that support the pregnancy in later gestation. The accessory or secondary CLs form from follicles that often luteinize without ovulating.[159] Pregnancy loss after day 35 can sometimes be associated with undetectable eCG concentrations in blood and the failure of endometrial cup formation, which is presumed to play an essential role in pregnancy maintenance. Pregnancy loss after day 35 may be associated with a positive or elevated eCG blood concentration following pregnancy loss, and affected mares often do not cycle properly because follicles fail to ovulate normally and luteinize. When ovulation does occur, the pregnancy rate is low, presumably because of a low-grade endometritis resulting from the presence of endometrial cups,[160-162] which have a life span of about 100 days and are therefore nonfunctional after about 150 days' gestation.[160] However, there have been reports of the prolonged survival of endometrial cups after abortion and even normal foaling.[161,162] The abnormal persistence of endometrial cups lasts an average of 18 months but could last up to 30 months. This has implications into the next breeding season, and mares showing aberrant ovarian activity should be investigated for the possibility of persistent endometrial cups (Fig. 19.18). Peak elevation of eCG occurs naturally between days 50 and 85 of gestation (15–125 IU/mL) and should be gone from the serum after 150 days' gestation.[162] Mares have been observed clinically to have abnormal, accessory CLs, retained endometrial cup tissue, and erratic cycles with eCG levels of 2 IU/mL, highlighting the importance of testing eCG in appropriate cases.

The measurement of serum or plasma concentrations of GnRH, gonadotropins (FSH and LH), and steroids appears to offer promise in the diagnostic assessment of the hypothalamic-pituitary-ovarian axis. The anterior pituitary gland produces FSH in response to GnRH release.[1,4,163,164] FSH is



FIG. **19.18** Hysteroscopic image of retained endometrial cups present in the base of what was the pregnant horn.

released in a bimodal pattern with peaks (35 ng/mL) in early and late diestrus. There are two major waves of follicular growth coincident with the two surges of FSH.[4,164-166] FSH starts to rise 4 to 5 days before an ovarian follicular wave. The peak of FSH is 3 days before the emergence of the wave, which then plateaus for 5 days. Sampling of circulating blood FSH would be performed in mares with inactive ovaries or erratic follicular waves to determine the cause.

GnRH release from the hypothalamus stimulates LH release from the anterior pituitary gland,[4,166] although there is an association between releases of oxytocin in estrous mares and LH release, which suggests that repeated sexual stimulation might also increase LH and advance ovulation.[167] LH concentrations are low during the midluteal phase (5 ng/mL) but rise a few days before estrus after progesterone decreases (<1 ng/mL) because of luteolysis. LH peaks (45 ng/mL) during estrus after ovulation and returns to midluteal levels over a few days.[166]

Little information is available regarding the existence of abnormalities in the hypothalamic-pituitary-ovarian axis and either their relationship with subfertility or their detection by measurement of hormone concentrations. Assessment of the production and release of either GnRH or FSH and LH presents practical difficulties, because this may require cannulation of the pituitary venous sinuses.[168] In addition, these hormones are released in a pulsatile manner, with pulse frequency and amplitude influencing function.[4,169] Finally, there are varying isoforms with differing bioactivity that may be difficult to distinguish on enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).[169,170]

Leptin is a protein synthesized by adipose (fat) tissue.[171,172] It acts as negative feedback to brain centers to control obesity (satiety centers) in times of nutritional abundance.[171-176] During starvation, reduced leptin levels cause changes in reproductive endocrinology that limit reproductive activity.[173] Short-term feed restriction (for 24 hours) significantly decreases leptin levels in mares but does not affect reproductive hormones.[176] There might be a seasonal influence on leptin levels because mares in good body condition experience a general decline in leptin concentrations during winter. The decline is not as great as that observed in mares in poor body condition, and the mares in good body condition continue to have estrous cycles through winter.[177]

Currently, the measurement of these and other hormones of the central nervous system appears to be restricted to research applications. Measuring these products may offer considerable benefits in the future, but additional research and development are required before it can be of use to field practitioners.

CONCLUSION

Following evaluation, interpretation of test results, and a review of the mare's history, a summary of the BSE findings should be made with the goal of identifying any abnormalities, providing a possible treatment plan, and discussing with the owner/client the prognosis for future reproductive health. There are conditions that may not have a treatment option (karyotype abnormalities, severe adhesions, and chronic incurable endometritis), but for all mares, regardless of the pathology, several options such as specific treatment plans, surgical correction (cervix, urethra, perineal body, and vulva), and advanced reproductive options (embryo transfer and ICSI) should be discussed. For some clients the BSE serves to ensure that an investment in the mare's prospective breeding career is not in vain, and a BSE with no abnormalities can provide useful information. It should be emphasized, based on the findings from that one examination, that a mare's prospective breeding status cannot definitively be labeled as fertile or infertile but that the mare meets or does not meet the normal parameters that one would expect for a satisfactory potential breeding animal. It is also important to recognize the legal implications of a BSE, and if the examiner is not comfortable or experienced enough to make a conclusion, then it is best to consult with an experienced practitioner or trained reproductive specialist.

Mare Reproductive Pathology

Maria R. Schnobrich

This section is a review of specific reproductive pathology in the nonpregnant mare. Some of this has been discussed as part of the findings of a BSE in the previous section, but this section will provide more details regarding pathogenesis, diagnosis, and treatment options.

VULVAR PATHOLOGY

The integrity of the vulvar lips and their anatomic relationship to the perineal area and anus are essential components of a mare's reproductive health, because they are the first barrier against uterine contamination from the external environment. The endocrine patterns associated with each stage of the estrous cycle and pregnancy can influence the disposition of the vulva, with increased estrogen relaxing vulvar tone and increasing vulvar length. Generally the vulva should have at least two thirds of its length below the pelvic brim, the slope of the vulva in relation to the vertical axis should not be greater than 10 degrees, and the vulvar lips should have an even and firm apposition. Absence (natural or acquired) of a normal perineal conformation can facilitate the entry of air (pneumovagina), feces, and potential pathogens into the reproductive tract, which jeopardizes the fertility of the mare as mentioned previously.[4,6,13]

Body Weight

Severe loss of body condition, as experienced by some pregnant mares not adequately supplemented during the winter or

during lactation, results in a sunken anus, an increased slope of the vulva, and loss of fat that maintains adequate conformation. An apparently normal perineal conformation noted in mares in good body condition may become less than adequate if loss of body weight is extreme. With weight loss the caudal barriers to the reproductive tract become less efficient and pneumovagina and contamination of the reproductive tract can occur. Contamination of the vagina with feces during mid to late gestation may lead to ascending bacterial placentitis, which is one of the leading causes of abortion and neonatal septicemia in the United States.[178]

Parity and Age

Aging of the mare associated with repeated foaling can cause stretching and loss of tone of the perineal muscles that allow the vulva to form an effective barrier. Injury to the mare during foaling aggravates this condition, resulting in loss of vulvar tone and apposition of the labia. Mares undergoing repeated episiotomies also may have permanent damage to vulvar structures.

Caslick Vulvoplasty

Surgical closure of the dorsal part of the vulvar labia is intended to correct poor perineal conformation. The Caslick procedure removes the vulvar mucosal surface at the mucocutaneous junction of the labia from the dorsal commissure to a level approximately level or below the brim of the pelvis. The vulvar opening is reduced significantly, and the opposing debrided labia are sutured together. Decreasing the length of the vulvar cleft, one decreases entry of air and potential pathogens into the vestibule and vagina in a mare otherwise susceptible to pneumovagina, fecal contamination, and associated complications. It has been reported that Caslick-operated mares with poor perineal conformation that successfully foal should be resutured immediately after parturition to minimize the chances of uterine contamination and consequent endometritis. This preventive measure is believed to contribute to adequate pregnancy rates and maintenance of pregnancy until term.[4,49,178-181]

This procedure, however, is overused in mares in which such intervention is not warranted. Paradoxically, a mare with successive Caslick surgeries may experience considerable loss of vulvar tissue, generating an abnormal perineal conformation. Another indirect complication associated with the Caslick vulvoplasty is the increased incidence of vulvar lacerations and dystocias in mares in which the vulva was not opened before foaling. Regardless of the originating cause, the Caslick operation is used to correct first-degree perineal laceration affecting only the perineal skin and vulvar mucosa. Second-degree (laceration of deeper tissues of the perineal body) and third-degree (a defect resulting in communication of the ventral rectum with the dorsal vagina) lacerations require more elaborate reconstructive surgery to correct.[180] As a rule, any corrective surgery of the perineal body and vulva should be delayed until inflammation and edema of the involved tissues have resolved. Mares with third-degree rectovestibular laceration invariably develop endometritis, but endometrial biopsy of mares affected with third-degree laceration have shown a rapid endometrial response to surgical repair of the laceration. Mares can be artificially inseminated by 2 weeks after surgery.[181]

Equine Coital Exanthema

Equine coital exanthema is a venereal disease caused by equine herpesvirus type 3 (EHV-3). Mares infected with equine coital exanthema develop pustules and ulcers in the vulvar mucosa and perineal area. Once the lesions start to heal (usually by 14 days after the onset of clinical signs), characteristic depigmented small circular areas (usually <1 cm) in the vulvar labia and perineal skin are visible.[182] Similarly to other herpesvirus-induced diseases, lifelong infection is the rule. Recrudescence may occur after stimuli such as stress, systemic disease, or trauma to the genital area. No specific treatment is recommended other than disinfection of pustules and ulcers to prevent secondary bacterial infections during the acute phase. Natural service should be avoided while active lesions are present to prevent transmission of the disease. One can artificially inseminate affected mares during the symptomatic stage of the disease or wait until 6 weeks after complete healing of equine coital exanthema–associated lesions before natural service.

Neoplasia

Melanoma is the most common disease of the vulva and perineum, affecting 80% to 100% of adult gray horses and, less frequently, aging horses of other colors. Common sites for melanoma include the anus, perineum, and vulva. No effective treatment is available for melanomas, but oral treatment with cimetidine (a histamine $_{H2}$ antagonist) has been reported with variable success, resulting in partial or complete regression of melanocytic nodules.[183] In addition, recent research has suggested autologous vaccination or other types of vaccines for melanomas may be effective.[184] Squamous cell carcinomas are less common than melanomas, and hemangiosarcomas have been reported.[185]

CLITORIS

The clitoris in the mare is an important reservoir for the bacterium *Taylorella equigenitalis,* which is the causative agent in mares affected with contagious equine metritis (CEM). The disease is highly contagious, and the organism can be harbored in the clitoral fossa and sinuses (especially the median sinuses) for prolonged periods. To test a mare for CEM, the practitioner should swab the median clitoral sinuses and then seed an Amies charcoal medium to be transported (preferably kept at 4°C) to a diagnostic laboratory.

Even in CEM-free areas, it is important to remember that the clitoral sinuses may function as nidi for uterine infection, especially infection that was iatrogenically induced during diagnostic procedures of the reproductive tract or AI. Careful asepsis of the perineal and vulvar area (including the clitoral fossa) with a mild disinfectant before any invasive procedures are performed minimizes the risk of introducing potential pathogens into the uterus. In rare cases, clitorectomy may be performed to eliminate this nidus of infection.

Congenital anomalies of the external genitalia occur in intersex animals that may have underdeveloped vulvar labia associated with an abnormally enlarged clitoris. Treating prepubertal mares or pregnant mares with anabolic steroids may lead to enlargement of the clitoris in the mare or foal exposed to androgens in utero, resulting in a partially and permanently exteriorized clitoris.

VAGINA

The vestibule is the area that separates the vulva and clitoris from the vagina proper. At the cranial border of the vestibule,

where it meets the vagina, lies the vaginovestibular fold. This folded mucous membrane acts as the second physical barrier between the uterus and the external environment. In young horses the hymen is usually a weak membranous extension of the vaginovestibular fold. Occasionally, a persistent hymen may be present in a maiden mare. A manual examination of the vagina is usually sufficient to rupture the persistent hymen. Some mares with a persistent hymen may accumulate fluid in the vagina proper and uterus (mucometra). Once the persistent hymen is disrupted, evacuation of the fluid is uneventful.

Pneumovagina

Listening for an inrush of air into the vagina when one gently parts the vulvar labia, or evidence of air in the vagina on transrectal ultrasound, can test the adequacy of the vaginovestibular fold as a physical barrier to external contaminants. A positive test (noticeable sound of air rushing in to the vagina, or air visualized in the vagina on transrectal ultrasound) indicates that the vestibular fold is not properly restricting the vagina proper from the outside environment.

Improper functioning of the first barrier (vulva) and second barrier (vaginovestibular fold) may lead to the constant or frequent entry of air into the vagina. The condition may be exacerbated during estrus, when the perineal body is more relaxed than in other stages of the estrous cycle. Accumulation of small amounts of a frothy fluid in the cranial vagina may indicate pneumovagina. Fecal staining and debris may also suggest this condition as well as the presence of air in the vagina and occasionally the uterus (pneumouterus), which is visible as hyperechoic particles seen in these areas during transrectal ultrasonographic examination.

In mares with severe alteration of the perineal conformation, vulvar closure using Caslick vulvoplasty may not correct the problem, and surgical reconstruction of the perineal body (perineoplasty) is recommended. In addition, in mares with poor body condition, weight gain may also be beneficial.

Urovagina

Urovagina, also known as *vesicovaginal reflux* or *urine pooling,* refers to the presence of urine in the cranial vagina and possibly in the uterus. As with pneumovagina, mares with marginal perineal conformation may be predisposed to accumulate urine in the vagina during estrus, when reproductive organs and the perineal body are relaxed. In older mares with splanchnoptosis, the reflux of urine into the genital tract may be permanent. Urovagina can cause vaginitis, cervicitis, endometritis, and placentitis, which ultimately result in infertility. Perineoplasty and urethral extension are common surgical procedures to correct this condition. In some cases fluid may only be appreciated in the uterus, and the fluid can be sampled and a creatinine concentration evaluated to determine whether urine is present. Generally values >2 mg/dL are considered consistent with urine contamination.

Varicose Veins

During estrus and especially during pregnancy, varicose veins may develop in older mares. Varicose veins can be present in any part of the vagina; however, they often are found in the vaginovestibular area. Bleeding may occur after natural service or spontaneously during mid to late gestation. Occasionally, persistent hemorrhage results in considerable blood loss, but the condition usually subsides with the end of pregnancy. Cautery or ligation of varicose veins is warranted if hemorrhage is persistent or frequent. There is anecdotal evidence of success with topical application of commercially available hemorrhoid creams containing phenylephrine HCl 0.25% for less severe cases.

Neoplasia

Vaginal neoplasms are not common in mares, but leiomyomas, squamous cell carcinoma, and melanomas have been reported.

Vaginitis

Minor tears, lacerations, and hematomas are common findings in the vaginas of mares after foaling. Medical treatment generally is not needed, and most mares do not show complications in the postpartum period. If more serious trauma occurs, then the mare may develop vaginitis. Mares with extensive vaginitis or a large hematoma may show signs of pain and refuse to stand quietly to nurse the foal. Vaginal abscesses can develop when a vaginal laceration becomes infected with local flora or fecal contamination.[108] Clinical signs caused by swelling from a hematoma or abscess include vulvar discharge, stranguria, and straining to defecate. Treatment with antibiotics and antiinflammatory drugs is warranted when extensive vaginitis or abscesses are found in the vagina. An abscess impinging on the urethra or interfering with defecation may require drainage. Occasionally, vaginal trauma may result in adhesions that can interfere with uterine drainage.

Rectovaginal Fistulas

During parturition the foot of the foal may be directed toward the dorsal vagina or ventral rectum and if unattended may cause a third-degree perineal laceration. However, if the dystocia is corrected in time, then the damage may be limited to a first- or second-degree rectovaginal tear or rectovaginal fistula. Surgical correction of the anatomic defect is necessary to restore fertility if the defect allows vaginal contamination to occur. Generally in the case of the complete loss of barrier between the rectum and vagina, the mare is evaluated 3 to 4 weeks after injury to allow the tissue to heal before surgical repair to prevent failure of corrective surgery.

Breeding Trauma

Vaginal lacerations may occur during natural service when the stallion's penis is disproportionately large compared with the mare's reproductive tract, or restraint of the mare prohibits appropriate coupling during mating. Depending on the location of the tear in relationship to the peritoneal reflection, a vaginal tear may communicate with the peritoneal cavity, likely resulting in peritonitis, or may be retroperitoneal. Treatment includes broad-spectrum antibiotics, antiinflammatory drugs, and tetanus prophylaxis. Peritoneal lavage may be beneficial if the tear communicates with the peritoneal cavity. A breeding roll positioned under the tail of the mare and dorsal to the penis of the stallion prevents the stallion from inserting the full length of its penis into the vagina and may help prevent mating-induced trauma. Some tears are large enough to require surgical correction, and any mare with excessive bleeding or discomfort after live cover should be evaluated.

CERVIX

The cervix is the last of the three physical barriers protecting the uterus from the external environment. Cyclic hormonal

changes dictate the tone of the cervix. During estrus the cervix is relaxed and open. High concentrations of progesterone during diestrus or pregnancy cause the cervix to be elongated, tubular, firm, and tightly closed. These changes are readily appreciated with transrectal palpation. Moreover, a vaginal examination during estrus reveals the cervix positioned low in the cranial vagina and relaxed, well lubricated, and easily dilated, allowing access to the uterus. During diestrus or pregnancy, a vaginal examination reveals a tightly closed cervical os that is pale and positioned high off the floor of the vagina.

Cervicitis

Inflammation of the cervix often accompanies vaginitis or endometritis. Cervicitis usually occurs in the postpartum period, especially after a dystocia. Severe cervicitis associated with metritis also may occur in mares infected with organisms, such as *T. equigenitalis,* that cause copious purulent discharge.[186] Infusion of certain chemicals (e.g., chlorhexidine, strong iodine solutions) into the uterus to treat endometritis may irritate not only the endometrium but also the cervix and vaginal mucosa. If the practitioner intends to use such solutions, care must be taken so that adhesions and fibrosis do not result as a sequela.

Trauma

Although cervical lacerations can occur during natural service, these lesions are usually small and resolve without major consequences. Occasionally, maiden mares are found in estrus with a tightly closed cervix that might suffer laceration during natural service, especially if the stallion is much larger than the mare. However, these tears are usually small and heal without further treatment. Most serious lacerations occur during parturition. They may result from normal parturition, or they may be iatrogenic, occurring during intervention to correct a dystocia by mutation or fetotomy.

Although one should examine the cervix digitally after a difficult foaling or dystocia (especially if a fetotomy is performed), the extent and severity of a cervical laceration are best evaluated once the cervical lesion has healed. The competency of the cervix should be evaluated during diestrus or when the mare is under the influence of exogenous progestogens so that the competency of the cervical canal can be accurately assessed. Diagnosis of cervical transluminal adhesions and anatomic defects is best performed by digital examination of the cervix rather than by vaginoscopy. Transrectal ultrasound and hysteroscopy can also be useful in detecting cervical defects. Because surgical correction is difficult and not always rewarding, the practitioner should take a biopsy sample of the uterus to assess the mare's ability to maintain a pregnancy before attempting surgical correction of a cervical laceration.

Generally any cervical defect that impairs cervical function should be addressed. In a retrospective study evaluating mares that had surgical repair of a cervical defect within 24 months, the postoperative pregnancy rate (67.24%) was greater than mares that did not undergo repair or waited longer than 24 months for repair (41.78%).[187]

Polyps or Cysts

Occasionally, pedunculated cystic structures are apparent on transrectal ultrasound, vaginoscopic, or digital examination of the cervix. These structures are often attached to the cervical os or emanate from the cervical lumen and protrude into the vagina or uterine body. Although their cause is unknown, they do appear to be associated with infertility, and removal by laser or ligation is recommended.

UTERUS

Ventral Sacculations

Aside from changes associated with pregnancy, other pathologic conditions cause focal enlargements in the ventral portion of the uterus. These uterine changes usually are associated with increased age and parity and invariably are found at the base of one or both of the uterine horns. Inexperienced veterinarians may mistake these enlargements for a pregnancy, especially if they do not use ultrasonography to confirm their palpation findings. Mechanisms contributing to formation of ventral uterine enlargements have been identified, including but not limited to endometrial atrophy, focal myometrial atonia, and lymphatic lacunae.[92] Furthermore, in older and multiparous mares the uterus may tilt ventrally in relation to the pelvic brim (uterine splanchnoptosis). Mares with ventral sacculations and uterine splanchnoptosis have a higher incidence of delayed uterine clearance than normal mares.[99]

Endometriosis

Endometriosis was once known as *chronic infiltrative endometritis* and currently refers to the presence of fibrosis in the stromal and periglandular compartments. The degree of endometriosis is associated closely with the ability of a mare to establish and maintain a healthy pregnancy until term. Parity and age contribute to degenerative changes occurring in the endometria of mares.[10,130]

Fibrotic changes may occur around the endometrial glands and in association with the basement membrane in the stratum compactum. The amount and pattern of distribution of the fibrotic tissue has been classified descriptively as slight (one to three layers of periglandular fibrosis), moderate (4 to 10 layers), and severe (more than 10 layers).[10] Cystic glandular dilation is another manifestation of endometriosis. Periglandular fibrosis, glandular epithelial hypertrophy, or inadequate lymphatic drainage may lead to dilation of the endometrial glands.

Other degenerative alterations in the endometrium that lead to endometriosis include lymphatic lacunae and angiosis.[130,188] Lymphatic lacunae are histopathologic indications of lymphangiectasia. Angiosis (a vascular pathologic condition) is associated with aging and parity, especially in uteri with ventral sacculations and associated venous congestion, which are pathogenic factors for angiosis.[188] No treatments exist for these anatomic and vascular degenerative changes in the uterus. Mares with lymphatic lacunae and disseminated uterine angiosis are at risk for infertility caused by delayed uterine clearance and persistent mating-induced endometritis.[130,188]

Pregnancy loss attributed to endometrial fibrosis more commonly manifests during the embryonic period. The secretion of histotroph by the endometrium is critical for proper embryonic development. The area of the endometrium in immediate contact with the conceptus has been shown to undergo specific changes in glandular density on days 16 through 30 of gestation; this is a period during which embryonic loss is commonly diagnosed in mares with endometrosis.[189] If pregnancy continues to progress beyond the initial 30 days of gestation, then abortion during the early fetal period may still occur if uterine fibrosis interferes with implantation of

the placenta. Microcotyledonary attachments begin to develop by 80 to 120 days' gestation.[190] Ultrastructural evaluation of the placenta in mares with chronic degenerative endometritis (CDE; endometriosis) has shown a delay in microcotyledon development and a reduction in the number of microcotyledons and villi per surface area. Endometrial atrophy may not result in abortion but can influence fetal growth. Fetal weights in mares with degenerative endometritis were lower than in normal mares.[191] Endometrial atrophy may be evident on inspection of the placenta after delivery.[192]

Endometritis

Whereas endometriosis reflects chronic structural changes associated with age and parity, endometritis encompasses endometrial changes associated with acute or chronic inflammation. The changes associated with endometritis are modulated by the action of a local immune system and influenced by the hormonal milieu. A transient endometritis normally occurs in all mares that were mated naturally or artificially inseminated. Mares mount an inflammatory reaction in response to the presence of semen in the uterus, but this apparently normal inflammatory response subsides (histologically) within 2 to 3 days.[193,194] Detection of intrauterine fluid by ultrasonography per rectum 24 hours after mating suggests delayed clearance. Persistent mating-induced endometritis is a clinical entity that has been recognized as a major cause of infertility in mares. A more detailed discussion on endometritis is found in later sections of this chapter.

Endometrial Biopsy

An endometrial biopsy, as covered in the section on BSE, is often considered a routine part of a complete BSE. Because an endometrial biopsy can aid in predicting the chances of a mare carrying a foal to term, one should consider the information provided by a biopsy before purchasing the horse or undertaking reproductive surgery such as repair of a cervical tear. Biopsies sometimes provide information that is useful in the diagnosis of infertility and may provide a basis for treatment. One must realize, however, that an endometrial biopsy alone is often not the most significant part of a BSE but must be considered in light of other information obtained from the history and reproductive examination.

Generally a biopsy specimen is taken from a site at the base of one of the uterine horns. When procuring a biopsy, the practitioner should take care not to obtain tissue from a site near the internal cervical os. Glands are less dense near the cervix, making a biopsy obtained from that area less representative of the uterus and more difficult to interpret. Moreover, accidentally taking a biopsy sample from the cervix can result in adhesions.

A single biopsy has been considered to be representative of the entire uterus, but studies have shown that variation by as much as an entire category may exist among sites.[125] Therefore one first should perform a thorough examination by palpation and ultrasonography to determine whether any areas of the uterus appear to be abnormal and also to rule out pregnancy before taking a biopsy. If one detects an abnormal area, then biopsy samples should be obtained from the abnormal and the normal areas. Repeated or multiple biopsies do not significantly affect fertility. A mare may become pregnant when bred just a few days after a biopsy specimen is taken.[195]

One must relay all pertinent history, including the estrous stage during which the biopsy sample was obtained, to the pathologist reading the sample. Periglandular fibrosis may appear worse in biopsy samples taken during anestrus because of the sparseness of glands. In addition, biopsy samples taken during anestrus or transition may have evidence of increased inflammation because the cervix has been in a relaxed state for a prolonged period as a result of the absence of progesterone.

Endometrial biopsies are classified into four categories as previously described (I, IIA, IIB, and III). A mare with a category I biopsy has an essentially normal endometrium. The likelihood of the mare becoming pregnant and carrying a foal to term, estimated at 80% to 90%, depends more on broodmare management than on the inherent fertility of the mare. Mares with a category III biopsy have severe pathologic changes in the endometrium and an estimated 10% chance of carrying a foal to term, even with good breeding management. Most mares are classified as a category IIA or IIB with an estimated 50% to 80% and 10% to 50% chance, respectively, of carrying a foal to term, reflecting a combination of management practices and the inherent fertility of the mare.

The clinician's primary concerns are the severity and distribution of inflammation and the presence of degenerative changes such as periglandular fibrosis, angiosis, and lymphatic lacunae (enlarged and dilated lymphatics). Degenerative changes carry a worse prognosis than inflammatory changes because they are considered permanent and progressive. No effective treatment for these conditions has been identified. The origin of such degenerative conditions is not known but widely presumed to be caused by repeated insults to the uterus. These conditions are more common in older mares.[130] Dilated lymphatics often indicate a uterine clearance problem. However, the practitioner can diagnose delayed clearance more reliably by ultrasonographic examination in the post-breeding period.

Although biopsy can reveal the presence of an inflammatory condition, other methods (e.g., examination of perineal conformation) are necessary to reveal the reason that the condition is present, and an endometrial culture is needed to identify the particular pathogen. A repeat biopsy after appropriate therapy may provide insight regarding the extent to which treatment was successful and aid in determining a prognosis for future fertility.[129]

Endometrial Cysts

Endometrial cysts often are cited as a cause of infertility, but a clear cause-and-effect relationship has not been established. The proportion of mares with endometrial cysts increases with age. Mares older than 11 years of age are more than four times as likely to have endometrial cysts compared to younger mares, and most mares older than 17 years of age have endometrial cysts. Reports that associate endometrial cysts with a lower pregnancy rate or increased embryonic loss often fail to account for the effect of advancing age. When one controls for confounding effects, such as parity and age, the assumption that cysts are causing infertility is not supportable. When confounding factors were accounted for in the analysis of nearly 300 mares, endometrial cysts did not have a statistically significant effect on establishing or maintaining pregnancy, although the time of initial pregnancy diagnosis was not controlled strictly.[94] Another report by a different group of researchers who did control the time of pregnancy diagnosis similarly found no difference in pregnancy loss between mares with cysts and those without, although mares with endometrial cysts tended to have a lower day-40 pregnancy rate. The effect of cysts on fertility appeared to be quantitative because

an effect was not evident until a mare had numerous cysts or the cysts were large. However, even then the effect of endometrial cysts on fertility was much less than that with delayed uterine clearance or intrauterine fluid accumulation. A quantitative effect of endometrial cysts could be caused by interference with embryonic mobility. It is well known that the equine embryo undergoes a period of mobility after entering the uterus and finally becoming fixed in place at approximately 16 or 17 days of gestation. If mobility is restricted during this period and the embryo is not permitted to come in contact with a sufficient portion of the endometrium, then maternal recognition of pregnancy may not occur, resulting in luteolysis and embryonic loss.

Rather than viewing endometrial cysts as a cause of infertility, one should consider them as an indication of underlying pathologic changes in the uterus. Large endometrial cysts are usually of lymphatic origin, and their occurrence may be associated with a disruption of lymphatic function.

Endometrial cysts are best diagnosed with ultrasonography. Cysts are identified as hypoechoic, immoveable structures, with a clear border, as opposed to intraluminal fluid, which is movable and has a less distinct shape or border. Endometrial cysts are usually multiple and most commonly found at the base of the uterine horns. Cysts may change in size and number between estrus, diestrus, and pregnancy.

Endometrial cysts can complicate a diagnosis of early pregnancy. They are often similar in size and appearance to an early conceptus. Cysts that appear spherical often are shown to have a more irregular shape if the ultrasound probe can be reoriented in relation to the cyst. To make the diagnosis of early pregnancy easier and more reliable, the practitioner should record the size and location of endometrial cysts using a diagram or by storing ultrasonographic images during a prebreeding examination. Even so, it may be necessary to repeat the pregnancy examination or delay confirmation in some mares with endometrial cysts. In most cases of endometrial cysts, no treatment is necessary other than recording their size and location for future reference during pregnancy examination. However, if the cysts are sufficient in size or number such that they pose a potential threat to embryonic migration, treatment can be aimed at facilitating the establishment of pregnancy by providing exogenous progestogen.

Progestogens, usually in the form of altrenogest (0.044 mg/kg/day, administered orally), can maintain pregnancy even when the signal for maternal recognition of pregnancy is lacking. Numerous studies have shown the ability of altrenogest to maintain pregnancy after luteolysis or in ovariectomized mares. It is important to note that if progestogen therapy is deemed necessary, then the correct dose and frequency of administration are required or the effort is wasted. For example, once-a-month administration of medroxyprogesterone is insufficient to maintain pregnancy. Therefore it would not be beneficial in mares with large or numerous endometrial cysts.

Alternatively, endometrial cysts may be removed surgically. Laser surgery is an ideal method if the equipment is available. Ligation and transection of the stalk of pedunculated cysts is an alternative. Merely puncturing and draining the cyst or incising its wall does not usually provide long-term remission.

Transluminal Adhesions

Trauma or severe infectious or chemically induced (e.g., after intrauterine infusion of irritating chemicals) endometritis may induce the formation of transluminal adhesions in the uterus. These lesions are usually asymptomatic and found during hysteroscopy. Uterine adhesions may cause the retention of endometrial secretions, resulting in mucometra or pyometra. Early embryonic motility, a phenomenon paramount for maternal recognition in horses, may be impaired by intraluminal adhesions. Treatment modalities include manual disruption of intraluminal adhesions or ablation by way of laser surgery.

Pyometra

Pyometra is an accumulation of purulent exudate in the uterus. Unlike cows, mares with pyometra do not necessarily have a persistent CL, and many may cycle normally. A cervical anatomic defect or persistent "hymen" that prevents the clearance of fluid from the uterus may predispose mares to pyometra. However, this condition also can affect mares without an apparent anatomic defect in the reproductive tract. One can diagnose pyometra readily by transrectal ultrasonography when intraluminal fluid with moderate echogenicity is visible in the uterus. Because most mares with pyometra are brought to the veterinarian's attention at an advanced stage, degenerative changes such as endometrial atrophy may preclude mares from returning to normal fertility after treatment. A biopsy sample of the uterus should be examined before treatment to determine the prognosis for potential fertility. Although medical evacuation of the uterus may be attempted, hysterectomy is an option for mares refractory to treatment or with advanced degeneration of the endometrium (severe endometriosis).

Neoplasia

Uterine neoplasms are uncommon in mares. Leiomyomas, often referred to as *fibroids,* are benign mesenchymal neoplasms derived from smooth muscle and often are associated with the presence of fibrous tissue. Leiomyoma is the most common neoplasm affecting the uteri of mares; small neoplasms do not necessarily result in reproductive failure. Leiomyosarcoma, lymphosarcoma, and adenocarcinoma are rare malignant neoplasms affecting mares.

Neoplasms affecting the equine uterus are usually discovered during rectal palpation and transrectal ultrasonography in broodmares during the breeding season. If a uterine neoplasm is suspected, then the clinician should perform uterine endoscopy and take a biopsy sample of the tissue for final diagnosis. Surgical excision of neoplasms is indicated when extensive hemorrhage and endometritis are present or when the presence of the neoplasia would be incompatible with establishing a pregnancy. Prognosis for future fertility is reduced, but pregnancy has been reported in mares with partial hysterectomy.[196]

Uterine Lacerations

Uterine lacerations can occur during unattended or assisted parturition. Rectal and vaginal palpation and abdominocentesis aid in diagnosis.[197] Uterine lacerations historically have been thought to occur most frequently in the dorsal wall of the uterine body, but more recent work has suggested an increased frequency in the uterine horn with the right side affected (73%) more often than the left.[198,199] Uterine lacerations, once confirmed, are surgically repaired because they can cause life-threatening peritonitis or persistent infertility. Adhesions involving the serosal surface of the uterus may occur after cesarean section or uterine tears. No effective treatment, other than attempted surgical excision, exists once the

adhesions form. Therefore some recommend daily palpation of the uterus to avoid adhesion formation following cesarean section.

Uterine Artery Rupture

There is conflicting evidence about what the predisposing factors are for uterine artery rupture. Age (>15 years) and parity have been shown to be contributing factors for mares at risk for uterine artery rupture.[5,200-202] Periparturient artery rupture can occur before foaling but most often occurs during or immediately after parturition. The mare may appear to have colic post foaling, or the hemorrhage may occur after uterine lavage or transrectal palpation several days later. In one study, mares with periparturient arterial rupture represented 1.5% of the total equine necropsy cases; there was no predilection for the left or right sides, and the uterine artery was the most commonly ruptured vessel.[201] Uterine vessels undergo extensive dynamic remodeling during pregnancy and the postpartum period, and this is thought to contribute to progressive degenerative vascular changes. If a mare is believed to be hemorrhaging, then the most common treatment regimen includes sedation and hemostatic support (aminocaproic acid and Yunnan Baiyao). Although many different treatments have proven to be effective, the most important goal is keeping the mare quiet and comfortable.[202,203] If a mare survives an acute episode of rupture of the uterine artery, then antimicrobial therapy is indicated to prevent the hematoma from becoming an abscess. Monitoring the mucous membranes and pulse for 30 minutes after foaling is indicated if the parturition was induced or assisted by a veterinarian and especially when an intervention to correct a dystocia is performed.[204] In mares that survive a ruptured uterine artery, fertility is usually not altered, but mares may be at increased risk for repeated fatal hemorrhage during the subsequent parturition. Further discussion of this condition can be found in the pregnant mare section.

UTERINE TUBES

As mentioned previously, mares are unique in that unfertilized oocytes are retained in the uterine tubes or oviducts and are not transported to the uterus. The mechanism accounting for this phenomenon (selective transport of fertilized versus unfertilized oocytes) has been demonstrated to involve embryo secretion of PGE_2.[8] A healthy oviduct responds to embryonic signals, resulting in proper timing of tubal transport. Diagnosis of pathologic conditions in the uterine tubes is difficult, but laparoscopic evaluation and patency tests have been described.

Postmortem examination of reproductive tracts revealed that salpingitis was common in mares: 37% had infundibulitis, 21% had ampullitis, and 9% had isthmitis. In that study 50% of mares were more than 15 years old, and 85% were older than 11 years of age. The infundibulum generally was found adhered to the uterus, mesovarium, or ovary. The incidence of adhesions on the right side was significantly higher than that on the left side.[205]

Postmortem analysis of uterine tubes has suggested that tubal patency is not a major problem in mares. Oviductal obstructions are less common in the mare than in the cow, although masses of collagen have been found in the oviducts of young maiden mares and pregnant mares and were observed more often in mares that are more than 7 years old.[206] An examination of 700 postmortem specimens, primarily from mares older than 11 years of age, found that almost all oviducts were patent, although more than 40% had adhesions involving the infundibula.[205] Only one uterine tube was found to be occluded among 1248 pairs of uterine tubes that were flushed postmortem.[205,206] Despite this research, there is evidence that oviductal treatment with PGE_2 topically through a laparoscopic approach and oviductal flushing have increased fertility in a certain population of mares that have unexplained infertility that includes a history of no early pregnancy seen despite what appears to be a normal reproductive tract.[207-211]

OVARY

The presence of an enlarged ovary in a mare may be normal or an indication of a pathologic ovarian condition. Consideration of the various possibilities and careful diagnostic procedures are necessary so that normal ovaries are not surgically removed. A thorough history review, including changes in behavior, estrous cycle characteristics, sexual behavior, and the last observed estrus, is important. Ultrasonography, palpation, and hormonal assays are helpful in reaching an accurate diagnosis. In some mares, sequential examinations are beneficial in determining changes in the size of ovaries or various structures on an ovary.

Discovery of an enlarged ovary may be an incidental finding during a normal reproductive examination or may be stimulated by specific clinical signs. Behavioral changes or signs of colic in a mare warrant examination of the reproductive tract, with special attention to the ovaries. Mares with a history of infertility often are suspected of having abnormalities of the ovaries and deserve a thorough examination before surgical removal of an enlarged ovary. The clinician must consider various factors, such as season and pregnancy status, when interpreting a finding of ovarian enlargement. Large ovaries may be normal during the transitional periods in the spring and fall and are expected during certain stages of gestation.

Neoplasia

Granulosa cell tumors (GCTs) or granulosa-theca cell tumors (GCTCs) are the most common tumors of the reproductive tract in mares. They are benign sex cord tumors that can occur in mares of any age; they also have been reported in foals and pregnant mares. Although the granulosa and the theca interna cell layers may be involved, the granulosa cell layer most commonly is affected.

Behavioral changes are common in mares with GCTs that secrete steroid hormones. Behavior may be stallion-like, or persistent estrus or anestrus may occur, depending on the steroid production of the tumor. In other cases of GCTs, behavior may be unchanged yet the mare may be showing signs of abdominal discomfort, lameness, anemia, or other signs seemingly unrelated to the reproductive system. Stallion-like behavior is the most commonly reported behavioral change observed. This is possibly because the change from previous behavior is obvious to the owner and causes an increased challenge in handling the mare. In one report of 63 mares diagnosed with GCTs, 20 exhibited anestrus, 14 were in persistent estrus, and 29 showed stallion-like behavior, which is usually associated with elevated serum testosterone (>45 pg/mL). However, persistent estrus has not been correlated with elevated estrogen.[147,212-214]

On rectal palpation the affected ovary is often enlarged, whereas the contralateral ovary is typically small and inactive. Atrophy of the contralateral ovary can be misleading during winter anestrus, when ovaries are small and minimal follicular growth occurs. Atrophy of the contralateral ovary is not absolute. Although unusual, GCTs have been reported in pregnant mares and in cyclic mares with a functional contralateral ovary and even in both ovaries.[212,213] The enlarged ovary may be smooth or knobby, hard or soft, and may feel as if multiple follicles are present as described previously. Typically, one cannot palpate the ovulation fossa on the affected ovary, although with any greatly enlarged ovary the fossa may be difficult or impossible to palpate. In one report a mare developed a GCT in the contralateral ovary 4.5 years after the initially affected ovary was surgically removed.[213] Ultrasonographic evaluation often shows the classic multiloculated appearance, with cystic structures having sharp rather than rounded borders. However, sometimes the tumor may appear solid or with a single larger cystlike hypoechoic area.[147,214] Although ultrasonography is a useful adjunct, it may not yield a definitive diagnosis as mentioned previously. The ultrasonographic image of GCTs can be similar to that of other ovarian abnormalities, especially ovarian hematomas. A variety of reported appearances makes diagnosis based solely on ultrasonography impossible in many instances.

Mares with GCTs have changes in the circulating hormone concentrations as previously described. Although testosterone often is elevated in mares with GCTs exhibiting stallion-like behavior, testosterone is within normal limits in 10% to 50% of cases. Testosterone in normal cycling mares is less than approximately 45 pg/mL and often is greater than 100 pg/mL in mares with stallion-like behavior. McCue reported that only 54% of mares with GCTs had elevated testosterone, yet 87% had elevated inhibin, leading to the conclusion that inhibin is a better indicator of the disease.[147,214] Inhibin suppresses FSH, leading to a decline in follicular growth, which explains the profound negative feedback effect on the contralateral ovary. Recent work, as mentioned in the previous section, has highlighted the increased sensitivity (98%) of an elevated AMH concentration for detecting the presence of a GCT.[149] At this time most practitioners will have the hormones AMH, inhibin, and testosterone tested, and an abnormal elevation in these concentrations, in conjunction with behavioral signs and abnormal ovarian architecture that persists >30 days, is considered sufficient evidence for the presence of a GCT and the recommendation for unilateral ovariectomy.[149,215]

Overall, the prognosis for life and reproductive function in a mare with a GCT is good. Depending on the time of year when the ovary is removed, the individual mare, and the length of time the tumor has been present, resumption of ovarian activity usually occurs 83 to 392 days after surgery, with a mean of 209 days. If the intended use of the mare is solely as a broodmare, then a reproductive examination, including a uterine biopsy, is recommended before surgery.

Teratomas, although uncommon, are the second most common ovarian tumors. They contain at least two, if not all three, germinal layers. Most teratomas found in mares are benign. They usually contain hair and also may contain bone, teeth, and neural tissue. Teratomas are usually an incidental finding because most are small and do not often cause significant ovarian enlargement. However, on occasion, large teratomas develop that result in ovarian enlargement. Teratomas do not affect the estrous cycle and, therefore, lack obvious outward clinical signs.

Serous cystadenomas are neoplasms of epithelial origin usually found in older mares. These tumors do not metastasize. Although they have been found in mares with high plasma testosterone,[216] behavioral changes are not characteristic. The contralateral ovary is not affected, continues to have normal activity, and does not atrophy; and affected mares usually continue to cycle. On the affected ovary the ovulation fossa is not obliterated and is palpable.

Dysgerminomas are highly malignant tumors that are also of germ cell origin. They metastasize rapidly to the abdominal and thoracic cavities and are considered the counterpart of the testicular seminoma. Because of their nature, dysgerminomas can affect other organ systems, and cases have been reported of associated hypertrophic pulmonary osteoarthropathy.[217] Clinical signs on presentation, therefore, often are unrelated to the reproductive system. These tumors carry a poor prognosis.

Ovarian Abscess

Ovarian abscesses often are attributed to procedures involving puncture of the ovary, such as biopsy or follicle aspiration. As assisted reproductive techniques became more successful and therefore more popular, the incidence of ovarian abscesses was thought likely to increase. However, this has not proved to be the case. Moreover, not all ovarian abscesses can be attributed to iatrogenic causes. Ovarian abscesses have been reported in mares that have had no such procedures performed on them. In these cases, abscesses are likely caused by the hematogenous spread of bacteria or may be associated with strongyle migration.

Affected mares may be febrile and anorectic with an elevated white blood cell count. On ultrasonographic examination the enlarged ovary typically has a thick-walled, fluid-filled structure. The fluid is usually heterogeneous and hyperechoic. Medical management with long-term antibiotic therapy has been successful in treating these cases. Surgical removal of the affected ovary is an alternative treatment, but one must take care that the abscess does not rupture in the abdominal cavity.

Ovarian Torsion

Ovarian torsion, a condition not uncommon in women, has been reported in a mare with a large GTCT that was showing signs of abdominal discomfort.[218] It might be suspected in mares with known ovarian enlargement if sudden signs of abdominal pain develop.

Non-neoplastic Ovarian Enlargement

An ovarian hematoma can form after follicular aspiration or as hemorrhage occurs into the follicular lumen after ovulation. On occasion this hemorrhage can be excessive, possibly because of an anticoagulant present in follicular fluid. Hematomas can be large, up to 20 cm in diameter or more. Although the ovulation fossa is still present, palpating it can be difficult if the hematoma is large. The ultrasonographic appearance varies, causing confusion with a GCT. Large fluid-filled cavities may be observed, or the hematoma may have a more solid appearance, sometimes with fibrin strands. A trait that is sometimes useful in differentiating a hematoma from a GCT is the responsiveness to administration of $PGF_{2\alpha}$. Hematomas that are at least 5 or 6 days old often respond to $PGF_{2\alpha}$ by decreasing in size because of the luteolytic effect. GCTs do not respond to $PGF_{2\alpha}$ treatment with any change in size, shape, or ultrasonographic appearance. Because the follicle wall still undergoes luteinization despite the presence of the hematoma, progesterone concentrations in blood may rise, and cyclicity

usually remains unaltered. Unless the hematoma obstructs the ovulation fossa, fertility is usually not compromised. However, because of the presence of a hematoma, the enlargement of the ovary may persist for several estrous cycles even though the life span of the luteal tissue is normal. Because this structure is a postovulatory phenomenon, the oocyte has been released and the mare may become pregnant if she is mated.

Anovulatory follicles are most common in mares near the end of the breeding season as they go through the autumn transition. These follicles grow to an unusually large size (70–100 mm) yet fail to ovulate. Instead, they fill with blood and develop a gelatinous consistency. A thick (compared with a normal follicle) wall commonly forms. The follicles become firmer and then regress over time, usually disappearing within a month. Free-floating echogenic spots are evident in the antrum of the follicle on ultrasonography, and these increase in number as the follicle grows. When the follicle stops growing, the contents become organized with an echogenic appearance and fibrin strands. Formation of luteal tissue around the periphery of the anovulatory follicle is usually minimal in a true autumnal follicle that occurs at the end of the season. The cause of anovulatory follicles is unknown, although they have been hypothesized to be caused by changes in the hormonal status of the mare that occur with autumn transition. This hypothesis does not explain the occasional occurrence of anovulatory follicles during the breeding season, however. Luteinized anovulatory follicles, although unusual during the ovulatory season, most often occur in older mares, mares with endometritis and mares administered luteolytic agents. Their response to induced luteolysis varies.

During gestation, ovarian enlargement is normal and should be expected when eCG levels are elevated and accessory CLs are present. Ovarian enlargement is associated with increased follicular activity and subsequent ovulation (secondary CLs) or anovulatory luteinization (accessory CLs). An increase in follicular growth begins before day 20 of gestation. New CLs form at approximately day 40 of gestation. Corpora hemorrhagica and hemorrhagic follicles are most common from day 40 to 60. Mares bred early in the season have greater follicular activity during the first 4 months of gestation than mares bred after July.

As described previously, mares undergo transition from winter anestrus to the normal ovulatory season during the spring. The spring transition is characterized by the development and regression of several follicular waves without ovulation, and the ovaries can be quite large, with multiple follicles on each ovary. This transitional period until ovulation occurs lasts for a variable time depending on the mare, photoperiod, and other undetermined factors. The ovaries can be large during the spring transition and may be mistakenly called *cystic*. No treatment is necessary, although a combination of progesterone and estrogen often is used to suppress follicular activity in an attempt to hasten the onset of ovulation and normal cyclicity.

Small and inactive ovaries normally are found in mares in deep anestrus, prepubertal mares, and pregnant mares in the last third of gestation when, curiously, the fetal gonads are larger than the ovaries of the dam. Mares subject to severe malnutrition, of advanced age, treated with anabolic steroids, and with chromosomal alterations leading to gonadal dysgenesis may have abnormally small and inactive ovaries. Mares administered GnRH vaccines have also been demonstrated to have prolonged periods of ovarian inactivity.[42,44]

This section has provided the basic approach to the most common pathologies associated with the reproductive tract of the nonpregnant mare. The following section serves as an in-depth discussion of endometritis, which is arguably one of the most important topics in reproductive management of the horse because it is the most common problem associated with establishing pregnancy in the mare.

Endometritis and Uterine Therapy

Charles F. Scoggin, Kirsten E. Scoggin

Endometritis is the leading cause of subfertility in broodmares. Identification of the cause(s), targeted therapy, and periodic monitoring are important components of successful management. In addition, some causes (e.g., sexually transmitted diseases [STDs]) have important regulatory considerations and can affect international trade and transport. This section will provide an overview of the pathophysiology, methods of diagnosis, and treatment strategies for endometritis.

INTRODUCTION

Inflammation of the uterus following breeding is considered a normal yet transient physiologic response in mares.[219] Most mares (~90%) are capable of managing this inflammation with little to no intervention; these individuals are considered reproductively normal or *resistant* mares. The other 10% either have or are prone to endometritis and are considered *susceptible* mares.[220,221] Causes of susceptibility include infiltration of pathogenic organisms, anatomic and functional defects, aberrant local immune response, and method of breeding.

PATHOPHYSIOLOGY OF ENDOMETRITIS

In clinical broodmare practice, the most important causes of endometritis are (1) infectious endometritis (IE), (2) post-mating-induced endometritis, (3) CDE, and (4) STDs. These diseases are not mutually exclusive and can occur in tandem.

Infectious Endometritis

Infectious endometritis occurs following colonization of the uterus with opportunistic bacteria. The most frequently encountered pathogens in mares are β-hemolytic streptococci (*Streptococcus equi* ssp. *zooepidemicus;* β-strep) and *E. coli*.[222,223] Less common but no less pathogenic are the bacteria *Pseudomonas aeruginosa* and *Klebsiella* spp. and the yeast *Candida* spp. and *Aspergillus* spp. As will be discussed later, these microbes are identified by microbiologic techniques, cytologic evaluations, and histologic studies of the endometrium.

Persistent Mating–Induced Endometritis

Equally as significant as IE is persistent mating–induced endometritis (PMIE). This condition occurs when mares have anatomic or physiologic abnormalities that lead to excessive intraluminal fluid accumulation and/or impairment of the uterine clearance mechanisms. Normal or resistant mares should typically have only a trace amount (e.g., <1 cm in depth) of anechoic fluid seen on transrectal ultrasound 24 to 36 hours after breeding.[224] In contrast, susceptible mares will

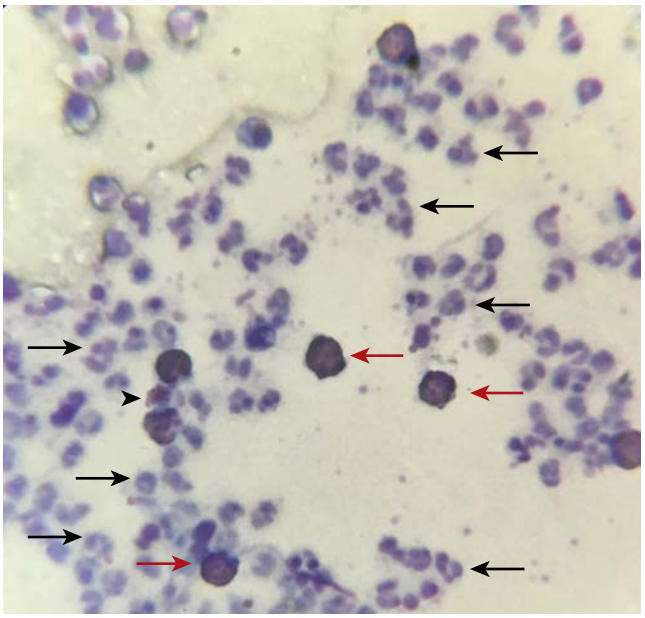


FIG. **19.19** Light micrograph of an endometrial cytology from a mare with persistent mating–induced endometritis (PMIE). Numerous neutrophils and eosinophils are present in this sample. Bacterial colonies are also evident.



FIG. **19.20** Cross-sectional transrectal ultrasonographic image of the uterus from a mare with persistent mating–induced endometritis (PMIE). Note the edema pattern present within the endometrium that is associated with the echogenic intraluminal fluid. (Courtesy P.M. McCue).

have a significant amount of echogenic fluid measuring several centimeters within the uterine lumen. This fluid is usually an accumulation of inflammatory cells, uterine secretions, and semen (Fig. 19.19). Endometrial edema is often present and excessive, indicating an exaggerated local inflammatory response (Fig. 19.20).

Chronic Degenerative Endometritis

Chronic degenerative endometritis is typically considered an age-related phenomenon.[225] Changes within the reproductive tract associated with CDE occur regardless of parity or past reproductive history. A recent review on this subject evaluated data from three different regions of the world with large Thoroughbred breeding programs.[226] On average, reproductive efficiency in broodmares increased steadily until ~10 years of age and remained relatively steady until ~14 years of age. Reproductive performance slowly but steadily declines thereafter and goes into a downward spiral by the late teens. Mares with CDE are readily diagnosed by endometrial biopsy. Common findings include increased fibrosis, cystic glandular distention, and lymphangiectasia (Figs. 19.21 and 19.22). Clinically, these changes put mares at risk for either IE or PMIE. In addition, age-related changes occur to other important parts of the reproductive system, such as the cervix and perineum.[227,228] The clinician is thus reminded to evaluate all structures of the reproductive tract when managing endometritis or other causes of subfertility.

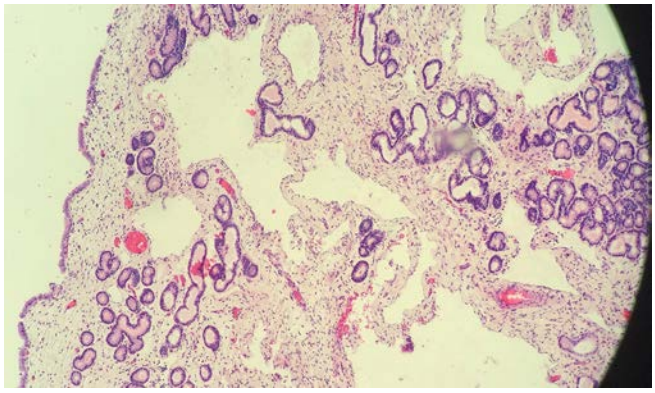


FIG. **19.21** Light micrograph of an endometrial biopsy showing age-related changes in the stratum compactum consistent with chronic degenerative endometritis (CDE). Note denuded epithelium, sparsity of glands, and foci of inflammatory cells. (Courtesy C.F. Scoggin).

Molecular and Gene Expression Studies

Recent studies have shown there are multiple and complex signaling pathways involved in both experimental and clinical cases of endometritis. Included in these events is release of proinflammatory and antiinflammatory cytokines. The interleukin (IL)-1 cytokine family (IL-1α, IL-1β, and IL-receptor antagonist [IL-1Ra]) plays an important role in immune regulation and inflammatory processes.[229] Several recent studies have focused on characterizing this cytokine signaling pathway in the mare reproductive tract in response to bacterial or breeding-induced endometritis (reviewed in Woodward and Troedsson[230]). Current experimental models use either bacteria (such as β-strep or *E. coli*) or spermatozoa to induce an inflammatory response. In these models, upregulation of proinflammatory cytokines—such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-8 (a neutrophil chemoattractant), as well as the antiinflammatory cytokine IL-10—was observed within 3 hours after challenge.[231] This same group also found a systemic increase in serum amyloid A (SAA) expression up to 12 hours after inoculation. A follow-up study comparing mares classified as resistant or susceptible to persistent endometritis found that both groups exhibited an immune response after

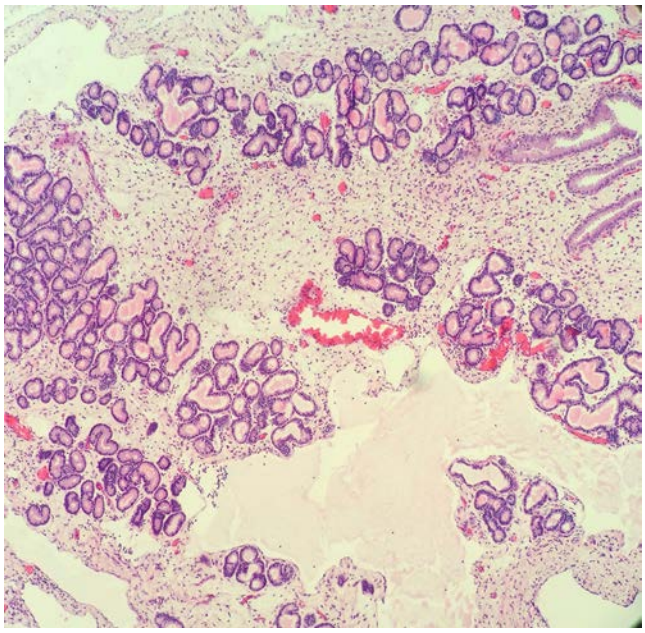


FIG. **19.22** Light micrograph of an endometrial biopsy of the stratum spongiosum. Age-related changes are evident and consistent with chronic degenerative endometritis. (Courtesy C.F. Scoggin).

E. coli infusion, but the susceptible mares showed a sustained cellular immune response (up to 72 hours).[232] The susceptible mares showed an increase in IL-1β and IL-8 expression at 24 hours, and IL-1β at 72 hours compared with resistant ones. In addition, susceptible mares had a decrease in IL-6 and TNF-α expression compared with resistant mares at 3 hours post infusion. In this study, there was no upregulation or downregulation observed for the inflammatory-modulating cytokine IL-10, but this may be caused by a lower dose of *E. coli* used for the bacterial challenge.

Endometrial cytokine gene expression was also analyzed in mares resistant and susceptible to persistent post-breeding endometritis. Fumuso et al.[233] examined the expression of IL-1β, IL-6, and TNF-α in resistant and susceptible mares 24 hours after AI and found that both groups detected an increase in these proinflammatory cytokines, but the susceptible mares had overall higher basal levels of these cytokines. The same research group subsequently found that susceptible mares had an increased expression of IL-8 (which mediates recruitment and leukocyte activation at sites of inflammation) and lower expression of the antiinflammatory mediator IL-10 compared with resistant mares 24 hours after insemination.[233,234] The cytokine response to killed spermatozoa was also compared between resistant and susceptible mares at multiple time points after insemination (0, 2, 6, 12, and 24 hours).[235] Both groups exhibited changes in cytokine expression, but significant differences between groups were only observed at the 6-hour time point. Six hours after insemination, susceptible mares had a lower expression of the antiinflammatory cytokines IL-1Ra and IL-10 and the proinflammatory cytokine IL-6 (which can stimulate the immune response and also has antiinflammatory properties[236] compared with resistant mares). Thus the inflammatory gene response may differ in mares with persistent breeding-induced endometritis. A better understanding of the molecular causes and pathways involved in the disease could lead to new treatments aimed at targeting these particular pathways. These include treatment with immune-modulating substances either locally or systemically and will be discussed in a later section pertaining to therapeutic options for endometritis.

Biofilm Infections

Over the past decade biofilm infections have received a great deal of attention as a cause of mare subfertility. Bacteria can exist in one of two states: The first is the free-floating or planktonic state, and the second is the biofilm state. This biofilm state contains a community of bacteria, and together they create an added barrier of protection against the host's immune system and antibiotic agents above and beyond the capability of planktonic bacteria. These biofilms are proposed as a cause for persistent and chronic endometrial infection, and research is actively being pursued on this topic.

Biofilm is made up of an exopolysaccharide matrix (EPS) that is produced by the bacterial community within the biofilm.[237] This EPS contains plasmids that can be shared among this community to confer resistance to outside stresses or an adverse environment. The EPS also allows for chemical signal transduction, allowing bacteria to coordinate their activities to increase efficiency and resiliency of the biofilm. The presence of biofilm has been found in various areas, including the bladder and skin wounds, and can lead to persistent infections, making treatment and resolution difficult. The actual presence of biofilm within the mare's uterus has not been confirmed, but research appears to be on the precipice of answering that question.[238,239]

Pathogenic bacteria implicated in biofilm formation in the mare are *E. coli, K. pneumoniae, P. aeruginosa,* and β-strep. A presumptive diagnosis of uterine biofilm infection can be made in mares that fail to respond to conventional treatments as determined by repeated "dirty" cultures and inflammatory cytologies. Current therapies are aimed at disrupting biofilm formation and will be discussed in a subsequent section.

Sexually Transmitted Diseases

There are several equine STDs that are of relevance from both clinical and regulatory standpoints. Readers are strongly encouraged to contact their local U.S. Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) official for updated information or whenever a reportable or foreign animal disease (FAD) is suspected. Questions pertaining to the handling of potentially infected gametes (e.g., spermatozoa and embryos) should be directed to these and other regulatory officials.

Contagious Equine Metritis

As the name implies, CEM is a contagious disease affecting the uterus of the mare. It is of increased significance because of its impacts on international transport of livestock. A true venereal disease, CEM is caused by the bacterium *Taylorella equigenitalis,* a gram-negative coccobacillus that is considered highly contagious.[240] Transmission via fomites also appears to be likely and was implicated as a cause for an outbreak in the United States from 2008 to 2009. Clinical signs vary from relatively unapparent to copious vulvar discharge originating from the uterus.[241] The majority of CEM-affected mares are otherwise clinically normal and will resolve the infection within 2 to 4 weeks. Unfortunately, by the time clinical signs are observed, at least one cycle is lost to endometritis, and spread of the disease is rapid to other mares and stallions. Inapparent or "silent" carrier stallions are the most common

source for disease transmission among horses. What ensues is a significant drop in productivity (as measured by per cycle pregnancy rates) and increased costs associated with management and veterinary care. As will be described later, treatment for *T. equigenitalis* is fairly straightforward and consists of careful cleansing of the internal and external genitalia and application of local antiinfective agents. Systemic antibiotics may be needed in severe cases. To the authors' knowledge, no vaccine exists for this disease. Because of the fairly recent outbreak of CEM, the United States has undergone a reclassification in its status as a CEM-free country. As this chapter goes to press, international transport of horses to and from the United States is permissible by most agricultural ministries provided proper screening and required treatments are performed prior to introduction of the animal into the country's breeding population.

Equine Viral Arteritis

Equine viral arteritis (EVA) is another disease that has important regulatory implications. It occurs following infection with equine arteritis virus (EAV), an enveloped, single-stranded virus.[242] Clinical signs vary in degree and intensity and include vasculitis, distal limb edema, pyrexia, rhinitis, conjunctivitis, and anorexia. Most important, it can cause abortion outbreaks and severe neonatal pneumonia in naive herds, causing significant economic damage and lost productivity. Modes of transmission include respiratory secretions and other body secretions, including semen, fetal fluids/tissues, and fomites. Most mares mount a significant immune response to the disease and are generally considered immune for several years following clinical infection. Current estimates suggest that 30% to 70% of exposed stallions will become persistently infected with the virus and are referred to as "shedder" stallions.[243] For a more complete review of EVA in stallions, the reader is referred to Chapter 8 and the section of this chapter related to diseases of the stallion. In mares, disease is usually self-limiting, and treatment consists primarily of supportive care and isolation strategies. A modified live virus vaccine is commercially available in the United States (Arvac; Zoetis, Inc., Parsippany-Troy Hills, NJ 07054) and has been approved for use in nonpregnant breeding stock. The authors have used it safely in mares at various stages of gestation. Control of disease is best accomplished by screening, isolation, and vaccination. Breeding with EAV-infected semen is permissible with proper documentation of the mare's vaccination status. Certain breed registries and state agricultural departments have restrictions regarding vaccination of breeding stock and use of EAV-infected semen. The reader is encouraged to compile and organize this information before vaccination or use of semen from a shedder stallion.

Equine Herpesvirus Type 3

EHV-3 is the causative agent of equine coital exanthema. It is not currently considered a reportable disease in the United States. This virus is transmitted primarily via coitus, but transmission can also occur via fomites, including shared examination gloves and vaginal specula. Clinical disease is primarily manifested as vesicular and painful lesions on the external genitalia of both mares and stallions. These lesions cause discomfort during reproductive evaluations and during live-cover matings, but aside from transient and often self-limiting pyrexia, infection typically has no lasting or impressionable effects on fertility.[244] Lesions will take 4 to 6 weeks to heal and are characterized by focal areas of depigmentation. Gentle cleansing of active lesions may soothe and reduce inflammation. Reports of antiviral treatments are sparse, and treatment is usually tincture of time. Mares can resume breeding once the lesions have healed. Recrudescence of this particular herpesvirus is unknown.[245] Control is aimed at early detection and temporarily removing infected animals from breeding until lesions have subsided. There is no commercially available vaccine against EHV-3 in the United States.

Dourine

Dourine is a serious STD of equids because of its high morbidity and mortality. The etiologic agent is the protozoa *Trypanosoma equiperdum*. Infection affects several body systems, and clinical signs include intermittent fever, swelling of and discharge from the genitalia, cutaneous plaques, and neurologic signs.[246] It is generally considered a chronic and progressive disease; the onset of recumbency usually indicates terminal disease. This disease has been eradicated in several developed countries, including the United States. It is still seen in Africa, the Middle East, South America, and Central America, but sporadic outbreaks have been reported in Asia and Germany.[240] Diagnosis is made via compatible clinical signs and a complement fixation (CF) test, which is prone to false-positives from other species of *Trypanosoma*. Newer methods of diagnostics, including ELISA and polymerase chain reaction (PCR) technologies, are currently being explored for both testing and screening purposes. If found in a particular area, then infected animals should be identified, and breeding activity should cease until positive animals have been removed, usually via culling. Treatment with antiprotozoal drugs has been performed in some endemic areas, but they are unproven and even discouraged because of the concern of generating inapparent shedding animals.

Piroplasmosis

Piroplasmosis is a disease that became prevalent in the United States earlier this decade after a fairly long period of absence. It is considered an FAD in the United States, but it is endemic in many other parts of the world. This disease is caused by the protozoa *Babesia caballi* and *Theileria equi* (formerly *Babesia equi*). Transmission occurs either via a vector (e.g., ticks) or mechanically (e.g., improperly sanitized instruments). It also has the potential to be transmitted venereally in cases of hemospermia. Piroplasmosis creates disturbances to the circulatory, musculoskeletal, abdominal, and nervous systems. Clinical signs can be nonspecific and may vary based on the severity of disease and degree of chronicity. Horses surviving the acute phase of the disease can become carriers. Diagnosis can be made based on observation of the organism on blood smears and serologic testing. Treatment with antiprotozoan medications is unproven, and recrudescence is a concern.[240] Similar to other reportable diseases, concerns about a potential FAD should be directed to the local USDA-APHIS official.

DIAGNOSTICS

History

Evaluation of past reproductive, medical, and management issues is tantamount to an effective workup of susceptible mares. Calculation of per cycle pregnancy, embryonic loss, and live foal rates are useful measures of reproductive

efficiency. Disturbances to other body organs may also factor into susceptibility. Chronic injuries to the musculoskeletal system can be particularly problematic because they can be a source of steady pain and ill-thrift. Metabolic disease can also affect fertility (e.g., PPID). This disease is covered more thoroughly in Chapter 16, but it can affect the reproductive system by leading to chronic infections and erratic cyclicity. Finally, consideration should be given to stallion fertility. Chances of conception are improved by selecting a stallion of known fertility. With respect to the phenomenon of *mare-stallion incompatibility,* it has never been proven in horses; nevertheless, the potential exists as suggested by numerous anecdotal reports of switching stallions and improving fertility rates in problem mares.

Reproductive Evaluations

The importance of a thorough physical examination cannot be overstated. As mentioned previously, abnormalities in other body systems can adversely affect reproductive efficiency in broodmares. These should be noted and incorporated into the future plan. Screening for infectious and metabolic disease should also be performed when indicated.

Reproductive evaluations consist of inspection of both the external and internal genitalia, including the mammary glands. Perineal conformation is judged by evaluating the declination and location of the vulva with respect to the anus and ischium. A more objective measure is calculating the Caslick index described by Pascoe.[49] In normal mares, the anus, vulva, and ischium lie in a relatively similar vertical plane, with about two thirds of the vulva lying dorsal to the ischium. The vulvar lips should form a tight seal enclosing the vestibule, urethral orifice, and clitoris. Abnormal findings include a forward-tilting pelvis, sunken-in anus, and more of the vulva lying dorsal to the ischium than ventral. Clinically, these abnormalities can lead to pneumovagina and urovagina, as well as fecal contamination of the vestibule and vagina.

Palpation per rectum should reveal two ovaries that are kidney-bean shaped with readily distinguishable ovulation fossae. The uterine horns, uterine body, and cervix should also be identified and felt for tone, relative turgidity, and location of the uterus relative to the pelvis. Sweeping these structures in a continuous motion can help identify adhesions and/or intramural masses. Susceptible mares often have a pendulous uterus, which can restrict uterine clearance. Rectal ultrasonography is a key component of mare reproductive examinations and allows for further characterization of the ovaries, uterus, cervix, and vagina. Archetypal findings of endometritis are echogenic, intraluminal uterine fluid exceeding 2 cm high by 2 cm wide, exaggerated endometrial edema using a subjective scoring system, and pneumovagina as evidenced by hyperechoic flecks within the vaginal vault.

Moving caudal to cranial, the three important barriers to the uterus are the vulva, the vestibulovaginal fold, and the cervix. Breakdowns in any of these barriers can lead to acute or chronic inflammation and/or contamination with pathogenic organisms. A vaginal examination can identify sources of vulvar discharge, the relative seal of the vestibulovaginal fold, character of the vagina, and integrity of the cervix. Most theriogenologists prefer to do both speculum and manual examinations because some abnormalities are best visualized, whereas other abnormalities are best palpated.

Finally, evaluation of the udder is recommended. Abnormalities are rare but can affect rearing and mothering ability, and they suggest endocrine disorders. Congenital defects, previous trauma, and infections can occur within the mammae. Admittedly, it is unusual for abnormalities of the mammae to be associated with endometritis. However, it is worthy to mention that milk production can be adversely affected in severe cases of endometritis that progress to metritis.

Assessment of the Endometrium

The past two decades have brought forth significant advancements in diagnostics used for identifying endometritis. This particular section will include both time-honored techniques (e.g., culture, cytology, and biopsy) that comprise a minimum database expected for evaluating mares with endometritis as well as discussion of new techniques that may aid in the diagnosis and management of endometritis. Table 19.6 provides a list of various clinicopathologic techniques available and their relative merit for diagnosing endometritis in the mare.

When samples are procured from the internal reproductive tract, the mare should be adequately restrained with her tail held or tied out of the way. Her rectum should be evacuated and her perineum thoroughly cleansed to remove feces, smegma, and other debris. The clinician should use sterile gloves, rods, sleeves, speculums, and swabs to maintain cleanliness.

Endometrial Culture

With respect to endometrial cultures, several means are available for acquiring samples. The most common method consists of using either a Kalayjian or double-guarded swab. The penetrating portion of the swab is protected within the palm or fingers of the clinician before entering the vaginal vault. The external os of the cervix is located, and the swab is fed through the cervix and into the lumen of the uterus. After contacting the endometrium, the swab is popped through the protective cap or sheath and gently rolled along the endometrium. Care must be taken to turn the swab in the same direction to prevent it from snapping off within the endometrium. The swab is then retracted back into its sheath(s) and placed in holding media, properly labeled, and submitted to the laboratory for culture and sensitivity. Aerobic cultures are most commonly submitted. In cases of suspected anaerobic or fungal infections, the clinician should communicate directly with the laboratory when preparing these cultures and attempting to identify the agent(s) involved. The major advantage of the swabbing technique is the relative ease by which it can be collected. Some clinicians prefer this method when using a speculum for accessing the vaginal cavity. The major downside of this method is that it involves sampling only a small portion of the endometrium. Focal infections can thus be missed, leading to a false-negative result.

Other means of obtaining uterine cultures are low-volume lavage (LVL), direct aspiration of intraluminal fluid, and biopsy specimens. The LVL is a popular method for evaluating cases of acute and chronic endometritis, especially in susceptible mares. It consists of feeding a Bivona catheter into the uterus and instilling a predetermined amount of physiologic saline or LRS. The uterus is gently massaged per rectum, and the effluent is collected via gravity. The retrieved fluid is first evaluated for its relative clarity and amount of mucus. Although subjective, visual assessment appears important because a cloudy efflux is highly correlated with the presence of bacteria.[247] This fluid is then transferred into sterile 50-mL conical vials and subsequently centrifuged at 400*g* for 10 minutes. The supernatant is poured off, and two sterile cotton swabs are placed

in the pellet: one for culture and one for cytology (see later). The primary advantage of the LVL is that it provides a more representative sample of the entire endometrium. The main disadvantage is that it is prone to false-positive bacteriologic results caused by potential contamination from the vagina.

Direct aspiration of intraluminal fluid entails collection of fluid already present (as diagnosed by ultrasound) within the lumen of the uterus. It can be accomplished by feeding a flexible insemination pipette into the uterus, attaching a syringe to the other end of the pipette, and under ultrasound guidance, gently aspirating fluid from the uterus. This fluid is submitted to the laboratory for both culture and cytology. Advantages to this method are that it does not require the addition of fluid into the uterus, and the recovered fluid has a high probability of containing inflammatory debris and/or pathogenic microbes. Disadvantages include the need for increased technical expertise and, in cases in which mares do not have a great deal of intraluminal fluid, unsuccessful retrieval.

A final means of obtaining an endometrial culture is the collection of an endometrial biopsy. A more thorough discussion of the technique and histologic evaluation was provided in an earlier section. Biopsy samples can also be used to obtain both microbiologic and cytologic samples. For bacteriology, the sample itself can be smeared on a blood agar plate, or a Culturette can be run over the specimen and placed into transport media before immersion into a fixative. Impression smears can also be made and evaluated with common staining techniques. The major benefits of this technique are improved sensitivity of bacteriology findings relative to the guarded swabs and cytologic evaluation, a high positive predictive value, and the potential to identify infectious organisms deeper within the stratum compactum.[222] Drawbacks of this method include an increased technical skill in obtaining the sample and relatively low negative predictive value.

Endometrial Cytology

As alluded to earlier, endometrial cytology is often performed in tandem with endometrial cultures. They are collected in the same manner as endometrial cultures, with clinician preference representing the deciding factor as to what method to use. When Kalayjian swabs are used, a cytology specimen can be obtained by gently rolling the outer sheath of the rod within the endometrium to allow the cap to collect a sample. It is important that the culture swab has been retracted back into the sheath before the rod is rolled to collect the cytologic specimen. Once collected, the rod can be withdrawn from the mare, the swab placed in transport media, and the cap gently "flicked" onto two separate slides, one for an eosin-nigrosin stain and one for a Gram stain. The specimen is then evaluated for the presence of inflammatory cells using bright-field microscopy. Other examples include the previously described LVL technique, mare cytology brushes, and impression smears from biopsy specimens. Mare cytology brushes are similar to those used in women for Papanicolaou (Pap) smears and attached to a plastic rod in a fashion similar to those of double-guarded swabs, and the procedure is identical. Biopsy specimens collected for histology can be used to make impression smears onto slides before immersion in fixative.

In addition to advancements in sample collection, our understanding and interpretation of results have also evolved. No technique is perfect, and each is prone to both false positives and false negatives, which influences the sensitivity (percent of disease-positive subjects that test positive) and specificity (percent of disease-negative subjects that are negative) of each test. Table 19.6 lists probability measures for each previously described technique used to diagnose endometritis. Uterine biopsy samples are the most consistent and serve as the "gold standard" in comparing the worthiness of other diagnostics for detecting endometritis. At least two studies have shown that bacteriology results obtained from endometrial biopsy samples are superior to guarded-swab techniques for positive identification of bacterial pathogens.[222,248] Additionally, these studies and another[106] demonstrated the importance of collecting both a culture and cytology. Indeed, cytology was at least twice as sensitive as culture in predicting the presence of endometritis.[106] The LVL technique has demonstrated improved sensitivity for detecting bacterial growth relative to the guarded swab but had lower specificities for culture and cytology than the swab method.[220] Consequently, it appears that LVL underestimates inflammation, which may be caused by dilution of retrieval fluid. However, it should be noted that when researchers factored in the character of the efflux, the specificity substantially improved.

Endometrial Biopsy and Histology

Histologic evaluation of biopsies is an invaluable tool and widely agreed to be the gold standard when measuring a mare's future reproductive capacity.[10] The procedure as described previously involves aseptic preparation of the perineum, and 60-cm uterine biopsy forceps are used to obtain the sample. Aside from proper restraint, mares generally do not react to the biopsy, and minimal aftercare is warranted. The instrument is then removed from the vagina, and the sample is immersed in a suitable fixative. The authors prefer Bouin solution over 10% neutral-buffered formalin as histologic architecture and luminal contents are better preserved, but samples must be transferred from Bouin solution into another fixative (e.g., water and alcohol) within 24 hours to avoid tissue disruption. Formalin is a suitable alternative and should be used if samples are not going to be processed immediately. If the sample is intended to be used for concurrent culture and cytology, then these samples should be obtained from the biopsy before immersion into a fixative. Multiple biopsies can be collected from different areas of the uterus, and focal abnormalities detected within the endometrium can be biopsied via ultrasound guidance or hysteroscopy. Samples can be submitted for processing and preparation. Each grade is associated with the likelihood of the mare carrying a pregnancy to term (expressed as a percentage) and has been previously described[249]:

Grade I: Normal endometrium or mild, focal inflammation or fibrosis; >80% of maintaining a pregnancy until term.

Grade IIA: Mild to moderate inflammation and/or multifocal fibrosis with one to three layers of fibroblasts surrounding glands or less than two fibrotic nests per 5-mm linear field; 50% to 80% chance of maintaining a pregnancy to term.

Grade IIB: Moderate inflammation and/or multifocal to diffuse fibrosis with four or more layers of fibroblasts surrounding glands or two to four fibrotic nests per 5-mm linear field; 10% to 50% chance of maintaining a pregnancy to term.

Grade III: Severe inflammation and/or diffuse fibrosis with five or more nests per 5-mm linear field; <10% chance of maintaining a pregnancy to term.

Review and interpretation of the sample by a theriogenologist are recommended to ascribe a grade and offer a treatment plan (i.e., epicrisis).

The above techniques represent a minimum database for evaluation of a subfertile mare. As a summary, these include a thorough history; physical examination; reproductive evaluation; and culture, cytology, and biopsy of the endometrium. Theriogenologists have gained an appreciation of the relative benefits and limitations of each test and how using them in tandem improves the reliability of the results and the inciting cause. However, there are some instances in which either routine diagnostics fail to identify the problem or prescribed treatment fails. The next section discusses advancements in diagnostics that are improving both the accuracy of results and response to therapy.

ADJUNCTIVE TECHNIQUES

Hysteroscopy

Hysteroscopy allows for complete visual assessment of the lumen of the uterus via video endoscopy. This imaging technique can identify transluminal adhesions, congenital abnormalities, persistent endometrial cups, uterine tears, foreign bodies, and focal areas of inflammation/infection. Ideally, this procedure should be performed while the mare is in diestrus so that the cervix is closed, allowing for proper insufflation. Priming with progesterone is an option when preparing a mare for hysteroscopy. The endoscope should be cold sterilized with an appropriate disinfectant and then rinsed thoroughly with sterile saline or LRS. The procedure is usually performed under standing sedation. The perineum is cleansed appropriately, and the distal end of the scope is guided into the vagina. Evaluation of the vaginal vault can be useful for detecting sources of persistent or periodic hemorrhagic discharge (e.g., varicose veins) or cervical abnormalities. The end is then passed through the cervix and into the uterus. Insufflation with air or a balanced polyionic fluid allows for visualization of the entire lumen as the uterus expands. The scope is then maneuvered for a complete evaluation of the body and both horns. Each horn should be examined along its entire length to visualize the papilla representing the uterotubal junction. A normal endometrium is pale pink in color with a mostly homogeneous smoothness. If the examination is performed during estrus, then endometrial edema will be grossly evident, characterized by folds and a glistening surface.[250] Video 19.1 provides an example of a normal endoscopic examination of the mare's reproductive tract. The endoscope was advanced through the cervix, and rapid insufflation distended both horns, allowing for complete visualization of the body and both horns of the uterus.

A hallmark sign of acute endometritis is cloudy, neutrophil-laden fluid. Video 19.2 is an example of an abnormal endoscopic examination with mucopurulent exudate diffusely throughout the endometrium. Subsequent biopsy specimens yielded a heavy growth of β-strep. Signs of chronic endometritis are more variable, but they would include focal changes in texture or color, erosions or ulcerations, and plaques or mucoid debris.[250] After hysteroscopy is completed, the uterus should be lavaged, and a luteolytic dose of $PGF_{2\alpha}$ is recommended to contract the uterus and open the cervix for proper drainage.

Hysteroscopy can also assist with treatment. When used in conjunction with an Nd:YAG laser system, it can be used to remove endometrial cysts, adhesions, and retained endometrial cups.[160] Additionally, it can facilitate ligation of vessels and removal of masses within the uterus or vagina. Other uses for endoscopy of the reproductive tract include low-volume insemination, fetal mummification or membrane extraction, hydrotubation of the oviducts, and foreign body removal.

Serum Amyloid A

Acute phase proteins (APPs), such as haptoglobin and SAA, are produced in the liver and stimulate the innate immune response and assist in the elimination of infection.[251] Their production is stimulated by proinflammatory cytokines, including IL-1, IL-6, and TNF-α, which help to modulate the local and systemic immune response. Studies in horses have measured SAA in monitoring the inflammatory response to various clinical conditions, such as castration,[252] endometritis and placentitis,[253,254] induced arthritis,[255] and neonatal illness.[256]

These proteins are also thought to play a role in the inflammatory cascade of the mare's reproductive tract. Studies in cattle have shown elevations in APPs associated with other clinical and diagnostic signs of endometritis[257,258] and could potentially be useful biomarkers in broodmares. SAA is a major protein that normally has low or undetectable levels in a healthy horse; however, plasma concentrations can increase more than 10-fold in response to injury or trauma.[259] SAA concentrations remain stable at baseline levels during the 4 months before parturition[260] and significantly increase within 36 hours postpartum.[261] Studies of mares experiencing early embryonic loss showed increased levels in peripheral SAA 72 hours after insemination and ovulation,[262] but a field study comparing SAA concentrations at days 0 and 15 between mares with early embryonic loss to matched mares that maintained pregnancy found no correlation between elevated SAA levels and pregnancy loss.[263] A study by Nash et al.[264] found that peripheral SAA levels did not increase at 24 hours post insemination with frozen/thawed semen, but a local response within the uterine was not tested. Recent studies have focused on the SAA response in equine endometritis using the bacterial-induced model to monitor changes in peripheral concentrations of SAA, as well as local changes, by measuring gene expression within in the endometrium. Uterine infusion of high doses of *E. coli* yielded a measurable increase in SAA, which significantly correlated with upregulation of SAA in endometrial biopsies at 3 and 12 hours post infusion.[231] Thus, selected markers of the systemic acute phase reaction were altered in the circulation in response to experimental IE. A subsequent study by the same group evaluated the effect of glucocorticoid therapy on SAA endometrial gene expression in susceptible mares after *E. coli* infusion. Researchers found a decrease in SAA concentrations in treated mares,[265] supporting previous clinical studies demonstrating the efficacy of preemptive steroid treatment in mares prone to PMIE.[266,267] More studies are necessary to further characterize the correlation and clinical usefulness of measuring SAA concentrations in cases of endometritis.

TREATMENT STRATEGIES

Antibiotics

The use of antibiotics in broodmare reproduction has a lengthy history. Local intrauterine infusions have been used for decades to treat or act as a prophylactic measure against

endometritis. Systemic antibiotics have gained popularity over the past few years for use either in conjunction with or as an alternative to intrauterine infusions. Doses, routes, and frequency for systemic antibiotics almost always follow published guidelines for administration of these drugs in horses (please see Chapter 2 for a detailed discussion). However, when choosing a systemic antibiotic, it is important to base the choice on sensitivity results and whether it can attain effective concentrations in the endometrium and other tissues of the urogenital system. These and other indications and options for antibiotic use in broodmares will be discussed next.

Intrauterine Antibiotics

In 1992, Pycock and Newcombe published a report in which they showed a significant association between postmating intrauterine antibiotics and improved pregnancy rates.[268] The authors hypothesized that these results were related to reducing or eliminating bacteria tracked into the uterus following breeding. In other words, because the act of mating, especially live cover, was viewed as an inherently dirty process, the use of intrauterine antibiotics assisted local immune defenses in treating opportunistic bacteria. Interestingly, intrauterine antibiotics used alone yielded pregnancy rates similar to those in mares treated solely with a single dose of oxytocin. However, when these two treatments were combined, pregnancy rates were significantly higher than in other treatment groups. This study gave credence to prophylactic intrauterine antibiotic infusions and popularized their use sufficiently where they have become a routine part of a postmating examination in live-cover operations regardless of other findings (or lack thereof). With concerns over antimicrobial resistance reaching near pandemic levels, this practice is no longer condoned. A more prudent and scrupulous course of action is to use antimicrobial agents when there is clinical and/or diagnostic evidence for treatment. Indeed, a recent review regarding the prophylactic use of intrauterine antibiotics concluded that despite justifiable pressures, use of antimicrobials in this manner was questionable, and the use of alternative treatments such as ecbolics and uterine irrigation may be more beneficial.[269]

When selecting an antimicrobial for intrauterine delivery, factors to consider include:

- Appropriate sensitivity pattern
- Stage of estrous cycle
- If it is relatively nonirritating to the endometrium and genitalia
- Compatibility of different agents if two different drugs are mixed

Although some clinicians discourage empiric therapy, there are instances in which delaying treatment or ignoring one's clinical intuition and other findings may have negative consequences. As such, broad-spectrum antibiotics can be chosen that meet the other requirements given earlier. With respect to the stage of the estrous cycle, past studies suggest that intrauterine treatment should only be performed while the mare is in estrus. Treatment during diestrus may have unintended consequences—namely, iatrogenic contamination and increased risk of fungal endometritis.[270] Selection should also depend on the chemical properties of the antibiotic and its respective diluent. Ideal solutions should be nonirritating, attain proper minimum inhibitory concentration (MIC) concentrations for the targeted microbe, and leave minimal residues. Other characteristics, such as -cidal or -static effects and concentration-dependent or time-dependent doses, can also be factored in. However, most doses are empiric. Dosages for various antibiotics for intrauterine delivery are listed in Table 19.7.

In a recent survey involving more than 200 equine practitioners, Dascanio et al.[271] reported that ceftiofur was the most commonly used antibiotic by a narrow margin over gentamicin (21% vs. 19%, respectively). These two were followed by ticarcillin and clavulanic acid (13%), ampicillin (12%), other (12%), amikacin (5%), procaine penicillin (3%), potassium penicillin (3%), and ticarcillin (3%). It was not specified if these selections were made based on culture and sensitivity. Regarding empiric therapy, one study showed that 100% of clinical isolates of β-strep were susceptible to both ampicillin and penicillin G, whereas 100% of *E. coli* were sensitive to gentamicin.[272]

Frequency and length of treatment are other important considerations. As shown in Table 19.7, most uterine infusions are performed once daily. The exception is ticarcillin and clavulanic acid, which, based on previous studies, indicates that multiple daily infusions may be more appropriate to achieve therapeutic concentrations than the standard once-daily dosing.[273] On the other hand, ceftiofur has been found to reach effective concentrations when delivered either intrauterine or IM.[274,275] A recent report demonstrated the potential benefit of a water-soluble solution of enrofloxacin for treating resistant infections.[276] Therapeutic concentrations were above MICs in half of the test mares, and no long-lasting effects on the endometrium were noted, indicating its relative safety. Studies of other antibacterial agents in the uterus are in need of evaluation to combat recurring infections and address antimicrobial resistance.

Systemic Antibiotics

The use of systemic antimicrobial agents to treat endometritis has gained significant momentum over the past decade. Dosages are listed in Table 19.7. The benefits of systemic antibiotics compared with those given via the intrauterine route are the ability to treat regardless of stage of the estrous cycle and the ability to maintain effective and steady therapeutic concentrations for a prolonged period. Additionally, they are not subject to the adverse environment of the uterine lumen and will not iatrogenically inoculate the reproductive tract, as would occur with repeated vaginal treatments.[220] Disadvantages are increased costs associated with higher volumes used and daily treatments. Less common but no less important risks are antibiotic-induced diarrhea, injection site reactions, thrombophlebitis, and anaphylaxis.

Systemic treatment should either be preceded by or performed concurrently with uterine irrigation to remove luminal inflammatory debris. Irrigation should be performed judiciously to reduce the risk of tracking bacteria into the reproductive tract. Systemic antimicrobials can also be used in conjunction with ecbolics, which are discussed in the following section. To the authors' knowledge, studies comparing the effects of systemic versus intrauterine or other therapies are lacking.

Ecbolics

Ecbolics are used to induce contractions of the uterus to promote uterine drainage. Oxytocin is a neuropeptide produced in the hypothalamus and released by the posterior pituitary. It is frequently given exogenously for its ecbolic effect, and its usefulness for uterine clearance has been previously documented.[268,277] Exogenous treatment is somewhat empiric, but consideration should be given to the stage of estrous

TABLE **19.7 Antibiotic Drugs, Systemic and Intrauterine Doses, and Clinical Considerations for Treatments of Endometritis in the Mare**[a]

Drug	Systemic Dose	Intrauterine Dose	Anticipated Spectrum of Activity	Comments
Amikacin sulfate	10–15 mg/kg, IV, q 24 h	1–2 g, q 24 h	Gram (–)	Buffer with bicarbonate for intrauterine use
Ampicillin	29 mg/kg, IV, q 12 h	1–3 g, q 24 h	Gram (+) and *Escherichia coli*	Dilute in high volumes (~200 mL) to prevent precipitation when using intrauterine dose
Ceftiofur sodium	2 mg/kg, IV or IM, q 12–24 h	1–2 g, q 24 h	Gram (+) and (–)	Dilute with sterile water only for intrauterine use (inactivated in saline)
Ceftiofur crystalline-free acid	6.6 mg/kg, IM, two doses 4 days apart	Not approved or studied for intrauterine use	Gram (+) and (–)	Can be given q 7 days for two more doses and maintain effective endometrial tissue concentrations[b]
Enrofloxacin	5–7.5 mg/kg, IV, q 24 h; 7.5 mg/kg, PO, q 12 h	50 mL of water-based 2.5% suspension, q 24 h[c]	Gram (–)	Only compounded formulations available for PO and intrauterine use
Gentamicin	6.6 mg/kg, IV or IM, q 24 h	1–2 g, q 24 h	Gram (+) and (–)	Buffer with equal volume bicarbonate solution and dilute further in sterile water or saline for intrauterine use
Neomycin	5–15 mg/kg, PO, q 24 h	4 g, q 24 h	Gram (–)	Infrequently used
Polymyxin B	5000 U/kg, IV, q 8–12 h	100,000 IU, q 24 h	Gram (–)	Dilute in 1 L fluids when administering systemically; dilute in 100–200 mL for intrauterine use
Potassium penicillin G	20,000–40,000 U/kg, IV, q 6 h	5 million units, q 24 h	*Streptococcus equi* subsp. *zooepidemicus*	Synergistic with aminoglycosides when given IV; adverse interaction when administered concurrently for intrauterine use[d]
Procaine penicillin G	20,000–40,000 U/kg, IM, q 12 h	5 million units, q 24 h	*S. equi* subsp. *zooepidemicus*	Possible intraluminal residues for intrauterine use
Sulfadiazine and trimethoprim	24 mg/kg, PO, q 12 h	Not approved or available in the United States	Gram (+) and *Nocardioform* species	Emerging resistance of *S. equi* subsp. *zooepidemicus*
Ticarcillin and clavulanic acid	50 mg/kg, IV or IM, q 6–8 h	3–6 g, q 12–24 h	Gram (+) and (–)	Dilute in 60–120 mL sterile water for intrauterine use

IM, Intramuscular; *IV,* intravenous; *PO,* orally.
[a]Adapted from LeBlanc MM. The chronically infertile mare. *Proc Ann Conv Am Assoc Equine Pract*. 2008; 54:391-407.
[b]From Scofield D, Black J, Wittenburg L, et al. Endometrial tissue and blood plasma concentration of ceftiofur and metabolites following intramuscular administration of ceftiofur crystalline free acid to mares. *Equine Vet J*. 2014; 46:606-610.
[c]From Schnobrich MR, Pearson LK, Barber BK, et al. Effects of intrauterine infusion of a water-based suspension of enrofloxacin on mare endometrium. *J Equine Vet Sci*. 2015; 35:662-667.
[d]From Wallace SM, Chan LY. In vitro interaction of aminoglycosides with beta-lactam penicillins. *Antimicrob Agents Chemother*. 1985; 28:274-281.

cycle when implementing a dosing strategy. For example, IV administration of 30 units to estrous mares was shown to reduce uterine contractions compared with lower doses of 5 to 10 units.[278] Interestingly, the density of oxytocin receptors is highest in late diestrus and decreases during estrus and early diestrus,[279] and the uterine response to oxytocin is inversely related to progesterone concentrations.[280] It thus seems prudent to adjust doses with respect to the stage of the estrous cycle. Current recommended doses are 10 to 20 units during estrus and 25 to 40 units after ovulation.[220] Routes of administration include IV, IM, and intrauterine. Oxytocin is not a "silver bullet," and its effects can be limited by anatomic (e.g., closed cervix, pendulous uterus) and mechanical (e.g., fibrosis aberrant contractions) deficits concurrently involving the reproductive tract. Carbetocin, a synthetic analog, has been available in countries outside the United States. Its plasma half-life is over two times that of oxytocin and may be useful in mares that do not respond to oxytocin.[281]

Oxytocin is directly responsible for the release of $PGF_{2\alpha}$ from the endometrium, as evidenced by a spike in its metabolite (PGFM) in the serum shortly after oxytocin administration.[279] Exogenous administration of $PGF_{2\alpha}$ can thus be used clinically as an ecbolic, and research suggests that the synthetic analog, cloprostenol, produces the best response

TABLE **19.8 Intrauterine Therapies and Lavage Fluid Additives**

Agent	Intrauterine Dose		Indications/Comments
NAC, 20%	30 mL in 150–250 mL saline, q 24 h		Mucolytic
Antimicrobial peptides (Ceragyn)	60 mL		Potential biofilm disruptor
Dimethyl sulfoxide (90%)	50 mL in 1 L saline, q 24 h		Mucolytic
Hydrogen peroxide (3% USP)	20 mL qs to 60 mL with saline	100 mL in 1 L lavage fluids	Potential biofilm disruptor, antiseptic, counterirritant
Kerosene	250–500 mL, once		Counterirritant; used for cases of CDE
Lactated Ringer's solution	1–6 L, q 12–24 h		Used for lavage to assist with uterine drainage and clearance of debris
10% povidone–iodine solution	5–15 mL in 1 L saline, q 24 h		Disinfectant
Saline (0.9%)	1–6 L, q 12–24 h		Lavage and diluent fluid
Tricide (EDTA-tris)	250–500 mL, q 24 h		Synergistic effect with aminoglycosides and clotrimazole, used in cases of chronic IE and suspect biofilm production
Vinegar (5%)	100 mL in 1 L saline, q 24 h		Yeast infections

CDE, Chronic degenerative endometritis; *EDTA,* ethylenediaminetetraacetic acid; *IE,* infectious endometritis; NAC, *N*-acetylcysteine; *qs,* as much as needed.

among the $PGF_{2\alpha}$ preparations studied.[282] The primary difference between oxytocin and $PGF_{2\alpha}$ is that the former produces strong and rapid uterine contractions over a relatively short period (~0 minutes). The latter produces lower amplitude contractions over a longer period (4–6 hours). These varying effects should be considered when implementing treatment. It is relatively unknown how much oxytocin and how many contractions are needed to obtain the ideal clinical effect. Dosing of oxytocin usually involves multiple daily dosing, but $PGF_{2\alpha}$ typically involves a single daily dose. There is some evidence that cloprostenol can interfere with early luteal development, but the clinical significance is unknown because fertility is not compromised in mares treated through the second day after ovulation.[283]

Other drugs potentially affecting uterine contractility are the α_2-agonist sedatives and nonsteroidal antiinflammatory drugs (NSAIDs). Detomidine causes contractions in normal non-pregnant mares, whereas NSAIDs, because of their inhibitory action on inducible prostaglandin release, may inhibit uterine clearance.

Lavage and Nonantibiotic Therapies

Uterine lavage or irrigation is an important component in the treatment and management of endometritis. In mares susceptible to PMIE, lavage can be performed before or after breeding. No detrimental effects on pregnancy rates were seen in mares lavaged with LRS before mating.[284] However, it appears that lavage should be withheld for a minimum of 4 hours after mating.[285] In mares with endometritis secondary to other disease processes (e.g., retained fetal membranes [RFMs], metritis, pyometra), lavage can be performed multiple times a day to evacuate septic uterine contents. Isotonic fluids, such as LRS, are preferred when performing uterine lavage because of the more physiologic pH, but saline can be substituted if needed.

Additives are frequently combined with lavage fluids. Examples are listed in Table 19.8. Both betadine and hydrogen peroxide have antiseptic properties, whereas dimethyl sulfoxide (DMSO) and *N*-acetylcysteine (NAC) are used for their mucolytic and antiinflammatory activities. Vinegar is added to fluids to increase acidity, which is commonly done for treatment of yeast infections. These treatments are followed by regular fluids or fluids without additives. Uterine lavage should be repeated daily until the effluent is clear. One common veterinary disinfectant that has been shown to cause substantial irritation to the uterus and should be avoided is a 2% chlorhexidine gluconate (CHG) solution.[286] Dilution to 0.25% CHG has been shown to be less irritating, but sanitizing properties were not evaluated.

NAC is a mucolytic agent that is useful when treating cases of endometritis. Treatment involves diluting a 20% (200 mg/mL) solution in 150 to 250 mL of saline to achieve a 3.3% solution and instilling it into the uterus.[287] This can be done 24 to 48 hours before or 6 to 24 hours after breeding. Serial uterine lavages are often performed 12 to 24 hours after infusion and repeated until the efflux is clear. In a clinical study, mares treated with NAC before breeding had higher fertility rates than those that were not, and treatment had no adverse effects on the endometrium as judged by uterine biopsy.[288]

Kerosene is a clear liquid formed by fractional distillation of petroleum. Primarily used as a fuel, it can also be used as a solvent for removing grease and tenacious mucilage, as well as a pesticide for killing lice and bed bugs. Intrauterine infusion of kerosene in subfertile mares was first reported by Bracher et al.[289] They reported relatively high early pregnancy (87.5%) and live foal (62.5%) rates in subfertile mares treated with kerosene on the cycle before mating. Despite inducing intense inflammation, no long-term side effects, such as cervical or uterine adhesions, were observed in mares treated with kerosene. Kerosene can be used at any stage of the estrous cycle, including diestrus and up to 48 hours before breeding. The only adverse effect observed was mild scalding on the gaskins of a small proportion (<5%) of mares. Mares who have gone more than 1 year barren, who have not responded favorably during multiple cycles of other intrauterine therapies, or who have severe intrauterine fluid accumulation are candidates for uterine infusion of kerosene. Treatment is thought to perform a chemical curettage of the endometrial epithelium, effectively stripping the epithelium of debris, inspissated material, and microorganisms.[290]

Recommendations for the volume of kerosene to be infused varies among theriogenologists. Some use moderate volumes (e.g., 90 mL), whereas others use much larger volumes (500 mL). Because of this rather wide variation in volume, some postulate that contact time with the endometrium may be just as important as (if not more than) the volume of the infusate. Unfortunately, this observation is purely subjective, because no studies exist comparing these variables. Approximately 12 to 16 hours post infusion, uterine lavage with LRS is performed. Lavage should be repeated daily until the effluent is clear. Effluent character can vary from mostly clear (yet strongly odorous of kerosene) to hemorrhagic with the consistency of curdled milk. Caution should be taken when handling and using kerosene because it is flammable, and it is recommended to turn mares out in the paddock overnight after infusion. The authors have found kerosene to be a reliable and successful treatment option in the management of CDE.

Other fluid additives include those targeting potential biofilm infections. Based on the current understanding of biofilm infections, treatment should target both the EPS and the bacteria within the biofilm community. Purported biofilm disruptors include the previously mentioned hydrogen peroxide, DMSO, chelating agents containing *tris*-EDTA, and Ceragyn (Purishield Life Sciences, LLC, Walnut Creek, CA 94597), which is a proprietary and commercially available antimicrobial peptide mimic. All of these agents have shown varying results in vitro for reducing biofilm mass and/or killing organisms within a biofilm.[239]

Long used as an antiseptic, hydrogen peroxide (H_2O_2) has shown a broad spectrum of activity against various microbes, including bacteria, virus, fungi, yeast, and spores. In mares, the recommended dose is 20 mL of a 3% H_2O_2 solution diluted to 60 mL in LRS.[287] An in vitro study demonstrated that 1% H_2O_2 was effective against most pathogens in both their planktonic and biofilm state, except for *P. aeruginosa,* which was capable of inactivating H_2O_2.[239] Bacterial resistance to H_2O_2 has been documented in human cases, but research is lacking in horses.

Buffered chelator solutions have proven useful in cases of refractory bacterial and fungal infections.[291] *Tris*-EDTA has been shown to potentiate the effects of certain antimicrobial agents, presumably by altering cell-wall permeability.[292] Aminoglycosides are one such example, and the two can be mixed together. β-lactam antimicrobials may precipitate in the presence of Tricide, which is thought to inactivate this class of antibiotics. In vitro studies evaluating the use of a similar buffered chelator solution, *tris*-EDTA, showed it to be highly effective on free-floating bacteria.[239] However, *tris*-EDTA was inconsistent in disrupting biofilm production from clinical isolates of *P. aeruginosa*[239]; thus it is by no means a silver bullet for suspected biofilm infections.

Naturally occurring antimicrobial peptides (AMPs) are produced and used by neutrophils to degrade bacteria. Ceragyn (Purishield Life Sciences, LLC, Walnut Creek, CA) is a commercially available wound-healing medical device that is marketed as an AMP mimic. Anecdotally, AMPs need only come in contact with the cell membrane to exert a bactericidal effect. Other attributes of AMPs include a broad spectrum of activity, less risk of developing resistance to microbial defense mechanisms, and direct activity against biofilm. Ceragyn is also being distributed as a uterine lavage device labeled for use either 4 hours before or 6 to 48 hours after breeding. Preliminary studies evaluating its activity against certain biofilm-producing bacteria have been promising. Ferris[239] showed that Ceragyn was effective in degrading biofilm biomass produced in vitro by *E. coli, K. pneumoniae, P. aeruginosa,* and β-strep, and it was also capable of killing β-strep within a biofilm. More research is necessary on this and other products to further evaluate their clinical efficacy. One thing for certain is that none appears to be a panacea for treating chronically dirty mares.

Adjunctive Therapies

Several other therapies exist for the treatment of endometritis. Most can be used quite effectively in conjunction with other therapies. As will be discussed next, some of these therapies are thought to assist with uterine drainage, whereas others act on the immune system to modulate the response to breeding.

Acupuncture

Acupuncture has its origin in Eastern medicine and has been used for a wide range of conditions affecting the mare's reproductive tract. For endometritis, acupuncture is thought to induce uterine contractions to help with the expulsion of intraluminal fluid. A retrospective study involving 44 broodmares showed a reduction in postmating fluid and improved pregnancy rates in mares with a history of susceptibility.[293] Controlled studies are necessary to compare this modality to more traditional treatments such as oxytocin and uterine lavage. Moreover, current protocols and methods used (e.g., "wet versus dry" needling, electrostimulation) are subject to individual practitioner preferences, and measuring response can be confounded by a lack of control subjects. As stated in a review on the use of acupuncture in equine reproduction: "Although acupuncture physiology and efficacy have yet to be thoroughly defined in a traditional Western medicine sense, current research demonstrates a case for continued use as well as further investigation."[294]

Immunomodulators

Influencing either systemic or local immune defense systems has been investigated in broodmares. Corticosteroids have been used to modulate the immune response of susceptible mares, with the primary benefits thought to be reduction in neutrophil margination into the uterus and countering lymphangiectasia. The clinical benefit of dexamethasone was described in a report by Bucca et al.[267] in which they reported improved pregnancy rates in susceptible mares treated with 50 mg (IM) of dexamethasone before breeding. Prednisolone (0.1 mg/kg, PO, b.i.d.) beginning 2 days before breeding has also been used to manage acute inflammation when breeding with frozen semen.[266] Attention should be paid to patient selection and length of dosing schedule because of potential untoward side effects of steroid administration in equids.

Modulation of local defense mechanisms has been explored as a means to improve fertility rates in susceptible mares. More specifically, improving the phagocytic activity of mononuclear cells and reducing the production of proinflammatory cytokines are possible benefits of altering uterine immune systems. Mares treated with a commercially available *Mycobacterium* cell-wall extract showed a quicker reduction in inflammation compared with controls.[295] A similar product containing *Propionibacterium acnes* was shown to increase pregnancy and live foal rates in barren mares treated with this product when combined with conventional management strategies.[296] Use of these agents for endometritis thus appears to warrant more consideration in the management of endometritis, especially PMIE.

Although commonly used to treat orthopedic conditions, regenerative therapies have been explored for their efficacy in the management of endometritis. Foaling mares treated with a combination intrauterine infusion of autologous plasma and antibiotics had higher pregnancy rates than control mares.[297] The purported benefit was thought to be improved opsonization of bacteria, but this treatment has not garnered wide-scale use. More recently, the use of platelet-rich plasma (PRP) was explored. Preliminary studies showed intrauterine infusion of PRP caused downregulation of inflammatory cytokines and improved pregnancy rates in susceptible mares.[298,299] Researchers at Colorado State University evaluated the use of autologous conditioned serum (ACS) and mesenchymal allogeneic stem cells (MSCs) in reproductively normal mares.[238] Mares treated with ACS 24 hours before a dead-sperm challenge had significantly lower neutrophil counts 6 and 24 hours after this challenge compared with control mares. Those treated with MSCs had significantly lower neutrophil counts 6 hours after sperm challenge relative to controls. More research and clinical studies are necessary to explore further the efficacy and indications for these treatments. Given the current costs, resources necessary, and foresight required to implement these therapies, they currently seem more of a novel or stopgap therapy in cases in which conventional therapies have repeatedly failed.

Exercise

The authors are advocates of providing breeding animals with as much exercise as possible. Farm and ranch management, climate, and overall health of the mare are factors dictating the type and amount of exercise. Farm practices in central Kentucky are such that horses are turned out in large multiacre fields with other broodmares for significant portions of the day. Turnout during inclement weather is at the discretion of the farm, but most horses are much more resilient to adverse environmental conditions than thought. Potential benefits of exercise include toning of the hindquarters to improve perineal conformation and improved uterine clearance associated with locomotion. In addition, exercise and socialization engage horses both mentally and physically. In humans, physical fitness has proven health benefits in staving off the ill effects of advancing age, which is the most significant factor affecting reproductive efficiency in broodmares.[226]

Supplements

A number of nutritional supplements are available to be fed to horses, some of which have either documented or anecdotal evidence supporting their use. Supplementation with omega-3 fatty acids (OM3FAs) has become popular, and a small body of research supports their use. Supplementing the diets of mares and stallions with OM3FAs has improved certain measures of reproductive performance. For example, mares fed a commercially available supplement containing docosahexaenoic acid (DHA)/OM3FA (Releira, Arenus, Fort Collins, CO 80524) was associated with a reduction in endometrial cytokine expression in mares bred with frozen semen as compared to control mares.[300] Supplementation with OM3FAs has also been postulated to improve follicular and luteal health to support a healthy pregnancy, but these assertions are in need of further study. With respect to stallions, those fed a DHA-enriched nutraceutical showed improved seminal quality judged by higher progressive motility after 24 and 48 hours of test cooling as well as significantly higher postthaw motility following freezing, compared with control stallions.[301] Although not yet fully evaluated, the use of OM3FAs may help decrease inflammation and improve the chance of maintaining a viable pregnancy.

Other supplements include antioxidants and hormone mimics. Antioxidant therapy may be beneficial in staving off sperm membrane damage from free radicals in the seminal plasma or uterus. Hormone-mimicking agents are purported to enhance breeding performance by improving libido, increasing sexual arousal, or removing inhibitions. Peer-reviewed and independent studies supporting the use of many of these products are lacking, so further research is necessary to determine the precise value of these products. Furthermore, caution should be exercised in using or mixing supplements because of potential adverse interactions.

FUNGAL ENDOMETRITIS

Endometritis secondary to colonization of the uterus with fungi is an uncommon (1%–5% of cases) but highly frustrating clinical condition in mares.[302,303] Fungal pathogens most commonly identified from uterine cultures are *Candida* spp. and *Aspergillus* spp. However, other organisms have been reported. For example, *Cladophialophora bantiana* was identified in one case report of a 15-year-old Standardbred mare that was subsequently euthanized because of the zoonotic potential of the pathogen.[304] Skin and feces are the most common sources of fungi, but fungal elements have also been found in the urethral fossa and penis of stallions, leading to potential (although unlikely) venereal transmission.[270,305] Factors predisposing mares to fungal endometritis include prolonged intrauterine antibiotic therapy, immunosuppressive therapy, endocrine disease, or foaling trauma.[270] The diagnosis of fungal endometritis is made based on a combination of aerobic culture and cytology, as described in a previous section. The presence of budding yeasts (Fig. 19.23) and/or branching hyphae

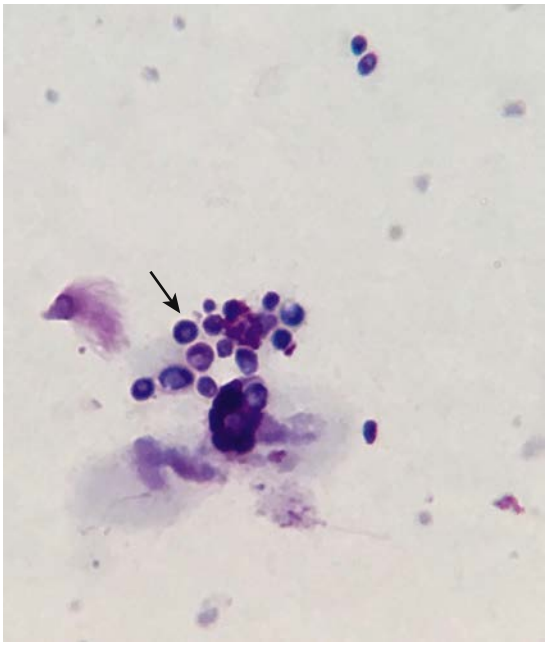


FIG. **19.23** Uterine cytology demonstrating the presence of budding yeast organisms using a modified Wright-Giemsa stain. (Courtesy C.F. Scoggin).

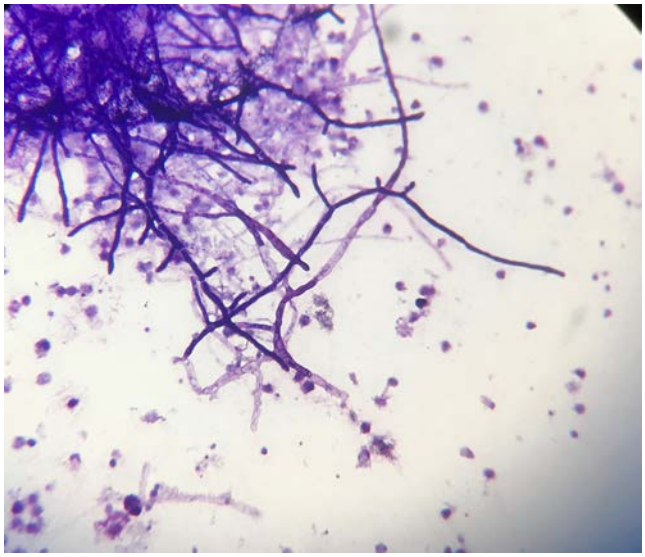


FIG. **19.24** Endometrial cytology from a mare demonstrating numerous and prolific branching fungal hyphae using a modified Wright-Giemsa stain. (Courtesy C.F. Scoggin).

(Fig. 19.24) is diagnostic for fungal endometritis. Endometrial biopsies have also been used to diagnose and/or confirm more invasive forms of fungal endometritis when cytology is inconclusive. Other methods, such as quantitative PCR, have been developed to detect and identify fungal DNA in equine endometrial samples. These can be used in conjunction with cytologic and/or histologic samples to confirm a diagnosis and further quantify the organism(s) present.[306]

Treatment for fungal endometritis has historically been based on individual case studies using empiric dosing schedules. Myriad treatments are available, but none of them appear to be particularly effective or carry a broad spectrum of activity. Various antifungal agents and their dosing regimens are provided in Table 19.9. Antifungal drugs are typically divided into two main categories: polyenes and imidazoles. Polyene antibiotics include amphotericin B, natamycin, and nystatin. The imidazole drugs include clotrimazole, ketoconazole, and miconazole.[307] Triazoles (fluconazole and itraconazole) are another group of synthetic antifungals used to treat yeast infections. Based on a retrospective study by Beltaire et al.,[308] polyenes are the best first choice for treatment of yeast infections (100% in vitro susceptibility), but care must be taken when using these antifungals because of their potential cytotoxicity. Consequently, these drugs are typically used locally as intrauterine infusions. Polyenes were also effective against most molds (69%–100%), whereas the imidazole drugs were only marginally effective (38%–46%) against molds and septated hyphae. All mold isolates were resistant to fluconazole. Reports such as these are beneficial for improving the efficiency and, potentially, the success of treatment. Moreover, although fungal endometritis is an uncommon problem, it is an area of research that could benefit from prospective studies that correlate clinical outcomes with fungal culture and treatment approach.

Another treatment for fungal infections often used in conjunction with antifungal agent(s) is uterine lavage with white wine vinegar. The acetic acid component can decrease the pH of the uterine environment, inhibiting growth of planktonic fungi. Typically used in an in-and-out fashion, regular fluids (e.g., LRS) are used for irrigation until the effluent is clear. The chitin inhibitor, lufenuron, has also been used to treat fungal infections.[309] Instillation of 540 mg of this drug diluted in 60 mL of physiologic saline has been used to resolve colonization of the uterus by both *Candida* spp. and *Aspergillus* spp. Despite these promising results, however, this treatment failed to develop steam as a viable treatment. Currently, treatment of fungal infections is deemed difficult, and resolution usually involves both a lengthy treatment schedule and tincture of time.

CONCLUSION

Endometritis is an important and ongoing issue in broodmare management. Numerous causes of endometritis have been identified, and the disease may manifest itself in many ways. No matter how innovative or novel a treatment is, it cannot replace the importance of first performing a thorough physical and reproductive examination. Careful evaluation of history, stallion fertility, perineal conformation, and reproductive organs will help identify problem areas that may need to be addressed before endometrial treatment. Goals of management include assisting with uterine drainage/clearance, addressing anatomic defects, implementing appropriate antimicrobial therapy, modulating the inflammatory response, and using other means to create a hospitable environment for sperm transport and a developing conceptus.

Hormonal Manipulation of the Mare

Carleigh E. Fedorka, Maria R. Schnobrich

This section will describe how hormonally based therapeutics are used to optimize reproductive efficiency in the mare. The background physiology and underlying targeted response or goal of each treatment will be described. The reader is referred to previous sections in this chapter for a review of the natural hormonal targets and physiology of the nonpregnant and pregnant mare.

VERNAL TRANSITION

Mares are seasonally polyestrous breeders, and during winter or shortened periods of daylight increased melatonin secretion from the pineal gland inhibits the release of GnRH. Because of this, the majority of mares experience a period of reproductive senescence when daylight is abbreviated. As day length increases in the early spring in the Northern Hemisphere, the suppression of the HPG axis is downregulated. The mare then enters the "vernal transition," which is characterized by a marked increase in FSH with low levels of luteinizing hormone (LH) and, therefore, a lack of ovulation as described previously.

Many hormonal regimens have targeted this transitional phase and are aimed at shortening the anovulatory period and hastening the onset of normal cyclicity. Increasing the exposure to light (to 14.5 hours a day, equivalent to a 100-W bulb in a stall, or "enough light to read a newspaper") beginning approximately 60 days before the start of the targeted first ovulation has proven to be the most widely used and effective means of shortening the time to the first ovulation of the season.[310] This

TABLE **19.9 Antimycotic Drugs, Doses, and Clinical Considerations for Treatment of Endometritis in the Mare**

Drugs	Systemic Dose	Intrauterine Dose	Anticipated Spectrum of Activity	Comments
Amphotericin B	0.3–0.9 mg/kg, IV, q 24–48 h	100–200 mg, q 24 h	Broad spectrum	Dilute to 1 mg/mL in 5% dextrose and administer over 1–2 h
Clotrimazole	Not reported	500–700 mg, q 24 h	Broad spectrum	—
Fluconazole	Loading dose of 14 mg/kg then 5 mg/kg, PO or IV, q 24 h	100 mg	Yeast	—
Itraconazole	5 mg/kg, PO or IV, q 12–24 h	Not reported	Yeast, *Aspergillus* spp., and dimorphic fungi	—
Ketoconazole	20 mg/kg (in 0.2 N HCl), NGT, q 12 h	Not reported	*Candida* spp.	Must be given via NGT to avoid irritation from acid
Lufenuron	Not reported	540 mg qs 60 mL with saline	*Candida* spp.	Chitin inhibitor
Miconazole	Not reported	500–700 mg, q 24 h	Broad spectrum	—
Nystatin	Not reported	0.5–2.5 million units, q 24 h	Yeast	Dilute in sterile water to avoid precipitation

HCl, Hydrochloric acid; *IV,* intravenous; *NGT,* nasogastric tube; *PO,* orally; *qs,* as much as needed.

is achieved through the use of outdoor paddock lighting, the stabling of horses for increased periods under lights, or the addition of lighted masks.[311] Along with the use of lights to effect the photoperiod, hormonal manipulations are often used in conjunction to hasten the onset of normal cyclicity.

Dopamine is secreted in synapses between dopaminergic neurons in the median eminence of the hypothalamus and hypothalamic GnRH neurons. Research has shown that inhibition of dopamine (D2 dopamine receptors) increases LH secretion, although the exact mechanism is unclear. It is believed to act by removing the inhibition on PRL secretion and possibly through the reduction on the negative feedback of estradiol on GnRH.[312] Many dopamine antagonists including sulpiride, domperidone, and perphenazine have been tested, but inconsistencies in dosage, treatment onset, and duration of studies have caused high variability in the outcomes of these studies, making comparisons difficult.

Domperidone significantly hastens the time to first ovulation (51 days vs. 129 days for controls).[313] When begun on January 15, and without the addition of a lighting program, treatment with domperidone results in increased LH, estradiol, and PRL concentrations, and six of eight treated mares resumed normal cyclicity after treatment. Perphenazine, another dopamine antagonist, is also able to shorten the anovulatory period by approximately 30 days when administered at a dosage of 0.08 mg/kg daily when used without the addition of a lighting program.[314]

Sulpiride is another commonly used dopamine antagonist. In a study by Mari et al. its effectiveness was compared with that of domperidone.[315] Mares were treated with 1 mg/kg sulpiride (Championyl), 1 mg/kg domperidone (Motilium), or a control daily for 25 days without the addition of a lighting program. Sulpiride significantly decreases the length of time to the first ovulation (36.9 days) compared with both domperidone (74.7 days) and control (81.4 days), and it hastens the establishment of pregnancy by an average of 20 days.[315] In a second study, mares were treated with sulpiride once the presence of a 25-mm follicle was noted. Daily treatment of 1 mg/kg sulpiride results in a hastened follicular development.[316]

Another strategy for manipulating the vernal transition is through the supplemental use of progestogens, in particular altrenogest. The mechanism of action is believed to be caused by negative feedback on the hypothalamic-pituitary axis, which when removed, results in an increase in LH. Daily administration of 27 mg of altrenogest (Regu-Mate) when a 20-mm follicle is present significantly decreases the length of time to ovulation compared with controls.[317]

Additionally, first ovulation can be hastened by use of GnRH analogs. Equine follicle-stimulating hormone (eFSH) at a dose of 12.5 mg IM (Bioniche) twice daily has been shown to hasten the time to first ovulation when administered to transitional mares once the presence of a 25-mm follicle is noted.[318] In addition, recombinant eFSH (reFSH) has been developed, and when it is administered at a dosage of 0.65 mg twice daily, it is able to stimulate follicular development in anestrous mares under lights. Unfortunately, these mares did return to anestrus after treatment and ovulated naturally at a similar time as control mares.[319] It can be difficult to reliably obtain eFSH; therefore this protocol, while effective, is not frequently used. Another protocol using a GnRH agonist consists of administration of 25 μg of buserelin twice daily (SQ) once the mare has developed a 20-mm follicle until ovulation occurs.[320] Ovulation induction is achieved in most GnRH analog protocols with the administration of human chorionic gonadotropin (hCG) at varying dosages of 1500 to 3000 IU, either IV or IM, once a follicle has reached the size of 35 mm or greater.[321]

SYNCHRONIZING THE ESTROUS CYCLE

To optimize breeding efficiency, the ability to predict estrus and ovulation becomes an important tool to successful reproductive management. Although mares show variability in their response to hormonal treatment, the concept of fixed-timed insemination, or "appointment breeding," has been successful in equine reproduction. Common reasons for estrous

synchronization include synchronization of a group of mares (e.g., in an embryo transfer program), manipulation of ovulation timing to best fit a popular stallion, limited show schedule for breeding, and strategic management of insemination with shipped or frozen semen.

To manipulate the estrous cycle of the mare with some precision, one must understand the basic endocrine events of the normal 21- to 22-day cycle of the mare as previously reviewed.[4] The day of ovulation is considered to be day 0 and is preceded by a period of estrus of variable length (5–7 days).

Most hormone regimens used to synchronize estrus are aimed at manipulating and shortening the luteal phase (diestrus) of the cycle. Perhaps the simplest method of synchronizing the cycle of a mare is through the use of synthetic preparations of $PGF_{2\alpha}$, which effectively and reliably shorten diestrus through inducing luteolysis and removal of the source of progesterone production. There was previously a belief that one had to wait until day 5 post ovulation to reliably induce luteolysis with a single dose of $PGF_{2\alpha}$.[322,323] Research has demonstrated that repeated doses of $PGF_{2\alpha}$ administered after ovulation prevented CL formation, and breeding on the subsequent estrus was not associated with a decrease in pregnancy rates.[324] The size of the follicle at the time of prostaglandin administration often dictates the duration to ovulation, with larger follicles ovulating sooner.

It is critical to evaluate the follicles present at the time of $PGF_{2\alpha}$ administration for two reasons: (1) to note if the mare has a large follicle, and therefore reexamination and breeding can be scheduled appropriately, and (2) because inducing estrus and breeding on a large diestral follicle is not optimal and waiting until the follicle regresses may be more ideal. Mares that were administered prostaglandin when follicles were larger than 30 mm had an approximately 20% lower per cycle pregnancy rate than mares bred on a natural estrus.[325] It has also been demonstrated that treatment of mares with a $PGF_{2\alpha}$ analog on day 0 (ovulation) or day 1 has a negative effect on the developing CL. Not only are the size and echogenicity of the CL reduced, but also a transient and significant suppression in progesterone levels occurs. Most important, pregnancy rates may be lower in treated mares.[326-328] Treatment with $PGF2_{\alpha}$ analogs increases the risk of AHFs, and this risk should be taken into consideration, most critically in mares that may be predisposed to developing AHF.[329]

A number of $PGF_{2\alpha}$ preparations have been used successfully for luteolysis in the mare. Side effects associated with $PGF_{2\alpha}$ administration include sweating, anxiety, diarrhea, and signs of colic. The severity of the side effects depends on the dose and preparation of $PGF_{2\alpha}$. A two-dose regimen at a much lower dose (0.5 mg $PGF_{2\alpha}$) is equally effective in inducing luteolysis as a higher single dose (50 mg $PGF_{2\alpha}$) but devoid of adverse effects.[330] Similarly, a single dose of 25-µg cloprostenol (Estrumate; Bayer Corp., Shawnee, KS), that is one tenth of the previously recommended dose, is effective in inducing luteolysis and does not cause adverse side effects.[331] Generally most practitioners use 10 mg of dinoprost (Lutalyse) or 250 µg of cloprostenol 5 days post ovulation. Cloprostenol is associated with fewer side effects and has also been shown to be as effective at inducing luteolysis as dinoprost, although cost may factor into the decision of which drug is used.

Treatment with progestogens with or without estrogens represents one of the oldest and most reliable means of synchronizing estrus in the mare, especially when coupled with appropriate light exposure and $PGF_{2\alpha}$ administration. Exogenous administration of progesterone simulates the diestrus period of the estrous cycle. Repositol progesterone, progesterone in oil (Sigma Chemical Co., St. Louis, MO), or oral progestin compounds (altrenogest [Regu-Mate], Intervet Inc., Millsboro, DE) are effective in suppressing estrus but not necessarily effective for inhibiting follicular development or ovulation.

When synchronizing mares, one protocol that is used commonly is to administer progesterone for a minimum of 10 days in cycling mares and 14 days in transitional mares. On the last day of progesterone treatment, a $PGF_{2\alpha}$ injection is administered to induce luteolysis of any remaining CL. As in treatment with prostaglandins alone, the mare should be evaluated at the time of prostaglandin administration because follicle growth may still occur with progestogen administration. Scheduling for breeding and predicting ovulation can be made based on the follicle size at the time of induced luteolysis. Generally it is believed that most mares come into heat 3 to 5 days after the last treatment of progesterone and ovulate an average of 7 days after treatment, but this depends on the size of the follicles present on the ovary at the time of luteolysis.

Addition of estradiol to a synchronization protocol inhibits follicular growth and allows better synchrony of ovarian structures at the time of luteolysis. Often referred to as "P and E" or "programming," this is done by administering daily injections (IM) of 10 mg of estradiol-17β and 150 mg of progesterone once the presence of a 20-mm follicle is noted. On the tenth day, the mare is administered $PGF_{2\alpha}$ and is evaluated at this time with transrectal ultrasound. Breeding is recommended once a dominant follicle is greater than 35 mm, and this tends to coincide with day 19 from the start of treatment. This protocol is considered superior by some because the addition of estradiol inhibits follicular activity; thus, at the time of prostaglandin administration all mares on this protocol should have minimal follicular activity and ovulate in a similar time period.

The oral progestogen preparation altrenogest is perhaps still the most popular progestin compound for use in mares. Altrenogest is easy to administer, not painful, and has few adverse effects. Although most commonly used for estrus suppression, it does not inhibit follicular development; therefore, it is rarely used when attempting to synchronize a mare's follicular growth. Injectable preparations of repositol altrenogest may be effective in suppressing estrus from a behavior standpoint, but they have not been thoroughly investigated for their ability to effectively synchronize follicular growth and ovulation.

For several reasons, all progestogen preparations should be used with caution in mares that are susceptible to delayed uterine clearance or uterine infections. Progesterone increases the tone of the cervix, and its use may inhibit the physical clearance of fluid. Progesterone has also been associated with lower immunoglobulin levels (especially IgG) in the uterus.[332] Thus the combination of adverse effects of most progestogens can lead to delayed uterine clearance, endometritis, pyometra, and other undesirable reproductive conditions.

SUPPRESSION OF ESTRUS

Although most hormonal regimens act to hasten the interval to estrus and ovulation, there are commonly requested behavioral and managerial reasons to suppress estrus. Altrenogest was designed to prevent show mares from exhibiting unwanted

estrus during performance, and it has proved to be effective in this regard, suppressing signs of estrus in 95% of mares that were given a daily dose of 0.044 mg/kg for 3 days. In an attempt to find a more economic and less time-consuming means of suppressing estrus in mares, progestin-estradiol implants (Synovex; Syntex Animal Health, Des Moines, IA) licensed for food animals have been investigated in mares. Although some mare owners report an improvement in the behavior of the mare, scientific reports have been less supportive of their use. McCue et al. found that mares treated with these implants did not show suppression of estrous behavior and cyclicity, even at 20 times the usual dose.[333] Vanderwall et al. have examined the effect of oxytocin administration during diestrus on prolonging time to luteolysis.[334] Mares that received oxytocin (60 IU twice daily IM) on days 7 through 14 after ovulation demonstrated prolonged luteal function up to 30 days after ovulation, suggesting that oxytocin administration may offer a means of long-term suppression of estrus. Medroxyprogesterone has also been suggested anecdotally to suppress estrus, but this has not been substantiated by any research. It was found to have no suppressive effects on follicular activity, although anecdotally many trainers claim benefits.[335]

Immunization against GnRH is another effective means of suppression of follicular development in the mare. When considering this treatment, it should be noted that a subset of mares may show estrus or sexual receptivity despite the absence of follicular activity. Mares vaccinated twice, 4 weeks apart, with a GnRH vaccine (Equity; Pfizer Animal Health, Sydney, Australia) did not return to follicular estrus during that breeding season but returned to cyclicity the next year. Foaling rates were not significantly different from control mares.[336] The vaccine is not commercially available worldwide, and there are reports of long-term infertility following vaccination.[337] Another form of immunization as a means for inhibiting fertile estrus is through immunocontraception. Joone et al. demonstrated that vaccinating pony mares with porcine zone pellucida (pZP) causes an extended anestrus in 86% (six out of seven) of mares and inhibited pregnancy in all vaccinated animals.[338] In addition to its suppression of follicular development, it was determined that this treatment was reversible, because all mares returned to normal cyclicity within 10 months post treatment. Unfortunately, the pZP vaccine is not available commercially, and these results should be critically reviewed due to small study size.

Another option for suppression of estrus is the BioRelease formulated progesterone or altrenogest (BET Pharm, Lexington, KY). These injectable drugs have been effective for estrus suppression using either a vehicle with a delayed release of hormone or microparticle formulation (used in absorbable sutures). A study by Storer et al. showed that altrenogest suspended in either the sustained-release vehicle at a dosage of 225 mg or 500 mg altrenogest suspended in the lactide-glycolide microparticle significantly increases the interovulatory interval (3.9 days from Lutalyse to ovulation compared with 33.5 days when the microparticle formulation was administered).[339]

Theoretically, if the LH surge has not yet occurred, then oral progestogen treatment should inhibit LH release and delay ovulation. Altrenogest at label dose or double the label dose is effective in delaying ovulation compared with control mares if an ovulation induction agent has not been administered.[340] Intraluminal fluid retention, possibly caused by decreased uterine contractility, has been observed in some treated mares.

Delaying the interval to first ovulation of postfoaling mares results in higher pregnancy rates for the first heat cycle. Although some breed on this "foal heat," it has been consistently demonstrated that delaying breeding until the second estrus, or "30-day heat," post foaling is associated with higher pregnancy rates and decreased pregnancy loss.[39] Breeding on foal heat or before 10 days post foaling is associated with an increase in pregnancy loss, which is likely because of a more hostile uterine environment, though this may be breed dependent.[39,341,342] To optimize the maintenance of those early pregnancies yet still increase the reproductive efficiency of the mare by breeding her back as soon as possible, delay of the first postpartum interval to ovulation has been attempted. Due to some of the adverse effects of progestogen administration this can be accomplished by delaying administration of an ovulation-inducing agent. The combination of 150 mg of progesterone and 10 mg of estradiol-17β (E2-17β; Sigma) has been suggested to prolong ovulation until at least day 10 post foaling.[343] Most experienced practitioners will evaluate the mare critically post foaling to determine whether a foal heat breeding is appropriate (additional time needed for involution, breed practice, availability of stud, pregnancy to carry, or embryo transfer), and this protocol is rarely used.

INDUCING OVULATION

Induction of ovulation is useful for assuring timely ovulation relative to insemination, to synchronize mares, and to shorten estrus length. Limitations of semen availability and longevity include stallions that are available only one day for live cover, semen being limited to a strict shipment schedule for cooled shipped semen, and the small time window of semen viability when using a single dose of frozen semen. A number of ovulation-inducing agents have been investigated and are used in practice. The most effective agents possess LH activity with varying degrees of FSH activity. The hCG (Chorulon; Intervet, Millsboro, DE), with its potent LH-like activity, is currently the least expensive and perhaps the most popular agent used for the induction of ovulation. Given IM, IV, or subcutaneously, hCG reliably induces ovulation within 48 hours in mares with a 35-mm or larger follicle, and it is effective at doses ranging between 1000 and 5000 IU.

Use of hCG in the mare has some limitations. First, with its repeated use, antibody development may occur.[344,345] Second, mare owners often complain about the apparent pain associated with administration of some hCG products, although this is a rare occurrence. Finally, the reliability of hCG in its ability to hasten the interval to ovulation, especially in the older or compromised mare, has been questioned. Regardless of its potential disadvantages, hCG remains a popular, inexpensive, and effective means of inducing ovulation in most mares.

Synthetic GnRH analogs also have proven to be effective in inducing ovulation because they cause endogenous release of LH, prompting ovulation.[346] Examples of synthetic GnRH analogs are deslorelin acetate (Ovuplant; Fort Dodge, Canada), buserelin (Intervet; Millsboro, DE), and Cystorelin (BET Pharm, Lexington, KY).[327-348] The use of Ovuplant, when left in place, has been associated with delayed return to estrus and in some cases may inhibit follicular activity for prolonged periods (anecdotally up to 3 months), but removal of the implant a few days after insertion can ameliorate this delay. Injectable deslorelin is available through compounding companies in the United States. One option that is approved in the United States

by the Food and Drug Administration is the injectable SucroMate, which contains 1.8 mg/mL deslorelin acetate. SucroMate has been shown to effectively induce ovulation within 48 hours when 1 mL is injected IM once the mare has developed a follicle larger than 30 mm. In a large clinical study done by Ferris et al., a higher number of mares ovulated when treated with SucroMate compared with hCG, and there was no difference in the interval of time between injection and ovulation for either treatment.[349] The general impression of practitioners is that the GnRH analogs are more reliable in inducing ovulation, especially in mares that are prone to ovulation failure (e.g., older mares, mares in vernal transition, mares concomitantly treated with prostaglandin-inhibiting agents such as many antiinflammatory drugs).

Ovulation-induction agents are reliable and effective only if given at the appropriate time during estrus. If endometrial edema and a large dominant softening follicle (>35 mm in diameter) are present, then hCG, deslorelin acetate, and buserelin are expected to induce ovulation in an average of 36, 41 to 48 hours,[344] and 24 to 48[348] hours, respectively, after administration. Samper reported that 98% of mares with maximal endometrial edema given hCG or deslorelin consistently ovulated within 48 hours of administration.[350] Most remarkably, most mares to which Ovuplant had been administered at the proper time ovulate 36 to 40 hours later, facilitating timely ovulation relative to a scheduled breeding.[351]

SUPEROVULATION

In an effort to increase the number of offspring produced in the lifetime of an animal or in any given season, researchers have investigated numerous means of superovulating to enhance the number of oocytes available for fertilization. Overall, attempts at reliable superovulation in mares have not been as successful as those in the food animal industry, and increasing the number of ovulations and the gain regarding embryos recovered have been minimal. Unlike the swine and bovine models, the administration of porcine FSH preparations is ineffective in mares.[352] Stimulation of endogenous FSH secretion by indirect and direct immunization against inhibin has not resulted in a superovulatory response in mares.[353] The administration of equine pituitary extract preparations in research trials increases the number of embryos recovered from 0.5 per cycle to 2.2 per cycle but with high variability among mares.[354,355]

Promising results have been achieved with the use of eFSH (Box 19.1). Treatment of cycling mares with eFSH in early diestrus (12.5 mg, administered twice daily) increased the pregnancy rate compared with untreated control mares (1.8 vs. 0.6, respectively).[356] In another study, in which Welsh et al. incorporated a "coast" period into the previously described experimental design, the ovulation and embryo recovery rate were 3.8 ± 1.2 ovulations and 1.7 ± 1.4 embryos, respectively.[357]

Because of the inability to obtain eFSH on the commercial market, investigators began examining recombinant proteins. The combination of recombinant LH (reLH) and reFSH proved efficient at stimulating numerous ovulations.[358] Although the protocol is intensive, it appears to be the most efficient in stimulating numerous ovulations. Treatment commences when a 22- to 25-mm follicle is noted, with IM injections occurring twice daily (1.3 mg reLH/0.65 mg reFSH). Luteal regression is stimulated by $PGF2_{\alpha}$ administration on the second day of reFSH treatment. Three days after the onset of treatment, reFSH dosage was reduced to once daily injections until a follicle of greater than 29 mm was present. This was followed by 1.3 mg reLH being administered three times daily until the majority of the follicles reached 35 to 38 mm, at which time hCG was administered to induce ovulation. Compared with only reFSH treatment, this dual treatment of reFSH/reLH significantly increased the number of ovulations and number of embryos retrieved but did not increase the number of anovulatory follicles observed.[358] The incidence of anovulatory follicles has been a concern in the majority of other superovulation protocols. Unfortunately, neither of the recombinant proteins is available commercially.

BOX 19.1 Recommended Protocol for Superovulation in Mares Using Equine Follicle-Stimulating Hormone

1. Determine the day of ovulation.
2. Monitor follicular development in early diestrus.
3. Begin eFSH therapy (12.5 mg IM twice daily) when the largest follicle is 22 to 25 mm in diameter.
4. Administer cloprostenol (259 μg IM) on the second day of eFSH therapy.
5. Discontinue eFSH treatment when follicle(s) are greater than 32 to 35 mm in diameter.
6. Administer hCG (2500 IU) to induce ovulation.
7. Breed or inseminate according to standard procedure.

eFSH, Equine follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *IM,* intramuscular.

Adapted from McCue PM, LeBlanc MM, Squires EL. eFSH in clinical equine practice. *Theriogenology.* 2007; 68:429-433.

CERVICAL DILATION

One of the more challenging conditions encountered when breeding mares is failure of the cervix to relax during estrus. Although not objectively studied, this condition is more common in older maiden mares and certain breeds. However, even normal maiden mares may not have the same degree of cervical relaxation during estrus that is typical of multiparous mares. This condition is also seen in mares with cervical trauma, adhesions, and fibrosis, which prevent normal relaxation. On transrectal palpation, the cervix may feel toned instead of soft in estrus, and transrectal ultrasound of the cervix may show increased echogenicity of the cervix, which can be evidence of fibrosis. Often it is not clear until the periovulatory period that this pathology exists, and it frequently results in fluid accumulation and increased incidence of PMIE.

Although this condition does not prevent breeding, it can prevent dilation of the cervix by the penis of the stallion in natural cover and the normal evacuation of fluid in the hours after breeding, setting the stage for delayed uterine clearance and persistent endometritis. A synthetic PGE_1 analog, misoprostol (Cytotec; Searle Corp., Chicago, IL), has been used successfully in women for cervical dilation. For years, clinicians have used this therapeutic to stimulate cervical relaxation in the horse and gotten promising results, but a controlled study by McNaughten et al. demonstrated that the application of misoprostol directly to the external cervical os had no effect on the tone, length, height, or degree of relaxation.[359] This treatment is still being used clinically, both to stimulate

cervical relaxation for breeding and during the induction of parturition, with successful anecdotal results. In addition, *N*-butylscopolammonium bromide combined with sterile gel and applied to the cervix has anecdotally resulted in cervical relaxation. In addition to relaxing the cervix around the time of breeding, these agents have also been used to soften the cervices of pregnant mares in preparation for induced parturition at various stages and may help prevent cervical trauma.[360]

OVIDUCTAL CONTRACTION

The early embryo secretes PGE_2, which is believed to play a role in the contractility of smooth muscle of the oviduct and allow selective transport of the embryo into the uterine lumen.[8] PGE_2 is also secreted during the high-mobility phase of the embryo within the uterus and is suspected to stimulate uterine contraction and enhance uterine tone.[361] Laparoscopic application of PGE_2 along the serosal surface of the oviduct can modify embryo transport. Interestingly, not only is embryo transport hastened, but also the unfertilized oocytes that are rarely recovered in the mare are released. Laparoscopic-guided PGE_2 application on the mare's oviduct has been reported to assist in clearing oviductal debris and improve the fertility in a small subset of mares suspected of having oviductal pathology.[362]

UTERINE CONTRACTILITY

One of the most common hormones used to induce uterine contractility is oxytocin. Naturally produced by the hypothalamus and stored in the posterior pituitary, it induces uterine contractions after sexual intercourse and during labor, aids in involution of the uterus, and promotes social bonding. Synthetic oxytocin increases intrauterine pressure in a dose-dependent manner from 2.5 to 10 IU in reproductively normal mares and mares suffering from delayed uterine clearance.[194] This is affected by the day of the cycle in which oxytocin is administered, with the periovulatory phase being more influenced by exogenous oxytocin than the postovulation phase.[280] This appears to be affected by season, because anestrous mares that have been primed with progesterone show minimal contractile response when oxytocin is administered, although uterine tone increases. Clinically, oxytocin is used to facilitate uterine clearance in mares suspected of experiencing abnormal fluid accumulation. When administered at a dosage of 10 to 20 IU IM two to four times daily in the periovulatory period, it is shown to be effective in promoting uterine clearance. It is recommended that exogenous oxytocin not be administered before 4 hours after breeding—and not immediately before breeding—to avoid any negative effect it may have on sperm transport to the oviduct.

The combination of PGE_2 and oxytocin is also used to induce parturition in the late-gestation mare. Mares pretreated with 2.0 mg of intracervical PGE_2 6 hours before IV administration of 15 IU oxytocin have increased cervical diameter, a decreased necessity for repeated oxytocin injections, and a hastened time to parturition compared with mares lacking PGE_2.[360] Mares pretreated with PGE_2 before oxytocin injection also produce foals that stand and nurse more swiftly than mares that are induced solely by oxytocin. Clinically, the addition of PGE_2 to the protocol has shown anecdotal evidence of hastening parturition as well as reducing the necessary dosage of oxytocin required to stimulate parturition.[363]

Prostaglandins have also been shown to induce uterine contractions, and the administration of the synthetic $PGF_{2\alpha}$ analog cloprostenol at a dosage of 250 µg has been shown to be effective at inducing more coordinated, longer effects than oxytocin (>6 hours compared with 20 minutes, respectively). The use of cloprostenol was also shown to be more effective at improving uterine clearance in a subset of mares.[282]

HORMONAL SUPPORT OF PREGNANCY

The equine pregnancy differs from other species in that progesterone is almost undetectable in the serum of pregnant mares in the latter half of pregnancy. Instead, in the second and third trimesters, the fetal-placental unit produces high concentrations of metabolites of progesterone, deemed progestogens, which inhibit uterine contractions and maintain cervical closure, promoting the optimum uterine environment for pregnancy. Despite conflicting evidence regarding the benefits of progestogen supplementation, progestogen supplementation throughout gestation is common. Recent studies have further elucidated the hormonal regulation of pregnancy and the proper supplementation protocols and should be reviewed by practitioners and breeders alike. This will also be discussed in more detail in the following sections describing management of the pregnant mare.

In the horse, the initial source of hormonal support for pregnancy is the progesterone derived from luteal cells of the primary CL. Progesterone secretion is sustained for nearly 30 days post ovulation because of the poorly understood mechanism of maternal recognition of pregnancy and subsequent suppression of luteolysis. A secondary surge of progesterone occurs roughly 35 days into gestation in response to the formation of endometrial cups, which actively synthesize eCG. Because of its dual LH and FSH functionality, it stimulates follicular growth and development in addition to ovulation and/or luteinization.[364] It is because of this dual functionality that accessory CLs are able to form and continue the luteal synthesis and secretion of progesterone. This increase in progesterone lasts until approximately 120 days of gestation, when the endometrial cups are destroyed by an endometrial lymphocytic immune response.

At approximately 120 days of gestation, the steroidal regulation of pregnancy transfers from ovarian to fetoplacental. At this time, uterine quiescence is thought to be maintained by metabolites of progesterone and pregnenolone, commonly referred to as the progestogens, and specifically 5α-pregnane-3,20-dione (5α-DHP) and 20α-hydroxy-5α-pregnane-3-one (20α-5P).[365-368] This hormonal support of pregnancy is made possible through synchrony of the fetal gonads, fetal adrenals, and the placenta functioning as complementary endocrine organs. Although these progestogens increase in concentration in maternal serum later in gestation, progesterone rapidly declines and remains at basal concentrations until the abrupt rise immediately before parturition.

In addition, the fetoplacental unit produces large quantities of dehydroepiandrosterone (DHEA) in late gestation, which rapidly are aromatized within the placenta into estrogens. In addition to estrone sulfate and estradiol, the equine-unique estrogens equilin and equilenin are also produced in high concentrations throughout late gestation.[368] Total estrogen concentrations rapidly rise starting at approximately 100 days'

gestation into thousands of nanograms per milliliters, peak roughly 2 weeks before parturition, and then promptly decline. Estrone sulfate has been used as a marker of fetal well-being in early pregnancy, because it represents the effective process of a conversion of the fetal gonadal production of estrogen.[155] Fetal estrogens are enzymatically converted to soluble estrone sulfate by the placenta. When estrone sulfate declines, it suggests that this conversion process has been disrupted, either from fetal demise or placental dysfunction.[155] Some have argued that estrogen supplementation in mares with low total estrogens during gestation may help, and there is a growing body of knowledge suggesting beneficial effects.[369] In contrast to other species, the horse has a rapid decline in total estrogen concentration before parturition, the exact reasoning for which has not been clearly determined. Esteller-Vico et al. did a study in which Letrozole, a potent aromatase inhibitor, was used to significantly suppress estrone sulfate in circulation in late-gestation mares.[369] Although estrone sulfate concentrations were suppressed to basal levels, gestational length was not affected, further indicating the lack of sufficient data and indicating the necessity for further work exploring estrogen supplementation in late gestation.

Poor uterine tone, the presence of abnormal uterine edema, low serum progesterone, and a small-for-age embryo are all indicators of a compromised pregnancy, which often lead to hormonal supplementation. Unfortunately, none of these conditions is well characterized, and their diagnosis and interpretation vary considerably among practitioners. In addition, low serum progesterone concentrations have been shown to correlate poorly with early embryonic loss.[370] Still, many practitioners supplement with progestogens throughout pregnancy. The two most common types of exogenous progestogens are oral altrenogest (Regu-Mate) or daily IM injections of progesterone in oil. In a study done by McKinnon et al., five different synthetic progestogens were evaluated for maintenance of pregnancy.[371] Only altrenogest, administered at the recommended dosage of 0.044 mg/kg daily, provided sufficient support for maintenance of pregnancy after luteolysis was induced using $PGF_{2\alpha}$. In addition to Regu-Mate, a long-acting progesterone formulation is currently available on the market (BioRelease P4 LA 150) that has been shown to be effective when administered once every 7 days (BioPharm). In addition, BioRelease also produces sufficient levels of progesterone for pregnancy maintenance in the absence of luteal tissue.[372] Although both provide adequate embryonic support in the absence of luteal functionality, the BioRelease formulation is less labor intensive, requiring weekly injections instead of daily.

Although it has been well documented that progesterone concentrations in serum are negligible in late gestation, supplemental progestogens are routinely administered. In cases of suspected placentitis, placental separation, placental insufficiency, inflammatory conditions, and supplemental progestogens are used to promote uterine quiescence and for their antiinflammatory properties. Altrenogest is recommended for the treatment of placentitis in addition to antimicrobials and antiinflammatories, which when combined significantly decrease the risk of abortion caused by experimentally induced placentitis.[373] Routine supplementation with altrenogest in late gestation is associated with clitoral enlargement in female offspring and an increase in serum LH in prepubertal fillies produced by mares that underwent treatment.[374] Foals born to mares that have received altrenogest in late gestation have been found to be immunocompromised, with a lower neutrophil/lymphocyte ratio than normal foals.[375] Although this may be caused by dysmaturity at birth, these results were contradicted by a second study, which found that supplemental altrenogest in late gestation has no antiinflammatory effects on the endometrium of mares post parturition.[376] A more comprehensive discussion of this topic will be covered in a later section.

INDUCTION OF LACTATION IN THE BARREN MARE

When the foal's dam has died, cannot lactate appropriately, or a suitable nurse mare or foster dam is unavailable, lactation-induction protocols are used to provide a lactating mare for the foal. Lactation can be induced in the barren mare, which can alleviate the need for producing an orphan foal from a nurse mare. Chavatte-Palmer et al. demonstrated that a hormonal manipulation of the mare, beginning at any time in her cycle, could induce lactation within days of treatment.[377] This protocol involves placement of an intravaginal sponge containing 500 mg altrenogest and 50 mg estradiol benzoate, and it has proved effective. A week after the onset of treatment, a second sponge is placed, containing 100 mg estradiol benzoate in addition to 50 mg/100 kg body weight sulpiride. In a second study, domperidone (1.1 mg/kg) was evaluated in replacement of sulpiride. Both treatments induce lactation within 7 to 12 days after the beginning of treatment, including the production of colostrum.

A second study found that routine IM injections of sulpiride (twice a day at a dose of 0.5 mg/kg) beginning during diestrus and continuing for 9 to 14 days effectively induced lactation.[378] Large mare-to-mare variability was seen when considering daily milk output. It has been noted that mares were only responsive to sulpiride when the ovaries were functionally performing steroidogenesis and producing estrogen and progesterone. Neither anestrous nor ovariectomized mares responded to treatment. Clinically, induction of lactation is assisted by daily milking and repeated oxytocin injections.

A follow-up study done by Korosue et al. demonstrated that foal size is not affected by the use of a lactationally induced surrogate mare.[379] Hormonal stimulation of lactation had no effect on subsequent reproductive performance, indicating that these foster mares may still achieve pregnancy.

Breeding Management to Optimize Pregnancy Rates

E. Bradecamp

There are several components of breeding management that can be manipulated and controlled in an attempt to optimize pregnancy rates. Fertility can be measured by per cycle pregnancy rate, which is the number of estrous cycles required to establish a pregnancy. The more concise the breeding management is, the more likely pregnancy rates will be maximized and the more efficient, both economically and timely, the breeding program will be.

It is imperative to recognize that breeding management can be divided into two categories, the mare factors and the

stallion factors. Additionally the human factor—the management of the mare and stallion that is directly affected by human decisions—cannot be overlooked as an important element in the success of a breeding program. Regardless of how fertile a mare or stallion may be, human factors that result in less than optimal management may yield decreased pregnancy rates.

STALLION MANAGEMENT TO OPTIMIZE PREGNANCY RATES

The gold standard for the measurement of stallion fertility is per cycle pregnancy rate. Semen analysis can provide a plethora of information and values regarding the quality of spermatozoa in an ejaculate. While a single in vitro test cannot reliably predict fertility, ejaculates with higher percentages of morphologically normal, progressively motile sperm yield higher pregnancy rates than ejaculates that exhibit poorer quality morphology and motility. Second, even if a stallion has excellent fertility, poor management of that stallion can result in poor pregnancy rates; therefore proper management is essential to optimize fertility. Depending on the breeding program and the method by which the mares are bred (live cover or AI with fresh, cooled shipped semen or frozen semen), there are important conditions that must be met to maximize pregnancy rates.

OPTIMIZING PREGNANCY RATES WITH FIELD BREEDING

Although less reported in scientific research and literature, field breeding remains the primary method of breeding for many stud farm operations around the world. In North America, many large ranches and pregnant mare urine (PMU) farms (farms that establish pregnancy in mares to recover estrogens from urine for commercial use) continue to rely on pasture breeding to achieve pregnancy or "settle" mares. It is important to realize that even with the many management techniques used in programs that utilize hand breeding or AI, higher pregnancy rates are not guaranteed and often are not achieved above those yielded by pasture breeding. At the same time, pasture breeding does have its limitations; mature stallions are often restricted to 20 to 35 mares with which they can breed live cover in a pasture setting. Placing a stallion with a herd of mares that is too large may have a negative effect on his ability to achieve optimal pregnancy rates, and it may be detrimental to the stallion's health. In breeding programs in which stallions are booked to greater than 100 mares per season, other methods of breeding must be used to optimize pregnancy rates.

When using field breeding, it is ideal to have a closed herd of mares that has had time to establish its order of dominance before introducing the stallion. The stallions are typically left with the mares for a period of time that allows every mare to pass through two consecutive heat cycles (45–60 days). The stallion is then removed from the herd, and pregnancy examinations are performed 21 or more days later. If a mare is bred on the day that the stallion is removed and does not ovulate for several days, this allows for every mare to be a minimum of 14 days pregnant at the time the pregnancy examinations are performed.

Ideally, once the herd is established and the stallion has been added, more mares should not be added to the herd because a new hierarchy will need to be established, and the stallion may not accept the new mare, even if she is in estrus. Injuries are more likely to occur when new horses are added to the herd. If a new mare must be added, then the stallion should be removed for a couple of days to allow the mares to reestablish their hierarchy, and then the stallion can be added back in the herd. If the mare is an outside mare (not native to the farm), then it is imperative that she has been appropriately quarantined before being added to the herd to prevent disease transmission that would negatively affect the herd health and fertility.

Pastures need to be maintained with safe fencing and ideally without direct contact with other horses across fence lines. The fields need to be large enough to allow horses to get away from more aggressive pasture mates. All horses should be unshod and have their feet trimmed to minimize injuries and lameness.

BSEs should be performed on both the mares and the stallion before the beginning of the breeding season to identify any need for treatment or management changes. An entire breeding season can be lost when mares that have been housed with a stallion for 45 to 60 days are not checked for pregnancy until several weeks after the stallion has been removed, and then reproductive pathology is discovered.

Several factors aid in maximization of pregnancy rates when using field breeding, including:

- For fertile stallions, a BSE should be performed before the breeding season, and ideally the stallion has a history of successful field breeding.
- Each fertile mare should have a BSE performed before the breeding season.
- Do not overextend a stallion and house him with more mares than he can breed successfully. A young, inexperienced stallion may not be able to breed as many mares as an older, more mature stallion.
- Maintain a closed herd to reduce the possibility of stress and introduction of diseases that could negatively impact pregnancy rates.
- Maintain good herd health practices, such as vaccination, deworming, hoof care, dental treatments, and an adequate plan of nutrition. These should all be addressed, in place, and up-to-date before the breeding window to minimize stress during this period.

OPTIMIZING PREGNANCY RATES WITH LIVE COVER IN HAND BREEDING

Many small stud farms rely on breeding mares live cover, and all Thoroughbreds bred to be registered with the Jockey Club are required to be bred live cover. The key factors affecting pregnancy rates in live-cover situations are ensuring that the stallion's book size is appropriate, based on the stallion's history and daily sperm production, and that the mares bred are suitable for mating and ovulate in a timely manner after breeding. Ovulation should occur within 48 hours of breeding or the mare should be bred again. Ideally the mare should be administered an ovulation-inducing agent to ensure ovulation occurs within 24 hours following breeding.

A more thorough discussion of how to calculate an appropriate book size based on the stallion's testes size and seminal parameters can be found in later sections. Briefly, Love et al.

described how to estimate a stallion's book size based on testes size and predicted or known daily sperm output (DSO).[380] If a stallion is booked to more mares than is appropriate for his DSO, then he may fail to meet an acceptable per cycle pregnancy rate, resulting in more mares needing to be rebred on subsequent cycles and compounding an already overestimated book size. Monitoring a stallion's ongoing per cycle pregnancy rate as the breeding season progresses aids in identification of any pathology and mismanagement or fertility issues and allows for necessary evaluations and adjustments to stallion management to be made to optimize fertility.

Research has also shown that for approximately 60% of stallions "reinforcement breeding" after the stallion has covered the mare results in improved pregnancy rates by approximately 10%.[381] Reinforcement breeding involves collecting the postejaculate semen that drips from the urethra following dismount, which is combined with an appropriate semen extender to be inseminated into the mare's uterus. Reinforcement breeding has been most beneficial to stallions that routinely dismount before completing ejaculation. One stallion the author worked with had a 30% increase in per cycle pregnancy rates when reinforced breeding was added to stallion management.

OPTIMIZING PREGNANCY RATES WITH ARTIFICIAL INSEMINATION WITH FRESH SEMEN

If the stallion and mare reside on the same farm, then instead of breeding via live cover, the stallion's semen may be collected and the mare bred with the fresh semen by AI. As with live cover, the mare should be programmed to ovulate within 24 to 48 hours post breeding. To optimize pregnancy rates, when the ejaculate is going to be used to breed more than one mare on a given day, each mare should be inseminated with a minimum of 500 million progressively motile sperm or 250 million progressively motile, morphologically normal sperm. Although some stallions can achieve acceptable per cycle pregnancy rates with fewer sperm per insemination dose, until a stallion's minimum sperm/dose threshold is determined, 500 million progressively motile sperm are considered an appropriate dose for fresh semen. To improve pregnancy rates with a subfertile stallion, one can increase the number of sperm per dose or perform deep-uterine-horn or hysteroscopic insemination techniques to deposit the semen closer to or on the oviductal papilla ipsilateral to the dominant follicle.

OPTIMIZING PREGNANCY RATES WITH ARTIFICIAL INSEMINATION WITH COOLED SHIPPED SEMEN

When one is breeding with cooled shipped semen, proper preparation of the semen is paramount to obtaining maximum pregnancy rates. In this program the semen is collected from the stallion, processed according to known standards of semen preparation, and placed in a cooling device (Equitainer or disposable shipper) to be maintained at 5°C until it arrives at the location for insemination. Generally most semen is shipped in this program overnight for arrival the next day, although this may vary depending on shipping options available. Once the semen arrives, the mare is artificially inseminated and ideally will ovulate within 24 hours post insemination. Human error in the preparation of the semen for shipment and delayed time from insemination to ovulation or missed ovulation are the most common detriments to establishing successful pregnancies.

Before the start of the breeding season a BSE should be performed on every stallion and mare considered for use in this program to identify pathology, establish ideal semen-processing techniques to optimize pregnancy rates, and minimize costs associated with multiple breedings. Regarding the stallion, a longevity analysis of sperm motility using different semen extenders and different conditions should be conducted to identify processing and packaging that optimally maintains sperm motility for 24 to 48 hours under cooled conditions. Generally semen processing for cooled shipped semen involves the following:

1. Immediately after the semen is collected it is extended to a minimum of 1:3 ratio of semen to extender (ideally 1:4 or greater), and kept at room temperature out of direct light until analysis and packaging are complete. The extender should be warmed to 37°C before collection and then allowed to passively cool to room temperature at the same rate as the semen to prevent any temperature shock of the semen when the two are combined.
2. The final concentration of the extended semen should be between 25 and 50 million spermatozoa per milliliter.
3. Industry standards have established that a minimum of 1 billion progressively motile sperm should be included in each dose of cooled shipped semen to optimize pregnancy rates.

The mare that is to be bred should be administered an ovulation-inducing agent 24 hours before breeding to ensure that she ovulates within 24 hours after breeding. When two doses of semen are available, reports conflict as to whether better pregnancy rates are achieved if both doses are inseminated at the same time or if the mare should be bred twice approximately 24 hours apart.[382,383] Unfortunately, both studies contained a limited number of stallions, and more research needs to be conducted in this area to answer this question. The fertility of the stallion and reproductive health of the mare and how well an individual stallion's spermatozoa tolerate cooling also factor into what management options are best.

OPTIMIZING PREGNANCY RATES WITH ARTIFICIAL INSEMINATION OF FROZEN SEMEN

When frozen semen is used for breeding, accurate timing of breeding in relationship to ovulation is imperative to achieve maximum pregnancy rates. Frozen-thawed semen does not remain viable in the mare (estimated 12 hours on average) as long as fresh or cooled semen (approximately 48 hours), potentially because of capacitation-like changes that occur during the cryopreservation process.[384] Recent studies have shown that similar pregnancy rates can be obtained with frozen and cooled shipped semen, and Loomis et al. reported first cycle pregnancy rates of 60% and 63%, respectively, in a study of three farms with a combined total of 36 stallions shipping either cooled or frozen semen to a total of 648 mares.[385] The number of doses available for insemination on a given cycle dictates whether the mare can be bred with two doses using a timed insemination protocol or if the mare is bred with one

dose once ovulation is detected. Similar pregnancy rates can be achieved with either method as long as insemination occurs no more than 12 hours before and 6 hours after ovulation. If two doses of semen are available, then the mare is administered an ovulation-inducing agent once a 35-mm follicle and maximal uterine edema are observed. Insemination occurs at 24 and 41 hours later, with ovulation being confirmed within 12 hours of the second ovulation. If only one dose of semen is available, then the ovulation-inducing agent is administered as described earlier, and starting 24 hours later transrectal ultrasonography is performed approximately every 6 hours until ovulation is detected, at which time the mare is bred. Frozen semen causes a more severe inflammatory reaction in the endometrium than fresh or cooled shipped semen because of the removal of seminal plasma and its antiinflammatory properties. Because of this, the decision to breed with one dose versus two is made based on the mare's reproductive health and history.

Proper handling of frozen semen is also important to ensure that maximal pregnancy rates are achieved. Frozen semen must be maintained in appropriate liquid nitrogen storage units until immediately before thawing and should not be exposed to ambient temperature air at any time for more than a couple of seconds. Exposure to ambient air will result in thawing and then refreezing when returned to the liquid nitrogen, resulting in damage to the spermatozoa. Frozen semen should be thawed according to instructions that are included with the semen when it is shipped to ensure that maximum viability is maintained. If no instructions are included then it is safest to thaw at 37°C for 30 seconds for 0.5-mL straws, with larger straws requiring a longer thawing time. Once thawed, the semen should be inseminated into the mare immediately. Although there is no set industry standard for the number of total or progressively motile sperm required for a dose of frozen semen, there are some guidelines. In a study of more than 30,000 mare inseminations in the French National Stud, it was concluded that when mares were inseminated with doses containing less than 750×10^6 total frozen-thawed sperm, the resulting per cycle pregnancy rates were lower.[386] Based on these results, the World Breeding Federation of Sport Horses currently recommends this as the accepted minimum number of sperm per dose of frozen semen. Additionally, Volkmann and van Zyl showed that per cycle pregnancy rates were significantly higher when the insemination dose of frozen-thawed semen was increased from 137 to 210×10^6 to 222 to 333×10^6 progressively motile sperm (44% vs. 73%, respectively).[387] Based on these studies, it has become industry standard that a commercial dose of frozen semen should contain 200 million progressively motile sperm and possess greater than 30% progressive motility on thawing. It should be remembered that, unlike in the bull, fertility varies greatly from stallion to stallion and may not always be highly correlated to motility.

OTHER FACTORS AFFECTING PREGNANCY RATES

In addition to proper management of the stallion and mare, there are other variables that affect pregnancy rates. Understanding, controlling, or adjusting management of the mare to address these variables when possible will ensure that optimal pregnancy rates will be achieved. In addition, it is important to understand how these factors, listed below, will affect expected pregnancy rates and allow the clinician to provide the owner with a realistic prognosis for reproductive success:

- Reproductive status of the mare (barren, foaling, or maiden)
- Age of the mare or stallion
- Time of year of breeding
- Presence of endometritis
- The effects of inflammatory cytology and positive culture on pregnancy rates
- Uterine and systemic treatments that can aid in improving pregnancy rates in mares with endometritis
  - Corticosteroids
  - Ecbolics
  - Uterine lavage
  - Intrauterine and systemic antibiotics
  - Antiinflammatories and mucolytics

Although these factors will not be discussed in this section, they are covered in detail in other sections. It is important to remember that appropriate management of these variables can aid in obtaining optimal pregnancy rates. Regarding the reproductive status of the mare, the highest per cycle pregnancy rates of age-matched mares under 9 years of age is in the maiden > foaling > barren mares, although exceptions can occur. Generally the highest pregnancy rates are achieved with young fertile animals using fresh or cooled semen. Per cycle pregnancy rates decrease, and increases in pregnancy loss occur when mares age.[388,389] Breeding outside of the natural breeding season, when daylight is short, can be associated with a decrease in fertility in both mares and stallions, with animals farther from the equator demonstrating a more dramatic, longer period of reproductive quiescence. Although it has been shown that endometritis, associated with the presence of fluid in the uterus, inflammatory cytology, and positive cultures all result in decreased pregnancy rates, it has also been shown that appropriate treatment of these pathologies can result in improvement in pregnancy rates.[390]

There exists a population of mares for which these traditional breeding programs do not meet acceptable expectations because of poor uterine health, repeated pregnancy loss, or inability to deliver a viable foal at term or the desire for more than one offspring per season. For these mares, assisted reproductive techniques such as embryo transfer and oocyte aspiration for ICSI are options that should be considered when consulting with the client.

Equine Embryo Transfer

E. Bradecamp

Embryo transfer as a means to produce offspring is used for several different reasons in the equine industry. As mentioned previously, it is the next option for mares that cannot generate viable offspring because of reproductive tract limitations (cervical damage, persistent endometritis, fibrosis, and repeated pregnancy loss), systemic issues (laminitis, pelvic fracture, and lameness), when the client would like to have multiple offspring in one season, or in a healthy performance mare that cannot carry a pregnancy because of competition. Briefly, equine embryo transfer involves breeding a "donor" mare with the desired genetics, monitoring ovulation, and recovering the embryo through a uterine flush 7 to 8 days post ovulation. The embryo is then transferred into a synchronized "recipient"

mare that will carry the pregnancy to term and raise the foal. A multitude of factors play a role in the success of an embryo transfer program, and a comprehensive understanding of the effects of donor mare fertility, recipient mare management, and embryo procurement and processing techniques is imperative to maximize the chances of obtaining a pregnancy. This section will provide an overview of the factors involved in managing donor and recipient mares and the techniques for flushing, transporting, and transferring equine embryos.

The first live foal produced by embryo transfer was reported by Oguri and Tsutsumi in Japan in 1974.[391] Initially, embryo transfers were performed surgically by using a standing flank laparotomy, by which the embryo was deposited into the uterine lumen through a small incision in an exteriorized uterine horn. The incision was then closed, the uterine horn returned to the abdomen, and the flank laparotomy site closed. By the late 1990s, with advancements in the technique of transcervical transfer, Foss et al. reported that pregnancy rates from nonsurgical embryo transfer could be achieved that were comparable or superior to surgical transfer.[392] Since that time, nonsurgical embryo transfer has become the industry standard of care.

Initially, it was hoped that embryo transfer would provide a modality by which to obtain foals from mares with various causes of infertility. Although successful in some of these cases, embryo transfer success depends on the cause of infertility and may not improve pregnancy rates for some infertile mares. The procedure has proven to be more successful in mares that become pregnant but fail to maintain their pregnancies than in mares in which no pregnancy has ever been diagnosed. Embryo transfer allows foals to be produced from show or race mares that the owner desires to keep in competition; mares that cannot carry a pregnancy because of systemic, musculoskeletal, or reproductive disease; 2-year-old mares; and mares that have foaled late in the breeding season. This allows them to remain open so that they can be bred back early the following year. Embryo transfer also allows embryos to be cryopreserved and stored for later use.

The success of an embryo transfer program is dependent on multiple factors, including donor mare fertility, recipient fertility and synchrony, semen quality, and expertise of the veterinarians managing the mares and performing the procedures. An extensive review of the factors affecting embryo transfer rates will be provided later in this section. The majority of embryos are shipped to recipient herds with proper synchronization and selection, but owner-provided recipient mares may also be used. Although a majority of breed registries (with the Jockey Club being one exception) now permit registration of foals produced by embryo transfer, mare owners need to check with their specific breed registry to familiarize themselves with the regulations of that organization. Some registries allow an unlimited number of foals to be produced from a mare in a given year, whereas some registries, such as the United States Trotting Association, only permit one foal per mare per year to be registered.

DONOR MARE

Factors Affecting Fertility

The fertility and age of the donor mare has a significant effect on the success of an embryo transfer program. The chances of obtaining an embryo is highest in young (less than 10 years of age), fertile mares, with Foss et al. reporting embryo recovery rates as high as 84.2% in show mares; 59.7% in multiparous, nonbarren mares; and as low as 30% in mares with a history of infertility.[392] McCue et al. reported that one or more embryos were recovered from 144 of 252 flushes (57.1%) from mares 15 years of age and younger and 93 of 236 flushes (39.4%) from mares older than age 15.[393] Marione et al. also showed that mare age had a significant effect on embryo recovery rate. When mares were divided into three age groups—fillies (3 and 4 years old), middle-aged mares (aged 5–10 years), and old mares (aged 13–25 years)—the embryo recovery rates were 171/244 (70.1%), 774/1081 (71.6%), and 385/701 (54.9%), respectively, with significantly fewer embryos recovered from the old mares.[394]

The type of semen used to breed the donor mare also has an effect on pregnancy rates and embryo recovery rate. Squires et al. reported that pregnancy rates were highest when fresh semen was used (60%–77%) and were significantly lower when chilled (44%) or frozen semen (46%) was used.[389] The quality of the sire's semen should be taken into careful consideration in an embryo transfer program. Stallions with poor per cycle pregnancy rates will subsequently yield poorer embryo recovery rates.

Both exercise and heat have the potential to negatively affect embryo recovery rates. This should be taken into consideration when attempts are being made to obtain embryos from mares that are concurrently in active training and/or competition. A study performed by Kelley et al. demonstrated that exercised mares had lower peak LH concentrations, higher cortisol levels, and altered follicular dynamics compared with nonexercised mares.[395] The exercised mares had longer interovulatory intervals, fewer 6- to 10-mm follicles, and more follicles 20 mm or greater on the day of deviation, suggesting delayed deviation. Additionally, when embryo transfer was performed on the exercised and control mares, significantly fewer embryos were recovered from the exercised mares. Mares were divided into three groups: nonexercised controls, mares that were exercised moderately for 30 minutes a day during the periovulatory period but rested after ovulation for 7 days, and mares that were exercised for 30 minutes daily from the periovulatory period until day 7 post ovulation. Embryo recovery rates were significantly higher in the controls (67%) than the partially exercised (44%) or fully exercised (43%) groups. Vascular perfusion of the periovulatory follicular wall was significantly decreased on the day before ovulation in both groups of exercised mares, and vascular perfusion of the follicular wall was correlated with the likelihood of recovering an embryo.[395] Mortensen et al. also demonstrated that when mares were exercised for 30 minutes daily under ambient conditions of over 30°C and over 50% humidity, an increase in rectal temperatures from 38°C to 39.9°C occurred. Embryo recovery rates in the exercised mares decreased by 50% compared with the control mares, and 50% fewer grade I embryos were recovered from the exercised mares.[396] Conversely, there have been studies that did not show any effect from exercise in hot, humid conditions on embryo recovery rates, but the mares in these studies experienced less elevation in rectal temperatures and may have been at a higher fitness level than the mares in other studies.

Managing the Donor Mare for Embryo Transfer

Management of the donor mare for embryo transfer is very similar to managing any mare that is being bred. The mare

should be in good body condition and health, and any health-related problems should be addressed and every attempt made to keep the mare pain and stress free. Mares should be bred to minimize and modulate the inflammatory process that naturally occurs in the reproductive tract with breeding, and the treatments required to do this may vary greatly from mare to mare. Young, healthy mares usually require minimal treatment, whereas older, subfertile mares may require more aggressive treatment modalities that aid in the evacuation of fluid from the uterus and minimize the development of postbreeding endometritis. It is imperative that the exact day of ovulation is documented to ensure that the embryo flush is performed on the correct day post ovulation. Embryo flushes are performed 7 to 8 days post ovulation. If the mare is less than 10 years of age and was bred with fresh or cooled shipped semen, then the flush may be performed on day 7 or day 8, depending on the clinician's preference. Embryo flushes are typically performed on day 8 in older mares and mares bred with frozen semen. The embryo appears to be slightly delayed in its early development and transport in older mares and when frozen semen is used, so recovery 1 day later allows an increased likelihood of recovering an optimally developed embryo.[355]

When multiple embryo flushes are going to be performed on a donor mare within one breeding season, it is imperative to be cognizant of the inflammation that is incited with each breeding and flush cycle. Research has shown that these inflammatory episodes are additive, and mares that have had multiple embryo flushes performed during a season have higher levels of inflammation and fibrosis observed on uterine biopsies compared with control mares. Carnevale et al. showed a positive correlation between repeated embryo recovery attempts and chronic inflammatory changes in the uterus.[397] Additionally, although there is anecdotal evidence that repeated embryo flushes over several years have a negative effect on the fertility of some mares, there are studies that do not show an alteration in embryo recovery rates with an increasing number of successive embryo collections.

RECIPIENT MARE

The ideal recipient mare should be 3 to 10 years of age, of good body size (15.2–16.2 hands) or as close to the same size of the donor mare as possible, systemically healthy, easy to handle, and reproductively sound. Standardbred and Quarter Horse–type mares have historically shown to be well suited as recipient mares, but the predominant type of mare within any individual recipient herd is often dictated by the types of mares available as recipients within that geographic region, and potentially the predominant types of donor mares are flushed. Although it has been well demonstrated that the size of the mare's uterus controls the size of the fetus, allowing the transfer of draft horse embryos into ponies to produce live foals without resulting in severe dystocia, it has been demonstrated that when there is a gross disparity (draft to pony) the fetus may not reach its full potential size as its siblings that are transferred into recipients of similar size to the donor. It should be noted that these studies examined the extremes of embryo transfer, and it is because of the mares' ability to control fetal size that embryo transfer can be performed so readily in mares and the offspring produced are able to mature to their genetic potential when light horse breeds are transferred into light horse breeds. Intrauterine growth restriction (IUGR), a potential for increased incidence of osteochondritis dessicans lesions, and changes in conformation have been attributed to large differences in the recipient and donor mares, and every effort should be made to best match the recipient to the size of the donor mare.

Detailed descriptions of the specific surgical and nonsurgical procedures involved in the collection, shipping, and transfer of equine embryos is beyond the scope of this text, and the reader is referred to other recent texts and publications for such information.

The Pregnant Mare

Igor F. Canisso, Maria R. Schnobrich

This section will review the physiology and relevant anatomic considerations for clinical management of the pregnant mare. In addition, management, treatment, and pathology associated with the pregnant mare and periparturient period will be discussed.

PHYSIOLOGY OF GESTATION

Maternal Recognition of Pregnancy

Once the oocyte is fertilized, the zygote begins its journey down the oviduct, reaching the uterine horn by days 6 to 7.[398-401] Older mares and mares bred post ovulation or with frozen semen are thought to have delayed embryonic vesicle transport through the uterine tubes by 1 or 2 days. It has been suggested that the early equine embryonic vesicle must be viable and signal its presence in the uterotubal junction to allow its passage into the uterine lumen.[4,402] This mechanism is thought to be mediated by embryonic production of PGE_2,[401] which is supported because PGE_2 administered in the oviduct hastens embryonic transport through the uterine tube.[8] Nonfertilized oocytes are thus retained in the mare's uterine tubes until complete degeneration occurs.[4] Detailed descriptions of the early development of the equine embryo are outside the scope of this text, and the reader is referred to elegant reviews published elsewhere.[4,401-403] Early embryonic development in the horse is characterized by the formation of a tough, elastic, unusual acellular mucin-like glycoprotein "capsule" covering the trophectoderm and the overlying zona pellucida.[400,403] This structure is first detected by day 7 post ovulation and completely envelops the spherical conceptus until as late as days 23 to 25 of gestation.[400,403] Although the term *embryo* is used routinely to describe the early conceptus, it is important to understand that what is generally being discussed is the embryonic vesicle as well as the embryo proper. These two structures will ultimately develop into the fetal membranes and fetus, respectively. Embryonic development has progressed sufficiently by day 40 of gestation, and the term *fetus* is used thereafter.[4]

The longitudinal arrangement of the endometrial folds is suggested to favor mobility of the spherical equine embryo.[4] After entering the tip of the uterine horn by days 6 to 7, the embryo moves down toward the uterine body by day 8.[404,405] The intrauterine mobility phase continues until days 15 to 17 post ovulation, and during this period the embryo may move between the horns 10 to 20 times each day.[399,401,404-406] An embryo-derived factor is suspected to signal the suppression of the uterine luteolysin ($PGF_{2\alpha}$) release, which interrupts the oxytocin-$PGF_{2\alpha}$ interaction in mares and allows early

pregnancy maintenance.[279,407-409] The mobility of the spherical equine conceptus is critical for this proposed blockage of luteolysis,[398,399,401] which is why large uterine cysts are thought to cause pregnancy loss.[135,410,411] If the enlarging vesicle cannot pass by a large lymphatic uterine cyst, then it may be unable to come into contact with all endometrial surfaces and inhibit $PGF_{2\alpha}$ production.[93] Historically, it has been suggested that maternal recognition of pregnancy starts by 10 days post ovulation in mares,[412] but a recent study reported successful establishment of pregnancies when day 10 embryos were transferred to recipient mares ovulating within 2 to 6 days of the donor mare.[413] This suggests that the luteolytic cascade does not begin until day 12 and that the critical window for maternal recognition of pregnancy in mares likely occurs between days 12 and 14 post ovulation.

If maternal recognition of pregnancy does not occur or the mare is not pregnant, luteolysis begins with release of $PGF_{2\alpha}$ by endometrial cells into the systemic circulation. This is in contrast to ruminants, in which $PGF_{2\alpha}$ is delivered directly to the ovary via the countercurrent exchange between the ovarian artery and uteroovarian vein.[414,415] If the mare is not pregnant or embryonic migration is impaired by day 15 of the cycle, then there is increased expression of cyclooxygenase-2 (COX-2) by endometrial epithelial cells compared with pregnant mares.[416,417] Therefore inhibition of the COX-2 enzyme is considered a key factor for maternal recognition of pregnancy in mares.[412,417] Interestingly, although this release of $PGF_{2\alpha}$ is stimulated by oxytocin produced by luteal cells in ruminants, there is no such luteal production of oxytocin in the mare, with the endometrium being its primary source.[4,418]

The embryonic vesicle typically becomes fixed at the base of one of the uterine horns with the embryonic pole of the vesicle opposite to the mesometrial attachment (antimesometrial).[419] This fixation is a result of the increasing embryonic vesicle diameter, uterine tone, and its arrival at the uterine bifurcation, which presumably has the smallest lumen diameter. An early vascular indicator of the future position of the embryo proper consists of a colored spot seen on Doppler imaging of the endometrium close to the wall of the embryonic pole. This finding may prove useful when differentiating between a cyst and a vesicle before the embryo proper becomes visible on the ultrasound image.[420,421] In postpartum mares, the embryo usually becomes fixed in the more involuted horn,[421-424] with higher pregnancy losses being associated with those embryonic vesicles that fix in the previously gravid horn by an unknown mechanism.[424]

Placentation

The trophoblast cells of the early embryo are programmed to form the absorptive placental contact with the endometrium.[4] The mare has a nondeciduate, epitheliochorial, diffuse, microcotyledonary placenta.[4,402,403,425-430] The entire maternal surface of the fetal membranes becomes covered with delicate, diffuse microvilli that interdigitate with the proliferating luminal epithelial cells to form intricately branched microcotyledons.[425-430] The interdigitation tends to be deeper at the tip of the nongravid horn, which may help explain the higher incidence of fetal membrane retention in that horn.[431-433] In a normal pregnancy the noninvasive allantochorion extends slowly to fill the uterus by days 80 to 85 of gestation, and its microcotyledonary architecture, which provides both hemotrophic and histotrophic nutrition for the growing fetus, is not fully established until days 120 to 140 of gestation.[4,161] During the second half of gestation, most of the mitotic activity is confined to the periphery of the microcotyledons, which are still growing.[434,435]

Vascular endothelial growth factor, a potent vasculogenic and angiogenic factor, and its two main receptors, Flt-l and KDR, are expressed on both the endometrium (i.e., maternal) and chorioallantois (i.e., fetal) throughout gestation in the mare.[436] These molecules are presumed to facilitate the continuing development of the prominent fetal and maternal capillary networks within the microplacentomes of the equine placenta.[436-438]

Degenerative changes (endometriosis) in the endometrium can adversely affect the ability of the microcotyledonary placenta to facilitate optimal hemotrophic nutrition and exchange of waste products.[437] If the pregnancy is maintained, dysfunctional placental attachment can lead to the birth of dysmature, weak foals through intrauterine growth restriction (IUGR), which may have detrimental consequences on the health and athletic performance of the mature horse.[437,439,440]

Failure of microcotyledon development results in the characteristic white, avillous portions on the maternal surface of aborted twin fetal membranes.[441,442] Although histotrophic nutrition (i.e., through secretions from endometrial glands and absorption by the chorionic cells) is likely to remain important throughout gestation, adequate hemotrophic nutrition is essential to support the rapid fetal growth that occurs during the latter part of gestation.[4] The morphology of endometrial blood vessels observed on uterine biopsy can vary considerably, depending on the age and reproductive status of the mare.[443] Mild to severe degenerative lesions may be observed in smaller and larger arterial and venous vessels in as many as 20.5% of the endometrial specimens examined.[188] Unaltered vessels were detected only in maiden mares.[188]

The incidence and severity of angiopathy increase with the number of previous pregnancies and with advancing age.[188,443] Changes in the endometrial vasculature of multiparous mares have been compared with the so-called pregnancy sclerosis of other species, with fraying and disruption of the elastic internal membrane, medial and adventitial elastosis and fibrosis, and calcification processes within the media.[188,443]

In multiparous mares, cycles of vascular growth during pregnancy and subsequent involution postpartum are thought to result in progressive degenerative vascular changes in the endometrium. Aging processes, chronic inflammation, and short foaling intervals have been implicated as additional detrimental factors.[188,437,438,443] Severe angiopathy is frequently combined with phlebectasia and lymphangiectasia, possibly indicating a reduced ability of the vessels to adapt to the varying demands of uterine circulation, with a decrease of uterine perfusion and lymph drainage.[188,443] In addition, mares with a grade III biopsy score, consistent with severe endometrial degenerative changes (Kenney and Doig classification[249]) had lower vascular perfusion during early pregnancy than mares with grades I and II (no or minimal endometrial fibrosis).[444] Angiosis in older, multiparous mares might therefore be intimately related to infertility, possibly because of detrimental effects on early embryo nourishment and subsequent placentation. Controlled studies have demonstrated the impact that uterine capacity and placental area can have on the size and birth weight of the foal and postnatal development.[445-448] In one study, pony embryos were transferred into draft mare recipients, and then the genetic dams were bred and allowed to carry a full sibling to term.[445] The embryo-transfer foals

were all larger than their siblings at birth, presumably due to the larger uterus in the draft mare recipients.[445] Although the increased milk production of the draft mares may have explained the subsequent faster growth rates, it was noted that all three draft-gestated foals were still heavier at 4 years of age.[445]

Recently a similar study demonstrated that pony foals born from draft recipient mares (i.e., a model for enhanced uterine environment) had insulin resistance postnatally, whereas Saddlebred foals born from pony mares (i.e., model for restricted uterine environment) had increased sensitivity to insulin and were considerably lighter.[447] A similar embryo transfer experiment used a Thoroughbred-in-Pony (Tb-P) and Pony-in-Thoroughbred (P-Tb) comparison, with Thoroughbred-Thoroughbred and Pony-Pony transfers acting as controls.[449] The Tb-P foals were lighter than the P-Tb foals and had lower placental weight, volume, and surface area and reduced postnatal adaptive responsive and hypercortisolemia.[448,449] Altogether, these studies were consistent with an earlier study in which a Shetland pony mare was inseminated with Shire semen and a Shire mare was bred with Shetland pony semen. The foal out of the pony mare was 50% of the weight of the half-sibling that was gestated in the Shire mare, with the marked size discrepancy remaining after maturity.[448] On the practical side, as mentioned in the discussion on embryo transfer, these studies demonstrated the importance in embryo transfer programs of having embryo recipients compatible with the body frame of the embryo donor to avoid effects of an enhanced or restricted uterine environment. It is worth noting that sudden weight loss may mimic the situation of a restricted uterine environment, which may result in deficient fetal growth and a weak newborn foal. This has been observed in clinical practice and demonstrated experimentally in Thoroughbred primiparous mares using a *Streptococcus equi* subsp. *equi* infection-mediating nutritional insults.[450]

Endocrinology of Pregnancy

Endocrinology of equine pregnancy has been elegantly reviewed elsewhere, and the reader is referred to those publications for more detailed description of all endocrinologic events that occur during pregnancy.[4,451] Regardless of whether the mare is pregnant or not, there is a rapid organization and vascularization of the corpus hemorrhagicum, which culminates in the formation of a CL and increased progesterone production.[4] There is controversy as to whether large (i.e., granulosa cells origin) and small luteal cells (i.e., internal theca origin) are steroidogenically active as in other domestic mammals. The lack of progesterone receptors and steroidogenic enzymes in small luteal cells substantiates the hypothesis that small luteal cells do not secrete progesterone.[452] It is interesting to note that after undergoing artificial insemination, mares that became pregnant had significantly higher progesterone concentrations as early as 5 days post ovulation when compared with mares that did not become pregnant.[453] Additionally mares experiencing pregnancy loss or premature luteolysis may have decreased progesterone concentrations by 10 to 15 days post ovulation.[454,455] This finding highlights the importance of checking progesterone concentrations in mares with recurrent embryonic death by 10 days post ovulation and at pregnancy diagnosis on day 15 for mares exhibiting signs of premature luteal regression (e.g., poor uterine/cervical tone, endometrial edema, absent/small CL, presence of a smaller than expected embryonic vesicle).[455]

After day 15 post ovulation, nonpregnant mares normally start undergoing luteolysis and return to estrus and ovulate at 20 to 22 days on average from the previous primary ovulation.[4] However, some normal mares (bred and nonbred) may experience prolonged luteal function caused by diestrual ovulation, endometrial lesions, or for unknown reasons.[4,66]

In pregnant mares, the chorionic girdle is a distinct and specialized structure that can be seen surrounding the top third of the embryonic vesicle by day 25 post ovulation.[398,399] At around 35 days post ovulation, invasive trophoblast cells originating from the chorionic girdle detach from the embryonic vesicle and successfully invade the endometrium.[4,456-460] The invasive cells aggregate to form the endometrial cups, which are endocrinologically active tissue that secrete large quantities of eCG.[456-460]

Endometrial cups become visible by day 38 to 40 as a ring of small, pale plaques in the endometrium that surround the developing conceptus.[4,401-403] Because fetal cells are provided by the sire and thus differ genotypically from the maternal cells, the fetal antigens invoke a cell-mediated immune response.[461,462] This influx of lymphocytes creates a pronounced, localized immunologic reaction, and by day 120 of gestation, the endometrial cups are sloughed.[461-463] The subsequent necrotic tissue and inspissated secretions form invaginations into the chorioallantois and may still be seen on the allantoic surface at delivery as allantoic pouches.[464]

Equine chorionic gonadotropin, a large-molecular-weight glycoprotein, has both FSH-like and LH-like activity, as previously described.[465] During early pregnancy, periodic surges (10–12 days apart) of pituitary FSH trigger the waves of ovarian follicular development.[466] The eCG also promotes luteinization and ovulation of ovarian follicles, forming accessory (secondary) CLs that augment the progesterone production from the primary CLs that developed at the time of conception.[4,401,402] This process is critical to ensuring pregnancy maintenance before the placenta is fully capable of supporting pregnancy at ~100 days' gestation.[4,401,402]

High concentrations of eCG may be detected in the blood of pregnant mares from 40 to 120 days' gestation.[4,401] Interestingly, mares losing pregnancies during this interval of gestation may have a prolonged and persistent presence of active endometrial cups that secrete eCG, causing erratic estrous behavior, unreliable follicular development, luteinization of immature follicles, and unpredictable ovulation. Anecdotally, the treatment of persistent endometrial cups can be attained through laser therapy, intrauterine infusion of kerosene, lavage of the uterus with hypertonic saline, and endoscopic removal of cysts, but these have not been critically evaluated.

The equine embryonic vesicle is capable of producing large quantities of estrogen.[451] Estrogen concentrations increase significantly in the yolk sac and uterine lumen by day 12 post ovulation, with estradiol becoming the predominant estrogen in the yolk sac by 18 to 20 days post ovulation.[467] Another unique feature of early gestation in the mare is that the corpora lutea produce increasing concentrations of conjugated estrogen (e.g., estrone sulfate) and testosterone in response to eCG.[468-471] Starting by 70 days post ovulation, there is a progressive increase in estrogen concentration (presumably of fetoplacental origin), peaking by 210 to 240 days post ovulation and progressively declining toward parturition.[451]

Fetoplacental estrogens are synthesized from androgens produced by the enlarged fetal gonads.[472] Bilateral fetal gonadectomy performed between days 197 and 253 of gestation

causes a precipitous drop in plasma estrogen levels that then remains basal until small foals are spontaneously delivered in 70 to 97 days.[473] After day 220 the enlarged gonads gradually shrink into an insignificant size by birth.[471] Two groups of estrogens can be found in pregnant mares: classic phenolic estrogens (estradiol, estrone, and their conjugated forms) and equine-specific steroid ring B unsaturated estrogens (equilin and equilenin).[472] DHEA and 7-dehydro DHEA serve as precursors for classic estrogens and for ring B unsaturated estrogens (equilin and equilenin), respectively.

Four separate components combine to produce the progesterone and biologically active 5α-reduced pregnanes needed to maintain pregnancy in the mare.[4,451,474] The primary CL is prolonged beyond its cyclical life span by the downregulation of endometrial oxytocin receptors to prevent activation of the luteolytic pathway. Its waning progesterone production is supplemented from day 40 of gestation by the formation of accessory CLs that develop in the maternal ovaries as a result of the gonadotropic actions of pituitary FSH and endometrial eCG.[4] The equine fetoplacental unit produces significant quantities of progesterone and C-21 progestogens.[4,474] The placenta (chorioallantois and endometrium) initially uses maternally derived cholesterol to metabolize into pregnenolone and then progesterone. A significant amount of progesterone originates from this source, starting from approximately day 70 of gestation.[4,474]

In normal pregnant mares, peripheral progesterone concentration starts to decline by 4 months of gestation, reaching baseline levels by days 150 to 180.[473-475] Progesterone may be undetectable in the serum by this time (<1 ng/mL), depending on the type of assay used, and is no longer indicative of what is happening at the uterine level, because the placenta secretes progesterone and progestogens directly to the endometrium and underlying myometrium. In the second half of gestation, most of the placental-derived progesterone is further metabolized into 5α-reduced pregnanes (5α-dihydroprogesterone or 5α-pregnane-3,20-dione, and 20α-5P or 20α-hydroxy-5α-pregnane-3-one, together with other 5α-reduced metabolites of progesterone and pregnenolone).[4,367,474] Even later in gestation, it has been suggested that another steroid, perhaps 5α-pregnane-3,20-dione (5α-DHP) and not progesterone, is an important precursor for the other progestin metabolites found in circulating plasma.[4,367,474] A recent study demonstrated that 5α-DHP binds to the progesterone receptor with at least the same affinity to progesterone and is thought to be responsible for pregnancy maintenance in the mare for the second and third trimester of pregnancy.[451,474] In the final months of gestation, the enlarging fetal adrenal gland secretes appreciable quantities of pregnenolone, which is then used by the placenta to synthesize progestogens.[4] Even at this late stage in equine gestation, there is insufficient adrenal 17α-hydroxylase activity to convert the pregnenolone into fetal cortisol, so it passes out in the umbilical vessels and is converted to 5α-reduced pregnanes in the placenta. Thus, unlike with ruminants, there is a significant increase in the mare's plasma progestogen concentrations during the last 4 to 6 weeks of gestation followed by a precipitous drop in concentrations at the time of parturition.[4,473] A premature rise in progestins has been observed in mares with chronic placentitis, and this phenomenon is thought to be associated with fetal stress and premature maturation of the pituitary adrenocortical axis and more progesterone escaping placental metabolization.[476] When pony mares were subjected to intrafetal injections of adrenocorticotropic hormone (ACTH) at day 300 of gestation, maternal plasma progestogen concentrations increased significantly.[477]

Physiology of parturition in the mare is not as well documented as in ruminants. The precise mechanism that couples the fetal hypothalamic-pituitary-adrenal axis in the foal that is carried to term remains to be determined.[451,478] Across species, under normal conditions parturition is triggered when the fetus becomes fully mature.[478-481] In the mare, the fetal cortisol concentrations remain basal until the last 2 days' gestation, when a marked rise culminates in parturition,[4,481] and enhanced adrenocortical activity in the fetus is also associated with onset of parturition in many species.[479,482] Final maturation of the fetus results in increased ACTH release from the pituitary and subsequent stimulation of the fetal adrenal cortex. However, it is not until the maturing adrenal gland attains 17α-hydroxylase capacity that the high levels of pregnenolone are metabolized into fetal cortisol.[4,483,484] Disruptions of these fetal maturational processes, with suppression of fetal adrenocortical activity, have been suggested to be key events in the pathophysiology of fescue toxicosis.[485] The normal fetal maturational change (pregnenolone conversion into fetal cortisol) causes the maternal progestogen levels to plummet, resulting in a rising estrogen-to-progestogen ratio, and in the final hours, estrogen ultimately becomes dominant in the prepartu-rient mare.[4,451,486]

Intrafetal injections of ACTH result in precocious maturation of the equine fetus and reduction in gestational length mediated through adrenal regulation of fetal maturation and production of maternal progestogens.[481] Further work is required to establish the optimal gestational age and dosage for maternal ACTH administration before clinical recommendations can be given for this therapy.[485]

Previously it was thought that transplacental transfer of cortisol was very minimal because of the presence of the enzyme 11α-hydroxysteroid dehydrogenase, which inactivates cortisol, converting it into cortisone.[487] However, work out of the United Kingdom demonstrated that healthy Thoroughbred mares receiving 100 mg of dexamethasone at days 315, 316, and 317 of gestation could deliver live foals.[488] This demonstrated that practitioners managing high-risk pregnancies may be able to rescue the foal or improve the likelihood of a viable foal being delivered alive if the above protocol is applied.

Relaxin is produced by the placental trophoblast cells and is speculated to be involved in softening the pelvic ligaments to facilitate fetal passage and to promote uterine quiescence until parturition is imminent.[4] The myometrium becomes more responsive to oxytocin and PG, and eventually the high concentrations of oxytocin and PGs may overcome the inhibitory effects of relaxin.[4,483,489] The sudden dominance of estrogen is thought to promote cervical production of PGE_2. $PGF_{2\alpha}$ promotes myometrial contractility by acting on intracellular calcium, but PGE_2 promotes cervical relaxation.[486]

Endocrinology of Parturition

The third trimester of pregnancy is characterized by increasing concentrations in $PGF_{2\alpha}$, promoted by estrogen.[490] Estrogen also increases expression of oxytocin receptors and myometrial gap junctions, facilitating the ability of the uterus to have coordinated contractions. Interestingly, pregnant mares experience an increase in estrogen and $PGF_{2\alpha}$ concentrations during the night,[473,491] which may explain the phenomenon of the majority of foalings occurring at night.[492] Together this

results in increased myometrial contractility, which facilitates normal parturition.[493] During stage I of parturition, the contracting myometrium forces positional changes in the fetus to prepare for expulsion, and the chorioallantoic sac begins to be pushed against the softening cervix, eventually resulting in rupture of the chorioallantois, which defines the onset of stage II of parturition. Once the foal is engaged in the birth canal, expulsive efforts during vaginal passage of the foal are driven by rising oxytocin and prostaglandin concentrations, which will reach peak concentrations during stage II of parturition. Contraction of the abdominal muscles (maternal straining) is almost always associated with large sustained uterine contractions. Mares with severe debilitating conditions (e.g., chronic diarrhea, neoplasia), advanced age with poor body condition score, and mares with abdominal bandage (e.g., following colic surgery, body wall rupture, hydrops) may be unable to have adequate abdominal contractions, resulting in a delayed second stage of parturition and dystocia. Such mares need assistance delivering their foals and may progress to a dystocia due to uterine inertia if not assisted.

Concentration of PRL increases in the last week before parturition; this increase is responsible for mammary development and the initiation of lactation.[4] Prolactin release from lactotrophs in the anterior pituitary is regulated by hypothalamic secretion of PRL-releasing factor. The PRL-inhibiting factor is thought to be dopamine.[4] Oxytocin is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. Milk is produced in the alveoli and expelled into the lactiferous ducts and teat cisterns when oxytocin causes contraction of the myoepithelial cells. It is assumed that nursing by the foal stimulates the release of oxytocin from the neurohypophysis.

Myometrial Activity

The myometrium of the uterus is composed of an inner circular and an outer longitudinal smooth muscle layer. This arrangement of muscle fibers permits regulation of luminal size (circular) and uterine length (longitudinal).[4] The uterus is palpably flaccid during and immediately after estrus and then increases in tone and becomes turgid if the mare is pregnant.[4,494] Myometrial activity is vitally important to uterine clearance following breeding.[494-496] Uterine contractions also play an integral role during the embryo mobility phase of early gestation.[496] Between at least days 9 and 16 post ovulation, the spherical equine conceptus migrates continuously throughout the uterine lumen, propelled by peristaltic myometrial contractions.[4,402] This unusually long period of intrauterine movement ensures that the conceptus delivers its antiluteolytic signal to the entire endometrium. Conceptus mobility is high between days 10 and 14 after ovulation but can be reduced immediately and markedly by an IV injection of flunixin meglumine.

This suggests that prostaglandins are the primary stimulus for the myometrial contractions that drive migration of the conceptus.[407] The embryo produces prostaglandins that act as myometrial stimulants, and then subsequent uterine contractions result in embryo mobility.[404] The conceptus produces estrogens by day 12, and these, along with embryonic production of PGE_2, are thought to play a role in stimulating uterine contractions and increasing uterine tone during the mobility phase.[4] Progesterone is vital for embryonic development, and it plays a role in the mobility, fixation, orientation, and maintenance of the equine conceptus.[497]

Because there is little, if any, progesterone in the maternal circulation during late gestation, the quiescence of the uterus is thought to be maintained by progestogens, which are uteroplacental metabolites of pregnenolone and progesterone.[367,498] These hormones are thought to play a significant role through a paracrine action within the fetus and uteroplacental tissues.[498] Administration of a progestogen (altrenogest) will prevent PG-induced abortion in the first trimester of pregnancy.[115] There is considerable controversy and confusion surrounding the use of progestogen supplementation in the later trimesters, which will be discussed later.

The cellular mechanisms involved in myometrial contractility have not been well characterized in the pregnant mare, and it may not be appropriate to extrapolate from studies in nonpregnant mares. The classic theory in many species is that myometrial excitation and uterine contractility are suppressed by the so-called progesterone block.[499] Progesterone and progestogens were once assumed to be necessary for myometrial quiescence in the pregnant mare, and it was also thought that binding of 5α-dihydroprogesterone to endometrial progesterone receptors controlled uterine prostaglandin production and inhibited myometrial activity.[500] It is now known that 5α-dihydroprogesterone binds to the progesterone receptor with equal affinity.[474] The oxytocin-neurophysin I gene is transcribed into mRNA in the endometrium of mares, and mRNA levels are negatively correlated with serum progesterone concentrations.[501] However, progestogens were ineffective at controlling myometrial contractility in vitro and did not inhibit the effects of oxytocin, raising questions about the accuracy of the progesterone block theory in the mare.[502]

A reduction in circulating progestogen levels by experimental blockade with the 3β-hydroxysteroid dehydrogenase inhibitor (epostane) did not increase myometrial activity (labor) in late-gestation pony mares as it does in other species of domestic animals.[368,503] However, despite a paucity of evidence that a deficiency of fetoplacental progestogen production is a cause of pregnancy loss in the mare, exogenous progestogen therapy is widely used as a form of preventive insurance in pregnant mares, in the belief that it will promote uterine quiescence and guard against the possibility of pregnancy failure. The progestogens may enhance the activity of the endometrial enzyme 15-hydroxyprostaglandin dehydrogenase, promoting rapid metabolism of prostaglandins into inactive metabolites.[368] Early cases of premature luteal regression can only survive if the mare is treated with progestogens, and this has been used as a reason to justify progestogen supplementation.[455]

Electromyographic activity in the uterus of pregnant mares increases during the last week before foaling, even though the circulating progestogen levels are still quite high.[451,493,504] There is a progressive, reversible rise in myoelectrical activity at night in the last 6 days preceding parturition.[505] The endocrine profile of the periparturient mare is characterized by increasing concentrations of progestogens and decreasing estrogens.[369] Despite this decline, estrogens are essential for normal parturition in the mare. Recent work suggests that nightly elevations in estradiol-17β levels may bring about changes in the uteroplacental tissues, which facilitate the PG and oxytocin release that promotes the onset of myometrial contractions.[369] Pashen and Allen demonstrated that if the fetal gonads are removed, then the pregnancy would continue, but there were weak ineffective myometrial contractions and significantly reduced prostaglandin production during labor.[490] However, a recent study failed to demonstrate the importance of estrogen in late-term pregnant mares; peripheral estrogen concentrations were reduced by 90% with letrozole (aromatase inhibitor), and

mares still delivered foals normally and no changes in vascular perfusion were observed.[369]

The high levels of relaxin in the preparturient mare may act by inhibiting myometrial contractions until rising oxytocin and PG concentrations become overwhelming.[479] The number of oxytocin receptors and myometrial gap junctions may not increase until just before parturition in the mare.[493] At that time the myometrium becomes remarkably sensitive to even low doses of oxytocin.[369] A rapid membrane depolarization will result in the onset of strong, coordinated uterine contractions that characterize the first stage of labor. In the last 6 hours before rupture of the chorioallantois, a significant increase in $PGF_{2\alpha}$ concentrations occurs before there is a significant increase in oxytocin concentration.[506] Even if the $PGF_{2\alpha}$ concentrations are reduced by the COX inhibitor flunixin meglumine (1.1 mg/kg), oxytocin administration can still facilitate an expedient delivery.[506,507]

Teratogenesis

Little is known about the embryotoxic, and possible teratogenic, effects of chemicals, drugs, and other agents that may be administered to pregnant mares. Goiters have been reported in two newborn Arabian foals after the mares had been supplemented with excess iodine during the final 24 weeks of pregnancy.[508] Phenothiazines, thiabendazole, and organophosphate anthelmintics have been reported to cause pregnancy loss.[6] Likewise, Sudan grass or sorghum pastures have been reported to be toxic to the fetus.[6,509] In recent years more definitive documentation has become available with respect to the toxic effects of some medications. When griseofulvin was used to treat dermatomycosis in a mare during the second month of pregnancy, the mare carried a male foal to 331 days' gestation.[510] The foal showed bilateral microphthalmia, severe brachygnathia superior, and palatocheiloschisis. The lesions were incompatible with life, and the animal was euthanized. Griseofulvin administration during pregnancy has been associated with similar lesions in other species. Given that the development of the eyes and facial bones in the horse occurs in the second month of pregnancy, the lesions described in this case most likely can be attributed to griseofulvin administration.

Three weak, recumbent neonatal foals with skin lesions, including a thin, wooly coat, were born to mares being treated for equine protozoal myeloencephalitis.[511] The foals were anemic, leukopenic, azotemic, hyponatremic, and hyperkalemic. The pregnant mares had received sulfadiazine or sulfamethoxazole-trimethoprim, pyrimethamine, folic acid, and vitamin E orally. Serum folate concentrations in the three foals and two mares were lower than those reported in the literature for clinically normal broodmares. At necropsy each foal had lobulated kidneys with thin cortices and a pale medulla. The spleen and thymus were small. Histologic examination revealed marked epidermal necrosis without inflammatory cells, thin renal cortices, renal tubular nephrosis, lymphoid aplasia, and bone marrow aplasia and hypoplasia. These observations indicated that oral administration of 2,4-diaminopyrimidines (pyrimethamine with or without trimethoprim), sulfonamides, and folic acid to mares during pregnancy is related to congenital defects in newborn foals. Further investigations are warranted to ascertain the toxic agent and to determine at what stage of gestation the fetus is most vulnerable. In general there is a paucity of research on known teratogens in the pregnant mare, but research into the known effects in the mare and effects in other species and the potential risk and necessity of the given treatment should always be considered prior to administration of any medication to a pregnant mare.

PREGNANCY DIAGNOSIS

Standard transrectal imaging technique will permit an experienced practitioner (using a 5-MHz ultrasound transducer under optimal lighting conditions) to detect up to 98% of embryos as early as day 11 after confirmation of ovulation.[512] However, the first pregnancy check is not usually performed prior to 14 days post ovulation, as asynchronous twins are more likely to be diagnosed and the increased vesicle size makes pregnancy confirmation easier. The black (anechoic) spherical vesicle is characteristic of the ultrasonographic appearance of the equine pregnancy at this stage. Novices should realize that bright white (echoic) lines at the upper and lower margins of the vesicle are the normal ultrasound artifact (referred to as "specular reflection") generated by the ultrasound waves penetrating and reflecting through a perfectly round fluid structure.[513] It also must be appreciated that the equine embryonic vesicle can be anywhere in the uterine lumen before fixation on day 16.

Diagnostic errors can easily be made unless the entire length of both horns and the uterine body down to the cervix are meticulously searched during an ultrasonographic examination. Confirmation of mobility, as well as the presence of the specular reflection, is useful in differentiating an embryonic vesicle from a cyst. At the time of fixation at the base of a uterine horn, the yolk sac has three germ layers (ectoderm, mesoderm, and endoderm) near the embryonic pole, and only two layers (ectoderm and endoderm) at the opposite pole.[4] The difference in rigidity between the three-layered ventral wall and the two-layered dorsal wall explains the characteristic guitar pick–shaped image on ultrasound by day 18. The thickest portion of the yolk sac wall (embryonic pole) rotates to a ventral position. The change from the previous spherical shape is caused by uterine turgidity and thickening of the dorsal uterine wall. The increased uterine tone is responsible for the failure of the embryonic vesicle's diameter to enlarge between days 18 and 26, thus creating the classic growth profile that plateaus during this period.[4]

By day 21 post ovulation, the amniotic cavity has completely formed, and the embryo can often be detected by ultrasound. As the allantoic sac enlarges, the embryo proper can be seen to be raised off the ventral floor by day 24. The embryo can be seen suspended on a thin echoic line that delineates the apposition of allantois and yolk sac.[513] This separating membrane tends to be horizontal, and thus it is a useful means for differentiating a singleton from twin embryonic vesicles. In the latter case the adjacent walls of the two vesicles tend to form a vertical separation. The primitive heartbeat can be detected between 22 and 25 days and is a useful indicator of embryonic viability. By day 40 the embryo (now called a fetus) has been elevated to the dorsal pole because the allantoic cavity has almost completely displaced the vestigial yolk sac. The membranes and blood vessels that separate the allantois and yolk sac give rise to the umbilical cord. The increasing size of the fetus causes the fetus and amniotic sac to gradually descend back to the floor of the chorioallantoic vesicle by day 48. The remnant of the yolk sac is incorporated into the umbilical cord. The site in which the umbilical cord attaches to the chorioallantois identifies the horn and site in which embryo

fixation originally occurred. The dorsal attachment ensures that the developing fetus will not compress this vital area.[402]

These gestational developments can be used by the practitioner to determine whether the pregnancy is developing normally. Generally each practitioner will have set gestational ages that pregnancy evaluations are performed to ensure normal development of the pregnancy. Most practitioners will perform ultrasound evaluations at the following times: 14 to 16 days to ensure normal size and identify the presence of twins, 28 to 30 days to confirm normal development, heartbeat and no twin before the formation of endometrial cups, 42 to 60 days to assess development and potential sex of the fetus, and later checks vary more after this period with either one or monthly evaluations thereafter. Generally mares with a history of pregnancy loss or abnormal ultrasound findings are evaluated and monitored more frequently to determine appropriate management and treatment.

Although ultrasonographic examinations have become standard practice for confirmation of pregnancy in mares, it is still essential that the veterinarian be competent at manual diagnosis. By 18 days after breeding, an experienced clinician may be able to perceive changes in uterine tone and consistency that, coupled with palpation of a narrow elongated cervix, are consistent with early pregnancy. However, it should be remembered that it is not uncommon for a persistent CL to provide a prolonged progesterone environment in a nonpregnant mare. It is important not to mistake the curvature at the base of the uterine horn for an embryonic vesicle.[4] An ultrasound image of the embryonic vesicle is required to definitively confirm pregnancy at this early stage. However, the gradually enlarging embryonic vesicle will eventually create a discernible ventral bulge that has been described as being the size of a hen's egg (day 30), goose's egg (day 35), and orange (days 40–45). Because there is minimal dorsal distention at this stage of pregnancy, it is essential that the ventral aspect of each uterine horn be examined. Errors can be made if the fingers are not passed far enough around the cranial aspect of the uterine horn to reach well under the uterine body and horns. A ventral bulge that is consistent with a 35- to 45-day pregnancy can be identified by the distinct margins that are palpable when the fingers are moved along the ventral aspect of the uterus. The conceptus becomes more oval as it expands during the third month of pregnancy. At 90 days, the chorionic vesicle is comparable in size and shape to a football. The mare's age and the number of previous foals will affect the rate of descent of the gravid uterus over the pelvic brim. Positive pregnancy diagnosis becomes progressively more difficult as the large, heavy uterus descends into the abdominal cavity. Normal pregnant mares should have a thin, pliable uterine wall, whereas pyometra cases have a thickened uterine wall and viscous purulent fluid in the uterus. The fetus itself is not always palpable before day 120 of gestation, but gentle ballottement will usually reveal the fetus after that time. Palpation of some part of the fetus should be able to confirm pregnancy status between 5 months and term.

Blood tests for pregnancy are sometimes indicated, especially in Miniature breeds.[514] Measurement of eCG levels verifies that the mare has endometrial cups but does not guarantee that a viable fetus is still present.[4] With this caveat in mind, eCG is suitable for determining pregnancy status in Miniature mares between 40 and 100 days after mating.[515] However, mares that have been diagnosed as pregnant should undergo a blood estrone sulfate test 100 or more days after mating to eliminate the possibility of a false-positive diagnosis. Determination of eCG concentration is used to confirm suspicious retention of endometrial cups in mares with history of losing a pregnancy in the first 100 days.

The fetoplacental unit secretes large quantities of estrogen.[4,451] Measuring blood estrone sulfate levels is recommended as the method of choice for determining pregnancy status in Miniature mares 100 or more days after mating.[515] Fecal estrone sulfate measurements provide a noninvasive alternative to blood testing from 150 days after mating. However, the discrimination between pregnant and nonpregnant estrone sulfate concentration is not as great in feces as it is in blood.[515] Determination of estrone sulfate and eCG concentrations can be used in some cases as confirmatory pregnancy diagnosis tests. For example, Miniature mares presented for pregnancy diagnosis are initially screened with an abdominal ultrasound. If signs of pregnancy are detected (fetal fluids, fetal heartbeat, and movements) then the mare is confirmed pregnant, but if one fails to identify these findings with ultrasound, then blood can be collected for eCG and/or estrone sulfate determination. Nonpregnant mares will have baseline concentrations for both hormones.

MANAGEMENT OF TWINS

In the mare it is assumed that most twin pregnancies arise from double ovulations, but occurrence of monozygotic and triplet pregnancies has been reported.[516-519] Presumably, monozygotic twin pregnancies may occur more often in mares following the transfer of a single embryo for unknown reasons.[516] There is a higher incidence of double ovulations in Thoroughbred and Warmblood mares, but mares of any breed may consistently double ovulate.[520-524]

In a large retrospective study involving more than 3000 cycles in 1581 Thoroughbred mares, multiple ovulation occurred in 29.3% of cycles.[523] Incidence of multiple ovulation increased with age (20.7% in 2- to 4-year-olds; 35.6% in 17- to 19-year-olds). Multiple ovulations may occur synchronously (same day or <2 days apart) or asynchronously (>2 days apart).[4,523] Asynchronous ovulation is termed *diestrous ovulation* because more than 2 days after the first ovulation the concentrations of progesterone will rise and the mare is in diestrus. Both synchronous and asynchronous ovulation may result in twin pregnancies, particularly if semen from a fertile stallion was used for breeding. This emphasizes the importance of counting the number of CLs present at the first (13–16 days) and second (23–28 days) pregnancy diagnosis, as well as meticulous ultrasound evaluation looking for the presence of a second embryonic vesicle, even if only one ovulation was detected. Epidemiologically, mares ovulating from different ovaries are more likely to have twin pregnancies than mares ovulating in the same ovaries. In addition, asynchronous ovulations within the same ovary are less likely to result in twin pregnancies compared with synchronous ovulations in the same ovary.[4,525] In a large study with Thoroughbred mares in the United Kingdom, 25.2% of multiple ovulations were apparent as multiple pregnancies and 37.8% as single pregnancies at 13 to 14 days post ovulation.[523] In a previous study of Thoroughbred mares in the United Kingdom, the rate of twin vesicles present at days 13 through 16 after ovulation was reported to be between 10.3% and 13.1%.[526] This was similar to a Swedish study (n = 430) in which the frequency of twin vesicles at days 14 through 15 was 10.5%.[527]

In the past, veterinarians often discouraged breeding of a mare when two large (>30 mm) follicles were palpated or to recheck the second follicle 10 to 12 hours after the first detected ovulation.[528] Because an ovulated oocyte is less likely to result in a viable embryo after this time, a delayed breeding could be performed in anticipation of the second ovulation. Today it is recommended to breed all eligible mares irrespective of the number of preovulatory follicles and perform a thorough pregnancy diagnosis between 13 and 16 days. The widespread adoption of early ultrasonographic pregnancy examinations has permitted the focus to be placed on embryonic vesicle reduction after the presence of a twin pregnancy is confirmed.

Manual Reduction

The increasing size of the embryonic vesicle, coupled with the increasing tone of the early pregnant uterus, will lead to the fixation of the conceptus at the base of one uterine horn by day 16.[4] When performing pregnancy diagnosis in mares, particularly during the first 100 days of pregnancy, it is essential that the ultrasonographic examination of the uterus is thorough, ensuring a complete evaluation of both uterine horns and the uterine body extending back to the cervix. This is especially important before day 16, because the vesicle moves freely within the lumen of both horns and the uterine body.[525] If twin vesicles are detected, then manually separating them before day 16 will be easier. Successful elimination of one vesicle is more likely at that time because the uterine walls are thin and minimal pressure is required to crush a vesicle.[529] A definite *pop* can often be felt when the vesicle ruptures, but success always should be confirmed by ultrasound. This sensation is attributable to the rupture of the embryonic capsule.[4,530]

The downside to this approach is that an early embryonic vesicle can easily be confused with an endometrial cyst. The embryo itself does not become readily identifiable until the fourth week of pregnancy (i.e., embryo proper and beating heart). Thus it is good practice to note the size and location of any cysts at the time the mare is being examined for breeding. Uterine cyst mapping is more easily performed post ovulation when the uterine horn becomes more toned because of the increasing progesterone concentrations. Interestingly, some cysts change shape according to the uterine tone. The printing of high quality pictures of the cysts or the ability to record them in a flash drive connected with the ultrasound machine has proven particularly useful in mares with excessive lymphatic cysts. If there is no record of cyst size and location, then it is virtually impossible to distinguish early twin vesicles from a singleton and a cyst with a single examination. This is especially true because asynchronous ovulations are likely to result in considerable size discrepancy between the two vesicles.[4]

If the clinician evaluates a mare at first pregnancy diagnosis and there is a suspicion of twins but no lymphatic uterine cysts mapped, it is recommended that the clinician record the size and location of each structure and reevaluate the mare in 1 or 2 days. In the next reproductive ultrasound, there will be little change in format of lymphatic uterine cysts, but embryonic vesicles should grow approximately 4 mm per day and are likely to move.[530] Early diagnosis of twin pregnancies is paramount for the best odds to successfully reduce one of the embryonic vesicles before fixation because delays may make separation of unilaterally fixed vesicles more difficult because of their ongoing growth and the increased uterine tone.

Manual reduction of bilaterally fixed vesicles requires less manipulation than with unilateral twins. It is a relatively easy procedure, and success rates exceeding 90% are not uncommon if the vesicle is crushed before day 16.[529,531] After fixation a success rate of 75% is still possible if the bilateral twin is crushed before day 30.[529] If the vesicles are unilaterally fixed, then the clinician should attempt to move the more proximal vesicle toward the tip of the uterine horn. At this location the manual reduction procedure is less likely to disrupt the remaining vesicle. The vesicle can be crushed by pinching it between the thumb and fingers. Alternatively, the vesicle is squeezed against the mare's pelvis using the ultrasound probe or fingers until it ruptures. If the twins can be separated before crushing, then the success rate may be similar to that for reduction of bilateral twins. If the unilateral twins cannot be separated or are greater than 20 days' gestation, then the success rate is lower.[532] After fixation and with unilaterally fixed vesicles (<20 days), it is the authors' preference to reduce twin pregnancies using the "snowflake" method, which consists of applying pressure with the transducer until hyperechoic spots (resembling snowflakes) are observed within the embryonic vesicle. A perceived advantage of this method is that there is less manipulation of the uterus and therefore less possibility of trauma to the other vesicle. Potentially the damaged vesicle that presented the snowflake appearance will release the fluid from the yolk sac slowly, which will be less likely to dislodge the viable vesicle and cause the loss of both embryonic vesicles.

Because squeezing an embryonic vesicle results in increased concentrations of 15-ketodihydro-PGF($_{2\alpha}$) and cortisol,[533] most clinicians commonly administer a combination of antiinflammatories (flunixin meglumine 1.1 mg/kg q 24 for 3 days) and progesterone (150–300 mg/day per mare) or altrenogest (0.044 mg/kg) until pregnancy diagnosis at 25 days post ovulation.

Transrectal ultrasound should be used to identify twin pregnancies and to maneuver the embryonic vesicle toward the tip of the uterine horn or against the cervix in exceptional cases. For a large majority of twin reductions, the authors recommend using the transducer of the ultrasound to identify, manipulate, and squeeze the embryonic vesicle to be reduced. In certain mares, one of the authors (IC) recommends moving the embryonic vesicle toward the tip of the uterine horn with ultrasound and then squeeze one vesicle with the hand without the transducer. This seems to be particularly useful for small embryonic vesicles (~10–12 mm). If the practitioner is not able to comfortably separate vesicles that are next to each other, then it is recommended to recheck the mare within 1 hour or during the next day if the embryonic vesicles are not likely to be fixed the next day. Rechecking the mare the next day may enable the practitioner to reduce one pregnancy manually because the embryonic vesicles have grown in diameter and might be located in an easier location to perform manipulation and manual reduction.

The likelihood of success improves with experience.[529,530] If the unilateral vesicles are not detected until after day 20, then manipulations can easily result in the disruption of both vesicles. If the practitioner is not experienced in performing manual twin reduction, then the unilaterally fixed vesicles should not be the ones used to gain experience. The best option in these cases may be to wait and see whether natural reduction occurs or refer to a more experienced colleague.

Natural Reduction

There is negligible natural reduction of twins before day 15, when the vesicles are close to the end of their mobility phase.[4]

Thus manual reduction is the treatment of choice if twins are diagnosed before fixation. Almost three quarters (70%) of twin embryonic vesicles become fixed unilaterally, with only 30% of twin vesicles becoming fixed bilaterally.[534,535] When twin vesicles are dissimilar in size, there appears to be a much higher incidence of unilateral fixation. The larger vesicle is thought to serve as an impediment to the continued mobility of the smaller vesicle.[402] Fortunately, natural reduction to a singleton is far more likely with unilaterally fixed vesicles. More than 80% of unilaterally fixed twins are likely to naturally reduce to a singleton, with over half of these occurring between days 16 and 20.[6,158,159] On the other hand, the majority of bilaterally fixed vesicles will continue to develop. The proposed mechanism for reduction has to do with loss of apposition between the embryo's trophoblastic surface and the endometrium, especially if the embryo proper is adjacent to another vesicle.[402,534-537]

Pregnancy Termination with Prostaglandin

The probability of natural reduction occurring decreases significantly by day 40.[534,536] If natural reduction does not occur, termination of the early pregnancy with a PG injection may be considered. This should lyse the CLs that resulted from the double ovulation, and the precipitous decline in progesterone will bring the mare back into estrus. However, treatment failures have been reported, and successful termination of the pregnancy should be confirmed by ultrasound.[538] This treatment must be given before day 35 because once the endometrial cups begin to form, repeated PG injections may be necessary to terminate the pregnancy.[498] Owners should be aware that the mare is unlikely to return to normal ovulatory cycles until the cups are sloughed. In the interim, they secrete eCG, a hormone that causes the development of accessory CLs. As mentioned previously, mares with elevated eCG demonstrate erratic estrous behavior, unreliable follicular development, and unpredictable ovulation.

If a mare carries twins into the latter half of gestation, then it is quite likely that abortion will occur between 7 and 9 months. Induced abortion is not without risk, and owners should be aware of the potential for dystocia, cervical lacerations, and fetal membrane retention.[529] Although several researchers have investigated induction of parturition at term, few protocols for early termination of an advanced pregnancy have been published. The natural PG analog cloprostenol has been used at various doses and frequencies, but results are inconsistent with respect to efficacy and duration to fetal expulsion. Daels et al. administered cloprostenol (250 µg) daily until fetal expulsion or for up to 5 days in mares that were 98 to 153 days pregnant. Fetal expulsion occurred after two to three cloprostenol administrations.[498] The addition of intracervical PGE_1 on the second day may help dilate the cervix and hasten delivery.

In Argentina, polo pony breeders do not want male colts; thus a large number of embryo-recipient mares undergo induced abortion to terminate pregnancies of male fetuses following fetal gender determination by 60 days post ovulation. Recently, a new technique was introduced that consists of administering one dose of 500 µg of cloprostenol diluted in 10 mL of saline intracervically; this technique resulted in 100% of the 104 mares aborted within 48 hours. A follow-up study reported that 84.4% (27 of 32) of mares aborted in 48 hours following a single uterine infusion with cloprostenol.[539] The same study reported that most mares underwent 1 to 2 anovulatory cycles (57.2%), whereas about 37.5% apparently ovulated normally and 6.2% progressed to anestrus. Following abortion, 18 mares were reused as an embryo recipient and became pregnant after embryo transfer (mean 58 ± 4.4 days).[539]

Transvaginal Ultrasound-Guided Twin Reduction

Even though the advent of transrectal ultrasonography has dramatically improved the ability of veterinarians to make an early diagnosis of twin pregnancies, diagnostic errors still occur. This could be caused by an early pregnancy diagnosis when the second vesicle was too small to detect, incomplete examination of the entire uterus, poor image quality, or an inability of the clinician to differentiate two embryonic vesicles that are closely apposed to each other. If natural reduction does not occur or the diagnosis of twins is not confirmed until after 30 days, then transvaginal aspiration of one vesicle is an option.[529,532,540] The results are best if the procedure is performed before day 35 and preferably before day 25. Although spontaneous reduction of twin pregnancies can occur even after day 40, the probability of this occurring is low. Natural twin reduction is more likely to occur if an obvious size discrepancy between the two vesicles is present at this time.

If a transvaginal reduction is to be attempted, the mare should be treated with flunixin meglumine. Many clinicians will also administer oral altrenogest. Because sedation causes significant uterine relaxation, most clinicians use a lidocaine enema to reduce straining.[541] The transvaginal aspiration technique uses a 5.0-MHz or 7.5-MHz endovaginal curvilinear transducer. The transducer and casing should be cold disinfected or sterilized before use. The assembled unit is then placed in a sterile transducer cover that has been filled with sterile lubricating gel. The transducer is advanced aseptically until its footplate contacts the cranial vaginal wall, lateral to the cervix. The clinician grasps the pregnancy rectally and advances a sterile, 60-cm, 18-gauge spinal needle with an echogenic tip along the needle guide in the transducer casing. A dotted line on the ultrasound screen can be used to select a path for the needle entry into the embryonic vesicle. A sharp jab of the needle penetrates the vaginal wall, peritoneal lining, uterus, and ultimately the allantoic or yolk sac. A 60-mL syringe is attached to the needle, and the embryonic fluid is aspirated. Aspiration should be stopped when there is a danger of damaging the adjacent vesicle of unilateral adjacent twins. If a bilateral twin is being eliminated, then the needle can be moved within the vesicle until all detectable fluid has been aspirated. The success rate is better for bicornuate twin reductions.[529] Death of the remaining twin is most likely to occur within 2 weeks of the procedure. A recent retrospective study compared two techniques for reducing twin pregnancies by 35 to 45 days post ovulation. Both techniques used the transvaginal ultrasound-guided approach to either stab one of the embryos/fetuses or to aspirate the yolk sac or allantoic sac, and no difference in the results was found between the two techniques.[541] Experienced operators may achieve a live singleton birth in about one third of cases.

Craniocervical Dislocation

Results of postfixation twin reduction techniques have been inconsistent regarding the production of a single healthy foal. A craniocervical dislocation method entails dislocating the first cervical vertebra from the cranium, disrupting the ligamentous

attachments, and severing the spinal cord either through a flank incision or transrectally.[529] A transrectal approach may be performed on a sedated mare between 60 and 90 days, but the mare's body frame and operator's arm length may limit the ability to perform the procedure safely. For the transrectal approach, propantheline bromide (30 mg IV) is recommended to relax the smooth muscles of the uterus and rectum, and flunixin meglumine (1.1 mg/kg) is administered to inhibit PG release. Daily altrenogest (0.044–0.088 mg/kg) is recommended for 3 to 4 weeks following the procedure or until the beating heart from only one fetus is present. A surgical procedure has been used most frequently on twin pregnancies of between 2 to 4 months' duration, and the twin closest to the tip of the horn is selected for elimination. This technique is performed with uterine and fetal manipulation through a flank incision, which enables one twin to be reduced before complete placenta formation has occurred. This allows the remaining fetus to use the entire endometrial surface and to grow to its full potential. Interestingly, a fetus was reported as having a beating heart for as long as 7 weeks following craniocervical dislocation.[529]

Transcutaneous (Abdominal) Ultrasound-Guided Fetal Puncture

In advanced twin pregnancies (up to approximately 5.5 months), it is possible to attempt reduction by a transabdominal approach.[532] Fetal intracardiac injection of potassium chloride (KCl) is effective but requires accurate placement of the KCl into the fetal heart. Best results are obtained when the pregnancy is between 115 and 130 days. At this stage experienced operators can achieve a 50% success rate.[532] Procaine penicillin G can cause fetal death when injected into either the fetal thorax or abdomen, but the effect is not instantaneous. The advantage of the latter treatment is that it does not require precise placement of the injection into the fetal heart. Procaine penicillin G also reduces the likelihood of bacterial infection, and the injection can be visualized on the ultrasound screen. Mares should be started on oral altrenogest, systemic antibiotics, and flunixin meglumine on the day of the procedure. The antibiotic coverage and antiinflammatory medication should be continued for 3 days in uncomplicated cases.

A 3.0-mHz transducer can be used to image the 90- to 130-day fetus in the caudal abdomen, just cranial to the udder, and best results may be obtained after day 115.[532,542] Once the mare has been sedated, the uterus will relax, and the location of the fetuses will shift cranially. A sedative–analgesic combination that works well for this procedure is acepromazine (10 mg), xylazine (100 mg), and butorphanol (10 mg). The smallest or most easily accessible fetus is selected for reduction. The ventral abdomen should be surgically prepared and local anesthetic infiltrated at the puncture site. An 18-gauge, 8-inch spinal needle with stylet can be used for most fetal injections, but the length of the needle is determined by the depth of the fetus from the abdominal wall. Specialized needles with echogenic tips are available to provide better visualization via ultrasound.[529] Once the location of the selected twin's thorax is confirmed, the needle is introduced through the aseptically prepared skin, abdominal wall, and uterus. If procaine penicillin G is to be injected, then the needle may puncture either the fetal thorax or abdomen. Up to 20 mL is typically injected into the fetus. Fetal death should be confirmed the next day.[529,532]

Although the benefits of supplemental progestin therapy are debatable, many clinicians suggest that the mare be medicated for at least 2 weeks if the initial twin reduction has been successful. It is essential that fetal viability is checked regularly because supplemental progestogen therapy may interfere with normal pathways that facilitate elimination of the dead fetuses if both die. Most abortions occur within 1 to 2 months after the reduction procedure.[532] If the operator is experienced in the technique, between 40% and 60% of cases reported have resulted in delivery of a live foal, although it should be noted that conservative estimates in clinical practice are more likely and often the remaining twin experiences some degree of IUGR.[529,532] The eliminated twin in these cases can be seen as a mummified remnant contained within an invaginated pouch that protrudes into the allantoic space of the viable foal's fetal membranes. One theory for the loss of both twins after an intrafetal injection has to do with the presence of vascular anastomoses between the two fetoplacental units.[532] It has been suggested that circulation of either the injected solution or other tissue degradation products could result in the death of the adjoining twin fetus. Small anastomosing vessels are present between twin vesicles as early as 40 to 60 days' gestation.

EARLY PREGNANCY LOSS AND ABORTION

Embryonic Loss

Pregnancy losses occurring prior to day 40 of gestation are defined as embryonic losses, whereas losses beyond this point are considered fetal losses or abortions (Table 19.10).[4] According to the stage of occurrence, pregnancy wastage may also be classified as stillbirth (delivery of a dead foal ≥300 days of gestation), abortions (losses from 40 to 300 days of gestation), and neonatal losses (deaths during the first week or 60 days of life).

Between 10% and 15% of mares undergo embryonic loss or abortion at some time in gestation, and most of these losses occur during the first 40 days' gestation, when the primary CL is the sole source of progesterone.[526,543] However, current evidence suggests that untoward luteolysis is not common in this period as suspected, and the losses that do occur have other underlying causes. Luteal insufficiency/premature luteal regression has been documented, however, in mares as described above.[453,454]

In a study of 376 Thoroughbred pregnancies, 12.2% experienced embryonic loss within 45 days after ovulation,[544] whereas a much larger study (n = 3373 mares) reported a loss of 7.2% to 8.0% by day 42.[526] Three quarters of the losses in the smaller study occurred between 16 and 25 days, and the loss was highest in previously barren mares, followed by aged mares (>15 years) and mares of more than 10 parities. These results were similar to those reported by Swedish investigators.[527] Increasing mare age was the single biggest limiting factor to an otherwise high rate of fertility in the larger study of well-managed Thoroughbreds in the UK.[526] Higher embryonic losses may be expected in mares that are bred during the first postpartum estrus and in those with cysts in the uterus.

It is not easy to differentiate between fertilization failure and embryonic loss before day 10 because this is the earliest stage of development in which ultrasonographic detection is possible under ideal research conditions. Fertilization failure rates and embryonic losses are higher in aged mares.[545-549] Fertilization rates in young, well-managed mares may exceed 90%

TABLE **19.10 Causes of Embryonic Losses in Mares**

Intrinsic	Endometrial disease	Endometritis will have deleterious effects on the embryo, which may result in prostaglandin production and early return to estrus Endometrial fibrosis causes improper embryonic nutrition because the horse is highly dependent on uterine milk secretions for survival during the embryonary period Large lymphatic uterine cysts in the uterine lumen may result in improper endometrial signaling resulting in failure of maternal recognition of pregnancy Large cysts present at the base of the uterine horn may result in embryonary death caused by improper embryo nutrition from endometrial glands
	Progesterone insufficiency	Premature luteal regression or improper CL formation is thought to cause early return to estrus, and low progesterone concentrations in plasma affect secretions of proteins in the uterine milk and, consequently, embryonic growth
	Maternal age	Older mares have defective oocytes, advanced stage of endometrial fibrosis, more lymphatic and endometrial gland uterine cysts, and endometrial fibrosis, which are associated with higher pregnancy losses
	Lactation	Lactating mares experience higher rates of pregnancy loss, particularly if under poor nutrition
	Foal-heat breeding	Mares bred in the foal heat have higher rates of embryonic losses, particularly if the mare ovulates before day 9 postpartum, resulting in arrival of the embryo in utero while the endometrium has not been completely regenerated
	Postovulation breeding	Breeding post ovulation results in higher pregnancy losses, probably related to oocyte aging
	Site of intrauterine fixation of the embryonic vesicle	Embryonic vesicles fixed at the uterine body, particularly the caudal body, present higher incidence of pregnancy loss; this condition is known as "body pregnancy"
	Maternal chromosomal abnormality	Mares carrying chromosome abnormalities are prone to producing defective embryos, which result in embryonic or early fetal loss
	Embryonic defects	Embryos presenting with morphologic abnormalities and underdeveloped for the stage of development result in higher embryonic losses
Extrinsic	Stress	Stress will result in an increase in cortisol concentration and potentially reduction of LH and luteal function, or it may result in ovulation failure
Nutrition		Poor nutrition is associated with reduced progesterone and higher rates of embryonic losses
Season/climate		Anecdotally severe weather (heat/cold) is thought to result in higher pregnancy loss Under experimental conditions mares exercised under intensive Texas weather had a reduced embryo recovery rate compared with mares without exercise, and they tended to present more grade II embryos
Embryo manipulation		Embryo transfer, in vitro–produced embryos, or embryos resulting from ICSI are thought to result in higher pregnancy rates

CL, Corpus luteum; *ICSI,* intracytoplasmic sperm injection; *LH,* luteinizing hormone.

and appear to be over 80% in aged mares.[547,550] Oocytes from aged mares may be more likely to result in nonviable embryos because of inherent morphologic defects.[547,551] Carnevale transferred oocytes from young and aged mares into young recipients so that fertilization and early embryonic development occurred in an optimal oviductal environment. The day-12 pregnancy rate in the recipients that received the oocytes from aged mares was significantly less than that achieved with the younger mares' oocytes.[552] Research has shown that embryo recovery rates are considerably lower in aged mares and that there are significant losses occurring before day 14 of pregnancy.[545,553-555]

The uterine environment may not be the only reason for subfertility in some mares. Embryos collected from normal mares resulted in similar pregnancy rates in both fertile and subfertile recipients (advanced stages of endometrial pathology) at day 28.[556] In a reversal of study design, embryos were collected from the oviducts of normal and subfertile mares 4 days after ovulation and then transferred into normal recipients. Pregnancy rates were lower in those normal mares that received embryos from the subfertile donors.[553] Thus, although the uterine environment may have a delayed effect on embryonic and fetal loss, it appears that oocyte quality and oviductal influences play a significant role in the problem of subfertility and early embryonic loss in mares.[557] In practice, embryonic losses that may be detected between days 14 and 40 can range between 8% and 15% in young, well-managed mares to 25% or 30% in aged mares.[526,550,558] The presence of endometrial inflammation and uterine fluid accumulation will have a detrimental effect on early embryo survival and can markedly increase the likelihood of early pregnancy loss.[83,85,544]

Early Fetal Loss

In one study of Thoroughbred mares, only 4% to 6% of pregnancy losses occurred between day 40 and day 150 gestation, and only a further 2% to 3% occurred before term.[526]

Formation of the endometrial cups is a defining moment related to early pregnancy loss in mares. If the embryo dies before day 35, the chorionic girdle cells do not invade the endometrium, and the endometrial cups do not form. These mares should return to normal estrous cycle activity and may be successfully bred again during the same breeding season. However, if the fetus is lost after day 40, the endometrial cups are irreversibly established.[4] Thus, mares that lose the pregnancy after day 40 following endometrial cup formation will have elevated eCG concentrations until those endometrial cups slough between days 120 and 140 of gestation. This will result in a false-positive blood test for pregnancy using an assay for eCG. Retention of the endometrial cups after fetal loss results in erratic estrous behavior, unreliable follicular development, and unpredictable ovulation.[161] Thus this unique physiologic mechanism typically prevents mares suffering fetal loss after endometrial cup formation from being bred back during the current breeding season.[160]

Pregnancy losses in mares may be multifactorial and difficult to determine. Beyond day 40 of gestation the secondary corpora lutea receive powerful luteotropic support from eCG, which results in increased progesterone concentrations. This makes the corpora lutea resistant to luteolysis as multiple exogenous doses of prostaglandin may be required to induce abortion. From day 80 to 100 until term the supply organ (placenta) and target tissues (endometrium and myometrium) are in direct contact with one another over their entire surfaces. Little evidence suggests that a deficiency of progesterone production is a cause of pregnancy loss in the mare.[543] Certainly, fetal death may follow uteroplacental insufficiency or an overwhelming sepsis.[559] In recent years there has been a general consensus that the inflammatory mediator $PGF_{2\alpha}$ may play an integral role in many cases of pregnancy loss. It is well known that PGs are luteolytic and increase uterine contractility. Thus, in the first 70 to 80 days, when the pregnancy depends on progesterone production by the corpora lutea (primary and accessory), the mare is especially susceptible to the luteolytic effects of PGs. It is important to remember, however, that repeated exogenous PG injections may be required to electively terminate a pregnancy once the endometrial cups have formed. This is because some of the immature accessory CLs may not be sufficiently developed to respond to the first PG injection. Another probable abortigenic feature of PGs may be myometrial hypermotility. This may be associated with placental inflammation or high systemic levels of PG.

An early pregnancy may be lost subsequent to PG-induced luteal deficiency associated with endotoxemia.[560,561] The detrimental effect of the endotoxin could be prevented only if a COX inhibitor (flunixin meglumine) was administered before clinical signs of endotoxemia were evident.[498,562,563] Thus, although gram-negative septicemia and endotoxemia associated with many gastrointestinal crises are known to result in elevated levels of inflammatory mediators, any pregnancy-sparing effect of PG inhibitors is likely to be effective only if antiinflammatory agents such as flunixin meglumine are administered in the acute phase of the disease.[559] Because a healthy fetoplacental unit can produce enough progesterone to sustain the pregnancy after 80 days' gestation, the concept of prophylactic altrenogest past 3 months of gestation is controversial.[371,498,543,563]

Although recent in vitro studies suggest that progesterone may not be the primary regulator of myometrial quiescence, in situations in which elevated PG levels are likely, there appears to be adequate clinical justification at present to provide exogenous progestogen support for high-risk pregnant mares.[502,564] Based on current knowledge, the administration of a double dose (0.088 mg/kg/day) of altrenogest is suggested during the acute phase of a medical or surgical condition when PG levels are likely to be elevated.[565] If oral medication is not possible, then short-term use of progesterone in oil (300 mg/day IV) is warranted. An IM injection of a compounded long-acting progesterone formulation (BioRelease P4 LA 150 mg/mL) every 7 days may also be an efficacious and suitable alternative to currently available progesterone formulations that require daily administration.[372] In the author's practice, LA P4 is used in situations where daily dosing is not possible or not reliable. Mares at high risk for imminent abortion should preferably receive short-acting progestogens.

Administration of supplemental progesterone is based on further work by Daels et al. demonstrating that progestin treatment could prevent PG-induced abortion at 3 to 5 months of gestation if a higher dose of progestin (0.088 mg/kg altrenogest) was administered.[498,563] Abortion did not occur in five of eight progesterone-treated mares and eight of eight altrenogest-treated mares, and endogenous $PGF_{2\alpha}$ secretion was inhibited, compared with values in aborting mares. It was concluded that circulating progestogen concentrations may play a role in the outcome of pregnancy during PG-induced abortion, which may occur after exposure to endotoxin.

It seems logical that separation of the chorioallantois from the endometrium will disrupt local endocrine function. The fetoplacental unit does attempt to compensate for this placental dysfunction by increasing progesterone production.[564,566] However, endotoxemia may harm placental circulation and disrupt vital steroid metabolism within the fetoplacental unit. Thus administration of flunixin meglumine to pregnant mares is indicated early in the course of any condition in which endotoxemia is possible.[567-569] If a mare develops a surgical colic condition during late pregnancy, then the fetus is at risk not only from the maternal endotoxemia that can be associated with gastrointestinal crises but also from any maternal hypoxic episodes that may occur during anesthesia.[568,570-572] Acute enteritis or colitis in a pregnant mare can also result in abortion caused by the effects of endotoxemia.[570,572] Because maternal hypoxia is a risk factor for abortion, intraoperative hypoxia must be avoided if a pregnant mare requires surgery. Approximately 16% to 20% of mares can be expected to abort after colic surgery, but superior intraoperative ventilation and oxygenation techniques may reduce this risk.[570,572] Stage of gestation and duration of anesthesia are less critical factors if maternal oxygenation is adequate. Apparently, aberrations in the cardiovascular and metabolic status of the mare and fetus are more detrimental to pregnancy maintenance than the actual medical or surgical condition.

Mare Reproductive Loss Syndrome

In the spring of 2001, an epidemic reproductive crisis affected the equine breeding industry in central Kentucky, southern Ohio, West Virginia, and Tennessee and ultimately cost hundreds of millions of dollars. Mares foaled prematurely with premature separation of the chorioallantois (late-term "red bag" abortions). Many mares that were approximately 45 to 80 days pregnant suffered acute fetal loss, and most affected mares failed to resume normal cyclic activity until after the official breeding season had closed because endometrial cups had been formed before the occurrence of mare reproductive

loss syndrome (MRLS).[573] The Appalachian region to the north also reported a high number of similar cases. Reports of weather patterns and an unusually high emergence of eastern tent caterpillars (ETCs; *Malacosoma americanum*) were identical in both the central Kentucky and southeast Ohio regions.[574] The epidemic that affected the horse industry in the Ohio Valley in late April and early May of 2001 and 2002 became known as MRLS.

During the initial outbreak, the state diagnostic laboratory was inundated with late-term aborted fetuses, and practitioners identified approximately 2000 early fetal losses.[575] Many of these were detected during what should have been a routine fetal gender determination. Fortunately no contagious infectious cause was identified, and this newly recognized disease became self-limiting as the breeding season progressed. Veterinarians and scientists involved in the epidemic considered the possibility of mycotoxins, ergot alkaloids, phytoestrogens, and even cyanide from wild cherry tree foliage.[576-578] A temporal correlation was established between MRLS and the presence of ETCs.[573,576,579-590] Mares inadvertently ingest ETCs when large numbers are present on pasture, hay, or in the water supply. Abortions consistent with MRLS can be induced by oral administration of whole ETCs or their exoskeletons, which contain hairs (setae). Ingested setae embed into the submucosa of the gastrointestinal tract. Fetal loss occurs when bacteria from the alimentary tract *(Streptococci, Actinobacilli)* establish infection in which the local immune system is compromised (the fetoplacental unit). Management strategies for controlling ETC have been proposed to reduce the risk of MRLS.[575,583] A similar abortion syndrome in Australia is associated with ingestion of hairy processionary caterpillars *(Ochrogaster lunifer)*.[590]

Placentitis

Placentitis is a major cause of pregnancy loss in late-term pregnant mares.[178,591,592] Inflammation of the placenta can affect the interchange of gases, nutrients, and waste products along with disruption of both fetal metabolic and endocrinologic pathways.[593] In recent years, controlled studies and clinical observations from various laboratories and practices across the world have contributed to major advancements in the recognition and treatment of equine placentitis. The reader is referred to recent comprehensive reviews for additional, more detailed, information.[594,595] Four types of placentitis are described based on macroscopic lesions: ascending (focal extensive), nocardioform (focal mucoid), diffuse (hematogenous), and multifocal.[594]

The disease tends to be a sporadic, individual mare problem that seldom has any lasting effect on mare fertility. Both bacterial (*Streptococcus zooepidemicus, S. equisimilis, E. coli, Enterobacter agglomerans, P. aeruginosa,* and *K. pneumoniae*) and fungal (*Aspergillus* spp.) organisms may be the cause. Overall, ascending placentitis is the most common type observed worldwide. [594] Ascending infection results in necrotizing and suppurative inflammation of the chorioallantois that may lead to detachment of the placenta in the area surrounding the cervical star and ventrally toward the uterine body. [594] At the time of diagnosis, the cervix is usually soft, and purulent exudate may be found draining into the vagina. Because the cervical star is the most common site of placentitis in mares, this part of the fetal membranes should be thoroughly and closely examined after abortion and at any foaling. The vestibular sphincter, cervix, and vulvar lips are important barriers to prevent ascending infections. Many aged mares require a Caslick procedure and cervical reconstruction to prevent the likelihood of ascending infection. Young mares, however, with apparently appropriate vulvar conformation and intact cervix may also develop ascending placentitis. The most commonly isolated bacteria are beta hemolytic streptococci (*Streptococcus equi* subsp. *zooepidemicus* or *equi*), *E. coli,* and *Enterococcus* spp., although a broad range of other bacteria have also been isolated from affected mares. In some horses, mixed bacterial and fungal infections occur; it has been suggested that the fungal infection occurs secondary to a primary bacterial infection.[594]

Focal mucoid placentitis is characterized by lesions located in the base of the uterine horns and uterine body.[596] It is commonly associated with nocardioform placentitis in which the fetal lungs are also affected with suppurative lesions, but other microorganisms (e.g., *Cellulosimicrobium cellulans*) can cause similar lesions.[597] Nocardioform placentitis was first diagnosed in mares in central Kentucky in 1986 as a sporadic cause of abortion and weak foals. Since that time, it has been described in mares in Florida, Italy, South Africa, and Australia.[594,596] The number of confirmed cases of nocardioform placentitis has varied widely from year to year, with more than 400 cases in central Kentucky in 2011.

Nocardioform placentitis is associated with gram-positive branching actinomycetes including *Crossiella equi* and *Amycolatopsis* spp. It has been suggested that these microorganisms may gain access to the uterus at breeding, but this unusual form of equine placentitis does not become apparent until the latter part of gestation.[598] The initial lesion is localized on the most dependent aspect of the chorion in the cranioventral aspect of the uterine body and subsequently extends cranially into the base of the horns and circumferentially around the placenta.[596] Outcomes vary from abortion to birth of a normal foal. Some foals are born prematurely, whereas others are delivered at term but may be stillborn or weak with an emaciated appearance.[594,596, 598] Vaginal discharge is not a feature of this condition because the region of the placenta including the cervical star is not involved. After the fetus has been expelled, affected mares rapidly clear the infection and usually experience no adverse effects on subsequent fertility.[594,596,598] When the fetal membranes are examined, there is an obvious line of demarcation between pathologic and normal tissue, with the affected area being covered by a characteristic thick, brownish, mucoid, viscous material.[594,596,598] The underlying chorionic villi are reduced in size, and in the central portion of the lesion, the chorionic surface may be completely denuded.[594,596,598]

Leptospira spp., the most common cause of hematogenous placentitis,[594] causes diffuse lesions with large numbers of spirochetes in the affected placental tissues.[596] Its occurrence is rather sporadic, but outbreaks have been described.[594,596] Late-term mares may abort with no premonitory clinical signs, and occasionally an infected premature or full-term weak, icteric foal may be delivered. The placenta is edematous, with a necrotic chorion covered with a mucoid exudate.[178,596,599] The gross placental lesions are associated with thrombosis, vasculitis, and inflammatory cell infiltrates. The spirochetes tend to be numerous and are readily demonstrated in the stroma and villi of the placenta.[596,599] A microscopic agglutination test (MAT) on fetal fluids (heart and body cavities) or maternal serum is likely to reveal a high titer (1:6400-1:819,200 or greater).[600] Leptospires may be detected in the fetus by the fluorescent antibody test (FAT), silver staining, or darkfield

microscopy.[600] Immunohistochemistry is more sensitive than silver staining and more specific than serology (MAT).[601,602] The fetal kidney should be submitted for a FAT because this tissue yields the highest percentage of positive results and is also the best tissue for culture.[596,603] If urine is to be submitted from an infected mare (FAT or darkfield analysis), it is important to obtain collection instructions and an appropriate transport medium from the diagnostic laboratory. The specimen must be obtained before any antimicrobial therapy has been administered. A recent retrospective study demonstrated that PCR is the most sensitive method in diagnosing *Leptospira* spp. in abortion cases.[600]

In the genus *Leptospira,* the species *L. interrogans* contains several pathogenic serogroups (common antigens) and serovars (specific strain). Serogroups consist of closely related serovars. The predominant serovar affecting horses varies with country and region. The *Bratislava* serovar is most commonly isolated from aborted fetuses in Northern Ireland, but in central Kentucky most leptospiral abortions have been associated with the *Pomona* serogroup, occasionally the *Grippotyphosa* serogroup, and rarely the *Hardjo* serogroup.[600,604] Equine leptospiral infections previously reported as being *L. pomona* are now thought to be more correctly identified as *L. interrogans* serogroup *Pomona* serovar *Kennewicki* and *L. kirschneri* serogroup *Grippotyphosa* serovar *Grippotyphosa*.[600,604] In North America pregnant mares are considered to be incidental hosts that become infected after exposure from maintenance hosts (i.e., wildlife such as skunks and raccoons for *Kennewicki* and *Grippotyphosa* and cattle for *Hardjo*).[600,604] Infected mares may shed leptospires in the urine for up to 14 weeks.[604] Thus therapy is aimed at prevention of urinary shedding and possibly prophylactic treatment of pregnant in-contact mares that have high titers. A combination of penicillin (10,000 to 15,000 IU/kg, administered intramuscularly) and streptomycin (10 mg/kg administered intramuscularly) every 12 hours for a period of 1 week has been recommended, but streptomycin is no longer widely used in equine practice. High doses of potassium penicillin G (20 million units IV every 12 hours) may be effective in preventing infection of a fetus if the mare has a high titer, but this recommendation has not been validated in controlled studies. The dosage and duration of treatment appear to be important. Oxytetracycline (5 to 10 mg/kg) has also been suggested, but it was less effective at preventing urinary shedding of leptospires in all cases tested (5 of 7 infected mares).[605] Anecdotally, doxycycline and enrofloxacin have been used in clinical practice as tools to prevent abortion in mares exposed to other mares aborting with leptospiral infections, but it is important to note that to date neither drug has been proven to be safe to the fetus or efficacious at treating and preventing placentitis in mares.

An approved vaccine for leptospirosis recently became available for horses in the United States, but its efficacy for prevention of abortion has not been assessed. Attempts should be made to prevent direct contact between maintenance hosts and pregnant mares and also to prevent exposure to urine infected by these species (e.g., contaminated water and feed).[594] It may be prudent to vaccinate mares with the equine vaccine if they reside on the same property as pregnant mares with confirmed leptospiral abortion and to isolate aborting mares.[594,596]

Rhodococcus equi has been incriminated as the cause of placentitis and abortion in mares.[606] *Mycoplasma* spp. have been associated with infertility, endometritis, vulvitis, and abortion in mares.[607] *Nocardia* spp. have been described as a cause of placentitis in two mares in Florida. Lesions were similar to nocardioform placentitis, but foals were born with suppurative lung lesions.[608] Cases of mycobacterial abortion and placentitis were confirmed by microbiologic techniques, PCR, and immunohistochemistry.[609] The atypical mycobacteria were within the nontuberculous group and classified as saprophytic and opportunistic microorganisms acquired from the environment (soil, water, and decaying vegetation).[609,610] Gross lesions observed within the submitted cases varied from none to those of a "nocardioform-like" placentitis. Affected fetuses exhibited varying degrees of malnourishment and chronic placentitis. Several fetuses had granulomatous to pyogranulomatous pneumonia, and one fetus without pneumonia contained disseminated granulomas in various organs.[609,610]

Cellulosimicrobium cellulans is a gram-positive branching bacillus that is an opportunistic microorganism found in the soil.[597] The nine described cases of equine abortion and placentitis resulting from *C. cellulans* had nocardioform-like placentitis lesions as well as granulomatous pneumonia. Because atypical *Mycobacteria* and *C. cellulans* organisms can cause similar gross and histologic lesions within the fetus and placenta, they should be considered as differential diagnoses when gross placental lesions suggestive of nocardioform placentitis are encountered.[597,609,610]

Protozoal Abortion

There is limited information available on the association between equine *Neospora* infections and abortions.[611-616] However, the apicomplexan protozoan parasite *Neospora caninum* has been recognized as a major cause of abortion in cattle.[616] Antibodies against *Neospora* spp. have been described in mares that recently aborted.[614] Using an agglutination test, researchers found that the number of animals with elevated (>80) anti-*Neospora* spp. antibody titer was higher in a group of 54 aborted mares than in a randomly chosen group of 121 mares ($P < 0.001$). *N. caninum* DNA was found in 3 of 91 fetal brains, 2 of 77 fetal hearts, and 1 of 1 placenta and was present in both brains and hearts of two fetuses. The mere presence of the organism in an aborted fetus does not necessarily implicate it as being the cause of the abortion.[616] Antibodies to *Neospora* spp. were detected in the sera from 11.9% of 800 asymptomatic horses in Israel and from 37.5% of mares that aborted.[617] Antibodies to *Neospora* spp. have also been detected in mares in Brazil.[612] Further investigations will be needed to determine whether there is an association between equine reproductive failure and *Neospora* spp. infection.[612,615]

Viral Abortion

Equine arteritis virus (EAV) and equine herpesvirus 1 (EHV-1) are the most important causes of viral abortion in horses in the world. Both viruses are associated with acute respiratory tract infection and are discussed in detail in Chapter 8.

EAV is transmitted through inhalation or venereally in the semen of asymptomatic (shedder) stallions.[618-623] Abortion may occur if pregnant mares become infected in the later stage of gestation (5–10 months).[619,622,624,625] Clinical signs are variable but may include fever, conjunctivitis, nasal discharge, and dependent edema that is associated with vasculitis.[618,620,626] Clinical signs are mild or subclinical in many horses and may be clinically indistinguishable from other respiratory infections. Viral myometritis with degeneration of myocytes and infiltration of mononuclear cells leads to transplacental

infection of the fetus.[627] Affected placentae are edematous, and degenerated fibroblasts may be observed in the subvillous layers. Lesions in the fetal tissue include an atrophy of the lymphoid follicles in the spleen and lymph nodes with degenerated lymphocytes. Immunofluorescence can detect EAV antigen in the myometrium and the endometrial glands in the dams, in the subvillous layer of the placentae, and in the aborted fetuses. The virus may be recovered from the uterus and fetus, but the placenta is likely to yield the greatest amount of the virus. The disease may be controlled by an effective vaccination program and screening tests.[618]

Most abortions associated with herpesvirus are caused by EHV-1, but occasional abortions are associated with other equine herpesviruses.[592,628] The number of abortions resulting from EHV-1 infection has declined over the past 20 years, and isolated abortions, rather than abortion storms, are now a more common feature of this disease.[629] This is due to the widespread adoption of stringent vaccination programs in combination with improved management practices on broodmare farms.[630,631] Pregnant mares should be vaccinated with an approved vaccine at 5, 7, and 9 months of gestation. Many farms also vaccinate at 3 months. New arrivals should be isolated for 3 weeks, and groups of pregnant mares should be isolated by stage of gestation. It is especially important to segregate pregnant mares from weanlings and other horses.[632]

EHV-1 can infect the fetus if a mare is viremic during pregnancy. The virus causes abortion as a result of the rapid detachment of the placenta.[633,634] Endothelial cells in the endometrium and allantochorion are often infected by the virus, with accompanying vascular lesions. The fetus can be infected by way of the chorionic vasculature or by inhalation of infected amniotic fluid.[635-637] Abortion may occur soon after the mare is infected or several weeks have elapsed. Therefore maternal serology is of little diagnostic value.[638] The aborted fetus will be fresh, with copious amounts of pleural and peritoneal fluid. The trachea may contain a fibrin clot. Small necrotic foci may be discernible on the swollen liver. A hyperplastic, necrotizing bronchiolitis may be seen in lung sections, and large intranuclear eosinophilic inclusion bodies are a characteristic histologic lesion. Although vaccination is widely practiced, owners should be aware that the protection is not absolute. If a pregnant mare is exposed to infected animals that have recently been to a show or that are returning from a training facility, then it is possible that any protective immunity conferred by the vaccine will be overwhelmed. Abortions have been associated with reactivation of latent virus that was induced by transport stress.[639] Therefore a history of regular vaccination of an aborting mare does not eliminate the possibility of a herpesvirus-related abortion. Neutralization and indirect immunofluorescence tests, as well as PCR and virus isolation, are used for EHV-1 diagnostics. Antigen detection, in combination with virus isolation and PCR from fetal lungs, gives reliable results. More detailed reviews have been published, and the reader is referred to these for a more in-depth discussion.[640,641]

A number of other acute viral infections have been associated with abortion in horses, especially if the horse suffers severe systemic illness at the time of initial infection. For example, mares may abort as a result of the systemic effects of acute infection with equine infectious anemia virus (EIAV), although it is not generally considered an abortigenic virus.[642] When widespread vaccination against West Nile virus was adopted in the United States, some suggested that the killed virus vaccine that was used might result in abortions, stillbirths, and deformities in foals. However, subsequent critical review clearly demonstrates that vaccination of pregnant mares during any period of gestation is not associated with an increased incidence of pregnancy loss.[643]

Treatment of Placentitis

Under field conditions, placentitis is diagnosed in mares presenting with compatible clinical signs of premature mammary gland development and vulvar discharge (with ascending placentitis). These clinical signs should prompt the practitioner to perform a transrectal palpation and ultrasonographic examination. Typically mares presenting with signs of placentitis will have demonstrable placental thickening and separation of the placenta from the endometrium.[594]

Placentitis is detrimental to the pregnancy not only because it disrupts nutrient exchange but also because of the inflammatory mediators (proinflammatory cytokines) that are released. The exact cause of fetal expulsion is not known, but ensuring myometrial quiescence is central to most therapeutic regimens.[505,644] Placentitis cases have substantially increased concentrations of $PGF_{2\alpha}$ and PGE_2 in the fetal fluids.[645,646] Thus antiinflammatory medication (flunixin meglumine at 1.1 mg/kg; phenylbutazone at 4 mg/kg, every 12 hours) is generally recommended,[647] although there are no controlled studies to specifically demonstrate their efficacy. [594] Placentitis associated with early abortions tends to be acute, with the fetus succumbing to bacteremia with no to minimal placental lesions.[648]

Broad-spectrum antibiotics that have been recommended for treatment of equine placentitis include trimethoprim-sulfadiazine (15–30 mg/kg PO every 12 hours), procaine penicillin (30,000 IU/kg/day) and gentamicin (6 mg/kg/day), and ceftiofur (1–5 mg/kg, every 12 hours).[647,649-651] Ceftiofur has been used for treatment of placentitis, but a recent study demonstrated that this drug does not cross the placenta and was ineffective for treatment of experimentally induced placentitis. [652] Gentamicin was undetectable in the plasma of newborn foals after mares were treated with the antibiotic (6.6 mg/kg) an hour before parturition, and it was initially believed that gentamicin does not cross the placenta of mares at term.[653] Murchie et al. monitored drug concentrations in the allantoic fluid of pregnant pony mares using in vivo microdialysis to determine whether this method could detect allantoic concentrations of drugs in normal mares and those with placentitis.[654] Pharmacokinetic comparisons indicated that potassium penicillin G (22,000 IU/kg q.i.d.) persists much longer in allantoic fluid than blood, whereas gentamicin (6.6 mg/kg/day) exhibited similar profiles in the two compartments. Flunixin meglumine (1 mg/kg b.i.d.) was not detected in allantoic fluid. In infected mares penicillin G achieved a similar peak concentration in allantoic fluid, whereas peak gentamicin concentration appeared to be reduced relative to drug concentrations in noninfected mares. Thus penicillin G and gentamicin appear to undergo effective placental transfer in pregnant mares, but transplacental drug transfer may be altered selectively if active placental infection is present.

If the localized production of high concentrations of prostaglandins stimulates the formation of gap junctions, then the subsequent myometrial hypermotility may impede placental blood flow as the uterus contracts.[505,644,655] This will reduce fetal oxygenation and increase fetal stress. Because it is known that progesterone inhibits gap junction formation,

it has been suggested that progestin supplementation may be beneficial when uteroplacental inflammation is suspected.[594] This is the rationale behind the current recommendation to treat suspected placentitis cases with a double dose of altrenogest (0.088 mg/kg/day).[594,647] Delaying premature labor long enough to allow accelerated fetal maturation may improve foal survival rates.[656]

A primary goal in the treatment of placentitis is to delay premature labor long enough to allow accelerated fetal maturation to occur, with the goal of improving foal survival rates.[594] The progestogens may enhance the activity of the endometrial enzyme 15-hydroxyprostaglandin dehydrogenase and thus promote rapid metabolism of PGs into inactive metabolites.[368,564] Although altrenogest is not metabolized to 5α-pregnanes, both 5α-DHP and 3β-5P levels may be increased as a result of supplementation.[657] However, Ousey and others have suggested that progesterone supplementation may be contraindicated because high levels could inhibit the placental enzyme 3β-hydroxysteroid dehydrogenase.[368,564,658] This enzyme produces progesterone from pregnenolone; thus inhibition could interfere with the normal fetoplacental steroidogenic pathway. At present this widely used therapy remains controversial, and even if it does no harm, the expense of long-term progestin supplementation may not be warranted in many cases. Essentially, the use of hormone therapies is subjective and to a large extent reflects lack of understanding about the endocrine relationships among the mare, placenta, and fetus.

The rationale for other treatment regimens is based on extrapolations from the human medical literature and application of sound reasoning for the potential efficacy of a particular drug. Scientific investigation in this area is crucial. Previously, an oral β_2-sympathomimetic drug (clenbuterol) has been suggested to suppress uterine motility in mares with placentitis.[647,659] In the United States the product is marketed as an oral formulation to treat chronic reactive airway obstruction. Research is needed to determine what oral dose of this bronchodilatory compound, if any, is actually effective on the gravid uterus. In countries in which an intravenous formulation is available, a 300-μg IV dose of clenbuterol will reduce uterine tone for approximately 2 hours.[660,661] However, daily IV administration of clenbuterol to mares showing maximal milk calcium levels was not effective in preventing the onset of myometrial contractions and delivery in normal foaling mares at term.[662]

The administration of pentoxifylline (8.5 mg/kg PO b.i.d.) is questionable. While it does not seem to improve perfusion in the mare reproductive tract,[663,664] some clinicians advocate it because it is used in humans to treat tissue ischemia, because of its ability to modulate the inflammatory process by downregulating proinflammatory cytokines, and because of its possible effect on erythrocyte deformability.[647,649,656] Pentoxifylline appears to have good uterine penetration and has been detected in the allantoic fluid of mares.[650,651] Despite these conflicting studies, a multipronged approach (i.e., antibiotics, antiinflammatory agents, altrenogest) to long-term therapy for placentitis may improve fetal survival and foal viability.

Noninfectious Causes of Abortion

Twins

In North America the incidence of twin abortions has decreased significantly because of early intervention after ultrasonographic diagnosis of a multiple pregnancy.[178,529,532] Before the widespread adoption of this technology, twin abortions were a major cause of fetal loss.[441,442] A large European study (n = 12,648 pregnancies) of Thoroughbred mares revealed a twinning rate of 3.5%, with 443 twins and only two triplets.[665] The type of placentation in the mare (diffuse, microcotyledonary) makes it highly unlikely that a twin pregnancy will be carried to term. Obviously there is a finite endometrial surface area available for allantochorion attachment. The more common unicornuate twin vesicles are a problem, because one conceptus is inevitably restricted to the proximal aspect of the gravid horn.[421] Thus the two conceptuses are literally in a deadly competition for adequate nourishment and subsequent placentation. If the twin pregnancy is maintained until the latter part of gestation, the nutrient demands of the rapidly growing fetuses outstrip the placental attachments. Fetal growth is such that nutrient demands may be met until the latter half of gestation, when the marked fetal development in the last trimester usually requires more exchange capability than the smaller placenta provides. The fetus becomes stressed as it becomes progressively emaciated and ultimately dies.[529,666] Death of one fetus or both fetuses is followed by abortion, with the characteristic avillous areas on the fetal membranes confirming the amount of placental disruption.[178,442,522] Affected mares develop premature mammary enlargement and may "run" milk before aborting. Transabdominal ultrasonographic evaluation may be useful to confirm the diagnosis at this late stage. Although the area of apposition of the two chorioallantoic membranes (twin membrane) may be seen, measurement of fetal thoracic diameters and heart rates can confirm the diagnosis of twins.[666] Twin abortions in the last few months of gestation are likely to cause a dystocia. Bicornuate twins are more likely to survive, because each membrane can attach to an entire horn and one side of the uterine body, but the resulting foals are likely to be stunted as a result of IUGR.[402,442,529] The live birth of twin foals is extremely uncommon, and many of these neonates do not survive, with as few as 14% of surviving foals reaching the second week of neonatal life.[442,531] The mares are prone to fetal membrane retention and may be difficult to rebreed. Thus it is not surprising that the equine breeding industry has always tried to prevent twin pregnancies. After such a diagnosis the owner is faced with three choices: attempt elimination of one fetus in utero, manage the pregnancy to term in the remote hope of two viable foals, or induce abortion. Management of a twin pregnancy was discussed in more detail in a previous section.

Umbilical Cord Compromise

On rare occasions, a large ossified remnant of the yolk sac may compromise blood flow through the umbilical cord and result in abortion.[667] The pathologic condition referred to as *umbilical cord torsion* may be defined as excessive twisting of the cord such that there is complete or partial occlusion of the umbilical vessels or urachus.[668] The two umbilical veins that return oxygenated blood from the placenta fuse within the distal aspect of the amniotic portion of the cord, and a single vein enters the fetal abdomen.[669] Strangulation may occur if the cord becomes tightly wrapped around a portion of the fetus. Pressure sites on or around fetal parts may be caused by the effects of constriction by prolonged tension on a strangulated umbilical cord.[670] Affected fetuses are not usually expelled immediately after death, so some degree of tissue autolysis should be expected.[671]

The length of the equine umbilical cord in normal gestation can be quite variable, and factors that affect cord length and the number of twists present are unknown. Lethal umbilical torsion appears to be a sporadic condition with no apparent increased risk for future problems in mares that abort as a result of fetal complications.[672] Whitwell has shown that some mares have three or more foals with abnormally long cords.[441] A study of 93 Standardbreds did not demonstrate an effect of sire on cord length, and much larger studies will be required to ascertain whether genetics are involved.[429] Although the possibility of a heritable component for cord length cannot be discounted, a more plausible explanation may be that abnormally long cords are associated with the amount of fetal movement.[669]

Ultrasonographic studies of fetal mobility have helped explain the characteristic twisting feature of the normal equine umbilical cord.[402,673-675] Differing from ruminants, the equine amnion floats freely within the allantoic fluid. Fetal rotation within the amniotic cavity and amniotic sac rotation within the allantoic cavity result in the characteristic twisting of the equine umbilical cord.[676,677] The lumen of both uterine horns becomes constricted (presumably by localized circular muscle contractions) between 5 and 7 months, and the allantoic fluid along with the fetus is contained within the confines of the uterine body.[402,673-676] Ginther has described that as the noncord horn remains closed, the cord horn gradually permits the entry of the hindlimbs between 7 and 9 months.[675] The limbs can enter the horn only when the fetus is in dorsal recumbency because the angle between the horn and body is so acute by this stage of gestation. Thereafter, the hindlimbs remain enclosed within the cord horn, and the hooves extend to the horn tip by the tenth month. It is interesting that the peak incidence of abortions due to umbilical torsions occurs when the hindlimbs can become permanently enclosed in the uterine horn. In some instances this may prevent the unraveling of those critical few rotations that can lead to circulatory compromise. There is no doubt that a longer umbilical cord predisposes the fetus to this condition.

Reports from the United Kingdom suggest that with the decline in twin abortions, excessive twisting of the umbilical cord has become the most frequently made diagnosis in some laboratories. These abortions were associated with excessively long cords, with many over 80 cm in length.[592,678] Because spiraling of the equine cord (four to five times over its length) is a normal feature, it is important to ensure that the fetal demise was caused by vascular compromise.[669,677] Evidence of pathologic twisting with tension and compressive forces on the affected portion of the cord may include aneurysms, tearing of the intima of vessels, hemorrhage, thrombosis of vessels, blanched constricted areas, local edema, and urachal dilations of varying sizes. Inadequate perfusion can cause intravascular thrombosis in the peripheral tissues of the chorioallantois and possible necrosis of the aspect of the placenta that is most distant from the attachment of the cord (the cervical pole of the body segment).[669,671] These findings are by no means definitive, and there may be variability in interpretation among laboratories. Other considerations should include general agonal changes and the potential effects of cord tension when a live fetus is expelled. Whitwell's morphologic studies have established normal metrics for equine fetal membranes. The average length of the umbilical cord in a Thoroughbred is 55 cm (95% confidence interval; 36–83 cm; $n = 143$).[679]

Williams reviewed 168 cases of umbilical cord torsion from the University of Kentucky Veterinary Diagnostic Laboratory, representing 6% of equine fetus submissions over a 5-year period.[672] The gestational age ranged between 5 and 10 months, with a mean of 7.5 months. Umbilical cord length varied between 62 and 125 cm, with an average length of 96 cm. The cords tended to be highly twisted, with areas of constriction, edema, hemorrhage, and fluid-filled sacculations. The fetuses were slightly to moderately autolyzed in a manner consistent with fetal death before abortion. Urinary bladder dilation was observed in some cases. The most consistent histopathologic finding was evidence of necrotic changes with secondary deposition of calcified material in the blood vessels of the chorioallantois.[591,672] A less common cause of abortion resulting from cord obstruction is when an excessively long amniotic portion of the cord becomes tightly wrapped around a portion of the fetus.[670,679,680] Although it may be more common for a fetal extremity to be involved, the author managed a case in a pony mare in which the tightly wrapped cord left a deep groove around the lumbar region, with evidence of local edema.[670]

GENDER DETERMINATION (FETAL SEXING)

The advent of fetal sexing has permitted early gender determination to influence the value of the pregnant mare. Factors that may vary depending on the predicted sex of the foal include choice of state for foaling, appraisals and insurance coverage, sales reserves, bookings for stallion service the next season, and retention or sale of the mare.[681] Accurate determination of the sex of the equine fetus can be made using either transrectal or transabdominal ultrasonography.[78,681-683] Fetal gender should be certified only when the identifying structures have been clearly delineated and the accuracy of the determination is guaranteed. Accurate determination of fetal sex may be difficult or impossible in some cases because of excessive mare or fetal movement or because the fetus is located too ventrally to permit adequate imaging. Although tranquilization (e.g., xylazine and butorphanol tartrate) is sometimes used, it may cause the uterus to relax and drop away from the examiner.[681] A 5.0-MHz linear array transducer is adequate for transrectal gender determination, but a 3.5-MHz transducer will ensure the depth of penetration that is required to obtain transabdominal images.

Gender determination is based on ultrasonographic assessment of the relative location of the genital tubercle, an embryologic structure that is initially located between the rear limbs in both sexes. The genital tubercle differentiates into either a clitoris or a penis and has an ultrasonographically distinctive, hyperechoic, bilobulated appearance in both sexes. Curran reported that the optimal time for gender determination is between days 59 and 68, and Holder concurs that a window between days 60 and 70 is ideal.[681,682] A second ideal period for gender determination may be between days 110 and 120, because the genitalia are now well developed and the fetus tends to be more accessible again. After this time the increasing depth of the uterus means that a diagnosis may not be possible if the fetus is in an anterior (cranial) presentation at the time of the examination.[681] If the transabdominal approach is used, then the optimal window of time in both sexes is between 100 and 220 days' gestation.[78,681,682] Thereafter, it may become increasingly difficult to identify the anatomic structures required to make an accurate gender determination.

Detailed instructions for fetal sexing by ultrasonography have been described.[78,681,682] Transabdominal gender identifications based on the presence of the penis or prepuce (or both) in males and mammary glands and teats or fetal gonads in females can be quite accurate.[78]

MONITORING PREGNANT MARES

Placentitis should be suspected in mares that develop premature mammary enlargement (with or without vulvar discharge). Varying blood markers have been recommended to monitor late-term pregnant mares.[594] Relaxin is a protein hormone produced by the placenta. Low concentration of peripheral relaxin has been associated with various causes of placental dysfunction including fescue toxicosis, oligohydramnios, and placentitis.[684,685] Currently, however, there is not a commercially available assay, and normal concentrations of relaxin are highly variable among mares.

Serum amyloid A and haptoglobin have been suggested to be useful diagnostic and prognostic markers for ascending placentitis.[253,261] Similarly, alpha-fetoprotein and estradiol 17β[472] have been shown to be useful markers for experimentally induced ascending placentitis. It remains to be determined, however, if these molecules are useful for diagnosis of naturally occurring placentitis.

Measurement of an equine fetal protein and estrone sulfate levels in maternal plasma has not proved useful for early detection of fetal stress associated with medical and surgical colics.[514,686] In central Kentucky, a commercial laboratory offers a test to measure "total estrogens" that purportedly measures different fractions of nonconjugated classic phenolic estrogens present in late-term pregnant mares. Mares with lower concentrations of total estrogen are supposedly prone to abortion.[687] Before day 310 of gestation, total estrogen levels of less than 1000 ng/mL may be indicative of fetal stress, and mares with levels of less than 500 ng/mL are likely to have a severely compromised or dead fetus. This assay has not been tested under controlled conditions.

It is important to remember that the circulating concentrations of hormones in maternal plasma represent a small percentage of the levels that are being metabolized by the fetoplacental unit.[368,564,688] The definitive studies on mare progestogens (progestins) were performed using gas chromatography-mass spectrometry (GC-MS) or liquid chromatography.[368,475,688] It is imperative that clinicians understand that common commercially available assays (e.g., RIA, ELISA) using an antibody raised against progesterone or estrogen usually cross-react with several other steroids present in plasma of pregnant mares. Thus the results typically reported by the laboratories as being progesterone, estradiol, or estrone represent a mix of different progestogens and estrogens, respectively. In clinical practice this might be of particular relevance when the practitioner tries to compare results from an assay presented by one laboratory to the results obtained from another laboratory. Assay results may not be readily comparable.

Although placental pathology has been associated with increased plasma progestin concentrations in some reports, others failed to detect differences in plasma progestin concentrations in mares with impending abortion and mares with normal pregnancies when monthly blood sampling was performed.[368,439,475,476,688] Therefore it is recommended that whenever possible, serial samples (e.g., daily for 3 days, then weekly) be obtained from a mare in which the condition is suspected; doing so may help the clinician identify a clinically useful trend in progestogen concentrations.[688] Unfortunately, in practical settings this might not be feasible, making progesterone measurement not applicable to all situations.

Plasma progestogen concentrations increase in normal mares as gestation progresses, and concentrations in the maternal plasma may range between 5 and 50 ng/mL or even higher as mares approach term. It is thought that chronic fetal stress may cause the increased progesterone and pregnenolone concentrations (as well as those of several metabolites) that have been observed in mares with placentitis.[688,689]

Ousey has described three abnormal progestogen patterns that may be clinically useful: a rapid decline, precocious elevation, and failure to increase at term.[368,688] A rapid decline is consistent with fetal death or imminent fetal expulsion and may be seen after a uterine torsion or colic.[367,688] Pregnant mares that have experienced colic or uterine torsion and have plasma progestogen concentrations below 2 ng/mL are at high risk for fetal loss.[686] Prolonged increase of progestogens for several weeks before delivery (especially if earlier than 305 days' gestation) is consistent with metabolic activity in the fetus and uteroplacental tissues despite the presence of placental pathology.[476,646,688-692] Although typically small with poor skeletal development, foals born after being exposed to chronic fetal stress (placentitis) tend to exhibit precocious maturation, even when delivered several weeks early.[693]

Transrectal ultrasonography provides an excellent assessment of the current status of the caudal allantochorion, and as such it is an invaluable aid when examining a mare in late pregnancy that exhibits signs of placentitis.[694,695] An image of the ventral placental tissues in the area adjacent to the cervical star provides the ability to accurately diagnose the early stages of ascending placentitis.[694,695] Experienced clinicians are able to observe abnormal tissue thickness and even evidence of placental separation with an associated pocket of inflammatory exudate. Early therapeutic intervention may provide the best chance for a successful outcome. In a normal placenta the chorioallantoic membrane and endometrium are intimately connected, making them ultrasonographically indistinguishable from each other. Thus a combined tissue measurement is used, and normal values for the combined thickness of the uterus and placenta (CTUP) have been established.[696] The amniotic membrane should not be included, and this should be remembered if the fetal limbs are active at the time of the examination. The area 1 to 2 inches cranial and ventral to the cervix provides the most consistent CTUP measurement in normal mares, and this is the recommended site for any measurements. The ultrasound image should be frozen once the landmark vessel (a branch of the uterine artery) in the ventral uterine wall is located, and caliper measurements are taken from the inner dorsal surface of the ventral uterine vessel to the edge of the allantoic fluid.[694] It is recommended that three individual measurements be recorded.

An increased CTUP at any point from midgestation until term may indicate placental disruption and pending abortion.[697] Transrectal CTUP measurements that are considered abnormal would be greater than 8 mm (between days 271 and 300), greater than 10 mm (between days 301 and 330), and greater than 12 mm after day 330.[694,696,698]

An increased CTUP measurement in the absence of other clinical signs constitutes only weak evidence of placentitis. While examining a mare suspected to have placentitis, the practitioner must be looking for further signs of placentitis,

such as placental separation, intracervical fluid accumulation, vulvar discharge, or premature mammary gland development.[594] Placental folds, amnion, and at times individual mare variation may be responsible for apparent increased CTUP in the absence of placentitis.

Most practitioners do not recommend vaginal examination of pregnant mares unless the mare is presenting with purulent vulvar discharge or has other clinical indications for performing the exam. If such an examination is warranted, however, it is important to employ strict hygienic procedures when performing a vaginal speculum examination on a mare with a high-risk pregnancy, because the first two barriers to the pregnant uterus will be breached (i.e., vulvar lips, vestibular sphincter). Mares at risk for abortion often have a moist, hyperemic, relaxed cervix. Even if vaginal discharge has not been reported, many of these cases have a purulent cervical discharge if placentitis is present. Whereas cervical softening and vaginal discharge may be present if the infection is localized around the cervical star, in the case of nocardioform placentitis, the lesion does not involve the cervical star, and vaginal discharge is conspicuously absent.[596,699,700] Although transrectal ultrasound is an extremely useful aid in diagnosing ascending placentitis, the site of the nocardioform lesion makes it of limited diagnostic value in these cases.[659] Transabdominal examination of the ventral uterus may reveal separation of the chorioallantoic membrane from the endometrium, often with evidence of hyperechoic fluid accumulation between the two surfaces. Placentitis and associated placental edema will result in a thickened uteroplacental image. The CTUP on a transabdominal ultrasound image should be between 7 and 12 mm.

Transabdominal ultrasonography has become a routine diagnostic aid for evaluating mid- to late-term mares. Although the 5.0-MHz linear array transducer is ideal for transrectal reproductive ultrasonography, some probes have shallow depth of penetration (approximately 10 cm) that limits their usefulness for transabdominal examinations in mares in late pregnancy. The author recommends use of a transducer that achieves 15- to 20-cm depth for optimal transabdominal examination. If the mare does not have a pronounced plaque of ventral edema, most modern ultrasound units with a 5.0-MHz transducer will be sufficient to image the uteroplacental unit and some of the fetal fluids and allow assessment of the fetal heart rate.

Either a 3.5- or 2.5-MHz curved linear array or sector scanner transducer is best for transabdominal examinations because these can penetrate to a depth of 20 or 30 cm, respectively. Although the 70- to 90-day fetus may be imaged from the ventral abdomen, just cranial to the mammary gland, the late-gestation gravid uterus extends along the ventral abdomen to the xiphoid.[694,701-704] By the ninth month of gestation, the fetus should be in anterior (cranial) presentation and dorsopubic or dorsolateral position[402,675,702]; thus in late gestation the fetal head should be positioned near the mare's pelvis. An abnormal presentation or the presence of twins is possible if a fetal head is detected along the ventral abdomen during late gestation. A more detailed examination is indicated in such cases. The posture of the extremities varies with fetal movement.[702]

A standardized methodology must be followed when scanning the uterus from the ventral abdomen, starting just cranial to the mare's mammary gland and moving cranially to locate the fetal thorax. The ribs cause multiple acoustic shadows that delineate the thoracic cavity. A complete examination of the fetus and uterus involves scanning cranially to the xiphoid in multiple parasagittal planes and then scanning from the left to right sides of the abdomen in multiple transverse planes.[704] Transabdominal ultrasound examination is an important diagnostic tool when attempting to identify the presence of twin fetuses during late gestation. Identification of the nongravid horn can be useful to help rule out the possibility of twins. Obvious size discrepancy often serves as confirmation that twins are present. In other cases one thoracic cavity does not contain a beating heart, confirming that one of the twins has already died.

In a normal pregnancy the majority of the fetal fluids are within the allantoic cavity. The amnion is imaged as a thin membrane that surrounds the fetus and lies in close contact with the fetus over much of its body. The amniotic membrane divides the imaged fetal fluid into two distinct cavities. It is most easily seen around the fetal neck, shoulder, thorax, and foreleg. The largest pocket of amniotic fluid is usually imaged at the point where the forelimb and neck meet the thorax.[704] The maximum vertical depth of amniotic and allantoic fluid and the echogenicity and character of amniotic and allantoic fluid are useful guides to fetal well-being. Any measurements of fluid depth should be made as perpendicular to the uteroplacental surface as possible. In the normal equine pregnancy, the maximum ventral fetal fluid pocket depth for amniotic fluid is 8 cm, and it is 13 cm for allantoic fluid.[694,703,704] Extremes in either direction are not normal. Obviously deficient amounts of fetal fluid indicate placental dysfunction, and excessive amounts suggest a hydrops condition. Fetal fluid quantities should be considered excessive if the maximal vertical amniotic fluid depth exceeds 14.9 cm or the maximum vertical allantoic fluid depth exceeds 22.1 cm. The quality of the fetal fluid is scored from 0 (clear) to 3 (echogenic fluid with numerous particles).[702] It is not unusual for echogenic particles to be noted in the fetal fluids, especially during periods of fetal activity. These represent sloughed cells and proteinaceous debris. The fetal skin releases vernix as the pregnancy advances, and these free-floating particles can increase the cloudiness of the amniotic fluid. Thus an increase in the number of echogenic particles in late gestation may not be abnormal.[702] However, if a high-risk pregnancy is being regularly monitored and a sudden increase in fluid turbidity (grade 3) is observed, the prognosis is not good.[702] The clinician should consider the possibility of inflammatory exudates, meconium passage by a compromised fetus, or even hemorrhage. It should be remembered that hippomanes (allantoic calculi) are a normal feature of the equine pregnancy. These structures may often be observed as free-floating echogenic accumulations on the ventral aspect of the allantoic cavity.[704]

An equine biophysical profile has been proposed as a guide to assessing fetal well-being and predicting perinatal morbidity and mortality.[694,702,705] Although a low score is definitely indicative of a negative outcome, higher scores do not guarantee the birth of a viable neonate. While establishing the biophysical profile of fetuses during pregnancy is an interesting concept, some clinicians question its validity for clinical practice as it is a time-consuming technique, and results are not necessarily useful in determining interventions. Fetal breathing, heart rate and rhythm, fetal tone, and general activity are useful guides when evaluating fetal health and well-being. Therefore chemical sedation of the mare is not recommended because commonly used drugs are likely to induce fetal bradycardia and suppress normal fetal activity.[706] Fetal breathing

is characterized by movement of the diaphragm between the thorax and abdomen in conjunction with rib cage expansion without any other movement by the fetus. Fetal breathing patterns should be monitored for at least 30 seconds.[694,704]

When fetal heart rate and rhythm are monitored, it is not appropriate to scan for only 10 to 15 seconds and then multiply by a correction factor to obtain the number of beats per minute. Beat-to-beat variations and observation of periodic accelerations are important. It is normal for the heart rate accelerations to occur in association with fetal activity. Multiple measurements of fetal heart rate and assessments of fetal heart rhythm should be made over a 30-minute period while evaluating the fetus, fetal fluids, and placenta. Ideally, three measurements should be obtained with the fetus at rest and another three after periods of activity. It is difficult to accurately monitor the heart rate during periods of fetal activity, unless M-mode echocardiography equipment is available. Fetal heart rates vary with the stage of gestation and the amount of fetal activity at the time of the examination.[694,707-709] The fetal heartbeat is normally regular and will decrease from greater than 120 beats/min in midgestation to between 60 and 90 beats/min in late gestation. Cardiac accelerations (20–40 beats/min above baseline) are normal if they are associated with fetal movement, but persistent tachycardia in the absence of fetal activity indicates fetal stress. A resting heart rate in excess of 104 beats/min indicates fetal stress in a late-gestation fetus.[702,704] A heart rate of less than 57 beats/min in a fetus that is less than 330 days' gestation and a rate of less than 50 beats/min in a fetus older than 329 days' gestation should be considered abnormal. A fetus suffering from hypoxia will have a slow heart rate, with minimal limb activity or fetal breathing, indicative of central nervous system depression. However, if the condition is chronic and ischemic conditions are developing, then the fetus will become tachycardic despite a lack of fetal activity. This is a prelude to fetal demise. In terminal cases extreme bradycardia ensues just before fetal death. Although failure to observe fetal activity may be caused by the stage of the normal rest-activity cycle, confirmation of a regularly beating heart at least verifies that the fetus is alive. This is a major advantage over transrectal fetal ballottement, in which failure to detect movement can raise unnecessary concerns about fetal health.

If Doppler ultrasound equipment is available, then the Doppler transducer is placed directly over the site in which the best image was detected by the ultrasound scan. Tracings of fetal heart rate and rhythm can be recorded over time (usually at intervals of 5 to 10 minutes). This makes analysis easier and serves as a permanent record of the fetal status at the time of the recording. If there is some question about the presence of twins after a transabdominal ultrasound examination, then fetal electrocardiogram (ECG) tracings may show two distinct fetal patterns.[708,709] Features of the ECG tracing that should be noted include fetal heart rate and rhythm, accelerations and decelerations, complex polarity changes, and beat-to-beat variation. In the last weeks of pregnancy fetal foals usually have a baseline heart rate in the range of 60 to 75 beats/min. Transient low heart rates of less than 60 beats/min are not uncommon. These troughs warrant concern only if they are not interspersed with accelerations. Likewise, transiently elevated rates in the order of 120 beats/min (occasionally >200 beats/min) are not abnormal, provided they return to baseline. If the fetal heart rate is found to be less than 60 beats/min or greater than 120 beats/min during an observation period, then more frequent monitoring is justified to determine whether the fetus is distressed. Beat-to-beat variations are normal, and a finding of no variability is an ominous sign. Maternal medications such as alpha-2 agonists (e.g., detomidine, xylazine) or opioids (e.g., butorphanol) will reduce fetal heart rate variability transiently.

The fetus is noted to have tone if it is observed to flex and extend the limbs, torso, or neck. Tone is poor or absent if the fetus appears flaccid. Fetal movements include partial to full rotation around the long axis of the fetus as well as less marked activity such as extension and flexion of the extremities. Fetal activity is rated on a scale from 0 to 3, with 3 being a very active fetus. A score of 0 indicates that no fetal movement was noted during the examination period. Long periods without noticeable fetal activity are cause for concern and should be evaluated in conjunction with information about the fetal heart rate and rhythm. The fetus may be distressed, suffering from advanced hypoxia and central nervous system depression.

Fetal aortic diameter is correlated with the weight of the pregnant mare and the final neonatal foal weight.[694,702,703] Thus the pregnant mare's weight can be used to estimate what the fetal aortic diameter should be, using the regression equation ($Y = 0.00912 \times$ pregnant mare's weight in pounds + 12.46), where Y is the predicted fetal aortic diameter (in millimeters).[702,703] The actual diameter of the fetal aorta should then be measured in the thoracic cavity as close to the fetal heart as possible. A smaller-than-predicted aortic diameter may indicate a dysmature or growth-retarded fetus (IUGR) or twins. The maximal thoracic diameter is measured from the spine to the sternum over the caudal part of the thorax, and in a late-gestation fetus it should be 18.4 ± 1.2 cm. It has been correlated with fetal aortic diameter and neonatal foal weight in high-risk pregnancies.[702, 703] Foal girth measurements and hip height are also correlated with fetal aortic diameter measurements.[704,710] Fetal biparietal measurements and orbital diameters have also been used to estimate fetal size.[694,710] Eye length (sclera to sclera) is a useful predictor of days before parturition in small ponies.[711] Decreased blood flow to the placental unit inhibits fetal growth, and some form of chronic placental insufficiency should be suspected when small fetal size is detected.

It is important to monitor fetal viability if a high-risk pregnancy is being maintained on altrenogest supplementation. Although most nonviable fetuses will be aborted, there is a report of a mummified fetus being retained when the mare was receiving long-term progestogen supplementation.[712]

COMPLICATIONS IN LATE GESTATION

Once confirmed to be at least 45 to 60 days pregnant, most mares can be expected to carry the fetus to term. The incidence of fetal loss after 100 to 120 days' gestation is low and accounts for only a small percentage of total pregnancy wastage. Fetal death and maceration are very uncommon in the mare. A set of macerated twins were seen by one of the authors (GF) in a draft breed mare that suffered no ill effects systemically. The mare was evaluated only after the owner noticed a foul-smelling vaginal discharge.[713]

Ventral body wall ruptures and uterine torsions are uncommon, and hydrops of the fetal membranes is an especially rare condition. Accurate diagnosis and appropriate management of these clinical cases can prevent the development of a life-threatening condition. If a ventral body

wall rupture or uterine torsion is present, then the birth of a viable foal may still be possible provided that the case is properly managed.[714] In referral hospitals, mares with body wall defects tend to be presented in advanced stage, making management more challenging. Outcomes for several diseases of the late-term pregnant mare can be improved by training farm personnel and horse owners to identify clinical problems. Owners should be trained to check late-term pregnant mares daily or weekly for signs of placentitis (e.g., vulvar discharge, premature mammary gland development) and areas of focal ventral edema. As described elsewhere in this text, ventral edema may be observed in some normal periparturient mares. Mares with expanding areas of edema in the ventral abdomen should be examined immediately by palpation and ultrasonography of the area and surrounding body wall. Muscle failure can frequently be felt and observed, and medical treatment, rather than surgical, may provide a better outcome for dam and fetus.[715]

Hydrops of the Fetal Membranes

Hydrops is a condition of the last trimester, with the pregnancy developing normally until somewhere between 7.5 months and term. Hydrallantois and hydramnios are rare conditions of pluriparous mares that involve a pathologic accumulation of fluid within the allantoic and amniotic compartments, respectively. Normal volumes of allantoic fluid in mares vary from 8 to 18 L at term. In documented cases of hydrops, the allantoic fluid volume ranged from 110 to 230 L. Just as with cows, hydrallantois accounts for most dropsical conditions in the mare.[716-719] The pathophysiology of hydrallantois in the cow has been related to a placental abnormality, whereas hydrops amnion has been associated with a fetal head anomaly that precludes swallowing.[200] Dysfunctional placentation may cause an increased production of transudate or disruption of transplacental fluid absorption. There does not appear to be any consistent abnormality of the fetus or fetal membranes that is characteristic of the condition in the mare. A mare with confirmed hydramnion delivered a viable foal.[720] A mild diffuse placentitis or endometrial vasculitis has been incriminated in some cases. A potential correlation between hydrallantois and *Leptospira* infection has been suggested, but causation has not been established.[720]

Generally there is a sudden onset of abdominal distention, and walking becomes difficult. The mare may exhibit variable degrees of colic. There is a progressive loss of appetite, and the mare may experience some difficulty in defecation. The increasing pressure on the diaphragm causes dyspnea, and the mucous membranes may appear cyanotic, especially when the mare is recumbent. On physical examination the rectal temperature is normal, but the heart rate will be increased. Transrectal palpation reveals characteristic findings. Copious lubrication and extreme caution should be used because passage of the forearm will be impeded by pressure from the large fluid-filled uterus. In advanced stages, the feces tend to be covered with mucus as a result of the prolonged passage through the lower gastrointestinal tract. The gross distention of the uterus means that the fetus is usually not palpable. Failure to detect the fetus by external ballottement further supports the diagnosis, but some mares may not tolerate ballottement. Transabdominal ultrasound may confirm the presence of excessive amounts of hyperechoic fluid. A thorough examination from both sides of the abdomen should be performed to rule out the possibility of twins.

Owners should be advised that this is a progressive condition, and it is extremely unlikely that the mare will be able to sustain the pregnancy and deliver a viable foal. Despite this, a recent report documented a case that was successfully managed until a viable foal was delivered at 321 days' gestation.[720] In that case the mare was provided with close monitoring of maternal and fetal health, in combination with care that included a supportive belly wrap, antiinflammatory medication, and altrenogest. A partial drainage technique is sometimes used in an attempt to manage cases that were diagnosed within 2 to 4 weeks of term. Affected mares receive abdominal support (belly band), IV fluids, broad-spectrum antibiotics, and antiinflammatory medication. The technique of slow, repeated drainage requires a major time commitment and would not be cost effective in many cases. Fetal death may occur as a result of placental separation. There also appears to be a considerable risk for iatrogenic fetal infection after contamination of the fetal fluids, despite attempts to perform the drainage technique in an aseptic manner. Despite heroic attempts in valuable mares, the fetus is likely to be lost in cases of hydrallantois. The prognosis for mare and foal survival may not be favorable, particularly when the mare is <300 days' gestation. Owners may opt to euthanize a mare without further effort, for economic reasons, if the foal has little chance of survival.

In most cases induction of parturition may be advisable before the mare's condition deteriorates further. Continued abdominal enlargement will predispose the mare to body wall tears or prepubic tendon rupture, and uterine rupture has also been reported.[721] Induction of parturition is not without risk (shock and dystocia), but the prognosis for survival of the mare is good provided appropriate supportive therapy is instituted, particularly at an early stage of disease. The prognosis for the mare's reproductive future may also be favorable if there are no untoward sequelae (cervical lacerations, retained fetal membranes, and metritis) or severe dystocia.

Application of PGE_1 to the cervix before induction may facilitate atraumatic fetal extraction.[360] Six of eight mares that had previously developed a hydrops pregnancy subsequently became pregnant and delivered normal healthy foals at term.

Before a therapeutic induction of parturition, the tail should be wrapped, the perineal area cleansed, and an indwelling IV catheter inserted. Large-volume IV fluid therapy may become necessary if hypovolemic shock develops as the allantoic fluid is discharged.[714] In some cases controlled drainage may be beneficial before inducing delivery. An added complication in hydrops cases is that the thickened, edematous chorioallantoic membrane may be difficult to rupture.[716] If digital pressure alone is unsuccessful, then an endometrial biopsy forceps can be used to create an opening in the chorioallantois. Some authors report that the lack of pressure from the atonic uterine wall will result in minimal release of fetal fluid from the punctured chorioallantoic sac, but in most cases there is a massive release of fluid once the chorioallantoic membrane is ruptured. If insufficient fluid release occurs, a sterile nasogastric tube can be introduced into the uterus to begin controlled siphoning of fluid. The authors recommend detaching the caudal pole of the chorioallantois and introducing a nasogastric tube through the cervix and the chorioallantois. An alternative technique is to introduce a thoracic trocar catheter through the cervix and employ a sharp puncture of the chorioallantois. This approach permits the excess fluid to be removed by controlled drainage. Administration of IV fluids with gradual

removal of the excess allantoic fluid will permit the mare's cardiovascular system to adapt.

Oxytocin and $PGF_{2\alpha}$ injections have been used in an attempt to abort these cases.[716-718] Although oxytocin is widely considered to be the most efficacious method for routine induction of parturition, in mares with hydrops conditions the distended uterine musculature may not contract effectively.[716,722] This primary uterine inertia is common, and gentle manual dilation of the cervix, or perhaps prior topical application of PGE_1, may be warranted.[360] A smooth induction and manual extraction of the foal was reported for one mare after two doses of cloprostenol administered 30 minutes apart.[723] Efficacy of ecbolics is likely more favorable in mares with mild to moderate distention of the uterus.

The abdominal musculature may be weakened by stretching, and thus the typical stage II abdominal contractions may be compromised. If an abdominal bandage is in place, many mares will not have abdominal contractions, and classic stage II of labor will not occur without intervention to manually extract the foal. Malpositioning and malpostures are not uncommon. The fetus may need to be extracted by assisted vaginal delivery, but care should be taken not to traumatize the cervix by overzealous traction. The expelled fetus will generally be alive, and humane euthanasia is usually warranted.

After delivery of the foal, oxytocin administered as a continuous rate infusion at 1.0 IU/min or as an intermittent intramuscular bolus at 5 to 10 units q 30 min should be included in the treatment plan to promote uterine involution and facilitate fetal membrane expulsion. Retention of the fetal membranes should be expected, and appropriate treatment for removal of these membranes and prevention of the metritis-laminitis complex is indicated. Uterine involution should be monitored by transrectal palpation and ultrasonography.

Ventral Body Wall Hernias and Prepubic Tendon Rupture

Apart from those with pathologic pregnancies, mares with ventral body wall defects are generally close to term.[715,724,725] Damage to the abdominal sling of the pregnant mare may involve rupture of the transverse abdominis and oblique muscles, the rectus abdominis muscles, and the prepubic tendon. In extreme cases the rupture may lead to hemorrhage, shock, and death. The prepubic tendon attaches to the cranial border of the pubis, and lordosis occurs if the tendon is ruptured. Although breed (draft breed and Standardbred mares) and age (older mares) may predispose a mare to development of the condition, in most cases there is no apparent predisposing cause. The extreme abdominal distention associated with the hydrops condition may cause rupture of the ventral musculotendinous support. Defects in the ventrolateral abdominal wall are more common than complete prepubic tendon rupture, and bilateral involvement of the abdominal wall seems to be most common.[9] In a retrospective study of 13 cases, only three were categorized as being caudal midline (prepubic tendon lesions), and all three had additional involvement of the body wall musculature.[715]

The most obvious clinical sign of an impending ventral body wall rupture is a thick plaque of ventral edema extending a variable distance cranial to the udder. However, ventral edema may be a normal consequence of late pregnancy, and it can indicate external trauma. A large ventral swelling may also occur with hematomas from external trauma and should be considered as a differential diagnosis. Mares in late pregnancy often develop a thick plaque of ventral edema that can extend from the udder to between the forelimbs. This is associated with the compressive weight of the gravid uterus on the venous and lymphatic drainage of the ventral abdomen. The presence of a hemorrhagic secretion in the mammary gland supports a diagnosis of tissue trauma rather than pregnancy edema. Unilateral edema is more indicative of damage to the ventrolateral body wall, but it may be associated with partial rupture of the prepubic tendon. The extreme pain associated with progressive enlargement of a ventral body wall rupture causes a marked tachycardia that may not be responsive to analgesics.

Pregnant mares with a ruptured prepubic tendon or abdominal wall may show signs of colic and generally are reluctant to move. If the prepubic tendon is completely ruptured, then the pelvis will be tilted such that the tailhead and tuber ischii are elevated and a lordosis will be present.[714,715,724,725] The mammary gland is often displaced cranially and ventrally because of the loss of the caudal attachment to the pelvis, and bloody secretion is usually present. A rent in the abdominal musculature may be complicated by bowel incarceration.

Confirmation of the tentative diagnosis is sometimes difficult. Because it is not always possible to be certain that a rupture has already occurred, mares with severe ventral edema should be confined to a stall with exercise restricted to hand walking. Rectal palpation of the defect is usually not possible because of the advanced stage of the pregnancy. External palpation is generally unrewarding because of the thickness of the edema, although some crepitation of the ventral abdominal wall may be noted. The mare is generally extremely sensitive and resists palpation of the area. Ultrasonographic examination of the posterior aspect of the ventral abdomen may be useful in some cases and can detect the presence of a bowel segment.[204,704,714,715] Rupture of the prepubic tendon can be identified as disruption of the tendon fibers immediately cranial to the pubis, whereas abdominal wall muscle tears are seen to be discrete tears within muscle fibers, often associated with hematomas.[715] An accurate assessment of the dimensions of the defect often cannot be made until the fetus and fetal fluids are expelled and the ventral edema has subsided. Often body wall tears can be found in the flank as well, and transcutaneous ultrasound in the flank region may be beneficial.

Depending on the degree of discomfort and stage of pregnancy, termination of the pregnancy may be the most humane treatment for the mare, given that further tissue damage is likely to occur to some degree until parturition. The possibility of a segment of intestine becoming incarcerated in the defect also should be considered. In extreme cases the mare may eventually become recumbent, especially if there is a rapidly expanding body wall defect. Owners should be aware that unless parturition is imminent, the prognosis for the foal is not good because fetal readiness for birth is difficult to predict. If it is determined that the goal for the owner is to save the fetus, the management plan should focus on supportive care to facilitate maintenance of the pregnancy to term. In these mares the treatment is essentially supportive, and the prognosis for the foal is good. Antiinflammatory drugs will help alleviate the mare's discomfort. An abdominal sling (belly band) made of canvas or padded leather or a snug abdominal bandage help provide support for the ventral abdominal wall. If a sling is used, the area over the back must be well padded to prevent pressure necrosis because the purpose of this support

is to transfer the weight of the gravid uterus to the vertebral column. Reducing the bulk of the ration and feeding a mild laxative may help reduce the degree of abdominal exertion associated with defecation.

Assistance with parturition should be available because the mare may experience difficulty in mounting sufficient abdominal pressure to expel the fetus, especially if the mare has an abdominal bandage.[715,725] However, one report suggests that some mares can position the foal and complete delivery unassisted.[715,724] Arrangements for an alternate source of colostrum should be made because ventral edema may prevent the foal from suckling. The owner should be informed that although in some cases surgical repair of the defect may be possible by mesh herniorrhaphy, rebreeding the mare with an expectation of carrying the foal to term is usually not advisable. Some mares with small, unrepaired defects may subsequently foal without assistance, but the possibility that future pregnancies may exacerbate the condition should be considered. Embryo transfer offers a viable alternative if this procedure is condoned by the relevant breed society.

Uterine Torsion

Uterine torsion accounts for 5% to 10% of all complicated obstetric conditions in the mare.[726,727] Neither mare age nor parity appears to be a significant risk factor.[728,729] The causes of uterine torsion in the mare are not well defined. The condition is much more common in cattle, and in that species a large term fetus has been implicated as a major risk factor. Most uterine torsions in cows occur at term and are thought to be a direct result of fetal positional changes during late first-stage and early second-stage labor. A striking difference between the mare and the cow is that more than 50% of uterine torsions in mares occur before the end of gestation.[730] It seems that most equine uterine torsions occur before term, and cases may be seen as early as 8 months of gestation.[714] One report documented a mare affected as early as 126 days' gestation.[731] A multicenter retrospective study of 63 cases reported that 59% of the mares were at less than 320 days of gestation.[728] Although Ginther et al. have shown that the fetus is locked in a dorsopubic position during the final months of gestation, it is still possible for the entire pregnancy (uterus and fetus) to rotate approximately 90 degrees on the lower maternal abdominal wall.[402] This occurs because any rotational movement of the caudal half of the fetus (pelvis and hindlimbs) by necessity will involve the close-fitting uterus. It seems likely that in extreme cases this rotating action can lead to a clinical uterine torsion. Owners who work closely with their mares may observe excessive fetal movements in the flank area 1 or 2 days before. In a recent study, 80% of term fetuses were in a dorsosacral position when the uterine torsion was corrected. This suggests that fetal righting reflexes may have played a role in creating the torsion.[726] Vigorous fetal movements during the latter stages of gestation are likely to be a significant factor in the etiology of this condition in the mare.

The clinical signs that attract the owner's attention are the result of abdominal pain and may include restlessness, sweating, anorexia, frequent urination, sawhorse stance, looking at flanks, and kicking at the abdomen. When the veterinarian is first contacted, the signs may have been present for a period ranging between 2 hours and 3 days or more, especially if signs are intermittent and moderate. In mares that are close to term, the owner may assume that the signs indicate impending parturition. In more extreme cases the signs are more severe and may be associated with concurrent involvement of the small or large colon. Veterinarians should always consider the possibility of uterine torsion when a mare in the last trimester of gestation exhibits a mild, persistent colic. Delay in making a definitive diagnosis increases the likelihood of fetal compromise. In one study of 63 cases, the mean time to admission was more than 20 hours.[728] Occasionally the condition may remain undiagnosed for several weeks.[732] In these instances an owner may have attempted treatment with analgesics that were prescribed for previous mild colic episodes.

Transrectal palpation is essential to determine whether a uterine torsion is present. All late-pregnant mares that display signs of mild to moderate colic warrant a thorough transrectal examination to rule out the possibility of uterine torsion. Although vaginal involvement in torsion is very common in the cow, uterine torsions in the mare seldom cause detectable changes in the vagina.[714,733,734] Thus vaginal examination is generally not diagnostically useful in mares.

On transrectal palpation the clinician should aim to carefully advance the forearm while palpating for a taut band on either side of the rectum. The broad ligament on the side of the torsion tends to be more caudal and is palpable as a tight vertical band. As the arm is advanced further, the opposite ligament will be palpable because it is pulled horizontally across the top of the uterus before being displaced ventrally. An accurate examination of the broad ligaments will confirm the diagnosis, determine the direction of the torsion, and give some idea of the severity of the torsion. One retrospective of 54 mares reported that 79% of referred cases were rotated no more than 180 degrees, and 59% of cases involved clockwise torsion.[728]

A transrectal ultrasound examination is useful to evaluate the condition of the fetal fluids and to note whether any placental detachment has occurred. The degree of uterine compromise can be gauged by noting the thickened uterine wall and distended vasculature. Compression of the veins and lymphatics occurs before occlusion of the arterial blood supply; thus the initial changes will be associated with pooling of fluid within the uterine wall. The compressive forces of the displaced broad ligaments may cause variable amounts of constriction of the small colon.[734,735]

Transabdominal ultrasonographic imaging may be used to assess fetal viability (heart rate and rhythm) and to evaluate the character of the fetal fluid. Compression of the uterine blood supply can cause fetal hypoxia and a fetal stress response, especially in more advanced pregnancies. Abdominocentesis may provide prognostic information and guide the clinician in choosing a mode of correction.[197] Because it may be difficult to obtain peritoneal fluid from a mare in late gestation, transabdominal ultrasonography is sometimes useful in locating a pocket of fluid.

Uterine rupture can be a complication of uterine torsion in the mare.[734,736] Mild uterine torsions, or those of short duration, do not alter the color, cellularity, or total protein content of the peritoneal fluid.[197] Mares with severe or chronic uterine torsion may develop significant uterine compromise that results in changes in the composition of the peritoneal fluid. Any alterations in the composition of the peritoneal fluid may indicate the presence of a compromised or ruptured uterine wall or concurrent gastrointestinal involvement.[3] A flank laparoscopic examination can confirm the condition of the uterine wall.[737] This information will facilitate an informed choice of surgical approach or perhaps support a decision for euthanasia if economic considerations preclude surgical intervention.

The stage of gestation and the mare's heart rate are important prognostic indicators for mare survival. A recent study reported that mares that died had significantly higher heart rates (mean 74 beats/min) at admission than survivors (mean 59 beats/min).[728] Increased fetal size and the weight of the gravid uterus make correction closer to term (>320 days) more difficult, and this may explain the poor mare survival rate (65%) compared with cases in less advanced pregnancies (<320 days), in which survival rates are excellent (97%). Owners should be advised that fetal survival rates are not as good, but a similar effect of stage of gestation applies. The chances of the foal surviving exceed 70% if the mare is at less than 320 days' gestation, but only about 30% survive when mares are closer to term.

If the mare is presented in stage I of labor and the cervix is sufficiently dilated to permit passage of a well-lubricated arm into the uterine body, then it may be possible to reach the fetus. It should be grasped ventrolaterally and then rocked back and forth until sufficient momentum is achieved to continue up in an arc. This reproductive maneuver may not be possible in mares with reproductive tract swelling because of a chronic, undiagnosed torsion. This manipulation should roll both fetus and uterus back into a normal position. More than 80% of term torsions may be corrected in this manner.[716,727] Options for management of a preterm uterine torsion are rolling the mare, flank laparotomy, or a ventral midline celiotomy.

In the controlled referral hospital environment, the method of correction is not associated with mare survival, but both rolling and midline celiotomy require general anesthesia and thus entail those additional risks.[728] There is a significant effect on foal survival if the mare is at less than 320 days' gestation, and using the standing laparotomy approach should provide a significantly better prognosis compared with the ventral midline approach. However, the final decision must be based on several factors, including the severity of pain being exhibited by the mare, client financial constraints, and surgeon preference. It should also be noted that a ventral midline celiotomy permits better assessment of uterine viability and evaluation of the gastrointestinal tract compared to a flank incision.

The anesthetized mare may be rolled in an attempt to rotate the mare's body around the stationary gravid uterus.[738] It is essential that the mare be positioned in lateral recumbency and rolled in the direction of the torsion. The aim of the procedure is to roll the mare such that the pelvis "catches up" with the displaced uterus. Correction by rolling the mare is controversial. Citations in the literature report on a limited number of cases.[730,738,739] Concerns with this approach include the following: Unsuccessful attempts to correct the torsion will prolong its adverse effects, misdiagnosis of the direction of the torsion means that rolling the mare may make the condition worse, the condition of the uterus cannot be evaluated, and displacement of the colon may result.[714] In addition, a higher risk of placental detachment and uterine rupture has been reported.[204,739,740] Another concern is that if general anesthesia is induced under less than ideal conditions, maternal hypoxia may cause fatal complications in the already compromised fetus.

In the standing flank approach, a grid incision is made on the same side as the direction of the torsion.[734] The torsion is corrected by placing the forearm under the uterus. The uterus and contents are rocked back and forth to gain momentum. A combination of lifting and rotating movements generally results in easy correction of the torsion. The presence of a live fetus greatly facilitates the detorsion manipulations. More difficulty may be experienced in mares that are close to term, and this may justify the more expensive ventral midline celiotomy approach. If the standing procedure is attempted, then an incision in the opposite flank will permit a second surgeon to assist by gently pulling across the top of the uterus as it is elevated from below. If the fetus is dead, then the mare should abort naturally once the uterine torsion has been corrected, removing the need for hysterotomy and any associated complications.[571,714] However, the mare should be closely monitored, and obstetric assistance must be available to correct any malposition or malposture. Mares experiencing intractable pain should receive general anesthesia during the operation. A ventral midline celiotomy is also indicated when significant uterine compromise is a concern or another problem coexisting in the abdomen is suspected.

The prognosis for mares with uterine torsion depends on the degree of vascular compromise. The severity and duration of the condition affect placental circulation and subsequent fetal viability.[714] In chronic cases in which there is significant uterine compromise, it is feasible to perform an ovariohysterectomy to salvage the mare for nonbreeding purposes.[732,741] If the fetus is alive and the uterine wall is not severely congested and edematous, then the prognosis for both the mare's survival and for the birth of a live foal at term is good. Progestogen supplementation for 3 to 5 days after the manipulations involved in correcting a uterine torsion may be indicated to promote myometrial quiescence and maintenance of the placental attachment. Although supplementation after a uterine torsion would be in the last 2 to 3 months of gestation, there are reports of mares retaining a nonviable fetus (one that died at 3–5 months' gestation) while receiving progestins.[712] Thus if progestin supplementation is administered to a mare after correction of a uterine torsion, then it is prudent to monitor fetal viability at regular intervals. There is probably little merit in continuing the supplementation once the mare has been discharged from the hospital. In one study 28 of 30 mares that were pregnant at the time of discharge subsequently delivered a live foal, and future fertility was good (24 of 29 were successfully rebred).[728]

Vaginal Hemorrhage

Visible blood on the tail hairs or hindlimbs of a pregnant mare warrants careful examination.[742] The integrity of placental attachment immediately cranial to the cervix should be evaluated using transrectal ultrasonography. A pocket of fluid may be evident if placental separation has occurred, and measurements of the CTUP should be made.[697] An increased CTUP measurement is indicative of placentitis.[594] Because two of the three barriers protecting the fetus (vulvar lips, vestibular sphincter, and cervix) will be entered when performing a vaginal speculum examination, it is essential to ensure stringent hygiene. Often, blood clots can be seen in the vestibule when the vulvar lips are parted. A sterile speculum should be covered with sterile lubricant and then gently inserted into the vagina. Some mares with bloody vulvar discharge may have experienced trauma inflicted by herdmates.[743]

In some mares there may be evidence of a serosanguineous discharge from the cervical os. Mares that are aborting a twin pregnancy may also develop a bloody vaginal discharge. However, the practitioner should be aware that the most common source of vaginal hemorrhage in pregnant mares is from varicose veins in the vestibulovaginal fold (hymen), not from an

impending abortion. In many cases no blood is visible in the cranial vagina. If the cervix is closed, pale, and covered with tenacious mucus, then it is unlikely that the blood is associated with the fetoplacental unit. Although the blood could be associated with cystitis or urolithiasis, the source of the hemorrhage usually is varicose vessels in the remnants of the hymen at the level of the vestibular sphincter or cranial vagina. It is not unusual to miss these as the speculum is inserted; thus particular attention should be paid to this area as the speculum is withdrawn.[742]

Vaginal varicosities are most likely to occur in older, multiparous mares during the latter months of gestation.[742,744] A large plexus of dilated veins can develop in the pedunculated mucosa at the level of the vestibulovaginal junction or less commonly in the cranial vagina. The veins can become engorged from 1 to 2 cm during late gestation. If the vessels are ulcerated, there may be intermittent, mild episodes of bleeding from the ventral commissure of the vulva. An owner may report seeing large blood clots in the bedding when the mare is fed in the morning. Although this quantity may be alarming to an owner, it can be explained as merely representing the discharge of pooled blood while the mare was recumbent during the night. Another explanation is that the pull of the rectal attachments when standing creates sufficient tension in the vaginal wall to control hemorrhage, whereas blood loss can occur when the mare is recumbent. In more severe cases there may be dried blood on the perineum, tail hairs, and hindlimbs. Overt vaginal hemorrhage may be sufficient to cause anemia.[744]

The etiology of vaginal varicosities in mares is unknown. The condition is not an uncommon complication of pregnancy in pluriparous women, and it has been proposed that a similar obstruction of venous return is responsible for the development of vaginal varicosities in the mare. Poor vulvar conformation with cranial displacement of the perineum may be involved in some cases.[49,742,744] It is possible that repeated stretching of the vestibulovaginal tissues and changes in the vulvar conformation of pluriparous mares create a physical impairment of venous return from the vestibular and vaginal components of the internal pudendal vein. The dilated veins are generally located on the cranial aspect of the vestibular fold and on the dorsal aspect of the caudal vaginal wall. Thus vaginal varicosities can be easily missed when a tubular speculum is introduced, and they may not be evident during withdrawal. In some cases a tri-valve metal Caslick speculum may provide better exposure so that the tissues of the vestibulovaginal fold can be everted and fully explored. If the diagnosis is still inconclusive, then the next logical step in the physical examination is ultrasound and endoscopic visualization of the vagina and cranial vestibulovaginal fold. The clinician should first advance the flexible scope toward the cervix and then angle the lens back to better evaluate the cranial aspect of the vestibulovaginal fold. If vaginal varicosities are present, usually no treatment is necessary, because the vessels will regress spontaneously after foaling (benign neglect).[742] However, if there is persistent or excessive hemorrhage or the owner is distressed, then the offending vessels can be cauterized (diathermy) or ligated.[742,745] Locating the source of the bleeding can be difficult. Traction on the adjacent vaginal mucosa may arrest the bleeding and help identify the site in such cases. Submucosal resection of a pedunculated plexus of varicose veins has been reported to be successful.[742,744] Some authors have had success with topical application of a human hemorrhoid cream to prominent or suspected vessels. Treatment is generally successful, and no recurrence has been reported.

It is important to consider bleeding from the urinary tract when vaginal hemorrhage is the presenting complaint. If an owner reports having seen bloody urine on the stall floor, then it should be remembered that normal equine urine contains pyrocatechin. This oxidizing agent can cause urine to turn red-brown after exposure to air.[742] Hematuria can result from several disorders of the urinary tract.[746] If blood is present throughout urination, then the lesion is likely to be in the kidneys, ureters, or bladder. If the endoscopic examination has not identified vaginal varicosities as the source of the hemorrhage, then the endoscope should be advanced down the urethra to visualize the bladder lumen (cystoscopy). Although uroliths are more common in geldings and stallions than in mares, a urolith may cause mucosal irritation and hemorrhage, resulting in hematuria.[747,748] Rectal palpation may reveal a firm mass in the bladder that can be imaged by ultrasonography. The pelvic portion of the urethra should also be palpated.[742] Although neoplasia of the urinary bladder is rare, mares are reported to be twice as likely as male horses to develop primary bladder tumors.[746] Squamous cell carcinoma is the most commonly reported bladder tumor, but transitional cell carcinomas can also occur. The condition has been reported as a cause of hematuria, often with blood clots.[749] A more detailed discussion of hematuria may be found in Chapter 14.

Fescue Toxicity and Agalactia

A wide range of reproductive problems (e.g., thickened placenta, abortion, prolonged gestation, dystocia, dead or weak foals, agalactia) have been attributed to the effects of the fungal endophyte (*Acremonium coenophialum,* now known as *Neotyphodium coenophialum*).[750-752] The endophyte produces a dopaminergic, vasoactive ergopeptine alkaloid (ergovaline). This alkaloid disrupts the fetoplacental production of progestogens, but the precise mechanism has not been established.[753, 754] Umbilical vein progestogen levels suggest that the disruption is not at the level of placental steroidogenesis, which is a remarkable observation when the fetal membranes are so edematous. Premature chorioallantoic separation and the failure of the membrane to rupture (red bag) are attributable to the edematous splanchnic mesoderm.[750] ACTH, T4, triiodothyronine, progestogen, and cortisol concentrations are lower in foals born to endophyte-exposed mares, suggesting that the effects are actually at the level of the fetal hypothalamic-pituitary axis, thyroid, and adrenal cortex.[753] This is likely to be the basis for the prolonged gestation and fetal dysmaturity that are associated with fescue toxicosis.

Ergovaline also inhibits prolactin secretion in affected mares by acting as a dopamine agonist at the maternal pituitary level.[754] Prolactin secretion can be inhibited experimentally by administering dopamine agonists such as bromocriptine.[755] Such prolactin-inhibiting treatment of pregnant mares results in agalactia and also mimics the other symptoms of fescue toxicosis (thickened placenta, prolonged gestation, and dystocia). An effect of fescue toxicosis in pregnant mares is a lowering of the circulating relaxin levels.[756] Clinical observations suggest that a one-time injection with fluphenazine improved pregnancy outcome by reducing the adverse effects of fescue toxicosis concomitant with a stabilization of plasma relaxin concentrations. (Caution should be exercised with the administration of fluphenazine to horses because this drug may cause severe extrapyramidal neurologic signs.) These data support

the hypothesis that systemic relaxin may be a useful biochemical means of monitoring placental function and treatment efficacy in the mare.[756]

Because mares in late pregnancy are so susceptible to the toxic effects of ergopeptine alkaloids, they should not be permitted to graze in endophyte-infected tall fescue pastures or ingest hay derived from such pastures. Short-term exposure by mares at 300 days' gestation results in a significant decline in both PRL and total progestogen concentrations within 48 hours. Fortunately, removal of pregnant mares (300 days' gestation) from infected pasture results in a significant increase in prolactin and progestogen levels within 3 days. This will prevent the development of the typical symptoms associated with fescue toxicosis.[753] Even when alternate feed sources are limited, every attempt should be made to remove pregnant mares from endophyte-infected fescue by 30 to 60 days before the expected foaling date. When this is not possible, prophylactic administration of the dopamine receptor antagonist domperidone (Equidone) at 1.1 mg/kg or sulpiride administered orally in the last month of gestation can prevent the negative effects of fescue toxicosis.[314,751,757-759]

FOALING MANAGEMENT

Gestation length is notoriously unpredictable in mares, and the greatest predictor of gestation length may be the mare herself.[760,761] Although the frequently recommended minimum gestation length for successful induction is 330 days, it must be remembered that many mares will carry a foal past 340 days and occasionally to 360 days and beyond. Some mares may carry and deliver a normal foal by 320 days of gestation. Light exposure affects gestation length in mares, particularly early in the foaling season when mares tend to have longer gestation lengths.[762] Mares foaling early in the season should be kept under lights for normal cyclicity postpartum and for normal gestation length. Mares bred in the fall will foal during the summer and will tend to have shorter gestation periods.

There also appears to be some breed variability in gestation length, with the mean for Friesians reported at 332 days, Lipizzaners reported at 334 days, Andalusians reported at 337 days, and Arabians reported at 340 days.[761,763,764] In a retrospective study of Standardbred mares, the mean duration of gestation was 343.3 days, and it was significantly greater for colt fetuses (344.4 days) than for filly fetuses (342.2 days).[765] An average gestation length of 344.1 ± 0.49 days was reported in a recent Thoroughbred study (n = 344 mares). Colt foal pregnancies were significantly longer (346.2 ± 0.72) than fillies (342.4 ± 0.65).[766] Colts were carried 1.5 days longer in a study of 495 Friesian mares.[763] The sire has been associated with duration of gestation, and gestation after mating with certain sires was consistently less than 340 days in duration, whereas duration after mating with other sires was consistently more than 350 days.[763,765] In Andalusians and Arabians, a delay of 1 month in breeding corresponded to a decrease of 3 days in the gestation length.[761]

Because gestation length is highly unpredictable in mares, several other methods have been recommended for use in routine foaling management. Every periparturient mare should be watched closely so that assistance is available at parturition to maximize chances of mare and foal survival. Assisted parturition allows early intervention and correction of simple to more complicated dystocias, allows the clinician to determine if the foal requires immediate veterinary attention, and allows intervention in cases of a "red bag" delivery (i.e., premature placental separation).

Although mammary development is a somewhat useful sign of approaching parturition in normal mares, monitoring changes in mammary secretion electrolyte concentrations and pH are useful tools to monitor time of imminent parturition.[767-769] An inversion in the sodium:potassium ratio followed by a rapid rise in calcium (Ca) concentrations in the last 24 to 48 hours has been correlated with fetal maturity in both mares and jennies.[767] Exact values may vary with the type of chemistry analyzer used by the diagnostic laboratory.

In a normal term pregnancy, the combined mammary secretion levels of Ca (>40 mg/dL), potassium (>30 mEq/mL), and sodium (<30 mEq/mL) are indicative of fetal maturity.[767] Serial assessment of these concentrations is most useful because of daily fluctuations that may occur. Calcium concentration of 10 mmol/L (40 mg/dL, 400 ppm) in mammary secretions is a reliable indicator of fetal "readiness for birth."[760,767,768]

Several stallside tests can measure the Ca ion (Ca^{2+}) concentration in mammary secretions based on a colorimetric change of pads on a test strip. Water hardness kits are also useful for determining the concentration of Ca in mammary secretions.[770-774] These involve titration of a diluted sample until an indicator dye changes color, and although they are more labor intensive than the test strips, they are reported to provide a more reliable guide for predicting the onset of parturition within the next 24 hours. However, it is important to ensure that the water hardness kit is measuring only Ca concentrations if it is being used to determine the safest time to induce parturition.[760] Many kits merely test for divalent cations, which include magnesium as well as Ca. Because magnesium levels peak earlier than Ca concentrations, misleading information about fetal maturity may be obtained.[768,771] If the intent is merely to predict the onset of spontaneous parturition, the type of test is not as critical.

A study has questioned the interpretation of calcium carbonate ($CaCO_3$) test kit data that has formed the basis of recommendations that 200 ppm or 250 ppm $CaCO_3$ should be used as the benchmark for readiness for birth. Paccamonti contends that because Ca in milk is not in the form of $CaCO_3$, any test that measures $CaCO_3$ levels in solution must be adjusted to account for this fact.[760] Because the molecular weight of $CaCO_3$ is 100 and that of Ca is only 40, the conversion factor is 2.5 (i.e., divide the $CaCO_3$ ppm by 2.5 to obtain the Ca ppm). Furthermore, because mammary secretions usually must be diluted before a water hardness test can be used to measure Ca, Paccamonti recommends that 1 mL of secretion be diluted in 4 mL of distilled water. Thus the final reading should be corrected by a factor of 5.[760] The division by 2.5 to convert $CaCO_3$ to Ca and the multiplication by 5 to correct for the dilution mean need only be doubled to provide an accurate level of Ca ppm. Using this logic, Paccamonti contends that the reports using 200 to 250 ppm $CaCO_3$ as an indication of readiness for birth are actually using Ca values of only 80 to 100 ppm. However, if these values are corrected for the reported test dilution (1:6) factor, then the $CaCO_3$ level being reported would have been 1400 to 1750 ppm (560–700 ppm Ca; 14–17.5 mmol/L). These corrected values are thus in excess of the 400 ppm (10 mmol/L) Ca concentrations reported by other researchers. It is obviously important to keep these calculations in mind because water hardness kits may vary and technicians may use different dilutions. Inappropriate application of the math could lead to an erroneous conclusion about fetal maturity and subsequent induction of a premature foal.[760]

Generally mammary secretion Ca concentrations are more reliable for predicting when a mare is unlikely to

spontaneously foal rather than for determining when she is likely to foal. Ley used a water hardness kit and reported that $CaCO_3$ levels greater than 200 ppm (see preceding discussion) indicated a 54% probability of spontaneous foaling within 24 hours, 84% probability of spontaneous foaling within 2 days, and 97% probability of spontaneous foaling within 3 days. A small percentage of mares foaled within 24 hours despite a $CaCO_3$ level below 200 ppm.[769] However, using Paccamonti's logic, this equates to a corrected value of less than 560 ppm Ca^{2+}. Because a value of 400 ppm Ca^{2+} indicates readiness for birth, it is not surprising that some of these mares foaled.

Although the fetus initiates parturition, the mare appears to be able to regulate the actual timing of delivery[775]; thus any untoward changes in the mare's environment may cause her to postpone the delivery and create discrepancies with predictions based on mammary secretion electrolyte concentrations. Calcium and other electrolyte concentrations can change rapidly during a single day, so testing secretions in the morning and evening may be useful. If a single test is to be performed, it is preferable to check the Ca levels late in the day. Generally the more rapid the rise in milk Ca levels, the more imminent is parturition. Primiparous mares can be especially difficult to monitor because no change in mammary secretion electrolyte composition may be detected until immediately before foaling.[770]

Serial assessment of pH from the mammary gland secretions in periparturient mares may also be useful in prediction of foaling.[776-778] A positive predictive value for foaling within 72 hours and a negative predictive value for foaling within 24 hours were 97.9% and 99.4%, respectively, using a pH strip test.[778] The pH of mammary gland secretions is highly and significantly correlated with electrolyte concentrations (Ca, Na, K) in those secretions. Mares that present a rapid reduction (0.5 to 0.8 pH units) from alkaline (e.g., 7.8) to slightly acidic pH (6.8 to 6.4) over 24 hours will typically foal within the next 24 hours.[777] Mares that present with a slow reduction in pH tend to foal when the pH reaches 6.4 to 6.2 and will have an acidic pH for several days prior to foaling. A subset of mares will have only a mild reduction in pH and may foal with an alkaline pH (7.4 to 7.8). These mares will not have a sodium/potassium inversion and will foal with low calcium concentrations in their mammary secretions.[777]

There are several methods to measure the pH of mare mammary secretions. While pH test strips are cheap and readily available, they may not be sufficiently sensitive to detect clinically relevant changes in pH. A hand-held pH meter that allows more accurate determination may be preferred.

Placentitis and other placental pathology are often associated with precocious mammary gland development and premature changes in mammary secretion electrolyte concentrations. Milk electrolyte changes are unreliable for assessing fetal readiness for birth in abnormal pregnancies (e.g., placentitis, impending twin abortion) or when domperidone for milk production has been administered. In cases of abnormal pregnancies there may be elevated mammary secretion calcium concentrations (>10 mmol/L; >400 ppm; >40 mg/dL) before day 310 of gestation.[476]

INDUCTION OF PARTURITION

Induction of parturition may be indicated as a clinical management procedure for some high-risk pregnancies, including mares with hydrops, ruptured prepubic tendon, and ventral herniation. These mares often require assistance with delivery because the stage II abdominal contractions may be compromised.[714] Induction of parturition should not be practiced for convenience alone but should only occur for reasons associated with the clinical well-being of the mare or foal. Owners should be advised that complications such as dystocia, premature placental separation, fetal hypoxia, and dysmaturity are not uncommon sequelae of the induction procedure.[770,779-781] The aim of a controlled foaling is not only to deliver a viable foal but also to prevent any injury to the mare that may compromise future fertility.

Induced foalings are sometimes indicated to ensure that optimal veterinary assistance is available when complications are expected and to optimize resuscitation attempts when a compromised fetus has been monitored in utero. When fetal stress is detected in a high-risk pregnancy, the clinician is faced with the dilemma of inducing delivery and attempting supportive care in a neonatal intensive unit or leaving the compromised fetus in utero. Owners should be informed that delivery is indicated only if the probability of extrauterine survival exceeds that for continued maternal support.

Experience suggests that an abnormal uterine environment is often more conducive to maintaining a fetal foal's life than a neonatal intensive care unit. A fetus that has been exposed to an adverse uterine environment for some time may be more tolerant of premature delivery.[565] Many clinicians administer a dose of corticosteroids to the mare if premature delivery appears inevitable. This may stimulate surfactant production and promote accelerated maturation of the fetal lung.

The normal physiologic processes in the prepartum mare and fetus were discussed in a previous section. There is clear evidence that the fetal hypothalamic-pituitary-adrenal axis initiates the final stages of fetal maturation, which initiates the hormonal cascade that culminates in parturition.[564] Final maturation of the fetus results in increased ACTH release from the pituitary and subsequent stimulation of the fetal adrenal cortex. It is not until the maturing adrenal gland attains 17α-hydroxylase capacity that the high levels of pregnenolone are metabolized into fetal cortisol. These vital changes lead to a fetal cortisol rise in the last 2 or 3 days before birth; thus the equine fetus is at a substantially increased risk of dysmaturity or prematurity if the induction is not carefully planned.[770] This planning has traditionally involved confirmation of gestation length, monitoring mammary development and milk or colostrum production, and ultimately evaluating the amount of cervical softening.[782] The fetus will usually be in the dorsopubic position, with neck and limbs flexed before induction. The incidence of posterior and transverse presentations is rare, but detection of these abnormalities by rectal palpation before induction would be reason to reevaluate the induction plans. Delivery by cesarean section may be a more prudent course of action, especially with transverse presentations.

The presence of cervical softening has traditionally been suggested as a prerequisite for optimal induction of parturition in the mare, and in a recent study mares with a relaxed cervix before induction had a more rapid delivery.[722] The same study found that foals delivered from mares with a preinduction relaxed cervix stood and nursed sooner and had fewer signs of intrapartum asphyxia (hypercapnia and maladjustment) than foals delivered from mares with a nondilated cervix. Mares that developed parturient complications (premature placental separation and dystocia) all had a closed cervix before induction. The status of the cervix is controversial because earlier reports suggest that inductions may proceed successfully even though the cervix is tightly closed and covered with mucus.[781]

Administration of intracervical PGE_2 (2.5 mg) before induction of parturition may aid in cervical relaxation and inducing parturition, though no difference was apparent in the mean interval from initial oxytocin treatment to rupture of the chorioallantois or to the delivery of the foal in mares pretreated with PGE_2 compared to mares administered oxytocin alone.[360] However, the impact on foal viability was positive in that foals delivered from PGE_2-treated mares suckled sooner. The application of intracervical PGE_2 may have some merit in termination of a pathologic pregnancy (e.g., hydrops) in which the induction is known to be premature and is aimed at salvaging the breeding prospects of the mare. Although there is no correlation between myometrial strip (in vitro) response to oxytocin treatment and gestational age, premature induction with oxytocin can take much longer (1–2 hours) than an induction in mares at term. This is consistent with the belief that a critical sequence of hormonal changes are required before fetal expulsion can occur. Because the aborted fetus will generally be alive and gasping for air, it is advisable to have ready some euthanasia solution to euthanize the nonviable neonate. After delivery, not all fetuses will have easily accessible blood vessels; intracardiac injection of euthanasia solution may be considered if immediate humane euthanasia is required.

Several experimental protocols have been reported for induction of parturition in the term mare, including glucocorticoids,[779,781,783-785] prostaglandins,[779,781,786,787] and oxytocin.[360,507,722,780-782] High (100 mg/day) and repeated doses (administered IM daily for 4 days) are required for glucocorticoid induction because this regimen has limited efficacy in the mare (which is not the case with ruminants).[781] However, a more recent study suggests that multiple injections of dexamethasone given to healthy mares at days 315 through 317 of gestation can induce precocious fetal maturation and delivery of viable foals within 5 to 7 days. The mares remained healthy with no evidence of laminitis.[783] Further studies are needed to determine whether dexamethasone treatment can be used safely in mares with compromised pregnancies. Prostaglandin induction is not very efficient in the mare. The synthetic products (e.g., cloprostenol) are more effective than natural prostaglandin, but results can be quite variable. Oxytocin is the preferred drug for induction of parturition in the mare. A wide range of protocols have been suggested over the years, including a bolus dose (20–75 units), low doses (2.5–20 units) repeated every 15 minutes to effect, and as a slow IV drip of 60 to 120 units total (1–2 units/min).

The choice of oxytocin regimen is less important for foal viability than appropriate case selection and adherence to criteria for induction.[722,770] A low-dose protocol has been recommended because it appears to work only in those mares that have a mature fetus.[507] Mares were diagnosed as being ready for birth by mammary secretion Ca strip test measurements. A single injection of 2.5 IU oxytocin (administered IV) was given between 1700 and 1900 hours and resulted in the delivery of a normal foal within 120 minutes in 95% of mares. In response to the first oxytocin injection, 24 of 38 (63%) treated mares foaled. Another 9 of 38 (24%) foaled the next evening in response to the second injection, and 3 of 38 (8%) foaled in response to the third treatment. It was concluded that the major advantage of injecting a daily low dose of oxytocin appears to be that such a low dose induces delivery in only those mares that are carrying a mature fetus and are ready to foal. It has been proposed that this low-dose, early-evening oxytocin protocol could be used as a reliable method to induce parturition or to predict that the mare would not foal that night, if parturition did not occur within 2 hours of treatment. However, even this promising protocol has limitations because it is still possible to occasionally induce a mare to deliver a premature foal.

Villani and Romano studied the effects of a daily oxytocin treatment (3.5 IU) on 174 full-term Standardbred mares that had mammary secretion Ca levels of ≥200 ppm.[788] In this study 69% of the mares foaled within 2 hours of treatment (51.3% responded to the first oxytocin administration, 14.2% to the second, and 3.4% to the third). No significant difference between treated and control mares was observed in the gestational length (340 ± 8 days vs. 337 ± 7 days), duration of foaling (10 ± 5.6 minutes vs. 11 ± 4.9 minutes), incidence of dystocia (1.4% vs. 1.7%), or failure of rupture of the allantochorion (0% vs. 0.6%). No significant difference was observed in the incidence of placental retention between treated and control groups (8.1% vs. 6.3%). Physical and behavioral characteristics were normal in foals of both groups. The authors concluded that daily injections of low doses of oxytocin in at-term mares showed only moderate efficacy for inducing parturition. However, the easy applicability and safety—for both mare and foal—of this method of foaling induction make it a useful tool to simplify the management of mares in commercial stud farms.

Because most inductions will be performed because complications are expected, the clinician should be well prepared before administration of the induction agent (low-dose oxytocin). Even if the induction is not being performed by an IV drip, it is recommended that an IV catheter be inserted. This will facilitate rapid induction of general anesthesia if obstetric difficulties ensue. A fully stocked obstetric kit and ample volumes of lubricant should be placed outside the stall. Neonatal resuscitation efforts should be anticipated, and appropriate supplies (e.g., oxygen delivery system) should be readily available. Premature separation of the placenta is not an uncommon complication of induced births. The clinician should immediately rupture the exposed chorioallantois and then assist with fetal delivery in conjunction with the mare's expulsive efforts. Overzealous traction at this time may cause a laceration of the cervix if it is not yet fully dilated. If necessary, an oxygen tube can be placed in the foal's nostril during the minute or two that may be required to complete the assisted delivery. Additional discussion of neonatal resuscitation and critical care is included in Chapter 20.

MANAGEMENT OF THE PREGNANT MARE

A series of manuscripts addressing the effects of nutrition on various aspects of equine reproduction have recently been published.[789-792] Copper is an overemphasized factor in the etiopathogenesis of osteochondrosis lesions. Supplementation of pregnant mares with copper had no significant effect on the concentration of copper in the liver of foals at birth or on the frequency or severity of lesions in articular cartilage at 160 days of age.[793] Regular exercise and routine hoof maintenance are important for broodmares. A regular anthelmintic program is essential to ensure the mare's well-being. It will also reduce the exposure of the foal to parasite eggs in the mare's feces and the transmammary transfer of *Strongyloides westeri*

larvae. Pregnant mares should be current for all vaccinations that are recommended for their particular geographic locations. In North America it is especially important to advise owners about the importance of a regular vaccination program for EHV. Owners and farm managers should be aware of the need to isolate pregnant mares from transient horses to reduce the risk of infectious disease, especially respiratory viral infections. A tetanus booster may be indicated 1 month before foaling. The American Association of Equine Practitioners has published a general guideline for vaccination of horses, including pregnant mares. The reader is referred to that publication for current best practices related to vaccination of horses.[794]

Ideally the mare should be transferred to the final foaling environment at least 1 month before the due date. Foals are born essentially agammaglobulinemic, and the neonate depends on passive transfer of colostral immunoglobulins to provide initial protection from environmental pathogens. If a vulvoplasty (Caslick surgery) has been performed, plans should be made to open this approximately 1 week earlier than the expected foaling date. If the mare has a history of a previous hemolytic foal (neonatal isoerythrolysis), plans should be made to prevent the neonate from suckling the mare until all the colostrum has been removed or plans for compatibility testing of the milk and foal blood should be made. The mare may be screened for the presence of antibodies to common equine blood antigens 2 weeks prior to the anticipated foaling date to identify mares at risk for producing incompatible colostrum. At-risk mares should be milked immediately postpartum and the foal not allowed to suckle its dam for 24 hours. Some practitioners recommend administering sulpiride or domperidone for early milk production and milking the mare prepartum to avoid colostrum formation. An alternate source of colostrum from an appropriate donor should be available for the foal and plasma supplementation considered.

Those responsible for monitoring the foaling process should understand that mammary development, followed by distention and waxing of the teats and then relaxation of the perineal area, indicates approaching parturition in the mare. The use of mammary secretion electrolyte concentrations and pH to predict foaling was discussed in a previous section. Although it is accepted that the fetus signals its readiness for birth, the mare can regulate the final timing of delivery. Electronic monitoring systems that can be used to signal the start of parturition are also available.

Inexperienced personnel should be counseled about normal foaling events and instructed in how to recognize when professional assistance is required. Inappropriate intervention by ill-informed individuals can jeopardize the foal's life and potentially cause life-threatening complications in the mare. Separation of the fetal membranes will deprive the fetus of oxygen, and this is the critical factor that must be considered when assessing an obstetric case that involves a live foal. Although most references suggest that fetal survival rates are very low if the foal is not delivered within 30 to 40 minutes of chorioallantoic rupture, one author reports having delivered live foals by cesarean section up to 90 minutes later.[795] Another study reports high foal survival rates in mares referred with dystocia for as long as 1.5 hours post rupture of the chorioallantois. These authors suggest that, because transverse presentation appears to be more common in draft mares, parturition does not progress as expected, mares stop having uterine contractions, and, as a consequence, there is minimal placental detachment. These are cases that were promptly presented to the veterinary hospital and that had minimal, if any, vaginal intervention at the farm. It is likely that limited vaginal intervention is less disruptive to the placental attachment, and these foals are not deprived of their oxygen supply. Keeping the mare on her feet, and walking if necessary, may help to reduce straining while professional assistance is sought. If a mare is located a great distance from a referral facility equipped to provide appropriate care, the mare should be sedated with detomidine, and oral clenbuterol should be administered prior to transport.

Normal Parturition

Terminology

The terms *presentation, position,* and *posture* are used to describe the orientation of the fetus as it enters the vaginal canal. Often a fetus is described as having been malpresented or malpositioned when the only anomaly present is postural, the most common cause of dystocia in the mare.[796] To prevent confusion, the all-encompassing term *fetal maldisposition* may be used to describe the combination of presentational, positional, and postural abnormalities that can contribute to a dystocia. *Presentation* describes the aspect of the fetus that enters the vaginal canal first and the orientation of the fetal spinal axis to that of the mare (anterior or posterior longitudinal; ventral or dorsal transverse). More recently, use of the terms *cranial* and *caudal presentation* has become more common. *Position* describes the relationship of the fetal dorsum (longitudinal) or head (transverse) to the quadrants of the mare's pelvis. The normal position for delivery is dorsosacral. A fetus that is still on its side would be right or left dorsoilial, and a fetus that is upside down would be dorsopubic. The terms *right* and *left cephaloilial* refer to the position of the fetal head relative to the mare's pelvic walls, and they imply that a transverse presentation was present. Posture is purely fetal and describes the relationship of the extremities (head, neck, and limbs) to the foal's body (flexed or extended).[200,796]

Fetal Kinetics

Fetal mobility has been discussed with respect to umbilical cord torsion and abortion. Fetal rotation within the amniotic cavity and amniotic sac rotation within the allantoic cavity result in the characteristic twisting of the umbilical cord.[676,677] A highly efficient mechanism ultimately guides most equine fetuses into a cranial, longitudinal presentation. Ultrasonographic studies have noted the percentage of anterior, posterior, and transverse presentations at 5 to 6 months to be 52%, 29%, and 19%, respectively, but the fetal presentation becomes predominantly anterior between 7 and 11 months.[673,675-677,682] Vandeplassche reported the incidence of anterior, posterior, and transverse presentations in the normal parturient mare population to be 98.9%, 1.0%, and 0.1%, respectively.[797] Ginther observed that muscular contractions close the lumen of both uterine horns between 5 and 7 months; thus the allantoic fluid (with the fetus) is confined to the uterine body.[673,674,676] During this time the fetus positions itself so that its head end points toward the mare's cervix (cranial presentation). It has been proposed that neurologic signals within the fetal inner ear may respond to the slope of the ventral uterine wall and guide the fetus to lie with its head elevated toward the cervix.[673] In most cases the noncord horn remains closed, whereas the cord horn gradually permits the entry of the hindlimbs between 7

and 9 months. The limbs can enter the horn only when the fetus is in dorsal recumbency because the angle between the horn and body is so acute by this stage of gestation. Thereafter the hindlimbs remain enclosed within the cord horn, and the hooves extend to the horn tip by the tenth month. Thus the selective closing and opening of the uterine horns, with subsequent trapping of the hindlimbs, is believed to be a key feature of the mechanism that ultimately directs fetal orientation into cranial presentation.[673,675] Entrapment of the hindlimbs within the uterine horn generally means that the caudal portion of the anteriorly presented fetus will be lying in a dorsopubic, and occasionally dorsoilial, position.[675] Ginther's ultrasonographic investigations substantiated the classic radiographic study demonstrating that the full-term equine fetus is initially lying in a dorsopubic position with the head, neck, and forelimbs flexed.[675,798]

In early pregnancy the mesometrial attachments suspend the uterine horns so that they point cranially and dorsally, but by late gestation the horn containing the hindlimbs comes to rest on the dorsal surface of the uterine body with the tip of the horn directed back toward the cervix.[402,675] The hooves and horn tip may be pushed so far caudally that they actually come to lie over the fetal head, meaning that when a rectal evaluation of a mare that is in late gestation is performed, the fetal hooves that are palpable may be attached to the hindlimbs. In some mares the vigorous, piston-like thrusts of the hindlimbs in association with elevation of the fetal rump may push the hooves past the cervix into the rectogenital pouch. This observation may explain the acute colic episodes that have been previously attributed to uterine dorsoretroflexion. Although the caudal aspect of the fetus is intimately associated with the uterine wall, the cranial portion has room to rotate within the uterine body itself. Ultrasonographic studies on mares close to term (>330 days' gestation) have shown that the cranial half of the fetus was in dorsopubic position approximately 60% of the time and in dorsoilial position about 40% of the time. The forelimbs and head were usually flexed (about 80%), but the remainder of the head or limbs was extended.[673] Postural changes are common; thus rectal palpation before the onset of first-stage labor is not a good predictor of impending dystocia. However, detection of a posterior or transverse presentation at this late stage is cause for concern, and appropriate plans should be made to manage the impending delivery.

Stages of Parturition

Behavioral changes that characterize the first stage of parturition include the mare looking at her flank, frequently lying down and getting up, stretching as if to urinate, and passing small amounts of feces. Patchy sweating may develop, and some mares will leak colostrum.[200,796] The restless behavior is similar to that of mild colic and is associated with the development of coordinated uterine contractions that increase uterine pressure and push the chorioallantoic sac (in the region of the cervical star) into the gradually dilating cervix. The increasing uterine tone during stage I of parturition may stimulate the fetus to extend its head and forelimbs up into the dilating pelvic canal.[402] Once the head and forelimbs are fully extended, they are unlikely to return to a flexed posture unless the foal reacts to manual intervention on the part of a foaling attendant. However, it is possible for the neck or a forelimb to develop a malposture if it is not correctly aligned when the mare begins an expulsive effort. Passage of the urine-like allantoic fluid ("water breaking") concludes the first stage of labor. Rupture of the chorioallantois and passage of the allantoic fluid does not occur until the fetlocks, or sometimes the knees, are at the level of the external cervical opening. If the chorioallantois does not rupture, then further separation from the endometrium can result in a red bag delivery, with the velvety red membrane appearing at the vulvar lips. In a normal delivery the chorioallantois is thought to remain attached to the endometrium until after the foal is delivered.[796]

Failure of the chorioallantois to rupture is a common complication of induced parturition.[722] If this happens, then continued separation from the endometrium will compromise transplacental oxygen exchange, and fetal hypoxia is likely.[796] Premature separation of the placenta, or red bag, is an emergency situation, and foaling attendants should be instructed to break the membrane and provide gentle traction in unison with the mare's expulsive efforts. Although the foal should be delivered as quickly as possible, injudicious traction at this time may cause a laceration in an incompletely dilated cervix.[796] Applying traction only in conjunction with the mare's expulsive efforts will reduce the likelihood of creating cervical trauma.

As parturition progresses, passage of the fetus into the pelvic inlet initiates a reflex release of oxytocin from the posterior pituitary (Ferguson reflex), enhancing uterine contractility.[200] Stage II is characterized by strong abdominal contractions that provide the expulsive force necessary to expel the fetus. Most mares will assume lateral recumbency once active straining commences. Many get up once or twice during stage II labor in what is believed to be a further attempt to correctly position the fetus.[796] Appearance of the translucent fluid-filled amnion at the vulvar lips can be expected to occur within 5 minutes of rupture of the chorioallantois.[799] Any delay in the stage II expulsion process increases the likelihood of fetal asphyxia or neonatal problems associated with hypoxia caused by placental separation. At least one hoof should be visible within the amniotic sac, and the other should be located approximately 2 inches behind it. If everything is progressing normally, then the soles of the hooves should face down toward the mare's hocks, and the foal's head should be resting between the carpi.

By the time the nose has reached the vulva, the cranial half of the torso should have rotated from a dorsopubic to a dorsoilial position. The mare probably assists the fetus to reposition itself by the characteristic side-to-side rolling each time she becomes recumbent. It is likely that some dystocias involving fetal malposition and malposture are caused by the failure of a compromised fetus to actively participate in the foaling process. Less vigorous or absent fetal righting reflexes early in the parturient process have been suggested by many authors as a cause of fetal maldisposition.[200,726,798] The observation that ventral deviation of the head and neck is more likely to be present if the fetus is in a dorsoilial position than in a dorsosacral position further substantiates the hypothesis that the fetal righting reflexes are compromised early in these cases.[726]

The second stage of labor in the mare is rapid, with the most forceful contractions occurring as the chest passes through the pelvic cavity. Most foals are delivered within 20 to 30 minutes after the chorioallantoic membrane ruptures. Primiparous dams generally require more time to expel the fetus than do multiparous dams.[200,799] The amniotic sac usually ruptures during these expulsive efforts. However, the equine amniotic sac is not attached to the chorioallantois, as is the case with a ruminant placenta, and the foal sometimes may be delivered with a portion of the sac wrapped around its head. Foaling

attendants should be instructed to promptly free the foal's head from the amniotic sac to prevent suffocation. Active straining ceases once the foal's hips are delivered, and the mare will rest in lateral recumbency. An active foal will extract the hindlimbs from the vagina as it struggles to stand. Stage III of parturition involves expulsion of the fetal membranes, which typically takes between 30 minutes and 3 hours.[200] Owners should be advised to seek veterinary assistance if passage of the membranes is delayed beyond 4 to 6 hours because life-threatening toxic metritis and laminitis are common sequelae of fetal membrane retention.

Etiology of Dystocia

The incidence of dystocia in the general horse population varies among breeds (Thoroughbreds 4%, Shetland ponies 8%, draft breeds 10%).[800] Dystocia is one of the few true emergencies in equine practice. Literally a matter of minutes can determine a successful outcome (i.e., the birth of a live foal). Perinatal asphyxia associated with dystocia is a major cause of equine reproductive loss.[591,592] The long fetal extremities (limbs and neck) predispose the mare to foaling problems. Alert, informed foaling attendants are essential to ensure that abnormalities are recognized early. Attendants should suspect that the mare is experiencing obstetric problems if either the first or the second stage of parturition is prolonged or not progressive.

Signs that a mare may be in dystocia include the failure of any fetal parts or of the amniotic membrane to appear at the vulvar lips for a prolonged period after rupture of the chorioallantois, appearance of only one hoof at the vulva, hooves upside down at the vulva, hooves and nose in an abnormal relationship, and nose but not hooves at the vulva. The most common impediments to delivery are malpostures of the fetal extremities (head and neck and limbs).[726] An experienced foaling attendant may be able to correct minor problems and facilitate a successful delivery, but inappropriate intervention can have potentially fatal consequences for the mare. Furthermore, obstetric manipulations can easily damage the uterus and cervix to the extent that the mare's reproductive future is jeopardized.

Management of Dystocia

When attending to a mare in dystocia, the veterinarian should make a rapid assessment of the mare's general physical condition, noting in particular mucous membrane color and refill time (hemorrhage, dehydration, and shock). A mare that is aborting in late gestation may experience a dystocia because the dead fetus cannot participate in the delivery process. A malodorous discharge and fetal hair in the hands of the clinician strongly suggest the presence of an emphysematous fetus. The perineal area should be inspected to determine the presence and nature of any vulvar discharge and the presence of fetal membranes and to identify any fetal extremities. Excessive hemorrhage or vulvar swelling may indicate that intervention by inexperienced individuals has caused trauma to the reproductive tract. Occasionally, a foaling mare will present with rectal prolapse, an everted bladder, or intestinal loops protruding from the vulvar lips. The intestines may be of fetal origin if there is incomplete closure of the ventral abdomen, but a ruptured vagina is more likely. In the later scenario the foal's foot may have ruptured the floor of the cranial vagina, but injudicious manipulations by an inexperienced attendant should not be discounted. If a rectal prolapse or the urinary bladder is evident, then an epidural anesthetic should be administered to prevent further straining. Alternately, the mare may be anesthetized to facilitate hoisting the hindquarters. The advantage of this approach is that the straining can be stopped immediately. This is especially important if the prolapse involves an intussuscepted colon (type IV). In these cases a palpable defect may extend several feet into the rectum, and avulsion of the mesentery can be a fatal complication that is not readily amenable to surgical correction.

Due to the unpredictable and potentially violent nature of mares in stage II of labor, the use of stocks for restraint is contraindicated. The initial examination can be performed on a standing mare with no more restraint than a twitch or lip chain if her demeanor permits this to be performed safely. It is important to ensure that the examination area is clean, with good footing. There should be ample space for the mare handler, obstetrician, and assistants to move to safety if necessary. Although most veterinary tranquilizers readily cross the placenta and can compromise the fetus, adequate restraint is essential for the safety of all involved.[801] Sedation with tranquilizers may be necessary for some uncooperative mares, and in extremely intractable cases it may be preferable to anesthetize the mare with a short-acting combination. In these cases a hoist should be available to elevate the hindquarters because manipulating the foal is sometimes difficult when the mare is in lateral recumbency. Although not essential, an initial rectal examination may help the practitioner rule out the presence of a term uterine torsion and determine the condition of the uterine wall (tears and spasm). It also may provide useful information regarding the disposition of the fetus. Before any vaginal examination the mare's tail should be wrapped and the perineal area thoroughly cleansed. The clinician's arms and hands should be scrubbed, and the clinician should wear sterile obstetric sleeves. Cleanliness and lubrication are the cornerstones of obstetrics.

The mare's vagina and cervix are easily traumatized by the friction associated with vaginal manipulations. Once the mucous membranes have been abraded, it is likely that adhesions and fibrosis will follow. Thus copious amounts of lubricant are vital to ensure that the soft tissues of the genital tract are not traumatized to preserve the mare's future fertility.[802] Lubricants include methyl cellulose, polyethylene polymer, white petrolatum combined with 10% boric acid, and mineral oil. Water-soluble lubricants are generally not as desirable, because they rapidly lose their lubricating abilities in the presence of fluids. If a large volume of lubricant is to be pumped around the fetus, then investigating the possibility of a uterine laceration is essential. In a referral situation, abdominocentesis should be performed. Serosanguineous to sanguineous fluid that contains elevated total protein levels and an increased white blood cell count is highly suggestive of a uterine rupture.[197] If a uterine tear is unlikely, obstetric lubricant may be gently instilled into the uterine lumen using a clean stomach tube and pump.[802] This is repeated as often as necessary during the procedure to keep the fetus and reproductive tract coated with lubricant.

The vagina, cervix, and accessible parts of the uterus should be carefully explored to ascertain the source of any hemorrhage. Lacerations should be noted and their presence discussed with owners or the attending personnel before any veterinary manipulations are attempted. Occasionally, the cause of the dystocia is a pelvic deformity (e.g., callus, neoplasia). It is important to determine the degree of cervical

dilation. If the mare has been in labor for some time, then it is possible that the uterus is relatively dry and tightly contracted around the fetus. This will make intrauterine manipulations much more difficult, especially because it becomes difficult to repel the fetus back into the uterus without risking rupture. When the uterus is contracted, warm lubricant tends to induce some uterine relaxation, and the volume expansion creates additional space in which to perform manipulations.[204,802] Myometrial contractions (uterine spasm) can be controlled by tocolytic drugs (isoxsuprine and clenbuterol) if they are available for veterinary use.[800] Some mares never stop having strong uterine contractions; general anesthesia is highly beneficial for these mares and allows the clinician to perform appropriate obstetric interventions.

Although fetopelvic disproportion is less common in the mare than the cow, it can be a factor in some equine dystocias.[445,796,803] The disposition of the fetus should be noted and fetal viability determined. Care should be exercised because active fetal response to manipulations can easily complicate an initially simple dystocia. Placement of a rope snare behind the ears and into the foal's mouth ensures that the clinician always has control of the head. This will facilitate correction of a potentially life-threatening development such as lateral deviation of the head and neck if the fetus pulls away from the clinician's manipulations. If the snare is placed around the mandible, then it is essential that only gentle traction be applied to guide the fetal head through the vaginal canal. Excessive force may cause a fracture of the mandible. When obvious fetal movement is absent, digital withdrawal may be initiated in response to pinching of the coronary band. Slight digital pressure over the eyelid onto the eyeball may arouse a response, as may stimulation of the tongue (swallowing). If the thorax can be reached, then the fetal heartbeat is definitive. In posteriorly presented cases the digital and anal reflexes are useful as indicators of fetal viability. Occasionally, it may be possible to reach the umbilical cord.

The clinician should inform the owner of the various options, costs, and prognosis once the current status of the foal is known and the cause of the dystocia has been determined. Ensuring that the owner is aware of the potential complications that may arise is especially important because postpartum medical care can become quite expensive. If delivery of a live foal is anticipated, then the clinician should consider the potential for fetal cardiovascular compromise before administering any tranquilizers to the mare. Light sedation of the mare with acetylpromazine (2–3 mg/100 kg IV) has minimal effect on the foal and may be useful in some cases.[801] Xylazine is preferable to detomidine if the fetus is viable because its depressant effects are of much shorter duration. However, neither xylazine nor detomidine should be used alone to sedate a dystocia case because some apparently sedated mares can become hypersensitive over the hindquarters.[706,801] The combination of xylazine and acetylpromazine provides good sedation in a quiet mare.[198] The author routinely uses a combination of xylazine (0.3–0.5 mg/kg IV) and butorphanol (0.01–0.02 mg/kg IV) for standing obstetric procedures if more sedation is required (e.g., fetotomy procedure). This provides good sedation and analgesia, and additional doses may be administered as necessary. Attendants are instructed to keep the lip chain loose and to tighten it only when instructed to do so. This will ensure that it retains its effectiveness when required to divert the mare's attention. LeBlanc suggested a xylazine (1.1 mg/kg) and morphine (0.1–0.2 mg/kg) combination for sedation of fetotomy cases but cautions that gastrointestinal stasis is a frequent complication.[801]

Although most dystocias can be resolved at the farm fairly quickly by brief manipulation and assisted vaginal delivery, the practitioner should consider the alternatives if resolution is likely to take more than 10 to 15 minutes. Prolonged, unproductive vaginal manipulations are contraindicated in equine obstetrics. Decisions on the next course of action should be based on the viability of the foal, the clinician's obstetric skills, the availability of equipment and facilities, and the financial constraints imposed by the owner.

An epidural does not prevent the mare's myometrial contractions or the abdominal press, and the time involved in administering an effective epidural anesthetic may make this form of restraint impractical when a live foal is present.[796,801] However, if the foal is dead, epidural anesthesia does reduce vaginal sensitivity and thus the mare's perception of vaginal manipulations (Ferguson reflex). Caudal epidural anesthesia should be used at the clinician's discretion, especially if general anesthesia or referral may become necessary. When an epidural is indicated, the author uses a combination of xylazine (0.17 mg/kg) and lidocaine (2–3 mL) diluted in saline, such that the final volume does not exceed 8 to 10 mL to reduce the likelihood of hindlimb weakness.[801,804] Excess volume can cause the mare to become ataxic. Short-term general anesthesia may be indicated when minor postural abnormalities are present and maternal expulsive efforts make correction difficult. Xylazine (1.1 mg/kg IV) followed by ketamine (2.2 mg/kg IV) provides a general anesthetic with a smooth, short (10–15 minutes) duration. Addition of the central-acting muscle relaxant guaifenesin (1 L of a solution of 5% guaifenesin in 5% dextrose) can provide an additional 10 to 20 minutes for fetal manipulation.[801]

In referral equine hospitals that are located close to well-managed broodmare farms, the fetus is often still alive when the mare arrives. A well-coordinated dystocia team that uses a defined protocol can minimize time spent nonproductively. Intrapartum intratracheal intubation and positive pressure ventilation of the fetus (ex utero intrapartum treatment [EXIT]) during resolution of the dystocia has been shown to improve survival rates. It should be remembered that positive pressure ventilation must be continued once begun because it promotes the conversion from fetal circulatory patterns to neonatal patterns, essentially eliminating the role of the umbilicus.[805] In referral hospitals it is common practice to anesthetize the mare after a brief vaginal intervention and then maintain the mare on halothane-oxygen with controlled ventilation. Because halothane anesthesia has been shown to compromise umbilical circulation, the concentration should be kept to a minimum if the foal is still alive.[806]

Total intravenous anesthesia (the so-called triple drip of ketamine, xylazine, and guaifenesin) may be preferable until the foal has been delivered.[801] The mare should be ventilated with oxygen, and IV fluids should be administered as required. If the hind-end elevation technique is used, almost three quarters of such cases can be resolved by controlled vaginal delivery. However, if the fetus is still alive and has not been delivered within 15 minutes, an immediate cesarean section is performed, with a 30% foal survival rate possible, provided the time from rupture of the chorioallantois to presentation at the veterinary hospital is minimal.[795,807] Fertility after cesarean section is quite good, and adverse reports probably pertain more to the extent of vaginal manipulation after surgery.[808,809]

If a hoist is available in the field, then hobbles can be placed on the hind pasterns and the hindquarters briefly elevated 1 to 2 feet. The combination of a relaxed uterus and the effects of gravity can facilitate fetal repulsion and manipulation. If attempts at mutation are successful, then the mare should be lowered into lateral recumbency to permit extraction of the foal. Prolonged dorsal recumbency results in compression of the aorta and vena cava and reduction in venous return, cardiac output, and blood pressure.[801] Hindlimb paresis may develop after prolonged hindquarter suspension and can complicate the recovery process. The use of pads to support the hindquarters will help take some weight off the limbs while the mare is suspended.

Mutation is an obstetric term that is used to describe manipulation of the fetal extremities, with correction of any positional abnormalities, so that assisted vaginal delivery can proceed.[200] Although extra space is available for manipulations when the fetus has been repelled into the uterus, the clinician should remain cognizant at all times that overzealous obstetric manipulations are a major cause of uterine rupture.[197,198,200,204,735,810-812] Repulsion of the fetus from the maternal pelvis is contraindicated if the uterus is contracted down around the fetus. In countries where they are legal, tocolytic agents are effective in relaxing a contracted uterus. If the fetus is dead, many cases may be amenable to correction by fetotomy provided that the clinician has the appropriate skills and equipment.[813-817] Although poor technique and inappropriate fetotomy cuts often lead to infertility, experienced clinicians can rapidly resolve a dystocia while preserving the mare's future breeding potential. The alternative is cesarean section.

Traction must be applied with careful regard for both maternal and fetal well-being. Often traction applied entirely by hand is all that is necessary. Obstetric straps or chains may provide a better grip, with one loop above the fetlock and a second loop encircling the pastern. In assisted vaginal deliveries, traction should be applied as an adjunct when the mare is exerting expulsive force and should be released when the dam stops straining, permitting rest and recovery. This approach is critical to permit adequate dilation of the caudal reproductive tract. Copious lubrication and slow traction with continuous monitoring of cervical dilation are especially important when a controlled vaginal delivery is performed on an anesthetized mare. Excessive use of force may be associated with fetal fractures (ribs, vertebrae, and limbs), maternal soft tissue trauma, and uterine prolapse.[818] No more than two or three people (depending on size and strength) should apply traction to the fetus.

Cranial (Anterior) Presentation

An anteriorly presented fetus that is in the dorsosacral position with head and forelimbs extended should require minimal traction to complete the delivery, assuming that the vaginal canal is well lubricated. By ensuring that slightly more traction is applied to one limb than the other, the practitioner reduces the width of the fetus across the shoulders and can successfully deliver most cases. If progress is not being made, then all traction should stop and the vaginal canal must be fully explored. There are three likely possibilities: elbow lock (incomplete extension of the forelimb), dog-sitting or hurdling posture, and occasionally a fetus that is too large.

Absolute or relative fetopelvic disproportion is uncommon in the mare even when the foal has been carried several weeks past the expected due date. In fact, some cases of prolonged gestation may involve a smaller than normal, dysmature fetus. In a referral hospital study, fewer than 2% of dystocias were attributed to this condition.[726] It is significant, however, that approximately 30% of referral hospital dystocia cases are in primiparous mares.[726,799] Primiparous mares were disproportionately represented in a report on dystocia and neonatal asphyxia from the central Kentucky area.[178] Although fetopelvic disproportion is not common in the mare, obstetric assistance (traction) is required much more often in primiparous mares.[200,730] Dystocia in these mares is further complicated by a tight vaginovestibular sphincter, which may predispose primiparous mares to lacerations and rectovaginal tears. If copious lubrication and gentle traction do not help, then cesarean section or partial fetotomy are the only alternatives.

Incomplete elbow extension may be unilateral or bilateral and should be suspected if the fetal muzzle lies at the same level as the hooves. In this posture the fetal elbows will be tucked back under the shoulder joint, causing increased depth and width of the fetus within the maternal pelvic inlet. Correction involves repelling the fetal trunk so that the forelimbs can be extended, raising the elbows up over the floor of the pelvic inlet.

In a dog-sitting or hurdling posture, there is either bilateral (dog-sitting posture) or unilateral (hurdling posture) hip flexion. This causes the fetal hooves (or hoof) to push against the pelvic brim during attempts at fetal extraction. The unilateral posture is more common.[726] Severe trauma can be inflicted on the mare if this malposture is not recognized and inappropriate amounts of traction are applied. Thus it is important to stop all traction and repel the fetus enough to sweep the floor of the pelvic inlet. In extreme cases the hindlimb may actually extend under the fetus and up into the vagina.[726] Although the hindlimb may be successfully repelled if the fetus is alive, it is a difficult procedure and is associated with some risk of uterine laceration. Judicious use of a snare or fetotome may facilitate safe repulsion of the hindlimb by looping the pastern and using the instrument to repel the hoof away from the pelvic brim. Repulsion should not be attempted on a standing mare if the fetus is dead, because the hindlimb may not return to its normal position. In such cases the hoof of the flexed hindlimb can puncture the ventral uterine wall as the foal is being extracted. If a dog-sitting or hurdling foal is dead, it is then recommended that general anesthesia and hoisting of the hindquarters be used to reduce the risk of ventral uterine rupture.[819] In experienced hands, partial fetotomy is a viable alternative to cesarean section.[813,814,817] A surgical alternative to a cesarean section involves manipulation of the hindlimb through a ventral midline celiotomy incision. An assistant may be able to extract the fetus through the vagina once the hindlimb has been grasped through the ventral incision. If successful, this technique will reduce the potential for contamination that may be associated with cesarean section. If a cesarean section is performed, some surgeons prefer to remove that portion of the foal that is protruding through the vulvar lips before withdrawing the hind end out through the surgical site.

In a foot-nape posture one or both of the forelimbs is displaced over the foal's head and pushed against the roof of the vagina.[802] To correct this malposture the fetus must be repelled into the uterus by applying pressure to the head. Once the forelimbs have been replaced under the head, fetal extraction can proceed quite uneventfully. If not corrected immediately, then the mare's straining can cause the fetal hoof to

lacerate the vaginal roof, and in extreme cases it can result in a rectovaginal fistula. A fistula is all that occurs if the foal withdraws its hoof from the rectum before delivery. A third-degree perineal laceration occurs if the strong expulsive efforts of the mare cause the limb that has penetrated the rectum to dissect through the caudal rectovaginal shelf and rupture through the anal sphincter, creating a cloaca.[820,821]

Carpal flexion is a common cause of dystocia. It may be unilateral or bilateral, and typically the affected carpus is located at the pelvic inlet.[802] To safely correct this malposture, the fetal body must first be repelled into the uterus. If the dystocia is prolonged, then the amount of uterine contraction may preclude meaningful repulsion of the fetus back into the uterus. Because the fetus is likely to be dead in these cases, a relatively simple fetotomy cut can be made at the level of the distal row of carpal bones. This permits safe delivery of the foal without the need to traumatize the reproductive tract and generally facilitates extraction within minutes.[813,815,817] If the foal is alive, then repulsion into the uterus will permit the flexed limb to be grasped at the level of the fetlock and pastern. By rotating the wrist, the obstetrician can rotate the carpus laterally while bringing the flexed fetlock medially and caudally into the birth canal. This maneuver allows maximal use of available space by obliquing the extremity through the pelvic inlet. The obstetrician should be aware that flexural deformities are considered to be the most common congenital anomaly of foals and that the rigid deformity often means that a cesarean section or fetotomy must be performed.[178,591,797,813,814] Limb contractures are generally bilateral. Contracture is more common in the forelimbs than the hindlimbs but can involve all four limbs.[797,802] Severely affected limbs cannot be straightened, and needless trauma can be inflicted on the genital tract by unrewarding attempts to manually correct the malposture. It is important that the clinician cup the hand over the bottom of the fetal hoof at all times while attempting to straighten the limb. Failure to do so may result in injury to the reproductive tract. Application of an obstetric chain or rope to the distal limb can be a useful aid. This permits traction to be applied to the distal limb while the hand covers and guides the hoof.

The single most common abnormality in referral hospital dystocia populations is a reflected head and neck.[726,797,816] Unfortunately, these malpostures are often iatrogenic in that a viable fetus has pulled back from the initial vaginal intervention that aimed to correct a minor postural problem. If the mare strains while the foal's head is pulled back, it is then possible for the muzzle to engage the wall or floor of the pelvic inlet. The mare's forceful expulsive efforts may then drive the head and neck ventrally or laterally along the thorax while the forelimbs are pushed farther into the vaginal canal. Ventral or lateral displacements of the head and neck can be very difficult to correct. The length of the foal's neck often makes it impossible to reach the head. Inexperienced clinicians should consider referral as soon as the condition is diagnosed, because prolonged unrewarding manipulations can easily jeopardize the mare's future fertility. A relatively simple fetotomy cut can resolve these cases quite atraumatically if the fetus is dead when the veterinarian arrives. The author believes that this approach is preferable to prolonged and often unsuccessful attempts at manual correction of this extremely difficult malposture.

If the fetus is alive, then an attempt can be made to place eye hooks or to loop a snare around the mandible. Some obstetricians even suggest applying a clamp on an ear to pull the head back enough to place a snare. The author prefers to use a snare whenever possible. If traction can be applied to the head, then the foal's body must be carefully repelled while attempts are made to bring the head and neck around into a normal posture for delivery. Factors influencing the successful outcome include uterine tonicity, clinician arm length and skill, and the presence or absence of torticollis and facial scoliosis. As with contracted tendons, it is essential that the practitioner consider the possibility of a wry neck. This condition is not amenable to correction by mutation, and needless trauma can be inflicted on the genital tract by unrewarding attempts at correction. Ventral deviation of the head is relatively easy to correct if the fetal nose is just below the brim of the pelvis (poll posture). It is generally easier to rotate the head laterally before attempting to bring the muzzle up over the pelvic brim. In more severe cases the neck is tucked down between the forelimbs and the head is often beyond reach (nape posture). If attempts to reposition the head and neck are unsuccessful, then cesarean section or fetotomy are indicated.

Shoulder flexion posture may be unilateral ("swimming" posture) or bilateral ("diving" posture). To gain access to the retained limb, it is usually necessary to repel the head and neck into the uterus. An immediate cesarean section may be preferable if the foal is alive, because correction of this malposture can be difficult and time consuming. If this is not an option, it is then recommended that a soft rope snare be placed on the fetal head (behind ears and into mouth) so that the head can be readily retrieved once the shoulder flexion has been corrected. If the limb can be reached, then correction of this malposture is performed in two stages. Initially, the shoulder flexion is converted to a carpal flexion when the limb is grasped in the area of the humerus working down to the distal radius. The limb is then pulled caudally and medially as the fetal body is repelled. The carpus is then hooked over the brim of the pelvis to create a carpal flexion, which is then corrected as previously described. It must be remembered that it is not always possible to repel the head sufficiently to gain access to the retained forelimb. In these cases cesarean section is the only option for delivery of a live foal. If the fetus is dead, then a fetotomy cut to remove the head and neck may provide sufficient room to correct the malposture.

Caudal (Posterior) Presentation

A foal in posterior presentation will have the soles of the hooves facing up. Although the author has seen dystocias in which an anteriorly presented foal was in a dorsopubic position with both forelimbs extended, this is a very unusual complication. Foaling attendants should be instructed to wash the mare's perineum and then to use a clean arm to check for the hocks somewhere in the vaginal canal. Gentle traction on the hindlimbs in conjunction with the mare's expulsive efforts may facilitate delivery of a live foal. However, approximately half of the fetuses may be malpositioned as well, and they often require veterinary assistance to permit an atraumatic delivery. Foals in a caudal (posterior) presentation are more likely to be in a dorsoilial position than foals in a cranial (anterior) presentation.[726] Although a normally positioned fetus in a caudal presentation may not be particularly difficult to deliver, it is more likely to suffer hypoxia because of compression of the umbilical cord under the fetal thorax or because of premature rupture of the umbilical cord.[802] Although only about 1% of foals are presented posteriorly, this malpresentation accounts for between 14% and 16% of referral hospital dystocia cases because any postural abnormalities create a major

complication.[726,797,800] Typically both hindlimbs are involved, and these types of dystocia cases (hock flexion and hip flexion) are extremely difficult to correct under field conditions. Hock flexion malposture accounts for about one quarter of referred posterior cases.[726,797,800] Correction of a hock flexion is dangerous because of the risk of perforation of the dorsal aspect of the uterus. The fetus must be repelled into the uterus while one hock is pushed dorsolaterally and the distal limb is directed medially. The procedure for obliquing the extremity into the birth canal is similar to that previously described for correction of a carpal flexion. An obstetric chain or strap can be used to apply traction to the limb while the hoof is cupped in the hand. Straightening a flexed hock entails considerable risk because the hock is invariably forced against the dorsal uterine wall. When the uterus is contracted, there is a real possibility of causing a laceration or perforation. Cesarean section may be preferable for delivery of a live fetus. The author strongly believes that fetotomy is a safer procedure than attempts at mutation if the fetus is dead. Approximately half of referred posterior presentation cases are breech (bilateral hip flexion posture).[726,797] A cesarean section is indicated if the fetus is alive because the manipulations involved in correcting this malposture are time consuming and extremely difficult. The comments for managing a hock flexion apply because if mutation is attempted, the hip flexion must first be converted into a flexed hock posture. A key point is to remember to flex both hocks before attempting to straighten a limb. If one limb is extended into the vaginal canal while the other hip remains flexed, then the fetal body will move back into the pelvic canal, and this will make it extremely difficult to access the retained limb. If the fetus is dead, the author recommends attempting to convert the bilateral hip flexion into a hock flexion posture, followed by correction with two fetotomy cuts through the distal row of tarsal bones. This may be safer and less traumatic than attempting to straighten the limbs, provided that the clinician is experienced in the use of a fetotome. Attempts to correct a bilateral hip flexion by fetotomy, without first creating a bilateral hock flexion, are often unrewarding because of the difficulty in correctly placing the fetotomy wire. Referral for cesarean section will often provide the best prognosis for future fertility in these cases.

Transverse Presentation

Only about 1 in 1000 foals is presented transversely. Successful resolution of these dystocia cases requires a significant amount of obstetric experience, which explains why these rare presentations account for between 10% and 20% of referral hospital dystocia cases.[726,797,809] Most transverse presentations are ventral transverse, with the abdomen and limbs of the fetus presented toward the birth canal. Although the widespread adoption of ultrasonography has markedly reduced the likelihood of a twin birth, this possibility must always be explored when more than two limbs are present in the birth canal.[178,591] In some instances it may be possible to repel the head and forequarters of the fetus while extending the two hindlimbs into the pelvic canal. If the manipulations are successful, the transverse presentation is thus converted into a posterior presentation for vaginal delivery. The likelihood of successfully resolving one of these cases is improved if the mare has been anesthetized and the hindquarters elevated.[802] Transverse presentations may be associated with flexural limb deformities, angular limb deformity, and spinal deformity. If the fetus is alive, then the delivery method of choice is cesarean section. Dorsal transverse presentations, with the spinal column of the fetus presented toward the birth canal, are very rare. These cases warrant an immediate referral for cesarean section even if the foal is dead. Although an experienced obstetrician may be able to deliver a transversely presented fetus by fetotomy, the owner should be advised that this will be a difficult and time-consuming procedure, with a high risk of trauma that will likely impede the mare's future fertility.[802,813,814]

Fetal Anomalies

Hydrocephalus is not uncommon in equine fetuses, especially in pony breeds.[726,797,822] The condition occurs when increased intracranial pressure causes the bones of the skull to enlarge, sometimes almost doubling the size of the head. The skull is often very thin, and many affected foals can be delivered after incising the soft portion of the skull with a finger knife, allowing the skull to collapse. The trunk of the hydrocephalic fetus is generally smaller than normal and seldom interferes with delivery. If the enlarged cranium is bony, then a fetotomy cut may be necessary to reduce the size of the head.

CARE OF THE POSTPARTUM MARE

The fetal membranes should be examined after every delivery to ensure that they have been passed intact and to check for any placental anomalies that may indicate impending problems in the neonate. The chorioallantois often has tears that can be misleading, especially if the mare has trod on the membranes repeatedly. Examining the allantoic side of the membrane may be helpful in that the blood vessels can be pieced together and will give some idea as to whether a portion is actually missing.[431] Ideally, all foaling mares should receive a brief physical examination within 24 hours of parturition. If the mare's attitude is normal and she displays typical maternal behavior toward the foal, then the udder should be checked and the perineal area inspected for evidence of trauma.

A detailed reproductive examination is usually unwarranted because it may unnecessarily disrupt the normal mare-foal bond that is developing at this time.[823] Routine postpartum use of oxytocin or prostaglandin (cloprostenol) therapy is not warranted because there is no apparent benefit for uterine involution in normally foaling mares.[824] If the mare has a foal that is unable to suckle from the mare, or the mare and foal must be housed in stalls with limited exercise, treatment with oxytocin (10–20 IU IM) every 6 hours should be considered. All mares should receive a thorough reproductive examination at the foal heat.

Occasionally an enlarged ovary is detected. This may be a GTCT that has enlarged during the course of the previous pregnancy or an ovarian hematoma due to intrapartum bleeding. Prompt diagnosis and surgical intervention may permit the mare to resume normal cyclicity and conceive during the current breeding season.

Abdominal discomfort in the peripartum mare may be due to uterine contractions, especially if the mare has been treated with oxytocin to promote passage of the fetal membranes. However, other causes of abdominal pain should not be discounted.[736,825-827] When a postpartum mare displays abdominal discomfort, transabdominal ultrasound and/or abdominocentesis may be indicated, especially if the mare does not respond to an analgesic dose of flunixin meglumine. The normal foaling process does not alter the composition of the peritoneal fluid from within the normal range. Even

a dystocia does not necessarily cause significant changes in the peritoneal fluid. If an experienced obstetrician performs the vaginal manipulations or fetotomy, then the fluid should remain normal.[197] If the peritoneal fluid is normal, then the mare should be monitored closely for signs of clinical deterioration. Repeated abdominocentesis or ultrasound may be indicated when clinical signs suggest that a parturient-related abdominal lesion may be present because the peritoneal fluid constituents can change within hours.[197] A single elevated peritoneal fluid value (total protein, white cell count, or percent neutrophils) may be an incidental finding. Elevation of two or more values often signals the onset of clinical abnormalities. If a postpartum peritoneal fluid sample has a total protein value above 3.0 g/dL in conjunction with a white blood cell count greater than 15,000 cells/μL and a white blood cell differential count greater than 80% neutrophils (especially if degenerative changes are present), then the presence of a potentially life-threatening lesion is likely.[197] The peritoneal fluid analysis should not be viewed in isolation and must be considered in conjunction with the history and clinical signs being exhibited by the mare. Detection of changes in the peritoneal fluid almost invariably indicates the presence of foaling-related trauma in either the reproductive or the gastrointestinal tract. An early diagnosis followed by appropriate medical or surgical intervention (or both) will often result in a favorable outcome. If treatment is not implemented until the affected mare has become depressed and febrile, with accompanying signs of shock and toxemia, then the prognosis may be more guarded.

Periparturient Hemorrhage

The arterial supply to the uterus is supported by the mesometrium (broad ligament). The major blood supply to the uterus is from the uterine artery, a branch of the external iliac artery. It forms a cranial branch that supplies the proximal uterine horn and a caudal branch that supplies the distal uterine horn and uterine body. The smaller ovarian artery gives off a uterine branch that anastomoses in the proximal horn with the cranial vessels from the uterine artery. The urogenital artery is a branch off the internal pudendal artery, and it gives rise to the caudal uterine artery along with vessels to the rectum, ureter, bladder, urethra, and vagina. The caudal uterine artery supplies the lateral side of the cranial vagina and continues past the cervix to ramify on the uterine body, in which it anastomoses with the caudal branch of the uterine artery.[4,828,829] Hemorrhage from these vessels, especially the large-diameter uterine artery, is a significant cause of periparturient colic signs and death in older multiparous mares.[811,826,829-831] However, a retrospective study of 73 cases suggests that periparturient hemorrhage can occur in mares of any age or parity, and the condition may occasionally occur before foaling.[832] In a study of 98 postpartum deaths, almost 40% were caused by uterine artery rupture.[826] The rupture may be anywhere along the vessel and is typically 2 cm or 3 cm in length and oriented parallel to the long axis of the vessel. Generally, there is no evidence of a predisposing aneurysm.[826] An association with low serum copper levels has been proposed as a reason for vessel fragility in aged mares.[833]

There appears to be a predilection for right-sided uterine vessel rupture. It has been suggested that the extent of cecal displacement of the gravid uterus to the left may be sufficient to place increased tension on the vessels in the right broad ligament.[826,830] Although the added stress of dystocia may increase the chances of arterial rupture, many cases occur in mares that appeared to have an uneventful delivery. Hemorrhage from the hypertrophied vessels that supply the gravid uterus may be rapidly fatal, especially if the artery ruptures directly into the peritoneal cavity. The mare may be found dead or moribund with pale mucous membranes, tachycardia (up to 140 beats/min), and tachypnea. Heroic attempts to administer blood transfusions, plasma expanders, and associated fluid therapy may save the life of some valuable mares, but costs may be prohibitive.[204]

If the bleeding is contained within the broad ligament, then the mare may be trembling and will exhibit signs of extreme pain (anxiety, sweating, and colic), presumably as a result of the stretching of the broad ligament as the hematoma develops.[204,811,826] The color of the mucous membranes may not change initially because of vascular compensation, and often these initial colic signs are mistaken for the typical discomfort experienced by postpartum mares as the uterus contracts. However, if significant hemorrhage is present, the color of the mucous membranes will eventually become pale, and capillary refill will be delayed. These mares must be monitored closely because the hematoma can subsequently rupture out of the mesometrium and lead to rapid exsanguination.[830]

If a ruptured artery is suspected, then the mare should not be disturbed any more than is necessary to perform an examination. In many cases, postponing, or even forgoing, rectal palpation may be prudent. Although an internal examination will reveal valuable diagnostic information, transabdominal ultrasound, abdominocentesis, and assessment of packed cell volume may be all that is necessary to confirm that an acute hemorrhagic episode has occurred. Transabdominal ultrasonographic evaluation will reveal free blood in the abdominal cavity if the hematoma has torn the broad ligament. If the broad ligament tears after a uterine artery rupture, then a bloody tap invariably results, with an elevated red blood cell count in the peritoneal fluid.[197] The centrifuged sample will have a pink or hemolyzed appearance if hemoperitoneum is present. A smear that reveals phagocytosed erythrocytes indicates hemorrhage rather than contamination during sampling. Even if a clot has contained most of the hemorrhage within the broad ligament, there is often considerable blood loss into the peritoneal cavity. The initial hemogram during an acute hemorrhagic episode can be confusing because the relative loss of erythrocytes and plasma may not alter the hematocrit immediately. Splenic contraction will also temporarily increase the hematocrit.

Ideally, mares suspected to have acute hemorrhage should not be transported because movement could destabilize the clot and prove fatal. Any supportive therapy should be administered at the stall until the mare has stabilized. The foal should be kept safely nearby so that the mare does not become unduly distressed.

Most recommendations for the management of postpartum hemorrhage in mares are based on the collective wisdom of experienced clinicians and from methodologies that have been extrapolated from the human trauma literature. The approach taken will be governed by the facilities and expertise available and financial constraints. In some instances an extreme hypotensive state may actually offer the best chance for survival (conservative approach), whereas in other cases an attempt to restore intravascular pressures and circulatory volume could be indicated. The conservative approach is to confine the hypotensive mare to a dark, quiet stall with minimal disturbances. In some cases a platelet–fibrin plug allows the rent in the vessel to be sealed once the arterial pressure

falls. Tranquilizers (especially acetylpromazine) should be used with caution, because any induced drop in blood pressure may exacerbate the hypovolemic shock. Some clinicians use hypotensive resuscitation by administering a vasodilating agent in conjunction with IV fluid therapy. The idea in this instance is to provide lifesaving volume replacement while maintaining a low mean arterial pressure. In life-threatening situations anything that may stabilize the mare is worthwhile, but the clinician should consider the possibility of impeding resolution of the hematoma by rapid expansion of blood volume and elevation of blood pressure. The need to support cardiac output and ensure oxygen delivery must be balanced against the prospect of the increased arterial pressure promoting further hemorrhage.

Although costly, an aggressive therapeutic approach can occasionally save the life of a valuable mare. If the mare presents in shock and appears to be deteriorating rapidly, then a large IV catheter should be inserted and substantial fluid therapy begun. Whole-blood transfusions must be given slowly and thus are of little benefit for resuscitative purposes when rapid volume expansion is required. One option is rapid administration of 2 to 3 L of hypertonic saline followed with 10 to 20 L of LRS over a period of 2 to 4 hours. An alternative to the hypertonic saline is the high oncotic pressure exerted by colloids (e.g., 3 L hydroxyethyl starch). Synthetic oxygen-carrying fluids are commercially available but extremely expensive. Supplemental oxygen can be provided by nasal insufflation at a flow rate of 5 to 10 L/min. If the hematocrit continues to drop to under 15%, then whole-blood transfusions (6–8 L over several hours) may be warranted. Benefits include provision of oxygen-carrying cells, clotting factors, and oncotic pressure (albumin).

A shock dose of corticosteroid is indicated. Because hemorrhagic shock can cause ischemic-reperfusion damage to the gut and lungs (multiple organ failure), broad-spectrum antibiotics, antioxidant drugs, and antiinflammatory medication may be warranted if the mare survives the initial hemorrhagic crisis. Flunixin meglumine (1.1 mg/kg) is administered to reduce the inflammatory cascades activated by ischemia and may help to alleviate the mare's discomfort. Low-dose (10–20 IU) oxytocin therapy is controversial, and some feel it aids uterine involution, reducing the weight supported by the ligaments. Higher doses should be avoided because an induced colic episode may precipitate a fatal hemorrhage. Antifibrinolytic drugs (aminocaproic acid and tranexamic acid) may assist with clot stabilization. Pentoxifylline increases erythrocyte flexibility and may increase oxygen delivery to ischemic tissues. It should be remembered that there is little refereed veterinary literature to validate the use of some of these medications in the horse. For instance, a conjugated estrogen product has been proposed on the basis of its ability to shorten prolonged bleeding times in humans. However, the benefit, if any, would not be realized until several days after the crisis has passed. Likewise, anecdotal reports suggest that naloxone (8 mg) may be efficacious, but the concept has been extrapolated from small animals, and controlled equine studies are lacking. A controversial historical therapy to promote hemostasis in the horse is the use of IV buffered 10% formalin, which is thought to enhance the activation of the clotting cascade. However, controlled studies were not able to demonstrate an effect on coagulation parameters or template bleeding times in normal horses.[203] Broad-spectrum antibiotics should be administered to prevent infection of the hematoma. Yunnan Baiyao, an herbal supplement, has been used by some practitioners to facilitate hemostasis.

If hemorrhage is contained within the wall of the uterus, then the intramural hematoma may be an incidental finding at the foal heat examination. However, some mares may exhibit variable signs of abdominal discomfort, even to the extent of warranting an exploratory celiotomy.[834-836] If an endometrial laceration severs an artery in the uterine wall, then substantial hemorrhage may ensue, often with blood escaping from the vagina. The mare should be confined to a stall and low-dose oxytocin therapy instituted. Uterine irrigation is contraindicated because it will disrupt clot formation and prolong the hemorrhagic episode. The internal pudendal artery, one of the terminal branches of the internal iliac artery, gives rise to the umbilical artery and the urogenital artery before terminating in branches to the perineal area and the vestibular bulb. The small cranial vesicular artery supplies the apex of the bladder before the remainder of the umbilical artery terminates into the cordlike round ligament of the bladder. The urogenital artery gives rise to a caudal uterine branch that runs cranially on the side of the vagina and ramifies with the caudal branch of the uterine artery on the body of the uterus. The urogenital artery also supplies branches to the rectum, ureter, caudal bladder, and urethra and continues as the vaginal artery to the caudal portion of the reproductive tract.[4,828,829] A hematoma arising from these vessels may dissect along the fascial plane within the pelvic cavity and present as a large unilateral vulvar swelling.[811] Affected mares typically will experience violent colic. Abscessation of a retroperitoneal hematoma can become a life-threatening complication after a dystocia; thus prophylactic broad-spectrum antibiotic coverage is warranted.[826] Mares with an infected retroperitoneal hematoma often develop signs of toxemia. In these cases the peritoneal fluid has an increased total protein content (3.0–5.0 g/dL) with a massive increase in the white blood cell count (often exceeding 100,000 cells/μL).[197]

Uterine Prolapse (Eversion)

Uterine prolapse, or eversion, is an uncommon complication of equine parturition that may occur up to several hours (and occasionally several days) after fetal delivery.[837] The condition may be complicated by bladder eversion (or prolapse), uterine rupture, or intestinal herniation and may be rapidly fatal if the uterine artery is ruptured.[200,800,812,826] If the mare is standing and personnel are available, then instructions should be given to place the uterus in a large plastic bag and elevate it to the level of the vulva. This may prevent further damage to the endometrium and, more important, will relieve the tension on the uterine vessels.[837] Fluid therapy may be indicated, and any calcium deficit must be corrected. Epidural anesthesia may reduce the amount of reflex straining provoked by vaginal manipulations, but it will not eliminate the mare's strong abdominal press.[801] General anesthesia may be necessary if the mare exhibits violent discomfort or if straining is excessive. Any uterine lacerations should be closed with absorbable sutures. The well-lubricated uterus is then pushed back through the vagina with a kneading motion. The fingertips can easily damage the edematous tissue, and manipulating the uterus through a plastic bag will reduce the likelihood of a finger rupturing the wall.[204] Ultrasonography is useful to evaluate any suspicious contents. A trapped bladder may be aspirated through a large-diameter needle, but a loop of bowel may require a ventral midline celiotomy.

The replaced uterus should be distended with sterile saline to ensure that the tips of both horns are fully extended. Repeated low doses (10–20 IU every 2 hours) of oxytocin should be administered to promote uterine involution. Failure to ensure complete extension of the uterine horns into a normal position within the abdomen may result in discomfort, straining, and recurrence of the prolapse.[204] Vulvar retention sutures should not be necessary provided that the uterus has been completely returned to its normal position, the calcium deficit has been corrected, and low-dose oxytocin therapy has been administered. Broad-spectrum antibiotics, NSAIDs, and tetanus prophylaxis are indicated. The mare should be closely monitored for evidence of internal hemorrhage. Affected mares may exsanguinate after the uterus has been replaced. Ischemic damage to trapped bowel is a potential complication. The clinician should be cognizant of the potential risks of endometritis-metritis, septicemia, endotoxemia, and laminitis. Between 2 and 3 days of intrauterine therapy may be warranted, depending on the condition of the exposed endometrium.

Partial Inversion (Intussusception) of the Uterine Horn

Injudicious traction on a retained fetal membrane remnant may invert the tip of the uterine horn, and this may progress to complete uterine prolapse.[204] If only the horn is affected, then compromised circulation and pressure on nerve endings may produce signs of abdominal discomfort; thus the tips of both uterine horns should be rectally palpated when a postpartum colic case is evaluated. The affected horn will be shorter than normal and extremely thickened.[800,812] Manual reduction by pressure from within the uterine lumen may be possible in some cases, and infusion of several liters of saline solution will usually ensure extension of the affected horn.[800] Oxytocin (10–20 IU) should then be administered and the fluid drained from the uterus as it contracts. Resolution of the problem should be confirmed by rectal palpation.

Uterine Rupture

In any dystocia case there is a risk for iatrogenic tears, and the uterus should always be checked for any obvious lacerations immediately after extraction of the fetus. Early recognition is important because the prognosis is worse once peritonitis develops.[197,810,811,826,838] However, obstetric intervention is not always the cause of uterine tears. Occasionally, the foal's hoof may be forced through the dorsal uterine wall during the mare's expulsive efforts, and the mare may be found with a loop of bowel protruding through the vulvar lips. The exposed bowel should be rinsed with sterile saline and replaced, but a ventral midline celiotomy may be warranted to fully evaluate intestinal damage and to repair the uterine laceration. A more common lesion in unassisted deliveries is a tear toward the tip of the gravid uterine horn.[199,838] Although the fetal hooves are covered with hard gel-like pads that presumably protect the placenta and uterine wall, the vigorous piston-like thrusts of the hindlimbs may occasionally cause a rupture.[402,675,839] Affected mares generally experience bouts of colic and become depressed, febrile, and anorectic as peritonitis develops. The interval from occurrence of the tear to diagnosis and initiation of therapy has a marked impact on the prognosis for survival.[197,199,204,811,826,838] It may not be possible to ascertain uterine integrity by vaginal palpation alone. The tips of the horns may be especially difficult to palpate from within the postpartum uterus. The changes in the peritoneal cavity depend on the duration of the condition, but generally one can expect to see serosanguineous to sanguineous fluid containing elevated total protein, increased white blood cell counts, and often extracellular and intracellular bacteria.[197,198,838,840]

Laparoscopic evaluation of the uterus may confirm the diagnosis and provide useful information when determining the need for surgery.[841] Complete perforation of the uterine wall is not necessary for peritonitis to develop if traumatic obstetric manipulations have damaged the uterine wall.[842] However, a fetotomy procedure does not alter the composition of the postpartum peritoneal fluid if it is performed correctly.[197]

If a uterine laceration is suspected (partial or full), then the mare should receive systemic broad-spectrum antibiotic coverage along with nonsteroidal antiinflammatory medications in an effort to prevent development of endotoxemia or other systemic infections. Oxytocin therapy (10–20 IU every 2 hours) will promote uterine involution. The dose can be increased if the mare does not become uncomfortable. Fluid therapy should be administered as necessary, ensuring that calcium levels are within the normal range. Intensive medical management may suffice for small dorsal uterine tears, but most warrant suturing if costs are not a limitation.[199,838,840,841] Opinions vary on the need for—and usefulness of—peritoneal lavage.[838,841] Large full-thickness tears warrant surgical intervention. In some instances a laceration in the uterine body can be sutured blindly in situ, but often a ventral midline celiotomy is the preferred approach.[204,736,810,840]

Retained Fetal Membranes and Toxic Metritis

Once the umbilical cord ruptures, there is a sudden cessation in blood flow through the capillary network in the placenta.[200] This causes a reduction in the tissue volume of the microcotyledons, and the rhythmic tubocervical contraction waves cause the membrane tips to separate and invaginate into the horn. The ongoing tubocervical detachment process causes the membranes to be passed inside out with the allantoic surface exposed. The membranes should be expelled within 3 hours after parturition, and the incidence of retention has been reported to range between 2% and 10% of foalings.[727,843,844] Membrane retention tends to be most commonly associated with the tip of the nongravid horn and appears to be associated with dysfunction of the initial separation process.[432]

In circumstances in which tissue inflammation is common (e.g., abortion, dystocia, cesarean section), it is more likely that membrane retention will occur. In these cases the endometrial edema may trap the microcotyledons within the endometrial crypts. Mares with membrane retention may have a significantly lower serum calcium level.[845] The number of endometrial mast cells observed during the puerperal period is significantly lower in the endometrium of mares with retained fetal membranes.[846] It is likely that some dysfunction of the normal endocrine-related maturational processes within the microcotyledons is involved. There may be an association between inbreeding and the high incidence of fetal membrane retention in Friesian mares.[763,847]

Appropriate management of a mare with fetal membrane retention varies depending on the time since foaling.[848] Although some mares, especially those foaling in a natural environment, may not experience any complications, prophylactic medication is recommended under intensive husbandry

conditions.[844,849] Bacterial contamination in this environment is highly likely. If a severe metritis develops, then inflammation of the uterine wall permits bacteria and toxins to enter the systemic circulation, producing septicemia and endotoxemia.[842,850] Laminitis is a frequent sequela.[204]

The approach to treating RFMs varies considerably, depending on the duration of membrane retention and the presence or absence of metritis with septicemia. In normal, unassisted foalings, one or two treatments with oxytocin may be all that is required to facilitate passage of the RFMs. The protruding placental remnants should be tied in a knot above the mare's hocks. An initial low dose (10–20 IU) of oxytocin is recommended because some postpartum mares are especially sensitive to this hormone and may experience a severe bout of colic within minutes of treatment. Higher doses are likely to be counterproductive because myometrial spasm will occur instead of the desired rhythmic tubocervical contractions. If colic does occur, then the mare should be sedated so that she does not roll and possibly injure the neonate. In these cases the next dosage of oxytocin should be reduced. Each mare's response to the initial treatment governs the subsequent dose recommendations of 10- to 20-IU incremental increases every 30 minutes to 2 hours.

Distention of the chorioallantoic sac with fluid, known as the *Burns technique,*[851] promotes membrane expulsion (5–30 minutes) in most postdystocia mares. A major advantage is that expulsion of the intact fetal membranes will remove any contaminants that may have been introduced by the obstetric procedures.[851] The technique works only if a sterile nasogastric tube can be passed beyond the torn distal fragments and is best performed while the membranes are still fresh. In more protracted cases the rapidly autolyzing chorioallantois becomes friable and generally tears once the fluid pressure increases. When the procedure is performed the exposed fetal membranes are held tightly around the tube while 12 to 15 L of solution are infused. The opening is then tied off with umbilical tape. The exact mechanism is unknown, but expansion of the uterine lumen may dilate the endometrial crypts such that the weight of the membranes can atraumatically pull the microcotyledons free. Endogenous oxytocin release can be supplemented to enhance uterine contractions.

Because the uterine response to oxytocin wanes during the postpartum period, the dose may be increased in small increments every 2 hours in mares that retain their fetal membranes despite the initial therapy. If a hospitalized mare is receiving IV fluids, then each oxytocin treatment can be added to the fluid line. Another option is to add oxytocin to the fluid bag at a dose that is calculated on the basis of the flow rate (1 IU/min).[204] However, a disadvantage of this approach is that these fluids must be discarded if the mare becomes uncomfortable yet still requires rehydration. The calcium ion plays a vital role in myometrial contractility, and it is important to ensure that calcium levels are within the normal range.[768,852] Supplemental calcium can markedly expedite the rate of passage, which suggests that uterine hypomotility is a component in some of these cases.[844] Controlled exercise is often beneficial in promoting uterine involution but is not always feasible if the mare is hospitalized or is being kept in a stall while a compromised neonate is being medicated.

It is widely accepted that excessive traction on the fetal membranes is contraindicated, but a recent study suggests that cautious manual removal of the membranes may not be as deleterious as previously thought.[853] When the membranes are extracted by force there is inevitably disruption of the epithelial barrier, making the traumatized uterine lining more susceptible to bacterial invasion and the development of metritis.[850] Endometrial trauma is also likely to contribute to the development of periglandular fibrosis. It is not uncommon for the membrane tip to tear off and remain firmly attached within the nongravid horn.[432] Injudicious traction on the membranes can also cause an inversion of the tip of the uterine horn, and this can progress to a complete uterine prolapse.

If the fetal membranes have not been expelled after 2 days of supportive therapy, then the autolytic tissue becomes less firmly embedded. A gentle twisting technique, with minimal traction, applied within the attached horn will often result in successful removal of the entire chorioallantois. This procedure works best while the uterus is being distended during a uterine lavage. A potentially effective treatment for RFMs in mares may be intraplacental injections of collagenase.[854,855]

If the membranes have been retained for 6 to 8 hours when the mare is first examined, then systemic antibiotic therapy is indicated.[204] Drugs that have been recommended for systemic administration include ampicillin, gentamicin, kanamycin, penicillin, ticarcillin, and trimethoprim-sulfamethoxazole. If a remnant is missing when the membranes are examined, then the approach to therapy should proceed as if the entire membrane were still present. Characteristic signs of toxic metritis are fever, depression, anorexia, tachycardia, and injected mucous membranes. The foal will not be receiving adequate milk intake, and many of these mares will have bounding digital pulses and evidence of laminitis.

Transrectal palpation will reveal a large, thin-walled, atonic uterus that contains moderate to large amounts of fetid fluid. A large volume of toxic, red-brown, watery fluid can accumulate within the pendulous postpartum uterus before any obvious vaginal discharge becomes evident. Often the history will reveal that the fetal membranes were discarded without being checked to ensure that they were passed intact.

Because the endometrium is likely to be necrotic, therapy should include broad-spectrum antibiotics, antiinflammatory drugs, and IV fluids if indicated. Tetanus prophylaxis is advisable. A combination of penicillin and gentamicin is widely used to provide broad-spectrum systemic coverage, especially against the coliforms that frequently contribute to endotoxemia and laminitis.[856] Flunixin meglumine should be administered to ameliorate the effects of endotoxemia. It is commonly administered IV at a reduced dose (0.25 mg/kg, t.i.d.).[204] Phenylbutazone (2–4 mg/kg) and provision of deep, soft bedding and padded shoe support are useful to alleviate pain when laminitis appears to be imminent. Radiographs can be useful to monitor changes in the position of the pedal bone. Vasodilators such as acetylpromazine maleate may be administered by IM injection (0.02–0.04 mg/kg every 4–6 hours).[856] Further discussion of the diagnosis and treatment of endotoxemia and laminitis are found in Chapters 10 and 12, respectively.

The uterus should be lavaged with sterile saline or very dilute ("weak tea") povidone-iodine solution. If povidone-iodine is used, then the final concentration should not exceed 0.1%; this is equivalent to 10 mL of 10% povidone-iodine solution (e.g., Betadine) in 1 L of saline. If costs are a concern, then 90 mL in a 9-L bucket of clean water may suffice. Extreme caution should be exercised to keep from puncturing the inflamed uterine wall with the tube. The lavage should be repeated until the returning fluid is relatively clear. The goal of

therapy is to eliminate toxins and to prevent the rapid proliferation of bacteria, especially coliforms and possibly anaerobes. The administration of intrauterine antibiotics in the postpartum mare is controversial because little scientific validation has been performed. Intrauterine administration of antibiotics and antiseptics may depress the phagocytic activity of uterine neutrophils, and many chemicals are known to irritate the endometrium in mares being infused for endometritis.

The pharmacokinetic properties of each drug will influence its efficacy in the postpartum uterus. Most of the studies of intrauterine therapy in mares have addressed therapy for endometritis in nonparturient animals. The efficacy of antibiotic formulations in the presence of the mixed bacterial population and tissue debris associated with fetal membrane retention in the mare remains to be established. The antibiotic of choice should be added to a large infusion volume (2–3 L) to ensure uniform distribution across the inflamed endometrial surface once a lavage has removed the toxic fluid and necrotic debris. Infusion of 2 g of amikacin after a uterine lavage is often clinically effective. Polymyxin B may have some merit because of its endotoxin-binding ability. Powdered and propylene glycol–based oxytetracycline formulations are known to be irritating when infused into the involuted uterus and should be avoided.[6,857] Other antibiotics that have been suggested for postpartum intrauterine therapy include ampicillin (3 g), ticarcillin and clavulanic acid (1–3 g), and gentamicin (2–3 g). Fewer than 60% of isolates from metritis fluid appear to be sensitive to ampicillin. The authors do not support intrauterine antimicrobial therapy in the postpartum mare.

Recently a technique has been described that is believed to cause immediate placental release. This technique consists of cannulating the umbilical cord vessels and using an adaptor to connect the catheter to a garden hose. Water is pumped into the mare uterus until the chorioallantois is detached from the endometrium.[858] A success rate of 93% for immediate release of the placenta is reported.

Gastrointestinal Complications

Prolonged straining during dystocia can lead to variable amounts of rectal mucosa being forced out through the anal sphincter (type I rectal prolapse). The tissue then becomes subject to trauma, contamination, and vascular compromise. If not promptly corrected, then pressure from the anal sphincter will cause venous congestion and swelling. This promotes more straining, and the condition can deteriorate rapidly. A type II prolapse involves all or part of the ampulla recti. An epidural anesthetic may help decrease straining. Topical glycerin or dextrose may be applied to the prolapsed tissue to reduce edema.[204] A purse-string suture can cause additional straining and will impede defecation.[859] Fecal softeners should be administered and the diet modified (e.g., pellets, pasture) to help produce soft feces.[569]

Chronic prolapses may warrant surgical resection of the devitalized mucosal mass. In a type III prolapse, there is a full-thickness rectal prolapse and an intussusception of the peritoneal rectum or small colon. In a type IV prolapse the intussuscepted bowel protrudes through the anus such that there is a palpable trench that may extend several meters into the rectum, depending on the length of the intussusception.[859-861] Midventral celiotomy is usually necessary to reduce the intussusception, although some smaller prolapses will reduce after an attendant has extracted the foal. The short mesentery that supports this section of bowel is often torn from the colon. These cases have a guarded prognosis, dependent on the vascular integrity of the affected small colon. This condition can develop when as little as 6 to 10 inches of bowel appear to be prolapsed. The avulsion most likely occurs when the mare's intermittent straining forces an extra 4 to 6 inches of bowel in and out of the rectum. Thus it is imperative to prevent straining as soon as possible. If the foal has not yet been extracted, it may be best to immediately anesthetize the mare and then elevate the hindquarters before attempting to correct the cause of the dystocia.

In rectal prolapse cases that have been managed conservatively, one of the first postpartum clinical signs may be discomfort attributable to impaction colic. If avulsion of the mesocolon has occurred, then ischemic necrosis of the affected bowel will cause a delayed peritonitis. Because early intervention is essential, sequential abdominocentesis is indicated when a type III or IV rectal prolapse is being conservatively managed. Initially, there may be negligible changes in the composition of the peritoneal fluid. However, if an avulsion has occurred, the compromised segment of bowel will soon lose its integrity, and a massively increased white blood cell count can occur within 24 to 48 hours as peritonitis ensues.[197] Laparoscopic evaluation of the abdomen can provide an immediate assessment of bowel integrity and permit an accurate prognosis to be given to the owner. The affected colon is not readily accessible for resection and anastomosis, so the prognosis in most cases is guarded.

Variable degrees of uncomplicated impaction are not uncommon in the postparturient mare, possibly as a result of localized perineal pain causing reluctance to defecate.[204] Astute managers will note an absence of fecal matter in the stall. Treatment with fecal softeners (e.g., mineral oil) and analgesics generally corrects the problem. Laxative feeds (e.g., bran mash) are effective in reducing the incidence of constipation in foaling mares.[800] Postpartum mares appear to be at an increased risk for development of a large colon torsion.[570,826,862] This condition presents as an especially violent colic with readily discernible abdominal distention. Extensive ischemic damage affects the prognosis, but early surgical intervention can increase the survival rate.[863] Bruising of the abdominal viscera can occur during foaling, with subsequent development of moderate to severe signs of impaction colic and peritonitis. Occasionally, the mesentery may be torn from a segment of intestine, leading to ischemic necrosis and peritonitis. An early diagnosis and prompt surgical intervention may save the mare's life.[197,737,825-827,864] A rent in the mesentery or broad ligament at the time of foaling may permit a segment of bowel to become incarcerated even weeks later.[568,820,826,827,865] Owners should be advised that surgical correction is feasible only if the segment of devitalized bowel is accessible.

Although mares tend to reduce their feed intake in the days leading up to foaling, ensuring a reduction in the amount of available roughage may help reduce the incidence of bowel rupture.[800] The tip of the cecum is the most likely site of a foaling-related rupture in the alimentary tract. On rectal palpation the inflamed serosal surfaces will feel roughened, with a discernible crepitus. Abdominocentesis will reveal dark green-brown gastrointestinal fluid that contains plant material and massively increased neutrophil numbers. Humane euthanasia is indicated because the leaking ingesta incites a severe peritonitis with accompanying septic shock, and the condition is likely to be rapidly fatal.[826,866-869] Diaphragmatic herniation has been reported as a rare parturient complication in heavily

pregnant mares.[735,870-872] Colic symptoms are attributable to strangulating obstruction or tension on the mesentery. Some mares may exhibit respiratory distress. Transthoracic ultrasonography can help confirm the presence of bowel within the thorax.[873] Surgical repair of the defect may not be possible, and assisted ventilation will be required.[871,872]

Vaginal Lacerations and Bladder Prolapse

Primiparous mares are especially susceptible to vaginal trauma. Vaginal lacerations are most likely to occur during injudicious attempts to relieve dystocia. Although most lacerations are retroperitoneal, they still may contribute to severe vaginitis, fibrosis, and possibly abscessation. If ventral trauma is present, then a urinary catheter should be passed to check for urethral integrity. In some instances ligation of a severed artery will be necessary. Emollient creams, tetanus prophylaxis, broad-spectrum antibiotics, and antiinflammatory drugs are indicated. A major concern is the possibility of herniation of intestine into the vagina if the tear is located just caudal to the cervix in the vicinity of the urogenital pouch.[826,874] If eventration has occurred, the bowel should be cleansed and examined for evidence of vascular compromise. If the involved intestine appears to be grossly normal, it should be rinsed with sterile saline and returned to the abdominal cavity. If vascular compromise is detected at the time of the initial examination, then the prognosis is guarded and a ventral midline celiotomy to facilitate resection is warranted. A bladder prolapse occurs when the bladder is forced up through a vaginal laceration. The viscus rapidly becomes distended as a result of the continued accumulation of urine from the ureters and an inability to void urine because of kinking of the urethra. The edematous serosal surface of the bladder may protrude through the vulvar lips. The exposed organ should be thoroughly cleansed and gently returned to the abdominal cavity. It may be necessary to administer an epidural and aspirate urine to facilitate replacement. If possible, the vaginal laceration should be sutured once any viscera have been returned to the abdominal cavity. In some cases the severity of the trauma precludes successful closure, and the wound must heal by second intention.[874] Placement of a Caslick suture reduces the possibility of bacterial aspiration. Mares may be cross-tied for several days to decrease the risk of eventration brought about by elevation of intraabdominal pressure as the mare lies down.[204] The mare should be treated for impending peritonitis (e.g., broad-spectrum antibiotics, NSAIDs). Tetanus prophylaxis is indicated. If severe colic symptoms develop, bowel compromise should be suspected.

Eversion of the Urinary Bladder

The mare's urethra has a large diameter, and occasionally the bladder may be everted up into the vagina after severe straining.[875] If the everted bladder protrudes through the vulvar lips, then the exposed mucosal surface will rapidly become edematous, and urine may be seen to drip from the ventral surface. Closer inspection will reveal that the urine is dribbling from the exposed papilliform openings of the ureters on the dorsal surface of the neck of the bladder.[876] A lip chain and epidural may provide adequate restraint to facilitate replacement. The mucosal surface should be thoroughly cleansed and any defects repaired. Sterile lubricant should be applied and the friable organ gently massaged back through the urethra. In some instances it may be necessary to incise the urethral sphincter if the bladder mucosa is especially thickened.[876] This incision should be closed once the bladder has been replaced. A Foley catheter can be inserted to lavage the bladder lumen and ensure complete repositioning. Broad-spectrum antibiotic coverage, NSAIDs, and tetanus prophylaxis are indicated.

Rupture of the Urinary Bladder

Occasionally, the bladder may rupture as a consequence of increased intraabdominal pressure in the foaling mare or because of direct trauma during parturition.[877-879] Clinical signs are delayed and are associated with electrolyte imbalances. Affected mares may be depressed and inappetent, with failure to void urine. Clinical examination will reveal tachycardia, tachypnea, and decreased gastrointestinal activity. Blood chemistry will reveal elevated serum levels of creatinine, blood urea nitrogen, and potassium in addition to decreased sodium and chloride levels. Evaluation of a peritoneal fluid sample helps confirm the diagnosis. The fluid will contain elevated urea and creatinine levels and calcium carbonate crystals. Cystoscopy is useful to evaluate the size and extent of the bladder injury. Once the mare's medical condition has been stabilized, surgical repair is indicated.[820,877-879] A standing vaginal approach eliminates the need for general anesthesia and allows excellent observation and repair of bladder tears in adult mares.[879]

Rectovaginal Fistulas and Perineal Lacerations

A first-degree perineal laceration involves the mucous membrane of the vestibule and the skin of the vulvar lips. In second-degree perineal lacerations, the deeper tissues of the perineal body are involved. Both of these conditions may be associated with unassisted delivery of a large foal or may be a sequela of dystocia. The laceration may be amenable to immediate repair and placement of a Caslick suture, or the clinician may elect to wait until the wound has granulated. The mare should be treated with broad-spectrum antibiotics, antiinflammatory medication, and tetanus prophylaxis. Provision of a bran mash diet and administration of mineral oil or stool softeners may facilitate defecation during the initial inflammatory period.

Third-degree perineal lacerations generally occur during unassisted foalings when the fetal hoof catches on the vaginal roof at the vestibulovaginal junction. Forceful straining by the mare can drive the hoof through the rectovaginal shelf such that the fetal hoof comes to lie within the rectum. If the fetus is viable, it may remove the affected limb and delivery will proceed unimpeded, but a rectovaginal fistula results. If the limb remains within the rectum, then continued passage of the fetus causes the trapped limb to tear out the perineal body and anal sphincter. The resulting defect is called a *third-degree perineal laceration.* These injuries do not respond well to immediate surgical intervention, and the general recommendation is to wait 4 to 6 weeks before attempting reconstructive surgery.[820,875,880] In the interim the mare should be treated with broad-spectrum antibiotics, antiinflammatory medication, tetanus prophylaxis, and fecal softeners.

Grade IV (full-thickness) rectal tears that communicate directly with the peritoneal cavity have a poor prognosis and can occur as a result of parturition.[881,882] Tears tend to occur just cranial to the caudal peritoneal reflection. Such cases warrant immediate intervention, and the rectum should be packed to prevent abdominal contamination during transport to a referral hospital. A standing technique that permits an easy and effective stapled primary closure repair has been described.[881]

Perineal Bruising and Vulvar Hematomas

Much of the swelling after prolonged obstetric manipulations is edematous. Fecal softeners such as oral mineral oil and bran mash are recommended to ease the passage of feces through the swollen and bruised perineal area.[735,806] Hematomas in the vaginal wall and vulvar lips are not uncommon, especially in primiparous mares and mares that have delivered an extremely large foal. It is important to differentiate a bulging vestibular hematoma from an everted or prolapsed bladder.[800,829] Needle aspiration of vulvar hematomas is not recommended because of the risk of abscessation. Broad-spectrum antibiotics and tetanus prophylaxis are indicated. Most hematomas will resolve uneventfully, but some vulvar, vaginal, or pelvic hematomas may warrant drainage in 7 to 10 days.[735]

Postpartum Eclampsia (Lactation Tetany)

Postpartum eclampsia, or lactation tetany, is extremely rare in mares but may occur in animals that are lactating heavily. The highest incidence is reported in draft breeds, but the author has encountered a case in a pony mare. Equine eclampsia is generally associated with some type of stress (e.g., change in surroundings). Early signs include restlessness, tachypnea, staring eyes, twitching, trembling, and clonic spasms (especially diaphragmatic). The clonic spasms gradually become more tonic, and eventually the mare may be unable to stand. The differential diagnosis is tetanus, but the nictitating membrane is not prolapsed. The condition responds well to intravenous calcium gluconate administration.[200]

Assisted Reproductive Techniques for the Mare

E.A. Bradecamp

The development of new assisted reproductive techniques for the mare has allowed production of offspring from mares that are infertile using standard breeding techniques or embryo transfer.

INTRACYTOPLASMIC SPERM INJECTION

Currently, intracytoplasmic sperm injection (ICSI) has become the most common assisted reproductive technique used to obtain pregnancies from mares that have failed to produce pregnancies themselves or via embryo transfer. ICSI is also used to obtain pregnancies from stallions that have very poor fertility or from which there is a limited amount of semen available. During ICSI a single sperm is selected, aspirated into a fine-bore needle, and injected into a mature oocyte. The injected oocyte can be transferred into a recipient's oviduct or cultured to allow embryo development[883] and then transferred into a recipient once it reaches blastocyst stage. Cochran et al. reported the first successful ICSI of an equine oocyte that was matured in vitro. Foals have been produced by ICSI using oocytes matured in vivo or in vitro.[884,885]

Currently, there are several commercial ICSI labs to which mares can be shipped for management for oocyte aspiration and ICSI, or oocytes can be collected at a remote location and shipped for ICSI. Subsequently, the fertilized oocytes are cultured until the blastocyst stage is reached. The embryos can then be transferred into on-site recipients, if available, or shipped to an off-site recipient herd.

Oocytes can be collected from preovulatory dominant or nondominant follicles. Oocytes from preovulatory follicles are collected between 24 and 36 hours after the administration of hCG (1500–2500 IU, IV) or deslorelin (1.5 mg, IM) to the donor, with the oocytes collected between 18 and 20 hours before anticipated ovulation. Therefore oocytes are probably at metaphase I or II. Criteria for hCG administration are as follows: (1) a follicle greater than 35 mm in diameter, (2) relaxed cervical and uterine tone, and (3) uterine edema or estrous behavior for a minimum of 2 days. Some mares, especially old mares, do not consistently respond to hCG. In these cases the authors use a combination of deslorelin acetate (1.5 mg, IM) and hCG (2000 IU, IV), with hCG administered between 4 and 5 hours after deslorelin. Oocytes have been collected from the follicles of mares by laparotomy,[886] colpotomy,[887] flank puncture,[888,889] and ultrasound-guided follicular aspiration.[890,891] Currently, most laboratories collect oocytes through the flank or with transvaginal ultrasound-guided punctures.

For the collection of oocytes using flank puncture, the trocar is placed through the flank ipsilateral to the preovulatory follicle at approximately the position of the ovary. The ovary is manipulated per rectum to position the preovulatory follicle against the end of the cannula. While stabilizing the ovary per rectum, the clinician places a needle (12–17 gauge) through the cannula and into the follicular antrum and removes the follicular fluid and oocyte by gentle suction and lavage of the follicle. Transvaginal ultrasound-guided follicular aspiration requires use of an ultrasound machine and a probe fitted to a special needle case. Linear, curvilinear, and sector transducers have been used. The transducer is placed in a casing containing a needle guide. Rectal contractions may be minimized by administration of propantheline bromide (0.04 mg/kg, IV)[892] or *N*-butylscopolamine or intrarectal use of lidocaine before the procedure. The clinician applies a nontoxic lubricant to the transducer and positions it against the anterior vaginal wall, lateral and dorsal to the external cervical os and ipsilateral to the follicle to be aspirated. The follicle must be carefully positioned through transrectal manipulations with the follicular apex juxtaposed to the needle guide. The needle is advanced through the needle guide to puncture the vaginal and follicular walls. In the authors' laboratory, a 12-gauge double-lumen needle is used. The follicular fluid is aspirated from the follicle using a pump set at approximately 150 mm Hg or with suction from a large syringe. After removing the follicular fluid, the clinician lavages the lumen with between 50 and 100 mL of flushing liquid. Typically, modified Dulbecco's phosphate-buffered solution or a sterile commercial embryo flush solution is used. Heparin (10 IU/mL) is added to the flush solution to prevent coagulation of blood in the aspirate. By use of this technique, oocytes were typically collected successfully from between 70% and 80% of the follicles in client donors.[892]

The major clinical advantage of ICSI is that limited sperm numbers or poor-quality sperm can be used to produce offspring, and pregnancies can be obtained from infertile mares that failed to produce embryos and pregnancies through embryo transfer.

OOCYTE TRANSFER

Although the first successful oocyte transfer was performed in 1988, the technique was not used for commercial transfers

until the late 1990s.[892-894] Oocyte transfer involves the transfer of an oocyte from a donor into the oviduct of a recipient, and the recipient is inseminated within the uterus. Fertilization, embryo development, and fetal development occur within the recipient, preventing problems associated with ovulation or the tubular genitalia of donors. The incidence of ovulatory failure increases with age and during the autumn months.[704,895] Prolonged exposure to an abnormal follicular environment results in aging and death of the oocyte. Some types of ovulatory failures can be detected with ultrasound as an atypical morphology of the follicle or ovulatory site. Mares that repeatedly fail to ovulate can provide oocytes for transfer successfully if oocytes are collected before deleterious changes occur within the follicle.[892]

Historically, the uterus has been considered the primary cause of reduced fertility in the mare. Mares with pyometra or persistent endometritis are expensive to treat and frequently do not provide embryos. Mares with cervical lacerations, cervical or uterine adhesions, or urine pooling often fail as embryo donors. Oviduct dysfunction is a rare impediment to fertility and can occur in young or aged mares. When the oviducts of old mares (>20 years) and young mares (2–9 years) were flushed between 1 and 4 days after ovulation, collection rates of recently ovulated oocytes or oviductal embryos were significantly higher in the young mares than in the old ones (26 of 27 [96%] vs. 17 of 29 [59%], respectively).[547] In subfertile mares pathologic changes of the oviducts were imaged using scanning electron microscopy, and significantly fewer sperm were detected in the caudal isthmus in subfertile mares than in fertile mares. Few sperm found in the oviducts of subfertile mares were motile, whereas oviducts of the normal mares contained highly motile sperm.[896] Obstructions of the oviductal lumen are postulated to be the cause of subfertility in some mares. Globular masses composed of type I collagen were found more frequently in older than in younger mares.[206] Oviductal masses were found in the oviducts in 73% (16 of 22) of mares between 2 and 22 years of age; in a small number of mares (3 of 43), the masses occupied and distended the oviductal lumen and could have resulted in infertility. The equine embryo remains in the oviduct for 5 or 6 days before entering the uterus; therefore oviductal problems such as inflammation could affect embryo viability.[897]

Oocyte Donors

Requirements for oocyte donors are minimal. Uterine infections in donor mares should be treated to prevent introduction of a pathogen into the abdominal cavity during transvaginal oocyte collections. Donors should have regular estrous cycles with growth of a preovulatory follicle. The age of the donor affects success rates. When oocytes were collected from the follicles of young donors (6–10 years) and old donors (20–26 years) and transferred into the oviducts of young recipients (3–7 years), significantly more oocytes from young than old donors developed into embryonic vesicles (11 of 12 [92%] vs. 8 of 26 [31%], respectively).[552] A higher incidence of morphologic anomalies was observed in oocytes from older than from younger mares.[551] Although younger mares are better candidates for oocyte donors, older (≥20 years) mares frequently are presented to commercial oocyte transfer programs, and pregnancies are obtainable through repeated transfers.[884]

Oocyte Culture and Transfer

Oocytes are sensitive to temperature changes; therefore the clinician should warm media and equipment for handling the oocyte to 38.5°C. On collection the flushing solution is poured into large search dishes and examined under a dissecting microscope to locate the oocyte. One can transfer oocytes collected at least 30 hours after hCG administration to the donor immediately into a recipient's oviduct. Oocytes are usually collected 24 hours after administration to the donor and between 12 and 16 hours before transfer. Most oocytes are cultured in medium similar to that first described by Carnevale and Ginther.[552] The timing of oocyte collection (24 vs. 36 hours after administration of hCG to donors) did not affect pregnancy rates.[898] A modification of these procedures was to collect oocytes 24 hours after hCG administration and immediately transfer them into the recipient's oviduct. Oocyte maturation was completed within the oviduct, and recipients were inseminated after oocyte maturation should have been completed (at 16 hours after transfer). Pregnancy rates were not statistically different for oocytes matured within the oviduct or within an incubator (43% vs. 57%).[899]

Because the reproductive tract of the recipient provides the environment for sperm transport, fertilization, and embryo development, recipient mares should be young (optimally 4–10 years) and have normal reproductive tracts. Cyclic and noncyclic hormone-treated mares have been used as oocyte recipients. When cyclic mares are used, recipients are synchronized with the donor, and the recipient's own oocyte is removed by transvaginal or flank aspiration before transfer of the donor's oocytes.[900] Anestrous and early transitional mares are used as recipients during the nonovulatory season.[892,901] During the breeding season a high dose of a GnRH agonist (4.2 mg deslorelin acetate)[902] or injections of progesterone and estrogen (150 mg progesterone and 10 mg estradiol)[894] have been administered to reduce follicular development in potential recipients. The endocrine environment of the cyclic mare is imitated in the noncyclic recipient with administration of estradiol (1.5–5 mg daily for 3–7 days) before transfer. Estradiol often is given to obtain a recipient with a relaxed, open cervix and moderate endometrial edema. Progesterone (150–200 mg daily) or a progestin is administered after transfer. Pregnancies are maintained through the administration of exogenous progesterone or progestins.[892]

Because oocytes are transferred surgically, adequate exposure of the oviduct is essential, and mares with short, thick flanks or short, broad ligaments are not good candidates for recipients. Most oocyte transfers are performed through a standing flank laparotomy. Tranquilization, preparation, closure, and aftercare of recipients are similar to previously described methods for embryo transfer.[903] The authors generally use a fire-polished glass pipette to transfer oocytes. The oviductal os is located by following the outline of the oviduct along the external surface of the infundibulum. The end of the structure is identified and the pipette containing the oocyte is inserted into the os, and the pipette is carefully advanced 2 to 3 cm. The oocyte and a minimal amount of medium (<0.1 mL) are deposited into the ampullary region of the oviduct, the ovary is gently returned to the abdominal cavity, and the surgical site is closed.

Insemination of Recipients

In a commercial oocyte transfer program, use of stallions with good fertility is essential for success, but cooled, transported semen frequently is provided from stallions of variable fertility.[892,904] The equine oocyte remains viable for approximately

12 hours after a natural ovulation.[4] Because of this limited life span, recipients must be inseminated before or directly after oocyte transfer or both. Pregnancies have occurred when recipients were inseminated only before[905,906] or after[899] the transfer of oocytes. However, for most experimental transfers, recipients were inseminated before transfer (approximately 12 hours) and after transfer (approximately 2 hours), with a total of 2×10^9 motile sperm. In a commercial program using older donors and cooled semen from numerous stallions of variable fertility, pregnancy rates when recipients were inseminated before or before and after oocyte transfer were significantly higher than when recipients were inseminated only after transfer (18 of 45 [40%], 27 of 53 [51%], and 0 of 10 [0%], respectively).[904] The results suggest that insemination of a recipient once before transfer with at least 1×10^9 progressively motile sperm from a fertile stallion is sufficient. However, if fertility of the stallion is not optimal, then insemination of the recipient before and after transfer could be beneficial.

After insemination and transfer, the uterus of the recipient should be examined with ultrasound to detect intrauterine fluid collections. Recipients with intrauterine fluid collections are treated like an ovulating mare, with oxytocin or PGs to stimulate uterine contractions or with uterine lavage and antimicrobial infusion.

Success of Oocyte Transfer

Pregnancy rates for commercial transfers using older donors and semen of variable quality ranged between 27% and 40% per transfer.[892,904] In contrast, experimental transfers under similar conditions using oocytes from young mares and fertile stallions resulted in pregnancy rates between 54% and 83% per transfer.[27] However, one or more pregnancies were obtained for more than 80% of donors during the breeding season in a commercial oocyte transfer program.[892] All mares in the program had histories of reproductive failure in breeding and embryo transfer programs, with a mean of 7 years (range of 3–15 years) from the last successful pregnancy or embryo collection.[892]

In Vitro Maturation and Fertilization of Oocytes

In vitro fertilization is not as repeatedly successful in the mare as it is in many other species, with only two foals born after in vitro fertilization.[907,908] One problem in trying to study procedures such as in vitro fertilization in the horse is the paucity of equine oocytes. Oocytes often are collected from the preovulatory follicles of live mares. Collection of oocytes from small follicles during diestrus results in reduced collection rates compared with collection of oocytes from preovulatory follicles.[909] In one study,[908] oocytes were collected from small follicles and preovulatory follicles. Oocytes collected from small follicles were matured in vitro for 36 to 38 hours before transfer, whereas oocytes collected from preovulatory follicles were transferred immediately into a recipient's oviduct. Embryo development rates after transfers were 9% for in vitro maturation and 82% for in vivo. In contrast, some laboratories have been very successful in developing methods to collect and mature oocytes from small follicles. Colleoni et al. reported an oocyte recovery rate of 58%, or 11 oocytes per ovum pick-up session.[910] At this time, in vitro embryo production is best achieved with oocyte aspiration, followed by ICSI to achieve fertilization, then embryo maturation in vitro, and transfer at the blastocyst stage into a recipient.

Research involving oocytes from excised ovaries is aimed at developing a method for salvaging gametes from the ovaries of valuable mares that have died or been euthanized. One can collect ovaries from mares immediately after death and ship them to a facility for oocyte recovery, maturation, and transfer. This technique was first attempted in 1999,[892] and a pregnancy was established that later underwent embryonic death. However, more recent attempts have resulted in pregnancies and healthy foals after shipment of ovaries from mares that were euthanized for various medical reasons.[911,912] Currently obtaining a late-term pregnancy or pregnancies from about one of four sets of ovaries that are shipped to a laboratory for oocyte recovery is anticipated.

Cryopreservation of Oocytes and Embryos

Cryopreservation of the equine oocyte results in the preservation of female genetics, whereas cryopreservation of the embryo results in the preservation of the female and male genome. The first foal was produced from a cryopreserved embryo in 1982.[913] Procedures for embryo cryopreservation have been reviewed.[914] Cryopreservation of small embryos (morulae or early blastocysts <300 μm) has consistently resulted in acceptable pregnancy rates close to 50%.[915,916] Cryopreservation of larger embryos (≥300 μm) is usually unsuccessful. Embryo collection is recommended on day 6 or 6.5 after ovulation.[914] Embryo donors are examined twice daily for ovulation, or embryo collections are timed from administration of an ovulation-inducing agent.[917] In recent years vitrification procedures have been used to successfully cryopreserve small equine embryos.[917-919] The advantage of vitrification over traditional slow-cooling methods include cryopreservation, which is a rapid procedure (<15 minutes) that requires minimal equipment and results in better pregnancy rates post thaw. Recently a technique that involves aspiration of fluid from the blastocele cavity before vitrification in larger embryos has been reported to have reasonable success rates.[920]

Although cryopreservation of the oocyte is difficult, successful fertilization of cryopreserved oocytes has been described.[921, 922] In 2001, the first foals were born after cryopreservation of oocytes.[906] Clinical use of oocyte cryopreservation has not been reported and is currently not used in a clinical setting.

ASSISTED REPRODUCTIVE TECHNIQUES FOR THE STALLION

Maximum fertility was obtained when fertile mares were inseminated every other day during estrus with 500×10^6 progressively motile sperm.[923] Insemination of low numbers of sperm would be beneficial for frozen semen that is of limited supply, semen from subfertile stallions with low sperm numbers, and insemination of sex-sorted sperm. The following discussion summarizes techniques for low-dose or assisted inseminations. A more complete discussion on stallion semen processing will be found in a later section.

Deep Intrauterine Insemination

Uterine contractions move sperm into the tips of the uterine horns within 20 minutes of routine AI.[924] The aim of deep uterine insemination (also known as "deep-horn insemination")

is to increase the number of sperm entering the oviduct ipsilateral to ovulation.[925-927] A flexible insemination pipette is passed through the cervix and into the uterine horn ipsilateral to the preovulatory follicle. Then rectal manipulation is used to advance and position the catheter at the tip of the uterine horn in which the sperm are then deposited. Fresh, cooled, and sex-sorted sperm in volumes ranging from 0.2 to 1.0 mL of commercial semen extender have been used for deep intrauterine inseminations. Pregnancy rates after deep intrauterine inseminations with 5×10^6 progressively motile sperm were between 30% and 50%,[923,928] and inseminations with 25×10^6 progressively motile sperm ranged between 57% and 63%.[929,930] However, in a study by Woods et al.,[930] control mares were inseminated with 25×10^6 progressively motile sperm in the uterine body, and pregnancy rates were not significantly different between standard and deep uterine inseminations. Because control inseminations were not done in many studies, the true benefit of deep uterine insemination has not been determined.

Hysteroscopic Insemination

Hysteroscopic insemination involves deposition of sperm directly onto the papilla of the uterotubal junction. A minute volume of extended sperm (approximately 0.05–0.25 mL) is desired for hysteroscopic insemination. Sperm are centrifuged through a density gradient to select a sperm population with a high percentage of motility. Numbers of fresh sperm inseminated ranged between 1 and 10×10^6 progressively motile sperm, with pregnancy rates between 40% and 75%.[928,931-933] Studies have been conducted using higher volumes[931] or lower sperm numbers,[931] but fertility was reduced.

With hysteroscopic insemination, semen is aspirated into an equine gamete intrafallopian transfer (GIFT) catheter (Cook Animal Health, Bloomington, IN) protected by an outer polypropylene cannula and loaded into the working channel of the videoendoscope. With a sterile gloved arm in the vagina of the mare, the operator guides the flexible endoscope (1.6 m in length) through the cervix and uterine lumen; directs the endoscope along the uterine horn ipsilateral to the preovulatory follicle; and on imaging the papilla of the uterotubal junction, extrudes the outer cannula and then the inner GIFT catheter containing the sperm suspension from the working channel of the endoscope. When the tip of the GIFT catheter touches the papilla, the operator bubbles the inseminate onto the surface of the papilla.[931]

Low-dose insemination with frozen-thawed sperm maximizes the use of a conventional dose of frozen sperm (800–1000×10^6 progressively motile sperm) by reducing the number of sperm needed for insemination. Using 5 or 10×10^6 frozen-thawed progressively motile sperm, different investigators obtained pregnancy rates between 33% and 47%.[932,934,935] Alvarenga et al.[936] inseminated client mares with 100 to 150×10^6 frozen-thawed sperm from 15 Warmblood stallions and obtained an overall pregnancy rate of 57%, demonstrating that hysteroscopic insemination can be applied immediately in the horse industry. Current rates for sorting sperm into X chromosome–bearing or Y chromosome–bearing populations are approximately 10 million sperm per hour, meaning that low-dose inseminations are necessary for sex-sorted sperm. Several studies have been conducted using hysteroscopic insemination of sex-sorted sperm, resulting in pregnancy rates between 25% and 44%.[932,934]

Gamete Intrafallopian Transfer

GIFT involves transfer of oocytes and sperm into the recipient's oviduct. Compared with oocyte transfer, GIFT requires low numbers of sperm. The first successful GIFT in the horse was reported in 1998.[901] GIFT sperm are often selected through a density gradient to select a population with a high percentage of morphologically normal, motile sperm, free of debris and seminal plasma, with 1 to 5×10^5 progressively motile sperm transferred with an oocyte into the recipient's oviduct. Optimal procedures for GIFT have probably not been established, although pregnancy rates between 27% and 82% have been reported.[899,937] GIFT is a potentially valuable technique to produce pregnancies from subfertile stallions, frozen semen, and sex-sorted sperm. However, the type of sperm and even extenders may have an effect on the success of GIFT. Pregnancy rates with GIFT were lower when cooled or frozen semen was used for GIFT (pregnancy rates of 25% and 8%, respectively) compared with pregnancy rates with fresh semen (82%).[938]

CONCLUSION

New assisted reproductive techniques have been developed that allow for the production of offspring from mares and stallions that would be considered subfertile or infertile when more standard breeding procedures are used. Although cost, expertise, and availability of these procedures may be limiting factors in their widespread use at this time, their clinical potential for preservation of valuable equine genetics is important to recognize.

The Stallion

Charles F. Scoggin

This section will describe the relevant anatomy, physiology, and management of the stallion and discuss pathology associated with the reproductive tract of male horses.

Medical conditions affecting stallions are generally no different from what we see in other horses, with the obvious exception being those pertaining to the reproductive organs. The incidence of problems associated with the genitalia or gonads is inherently low because there are proportionally fewer intact male horses compared with female horses and geldings. Nonetheless, on the vast majority of farms and ranches, stallions are one of—if not the—most valuable assets of the breeding operation and represent a very large financial investment. Time off in the breeding shed because of illness or injury can have significant and negative effects on both the stud farm and the stallion. Problems must thus be addressed efficiently and appropriately to minimize financial losses and maximize fertility. Understanding the relationship between the reproductive system and other organs in the stallion allows for a thorough evaluation with the intent of achieving a timely diagnosis and therapeutic plan.

Stallions are considered by some to be the most coveted and financially relevant assets of a breeding farm. Consequently, the importance of keeping them happy and healthy cannot be overemphasized. Understanding the various anatomic and physiologic attributes of breeding stallions allows for proper reproductive management and health care.

REPRODUCTIVE ANATOMY

Internal Genitalia

Testicles

Reproductively normal stallions should have paired testes that are readily palpable within the scrotum. Normal equine testes are relatively ovoid in shape. Each is oriented mostly in the horizontal plane, with the head *(caput)* and tail *(cauda)* of the epididymis located on the cranial and caudal poles, respectively, of the testis. The body *(corpus)* of the epididymis runs along the dorsal aspect of each testis. Occasionally, the testes may rotate up to 180 degrees without any apparent signs of discomfort or swelling. Such rotation is usually temporary but may occasionally become permanent. It should also be distinguished from spermatic cord rotation, which is a pathologic condition described later. This condition strays from normal and may adversely affect perfusion to the testis. As such, this finding should be taken into consideration when performing a reproductive evaluation on a breeding stallion and duly noted within the records. An important landmark is the *proper ligament of the tail of the epididymis,* which is a remnant of the fetal gubernaculum. The proper ligament attaches the tail of the epididymis to the caudal pole of the testis. Clinically, this ligament is palpable as a fibrous nodule that can be large in newborn colts and mistaken for the testis, and it may also be a useful reference in determining the relative orientation of the testis. The *ligament of the tail of the epididymis* is another gubernacular remnant that attaches the tail of the epididymis to the parietal vaginal tunic of the scrotum and anchors the testis and epididymis to the scrotum.

Each testicle is encapsulated by the tunica albuginea. This tough collagenous membrane contains smooth muscle that sends supporting trabeculae into the testicular parenchyma, dividing the testis in adjoining lobules. Functionally, this muscular investment is thought to assist with sperm transport and determine the relative tone and turgidity of the testicle.[939] Normal testes should be firm but resilient on palpation. Soft or hard testes are abnormal and may be associated with myriad conditions, including neoplasia, degeneration, infection, and trauma.

Each testis is composed of several different cell types. Some of these have endocrine functions, whereas others have exocrine or homeostatic functions. The parenchyma of the testis accounts for 85% to 90% of the testicular volume. It is composed of the seminiferous tubules and the interstitial tissue. These work in tandem to manufacture, mature, and deliver spermatozoa for fertilization of female gametes.

The seminiferous tubules are lined with an epithelial layer (seminiferous epithelium) composed of germ cells and Sertoli cells. Germ cells will be present at various stages of development, from early spermatogonia to mature spermatids, and are the most prominent cell types. The interstitium is formed by Leydig and myoid cells and comprises ~15% of the parenchyma.[940]

Because of the complexities of the spermatogenic process, it and the various stages of germ cells involved in spermatogenesis will be covered in a separate section. What follows immediately is a brief description of the other cells involved in testicular function.

Sertoli Cells. Sertoli cells are pivotal for germ cell maturation, serving as nurse cells and forming the blood-testis border. These cells help nurture and support developing germ cells through their exocrine secretions that bathe the developing germ cells. These cells also have important endocrine functions, and by secreting androgen binding protein (ABP), activin, and inhibin, they can affect the hormonal milieu of the testis. The former's role is to bind dihydrotestosterone (DHT; the bioactive form of testosterone) to maintain proper concentrations of DHT in the seminiferous tubules and epididymis, whereas the latter two are responsible for stimulating or suppressing FSH, respectively. Sertoli cells also produce other proteins that are necessary to support and regulate spermatogenesis. Examples include ceruloplasmin and transferrin, which act as carrier proteins for copper and iron, respectively, and are necessary for proper testis function.

The blood-testis barrier divides the seminiferous epithelium into basal and adluminal compartments via tight gap junctions formed between Sertoli cells. This barrier functions primarily to protect the haploid spermatozoa found in the seminiferous tubules from the host immune system that would recognize them as foreign; thus its primary function is one of providing immune privilege to the adluminal compartment of the seminiferous tubules.

The number of spermatogenic cells and ultimately the total sperm production of a given stallion are determined by the number of cells that can be accommodated between the tight gap junctions between two Sertoli cells. Sertoli cell numbers increase steadily during puberty and continue to rise with maturity. The number of Sertoli cells within the adult stallion's testes was once thought to be finite. However, recent research suggests that Sertoli cells are not stable; instead, their numbers fluctuate with season.[941]

Leydig Cells. Leydig, or interstitial, cells are the main source of testosterone production. Androgen production is mediated primarily via pituitary secretion of LH, and Leydig cells contain an abundance of LH receptors. Through steroid production, Leydig cells provide the feedback mechanisms on the pituitary necessary to maintain spermatogenesis, secondary sex characteristics, and libido. Testosterone concentration in the testicular microcirculation is at least 10 times higher than that in the systemic circulation. Season and not age appear to affect the testosterone production in adult stallions, which is mediated through a change in total number of Leydig cells rather than total volume of cells per testis. In contrast to Sertoli cells, Leydig cell numbers do not increase dramatically with age, but both number and size of these cells can vary with season.[942]

Myoid Cells. Myoid cells appear essential for proper testicular function. They provide architectural support to the seminiferous tubules and likely play an important role in intratesticular movement of sperm. In addition, myoid cells also modulate Sertoli and Leydig cell function via paracrine factors. One example is production of the mesenchymal factor PmodS, which has been shown in vitro to stimulate release of transferrin from Sertoli cells.[943]

Germ Cells. In contrast to mares, normal stallions continually produce germ cells. This process of development of a diploid germ cell to a haploid spermatozoa capable of fertilization requires approximately 57 days, and the number of germ cells produced is directly correlated with the total volume of the testes.[944] Germ cells line the seminiferous tubules and can be found in different stages of maturation from early diploid stem cell spermatogonia to almost fully mature haploid spermatids. The process by which these primordial cells transform into spermatozoa is referred to as *spermatogenesis.*

Spermatogenesis follows a chronologic order in the development of germ cells yet is also a highly complex process. The spermatogenic process will be reviewed later in the discussion of stallion physiology.

Testis Cell Interactions. The importance of the relationship between the individual cells within the testes cannot be overstated. For example, Sertoli, or "nurse," cells have a direct interaction with germ cells, and Leydig cells interact with Sertoli and germinal cells through hormonal signals. The myoid cell produces at least one paracrine factor that interacts directly with the Sertoli cell. Paracrine and autocrine modulating factors, products of the peritubular cell, appear to have an effect on ABP function. In turn, the myoid cell is under the regulatory influence of transforming growth factors α (stimulatory) and β (inhibitory). Other factors involved in cell-to-cell communication of the cells of the testis include collagen, plasminogen activator, vitamin A, pyruvate, and carbohydrates.[945] Most of these products and mechanisms have not been investigated extensively in the stallion.

Epididymides

The specific absorptive and secretory functions of each segment of the stallion epididymis remain the subject of considerable debate and investigation. The histologic structure of the epididymis changes as it continues through its different regions, with epithelial height being greatest proximally and smooth muscle components greatest distally.[946] As spermatozoa are transported from the excurrent ducts into the head, along the body, and into the tail, they undergo a number of morphologic and physiologic changes that ultimately render them motile and fertile. Specific maturational changes that occur include (1) acquiring the capacity for progressive motility, (2) shedding of the cytoplasmic droplet, (3) plasma and acrosomal membrane alterations, (4) DNA stabilization, and (5) metabolic changes.[947] All these changes occur primarily at the level of the mid to distal corpus.[947,948] The tail of the epididymis serves primarily as a storage site for nearly competent spermatozoa that are ready for ejaculation.

Fluid resorption occurs throughout the epididymis and at a steady rate, which results in a significant increase in sperm concentration. Stallions with high-volume ejaculates with relatively low sperm concentration and poor sperm morphology may have epididymal dysfunction. However, the association has yet to be clearly defined.

Spermatic Cord

The spermatic cord supplying each testis is enveloped in the parietal layer of the vaginal tunic, which extends distally from the internal inguinal ring. Within each cord are the corresponding deferent duct, testicular artery, testicular veins, lymphatic vessels, and nerves. The cremaster muscle is situated in the caudolateral borders of each spermatic cord. The testicular artery, a branch of the abdominal aorta, descends through the inguinal ring into the cranial border of the spermatic cord in a tortuous manner and divides near the testis into several branches to supply the testis and epididymis. These small branches, embedded in the tunica albuginea, enter the parenchyma by way of the trabeculae and septae of the testis. A corresponding network of veins leaves the testis and surrounds the testicular artery in a tortuous manner, forming the *pampiniform plexus.* This arrangement of artery and veins serves a critical role in thermoregulation of the testis in the stallion. The pampiniform plexus allows for heat exchange from the testicular artery to the venous side, resulting in testicular arterial blood being several degrees cooler than systemic blood temperature. Abnormal distention of the veins of the pampiniform plexus is termed a *varicocele* and is an uncommon condition in stallions. Palpation of the spermatic cord of an affected stallion reveals the dilated and often tortuous vessels. Varicoceles are usually not painful but can result in fluid accumulation around the vaginal tunics, most often involve only one side of the spermatic cord, and usually are diagnosed by the observation of the dilation of the vessels from the pampiniform plexus with ultrasonography. The condition has been identified in stallions with normal semen parameters.[949]

Accessory Sex Glands

In the stallion, the *accessory sex glands* are composed of the bulbourethral glands, prostate gland, seminal vesicles, and ampullae. Their secretions produce the seminal plasma that makes up most of the ejaculate volume. The ampullae, which are dilations of the vas deferens before opening in the colliculus seminalis, are also considered a storage site for sperm.

Short exposure to seminal plasma appears to be important for sperm function, but long-term exposure to seminal plasma components may be detrimental to spermatozoa survival for some stallions. Often termed "toxic" seminal plasma, spermatozoa exposed to certain stallions' seminal plasma quickly lose their viability, leading to subfertility. AI programs deal with this potential detrimental effect by dilution of semen with semen extenders in fresh or fresh-chilled programs and by centrifugation to remove seminal plasma in frozen semen programs and some chilled-shipped programs. Live-cover operations may counter toxic seminal plasma by infusing semen extender into the mare's uterus before or after mating.

Seminal plasma appears to suppress the inflammatory response of the endometrium of the mare to sperm after insemination or natural mating. Although the functions of the specific components of the seminal plasma remain rather obscure, the fluid suspends the ejaculated sperm and also is thought to be a source of energy, protein, and other macromolecules required for sperm functions and metabolism.[950-952]

Next is a brief discussion on the anatomy and functionality of the stallion's accessory sex glands.

Bulbourethral Glands. Multiple ductules from the bulbourethral glands enter the dorsomedial wall of the pelvic urethra caudal to the prostatic ductules. Bulbourethral gland secretions compose most of the presperm or first fraction of the ejaculate and serve as a cleanser and pH stabilizer in the urethra before ejaculation.

Prostate Gland. In the stallion the prostate is formed by a central isthmus and two lateral lobes located on the caudolateral borders of each vesicular gland. Multiple ductules from the prostate enter the lumen of the pelvic urethra in the dorsal urethral wall lateral to the colliculus seminalis. The watery secretions of the prostate contribute to the presperm fraction of the ejaculate.

Ampullae. The ampullae are the enlarged distal portions of the deferent ducts. They converge caudad and pass beneath the prostate gland but lie dorsal to the pelvic urethra. At their distal ends they continue through the dorsal wall of the urethra, opening into the colliculus seminalis alongside the excretory ducts of the seminal vesicles. The ampullae, in addition to serving as a sperm storage area, have many branched tubular glands located within the thickened wall.[953]

The ampullae are a common site for blockage caused by stasis of sperm (as would be seen in a sexually inactive stallion) or an inability to effectively void spermatozoa. This condition is referred to as *spermiostasis* or *plugged ampullae* and can cause subfertility if not properly managed. This condition will be discussed later.

Seminal Vesicles. The seminal vesicles or vesicular glands are paired, pyriform, and thin-walled structures lying lateral to the ampullae. On occasion they may extend far cranially to hang over the brim of the pelvis. The caudal ends of the glands converge, passing under the prostate as they lie parallel to the ampullae, terminating in the seminal colliculus in the dorsal wall of the urethra. The excurrent ducts of the vesicular glands open lateral to the excurrent ducts of the ampullae at the colliculus seminalis of the urethra. Secretions of the vesicular glands compose the gel fraction of the ejaculate. Higher gel volumes can be collected with increased sexual stimulation and season. The specific function of the gel fraction is unclear, and it should be removed when processing semen for evaluation or AI. The seminal vesicles are the glands that are most prone to bacterial infections. Diagnosis is based on the cytologic evaluation of the semen with the presence of white blood cells. Treatment and management typically involve both local and systemic antibiotics based on culture and sensitivity results.

External Genitalia

The penis and scrotum comprise the extent of a normal stallion's external genitalia. The penis is comprised of smooth muscle and encased in a sheath of tissue, often referred to as the prepuce. The penis of the stallion is composed of a root, a body, and a glans penis and is musculocavernous. The penile base arises at the ischial arch in the form of two crura that fuse distally to form the single dorsal corpus cavernosum penis (CCP) enclosed by a thick tunica albuginea. The corpus cavernosum, corpus spongiosum, and corpus spongiosum glandis are the three spaces that make up the erectile tissue of the penis. Engorgement of these spaces with blood from branches of the internal and external pudendal arteries and obturator arteries is responsible for an erection. The cavernous spaces within the penis are continuous with the veins responsible for drainage. The corpus spongiosum originates in the pelvic area and surrounds the penile urethra within a groove on the ventral side of the penis and forms the corpus spongiosum glandis at the distal end of the penis.[954] The corpus spongiosum glandis creates the distinct bell shape of the stallion penis after ejaculation.

The distal end of the urethra contains a distinct urethral process, which is visible within the center of the glans penis and surrounded by an invagination known as the fossa glandis. Within the fossa glandis are diverticulae and a urethral sinus. Accumulation of secretions in these areas can occur to form "smegma," and further coalescence can form semifirm concretions ("beans") within one or all of these sites. Therefore careful examination and cleansing of this area are imperative during the reproductive evaluation of a stallion or before breeding.

The bulbospongiosus muscle and two retractor penis muscles are located ventrad on the stallion penis and run the length of the organ. The former is responsible for providing rhythmic contractions or pulsations to assist in moving the penile urethral contents (semen and urine) distally during ejaculation; the latter are responsible for returning the penis to the sheath after detumescence.

The prepuce is formed by a double fold of skin that is hairless and well supplied with sebaceous and sweat glands. The primary preputial function is to contain and protect the nonerect penis. The external part of the prepuce, or sheath, begins at the scrotum and is continuous with the scrotal raphae. The internal layer of the prepuce extends caudally from the orifice to line the internal side of the sheath and then reflects cranially toward the orifice again before reflecting caudally to form the internal preputial fold and preputial ring. This additional internal fold allows the considerable lengthening (approximately 50%) of the penis during erection. During erection the preputial orifice is visible at the base of the penis just in front of the scrotum, and the preputial ring is visible at approximately the midshaft of the penis.

The penis and prepuce of a breeding stallion can best be examined after teasing with an estrous mare, when one can observe the stallion drop the penis and attain a full erection. The prepuce and penis should be free of vesicular, proliferative, or inflammatory lesions such as those found in horses with coital exanthema, squamous cell carcinoma, or cutaneous habronemiasis. Removal of smegma accumulations is necessary for a complete examination of the skin surfaces.

REPRODUCTIVE PHYSIOLOGY OF THE STALLION

Sexual Differentiation

Mammalian sexual differentiation is a complex process. Timing is crucial, and an intricate series of intracellular and extracellular signaling events during specific periods of embryonic and fetal development must occur for sexual differentiation to proceed normally. Sexual differentiation is established by the chromosomal sex at fertilization when oocytes comprising the X chromosome encounter the X-bearing or Y-bearing sperm. Ovarian or testicular development pathways are initiated by the bipotential embryonic gland, which is a diverse population of somatic cells and germ cells. The testis-determining factor, located on the Y-region, is known as the sex-determining region (*SRY* in humans, and *Sry* in horses and other mammals). The presence of *SRY* activates the testis-specific pathway and suppresses the ovary-specific genetic development pathway in most mammals, and the reader is referred to more comprehensive reviews on this topic.[955-959] The protein product SRY is expressed in XY somatic cell precursors and in conjunction with steroidogenic factor 1 *(SF1)* acts as a molecular switch specifically to upregulate expression of *SOX9* in the *SOX* (*SRY*-related high-mobility group box) gene family.[957] Initially, *SOX9* is expressed in the bipotential gonad of both sexes, whereas *SOX9* expression in XX gonads is downregulated, and expression of *SOX9* in XY gonads is significantly upregulated. On reaching a certain threshold, somatic cell precursors differentiate into testis-specific Sertoli cells.[957] Upregulation of *SOX9* protein expression also leads to a series of molecular events to advance testis differentiation by promoting cellular and structural organization. Protein expression of *SOX9* is also responsible for a negative-feedback loop to repress *SRY* expression. Despite cessation of *SRY* expression, expression of the *SOX9* and *SF1* proteins continues and is maintained at high levels in Sertoli cells to further promote Sertoli cell development. Factors and signaling pathways that also play an important role in Sertoli cell differentiation include the signaling molecule *FGF9* (fibroblast growth factor) and its

receptor *FGFR2,* as well as prostaglandin D2 (PGD2). Expression of *FGF9* is activated downstream of *SOX9* and *SRY.* Both *FGF9* and *SOX9* work together in a positive feedforward loop in which SOX9 protein is required to upregulate *FGF9* expression. This then maintains *SOX9* expression to further promote differentiation into Sertoli cells.[960] In the absence of *FGF9* or *FGFR2* expression in XY mice, *SOX9* expression was lost and Sertoli cells did not develop, resulting in sex reversal.[961,962] Upregulation of *FGF9* during testis development also actively represses the female gene wingless-related MMTV integration site 4 *(WNT4),* which is required for ovarian development.[963] Thus *FGF9* expression is critical for testis development.

Another autoregulatory loop that works independently from *FGF9* and promotes Sertoli cell development is the PGD2-mediated signaling pathway. Sertoli cells secrete PGD2, causing neighboring somatic cell precursors to express and accumulate *SOX9,* spawning more Sertoli cells,[964] but this particular signaling pathway is not essential for testis development.

Testicular Descent

Normal descent of the testes into the scrotum is somewhat variable and typically occurs between the last 30 days' gestation and the first 10 days of birth. In some colts the testes may descend into the inguinal region and remain there for some time before fully descending. Both endocrine and anatomic events must occur for proper migration of the testes into the scrotum. Androgens and AMH are involved in the signaling for testicular descent.[965-968] Traction of the gubernaculum, which attaches the caudal pole of the testis to the parietal vaginal tunic of the scrotum, is believed to draw the developing testis and epididymis into and through the inguinal ring.[2]

Cryptorchidism is the condition in which one or both testes fail to descend into the scrotum by 2 years of age. It is one of the most common congenital defects occurring in colts, yet the causes remain obscure and are presumably multifactorial. A true genetic link has not been found, but anecdotal evidence abounds about certain family lines in particular breeds whose males are prone to the condition. Diagnosis and management of this condition will be discussed later.

Puberty

This period is defined as the age at which a colt is able to mount, copulate, and successfully impregnate a mare and generally occurs during the second spring after the year of birth in the Northern Hemisphere. It should be noted that in some cases colts as young as 8 months have been able to achieve pregnancies. In some large breed horses (e.g., draft breeds) there may be a delay in puberty. Puberty should not be confused with sexual maturity. Whereas puberty signifies the time in which a male can successfully impregnate a female, sexual maturity indicates the time period after which a stallion has fully developed secondary sexual characteristics and fully developed genitalia. Puberty occurs earlier (e.g., ~2 years of age), whereas sexual maturity is reached at approximately 5 years of age.

The hypothalamic pulse generator is a group of cells located in the arcuate nucleus of the hypothalamus.[965] Neuroendocrine activity from this generator stimulates the onset of puberty via pulsatile secretion of GnRH from the hypothalamus, which stimulates the secretion of LH and FSH from the anterior pituitary. Season, age, breed, nutritional status, and external hormones affect puberty. In horses, onset of puberty occurs around 12 to 18 months and is complete by 18 to 24 months, as previously mentioned.[969]

Endocrinology

The pineal gland plays a significant role in the seasonality of the horse. Specifically, the retina captures photoperiod information and transports it by way of nerve fibers to the pineal gland. In horses, photoperiod stimulation (i.e., long days) inhibits the production of melatonin. Because melatonin inhibits GnRH release in horses, suppression of melatonin leads to increased pulses of GnRH from the hypothalamus. Unlike mares, stallions do not undergo a complete reproductive quiescence during short days and will continue to produce sperm year round. The cause for this partial refractoriness of the stallion to changes in photoperiod is not well understood but likely clinically insignificant in normal fertile stallions.

The hypothalamus, pituitary, and testes (HPG axis) must work in synchrony for a stallion to be able to start and sustain sperm production. The primary role of the hypothalamus is the production of the 10-amino-acid peptide GnRH, which is secreted in multiple daily pulses and then transported via the hypothalamic-pituitary portal system to the anterior pituitary. In addition to melatonin-mediated stimuli, the hypothalamus responds to tactile, olfactory, and visual stimuli.[970]

The pituitary, which is connected to the hypothalamus by neural fibers, has two lobes. The anterior lobe possesses GnRH receptors. Binding of GnRH activates these receptors and induces secretion of FSH or LH. FSH and LH act on the Sertoli and Leydig cells, respectively, stimulating production of steroids and other protein hormones. The peptide hormones inhibin and activin regulate FSH secretion at the level of the pituitary. Steroid hormones produced in the testis (e.g., estradiol, testosterone) feed back at the level of the pituitary to inhibit FSH and LH secretion and to regulate GnRH secretion from the hypothalamus.

Because of the complexity of interactions among hormones, their target tissues and other important physiologic events, current methods do not exist to accurately predict infertility in stallions. As will be discussed later, pharmaceutical therapy, particularly supplementation with exogenous hormones, is empiric, somewhat unreliable, and only of benefit in certain instances.

Spermatogenesis

Spermatogenesis describes a series of chronologic changes occurring within the seminiferous tubule that transforms a large, round diploid spermatogonium into a haploid spermatozoon capable of fertilization. This process takes approximately 57 days in stallions and is not affected by frequency of ejaculation or sexual stimulation. Next will be a brief review of the spermatogenic process. However, many of the complexities are beyond the scope of this chapter, so the reader is directed to reviews on this subject for more in-depth information.[939,949,971]

The process starts when an A_1 stem cell spermatogonia undergoes mitosis, giving rise to (1) a second A_1 spermatogonia to maintain a constant population of stem cells and (2) an A_2 spermatogonia. In turn, the A_2 spermatogonia gives rise to A_3, A_3 to B_1, and B_1 to B_2. Spermatogenesis can be divided into three phases of similar length: *spermatocytogenesis, meiotic divisions,* and *spermiogenesis.*

Spermatocytogenesis is characterized by the mitotic divisions of A and B spermatogonia. Meiotic divisions result in the

formation of primary spermatocytes from B_2 spermatogonia, followed by formation of secondary spermatids. Leptotene spermatocytes that form immediately after the first meiotic division are shielded from the host's immune system by the blood-testis barrier. Spermiogenesis is characterized by the transformation of round spermatogonia to elongated spermatids and, ultimately, spermatozoa. During spermiogenesis, the acrosome arises from the Golgi complex, and compaction of DNA occurs because of expression of protamine genes at the spermatid stage. The end of spermatogenesis is characterized by *spermiation,* which is the release of elongated spermatids into the lumen of the seminiferous tubule and represents the transition to a spermatozoa.[972]

Seasonality

Stallions are subject to seasonal influences. For example, concentrations of LH and testosterone, DSO, and libido all increase during times of the year that coincide with periods of longer exposure to light, or "long days."[973-975] However, stallion reproduction does not appear to be as tightly regulated by season as that of mares. When given the opportunity, stallions will breed year round and are capable of ejaculating normal sperm and achieving adequate pregnancy rates.[973]

Because stallions are seasonal long-day breeders, exposure to artificial lighting programs has been used in the management of commercial breeding stallions. It is thought that it can hasten the onset of improved breeding behavior and sperm production. Such strategies are used on several commercial stud farms in the United States. Two studies reported no detrimental effect on photostimulated stallions.[975] Another study demonstrated that light therapy increased testicular size and sperm production, but changes were transient.[976,977] In addition, some stallions experienced a reduction in testis size and circulating testosterone concentrations by June, which could potentially have a negative effect on reproductive efficiency, especially in stallions of already questionable fertility.

Sexual Behavior

Stallions will demonstrate sexual behavior in many ways. Vocalization, snorting, bowing of the neck, biting/nipping, striking, and the flehmen response are all typical signs associated with normal breeding behavior. Depending on the individual stallion, these precopulatory signs will be expressed with various frequencies and intensities. *Libido* or "sex drive" is the vigor with which stallions display breeding behavior. Strength of libido is dependent on numerous factors such as genetics, farm and breeding management, season, and external stimuli (olfactory, visual, and auditory) and is highly variable among individual stallions. When exposed to an estrous mare, stallions should drop their penis within 2 minutes, which is referred to as the "latency to erection" period, and attempt to mount the mare or phantom mount in 3 to 5 minutes. Once intromission occurs, stallions give several (five to eight) vigorous pelvic thrusts, followed by three to five short thrusts immediately before ejaculation. External signs of ejaculation that are used to determine whether ejaculation has occurred are rhythmic and frequent urethral pulsations in the penis (fremitus), flagging of the tail, and head relaxation. A single stallion tends to be consistent in his breeding behavior, provided that the conditions under which he usually mates are the same.[978] Exceptions to these rules are stallions that have never bred before (i.e., *novice stallions*). Their behavior can be somewhat erratic because of inexperience but will evolve and stabilize with increased breeding frequency. Management of the abnormal behavior and the novice stallion will be discussed later.

CLINICAL EVALUATION OF THE STALLION

Assessments of stallions for their suitability as breeding animals are called breeding soundness evaluations (BSEs). However, the author prefers the term *stallion reproductive evaluations* (SREs) because BSE is commonly associated with examination of bulls for their relative virility. The criteria used to determine the value of and measure the reproductive performance of males are vastly different between the two species. Thus it seems reasonable to use a separate term to avoid confusion.

Many SREs are performed as either part of a prepurchase examination or as annual evaluations (typically performed before the breeding season) to monitor reproductive capacity. Other indications for SREs would be a precipitous drop in fertility or as partial fulfillment of an insurance examination. An example of a typical form to record findings from an SRE is shown in Fig. 19.25.

Recommendations for determining whether a stallion met the criteria to be considered *satisfactory* for breeding were outlined in the (no longer available) *Manual for Clinical Fertility Evaluation* published by the Society for Theriogenology in 1983.

Standards by which a stallion is judged to be a satisfactory breeder include the ability to

1. Render 75% of 40 mares pregnant via live cover (or 120 mares by AI) in one breeding season;
2. Produce ≥1 billion progressively motile, morphologically normal sperm in the second of two ejaculates collected an hour apart;
3. Have a total scrotal width of 8 cm; and
4. Be free of undesirable/hereditable defects.

Stallions failing to meet these requirements are given either *differed* or *unsatisfactory* status based on findings from the evaluation. Much advancement in our knowledge of reproductive physiology and breeding management has been made since this manual's publication, and some theriogenologists have acknowledged the need for updating the requirements.[979] Moreover, problems often arise when trying to quantify biologic systems, especially when there is more "skin in the game" than just finances. For example, there are certain intangibles a stallion may represent that prove nearly impossible to calculate. Farm management practices, whether knowingly or unknowingly, can also influence reproductive efficiency. Therefore data obtained from the SRE should always be interpreted with respect to individual farms and stakeholders.

Breeding Records

The importance of maintaining accurate and current breeding records cannot be overstated. Both computer-generated and manual records can be used to evaluate and track a stallion's reproductive efficiency. In addition, these records are invaluable to clinicians when asked to assess a stallion for his reproductive performance.

In central Kentucky, "Horse Farm Management," provided by the Jockey Club Information Systems (Jockey Club Information Systems, Lexington, KY) is an example of a digital

program that can track and compile data about past and current reproductive parameters. For example, this program can generate reports on the numbers of mares bred, mare information, and seasonal summary reports for individual stallions or the stud farm as a whole. This information can then be tallied to evaluate breeding efficiency and/or factors associated with poor performance.

Physical Examination

Stallions should be sound in both mind and body to be suitable for breeding. Organ systems should be evaluated for signs of diseases or disorders that could adversely affect breeding performance. In particular, diseases of the musculoskeletal system can significantly impede reproductive efficiency, and chronic disorders (e.g., arthritis, laminitis) can drastically shorten a stallion's breeding career. When or if possible, baseline radiographs of the distal limbs can prove useful for screening, monitoring, and treating orthopedic issues.

Careful attention should be paid to the external genitalia. Visual and manual inspection of the entire penis, sheath, and scrotum for size, texture, consistency, and signs of dermatitis are indicated. Measurement of total scrotal width using calipers or ultrasonography is useful for measuring reproductive efficiency. As will be discussed in the next section, ultrasonography is a highly useful means to assess the internal anatomy of the stallion, especially the testes.

Evaluation of the External Genitalia

Ultrasonography of the testes is a useful diagnostic tool for screening for pathology, monitoring progression of disease, and predicting reproductive capacity. Pathology of the testes will be discussed later, but at a minimum ultrasound and

ROOD & RIDDLE EQUINE HOSPITAL

LeBlanc Reproduction Center
2150 Georgetown Road
Lexington, KY 40511

Stallion Reproductive Evaluation Form

Stallion Information	
Name: Choose an item.	Year of Birth: Choose an item.
Breed: TB	Color: Choose an item.
Current Use: Choose an item.	Tattoos or Microchip: Click here to enter text.
Location of Evaluation:	
Evaluator:	

Health History	
Vaccinations	Deworming History
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Last PEC: Click here to enter a date. Result of PEC: Click here to enter a text. Last Dewormed: Click here to enter a date. Deworming Product: Choose an item.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Last Coggins: Click here to enter a date. Result: Choose an item. Lab and it: Click here to enter a text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Last CBC/Fib: Click here to enter a date. Result/Interpretation:
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Last Biochemistry: Click here to enter a date. Result/Interpretation: Click here to enter a text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Last Endocrine Testing: Click here to enter a date. Result/Interpretation: Click here to enter a text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Aerobic Bacterial Cultures Penis: Click here to enter text. Fossa Glandis: Click here to enter text. Pre-Ejaculatory Urethra: Click here to enter text. Post-Ejaculatory Urethra: Click here to enter text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Past-Lameness Issues: Click here to enter text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Past Surgeries: Click here to enter text.
Diseases: Choose an item. Product: Choose an item. Date Given: Click here to enter a date.	Current Medications: Click here to enter text.

FIG. **19.25** Example of a stallion reproductive evaluation (SRE) form.

Continued

ROOD & RIDDLE EQUINE HOSPITAL

Reproductive History	
Previous Season	Past Reproductive Issues
Total No. Mares Bred = Click here to enter text.	Issue #1: Click here to enter text.
Total No. Covers = Click here to enter text.	Issue #2: Click here to enter text.
Seasonal Pregnancy Rate = Click here to enter text.	Issue #3: Click here to enter text.
Pregnancy Rate / Cycle = Click here to enter text.	Issue #4: Click here to enter text.
No. Covers / Pregnancy = Click here to enter text.	Past Breeding Behavior: Choose an item.
No. Covers / Mare = Click here to enter text.	Typical No. Jumps per Mare: Choose an item.

External Genital Examinations				
Date Performed: Sunday, March 06, 2016				
Palpation and Visual Inspection				
	Penis/Prepuce	Scrotum	Left Testis	Right Testis
Size	Choose an item.	Choose an item.	Choose an item.	Choose an item.
Cinsistency	Choose an item.	Choose an item.	Choose an item.	Choose an item.
Other Findings	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Ultrasonographic Findings		
	Left Testicle	Right Testicle
Length (cm)	0	0
Width (cm)	0	0
Height (cm)	0	0
Volume (cm^3)	0.0	0.0
DSO ($x10^3$)	-1.26	

Accessory Sex Gland Examination	
Date Performed:	
Method Used: Choose an item.	
Bulbourethral Glands	Click here to enter text.
Prostate	Click here to enter text.
Seminal Vesicles	Click here to enter text.
Ampullae	Click here to enter text.
Bladder	Click here to enter text.

FIG. **19.25, Cont'd**

palpation of the testes should be performed to determine the size, tone, orientation, and character of the testes and associated structures. Measurements of the *height, width,* and *length* of each testis can be obtained with a standard 5-MHz or 7.5-MHz linear rectal probe. These values can be used to calculate testicular volume and then the estimated DSO[980] and breeding frequency expected for testes of that size.[981] Table 19.11 shows the relationship among testicular volume, DSO, and expected number of daily breedings determined in Thoroughbred stallions performing in a commercial live-cover program.

Specific techniques for examination of the testis have been previously discussed.[982,983] Beginning at the proximolateral aspect of the scrotal neck with the long axis of the probe parallel to the ground, the spermatic cord can be imaged. The arrangement of the pampiniform plexus results in the mottled, heterogeneous appearance of the spermatic cord, and the testicular artery and veins are identifiable in cross-sectional images. If the ultrasound machine is equipped with a power Doppler function, then evaluation of the vasculature can be performed and compared with previously established values.[984,985] Slowly moving distal and cranial toward the cranial pole of the testis, the probe can then be oriented in a vertical position (i.e., long axis perpendicular to the ground) and moved caudad. Visualization of the scrotum reveals a thin, echogenic uniform layer. Minimal, if any, fluid is visible between the scrotal skin and testicular parenchyma in the normal stallion. In the cranial third of the scrotum, the head of the epididymis, testicular parenchyma, blood vessels of the spermatic cord, and central vein of the testis are visible.

As the probe is moved caudal, the spermatic cord vessels disappear, and the head of the epididymis continues into the body of the epididymis. The head and body of the epididymis appear as heterogeneous areas just below the spermatic cord. Further caudad, the body of the epididymis becomes indistinct. With the exception of the central vein, the testicular parenchyma appears uniformly echogenic and homogeneous. The central vein appears as a small anechoic area within the testicular parenchyma at the cranial third of the testis and should not be mistaken for a pathologic lesion. Dilation of the central vein may be visible in cases of varicocele or spermatic cord torsions and usually is accompanied by detectable dilations of the vessels of the spermatic cord. Well-defined and hypoechoic or hyperechoic lesions within the parenchyma suggest testicular tumors.

ROOD & RIDDLE EQUINE HOSPITAL

Semen Collection and Evaluation			
Date Performed: Click here to enter a date.			
Method of Semen Collection: Choose an item.			
Use of Jump Mare or Phantom: Choose an item.			
Method of Concentration Determination: Choose an item.			
Method of Motility Evaluation: Choose an item.			
Use of Semen Extender: Choose an item.		Semen Extender Used: Choose an item.	
	Ejaculate 1	Ejaculate 2	Ejaculate 3
Date and Time			
Libido	Choose an item.	Choose an item.	Choose an item.
Erection	Choose an item.	Choose an item.	Choose an item.
No. Mounts	Choose an item.	Choose an item.	Choose an item.
Breeding Ability	Choose an item.	Choose an item.	Choose an item.
Gross Appearance	Choose an item.	Choose an item.	Choose an item.
Volume Gel			
Volume (gel free; mL)			
Concentration (x 10^6/mL)			
Total No. Sperm			
Total Motility (%)			
Progressive Motility (%)			
Velocity			
Sperm Morphology			
Method of Morphology: Choose an item.			
	Ejaculate 1	Ejaculate 2	Ejaculate 3
%Normal Sperm			
%Detached Heads			
%Abnormal Heads			
%Abnormal Acrosomes			
%Abnormal Midpieces			
%Tail Bent			
%Tail Coiled			
%Proximal Droplets			
%Distal Droplets			
%Round Cells			
%Other			
Sperm Longevity			
Ejaculate 1		Ejaculate 1	
Method Used: Choose an item.		Method Used: Choose an item.	
Date/Time		Date/Time	
Total/Progressive Motile		Total/Progressive Motile	
Date/Time		Date/Time	
Total/Progressive Motile		Total/Progressive Motile	
Date/Time		Date/Time	
Total/Progressive Motile		Total/Progressive Motile	
Adjunctive Tests			
Scanning Electron Microscopy			
Membrane Integrity			
Sperm Chromation Structure Assay			
Acrosomal Status			
Semen Cytology			

FIG. **19.25, Cont'd**

Continued

After reaching the most caudal aspect of the testis, the clinician rotates the probe to face cranially in a vertical position to allow examination of the tail of the epididymis. This structure appears as a heterogeneous area with a Swiss cheese–like appearance. Identification of the epididymal tail may assist in diagnosis of testicular rotations. In horses with 360-degree torsions, the tail of the epididymis, although in its caudal position, is more dorsal because of the tension on the ligament of the tail of the epididymis by the deferent duct.

ROOD & RIDDLE EQUINE HOSPITAL

Overall Evaluation
Based upon the above findings, including—but not limited to—a careful physical examination, a thorough review of past and present breeding ability, and a systematic evaluation of the genitalia and seminal characteristic of this stallion, it is my professional opinion that this stallion:

Sincerely,

FIG. **19.25, Cont'd**

TABLE **19.11** **Relationship Between Testicular Volume, Daily Sperm Output, and Expected Daily Breeding Frequency in Thoroughbred Stallions in a Commercial Live-Cover Breeding Operation**[981]

	Estimating Breeding Book Size Based on Testicular Measurements	
Testicular Volume (mL)	Range in Predicted Daily Sperm Output (x 10^9)	Number of Covers per Day
200	3.54–4.04	2.36–2.69
250	4.74–5.24	3.16–3.49
300	5.97–6.44	3.96–4.29
350	7.14–7.64	4.76–5.09

Some stallions may have only a single testicle because the other one is either retained or removed. Most of these stallions can perform adequately as breeding stallions. The functional testis will grow in size (i.e., hypertrophy) and usually produce adequate numbers of sperm. The developmental disorder of *cryptorchidism* will be discussed in a later section.

Evaluation of the Internal Genitalia

The majority of the internal reproductive structures of interest can best be evaluated and imaged using rectal palpation and ultrasonography. Most stallions tolerate transrectal palpation well with adequate restraint. The important structures are located relatively close to the anus in the caudal pelvic region, usually requiring the operator to insert only the hand into the rectum. The accessory sex glands, ampullae, and pelvic urethra are much easier to palpate and visualize on sexually stimulated stallions. Next are descriptions of the appearance of these structures, and the reader is referred to several publications for more detailed information.[986] Thorough evaluation of the urethra is best accomplished using endoscopy, although ultrasonography can be used to supplement endoscopic findings.

Bulbourethral Glands. The paired bulbourethral glands, although not usually palpable rectally because of the urethralis and bulboglandularis muscles close to the ischiatic arch, are easy to evaluate by ultrasonography. They are located 3 to 4 cm cranial to the anus, lateral to the pelvic urethra, and will appear as variably sized yet distinct ovoid structures with multiple hypoechoic regions throughout the parenchyma.

Prostate Gland. In the stallion, the prostate is formed by a central isthmus and two lateral lobes located on the caudolateral borders of each vesicular gland. Although not always palpable rectally, the prostate is lobulated or nodular and firm, distinguishing it from the smooth, thin-walled vesicular glands lying medial to the prostatic lobes. Each prostatic lobe measures 5 to 9 cm long, 2 to 6 cm wide, and 1 to 2 cm thick. They appear as two symmetric and homogeneously echogenic lobes visible lateral to the area in which the penile urethra merges with the neck of the bladder. Hypoechoic dilations within the gland's parenchyma are usually evident in a teased stallion.

Ampullae. The ampullae are the enlarged distal portions of the deferent ducts measuring 1 to 2 cm in diameter and 10 to 25 cm in length. Palpable along the midline of the pelvic floor over the neck of the bladder, they converge caudally and pass beneath the prostatic isthmus to terminate in the seminal colliculus in the dorsal wall of the pelvic urethra. Because of the longitudinal orientation of the ampullae, sometimes they are easier to find on rectal palpation. One can identify them by ultrasonography by their hypoechoic central lumen surrounded by a uniformly echogenic wall and a hyperechogenic outer muscular layer. Orienting the transducer in a transverse position inside the rectum can provide a good cross-sectional image of the ampullae.

Seminal Vesicles. Palpation of the vesicular glands may be easier after considerable teasing of the stallion. With adequate stimulation, the vesicular glands can be felt as dilated and elongated structures measuring 12 to 20 cm long and 5 cm in diameter. The glands also are readily palpable in instances of pathologic enlargement. With ultrasonography the vesicular glands appear in a longitudinal section as a flattened oval to triangular sacs, depending on the degree of sexual stimulation. A thin echogenic wall surrounds a lumen containing fluid of

variable echogenicity. Increased echogenicity of vesicular gland fluid is associated with the highly viscous gel fraction produced by some stallions. The seminal vesicles are the accessory sex glands most prone to bacterial infections. Diagnosis is based on the cytologic evaluation of the semen with the presence of leukocytes, which are often most elevated in the gel fraction of the ejaculate.

Pelvic and Penile Urethra. Although the pelvic urethra can be evaluated by transrectal ultrasonography, in most cases little to no pathology is identified. Endoscopic examination is often more reliable in identifying sites of hemorrhage, rents, or abnormal mucosal surfaces, as well as allowing excellent visualization of the bladder.[987,988] The procedure is performed by gently passing a 1-m endoscope with an outer diameter of 8 to 9 mm into the urethra of a sedated horse. The penis is washed, and lidocaine gel and/or sterile lubricant are applied to the endoscope to facilitate passage. The clinician applies gentle and constant pressure to pass 70 to 80 cm of the endoscope into the urethra. Care must be taken not to inflate the bladder with too much air because of a slight risk of rupture.

The ductules to the bulbourethral glands, which are conduits from the gland to the urethral lumen, are arranged as two rows of 6 to 10 small openings dorsal and abaxial along the urethra. The prostatic ductules are arranged in a similar way and are visible as two groups of small openings lateral to the ejaculatory orifices of the seminal colliculus. The ductules to the bulbourethral gland are grouped about 2.5 to 3 cm caudal to the prostatic openings.

One can identify the colliculus seminalis as a rounded prominent structure found on the medial aspect of the dorsal wall of the urethra approximately 5 cm caudal to the internal opening of the urethra from the bladder. On either side of the colliculus is an ejaculatory duct orifice, which is a small slit-like diverticulum within which the ampullary ducts and ducts of the seminal vesicles open. By passing the endoscope into this orifice, one can visualize and evaluate the seminal vesicles. Samples can be collected for culture in cases of suspected seminal vesiculitis.[989] Ventral and cranial to the seminal colliculus are the openings of the urethral glands. These are visible laterad on the widened pelvic portion of the urethra at the level of the prostatic gland openings.

Endoscopic examination of the urethra is indicated in stallions with clinical signs suggestive of pathology of the urethra and accessory sex glands. In cases of hemospermia the bleeding area may be visualized with the endoscope. These lesions are most readily identified in the region of the ischiatic arch and distal urethra. One should take care to assess the urethral mucosa as the endoscope is passed forward because some irritation and erythema of the mucosal lining often results from the endoscopic examination. A false diagnosis of urethritis may result if one assesses the mucosa while withdrawing the endoscope.

Semen Collection and Evaluation

When a reproductive evaluation of a stallion is performed, two ejaculates are collected approximately 1 hour apart. Semen samples are collected with the use of an artificial vagina (AV). Ejaculates are collected either with the stallion mounted on an estrous mare or a breeding mount/phantom. Some stallions will not ejaculate into an AV, in which case they are allowed to breed a mare in estrus, and the drippings are collected from the stallion as he dismounts the mare. Other means of semen collection include aspiration of semen from the uterus and vagina of a recently bred mare, use of a large condom, and pharmacologically induced ejaculation.

Artificial Vaginas

Several different types of AVs are commercially available. Considerations for selecting an AV include size, cost, frequency of use, and stallion preference. Temperature and pressure of the AV are two important factors, and these can be adjusted accordingly for individual stallions. Most stallions favor warmer temperatures, such as 44°C to 48°C, for adequate stimulation. Adequate pressure and nonspermicidal lubrication are also necessary and important factors for successfully collecting semen.

If experiencing difficulty in obtaining an ejaculate, troubleshooting the various aspects of the process is useful for identifying the problem(s). Some instances can be caused by incorrect positioning of the AV, which places the penis at an awkward angle. Other instances include low AV temperature, poor lubrication, or inadequate size. Manual pressure to the glans and/or base of the penis or use of a warm compress can increase stimulation. Jump mare selection can also be important in that some stallions may prefer a mare in natural estrus as opposed to an ovariectomized mare receiving exogenous estrogen.

Colorado State University Model Artificial Vagina. The Colorado State University (CSU) model is essentially a large piece of PVC piping with a water bladder and a removable liner. It has an inline filter to remove debris and the gel fraction from the ejaculate. Advantages include its size and ability to maintain proper temperatures in cold weather and for extended periods. Additionally, the relative rigidity of the CSU model provides the operator better control over particularly aggressive stallions. However, the size is also a disadvantage, making it heavy and cumbersome. Heat shock is also a concern when using this model because ejaculated semen is exposed to the liner inside the AV (~46°C), which is hotter than normal and enough to cause thermal damage to spermatozoa. Finally, the CSU model requires fairly precise assembly before use, causing it to be somewhat higher maintenance than other models discussed below.

Missouri Model Artificial Vagina. The Missouri equine AV is likely the most popular type of AV in the United States. Unlike the CSU model, it requires less assembly and is made by two molded layers of latex forming a water jacket. It also can be fitted with an inline filter and is held in place with a leather or neoprene case. The Missouri model is much less rigid than the CSU model, and most stallions will ejaculate beyond the water jacket, preventing semen from being exposed to increased temperatures. This AV also allows for application of pressure on the glans or base of the penis, which can be difficult with the CSU model due to its relative rigidity.

Hanover Model Artificial Vagina. The Hanover model AV is more common in Europe, is shorter and smaller in diameter than the CSU AV, and is made of a hard rubber casing and inner rubber liner. This AV should work well for most stallions. Ejaculation occurs at or near the end of the water jacket. Pressure of the Hanover model AV is critical. This AV has an eccentric opening. If the AV is too loose, then the nonengorged glans can come through the ring, and glans dilation occurs on the other side of the opening, which is painful for the stallion.

Polish or Open-Ended Model Artificial Vagina. The Polish model is substantially different from other models on the market. Using the open-ended AV, one can visualize the process of

ejaculation and can collect individual jets of presperm, sperm-rich, or gel fraction of the ejaculate. This AV has been valuable in the diagnosis of hemospermia, urospermia, internal genital tract infections, and ejaculatory failure.[990] Additionally, this AV has been useful in obtaining semen for commercial use from stallions with hemospermia and urospermia, because most of these affected stallions ejaculate the blood or urine after the initial jets of sperm-rich semen. The open-ended AV also has been useful in cryopreservation programs for obtaining sperm-rich and bacteria-free ejaculates from stallions. This method of collection is also used to obtain "clean" ejaculates from stallions that are untrained and intolerant of penile washing. The Polish AV also allows the use of high internal AV temperatures without the risk of sperm cell damage because the ejaculate usually is emitted directly into a funnel with an attached receptacle held by a second person. Open-ended AVs are not currently available in the United States but can be made by hand from plastic or polyvinyl chloride tubing or by removing the coned portion of the Missouri University model AV and using only the innermost rubber liner to form a water jacket.

Disposable Artificial Vagina Liners. Sterile, plastic disposable liners have become commercially available for most types of AVs. These can be useful to reduce the risk of semen being exposed to chemical residues or latex. Many stallions object to these liners, however, and the number of mounts per ejaculation increases. Breakage of the plastic liner may occur during thrusting, and complete eversion of the liner may occur during dismount. If stallions ejaculate on first entry into an AV fitted with a disposable liner, the bacterial contamination of semen is reduced sharply. As the number of entries into the AV or the number of thrusts in the AV increases, however, the bacterial contamination of semen also increases. As such, ensuring that the stallion is properly stimulated and prepared to breed is useful for limiting the number of mounts.

Care of Artificial Vaginas. The AV should be cleaned immediately after each use, rinsed thoroughly with hot water, and wiped clear of dirt, debris, and smegma. If disposable liners are not used, then the rubber liners should be immersed in 70% alcohol for 1 hour or more, rinsed thoroughly with hot water, and hung in a dust-free, dry environment. To prevent the accumulation of chemical residue, soaps and disinfectants should not be used on the rubber equipment. If disposable AV liners are not used or the AV and its liners are not thoroughly cleaned, then the AV may become an effective fomite for spread of infectious or venereal disease. For these reasons, many farms maintain an individual AV for each stallion at the breeding farm.

Pharmacologically Induced Ejaculation

Several protocols have been published for the *ex copula* ejaculation of stallions using various pharmacologic agents alone or in tandem. The most commonly used drugs include xylazine, imipramine, and prostaglandins.[991-994] Semen collected in this fashion is generally of low volume and high concentration because of the decreased contribution from the accessory sex glands. One can use the resulting ejaculate for evaluation, AI, or cryopreservation. The inability to obtain ejaculates on a predictable schedule limits the commercial usefulness of these methods. In one experiment, ponies were collected in 10 of 24 attempts using imipramine and xylazine.[992] In selected cases in which the stallion is physically unable to mount and copulate, it is possible to obtain semen specimens with the aid of pharmacologic agents. Under farm conditions, semen is obtained in 25% to 30% of attempts. Keeping the stallion quiet and undisturbed is important, especially after xylazine has been administered.

A common protocol is to administer imipramine (2.2 mg/kg, PO, once) approximately 2 hours before anticipated semen collection.[995] Xylazine (0.2–0.3 mg/kg, IV) is then given. Ejaculation typically occurs shortly after xylazine administration (e.g., 1–3 minutes) or as the stallion begins to wake up from sedation (15–20 minutes). Although successful about 25% of the time, the procedure is time consuming and unpredictable for mare owners. The success rate may increase if the dosages are altered for individual stallions.

Miscellaneous Methods of Semen Collection

Condoms have been used in stallions that have an aversion to ejaculating into an AV. Condoms should be large and nonspermicidal. Disadvantages of this method are the high risk of the condom rupturing and contamination with debris. Aspiration from the uterus and vagina of recently bred mares is another option, but it is prone to contamination and does not lend well for achieving an accurate volume of the ejaculate.

Semen Evaluation

This process involves the collection of stallions' ejaculates and analysis of various seminal parameters as follows:

1. Volume and color: One should record the color and volume (in milliliters) of the ejaculate. Generally the color of the normal ejaculate ranges from watery to creamy white opalescent and depends on the sperm concentration per milliliter. Abnormal colors or volumes can indicate contamination of the ejaculate with blood, urine, or white cells. Normal volumes of light horse ejaculates range from 20 to 250 mL, with an average of 50 to 60 mL.[996] Factors influencing the volume are degree of sexual stimulation before collection, breeding conditions, and foreign material in the ejaculate. The volume of the ejaculate does not always reflect the quality of the ejaculate, and other parameters should be considered in light of the volume. When possible, distinction should be made between the volume of the gel and gel-free portions of the ejaculate. Most AVs can be fitted with an inline filter to separate these two fractions.
2. Sperm concentration: The number of sperm per milliliter must be accurately estimated to calculate total sperm numbers in the ejaculate and how best to process semen for further use. Sperm concentration, or density, is reported in millions of sperm cells per milliliter. Average counts are between 100 and 300 million, but there are many factors that can influence concentration, including season, frequency of collection, testicular size, and age. Although subject to as high as 10% variation, the Neubauer chamber or hemocytometer is regarded as the gold standard for evaluation of sperm concentration.[997] Even though the hemocytometer is regarded as the most accurate method to measure sperm concentration, it is time consuming and therefore not regularly used for routine counting of raw semen. The NucleoCounter SP-100 (Chemometec, Allerod, Denmark) counts sperm cell nuclei stained with the DNA-specific fluorescent dye propidium iodide.[997] The primary advantage to the NucleoCounter is that it retains its accuracy even when samples are contaminated with other debris, such as blood or urine. It is also capable of evaluating sperm viability via

detection of nonmembrane intact spermatozoa. The main disadvantage of this system is its significant cost. Other systems, such as the SpermaCue (MiniTube, Tieffenbach Germany), Equine Densimeter (Animal Reproduction Systems, Chino, CA,USA, or the Accucell (IMV Technologies, L'Aigle, France), are used. With all these systems it is critical to have a very clean sample free of foreign particles, debris, or extender. All these systems are accurate and repeatable at the 100 to 300 million sperm range, but their accuracy diminishes out of this range.[997] The computer-assisted sperm analysis (CASA) systems can also be used for concentration determination with an accuracy comparable to densimetry methods. In addition to providing sperm numbers, the CASA system reports sperm motility and the number of fast-moving and progressively motile sperm.

3. Osmolarity and pH: Osmolarity of stallion semen ranges between 290 and 310 mOsm. Values greater than 350 mOsm may indicate urospermia, and one should measure the level of creatinine (normal semen creatinine concentration <2 ng/mL). Values less than 250 mOsm suggest water contamination, and values over 500 mOsm reflect possible lubricant contamination. Seminal pH ranges between 6.9 and 7.5, and values higher than those should warn the clinician that there may be extraneous material in the ejaculate or an infectious process in the reproductive tract of the stallion.
4. Spermatozoal motility: Sperm motility has long been used as a method to estimate the percentage of viable sperm in the ejaculate. Several methods have been used to evaluate sperm motility, but all are used to determine the percentage of *total motile sperm* (the percentage of sperm in the ejaculate with any movement) and the percentage of *progressively motile sperm* (the percentage of sperm in the ejaculate moving in a linear or progressive line). The CASA system is the gold standard by which equine spermatozoal motility is evaluated. This modality generates objective values for sperm motility by counting and measuring individual cells in set frames. It also provides data on sperm characteristics such as linear velocity, linearity, path velocity, and lateral head displacement. The relative expense of the CASA system may preclude its use by some practitioners. Another common method is visual assessment of sperm motility. This involves placing a drop of semen on a microscope slide with a coverslip and evaluating cell movement at 200 to 400× magnification using phase contrast microscopy. This is the most widely used assay for evaluating sperm motility because of its simplicity and low cost. However, many factors can influence this mode of evaluation: individual judgment, volume of the drop used, concentration of sperm in the ejaculate, degree of contamination, degree of agglutination, and temperature. Even under the most tightly controlled conditions, repeatability of visual motility assessment is poor between technicians and laboratories. Visual motility estimates of freshly ejaculated stallion sperm have been reported to account for only 50% to 70% of the variation of fertility in that sample. The correlation is even worse ($r = 0.3$) when trying to predict fertility of a frozen-thawed sample of semen based on postthaw motility.[998] Although analysis of sperm motion with a computer is more objective and provides a highly consistent way of evaluating spermatozoa, the motility is but one factor influencing stallion fertility and should not be overinterpreted. In addition the formulas or settings for calculating the various motility parameters can differ between CASA systems, so analysis of one ejaculate may differ between the CASA systems used for each ejaculate.
5. Longevity of sperm motility: Duration of sperm motility can be determined using raw, undiluted semen or extended semen. For raw semen, a sample should be held at room temperature and evaluated every hour until <10% motility is observed. Semen from most normal stallions will maintain >10% motility for at least 4 hours. For extended semen, it should be processed, cooled, and stored appropriately. It can then be evaluated every 24 hours for the percentage of total and progressively motile spermatozoa.
6. Sperm morphology: For any sperm morphology evaluation, a minimum of 200 cells should be counted. Normal sperm cells, as well as those with acrosomal, head, midpiece, droplet, and tail defects, should be recorded, with the specific type of defect noted for each part. Although only 200 cells are counted routinely, sperm cells with more than one defect should be recorded as such to help the clinician evaluate the incidence of defects in a particular semen sample. One can evaluate cells as wet mounts under phase-contrast microscopy or differential interference contrast (DIC) microscopy after fixation in buffered formal saline or 4% glutaraldehyde. An antibiotic should be added to the fixative if samples are to be preserved for longer periods. Alternatively, cells can be evaluated after staining. One drop of semen is well mixed with the stain and smeared on a glass slide. Common stains currently used include India ink, eosin-nigrosin, eosin-aniline blue, Giemsa, Wright, and several others. The clinician should be aware that severe changes in the osmolarity of the stain as well as mechanical damage to the sperm could alter the normal morphology of the cells. Morphologic assessment of the sperm may aid in the diagnosis of certain pathologies because some defects are associated with different anatomic regions or suggest a specific reproductive pathology. Several attempts have been made to try to correlate the percentage of morphologically normal sperm present in a given ejaculate with fertility. Unfortunately, there appears to be a lack of consistency among clinicians in reporting sperm morphology. Among the problems that clinicians encounter are the definition of normal and abnormal in light of the tremendous range of normality and the fact that little knowledge exists regarding specific sperm defects that interfere with fertility. This problem is even more vexing when a clinician is trying to interpret the results from a referring veterinarian or a veterinary technician. Some of these inconsistencies can be avoided by recording specific morphologic defects rather than grouping defects into primary and secondary, because this last method erroneously assumes the origin of sperm defects (testis and posttesticular, respectively).

Semen evaluation thus provides a slew of data to supplement other examination findings. Calculation of the ejaculate's total volume and concentration will yield the total number of sperm cells in the ejaculate. Determination of concentration can be determined by manual, photometric, and fluorescence-based studies. Assessment of individual sperm cells (i.e., morphology) is performed to determine the number of normal looking sperm cells. Longevity studies are useful when planning matings and frequency of collections, but caution should be exercised in interpreting results because they do not adequately represent the environment within the mare's reproductive tract.

More advanced techniques exist for evaluation of stallion spermatozoa. These include testing the function of spermatozoa using the sperm chromatin structure assay, hyperosmotic swelling test, acrosomal reaction, and mitochondrial activity. Alternatively or in addition, transmission electron microscopy is useful for ultrastructural evaluation of individual sperm. It is capable of identifying subtle yet serious lesions within individual spermatozoa.

Hematologic and Endocrine Testing

Blood is commonly collected for evaluation as part of the BSE and typically consists of a complete blood count (CBC), fibrinogen, biochemistry profile, and testing for equine arteritis virus (EAV) if indicated. Endocrine testing is also commonly performed and concentrations of resting testosterone, estradiol, FSH, and LH are evaluated. In addition to screening for underlying diseases or disorders, these tests are also useful for establishing a baseline of normal values should an illness arise at some separate point in time.

Endocrine testing is often considered an adjunctive test but is nonetheless useful in the diagnosis of specific disorders. In particular, it allows for detection of primary testicular issues such as testicular degeneration. Because spermatogenesis relies on an intricate network of various autocrine, paracrine, and endocrine signals, aberrations in circulating gonadotropins and sex steroids may signal the onset of testicular dysfunction. Shown in Table 19.12 are examples of how serum concentrations of LH, FSH, testosterone, and estrogen conjugate can differ by season in normal stallions. Differences are also seen when comparing the concentrations of these hormones between fertile and subfertile stallions. Subfertile stallions have higher circulating gonadotropin concentrations, whereas estrogen conjugate is lower and testosterone remains relatively steady regardless of season.[999]

Microbiologic Testing

Screening for STDs should also be performed. Specific disease screening recommendations depend on the country where the horse has lived, currently resides, or where the horse may be shipped following the evaluation. Generally culture swabs of the penis/prepuce, urethral fossa, and urethra (both preejaculation and postejaculation) are taken and submitted for aerobic culture as a minimum in an SRE. During the breeding season, serial testing is performed to monitor for potentially pathogenic agents, such as *Klebsiella* spp. and *P. aeruginosa.* In certain areas of the United States, novice stallions will need a negative EVA serum titer either periodically or immediately before vaccination. Recently imported stallions will be subject to testing mandated by the USDA-APHIS as described earlier. Imported stallions are routinely tested for CEM by collecting swab samples of the external genitalia. In accordance with the USDA-APHIS, previously active breeding stallions also require test breeding of at least two mares, from which serial swabs are collected before and after breeding. Twenty-one days after the last test breeding, serum is collected for a CF test. The reader is directed to the USDA-APHIS *Contagious equine metritis* fact sheet[1000] for more information on the testing protocol. Again, the clinician is referred to the country's regulating body and regulations regarding sexually transmissible diseases of stallions when it comes to determining what testing must be performed.

Cytogenetic Testing for Markers of Fertility

This particular means of evaluating the breeding potential and soundness of stallions is still in its infancy. Since the horse genome project was completed in 2007, equine researchers are now discovering key relationships between DNA and the onset of certain diseases and disorders. This has allowed for the development of sensitive testing techniques to monitor or diagnose various hereditary diseases. These will all be discussed in another chapter, but one important disorder is a genetic link that has been found for impaired acrosome reaction (IAR), which is a known cause of infertility in many mammalian species. Researchers at Texas A&M University recently identified a susceptibility gene in horse chromosome 13 in FK506 binding protein 6 *(FKBP6)* in horses with IAR.[1001] This test can be useful as either a screening test for a prospective stallion or a diagnostic test in cases of unknown subfertility. The author currently uses the Animal Genetics Inc. (Tallahassee, FL) laboratory for most genetic testing, whereas the testing for IAR is done exclusively at the Department of Integrative Biosciences, Texas A&M University (College Station, TX).

TABLE **19.12** **Seasonal Effects of Luteinizing Hormone, Follicle-Stimulating Hormone, Testosterone, and Estrogen Conjugate in Fertile and Subfertile Stallions**

	Fertile (n = 8)		Subfertile (n = 6)	
Hormone (ng/mL)	**Nonbreeding Season**	**Breeding Season**	**Nonbreeding Season**	**Breeding Season**
Luteinizing hormone	2.4 ± 0.8	6.6 ± 0.8	9.2 ± 1.4	12.6 ± 2.8
Follicle-stimulating hormone	4.7 ± 0.5	6.1 ± 0.7	13.6 ± 3.1	14.5 ± 3.3
Testosterone	0.69 ± 0.14	1.14 ± 0.17	0.75 ± 0.08	0.88 ± 0.13
Estrogen conjugate	232 ± 29	216 ± 20	169 ± 45	186 ± 59

Values are presented as the mean ± SEM.
Adapted from Roser JF, Hughes JP. Seasonal effects on seminal quality, plasma hormone concentrations, and GnRH-induced LH response in fertile and subfertile stallions. *J Androl*. 1992;13:214-223.

Summary and Epicrisis for Stallion Reproductive Evaluations

As mentioned at the beginning of this section, stallion reproduction involves many different factors. Attempting to account for all of them and predict fertility is currently impossible. Consequently, it now seems common practice not to assign stallions a particular status (e.g., satisfactory, unsatisfactory, status deferred) but to summarize the findings and offer recommendations for future management.

MANAGEMENT OF BREEDING STALLIONS

Nutrition

Specific feeding programs are at the discretion of the stud farm. General horse husbandry practices apply, and it is important to account for the method of breeding (e.g., in-hand, pasture, collection with an AV). Stallions in live-cover programs with book sizes of 60 or more mares may be asked to breed two to three times a day for several days in a row, creating a significant caloric demand. Providing adequate forage, a balanced concentrate, and fresh water ad libitum is the principal concern in the management of stallions. Average breeding stallions (500–600 kg) will usually consume 6 to 10 quarts of concentrate (12%–14% protein) a day. New rations and supplements should be introduced gradually to avoid gastrointestinal upset.

Healthy and active stallions should enter the breeding season approximately one body condition score higher (on a scale of 1–9) than normal, realizing they shed this weight quickly. For most stallions, an ideal body score is 5 to 5.5 of 9, so starting out at 6 to 6.5 of 9 is generally acceptable and unlikely to have ill effects if the stallion is otherwise healthy.

Care should be taken not to overfeed stallions. Obesity can have negative and often serious consequences (e.g., predisposition to colic, laminitis, metabolic disease) that can adversely affect a stallion's breeding performance. Moreover, stallions should not experience extreme weight fluctuations because dramatic swings can hinder both health and fertility.

There is some evidence supporting the efficacy of certain supplements to improve seminal quality in stallions. Feeding OM3FAs is supported by evidence-based research demonstrating their positive effects. For example, Brinsko et al. reported improvement in sperm motion characteristics in cooled and frozen semen after stallions were fed a DHA-enriched nutraceutical for 14 weeks.[301] Another study demonstrated increased concentrations of OM3FAs in seminal plasma and total sperm output in stallions fed an OM3FA supplement but no improvement in sperm motility. Both of these studies used a marine-based source of OM3FAs.[1002] Algae-based supplements and flaxseed are other sources of OM3FAs and, at least anecdotally, appear more palatable than those that are marine based. Studies describing a direct relationship of feeding OM3FAs and improved fertility are lacking.

Other nutritional supplements used in the breeding management of stallions are antioxidants (e.g., vitamins C and E) and acetyl-L-carnitine. The former may be beneficial in reducing oxidative stress on spermatozoa, whereas the latter is thought to enhance motility by stimulating β-lipid oxidation in sperm mitochondria. Scientific evidence supporting the use of these products is lacking, but anecdotal reports are mostly favorable. The efficacy of antioxidants is somewhat curious in that one study showed that the most fertile ejaculates from dismount samples collected from live-cover matings were those with the highest level of oxidative stress.[1003] Nevertheless a plasma membrane surrounds the entire spermatozoon, so the relative intactness of this membrane is thought to be closely related to sperm quality. Because antioxidants may suppress or quench the damaging effects of free radicals on lipid peroxidation,[1004] supplementation, in theory, may assist in improving sperm quality.

Exercise

Exercise is an important yet sometimes overlooked part of stallion management. Programs should be based on the individual stallion and account for his current and desired weight, soundness, anticipated book size, environment, and other health issues that may preclude the stallion from receiving adequate exercise. Methods for exercise include forced (e.g., riding, lunging, mechanized walkers) and passive (e.g., turnout in a large paddock for several hours a day). The means of exercise will vary from farm to farm, but the objective should be to keep stallions fit and their mind occupied, because exercise engages them both mentally and physically. In humans, physical fitness has proven advantages in maintaining vigor and preventing disease. It thus seems logical, albeit a bit anthropomorphic, that horses can reap the same benefits.

Length of forced exercise is usually predicated on the horse's level of fitness, whereas turnout should be kept on a regular schedule and consist of several hours of free grazing. Numerous large-scale breeding operations keep their stallions in training or continue to exercise them throughout their stud careers. One experiment showed stallions subjected to a moderate amount of exercise had a decline in number of morphologically normal spermatozoa post exercise compared with those obtained preexercise. No other seminal parameters (e.g., concentration, gel volume, total sperm) differed between the preexercise and postexercise samples.[1005]

If farm management practices or the horse's physical condition preclude active exercise, then paddock or round-pen turnout can be used. Paddock size is usually relegated to no more than a few acres. It can be helpful to place water and feed buckets in different areas to encourage increased movement. Turnout during inclement weather is at the discretion of the farm, but most horses are very resilient to adverse environmental conditions.

Herd Health Management

Routine hoof and dental care, as well as targeted vaccinations and dewormings, provide a solid foundation to manage the overall health of stallions. Similar to the previous considerations, herd health management will vary based on the particular farm and location.

Timing of vaccinations is important when managing active breeding stallions. Although a relatively low risk, especially considering the advancements made in vaccine quality over the past two decades, adverse events can still occur following immunizations. Untoward effects, whether local or systemic in nature, can hinder breeding performance. For example, local swelling at the site of an injection can cause soreness and reluctance to breed. Alternatively or in addition, a fever can negatively affect spermatogenesis because of secondary thermal stress.

Some practitioners choose to limit or postpone vaccinations during the breeding season. The author usually gives booster

vaccines in late December or early January so that any issue resulting from vaccination can be resolved before the beginning of the breeding season (mid-February). Boosters are also given, but evenly spaced out, in the summer and fall. Since stallions are at risk for acquiring infectious diseases because of contact with outside mares, vaccination against equine rhinopneumonitis can be performed every 2 to 3 months.

Vaccination against EAV, the causative agent of EVA, is required in some states for active breeding stallions because this virus can be shed in bodily fluids such as semen and respiratory secretions. The most pressing concern with EAV is the potential to cause abortion storms or neonatal death in naive pregnant mares.[243] The reader is encouraged to contact his or her state veterinarian's office for rules and requirements pertaining to vaccination against EVA in breeding stallions. Some stallions can transmit EAV in their semen (i.e., shedding stallions), so the stud farm should disclose if a stallion is a chronic shedder of EAV. Guidelines for breeding a mare to an EAV-shedding stallion are provided by the American Association of Equine Practitioners.[1006]

Breeding Methods

Live Cover

Also referred to as *natural service* or *in-hand* mating, live-cover breedings are commonly performed in commercial Thoroughbred operations throughout the world. Briefly, when an estrous mare is identified and deemed suitable for mating, she is brought to the breeding shed where she is teased and prepared routinely for breeding by washing of the perineum. Mares are usually restrained with a lead shank and a nose twitch, and padded leather "kick" boots are placed on the hind feet. Some sheds apply a neck shield or "cape" around the mare's cervical spine to provide extra protection. An example of typical preparation of a mare for a live-cover mating is shown in Fig. 19.26. The stallion is presented to the mare and teased until properly stimulated. He is then given a cue to mount, and, on doing so, an attendant will direct his penis into the mare's vagina. When ejaculation occurs, the attendant will apply his fingers to the base of the penis to feel for characteristic pulsations and fremitus of emission and ejaculation. As the stallion dismounts, the attendant will collect the drippings from the penis, which will undergo microscopic evaluation for the presence of live sperm, offering further confirmation of ejaculation. The sequence of events is shown in Fig. 19.27.

Semen Collection Using a Live Mare as a Mount

Selection of a suitable mount or "jump" mare depends on the experience and breeding mannerisms of the stallion and farm resources. For example, the inexperienced stallion may need to be taught to mount the mare from the rear quarters. This training requires a disciplined, cooperative mare that will tolerate being mounted from the side. Some stallions vocalize loudly in the breeding shed and may frighten maiden or timid mares. The mount mare needs to tolerate a certain amount of nipping and biting of the neck, shoulders, flank region, and hocks to be suitable for some stallions. Mares with foals at their sides are frequently protective of their foals and less cooperative than barren mares. The mount mare also should be an appropriate size match for the stallion. For routine breeding farm activities, the reliance on an estrous mare as a mount has significant shortcomings because the mare must be followed with



FIG. **19.26** Example of a mare being restrained for a live-cover mating.

teasing and ultrasound to ensure she is in estrus. Additionally, in a cooled, shipped semen program, the breeding farm may not have access to nonpregnant mares, particularly at the end of the breeding season. Therefore some breeding farms maintain one or more ovariectomized mares as mount mares. One should select from these ovariectomized mount mare candidates based on their size, tolerant attitude toward handling, and strong estrous behavioral signs as intact mares. A mare with gonadal dysgenesis, karyotype (XO), may be a good mount mare candidate without requiring an ovariectomy. Most ovariectomized mares perform well as mount mares while being restrained with a twitch or lip chain placed on the upper gum. In some cases it may be necessary to administer a low dose of estradiol cypionate (0.5–4 mg, IM) at intervals of 3 days to 3 weeks to maintain receptivity by the mare.

During the semen collection process, the mount mare usually is restrained using a twitch. Hobbles also may be applied to rear pasterns or hocks, but there is risk of the stallion becoming entangled in the hobbles if the collection procedure goes awry. Protective boots can also be placed on the hind feet to minimize damage if the mare does kick, and hind shoes should always be removed. The long tail hairs at the base of the mount mare should be wrapped to prevent the tail from interfering with deflection and entry of the penis into the AV.

Breeding Phantom

The breeding phantom, dummy, or mount consists of a structure suitable for stallion mounting that is usually a cylindrical barrel or tube mounted in a stationary position that is the approximate size of the body of a mare the stallion would breed (Fig. 19.28). The barrel is usually covered with 1 to 2 inches of firm padding. The padded cylinder then is covered by a tough, nonabrasive cover that is free of wrinkles. Stallions that repeatedly mount and dismount a phantom can abrade the medial aspects of their forearms and carpi. Wraps can be applied to minimize trauma. The stallion should be taught to dismount the phantom in a controlled manner by backing off of the mount rather than making a side dismount. The diameter, length, and height of the phantom should be dictated by stallion size and preference, with most light horse stallions tolerating a total diameter of 20 to 24 inches. The struts of the phantom should be far enough from the mounting end to prevent injury to the hindlegs of the stallion during mounting and



FIG. **19.27** Sequence of events for a live-cover mating.

dismounting. The height and the angle of the phantom should be adjustable to accommodate older stallions, stallions with hindlimb lameness or musculoskeletal issues, and stallions of varying stature.[970,1007]

Adequate space should surround the phantom for the safety of the handlers and to allow a teaser mare to be positioned alongside or in front of the phantom if additional stimulation is needed. Many stallions are trained to mount the phantom even when the teaser mare is not close to it.

When a phantom is used to collect semen from a stallion, the stallion should approach the mount in a controlled fashion, mount the rear of the phantom, and use his forelimbs to stabilize himself by grasping the padded barrel of the mount. Once the stallion is properly situated, the operator approaches from the left or near side of the stallion, deflects the penis into the AV using a right, overhand grip, and stabilizes him- or herself on the near side of the phantom as the stallion services the AV. While on the left side of



FIG. **19.28** Example of a breeding phantom used for collection of stallion semen in an artificial insemination program. The phantom is secured in the ground by a single and sold strut. The body or barrel of the phantom is reinforced with soft, nonabrasive material for comfort.

the stallion (and phantom), the operator deflects and stabilizes the base of the penis with the right hand. This practice minimizes potential injury to the penis and prepuce during thrusting by the stallion. Some phantom mounts are fitted with an AV installed within the posterior end, which works well for some stallions and requires only one person for the collection procedure. The only disadvantage of indwelling AVs is that occasionally the stallion's penis can be traumatized by incorrect insertion. In addition, some stallions need manual stimulation of the glans penis, which is easier to provide when the operator has control of the AV. Stallions regularly used for live-cover breedings can be difficult to train to a phantom. For this reason, certain circumstances may require access to an estrous mare to facilitate semen collection.

Measuring Reproductive Performance

Formulas for evaluating reproductive efficiency of stallions are presented below. These measures are useful for comparing stallions among and within farms. They also offer insight into breeding and farm management and can help identify areas of concern.

$$\text{Per Cycle Pregnancy Rate} = [(\text{No. in Foal}) / (\text{No. Covers}) (\text{No. Doubles})] (100\,\%)$$

$$\text{Seasonal Pregnancy Rate} = [(\text{Total No. Pregnancies}) / (\text{Total No. Mares Bred})] (100\,\%)$$

$$\text{First Cycle Pregnancy Rate} = [(\text{No. in Foal on First Cover}) / (\text{Total No. Mares Bred on First Cycle})] (100\,\%)$$

$$\text{Covers Per Pregnancy} = \frac{\text{Total No. Covers}}{\text{Total No. Pregnancies}}$$

Organization and tabulation of these data are most commonly done electronically. Computerized spreadsheets can assist with data collection and analysis. Charts and graphs comparing various reproductive parameters are useful for summarizing and comparing findings in a concise yet comprehensive manner and can be used to monitor patterns of breeding performance.

Novice or Maiden Stallion

The arrival of a new stallion to a breeding farm is usually an exciting time. These stallions have proven superior in their respective disciplines and are thus of high value both financially and emotionally. Although the urge exists to "see what they're made of" with respect to breeding behavior, good managers believe it is best to give them time to familiarize themselves with their surroundings before initiating breeding activity. Documenting and addressing previous health issues or injuries should also be done so that the stallion remains fit and sound for breeding. This transition, or *let-down*, period is variable (2 weeks to 2 months) and should remain fluid to allow the stallion to settle into his new career. The let-down period typically includes the following:

- Quarantine to reduce risk of transmissible disease
- Conditioning and acclimatization to new surroundings
- Establishment of a routine
- Diagnostic testing

Most stallion managers are also cognizant of the various mental adjustments that a stallion goes through as he transitions to a new environment. Moreover, each stallion is an individual and should be treated as such. Stallions must remain respectful to prevent aggression toward their handlers and other horses.[1008] Novice stallions must be treated with patience, positive reinforcement, and in some instances, perseverance. Unnecessary punishment and rough handling can aggravate a problem and may result in breeding disinterest.

Following the let-down period, maiden stallions are introduced to mating via test breeding of estrous mares. This process allows for conditioning of the stallion to the farm's breeding procedure and for evaluation of the stallion's breeding behavior and seminal characteristics. A test mare recently screened for EVA and CEM is prepared as for a normal live cover. The stallion is introduced to the mare and allowed to sniff and interact with the mare in a respectful manner. Both patience and repetition are required during the test breeding process because mating is a new experience for these stallions. Some stallions may require only a few breeding sessions before management is adequately satisfied that the stallion is trained; others may require several attempts before they display any interest in breeding. Dismount samples are routinely evaluated for the presence of live, motile, and morphologically normal spermatozoa. A stallion is considered "trained" once he willingly breeds a mare in an efficient and proper manner. Shown in Video 19.3 is a novice stallion presented to an estrous mare for the first time. This stallion displayed excellent libido and bred the mare in the relatively short period. For stallions destined for live-cover operations, the author prefers to train stallions to the mare first. The collection of entire ejaculates via an AV can be performed at a later date.

Starting novice stallions under lights is used in some programs. No proven benefits have been demonstrated from this practice. Anecdotally, and as discussed previously, the additional photoperiod may stimulate reproductive function and prepare the stallion for success early in the breeding season.

DISEASES AND DISORDERS OF INTACT MALE HORSES

Abnormal Behavior

Manifestations of abnormal behavior are numerous. Examples are provided in Table 19.13 along with potential causes for deviant sexual behavior. McDonnell reviewed the incidence of behavioral problems in 250 stallions over a 5-year period.[978] More than 50% of the cases had complaints related to poor libido or excessive aggressiveness. Of those, nearly half were described in stallions with no previous sexual experience. The rest were divided evenly between experienced stallions with low sexual interest and those with increased aggressiveness. Mounting and erection dysfunction accounted for 11% of complaints, whereas ejaculatory problems accounted for 25% of the total cases. Other problems, such as self-mutilation and various stereotypies accounted for 11% of the reported cases.[978]

For clinicians, it is important that dysfunctional behavior is interpreted relative to the expectations of the breeding outfit.[1009] For example, stallions requiring 30 minutes or more to mount and ejaculate or those needing several mounts before ejaculation may be considered unacceptable in some intensive management situations. On the other hand, a stallion that takes several hours to achieve an erection, mount, and ejaculate may be considered normal if he breeds only two or three mares during the entire breeding season in pasture conditions.

Low Libido

Lack of adequate sex drive is the most common behavioral issue in breeding stallions.[978] Many factors can be at play, including management practices and environmental cues. For example, timid stallions housed next to dominant stallions may show a lack of libido because of perceived subordination to the dominant stallion. Other stallions may have an aversion to or preference for a particular color of mare. Lack of libido may be observed in experienced stallions toward the end of the breeding season, particularly in heavily used or overused animals. This problem can be corrected by decreasing the frequency of service or collection. Stallions that have been kicked by mares or negatively reinforced for displaying sexual behavior during performance may demonstrate reduced sexual desire. It is thus prudent to consider the history, health, and management of the stallion from many different perspectives when determining the cause of low libido.

Treatment of a stallion with low sexual drive is best directed at correction of the underlying problem, but assessment of the nature of the problem is often difficult. One may try several alternatives, including

- Change of stimulus mare or environment
- Breeding or collecting another stallion in the presence of the low-libido animal
- IV administration of GnRH, 50 μg, 1 to 2 hours before breeding or hCG, 5000 to 10,000 IU, 1 hour before breeding
- IV administration of diazepam or midazolam at a dosage of 0.05 mg/kg (maximum 20 mg) 10 to 15 minutes before breeding to reduce inhibition of sexual behavior in some stallions
- Single injection of a short-acting testosterone

The efficacy of most of these treatments is dependent on the individual stallion and the primary problem(s). Some of these treatments may have adverse effects. For example, chronic administration of steroids, particularly androgens, is well documented to negatively affect spermatogenesis.[970] Therefore treatment of stallions with exogenous steroids and particularly androgens to improve libido should be measured carefully with respect to the risks and potential benefits. Consequently, treatment is typically reserved for use when other means of increasing arousal have failed. Current recommendations are treatment with an initial dose 80 to 100 mg (IM) of testosterone cypionate and maintaining serum concentrations at 2 to 4 ng/mL. The effect typically lags a significant time following treatment (5–8 days). Anecdotal reports of topical application of testosterone cream to the penis to improve tactile stimulation have been reported but not rigorously studied.

TABLE **19.13 Signs of Abnormal Stallion Behavior and Potential Causes**

Signs	Causes
Low libido	Pain
Paraphilia	Secondary health issues
Timidness	Mare preference
Erectile failure	Environmental changes
Ejaculatory failure	Management changes
Self-mutilation	Previous adverse experience

Erection Failure

The inability of a stallion to develop and maintain a normal erection despite normal libido may be related to anatomic issues. Possible problems include vascular damage associated with traumatic injuries or neurologic problems associated with other penile or lumbosacral compromise.[991]

Medical (e.g., administration of vasoactive substances) and surgical (e.g., penile implants) options may be considered. Unfortunately, evidence-based research supporting these methods is lacking. Sildenafil (Viagra, Pfizer, New York, NY) has long been discussed but does not currently have a reported dose or effect in horses.

Ejaculatory Failure

Some stallions show normal precopulatory behavior, mounting, and intromission but fail to ejaculate. These stallions may become exhausted or frustrated, displaying aggressiveness to the mare or handler. For this condition it is important to rule out musculoskeletal or orthopedic pain such as degenerative joint disease. If suspected, trial treatment with an appropriate dose of a nonsteroidal agent can be of benefit. Circulatory disorders, such as iliac thrombosis, have also been associated with ejaculatory failure.

Despite being difficult to diagnose, psychological problems can lead to ejaculatory dysfunction. In many instances, a history of a traumatic incident while breeding is closely associated with the onset of ejaculatory failure. If the stallion refuses to ejaculate only under specific circumstances, such as into an AV or when breeding a mare, then a systematic approach is important to determine the problem.

A variety of behavioral and managerial aids have been used to help stallions ejaculate.[1010] One should adapt these aids according to the physical condition of the stallion (i.e., stallions that cannot achieve a full erection; stallions that have difficulty mounting; stallions that refuse to ejaculate after normal erection, mounting, thrusting, and belling of the glans).

Neither mounting nor full erection is necessary for ejaculation. Stallions that have difficulty mounting can be taught to ejaculate on the ground by manual stimulation of the penis or with an AV. Stallions with erection problems can ejaculate if proper stimulation is given to the penis. One can achieve proper stimulation to the penis by raising the temperature of the AV or by applying hot towels to the base of the penis during thrusting. It is also important to consider changes in footing and surroundings, stimulus mare, and handlers before implementing pharmacologic therapy.

Therapeutic regimens for ejaculatory dysfunction are empirical and include those already mentioned for the treatment of low-libido stallions. In addition, PGs, oxytocin, and xylazine have been used to aid stallions in the process of ejaculation. Oxytocin and PG also have been used to treat azoospermia caused by ampullary blockage.[1011] The tricyclic antidepressant imipramine can be given to lower the threshold for ejaculation in stallions.[995] As discussed previously, varying success rates are obtained when using these agents for either short-term or long-term management of ejaculatory failure.

Self-Mutilation

Self-mutilation is a highly complex behavioral syndrome that can include self-biting, stomping and kicking, rubbing, and lunging at objects. According to McDonnell, three distinct types of self-mutilation occur.[1012] Type I represents a normal behavioral response to continuous or intermittent unrelieved physical discomfort. Type II, seen in stallions and geldings, can be recognized as self-directed intermale aggression. This behavior includes certain elements of the natural interactions typical of encounters between two stallions, except that the stallion himself is the target of his intermale behavior. Type III involves a quieter, often rhythmically repetitive or methodical behavioral sequence of a stereotypy (e.g., nipping at various areas of the body in a relatively consistent pattern, stomping or kicking rhythmically against an object). Some researchers have speculated that self-mutilation has a genetic component. The prevalence of self-mutilation of one form or another has been observed in as many as 2% of domestic stallions.[1013] Among and between stallions, self-mutilation varies in frequency and intensity and can reach levels that threaten the stallion's fertility and even his life.

Careful evaluation of the horse's behavior is often necessary to distinguish the specific type of self-mutilation. Type I self-mutilation can be eliminated by relieving the source of discomfort. For types II and III, understanding of intermale interactive behavior of horses and the environmental factors triggering the self-mutilating form can be useful in guiding humane management or behavior modification. Pharmacologic manipulation and nutritional changes may be useful adjuncts in the management of this condition.

Although self-mutilation is usually limited to postpubertal horses in type II or type III, it is not limited to confined animals like some stereotypies. In some animals the problem is exacerbated on presentation of a mare to a confined stallion or breeding in the presence of another stallion.[1014] The compulsive behavior seems to be more dramatic during the breeding season.

Self-mutilation may be a problem exacerbated by olfactory stimuli. In some horses, smelling their manure triggers this behavior. The stallions often mistakenly recognize themselves as a threat, triggering the compulsive behavior.

Therapy of this complex syndrome depends on the type of self-mutilation and its underlying causes. Potential strategies include pain management, surgery, regular exercise, stall toys, and companion animals. Products to reduce the olfactory stimuli can be used, and diet modification to decrease carbohydrates have been used with some success. Treatment with behavior supplements, such as a milk-protein–based product (Zylkene; Vetoquinol USA, Inc., Fort Worth, TX) or pheromone mimic (Confidence EQ; Ceva Animal Health, Lenexa, KS) may help some stallions, but their efficacy is unproven. Physical restraints such as head cradles or muzzles most likely lead to development of another self-mutilating behavior. In extreme, inhumane, and refractory cases, castration of the stallion has eliminated the problem for type III self-mutilation but not for the other types. Therefore an accurate diagnosis is a critical component of the treatment protocol.

Other Behavioral Problems

Some stallions may have behavioral traits or vices that adversely affect their breeding ability. Overly aggressive stallions may be too dangerous to handle because of savaging the handlers or the mare. This aggression may be caused by frustration, past breeding experience, and/or potentially paraphilia. Aggression is best managed with an experienced and patient handler and identification and elimination of potential management and environmental cues that lead to adverse behavior.

Stereotypies can also affect reproductive performance. Cribbing, weaving, stomping, and wall kicking are all examples that can lead to secondary health issues (e.g., colic, weight loss, lameness). Because these are thought to be born of boredom, providing mental stimulation via enrichment activities (e.g., rubber ball, companion animal) may help. Some stereotypies are a result of physical discomfort (ulcers, laminitis, and neuropathic pain), and general health causes for discomfort should always be ruled out.

Abnormalities of the Penis and Prepuce

Clinicians must be able to accurately identify pathologic conditions of the external genitalia to make sound decisions regarding the therapy and management of stallions. Acquired conditions may be reversible by surgical or other means, but diagnosis of irreversible or terminal conditions must be well substantiated because they could have a significant effect on the viability of the breeding operation. Ethical considerations are also pertinent because some genetic diseases can have a significant effect on the breed as a whole.

Developmental Conditions

As reviewed previously, the sexual development of the equine embryo to a mature normal stallion capable of successfully breeding requires multiple genetic factors, pathways, and endocrine events to occur and proceed normally. Horses with genetic abnormalities can vary in their genotype, anatomic features, and behavior. The most common causes of congenital defects are hermaphroditism, XY sex reversal, and testicular feminization or androgen insensitivity. Previously described as intersex conditions, they are now referred to as *disorders of sexual development* (DSD).[1015,1016]

Hermaphrodites are classified on the basis of the type of gonadal tissue present. True hermaphrodites have testicular and ovarian tissue, whereas pseudohermaphrodites have either testes (male pseudohermaphrodite) or ovaries (female pseudohermaphrodite). Various combinations of male and

female internal and external reproductive organs can exist and have been described.[1017,1018] The most commonly diagnosed DSD is the male pseudohermaphrodite, which has a mostly female phenotype but testes instead of ovaries. A common clinical presentation is a "mare" with a small vulva and an extremely large clitoris or what appears to be a glans penis with an increased anogenital distance. Behaviorally, the horse may display stallion-like behavior because of the presence of testicular tissue. Female pseudohermaphrodites are much less common and are characterized by a male phenotype, but the animal's internal gonads are ovaries instead of testes.

Horses with XY sex reversal syndrome are characterized by female external genitalia but a normal 64XY karyotype. Androgen insensitivity or testicular feminization syndrome is a well-characterized genetic disease in human beings and in some domestic species. Animals have a normal XY karyotype and display male behavior but have a female phenotype. The syndrome has two possible causes: a mutation at the level of the gene that codes for the androgen receptor or a deficiency in 5α-reductase, the enzyme responsible for conversion of testosterone to the active androgen DHT.[1019] In either situation the reproductive tract is underdeveloped and the female phenotype predominates.

Genetic defects can be tentatively diagnosed by visual inspection of the external genitalia, rectal palpation, and ultrasonography. However, a definitive diagnosis requires cytogenetic evaluation by a commercial molecular and cytogenetics laboratory.

Segmental aplasia of the vas deferens or the epididymal duct has also been reported.[1020] These conditions can render the stallion sterile in the case of bilateral aplasia, due to the inability to emit and ejaculate spermatozoa. Other aberrations in gonadal development can potentially lead to improper germ cell development and/or maturation.

Phimosis is the inability to protrude the penis and can be congenital. In neonatal colts, the penile frenulum is commonly broken down early in life but can occasionally persist. The most common presenting complaint is an abnormal urine stream; inspection of the colt's prepuce and penis reveals a palpable frenulum and the glans penis is reflected caudally. This condition is usually self-limiting, but transection of the band can be performed to hasten resolution.

Acquired Conditions

Paraphimosis occurs when the penis is unable to retract back into the sheath. This condition is seen in cases of severe malnourishment or exhaustion, neurologic disease, administration of certain psychoactive drugs, and trauma. Whether alone or in combination, these causes culminate in chronic flaccidity and extensive edema of the penis.[1021,1022] Because venous return is impaired, the condition can progress to balanoposthitis, which includes the development of skin ulcerations, secondary infections, and penile necrosis. Treatment aims are to restore venous blood flow and reduce swelling and inflammation. Hydrotherapy, massage, application of topical emollients, antiinflammatory drugs, and diuretics can all be useful therapies. Antimicrobials and corticosteroids may also be indicated to prevent secondary infections and manage the edema. Support of the penis and prepuce are important to prevent microthrombi from forming and can be accomplished using mesh support, a probang device, or purse-string suture (as shown in Fig. 19.29).[1023,1024] In some instances flushing of the CCP with heparinized saline or intracavernous injection of phenylephrine may aid in blood drainage and penile retraction. Traumatic accidents involving the penis are not uncommon. Cuts with mare tail hair, poorly constructed breeding phantoms with fixed AVs, and severe bending of the erect penis may result in abrasions, lacerations, and severe hematomas. These wounds are treated with supportive therapy and surgery, when indicated. Prevention of secondary contamination and adhesion formation is important. It is also essential in cases of paraphimosis that the stallion's ability to urinate is assessed and addressed, because this can quickly become a life-threatening condition if the damage to the penile urethra prevents urination.



FIG. **19.29** Example of a penile sling using women's undergarments. Lacy material is ideal to allow for passage of urine and exudate. The sling is secured with rubber tubing.

Acquired conditions causing *phimosis,* or the inability to extrude the penis from the prepuce, include those conditions that cause stenosis of the preputial orifice. Strictures can arise from trauma or neoplastic growths. Horses experience difficulty urinating or have an inappropriate urine stream. Surgical resection of the stricture and/or debulking of the growths and lesions can help resolve the condition. Some breeding stallions may achieve an erection before full protrusion from the sheath. This condition can cause mild discomfort and even self-trauma. This condition is resolved by calming the stallion via removing the sexual stimulus and allowing the penis to detumesce.

Priapism is a persistent erection in the absence of sexual stimulation. It has been commonly associated with phenothiazine-derivative tranquilizers,[1021,1025,1026] but it can occur following the administration of any sedative or tranquilizer (though this is uncommon). Medical management with the IV administration of benztropine mesylate (8 mg, IV) or direct injection of the CCP with 1% phenylephrine (10 mg diluted in 10 mL physiologic saline) have been used successfully to resolve this condition. Surgical treatment is usually instituted when medical therapy fails. It consists of evacuating and flushing the CCP with heparinized saline. A partial phallectomy can also be used to manage this condition in geldings.

Neoplastic Conditions

The most common form of neoplasia affecting the penis is squamous cell carcinoma. Smegma accumulation on the penis may be a predisposing factor for this type of neoplasia.[1027] The

tumors start as small, keratinized plaques that slowly progress into necrotic foci with foul-smelling exudate from secondary bacterial contamination. The papillomavirus has been suggested as a predisposing factor, which is a hypothesis that warrants further investigation.[1028] Conclusive diagnosis is based on histopathologic examination of tissue from the affected region. The condition is treated with cryosurgery, reefing, or phallectomy.

Warts or squamous papillomas, sarcoids, melanomas, fibromas, and lipomas may also occur on the penis. Cryotherapy, autogenous vaccines, chemotherapeutic agents, and surgical debulking have been used with varying degrees of success to treat these conditions.

Infectious Conditions

The most common infections involving the penises of stallions are habronemiasis, EHV-3, and bovine papillomavirus (BPV). Dermatologic lesions secondary to *Habronema* spp. and the microfilaria *Onchocerca cervicalis* result in characteristic lesions commonly referred to as "summer sores." Diagnosis of habronemiasis is usually straightforward by observing characteristic lesions on the urethral process, glans, and sheath. Enteral and topical therapy with ivermectin is often useful. Affected horses are prone to recurrence, and severely affected horses may benefit from treatment with corticosteroids. While the occurrence of habronemiasis waned with the advent and ubiquitous use of avermectin anthelmintics, this disease has seen a mild reemergence in recent years that has been attributed at least in part to possible resistance of the organisms to common dewormers.

Coital exanthema caused by EHV-3 is a contagious venereal disease affecting stallions and broodmares. It causes characteristic lesions and can be readily transmitted by both horses and fomites. EHV-3 and other venereal diseases are covered in a separate section.

Infection with BPV is an interesting concept because it has been implicated in the onset of equine sarcoids.[1029] Consequently, treatment with topical antiviral agents (e.g., acyclovir) may be of benefit in arresting and even reducing growth.

Bacterial infections of the penis should always be interpreted in light of both clinical signs and breeding records. For example, the external genitalia of commercial Thoroughbred stallions are cultured routinely to screen for potential pathogens. It is not uncommon to obtain positive growth of various organisms, including *E. coli, S. zooepidemicus, S. equisimilis, Staphylococcus aureus, Bacillus* spp., *K. pneumoniae,* and *P. aeruginosa.*[1030] Despite positive growth, many stallions will remain unaffected as evidenced by a lack of clinical signs and adequate pregnancy rates. However, when the normal flora is disrupted, potentially pathogenic bacteria, particularly *P. aeruginosa* and *K. pneumoniae,* can colonize the penis and prepuce. These organisms rarely produce clinical disease in stallions, but controversy exists regarding the risk of transmission to the mare at the time of breeding and the potential for causing IE and associated subfertility. Factors contributing to the colonization of the penis by these bacteria have not been clearly determined. Normal bacterial microflora of the external genitalia of the stallion may combat proliferation of pathogens, and frequent washing of the penis, especially with soaps, may remove these nonpathogenic resident bacteria, increasing the susceptibility of the penis and prepuce to colonization by pathogenic organisms.[1031] Others dispute this concept, asserting that repeated washing of the external genitalia alone does not contribute to overgrowth of pathogenic microorganisms. The environment in which a stallion is housed may influence the types of organisms harbored on the external genitalia. The stallion also can acquire these organisms at the time of coitus with a mare that has a genital infection.[1008] Diagnosis of pathogenic colonization of the stallion's penis begins with careful evaluation of breeding records and early pregnancy rates. A sudden and unexplained drop in reproductive efficiency should warn the stallion manager about a possible problem. Definitive diagnosis requires isolation of the pathogenic microorganisms from cultures of the genitalia. In addition, isolation of the same microorganism with a similar sensitivity pattern from recently bred mares may help confirm the diagnosis.

Treatment of pathogenic bacterial colonization of the penis depends on the type of bacteria isolated and method of breeding. For stallions breeding by AI, a thorough penile wash before semen collection is recommended. Collected semen should be filtered and diluted appropriately in extender containing an antibiotic to which the bacteria is sensitive. Stallions breeding by live cover should have their penis washed and dried before or after mating. Aggressive management of the mare may also be pursued (e.g., uterine lavage, antibiotic infusions, and/or systemic antimicrobials) if growth is of significant concern and/or the mare is susceptible to postmating-induced endometritis. Stallions with penile colonization by *Klebsiella* or *Pseudomonas* spp. can be washed with a weak solution of HCl or sodium hypochlorite. Systemic treatment is not recommended because it has proved unrewarding in most cases.

Abnormalities of the Scrotum

The scrotum in the horse is a pliable and thin-skinned pouch with a fine layer of short hair, numerous sweat glands, and the thick muscular layer (tunica dartos). The scrotum is usually darkly pigmented. Functional integrity of the scrotum is vital because of its role in thermoregulation of the testes.[1032] Abnormal conformation, trauma, and increased thickness can have a dramatic and deleterious effects on spermatogenesis. The scrotum is easily accessible for examination by visual inspection and palpation, and most stallions allow evaluation and palpation with mild restraint.

Developmental Conditions

The most common developmental condition of the scrotum is a reduction in size secondary to unilateral or bilateral cryptorchidism (discussed later). Stallions affected with DSDs may also display smaller than expected scrotal size or an unusual location of the scrotum.

Acquired Conditions

Traumatic insults to the scrotum or the scrotal area and other inflammatory processes in the genital area often result in scrotal edema. The clinician must identify the causes of the swelling to initiate adequate therapy. Chronic edema of the scrotum can result in abrasions or wounds that can be complicated further by bacterial contamination. Differential diagnoses for scrotal swelling include systemic infectious processes, scrotal herniae, spermatic cord torsion, scrotal abscess or hematoma, hemorrhagic processes associated with the testes or spermatic cords, and ascites or peritonitis.

An ultrasonographic study is indicated in stallions with scrotal edema or swelling to evaluate the scrotal tissues and

contents. Percutaneous aspiration of fluid in the scrotal tissue or between the visceral and parietal vaginal tunics can prove useful for further characterization of the fluid but does carry risk of iatrogenic infection.

Therapy is directed toward removing the primary cause of scrotal swelling and controlling any ongoing inflammation and edema. Antiinflammatory drugs (e.g., NSAIDs, corticosteroids), diuretics, and frequent hydrotherapy are all measures that should be implemented to counteract swelling and reestablish circulation. Broad-spectrum antibiotic therapy can be instituted, and application of lubricants and emollients is useful for preventing dermatitis and countering secondary bacterial infection.[1033]

Quick resolution of scrotal edema and swelling is of paramount importance. Acute systemic (e.g., fever) or local inflammation can lead to thermal insult of the testes, which can adversely affect spermatogenesis and translate into poor semen quality and reproductive efficiency. Chronic inflammation can also be detrimental, leading to fibrosis and testicular degeneration.

Neoplastic Conditions

Cancers of the scrotum are similar to those affecting the skin. Sarcoids, squamous cell carcinoma and papillomas, and melanoma have all been reported.[1034] Diagnosis is made based on both clinical signs and histopathology of the affected tissue. Combination therapy with surgical removal and chemotherapeutic agents appears to be the most common mode of treatment.[1035]

Infectious Conditions

EIA or other infectious diseases causing severe hypoproteinemia can result in scrotal edema and affect stallion fertility. The acute phase of EVA can cause different degrees of ventral or dependent abdominal edema that may involve the scrotum. Coital exanthema caused by EHV-3 can also affect the scrotum and is discussed later.

Infection with *T. equiperdum,* the causative agent of dourine, can also cause scrotal edema. It is considered an FAD in the United States but is found in equids located in parts of Africa, Asia, and possibly South America.[246] As will be discussed later, dourine is transmitted almost exclusively by coitus. Habronemiasis is another parasitic disease that infrequently affects the scrotum and can be managed as described for similar lesions affecting the penis.

Abnormalities of the Testes

As previously reviewed, the major cell types of the testes are Sertoli, Leydig, myoid, and germ (sperm) cells. Congenital, acquired, neoplastic, and infectious conditions affecting these cells, whether alone or in combination, can have significant consequences on fertility. Early diagnosis and management are key to a resolution.

Developmental Conditions

Discussed next are various developmental disorders of the testes. In addition to these, monorchidism, anorchidism, and polyorchidism have been described in stallions.[1036]

Ectopic Testis. A gonad that fails to reach the scrotum and deviates from the normal path of descent is termed an *ectopic testis.* These testes may be located in subcutaneous tissues within the inner thigh, abdomen, or perineal region. Cryptorchid testes are sometimes referred to as ectopic testes.[1037] Splenic-testicular fusion has also been reported and represents an extreme case of ectopic testis.

Cryptorchidism. This condition refers to the failure of one (unilateral) or both (bilateral) testes to fully descended into the scrotum by 2 years of age. Cryptorchidism is a fairly common congenital defect of horses and is further characterized by the location of the retained testis (inguinal or abdominal). Two retrospective studies documented 350 cases over a 14-year period[1038] and 500 cases over a 23-year period.[1039] Diagnosis is fairly straightforward and involves both palpation and ultrasonography. The former is useful for confirming the lack of a testis or testes, whereas the latter aids in locating the retained testicle, which is beneficial when planning a particular surgical approach.

When the castration status of a horse is unknown, endocrine analyses are relatively sensitive methods for detecting the presence of testicular tissue. Evaluation of resting testosterone concentration, hCG stimulation test, estrone sulfate concentrations, and AMH serum concentrations have all been described. The most common endocrine test performed to evaluate whether testicular tissue is present is baseline testosterone concentrations, with values <50 pg/mL suggesting an absence of functional testicular tissue, 50 to 100 pg/mL being inconclusive, and values >100 pg/mL suggesting the presence of functional testicular tissue. If this test is inconclusive, then an hCG stimulation test is performed that induces an increase in testosterone secretion and production from the Leydig cells within the testes. This test involves collection of a baseline serum sample, followed by IV administration of 5000 to 10,000 IU hCG and collection of serum at 24 and 48 hours later. A 2- to 3-fold increase in circulating testosterone concentration from baseline indicates the presences of active testicular tissue. Unfortunately, this test can yield equivocal results when posttreatment samples are collected too early.[1040] The current recommendation for those using this test is to collect the posttreatment sample 2 days after hCG stimulation.[1041] Detection of AMH is proving to be just as—if not more—sensitive for the diagnosis of cryptorchidism.[968] This test only requires a single serum sample but is subjected to seasonal influences.[1040]

Nonsurgical treatment for cryptorchidism should be initiated early and cases selected appropriately to increase the likelihood of success.[1042] When the testis is located within the abdomen, surgical removal is the only means of removal. Retained testicles should be dealt with at an early age for the following reasons:

1. Trainers believe the retained testicle can be a periodic source of discomfort.
2. A retained testicle, while unlikely to produce viable spermatozoa, remains hormonally active, which can have suppressive effects on the descended testis.
3. Retained testes can become neoplastic with advancing age.

To further the last point, the relative incidence of testicular cancer in stallions is difficult to precisely quantify because of the relatively low number of older intact males. Nevertheless, neoplasia of the testes can have serious consequences on breeding efficiency and overall health. Surgical removal is often curative, with the risk of metastatic disease relatively low, although not entirely uncommon.

Breeding stallions with only one functional testis can often be managed successfully. Generally speaking, the functional testis will hypertrophy. Young fertile stallions can often perform adequately with only one testicle. Appropriate

management of book size and selecting young mares can all favor a stallion, especially early in his career.

Gonadal Hypoplasia. Small testes may result from a number of underlying complex processes such as spermatogenic arrest and germ cell deficiencies. Testicular hypoplasia is the failure of the gonads to reach their full adult size and must be differentiated from gonadal atrophy or testicular degeneration, which is the reduction in testicular size after the gonad has reached full adult size. The cause of the hypoplastic gonad is complex and is thought to be congenital or acquired. Although not clear for horses, a genetic component has been identified in other species.[1016] Generally testicular hyperthermia, malnutrition, and endocrine imbalances—particularly in steroid-treated young stallions—can negatively affect testicular size. It is important to remember that the testes do not start to enlarge until 15 to 18 months of age, and they continue to increase in size until 4 to 5 years of age. Testicular hypoplasia should not be confirmed before the stallion is 2 to 3 years old. The breeding of mares to stallions with hypoplastic gonads is discouraged, but the value and performance record often trump breeding soundness. In these cases, implementation of managerial practices to maximize the reproductive performance of the animal is important and should include reducing the number of mares in the book and breeding mares only once when they are close to ovulation.

Gonadal Atrophy. Also known as *testicular degeneration,* gonadal atrophy is most common in the mature stallion and can lead to profound disruptions in spermatogenesis. Gonadal atrophy and gonadal hypoplasia must be differentiated because they represent distinct disease processes. A thorough reproductive examination and an accurate history are fundamental in differentiating the two conditions. One should note inconsistencies between measured sperm output and estimated sperm output (DSO) based on testis size. Small testes in relation to the epididymis indicate atrophy. However, developmental abnormalities such as a small penis and enlarged inguinal rings associated with small testicular size often indicate hypoplasia. Atrophy or degeneration is considered an acquired condition that may or may not be reversible, depending on the cause.

Testicular atrophy can be caused by exogenous hormone administration. Historically, colts or stallions given anabolic steroids to improve recovery and muscle mass experienced reduction in testicular size caused by suppression of endogenous testosterone production. The use of anabolic steroids is no longer common in the United States. The synthetic progestin, altrenogest, can also cause testicular atrophy and a reduction in seminal quality and quantity.[1043] The detrimental effects on spermatogenesis are caused by an increase in the circulating levels of androgens, which in turn provide negative feedback on LH secretion by the pituitary with a consequent decline in testosterone production. As a consequence, testicular function is compromised, leading to a significant reduction in sperm production. Fortunately, the negative effects of steroids on testicular function are generally considered to be reversible, at least with respect to short-term use.[123] Long-term effects are unknown and need further study.

Diagnosis of gonadal atrophy requires a multimodal approach consisting of an accurate history, physical examination, evaluation of the external genitalia, semen analysis, and endocrine testing. An early indication of testicular degeneration is poor spermatogenic efficiency or decreased sperm output relative to what is expected based on calculations from testis size. More specifically, these stallions can have adequate-sized testes, but the quantity and/or quality of spermatozoa within the ejaculate is low or unusually poor, and during the initial phases the testes can feel softer. With chronicity, the testicular parenchyma is replaced by connective tissue, which makes the testes feel firmer. Leydig and Sertoli cells and spermatogonia and spermatozoa are more resistant to degeneration than cells of the intermediate stages of spermatogenesis; therefore semen analysis varies depending on the extent of damage. In most cases gonadal atrophy does not affect libido.

Endocrine testing can help confirm a diagnosis of gonadal atrophy. In many instances stallions will have increased circulating FSH concentrations but decreased concentrations of other sex hormones, such as LH, estradiol, testosterone, and inhibin.[1044] The use of AMH is an interesting concept, but to the author's knowledge, the usefulness of this endocrine assay in evaluating stallions with possible testicular degeneration has yet to be determined.[968]

Treatment of gonadal atrophy revolves around determining the cause of degeneration. Resumption of normal testicular function can be expected in instances in which damage was mild and relatively short lived. Chronic disease and idiopathic causes carry a more guarded prognosis. Reduction in book size, maintaining a selective book, and advanced semen processing and insemination techniques may help achieve acceptable pregnancy rates. Administration of GnRH has anecdotally been reported to be of benefit in early cases but is unlikely to be of benefit in reversing degenerative changes. For further information on management strategies for gonadal atrophy the reader is directed to an excellent review.[1045]

Acquired Conditions

Any condition causing hyperthermia or hypothermia can lead to damage and abnormal testicular function. Examples of conditions implicated as possible causes of testicular degeneration include prolonged recumbency, trauma, torsions of the spermatic cord, disruption of the blood-testis barrier and the consequent production of antisperm antibodies, inappropriate or prolonged steroid therapy, accumulations of fluids such as in hydroceles, and advanced age. Scrotal lesions that impair the normal testicular thermoregulation may be a significant factor causing gonadal atrophy. Other acquired disorders of the testes include radiation exposure; nutritional disorders, particularly those of vitamin A and zinc; and toxicity with heavy metals or nitrogen, phosphorus, and halogenated compounds.

Immune-mediated orchitis is rare in stallions. As soon as spermatogonia enter the leptotene stage during the meiotic phase, they become isolated from the general immune system by tight junctions between adjacent Sertoli cells. As mentioned previously, these gap junctions form the blood-testis barrier. A fine balance is maintained so that maturing spermatids can migrate toward the adluminal compartment without eliciting an immunologic response. In addition to the blood-testis barrier, local immunosuppressors are present in the testicular interstitium. Factors that disrupt the blood-testis barrier with the consequent formation of antisperm antibodies include tumors, trauma, biopsies, and spermatic cord torsions of more than 360 degrees. The association between antisperm antibodies and infertility, although reported for the stallion, warrants further investigation.[1046]

Hypertrophy refers to a condition in which the individual cells of the testes increase in size. The most common cause of testicular hypertrophy is removal of one testis or the abdominal

retention of one that triggers a compensatory growth of the contralateral gonad. *Hyperplasia* refers to an increase in the number of cells and can be focal or generalized. Testicular hyperplasia is rare in stallions.

Neoplastic Conditions

With an estimated frequency of approximately 4%, testicular cancer in stallions is relatively rare. The low occurrence is likely related to the relatively low number of sexually mature intact males compared with females. Teratomas were the most common (37%), followed by interstitial cell tumors (30%), seminomas (23%), lipomas (7%), and mast cell tumors (3%).[1047] Teratomas are commonly found in juvenile colts at the time of castration, which may explain their overrepresentation in incidence. Cryptorchid testes appear to have a predisposition for neoplasia, especially germ cell tumors, such as seminomas.[1047] Risk of metastasis is generally considered low, but one author has known two stallions that experienced secondary complications and subsequent death from malignancies arising from confirmed seminomas. Consequently, ensuring adequate excisional margins by histopathologic evaluation of excised tissue and evaluating regional lymph nodes provide useful information related to prognosis for that stallion.

Diagnosis of testicular neoplasia is based on a careful examination of the testes and related structures. One should palpate the scrotal contents and note soft or firm spots, nodules, or asymmetry. Ultrasonographic examination is crucial for the identification of fluid-filled or solid nonpalpable lesions embedded deep in the testicular stroma. The spermiogram often yields nonspecific findings but can demonstrate a high incidence of teratozoospermia (abnormal morphology), asthenozoospermia (poor motility), and/or the presence of round spermatids and other testicular cells. Abdominally retained testes can be diagnosed by transrectal and transabdominal palpation and ultrasonography. Unilateral or bilateral orchiectomy is the treatment of choice regardless of the type of tumor. Depending on the size and location of the testis, an inguinal, flank, or ventral midline incision is recommended. Ligation and removal of as much of the cord as possible are strongly recommended, and sampling of regional lymph nodes can be useful for determining the risk of metastatic disease. The gross appearance of testicular neoplasia can vary with the primary cell type. Interstitial cell tumors are usually soft, orange, and nodular, with no clear demarcation with the adjacent testicular tissue. Seminomas can vary in color from white to dark gray with a glistening appearance; the neoplastic area frequently bulges above the adjacent testicular tissue. Fluid-filled cysts often are present. Sertoli cell tumors are usually firm, nodular, and pale gray. Teratomas are easily identifiable by the presence of tissue of different origins (e.g., bone, hair). Ultimately, diagnosis is confirmed by histopathologic examination of the affected tissue.

Infectious Conditions

Inflammation of one or both testes is referred to as *orchitis.* Causes may be bacterial, viral, parasitic, or aseptic after trauma. Orchitis can be primary or secondary to another disorder. It is important to differentiate this condition from the more common conditions of periorchitis or scrotal edema, although the two conditions may be present simultaneously. Bacterial orchitis in horses may be caused by *Brucella abortus, Actinobacillus equuli, Pseudomonas pseudomallei, S. zooepidemicus, S. equisimilis, Salmonella* spp., *E. coli,* and *Staphylococcus* spp.[1048] The viral diseases EVA and EIA affect the testes because of associated fevers and edema. These agents can also be shed in the semen of affected stallions. Focal lymphocytic infiltrations may be observed in affected stallions. Parasitic orchitis is usually a sequela of migratory larvae of the parasite *Strongylus* spp.[1049] The condition can affect descended or undescended testicles and the tunics and spermatic cords. A possible secondary lesion associated with larvae is known as focal lymphocytic orchitis, which occurs around the seminiferous tubules.[1050] It is different from the condition of autoimmune orchitis reported for the mouse in which foci of lymphocytes are localized exclusively at the rete testis and efferent ductules. Initial diagnosis of the granulomatous-type lesions in the testicle caused by strongylosis sometimes can be done by ultrasonography, but histopathologic identification is necessary for a definitive diagnosis. Regular deworming programs with ivermectin can help control both of these conditions.

Abnormalities of the Epididymides

Congenital Conditions

Blind-ended ductules can form within the epididymis and likely represent failure of fusion of the ducts during embryonic or fetal development. If sufficient numbers of tubules are blocked, then the condition may lead to spermiostasis with development of cystic dilations, formation of epididymal sperm granulomas, and reduction of fertility. These cystic dilations may be diagnosed by palpation and ultrasonography.

Segmental aplasia of the mesonephric duct or epididymal agenesis is rarely reported in horses. Interestingly, the epididymis in the stallion is not fused completely with the testicle as in other species, which may predispose them to epididymal pathology such as spermatoceles and cystic dilations. Other, less common conditions include adenomyosis and tumors.[1051] Palpation and ultrasonography may be useful in diagnosis of these conditions.

Acquired Conditions

Other causes of epididymal dysfunction can manifest as abnormal accumulation of sperm in the cauda epididymides or generalized dysfunction of the epididymal epithelium associated with deficiencies in electrolyte, protein, or steroid secretion or resorption. Accumulated material may become inspissated and clog the normograde flow of sperm. Epididymal dysfunction, whether primary or secondary, can create changes in pH and osmolarity of the seminal plasma that might adversely affect maturation and viability of sperm. These conditions can be diagnosed by frequent semen collections (once or twice daily for 7–10 days) and evaluation of sperm morphology and motility, as well as seminal pH and metabolite analysis. Oligospermia and varying degrees of teratospermia are common. Morphologies from stallions with plugged cauda epididymides often have an unusually high percentage of detached heads and clumps of spermatozoa. Normal stallions ejaculate semen with a pH of 7.4, whereas those with decreased circulating testosterone and estradiol concentrations tend to have higher pH levels.[1052] Because the accessory sex glands contribute a significant portion of volume to semen, disorders affecting these structures also need to be ruled out.

Infectious Conditions

Bacteria or trauma to the scrotal area may cause inflammation of the epididymides. Infectious epididymitis as a primary

disease is rare in stallions and is considered a sequela to orchitis or to deep lacerations of the scrotal area. Migration of *Strongylus edentatus* larvae also may cause epididymitis with the consequent formation of granulomas.[1053] One confirms a diagnosis of epididymitis by palpation, ultrasonography, the presence of inflammatory cells in the ejaculate, and bacterial growth on culture.

Abnormalities of the Spermatic Cord

Congenital Conditions

The terms *inguinal hernia* and *scrotal hernia* are often used interchangeably. These involve protrusion of intestines or omentum through the vaginal ring and into the vaginal cavity. Ruptures of the vaginal sac can also occur. Inguinal hernias are more common in intact males because of the rapid closure of the rings following castration. Congenital and hereditary causes have been identified,[1054] and one of the authors (CFS) has seen them most often in Standardbred horses. Percutaneous and transrectal palpation and ultrasonography are ideal for diagnosing inguinal hernias and ruptures. In foals, many will spontaneously resolve by 6 months of age. Application of a truss or frequent manual reduction may hasten resolution in some affected horses. Occasionally, the bowel may become incarcerated and undergo ischemic insult, necessitating surgical closure of the rings and possible resection of bowel.

Acquired Conditions

Vascular problems associated with the cord include torsion, varicoceles, and thrombosis.[1055,1056] Torsion of the cord is significant when the cord has rotated more than 180 degrees. Torsions of less than 180 degrees are usually an incidental finding in young horses and are usually transient but occasionally permanent. Oftentimes torsions of less than 180 degrees are of little clinical significance, especially when no other clinical signs are evident. Torsions greater than 270 degrees are associated with scrotal swelling, pain on palpation, an abnormal gait, and acute abdominal pain. Diagnosis is made by a combination of clinical signs, palpation, and ultrasonography. The latter modality is very useful in differentiating spermatic cord torsions from scrotal hernias. The treatment of choice in spermatic cord torsion is hemiorchiectomy because the affected testicle will likely necrose and become nonfunctional.

Varicoceles in stallions are of questionable significance, especially if unilateral. Nevertheless, they have been associated with poor seminal quality and subfertility in other species.[1057,1058] If severe enough, the function of the pampiniform plexus (the mechanism responsible for countercurrent heat exchange of blood entering and exiting the testes) can be altered, predisposing the developing sperm cells to hyperthermia. Thrombosis of the cord is a more serious condition. The clinical signs resemble those of pathologic spermatic cord torsions, and unilateral castration is recommended. Ultrasonography is a useful means of diagnosis, and color flow Doppler can be used to evaluate blood flow when restriction, thrombosis, or infarction is suspected.

Infectious Conditions

Diseases and disorders of the spermatic cord involving older horses are primarily limited to infections and vascular issues. In intact horses, the most common infectious causes would be aberrant parasite migration and vasculitis secondary to local or generalized sepsis. Infection of the spermatic cord can occur following castration and is referred to as *septic funiculitis, scirrhous cord,* or *champignon.* Treatment consists of systemic broad-spectrum antibiotic therapy and supportive care. Surgical debridement and removal of the necrotic foci are also necessary because most cases do not resolve with medical management only.

Aberrant parasite migration into the pampiniform plexus can occasionally occur. However, it is now uncommon because of the widespread use of the avermectins, which have effectively resolved large strongyle infections.

Neoplastic Conditions

Neoplastic conditions are rare and usually secondary to invasion or metastases. Seminomas can invade the cord, and blood-borne cancers such as lymphoma may also metastasize to the spermatic cord. Diagnosis is made based on accompanying clinical signs, imaging, and histopathology.

Abnormalities of the Accessory Sex Glands

Congenital Conditions

Congenital disorders of the accessory sex glands in intact male horses are rare. In cases of DSDs, infantile or hypoplastic glands may be noted in the absence of androgen stimulation.

Acquired Conditions

The most common acquired condition of the accessory sex glands is spermiostasis or plugged ampullae. Stallions with this condition are referred to as *sperm accumulators.* Current theory holds that these stallions, when subject to prolonged periods of sexual rest, accumulate an abnormal amount of sperm and seminal secretions in their ampullae and deferent ducts. The incidence is estimated at ~25% (DD Varner, personal communication, 2015), and Thoroughbred stallions are overrepresented in most estimates. Possible causes include stallions failing to void "stale" sperm in their urine (unconfirmed observation) and/or anatomic variations causing narrowing of the lumen of the deferent ducts.[83,86]

Diagnosis entails collection of sequential semen samples and evaluation of the spermiogram. Ejaculates can range from oligospermic to containing unusually high (>30 billion) total sperm cells. In stallions producing a large amount of sperm, a characteristic finding is a high frequency of detached heads as determined by DIC microscopy. "Clumps" of sperm aggregates may also be seen. Ultrasonography per rectum can also be used to evaluate the accessory sex glands. Blocked ampullae can have a distended lumen and even hyperechoic regions proximal to the seminal colliculus, suggesting inspissation of seminal material.

The most common treatments for spermiostasis are frequent and consistent collections or breedings. Stallions initially diagnosed with this condition may take 1 to 2 weeks for seminal quality to improve. It is important to maintain accumulating stallions on a regular breeding schedule when possible. Other treatment strategies include administration of ecbolic agents (oxytocin [10–20 IU, IM/IV] or $PGF_{2\alpha}$ [cloprostenol; 25–250 µg, IM]) and manual massage of the ampullae per rectum. Severe cases can be treated with normograde or retrograde flushing of the ampullae and deferent ducts. One author (CFS) has used phenylephrine (20 mg, diluted in 1 L LRS, IV) in a horse with refractory plugged ampullae with positive results. This treatment, however, did cause moderate colic-like signs and intense full-body contractions during and shortly after

treatment and should only be administered with caution and informed consent from owners.

Neoplastic Conditions

Neoplasia of the accessory sex glands is rare. A recent report from the University of Pennsylvania described two cases of prostatic masses that were confirmed as either prostatic cystadenoma or adenocarcinoma.[1059] Surgical debulking was performed in one horse, but both horses died within 9 months of diagnosis.

Infectious Conditions

Although infections of the accessory sex glands are uncommon, unilateral or bilateral seminal vesiculitis can occur. Bacterial infection of the seminal vesicles is usually not accompanied by any external clinical signs. The ejaculate, however, is often turbid and discolored, and evaluation reveals a significant number of degenerate white blood cells. The glands may be enlarged, firm, and painful on palpation if the condition is acute. Ultrasonography can be used, but results need to be interpreted with caution because the size of the seminal vesicle can vary with breed, season, and sexual stimulation. Diagnosis of seminal vesiculitis is best made by rectal palpation, observation of large numbers of neutrophils in the semen (gel fraction), bacterial culture of semen and/or gel fraction, and endoscopy of the urethra and seminal vesicles. Direct culture of the seminal vesicles during endoscopy increases the clinician's confidence in the significance of organisms cultured. Treatment is difficult, and the prognosis is guarded. Reported treatments include systemic and local antibiotic therapy as well as the addition of extenders containing appropriate antibiotics to the semen of the affected stallion. Endoscopic evaluation is highly useful and can be followed by lavage and local antibiotic infusion.

Sexually mature stallions can harbor EAV in their accessory sex glands. This leads to chronic shedding and transmission of the virus to mares. This virus and its disease (EVA) will be discussed later in a separate section on equine venereal disease.

Abnormalities of Semen and Spermatozoa

Azoospermia and *oligospermia* describe the absence or abnormally low numbers of spermatozoa in an ejaculate, respectively. *Teratospermia* refers to the presence of an unusually large number of individual sperm defects, whereas *asthenospermia* refers to sperm with poor motility. Causes can be characterized as developmental, acquired, and infectious.

Developmental Conditions

Azoospermia is usually secondary to a gonadal or epididymal disorder. Cryptorchidism, anorchidism, and testicular hypoplasia secondary to a DSD can lead to low sperm production. Hypoplasia of the epididymides and deferent ducts leading to outflow obstructions can also lead to little to no sperm cells within an ejaculate. Diagnosis and treatment of cryptorchidism have previously been discussed. Developmental abnormalities are often diagnosed via exclusion and genetic testing (e.g., karyotyping and molecular studies).

Infectious and Acquired Conditions

Pyospermia. The hallmark of this condition is the presence of purulent debris, white blood cells, and pathogenic organisms in an ejaculate. This condition is usually secondary to infection or inflammation of the deferent ducts, accessory sex glands (e.g., seminal vesicles), and/or urethra. Following a live-cover, normal dismount, samples may be "contaminated" with cellular debris and microorganisms. Caution should thus be exercised not to overinterpret the findings in these samples, which should be analyzed with respect to current reproductive performance. Aerobic cultures of the stallion's penis, fossa glandis, urethral sinus, and preejaculate and postejaculate urethra can help with localization of the infection. Further characterization of the source can be aided by fractionation of the ejaculate using an open-ended AV. If the clinician suspects that the stallion is the cause of bacteria-related fertility problems in a group of mares, then it is important to culture the mares to determine whether the mares are harboring the same pathogens as the stallion. Giemsa, Wright, or Diff-Quik stains of the semen aid in identifying the type of white blood cell present. Treatment of pyospermia consists of resolving the primary issue, use of semen extenders with appropriate antibiotics, and isolation of the sperm-rich fraction using centrifugation or filtration.

Urospermia. General causes of urospermia include neurologic disease, orthopedic conditions, psychogenic or behavioral problems, and idiopathic causes. Although uncommon, retrograde ejaculation can also occur.[1060] Urine-contaminated semen is often readily detectable because of changes in color (yellow), odor (urea), and volume (increased). However, when urine is not obvious but suspected to be a contaminant, the concentrations of urea nitrogen >30 mg/dL and creatinine >2 mg/dL in the semen are diagnostic of urospermia. The effect of urine on the sperm cells is not well documented. However, the reduction in motility and fertility is significant because of the effects of hyperosmotic medium and water removal from the sperm cells. One author (JCS) has observed several cases of urospermia associated with self-mutilating stallions. Pharmacologic therapy of the problem is purely empiric and limited to drugs that act to close the neck of the bladder, such as β-blockers. A more common approach is to use managerial procedures such as encouraging urination before semen collection and the collection of semen directly into an extender and immediate centrifugation. The ideal procedure is to fractionate the ejaculate during collection with an open-ended AV. In breeds for which AI is not allowed, an extender may be infused into the uterus and flushed out 4 to 6 hours later. Holding stallions in a freshly bedded stall or placing feces from another stallion in their stall before semen collection can sometimes stimulate the stallion to void urine and can thus be used to limit the risk of urospermia.

Hemospermia. Hemospermia is typified by an unusually large amount of blood (fresh or dried) in an ejaculate. The presence of erythrocytes in semen can adversely affect seminal quality and subsequent fertility.[1061] Decreased progressive motility and plasma membrane integrity were lower in ejaculates incubated in whole blood compared with controls. In a recent report on eight cases, causes of hemospermia included urethral rents (3), urethral process lesions (2), squamous cell carcinoma (1), kick to the penis (1), and unknown origin (1).[1062] Penile lacerations and habronemiasis are other potential causes of hemospermia. Interestingly, stallions collected for AI were overrepresented in reviews on this condition.[1062,1063] If the bleeding site cannot be found externally, another common site is where the pelvic urethra folds over the ischium. Urethroscopy performed immediately after ejaculation can be used to visualize the penile and pelvic urethra and identify the

location of a rent. If the source of bleeding can be identified in the urethra, a common approach to treatment is sexual rest to allow for tissue remodeling. Alternatively, a urethrostomy of the area is indicated for horses that fail to respond with conservative therapy. The urethrostomy relieves pressure and allows the rent in the urethra to heal by second intention. Some of the management procedures described for urospermia, such as fractionation and extension, also may be considered.

Sexually Transmitted Diseases

There are several STDs that are relevant from both clinical and regulatory standpoints. Readers are strongly encouraged to contact their local regulatory official for updated information or whenever a reportable or foreign animal disease (FAD) is suspected. Questions pertaining to handling of potentially infected gametes (e.g., spermatozoa, embryos) should be directed to local regulatory officials.

Contagious Equine Metritis

CEM is a true venereal disease caused by the bacterium *T. equigenitalis,* a highly contagious gram-negative coccobacillus.[240] Transmission via fomites is possible and was implicated in a recent outbreak in the United States.[1064] Clinical signs vary from relatively inapparent to copious vulvar discharge originating from the uterus.[241] Stallions rarely display clinical signs but are unapparent carriers and are the most common means of transmission because they are much more sexually active. Significant drops in reproductive efficiency ensue, leading to increased costs associated with management and veterinary care. Diagnosis is made by isolation of the organism. Because of the organism's rather fastidious nature, samples should be sent to laboratories familiar with culturing techniques. Treatment for *T. equigenitalis* is fairly straightforward and consists of careful cleansing of the internal and external genitalia and application of local antiinfective agents. The United States has undergone a reclassification in its status as a CEM-free country because of the previously mentioned outbreak. Readers are thus encouraged to contact their local USDA official for current recommendations. All breeding activity should be withheld until proper screening and prophylactic treatments have been performed before introduction of the animal into a local breeding population.

Equine Viral Arteritis

EVA is another disease that has important regulatory implications. The causative agent is EAV, which is an enveloped, single-stranded RNA virus.[242] Clinical signs include vasculitis, distal limb edema, pyrexia, rhinitis, conjunctivitis, and anorexia. The virus can also cause abortion outbreaks and severe neonatal pneumonia in naïve herds. It is transmitted via respiratory secretions and other body fluids, including semen and fetal fluids/tissues, and fomites. Current estimates suggest that 30% to 70% of exposed stallions will become persistently infected when exposed to the virus, and they are referred to as shedder stallions.[243] Consequently, prevention of the disease is key and has even been nearly eradicated in commercial North American Thoroughbreds by screening and vaccination. A modified-live virus vaccine is commercially available in the United States (Arvac; Zoetis, Parsippany, NJ) and approved for use in breeding stallions. In some breeds that allow AI, breeding with EAV-infected semen is permissible with proper documentation of the mare's vaccination status. Certain breed registries and state agricultural departments have restrictions regarding vaccination of breeding stock and use of EAV-infected semen. State requirements can be obtained by contacting local regulatory officials.

Equine Herpesvirus Type 3

EHV-3 is the causative agent of *equine coital exanthema,* characterized by vesicular or poxlike lesions on the genitalia that eventually progress to depigmented regions of skin. It is not currently considered a reportable disease in the United States. Transmission is most common via coitus but can also occur via fomites. Clinical disease is primarily manifested as vesicular and painful lesions on the external genitalia of both mares and stallions. During the acute phase, these lesions can cause discomfort during reproductive evaluations and during live-cover matings, but infection has no lasting or impressionable effects on fertility.[244] Lesions will take 4 to 6 weeks to heal and are characterized by focal areas of depigmentation. Gentle cleansing of active lesions may soothe and reduce inflammation. Reports of antiviral treatments are sparse. One author (CFS) has used docosanol cream (Abreva; Zoetis, Florham Park, NJ) in four cases with seemingly good results in resolving the acute inflammation associated with the lesions. However, no comparison was made regarding the length of the convalescent period between treated and control groups. Transmission to other animals is a concern and seems to be highest during the acute phase of this disease. A period of 4 to 6 weeks of sexual rest is recommended to prevent further transmission and allow the lesions to heal. Resumption of breeding activity can occur once the lesions have healed. Natural recrudescence of this particular herpesvirus is unknown,[245] but experimental administration of dexamethasone has been shown to cause reactivation of the virus.[1065] Control is aimed at early detection and sexual rest until lesions have subsided. There is no commercially available vaccine against EHV-3 in the United States.

Dourine

Dourine is a venereal disease of significance because of its high level of morbidity and its effect on international transport of equids. The etiologic agent is the protozoa *T. equiperdum.* Clinical signs include intermittent fever, swelling of and discharge from the genitalia, cutaneous plaques, and neurologic signs.[246] Generally considered a chronic and progressive disease, the onset of recumbency usually indicates terminal disease. Dourine has been eradicated in the United States, but it is still present in Africa, the Middle East, South America, and Central America. Sporadic outbreaks have been reported in Asia and Germany.[240] Diagnosis is made via compatible clinical signs and laboratory testing. The CF test is prone to false positives from other species of *Trypanosoma,* and newer methods of diagnostics, including ELISA and PCR technologies, are currently being explored for both testing and screening purposes. Infected animals should be removed from breeding and culled. Treatment with antiprotozoal drugs has been performed in some endemic areas, but they are unproven and even discouraged because of concern about inapparent shedding animals. Suspected cases of dourine should be reported immediately to local regulatory officials.

Piroplasmosis

Previously eradicated in the United States, cases of piroplasmosis have sporadically occurred over the past few years. This disease is caused by the protozoa *Theileria equi* (formerly *Babesia equi*) and *B. caballi.* Transmission occurs either via

an insect vector (e.g., ticks) or mechanically (e.g., improperly sanitized instruments). It also has the potential to be transmitted venereally in cases of hemospermia. Piroplasmosis creates disturbances in several body systems. Clinical signs are often nonspecific and vary based on the severity of disease and chronicity. Horses surviving the acute phase can become carriers. Diagnosis is confirmed based on observation of the organism on blood smears and serologic testing. Treatment with antiprotozoan medications is unproven, and recrudescence is a concern.[240] Similar to other reportable diseases, suspect cases should be directed to the local USDA-APHIS office.

Opportunistic Pathogens

Several aerobic bacteria have been associated with poor reproductive efficiency. These include β strep, *E. coli, P. aeruginosa,* and *Klebsiella* spp. Although rarely a cause for clinical disease,[1066] overgrowth of these bacteria may occasionally influence pregnancy rates in, for example, susceptible mares. Serial collection of aerobic cultures of the external genitalia (e.g., penis/prepuce, urethral fossa, and preejaculate and postejaculate urethra) is common to monitor for pathogens. Cleansing with lukewarm water and drying the penis before and after breeding are useful preventive measures, and they also allow for inspection of the genitalia for abnormalities. Treatment with local and/or systemic antibiotics is usually not indicated for fear of disrupting the normal flora on the genitalia. Washing with soaps or antiseptics may remove the normal flora and select for more resistant and pathogenic strains. The environment in which a stallion is housed may influence the types of microorganisms harbored on the external genitalia and should also be considered. When there is concern about transmission of potentially pathogenic bacteria, breeding strategies can be implemented to abate the risk. In the case of stallions used for live cover, semen extender containing an appropriate antibiotic can be infused into the uterus before or after mating. Semen from stallions used for AI should be screened for microorganisms and extended in an appropriate semen extender. For mares bred to these stallions, postbreeding management could entail uterine lavages, infusions, and ecbolics.

Conclusion

Whether developmental or acquired in nature, there are many conditions that can affect the reproductive tract of intact male horses. Careful evaluation of all body systems is indicated when disorders of the reproductive tract are suspected because of its interrelationship with other organs. This also holds true when examining a breeding stallion for fertility issues. In these particular cases, the sense of immediacy is ratcheted up a few notches to obtain a timely diagnosis and implement effective management strategies to improve reproductive efficiency. Clinical examination, diagnostic imaging, endocrine testing, and semen evaluation are all useful in meeting these goals.

REFERENCES

1. Senger PL. *Pathways to Pregnancy and Parturition*. 2nd ed. Pullman: Current Conceptions; 2003.
2. Dyce KM, Sack WO, Wensing CJG. *Textbook of Veterinary Anatomy*. 3rd ed. Philadelphia: Saunders; 2002.
3. Budras KD, Sack WO, Rock S. *Anatomy of the Horse, An Illustrated Text*. 4th ed. Hannover: Schultersche GmbH&Co; 2003.
4. Ginther OJ. *Reproductive Biology of the Mare: Basic and Applied Aspects*. 2nd ed. Cross Plains: Equiservices; 1992.
5. Blanchard TL, Varner DD, Schumacher J, et al. *Manual of Equine Reproduction*. 2nd ed. St. Louis: Mosby; 2003.
6. Youngquist RS, Threlfall WR. *Current Therapy in Large Animal Theriogenology*. 2nd ed. St. Louis: Saunders; 2007.
7. Battut IGdR, A. Nicaise JL, Fieni F, et al. When do equine embryos enter the uterine cavity? An attempt to answer. *5th Int Symp Equine Embryo Transfer Havemeyer Found Monogr Ser No 3*. 2001:66–68.
8. Weber JA, Freeman DA, Vanderwall DK, et al. Prostaglandin E2 hastens oviductal transport of equine embryos. *Biol Reprod*. 1991;45:544–546.
9. Buergelt CD. *Color Atlas of Reproductive Pathology of Domestic Animals*. St. Louis: Mosby; 1997.
10. Kenney RM. Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. *J Am Vet Med Assoc*. 1978;172:241–262.
11. Kainer RA. Internal reproductive anatomy. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
12. Sisson S, Grossman JD. *The Anatomy of the Domestic Animals*. 4th ed. Philadelphia: Saunders; 1953.
13. Bradecamp EA. Pneumovagina. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 4th ed. West Sussex: Wiley-Blackwell; 2011.
14. Duquesne F, Pronost S, Laugier C, et al. Identification of *Taylorella equigenitalis* responsible for contagious equine metritis in equine genital swabs by direct polymerase chain reaction. *Res Vet Sci*. 2007;82:47–49.
15. Burkhard J. Transition from anoestrus in the mare and the effects of artificial lighting. *J Agr Sci*. 1946;37:64–68.
16. Huszenicza G, Nagy P, Juhasz J, et al. Relationship between thyroid function and seasonal reproductive activity in mares. *J Reprod Fertil Suppl*. 2000;56:163–172.
17. King SS, Douglas BL, Roser JF, et al. Differential luteolytic function between the physiological breeding season, autumn transition and persistent winter cyclicity in the mare. *Anim Reprod Sci*. 2010;117:232–240.
18. Weedman BJ, King SS, Neumann KR, et al. Comparison of circulating estradiol-17B and folliculogenesis during the breeding season, autumn transition and anestrus in the mare. *J Equine Vet Sci*. 1993;13:502–505.
19. Sharp DC, Grubaugh WR. Use of push-pull perfusion techniques in studies of gonadotrophin-releasing hormone secretion in mares. *J Reprod Fertil Suppl*. 1987;35:289–296.
20. Davis SD, Grubaugh WR, Weithenauer J. Follicle integrity and serum estradiol 17B patterns during sexual recrudescence in the mare. *Biol Reprod Suppl*. 1987;36:143.
21. Donadeu FX, Ginther OJ. Follicular waves and circulating concentrations of gonadotrophins, inhibin and oestradiol during the anovulatory season in mares. *Reproduction*. 2002;124:875–885.
22. Sharp DC. Vernal transition into the breeding season. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
23. Tucker KE, Cleaver BD, Sharp DC. Does resumption of follicular estradiol synthesis during vernal transition involve a shift in steroidogenic pathways? *Biol Reprod Suppl*. 1993;1:517.
24. Newcombe JR. The follicle: practical aspects of follicular control. In: Samper JC, Pycock JF, McKinnon AO, eds. *Current Therapy in Equine Reproduction*. St. Louis: Saunders; 2007.
25. McCue PM, Scoggin CF, Lindholm ARG. Estrus. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
26. Ginther OJ, Gastal EL, Rodrigues BL, et al. Follicle diameters and hormone concentrations in the development of single versus double ovulations in mares. *Theriogenology*. 2008;69:583–590.
27. Pelehach L. Relationship between uterine edema and estrogen in pony mares. *Biol Reprod*. 1999;60:206.

28. Bastos HB, Kretzmann NA, Santos GO, et al. Gene expression of matrix metalloproteinases and LH receptors in mare follicular development. *Theriogenology*. 2014;82:1131–1136.
29. Martinez-Bovi R, Cuervo-Arango J. Intrafollicular treatment with prostaglandins PGE2 and PGF2α inhibits the formation of luteinised unruptured follicles and restores normal ovulation in mares treated with flunixin-meglumine. *Equine Vet J*. 2016;48:211–217.
30. Pycock JF. Treatment of fluid accumulation. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
31. Pycock JF, Newcombe JR. The relationship between intraluminal uterine fluid, endometritis, and pregnancy rate in the mare. *Equine Pract*. 1996;18:19–22.
32. McDonnell S. The Equid Ethogram: A Practical Field Guide to Horse Behavior. Eclipse Press, Lexington, KY.
33. Hughes JP, Couto MA, Stabenfeldt GH. Luteal phase ovulations: what are the options? *Proc Annu Meeting Soc Theriogenology*. 1985:123–125.
34. Taverne MAM, van der Weyden GC, Fontijne P, et al. In-vivo myometrial electrical activity in the cyclic mare. *J Reprod Fert*. 1979;56:521–532.
35. Troedsson MHT, Wistrom AOG, Liu IKM, et al. Registration of myometrial activity using multiple site electromyography in cyclic mares. *J Reprod Fert*. 1993;99:299–306.
36. King SS. Autumnal transition out of the breeding season. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
37. Gygax AP, Ganjam VK, Kenney RM. Clinical, microbiological and histological changes associated with uterine involution in the mare. *J Reprod Fertil Suppl*. 1979:571–578.
38. McKinnon AO, Squires EL, Harrison LA, et al. Ultrasonographic studies on the reproductive tract of mares after parturition: effect of involution and uterine fluid on pregnancy rates in mares with normal and delayed first postpartum ovulatory cycles. *J Am Vet Med Assoc*. 1988;192:350–353.
39. Blanchard TL, Thompson JA, Love CC, et al. Influence of day of postpartum breeding on pregnancy rate, pregnancy loss rate, and foaling rate in Thoroughbred mares. *Theriogenology*. 2012;77:1290–1296.
40. Turner DD, Garcia MC, Miller KF, et al. FSH and LH concentrations in periparturient mares. *J Reprod Fertil Suppl*. 1979;27:547–553.
41. Sargent GF, Pope NS, Kesler DJ. Postpartum LH profile characteristics, estrus and ovulation. The influence of short-term foal removal. *J Equine Vet Sci*. 1988;8:156–160.
42. Botha AE, Schulman ML, Bertschinger HJ, et al. The use of a GnRH vaccine to suppress mare ovarian activity in a large group of mares under field conditions. *Wildlife Research*. 2008;35:548–554.
43. Robinson SJ, McKinnon AO. Prolonged ovarian inactivity in broodmares temporally associated with administration of Equity. *Aust Equine Vet*. 2006;25:85–87.
44. Schulman ML, Botha AE, Muenscher SB, et al. Reversibility of the effects of GnRH-vaccination used to suppress reproductive function in mares. *Equine Vet J*. 2013;45:111–113.
45. Greenhoff GR, Kenney RM. Evaluation of reproductive status of nonpregnant mares. *J Am Vet Med Assoc*. 1975;167:449–458.
46. Hughes J. Clinical examination of the brood mare. *J Reprod Fert Suppl*. 1982;32:637.
47. Sessions-Bresnahan DR, Carnevale EM. The effect of equine metabolic syndrome on the ovarian follicular environment. *J Anim Sci*. 2014;92:1485–1494.
48. Vick MM, Sessions DR, Murphy BA, et al. Obesity is associated with altered metabolic and reproductive activity in the mare: effects of metformin on insulin sensitivity and reproductive cyclicity. *Reprod Fertil Dev*. 2006;18:609–617.
49. Pascoe RR. Observations on the length and angle of declination of the vulva and its relation to fertility in the mare. *J Reprod Fertil Suppl*. 1979;27:299–305.
50. Plumb DC. *Plumb's Veterinary Drug Handbook*. 8th ed. Wiley-Blackwell; 2015.
51. Carleton C. Atypical, asymmetrical, but abnormal? Large ovary syndrome. *Mare Reprod Symp, Annu Conf Theriogenology, Soc Theriogenology*. 1996.
52. Hughes JP, Stabenfeldt GH, Kennedy PC. The estrous cycle and selected functional and pathologic ovarian abnormalities in the mare. *Vet Clin North Am Large Anim Pract*. 1980;2:225–239.
53. Stabenfeldt GH, Hughes JP, Kennedy PC, et al. Clinical findings, pathological changes and endocrinological secretory patterns in mares with ovarian tumours. *J Reprod Fertil Suppl*. 1979;27:277–285.
54. Chopin JB, Chopin LK, Knott LM, et al. Unusual ovarian activity in a mare preceding the development of an ovarian granulosa cell tumour. *Aust Vet J*. 2002;80:32–36.
55. Hinrichs K, Watson ED, Kenney RM. Granulosa cell tumor in a mare with a functional contralateral ovary. *J Am Vet Med Assoc*. 1990;197:1037–1038.
56. Bailey MT, Troedsson MH, Wheato JE. Inhibin concentrations in mares with granulosa cell tumors. *Theriogenology*. 2002;57:1885–1895.
57. Claes AN, Ball BA. Biological functions and clinical applications of anti-Mullerian hormone in stallions and mares. *Vet Clin North Am Equine Pract*. 2016;32:451–464.
58. Piquette GN, Kenney RM, Sertich PL, et al. Equine granulosa-theca cell tumors express inhibin α- and βA-subunit messenger ribonucleic acids and proteins. *Biol Reprod*. 1990;43:1050–1057.
59. White RA, Allen WR. Use of ultrasound echography for the differential diagnosis of a granulosa cell tumour in a mare. *Equine Vet J*. 1985;17:401–402.
60. Hinrichs K, Hunt PR. Ultrasound as an aid to diagnosis of granulosa cell tumour in the mare. *Equine Vet J*. 1990;22:99–103.
61. Ginther OJ, Gastal MO, Gastal EL, et al. Induction of haemorrhagic anovulatory follicles in mares. *Reprod Fertil Dev*. 2008;20:947–954.
62. McCue PM, Squires EL. Persistent anovulatory follicles in the mare. *Theriogenology*. 2002;58:541–543.
63. Ginther OJ, Gastal EL, Gastal MO, et al. Incidence, endocrinology, vascularity, and morphology of haemorrhagic anovulatory follicles in mares. *J Equine Vet Sci*. 2007;27:130–139.
64. Ginther OJ, First NL. Maintenance of the corpus luteum in hysterectomized mares. *Am J Vet Res*. 1971;32:1687–1691.
65. Neely DP, Kindahl H, Stabenfeldt GH, et al. Prostaglandin release patterns in the mare: physiological, pathophysiological, and therapeutic responses. *J Reprod Fertil Suppl*. 1979;27:181–189.
66. Stabenfeldt GH, Hughes JP, Evans JW, et al. Spontaneous prolongation of luteal activity in the mare. *Equine Vet J*. 1974;6:158–163.
67. Stabenfeldt GH, Hughes JP, Wheat JD, et al. The role of the uterus in ovarian control in the mare. *J Reprod Fertil*. 1974;37:343–351.
68. Gajos K, Kozdrowski R, Nowak M, et al. Altered secretion of selected arachidonic acid metabolites during subclinical endometritis relative to estrous cycle stage and grade of fibrosis in mares. *Theriogenology*. 2015;84:457–466.
69. Chandley AC. Chromosome abnormalities as a cause of infertility in mares. *J Reprod Fert Suppl*. 1975;23:377–383.
70. Hughes J. Sex chromosome abnormalities. *J Reprod Fert Suppl*. 1982;32:642.
71. Hughes JP, Benirschke K, Kennedy PC, et al. Gonadal dysgenesis in the mare. *J Reprod Fertil Suppl*. 1975;23:385–390.
72. Bugno M, Klukowska J, Slota E, et al. A sporadic case of the sex-reversed mare (64,XY; SRY-negative): molecular and cytogenetic studies of the Y chromosome. *Theriogenology*. 2003;59:1597–1603.

73. Buoen LC, Zhang TQ, Weber AF, et al. SRY-negative, XX intersex horses: the need for pedigree studies to examine the mode of inheritance of the condition. *Equine Vet J*. 2000;32:78–81.
74. Davies TG. Turner's syndrome (karyotype 63 XO) in a Thoroughbred mare. *Equine Vet Educ*. 1995;7:15–17.
75. Long SE. Chromosome anomalies and infertility in the mare. *Equine Vet J*. 1988;20:89–93.
76. McFeely RA. A review of cytogenetics in equine reproduction. *J Reprod Fert Suppl*. 1975;23:371–374.
77. Villagomez DA, Lear TL, Chenier T, et al. Equine disorders of sexual development in 17 mares including XX, SRY-negative, XY, SRY-negative and XY, SRY-positive genotypes. *Sex Dev*. 2011;5:16–25.
78. Bucca S. Equine fetal gender determination from mid- to advanced-gestation by ultrasound. *Theriogenology*. 2005;64:568–571.
79. Makinen A, Hasegawa T, Makila M, et al. Infertility in two mares with XY and XXX sex chromosomes. *Equine Vet J*. 1999;31:346–349.
80. Bugno M, Slota E, Wieczorek M, et al. Nonmosaic X trisomy, detected by chromosome painting, in an infertile mare. *Equine Vet J*. 2003;35:209–210.
81. Halnan CRE, Watson JI, Pryde LC. Detection by G- and C-band karyotyping of gonosome anomalies in horses of different breeds. *J Reprod Fert Suppl*. 1982;32:626–627.
82. Schnobrich MR, Bradecamp EA, Lear TL. Reproductive findings in two trisomy X (65, XXX) Thoroughbred mares. *Clin Therio*. 2016;8:340.
83. Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. *Theriogenology*. 1992;37:1101–1115.
84. McKinnon AO, Squires EL, Carnevale EM, et al. Diagnostic ultrasonography of uterine pathology in the mare. *Proc Am Assoc Equine Pract*. 1987;33:605–622.
85. Adams GP, Kastelic JP, Bergfelt DR, et al. Effect of uterine inflammation and ultrasonically-detected uterine pathology on fertility in the mare. *J Reprod Fertil Suppl*. 1987;35:445–454.
86. Watson ED, Stokes CR, David JS, et al. Concentrations of uterine luminal prostaglandins in mares with acute and persistent endometritis. *Equine Vet J*. 1987;19:31–37.
87. Brinsko SP, Rigby SL, Varner DD, et al. A practical method for recognizing mares susceptible to post-breeding endometritis. *Proc Am Assoc Equine Pract*. 2003;49:363–365.
88. Ginther OJ, Garcia MC, Bergfelt DR, et al. Embryonic loss in mares: pregnancy rate, length of interovulatory intervals, and progesterone concentrations associated with loss during days 11 to 15. *Theriogenology*. 1985;24:409–417.
89. Pycock JF. Management of the problem breeding mare. *Proceedings of the Annual Conference, The Society for Theriogenology*. Nashville, TN; 1999.
90. Troedsson MH, Madill S. Clinical examination of the reproductive tract of the mare. *Proceedings of the Annual Conference, The Society for Theriogenology*. Nashville, TN; 1999.
91. Zent WW, Troedsson MH, Xue J. Postbreeding uterine fluid accumulation in a normal population of thoroughbred mares: a field study. *Proc Am Assoc Equine Pract*. 1998;44:64–65.
92. Kenney RM, Ganjam VK. Selected pathological changes of the mare uterus and ovary. *J Reprod Fertil Suppl*. 1975;23:335–339.
93. McDowell KJ, Sharp DC, Grubaugh W, et al. Restricted conceptus mobility results in failure of pregnancy maintenance in mares. *Biol Reprod*. 1988;39:340–348.
94. Eilts BE, Scholl DT, Paccamonti DL, et al. Prevalence of endometrial cysts and their effect on fertility. *Biol Reprod Mono*. 1995;1:527–532.
95. Scherrer N. Treatment of uterine cysts with diode laser photoablation in a Thoroughbred brood mare population. *Proc Am Assoc Equine Pract*. 2015;61:469.
96. Hughes JP, Stabenfeldt GH, Kindahl H, et al. Pyometra in the mare. *J Reprod Fertil Suppl*. 1979;27:321–329.
97. Brown JA, Hodder AD, Benak J, et al. Uterus unicornis in two mares. *Aust Vet J*. 2007;85:371–374.
98. Bracher V, Stone R, Allen WR. Transendoscopic Nd:YAG laser surgery for treatment of intrauterine adhesions in 4 mares. *Equine Vet Educ*. 1994;6:22–26.
99. LeBlanc MM, Neuwirth L, Jones L, et al. Differences in uterine position of reproductively normal mares and those with delayed uterine clearance detected by scintigraphy. *Theriogenology*. 1998;50:49–54.
100. Easley KJ. Diagnosis and treatment of vesicovaginal reflux in the mare. *Vet Clin North Am Equine Pract*. 1988;4:407–416.
101. Bohn AA, Ferris RA, McCue PM. Comparison of equine endometrial cytology samples collected with uterine swab, uterine brush, and low-volume lavage from healthy mares. *Vet Clin Pathol*. 2014;43:594–600.
102. Cocchia N, Paciello O, Auletta L, et al. Comparison of the cytobrush, cottonswab, and low-volume uterine flush techniques to evaluate endometrial cytology for diagnosing endometritis in chronically infertile mares. *Theriogenology*. 2012;77:89–98.
103. Kozdrowski R, Sikora M, Buczkowska J, et al. Effects of cycle stage and sampling procedure on interpretation of endometrial cytology in mares. *Anim Reprod Sci*. 2015;154:56–62.
104. Threlfall WR. Broodmare uterine therapy. *Comp Cont Educ Pract Vet*. 1980;11:S246–S254.
105. Burleson MD, LeBlanc MM, Riddle WT, et al. Endometrial microbial isolates are associated with different ultrasonographic and endometrial cytology findings in Thoroughbred mares. *Proc Am Assoc Equine Pract*. 2010;56:317.
106. Riddle WT, LeBlanc MM, Stromberg AJ. Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. *Theriogenology*. 2007;68:395–402.
107. Ferris RA, Veir JK, Lappin MR, et al. Development and comparison of sampling techniques for a broad range, semiquantitative polymerase chain reaction assay for detection of bacterial DNA in the equine uterus. *J Equine Vet Sci*. 2014;34:687–693.
108. Hinrichs K, Cummings MR, Sertich PL, et al. Clinical significance of aerobic bacterial flora of the uterus, vagina, vestibule, and clitoral fossa of clinically normal mares. *J Am Vet Med Assoc*. 1988;193:72–75.
109. Petersen MR, Skive B, Christoffersen M, et al. Activation of persistent *Streptococcus equi* subspecies *zooepidemicus* in mares with subclinical endometritis. *Vet Microbiol*. 2015;179:119–125.
110. Blanchard TL, Garcia MC, Hurtgen JP, et al. Comparison of two techniques for obtaining endometrial bacteriologic cultures in the mare. *Theriogenology*. 1981;16:85–93.
111. Millar R, Francis J. The relation of clinical and bacteriological findings to fertility in thoroughbred mares. *Aust Vet J*. 1974;50:351–355.
112. Stratton LG, Corstvet R, Brown J, et al. Isolation of *Klebsiella pneumoniae* from the urogenital tract of experimentally infected mares. *J Reprod Fertil Suppl*. 1979;27:317–320.
113. Conboy HS. Diagnosis and therapy of equine endometritis. *Proc Am Assoc Equine Pract*. 1978;24:165–171.
114. McCue PM, Hughes JP, Jang SS, et al. Antimicrobial susceptibility patterns for equine endometrial isolates. *Calif Vet*. 1991;45:23–26.
115. Ricketts SW, Young AME. Uterine and clitoral cultures. In: McKinnon AO, Voss JL, eds. *Equine Reproduction*. Philadelphia: Lea & Febiger; 1993:234–245.
116. Woolcock JB. Equine bacterial endometritis. Diagnosis, interpretation, and treatment. *Vet Clin North Am Large Anim Pract*. 1980;2:241–251.
117. Knudsen O. Endometrial cytology as a diagnostic aid in mares. *Cornell Vet*. 1964;54:415–422.
118. Riddle WT, LeBlanc MM, Pierce SW, et al. Relationships between pregnancy rates, uterine cytology, and culture results in a Thoroughbred practice in central Kentucky. *Proc Am Assoc Equine Pract*. 2005;51:198–201.

119. Asbury AC. Endometrial biopsy. In: Colahan P, Mayhew I, Merritt A, et al., eds. *Equine Medicine and Surgery*. 5th ed. St. Louis: Mosby; 1999:1097–1100.
120. Couto MA, Hughes JP. Technique and interpretation of cervical and endometrial cytology in the mare. *J Equine Vet Sci*. 1984;4:265–273.
121. Crickman JA, Pugh DG. Equine endometrial cytology: a review of techniques and interpretations. *Vet Med*. 1986;81:650.
122. Ricketts SW. Endometrial biopsy as a guide to diagnosis of endometrial pathology in the mare. *J Reprod Fertil Suppl*. 1975;23:341–345.
123. Ricketts SW. The technique and clinical application of endometrial biopsy in the mare. *Equine Vet J*. 1975;7:102–108.
124. Carleton CL. Basic techniques for evaluating the subfertile mare. *Vet Med*. 1988;83:1253–1261.
125. Dybdal NO, Daels PF, Couto MA, et al. Investigation of the reliability of a single endometrial biopsy sample, with a note on the correlation between uterine cysts on biopsy grade. *J Reprod Fertil Suppl*. 1991;44:697.
126. Bergman RV, Kenney RM. Representativeness of a uterine biopsy in the mare. *Proc Am Assoc Equine Pract*. 1975;21: 355–362.
127. Held JP, Rohrback B. Clinical significance of uterine biopsy results in the maiden and non-maiden mare. *J Reprod Fertil Suppl*. 1991;44:698.
128. Kenney RM. Prognostic value of endometrial biopsy of the mare. *J Reprod Fertil Suppl*. 1975;23:347–348.
129. Ricketts SW, Alonso S. Assessment of the breeding prognosis of mares using paired endometrial biopsy techniques. *Equine Vet J*. 1991;23:185–188.
130. Ricketts SW, Alonso S. The effect of age and parity on the development of equine chronic endometrial disease. *Equine Vet J*. 1991;23:189–192.
131. Kilgenstein HJ, Schoniger S, Schoon D, et al. Microscopic examination of endometrial biopsies of retired sports mares: an explanation for the clinically observed subfertility? *Res Vet Sci*. 2015;99:171–179.
132. Woodward EM, Christoffersen M, Campos J, et al. Susceptibility to persistent breeding-induced endometritis in the mare: relationship to endometrial biopsy score and age, and variations between seasons. *Theriogenology*. 2012;78: 495–501.
133. Schiemann V. Studies on the uterine distension and intrauterine pressure development during the hysteroscopy of the horse. *School of Veterinary Medicine*. Hannover: University of Hannover; 2001.
134. Bracher V, Allen WR. Videoendoscopic evaluation of the mare's uterus: I. Findings in normal fertile mares. *Equine Vet J*. 1992;24:274–278.
135. Bracher V, Mathias S, Allen WR. Videoendoscopic evaluation of the mare's uterus: II. Findings in subfertile mares. *Equine Vet J*. 1992;24:279–284.
136. Perkins NR, Threlfall WR, Ottobre JS. Pulsatile secretion of luteinizing hormone and progesterone in mares during the estrous cycle and early pregnancy. *Am J Vet Res*. 1993;54: 1929–1934.
137. Perkins NR, Threlfall WR, Ottobre JS. Absence of diurnal variation in serum progesterone concentrations in mares. *Theriogenology*. 1993;39:1353–1365.
138. Ginther OJ. Embryonic loss in mares: incidence, time of occurrence, and hormonal involvement. *Theriogenology*. 1985;23:77–89.
139. Knowles JE, Squires EL, Shideler RK, et al. Relationship of progesterone to early pregnancy loss in mares. *J Equine Vet Sci*. 1993;13:528–533.
140. Morgenthal JC, van Niekerck CH. Plasma progestogen levels in normal mares with luteal deficiency during early pregnancy, and in twinning habitual aborters. *J Reprod Fertil Suppl*. 1991;44: 728–729.
141. Nagy P, Huszenicza G, Reiczigel J, et al. Factors affecting plasma progesterone concentration and the retrospective determination of time of ovulation in cyclic mares. *Theriogenology*. 2004;61:203–214.
142. Shideler RK, Squires EL, Voss JL, et al. Progestogen therapy of ovariectomized pregnant mares. *J Reprod Fertil Suppl*. 1982;32:459–464.
143. Buff PR, Messer NTt, Cogswell AM, et al. Seasonal and pulsatile dynamics of thyrotropin and leptin in mares maintained under a constant energy balance. *Domest Anim Endocrinol*. 2007;33:430–436.
144. Lowe JE, Baldwin BH, Foote RH, et al. Equine hypothyroidism: the long term effects of thyroidectomy on metabolism and growth in mares and stallions. *Cornell Vet*. 1974;64: 276–295.
145. Fitzgerald BP, Davison LA. Thyroxine concentrations are elevated in mares which continue to exhibit estrous cycles during the nonbreeding season. *J Equine Vet Sci*. 1998;18:48–51.
146. Gutierrez CV, Riddle WT, Bramlage LR. Serum thyroxine concentrations and pregnancy rates 15 to 16 days after ovulation in broodmares. *J Am Vet Med Assoc*. 2002;220:64–66.
147. McCue PM. Equine granulosa cell tumors. *Proc Am Assoc Equine Pract*. 1992;38:587–593.
148. Almeida J, Ball BA, Conley AJ, et al. Biological and clinical significance of anti-Mullerian hormone determination in blood serum of the mare. *Theriogenology*. 2011;76:1393–1403.
149. Ball BA, Almeida J, Conley AJ. Determination of serum anti-Mullerian hormone concentrations for the diagnosis of granulosa-cell tumours in mares. *Equine Vet J*. 2013;45:199–203.
150. Ball BA. Applications of anti-Mullerian hormone (AMH) in equine reproduction. *Clin Therio*. 2016;8:278.
151. Allen WR, Mathias S, Lennard SN, et al. Serial measurement of peripheral oestrogen and progesterone concentrations in oestrous mares to determine optimum mating time and diagnose ovulation. *Equine Vet J*. 1995;27:460–464.
152. Daels PF, Ammon DC, Stabenfeldt GH, et al. Urinary and plasma estrogen conjugates, estradiol and estrone concentrations in nonpregnant and early pregnant mares. *Theriogenology*. 1991;35:1001–1017.
153. Monfort SL, Arthur NP, Wildt DE. Monitoring ovarian function and pregnancy by evaluating excretion of urinary oestrogen conjugates in semi-free-ranging Przewalski's horses (*Equus przewalskii*). *J Reprod Fertil*. 1991;91:155–164.
154. Schwarzenberger F, Mostl E, Palme R, et al. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Anim Reprod Sci*. 1996;42:515–526.
155. Kasman LH, Hughes JP, Stabenfeldt GH, et al. Estrone sulfate concentrations as an indicator of fetal demise in horses. *Am J Vet Res*. 1988;49:184–187.
156. Sist MD, Williams JF, Geary AM. Pregnancy diagnosis in the mare by immunoassay of estrone sulfate in serum and milk. *J Equine Vet Sci*. 1987;7:20–23.
157. Henderson KM, Perkins NR, Wards RL, et al. Enzyme immunoassay of oestrone sulphate concentrations in faeces for non-invasive pregnancy determination in mares. *N Z Vet J*. 1999;47:61–66.
158. Ball BA. New diagnostic biomarkers to evaluate late term pregnancy in mares. *Clin Therio*. 2016;8:271.
159. Allen WR. Hormonal control of early pregnancy in the mare. *Vet Clin North Am Large Anim Pract*. 1980;2:291–302.
160. Steiner J, Antczak DF, Wolfsdorf K, et al. Persistent endometrial cups. *Anim Reprod Sci*. 2006;94:274–275.
161. Allen WR, Kolling M, Wilsher S. An interesting case of early pregnancy loss in a mare with persistent endometrial cups. *Equine Vet Educ*. 2007;19:539–544.
162. Crabtree JR, Chang Y, de Mestre AM. Clinical presentation, treatment and possible causes of persistent endometrial cups illustrated by two cases. *Equine Vet Educ*. 2012;24:251–259.

163. Meinert C, Silva JF, Kroetz I, et al. Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin. *Equine Vet J*. 1993;25:65–68.
164. Irvine CH. Endocrinology of the estrous cycle of the mare: applications to embryo transfer. *Theriogenology*. 1981;15:85–104.
165. Evans MJ, Irvine CH. Measurement of equine follicle stimulating hormone and luteinizing hormone: response of anestrous mares to gonadotropin releasing hormone. *Biol Reprod*. 1976;15:477–484.
166. Ginther OJ, Wentworth BC. Effect of a synthetic gonadotropin-releasing hormone on plasma concentrations of luteinizing hormone in ponies. *Am J Vet Res*. 1974;35:79–81.
167. Alexander SL, Irvine CHG, Shand N, et al. Is luteinizing hormone secretion modulated by endogenous oxytocin in the mare? Studies on the role of oxytocin and factors affecting its secretion in estrous mares. *Biol Reprod Mono*. 1995;1:361–371.
168. Irvine CH, Alexander SL. A novel technique for measuring hypothalamic and pituitary hormone secretion rates from collection of pituitary venous effluent in the normal horse. *J Endocrinol*. 1987;113:183–192.
169. Alexander SL, Irvine CH. Secretion rates and short-term patterns of gonadotrophin-releasing hormone, FSH and LH throughout the periovulatory period in the mare. *J Endocrinol*. 1987;114:351–362.
170. Alexander SL, Irvine CH, Turner JE. Comparison by three different radioimmunoassay systems of the polymorphism of plasma FSH in mares in various reproductive states. *J Reprod Fertil Suppl*. 1987;35:9–18.
171. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. *Nature*. 1996;382: 250–252.
172. Cunningham MJ, Clifton DK, Steiner RA. Leptin's action on the reproductive axis: perspectives and mechanisms. *Biol Reprod*. 1999;60:216–222.
173. Barb CR, Kraeling RR. Role of leptin in the regulation of gonadotropin secretion in farm animals. *Anim Reprod Sci*. 2004;82-83:155–167.
174. Smith GD, Jackson LM, Foster DL. Leptin regulation of reproductive function and fertility. *Theriogenology*. 2002;57:73–86.
175. Barash IA, Cheung CC, Weigle DS, et al. Leptin is a metabolic signal to the reproductive system. *Endocrinology*. 1996;137:3144–3147.
176. McManus CJ, Fitzgerald BP. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. *Domest Anim Endocrinol*. 2000;19:1–13.
177. Gentry LR, Thompson Jr DL, Gentry Jr GT, et al. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J Anim Sci*. 2002;80:2695–2703.
178. Giles RC, Donahue JM, Hong CB, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). *J Am Vet Med Assoc*. 1993;203:1170–1175.
179. Hemberg E, Lundeheim N, Einarsson S. Retrospective study on vulvar conformation in relation to endometrial cytology and fertility in thoroughbred mares. *J Vet Med A Physiol Pathol Clin Med*. 2005;52:474–477.
180. Trotter GW, McKinnon AO. Surgery for abnormal vulvar and perineal conformation in the mare. *Vet Clin North Am Equine Pract*. 1988;4:389–405.
181. Schumacher J, Schumacher J, Blanchard T. Comparison of endometrium before and after repair of third-degree rectovestibular lacerations in mares. *J Am Vet Med Assoc*. 1992;200: 1336–1338.
182. Studdert MJ. Comparative aspects of equine herpes viruses. *Cornell Vet*. 1974;64:94.
183. Goetz TE, Ogilvie GK, Keegan KG, et al. Cimetidine for treatment of melanomas in three horses. *J Am Vet Med Assoc*. 1990;196:449–452.
184. Phillips JC, Lembcke LM. Equine melanocytic tumors. *Vet Clin North Am Equine Pract*. 2013;29:673–687.
185. Gumber S, Baia P, Wakamatsu N. Vulvar epithelioid hemangiosarcoma with solar elastosis in a mare. *J Vet Diagn Invest*. 2011;23:1033–1036.
186. Katz JB, Evans LE, Hutto DL, et al. Clinical, bacteriologic, serologic, and pathologic features of infections with atypical *Taylorella equigenitalis* in mares. *J Am Vet Med Assoc*. 2000;216:1945–1948.
187. Makloski-Cohorn CL, Embertson RM, Payton ME, et al. Postoperative fertility in mares with cervical defects. *J Equine Vet Sci*. 2014;34:137–138.
188. Gruninger B, Schoon HA, Schoon D, et al. Incidence and morphology of endometrial angiopathies in mares in relationship to age and parity. *J Comp Pathol*. 1998;119:293–309.
189. Lefranc A, Allen WR. Endometrial gland surface density and hyperaemia of the endometrium during early pregnancy in the mare. *Equine Vet J*. 2007;39:511–515.
190. Bracher V, Mathias S, Stocker M. Ultrastructural evaluation of placentation in mares with chronic degenerative endometritis. In: *Second International Conference on Veterinary Perinatology*. Cambridge, England; 1990.
191. Bracher V, Allen WR, McGladdery AJ. Ultrastructural evaluation of naturally occurring and experimentally induced placental pathology in the mare. In: *International Meeting on Disturbances in Equine Foetal Maturation: Comparative Aspects*. Naples, FL; 1991:34.
192. Asbury AC. *Relationship of abnormality of the equine placenta to size, health and vigor of the foal*. In: Annual Meeting of the Society of Theriogenology; 1988:306–310.
193. Katila T. Post-mating inflammatory responses of the uterus. *Reprod Domest Anim*. 2012;47(suppl 5):31–41.
194. Cadario ME, Merritt AM, Archbald LF, et al. Changes in intrauterine pressure after oxytocin administration in reproductively normal mares and in those with a delay in uterine clearance. *Theriogenology*. 1999;51:1017–1025.
195. Watson ED, Sertich PL. Effect of repeated collection of multiple endometrial biopsy specimens on subsequent pregnancy in mares. *J Am Vet Med Assoc*. 1992;201:438–440.
196. Santschi EM, Slone DE. Successful pregnancy after partial hysterectomy in two mares. *J Am Vet Med Assoc*. 1994;205:1180–1182.
197. Frazer G, Burba D, Paccamonti D, et al. The effects of parturition and peripartum complications on the peritoneal fluid composition of mares. *Theriogenology*. 1997;48:919–931.
198. Brooks DE, McCoy DJ, Martin GS. Uterine rupture as a postpartum complication in two mares. *J Am Vet Med Assoc*. 1985;187:1377–1379.
199. Sutter WW, Hopper S, Embertson RE, et al. Diagnosis and surgical treatment of uterine lacerations in mares (33 cases). *Proc Am Assoc Equine Pract*. 2003;49:357–359.
200. Roberts SJ. *Veterinary Obstetrics and Genital Diseases (Theriogenology)*. 3rd ed. Woodstock: S.J. Roberts; 1986.
201. Williams NM, Bryant UK. Periparturient arterial rupture in mares: a postmortem study. *J Equine Vet Sci*. 2012;32:281–284.
202. LeBlanc MM. Common peripartum problems in the mare. *J Equine Vet Sci*. 2008;28:709–715.
203. Taylor EL, Sellon DC, Wardrop KJ, et al. Effects of intravenous administration of formaldehyde on platelet and coagulation variables in healthy horses. *Am J Vet Res*. 2000;61:1191–1196.
204. Perkins NR, Frazer GS. Reproductive emergencies in the mare. *Vet Clin North Am Equine Pract*. 1994;10:643–670.
205. Vandeplassche M, Henry M. Salpingitis in the mare. *Proc Am Assoc Equine Pract*. 1977;23.
206. Liu IKM, Lantz KC, Schlafke S, et al. Clinical observations of oviductal masses in the mare. *Proc Am Assoc Equine Pract*. 1990;37:41–45.
207. Arnold CE, Love CC. Laparoscopic evaluation of oviductal patency in the standing mare. *Theriogenology*. 2013;79:905–910.

208. Bennett SD, Griffin RL, Rhoads WS. Surgical evaluation of oviduct disease and patency in the mare. *Proc Am Assoc Equine Pract*. 2002;48:347–349.
209. Zent WW, Liu IK, Spirito MA. Oviductal flushing as a treatment for infertility in the mare. In: *Third International Symposium on Equine Embryo Transfer.* Buenos Aires; 1993.
210. Ortis HA, Foss RR, McCue PM, et al. Laparoscopic application of PGE(2) to the uterine tube surface enhances fertility in selected subfertile mares. *J Equine Vet Sci*. 2013;33: 896–900.
211. Inoue Y. Hysteroscopic hydrotubation of the equine oviduct. *Equine Vet J*. 2013;45:761–765.
212. Turner TA, Manno M. Bilateral granulosa-cell tumor in a mare. *J Am Vet Med Assoc*. 1983;182:713–714.
213. Frederico LM, Gerard MP, Pinto CRF, et al. Bilateral occurrence of granulosa-theca cell tumors in an Arabian mare. *Can Vet J-Rev Vet Can*. 2007;48:502–505.
214. McCue PM. Neoplasia of the female reproductive tract. *Vet Clin N Am-Equine Pract*. 1998;14:505–515.
215. Sherlock CE, Lott-Ellis K, Bergren A, et al. Granulosa cell tumours in the mare: a review of 52 cases. *Equine Vet Educ*. 2016;28:75–82.
216. Hinrichs K, Frazer GS, Degannes RVG, et al. Serous cystadenoma in a normally cyclic mare with high plasma testosterone values. *J Am Vet Med Assoc*. 1989;194:381–382.
217. van der Kolk JH, Geelen SNJ, Jonker FH, et al. Hypertrophic osteopathy associated with ovarian carcinoma in a mare. *Vet Rec*. 1998;143:172–173.
218. Sedrish SA, McClure JR, Pinto C, et al. Ovarian torsion associated with granulosa-theca cell tumor in a mare. *J Am Vet Med Assoc*. 1997;211:1152–1154.
219. Troedsson MHT. Breeding-induced endometritis in mares. *Vet Clin N Am-Equine Pract*. 2006;22:705–712.
220. LeBlanc MM, Causey RC. Clinical and subclinical endometritis in the mare: both threats to fertility. *Reprod Domest Anim*. 2009;44:10–22.
221. Hughes JP, Loy RG. Investigations on the effect on intrauterine inoculation of *Streptococcus zooepidemicus* in the mare. *Proc Am Assoc Equine Pract*. 1969;15:289–292.
222. Nielsen JM. Endometritis in the mare: a diagnostic study comparing cultures from swab and biopsy. *Theriogenology*. 2005;64:510–518.
223. Davis HA, Stanton MB, Thungrat K, et al. Uterine bacterial isolates from mares and their resistance to antimicrobials: 8,296 cases (2003-2008). *J Am Vet Med Assoc*. 2013;242: 977–983.
224. Troedsson MHT. Uterine clearance and resistance to persistent endometritis in the mare. *Theriogenology*. 1999;52: 461–471.
225. Allen WR. Proceedings of the Hughes, John, P. International Workshop on Equine Endometritis - Summary. *Equine Vet J*. 1993;25:185–193.
226. Scoggin CF. Not just a number: effect of age on fertility, pregnancy and offspring vigour in thoroughbred brood-mares. *Reprod Fertil Dev*. 2015;27:872–879.
227. Caslick EA. The vulva and vulvo-vaginal orifice and its relation to genital health of the Thoroughbred mare. *Cornell Vet*. 1937;27:178–187.
228. Pycock JF. How to maximize the chances of breeding successfully from the older maiden mare. *Proc Am Assoc Equine Pract*. 2006;52:245–249.
229. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity*. 2013;39:1003–1018.
230. Woodward EM, Troedsson MH. Inflammatory mechanisms of endometritis. *Equine Vet J*. 2015;47:384–389.
231. Christoffersen M, Baagoe CD, Jacobsen S, et al. Evaluation of the systemic acute phase response and endometrial gene expression of serum amyloid A and pro- and antiinflammatory cytokines in mares with experimentally induced endometritis. *Vet Immunol Immunopathol*. 2010;138:95–105.
232. Christoffersen M, Woodward E, Bojesen AM, et al. Inflammatory responses to induced infectious endometritis in mares resistant or susceptible to persistent endometritis. *BMC Vet Res*. 2012;8:41.
233. Fumuso E, Aguilar J, Giguere S, et al. Interleukin-8 (IL-8) and 10 (IL-10) mRNA transcriptions in the endometrium of normal mares and mares susceptible to persistent post-breeding endometritis. *Anim Reprod Sci*. 2006;94:282–285.
234. Fumuso EA, Aguilar J, Giguere S, et al. Immune parameters in mares resistant and susceptible to persistent post-breeding endometritis: effects of immunomodulation. *Vet Immunol Immunopathol*. 2007;118:30–39.
235. Woodward EM, Christoffersen M, Campos J, et al. Endometrial inflammatory markers of the early immune response in mares susceptible or resistant to persistent breeding-induced endometritis. *Reproduction*. 2013;145:289–296.
236. Scheller J, Chalaris A, Schmidt-Arras D, et al. The pro- and antiinflammatory properties of the cytokine interleukin-6. *Biochim Et Biophys Acta-Mol Cell Res*. 2011;1813:878–888.
237. Ikuma K, Decho AW, Lau BLT. The extracellular bastions of bacteria - a biofilm way of life. *Nat Educ Knowledge*. 2013;4:2.
238. Ferris RA, Frisbie DD, McCue PM. Use of mesenchymal stem cells or autologous conditioned serum to modulate the inflammatory response to spermatozoa in mares. *Theriogenology*. 2014;82:36–42.
239. Ferris RA. Bacterial endometritis: a focus on biofilms. *Clin Therio*. 2014;6:315–319.
240. Metcalf ES. Venereal disease. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
241. Timoney PJ. Contagious equine metritis. *Comp Immunol Microbiol Infect Dis*. 1996;19:199–204.
242. Balasuriya UBR, Snijder EJ, Heidner HW, et al. Development and characterization of an infectious cDNA clone of the virulent Bucyrus strain of Equine arteritis virus. *J Gen Virol*. 2007;88:918–924.
243. Holyoak GR, Balasuriya UBR, Broaddus CC, et al. Equine viral arteritis: current status and prevention. *Theriogenology*. 2008;70:403–414.
244. Pascoe RR. The effect of equine coital exanthema on the fertility of mares covered by stallions exhibiting the clinical disease. *Aust Vet J*. 1981;57:111–114.
245. Lu KG, Morresey PR. Infectious diseases in breeding stallions. *Clin Tech Equine Pract*. 2007;6:285–290.
246. Health TCfFSaP. *Dourine*. Ames, Iowa: Iowa State University; 2015.
247. LeBlanc MM, Magsig J, Stromberg AJ. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. *Theriogenology*. 2007;68:403–412.
248. Nielsen JM, Troedsson MH, Pedersen MR, et al. Diagnosis of endometritis in the mare based on bacteriological and cytological examinations of the endometrium: comparison of results obtained by swabs and biopsies. *J Equine Vet Sci*. 2010;30:27–30.
249. Kenney RM, Doig PA. Equine endometrial biopsy. In: Youngquist RS, Threlfall WR, eds. *Current Therapy in Theriogenology*. 2nd ed. St. Louis: W.B. Saunders; 1986.
250. Card CE. Endoscopic examination. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
251. Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. *Comp Med*. 2009;59:517–526.
252. Jacobsen S, Jensen JC, Frei S, et al. Use of serum amyloid A and other acute phase reactants to monitor the inflammatory response after castration in horses: a field study. *Equine Vet J*. 2005;37:552–556.
253. Canisso IF, Ball BA, Cray C, et al. Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. *Am J Reprod Immunol*. 2014;72:376–385.

254. Kavak A, Tuppits U, Orro T, et al. Influence of the level and duration of local inflammatory reaction of mares' uterus to the systemic plasma concentrations of acute phase proteins. *Reprod Domest Anim*. 2012;47. 483–483.
255. Hulten C, Gronlund U, Hirvonen J, et al. Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and α(2)-globulins during induced noninfectious arthritis in the horse. *Equine Vet J*. 2002;34:699–704.
256. Stoneham SJ, Palmer L, Cash R, et al. Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Vet J*. 2001;33:599–603.
257. Sheldon IM, Noakes DE, Rycroft A, et al. Acute phase protein responses to uterine bacterial contamination in cattle after calving. *Vet Rec*. 2001;148:172–175.
258. Brodzki P, Kostro K, Krakowski L, et al. Inflammatory cytokine and acute phase protein concentrations in the peripheral blood and uterine washings of cows with subclinical endometritis in the late postpartum period. *Vet Res Commun*. 2015;39: 143–149.
259. Jacobsen S, Andersen PH. The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Vet Educ*. 2007;19:38–46.
260. Nunokawa Y, Fujinaga T, Taira T, et al. Evaluation of serum amyloid A protein as an acute phase reactive protein in horses. *J Vet Med Sci*. 1993;55:1011–1016.
261. Coutinho da Silva MA, Canisso IF, MacPherson ML, et al. Serum amyloid A concentration in healthy periparturient mares and mares with ascending placentitis. *Equine Vet J*. 2013;45:619–624.
262. Krakowski L, Krawczyk CH, Kostro K, et al. Serum levels of acute phase proteins: SAA, Hp and progesterone (P4) in mares with early embryonic death. *Reprod Domest Anim*. 2011;46:624–629.
263. Schnobrich MR, Howard CR, Bradecamp EA. Comparison of serum amyloid A concentrations in Thoroughbred mares experiencing embryonic loss and mares maintaining pregnancy. *Clin Theriogenology*. 2014;6:376.
264. Nash DM, Sheldon IM, Herath S, et al. Markers of the uterine innate immune response of the mare. *Anim Reprod Sci*. 2010;119:31–39.
265. Christoffersen M, Woodward EM, Bojesen AM, et al. Effect of immunomodulatory therapy on the endometrial inflammatory response to induced infectious endometritis in susceptible mares. *Theriogenology*. 2012;78:991–1004.
266. Dell'Aqua JA, Papa FO, Lopes MD, et al. Modulation of acute uterine inflammatory response after artificial insemination with equine frozen semen. *Anim Reprod Sci*. 2006;94: 270–273.
267. Bucca S, Carli A, Buckley T, et al. The use of dexamethasone administered to mares at breeding time in the modulation of persistent mating induced endometritis. *Theriogenology*. 2008;70:1093–1100.
268. Pycock JF, Newcombe JR. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. *Vet Rec*. 1996;138:320–323.
269. Cooke CD. Prophylactic intra-uterine antibacterial therapy. *Equine Vet Educ*. 2015;27:554–555.
270. Stout TAE. Fungal endometritis in the mare. *Pferdeheilkunde*. 2008;24:83–87.
271. Dascanio JJ. How and when to treat endometritis with systemic or local antibiotics. *Proc Am Assoc Equine Pract*. 2011;57:24–31.
272. Albihn A, Baverud V, Magnusson U. Uterine microbiology and antimicrobial susceptibility in isolated bacteria from mares with fertility problems. *Acta Vet Scand*. 2003;44:121–129.
273. Van Camp SD, Papich MG, Whitacre MD. Administration of ticarcillin in combination with clavulanic acid intravenously and intrauterinely to clinically normal oestrous mares. *J Vet Pharmacol Ther*. 2000;23:373–378.
274. Bermudez V, Sifontes L, Navarro N, et al. Effects of intrauterine infusion of sodium ceftiofur on the endometrium of mares. *Proc Am Assoc Equine Pract*. 1995;41:261–263.
275. Scofield D, Black J, Wittenburg L, et al. Endometrial tissue and blood plasma concentration of ceftiofur and metabolites following intramuscular administration of ceftiofur crystalline free acid to mares. *Equine Vet J*. 2014;46:606–610.
276. Schnobrich MR, Pearson LK, Barber BK, et al. Effects of intrauterine infusion of a water-based suspension of enrofloxacin on mare endometrium. *J Equine Vet Sci*. 2015;35:662–667.
277. LeBlanc M, Neuwirth L, Mauragis D, et al. Oxytocin enhances clearance of radiocolloid from the uterine lumen of reproductively normal mares and mares susceptible to endometritis. *Equine Vet J*. 1994;26:279–282.
278. Campbell MLH, England GCW. A comparison of the ecbolic efficacy of intravenous and intrauterine oxytocin treatments. *Theriogenology*. 2002;58:473–477.
279. Sharp DC, Thatcher MJ, Salute ME, et al. Relationship between endometrial oxytocin receptors and oxytocin-induced prostaglandin F-2α release during the oestrous cycle and early pregnancy in pony mares. *J Reprod Fertil Suppl*. 1997;109:137–144.
280. Gutjahr S, Paccamonti DL, Pycock JF, et al. Effect of dose and day of treatment on uterine response to oxytocin in mares. *Theriogenology*. 2000;54:447–456.
281. Schramme AR, Pinto CRF, Davis J, et al. Pharmacokinetics of carbetocin, a long-acting oxytocin analogue, following intravenous administration in horses. *Equine Vet J*. 2008;40:658–661.
282. Combs GB, LeBlanc MM, Neuwirth L, et al. Effects of prostaglandin F-2α' cloprostenol and fenprostalene on uterine clearance of radiocolloid in the mare. *Theriogenology*. 1996;45:1449–1455.
283. Nie GJ, Johnson KE, Wenzel JGW, et al. Effect of periovulatory ecbolics on luteal function and fertility. *Theriogenology*. 2002;58:461–463.
284. Vanderwall DK, Woods GL. Effect on fertility of uterine lavage performed immediately prior to insemination in mares. *J Am Vet Med Assoc*. 2003;222:1108–1110.
285. Brinsko SP, Varner DD, Blanchard TL. The effect of uterine lavage performed four hours post insemination on pregnancy rate in mares. *Theriogenology*. 1991;35:1111–1119.
286. Jackson PS, Allen WR, Ricketts SW, et al. The irritancy of chlorhexidine gluconate in the genital tract of the mare. *Vet Rec*. 1979;105:122–124.
287. LeBlanc MM, McKinnon AO. Breeding the problem mare. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
288. Gores-Lindholm A, Ahlschwede S, Causey R, et al. Effect of intra-uterine infusion of diluted N-acetylcysteine on equine endometrium. *Proc Am Assoc Equine Pract*. 2009;55:326.
289. Bracher V, Neuschaefer A, Allen WR. The effect of intrauterine infusion of kerosene on the endometrium of mares. *J Reprod Fertil*. 1991:706–707.
290. Bradecamp EA, Ahlschwede SA, Cooke JL. The effects of intrauterine kerosene infusion on endometrial epithelial cilia concentration. *J Equine Vet Sci*. 2014;34:134.
291. Lyle SK, LeBlanc MM, Staempfli SA, et al. How to use a buffered chelator solution for mares with chronic endometritis. *Proc Am Assoc Equine Pract*. 2011;57:16–18.
292. LeBlanc MM. The chronically infertile mare. *Proc Am Assoc Equine Pract*. 2008;54:391–407.
293. Rathgeber R. Acupuncture therapies for equine reproductive disorders. *Hagyard Bluegrass Symposium*. Lexington: Hagyard Equine Medical Institute; 2000.
294. Schofield WA. Use of acupuncture in equine reproduction. *Theriogenology*. 2008;70:430–434.
295. Rogan D, Fumuso E, Rodriguez E, et al. Use of a mycobacterial cell wall extract (MCWE) in susceptible mares to clear experimentally induced endometritis with Streptococcus zooepidemicus. *J Equine Vet Sci*. 2007;27:112–117.

296. Rohrbach BW, Sheerin PC, Cantrell CK, et al. Effect of adjunctive treatment with intravenously administered *Propionibacterium acnes* on reproductive performance in mares with persistent endometritis. *Javma-J Am Vet Med Assoc*. 2007;231:107–113.
297. Pascoe DR. Effect of adding autologous plasma to an intrauterine antibiotic therapy after breeding on pregnancy rates in mares. *Biol Reprod Mono*. 1995;1:539–543.
298. Metcalf ES, Scoggin KE, Troedsson MH. The effect of platelet-rich plasma on endometrial pro-inflammatory cytokines in susceptible mares following semen deposition. *J Equine Vet Sci*. 2012;32:498.
299. Metcalf ES. The effect of platelet-rich plasma (PRP) on intraluminal fluid and pregnancy rates in mares susceptible to persistent mating-induced endometritis (PMIE). *J Equine Vet Sci*. 2014;34:128.
300. Brendemuehl JP, Kopp K, Altman J. Influence of dietary algal N-3 fatty acids on breeding induced inflammation and endometrial cytokine expression in mares bred with frozen semen. *J Equine Vet Sci*. 2014;34:123–124.
301. Brinsko SP, Varner DD, Love CC, et al. Effect of feeding a DHA-enriched nutriceutical on motion characteristics of cooled and frozen stallion semen. *Proc Am Assoc Equine Pract*. 2003;49:350–352.
302. Dascanio JJ, Schweizer C, Ley WB. Equine fungal endometritis. *Equine Veterinary Education*. 2001;13:324–329.
303. Coutinho da Silva MA, Alvarenga MA. Fungal endometritis. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
304. Rantala M, Attia S, Koukila-Kahkola P, et al. *Cladophialophora bantiana* as an emerging pathogen in animals: case report of equine endometritis and review of the literature. *J Clin Microbiol*. 2015;53:3047–3053.
305. Rota A, Calicchio E, Nardoni S, et al. Presence and distribution of fungi and bacteria in the reproductive tract of healthy stallions. *Theriogenology*. 2011;76:464–470.
306. Ferris RA, Dern K, Veir JK, et al. Development of a broad-range quantitative polymerase chain reaction assay to detect and identify fungal DNA in equine endometrial samples. *Am J Vet Res*. 2013;74:161–165.
307. Carter GR, Chengappa MM. Introduction to the fungi and fungus infections. In: Carter GR, Changappa MM, Roberts AW, eds. *Essentials of Veterinary Microbiology*. Philadelphia: Williams & Wilkins; 1995.
308. Beltaire KA, Cheong SH, da Silva MAC. Retrospective study on equine uterine fungal isolates and antifungal susceptibility patterns (1999-2011). *Equine Vet J*. 2012;44:84–87.
309. Hess MB, Parker NA, Purswell BJ, et al. Use of lufenuron as a treatment for fungal endometritis in four mares. *J Am Vet Med Assoc*. 2002;221:266–267. 240.
310. Tibary A. Dopamine antagonists. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
311. Murphy BA, Walsh CM, Woodward EM, et al. Blue light from individual light masks directed at a single eye advances the breeding season in mares. *Equine Vet J*. 2014;46:601–605.
312. Ciechanowska M, Lapot M, Malewski T, et al. Implication of dopaminergic systems on GnRH and GnRHR genes expression in the hypothalamus and GnRH-R gene expression in the anterior pituitary gland of anestrous ewes. *Exp Clin Endocrinol Diabetes*. 2008;116:357–362.
313. Brendemuehl JP, Cross DL. Influence of the dopamine antagonist domperidone on the vernal transition in seasonally anoestrous mares. *J Reprod Fertil Suppl*. 2000;56:185–193.
314. Bennett-Wimbush K, Loch WE, Plata-Madrid H, et al. The effects of perphenazine and bromocriptine on follicular dynamics and endocrine profiles in anestrous pony mares. *Theriogenology*. 1998;49:717–733.
315. Mari G, Morganti M, Merlo B, et al. Administration of sulpiride or domperidone for advancing the first ovulation in deep anestrous mares. *Theriogenology*. 2009;71:959–965.
316. Panzani D, Zicchino I, Taras A, et al. Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares. *Theriogenology*. 2011;75:138–143.
317. Webel SK, Squires EL. Control of the oestrous cycle in mares with altrenogest. *J Reprod Fertil Suppl*. 1982;32:193–198.
318. Raz T, Carley SD, Green JM, et al. Evaluation of two oestrus synchronization regimens in eFSH-treated donor mares. *Vet J*. 2011;188:105–109.
319. Meyers-Brown GA, McCue PM, Troedsson MH, et al. Induction of ovulation in seasonally anestrous mares under ambient lights using recombinant equine FSH (reFSH). *Theriogenology*. 2013;80:456–462.
320. Levy I, Duchamp G. A single subcutaneous administration of buserelin induces ovulation in the mare: field data. *Reprod Domest Anim*. 2007;42:550–554.
321. Turner JE, Irvine CH. The effect of various gonadotrophin-releasing hormone regimens on gonadotrophins, follicular growth and ovulation in deeply anoestrous mares. *J Reprod Fertil Suppl*. 1991;44:213–225.
322. Samper JC, Geertsema H, Hearn P. Rate of luteolysis, folliculogenesis and interval to ovulation of mares treated with a prostaglandin analogue on d 6 or 10 of the estrous cycle. *Proc Am Assoc Equine Pract*. 1993;39:169–170.
323. Coffman EA, Pinto CR. A review on the use of prostaglandin F-2α for controlling the estrous cycle in mares. *J Equine Vet Sci*. 2016;40:34–40.
324. Coffman EA, Pinto CRF, Snyder HK, et al. Antiluteogenic effects of serial prostaglandin F-2α administration in cycling mares. *Theriogenology*. 2014;82:1241–1245.
325. Agnew ME, Schnobrich MR, Stromberg A, et al. Pregnancy outcomes in Thoroughbred mares administered different doses of cloprostenol. *Clin Therio*. 2015;7:335.
326. Peltier MR, Robinson G, Sharp DC. Effects of melatonin implants in pony mares. 2. Long-term effects. *Theriogenology*. 1998;49:1125–1142.
327. Brendemuehl JP. Effect of oxytocin and PGF2α on luteal formation, function and pregnancy rates in mares. *Proc Am Assoc Equine Pract*. 2001;47:239–241.
328. Brendemuehl JP. *Influence of oxytocin, PGF2α and cloprostenol administered in the immediate postovulatory period on luteal formation and function in the mare*. San Antonio: Society for Theriogenology Annual Conference; 2000:267.
329. Cuervo-Arango J, Newcombe JR. The effect of hormone treatments (hCG and cloprostenol) and season on the incidence of hemorrhagic anovulatory follicles in the mare: a field study. *Theriogenology*. 2009;72:1262–1267.
330. Irvine CHG, McKeough VL, Turner JE, et al. Effectiveness of a two-dose regimen of prostaglandin administration in inducing luteolysis without adverse side effects in mares. *Equine Vet J*. 2002;34:191–194.
331. Nie GJ, Goodin AN, Braden TD, et al. How to reduce drug costs and side effects when using prostaglandins to short-cycle mares. *Proc Am Assoc Equine Pract*. 2004;50:396–398.
332. Asbury AC. Uterine defense mechanisms in the mare: the use of intrauterine plasma in the management of endometritis. *Theriogenology*. 1984;21:387–393.
333. McCue PM, Lemons SS, Squires EL, et al. Efficacy of progesterone/estradiol implants for suppression of estrus in the mare. *Proc Am Assoc Equine Pract*. 42;42:195–196.
334. Vanderwall DK, Rasmussen DM, Woods GL. Effect of repeated administration of oxytocin during diestrus on duration of function of corpora lutea in mares. *Javma-J Am Vet Med Assoc*. 2007;231:1864–1867.
335. Gee EK, DeLuca C, Stylski JL, et al. Efficacy of medroxyprogesterone acetate in suppression of estrus in cycling mares. *J Equine Vet Sci*. 2009;29:140–145.

336. Card C, Raz T, LeHeiget R, et al. GnRH immunization in mares: ovarian function, return to cycling, and fertility. *Proc Am Assoc Equine Pract*. 2007;53:576–577.
337. Gupta SK, Shrestha A, Minhas V. Milestones in contraceptive vaccines development and hurdles in their application. *Hum Vaccin Immunother*. 2014;10:911–925.
338. Joone CJ, Bertschinger HJ, Gupta SK, et al. Ovarian function and pregnancy outcome in pony mares following immunocontraception with native and recombinant porcine zona pellucida vaccines. *Equine Vet J*. 2017;49:189–195.
339. Storer WA, Thompson DL, Gilley RM, et al. Evaluation of injectable sustained release progestin formulations for suppression of estrus and ovulation in mares. *J Equine Vet Sci*. 2009;29:33–36.
340. James AN, Vogelsang MM, Forrest DW, et al. Efficacy of short-term administration of altrenogest to postpone ovulation in mares. *J Equine Vet Sci*. 1998;18:329–331.
341. Loy RG. Characteristics of postpartum reproduction in the mare. *Vet Clin North Am Equine Pract*. 1980;2:345–359.
342. Camillo F, Marmorini P, Romagnoli S, et al. Fertility at the first postpartum estrous compared with fertility at the following estrous cycles in foaling mares and with fertility in non-foaling mares. *J Equine Vet Sci*. 1997;17:612–616.
343. Sexton PE, Bristol FM. Uterine involution in mares treated with progesterone and estradiol-17-β. *J Am Vet Med Assoc*. 1985;186:252–256.
344. McKinnon AO, Perriam WJ, Lescun TB, et al. Effect of a GnRH analogue (Ovuplant), hCG and dexamethasone on time to ovulation in cycling mare. *World Equine Vet Rev*. 1997;2:16–18.
345. Roser JF, Kiefer BL, Evans JW, et al. The development of antibodies to human chorionic gonadotrophin following its repeated injection in the cyclic mare. *J Reprod Fertil Suppl*. 1979;27:173–179.
346. Green JM, Raz T, Epp T, et al. Relationships between uteroovarian parameters and ovulatory response to human chorionic gonadotrophin (hCG) in mares. *Proc Am Assoc Equine Pract*. 2007;53:563–567.
347. Mumford EL, Squires EL, Jochle E, et al. Use of deslorelin short-term implants to induce ovulation in cycling mares during 3 consecutive estrous cycles. *Anim Reprod Sci*. 1995;39:129–140.
348. Barrier-Battut I, Le Poutre N, Trocherie E, et al. Use of buserelin to induce ovulation in the cyclic mare. *Theriogenology*. 2001;55:1679–1695.
349. Ferris RA, Hatzel JN, Lindholm ARG, et al. Efficacy of deslorelin acetate (SucroMate) on induction of ovulation in American Quarter Horse mares. *J Equine Vet Sci*. 2012;32:285–288.
350. Samper JC. Ultrasonographic appearance and the pattern of uterine edema to time ovulation in mares. *Proc Am Assoc Equine Pract*. 1997;43:189–191.
351. Samper JC, Hankins K. Breeding mares with frozen semen in private practice. *Proc Am Assoc Equine Pract*. 2001;47:314–318.
352. Squires EL, Garcia RH, Ginther OJ, et al. Comparison of equine pituitary extract and follicle stimulating hormone for superovulating mares. *Theriogenology*. 1986;26:661–670.
353. McCue PM, Hughes JP, Lasley BL. Effect on ovulation rate of passive immunisation of mares against inhibin. *Equine Vet J*. 1993;25:103–106.
354. Scoggin CF, Meira C, McCue PM. Use of twice-daily step-down dosage of equine pituitary extract for induction of multiple ovulations in mares. *Theriogenology*. 2002;57:771.
355. Squires EL, McCue PM, Vanderwall D. The current status of equine embryo transfer. *Theriogenology*. 1999;51:91–104.
356. Squires EL, McCue PM, Niswender K, et al. A review on the use of eFSH to enhance reproductive performance. *Proc Am Assoc Equine Pract*. 2003;26:360–362.
357. Welch SA, Denniston DJ, Hudson JJ, et al. Exogenous eFSH, follicle coasting, and hCG as a novel superovulation regimen in mares. *J Equine Vet Sci*. 2006;26:262–270.
358. Jennings MW, Boime I, Daphna-Iken D, et al. The efficacy of recombinant equine follicle stimulating hormone (reFSH) to promote follicular growth in mares using a follicular suppression model. *Anim Reprod Sci*. 2009;116:291–307.
359. McNaughten J, Pozor M, Macpherson M, et al. Effects of topical application of misoprostol in mares. *Reprod Domest Anim*. 2014;49:1057–1062.
360. Rigby S, Love C, Carpenter K, et al. Use of prostaglandin E-2 to ripen the cervix of the mare prior to induction of parturition. *Theriogenology*. 1998;50:897–904.
361. Gastal MO, Gastal EL, Torres CA, et al. Effect of PGE2 on uterine contractility and tone in mares. *Theriogenology*. 1998;50:989–999.
362. Allen WR, Wilsher S, Morris L, et al. Laparoscopic application of PGE(2) to re-establish oviducal patency and fertility in infertile mares: a preliminary study. *Equine Vet J*. 2006;38:454–459.
363. Witkowski M, Pawlowski K. Clinical observations on the course of oxytocin- or prostaglandin E2/oxytocin-induced parturition in mares. *Pol J Vet Sci*. 2014;17:347–351.
364. Cole HH, Saunders FJ. The concentration of gonad-stimulating hormone in blood serum and of oestrin in the urine throughout pregnancy in the mare. *Endocrinology*. 1935;19:199–208.
365. Holtan DW, Nett TM, Estergreen VL. Plasma progestogens in pregnant mares. *J Reprod Fertil Suppl*. 1975;23:419–424.
366. Short RV. Progesterone in blood. IV. Progesterone in the blood of mares. *J Endocrinol*. 1959;19:207–210.
367. Ousey JC, Forhead AJ, Rossdale PD, et al. Ontogeny of uteroplacental progestogen production in pregnant mares during the second half of gestation. *Biol Reprod*. 2003;69:540–548.
368. Ousey JC. Hormone profiles and treatments in the late pregnant mare. *Vet Clin North Am Equine Pract*. 2006;22:727–747.
369. Esteller-Vico A, Troedsson MHT, Squires EL, et al. Inhibition of estrogen synthesis during the last trimester of gestation: changes in endocrine patterns, fetal growth and uterine artery hemodynamics in mares. *J Equine Vet Sci*. 2014;34:207.
370. Irvine CHG, Sutton P, Turner JE, et al. Changes in plasma progesterone concentrations from days 17 to 42 of gestation in mares maintaining or losing pregnancy. *Equine Vet J*. 1990;22:104–106.
371. McKinnon AO, Lescun TB, Walker JH, et al. The inability of some synthetic progestogens to maintain pregnancy in the mare. *Equine Vet J*. 2000;32:83–85.
372. Vanderwall DE, Williams JL, Wood GL. *Use of a compounded proprietary long-acting progesterone formulation for maintenance of pregnancy in mares*. Columbus, OH: Society for Theriogenology; 2003.
373. Bailey CS, Macpherson ML, Pozor MA, et al. Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. *Theriogenology*. 2010;74:402–412.
374. Naden J, Squires EL, Nett TM. Effect of maternal treatment with altrenogest on age at puberty, hormone concentrations, pituitary-response to exogenous GnRH, estrous-cycle characteristics and fertility of fillies. *J Reprod Fertil*. 1990;88:185–195.
375. Neuhauser S, Palm F, Ambuehl F, et al. Effect of altrenogest-treatment of mares in late gestation on adrenocortical function, blood count and plasma electrolytes in their foals. *Equine Vet J*. 2009;41:572–577.
376. Palm F, Walter I, Nowotny N, et al. Progestin treatment does not affect expression of cytokines, steroid receptors, oxytocin receptor, and cyclooxygenase 2 in fetal membranes and endometrium from pony mares at parturition. *Theriogenology*. 2013;79:59–68.
377. Chavatte-Palmer P, Arnaud G, Duvaux-Ponter C, et al. Quantitative and qualitative assessment of milk production after pharmaceutical induction of lactation in the mare. *J Vet Intern Med*. 2002;16:472–477.

378. Guillaume D, Chavatte-Palmer P, Combarnous Y, et al. Induced lactation with a dopamine antagonist in mares: different responses between ovariectomized and intact mares. *Reprod Domest Anim*. 2003;38:394–400.
379. Korosue K, Murase H, Sato F, et al. Successful induction of lactation in a barren Thoroughbred mare: growth of a foal raised on induced lactation and the corresponding maternal hormone profiles. *J Vet Med Sci*. 2012;74:995–1002.
380. Love CC, Garcia M, Riera FR, et al. Use of testicular volume to predict daily sperm output in the stallion. *Proc Am Assoc Equine Pract*. 1993;36:15–21.
381. Blanchard TL, Love CC, Thompson JA, et al. Role of reinforcement breeding in a natural service mating program. *Proc Am Assoc Equine Pract*. 2006;52:384–386.
382. Shore MD, Macpherson ML, Combes GB, et al. Fertility comparison between breeding at 24 hours or at 24 and 48 hours after collection with cooled equine semen. *Theriogenology*. 1998;50:693–698.
383. Squires EL, Brubaker JK, McCue PM, et al. Effect of sperm number and frequency of insemination on fertility of mares inseminated with cooled semen. *Theriogenology*. 1998;49: 743–749.
384. Ellington JE, Ball BA, Blue BJ, et al. Capacitation-like membrane-changes and prolonged viability in-vitro of equine spermatozoa cultured with uterine tube epithelial-cells. *Am J Vet Res*. 1993;54:1505–1510.
385. Loomis PR. Clinical fertility data for mares inseminated with frozen semen: effects of timing and frequency of insemination. *Havemeyer Found Monogr Ser*. 2003;12:77–80.
386. Vidament A. French field results (1985-2005) on factors affecting fertility of frozen stallion semen. *Anim Reprod Sci*. 2005;89:115–136.
387. Volkmann DH, van Zyl D. Fertility of stallion semen frozen in 0.5-ml straws. *J Reprod Fertil Suppl*. 1987;35:143–148.
388. Sanderson MW, Allen WR. *Reproductive efficiency of thoroughbred mares in the United Kingdom*. Sydney, Australia: Bain Fallon Memorial Lectures; 1987:31–41.
389. Squires E, Barbacini S, Matthews P, et al. Retrospective study of factors affecting fertility of fresh, cooled and frozen semen. *Equine Vet Educ*. 2006;18:96–99.
390. Christoffersen M, Soderlind M, Rudefalk SR, et al. Risk factors associated with uterine fluid after breeding caused by Streptococcus zooepidemicus. *Theriogenology*. 2015;84:1283–1290.
391. Oguri N, Tsutsumi Y. Non-surgical egg transfer in mares. *J Reprod Fertil*. 1974;41:313–320.
392. Foss RR, Wirth N, Schlitz P, et al. Nonsurgical embryo transfer in a private practice (1998). *Proc Am Assoc Equine Pract* 999;45:210–212.
393. McCue PM, Wall JJ, Brink ZA, et al. Live foal rate: effects of embryo transfer and donor mare age. *Reprod Fertil Dev*. 2010;22:247–247.
394. Marinone AI, Losinno L, Fumuso E, et al. The effect of mare's age on multiple ovulation rate, embryo recovery, post-transfer pregnancy rate, and interovulatory interval in a commercial embryo transfer program in Argentina. *Anim Reprod Sci*. 2015;158:53–59.
395. Kelley DE, Gibbons JR, Smith R, et al. Exercise affects both ovarian follicular dynamics and hormone concentrations in mares. *Theriogenology*. 2011;76:615–622.
396. Mortensen CJ, Choi YH, Hinrichs K, et al. Embryo recovery from exercised mares. *Anim Reprod Sci*. 2009;110:237–244.
397. Carnevale EM, Ramirez RJ, Squires EL, et al. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. *Theriogenology*. 2000;54:965–979.
398. Betteridge KJ, Eaglesome MD, Mitchell D, et al. Development of horse embryos up to 22 days after ovulation - observations on fresh specimens. *J Anat*. 1982;135:191–209.
399. Allen WR, Wilsher S. A review of implantation and early placentation in the mare. *Placenta*. 2009;30:1005–1015.
400. Freeman DA, Weber JA, Geary RT, et al. Time of embryo transport through the mare oviduct. *Theriogenology*. 1991;36:823–830.
401. Allen WR. The physiology of early pregnancy in the mare. *Proc Am Assoc Equine Pract*. 2000;46:338–354.
402. Ginther OJ. Equine pregnancy: physical interactions between the uterus and conceptus. *Proc Am Assoc Equine Pract*. 1998;44:73–104.
403. Betteridge KJ. Embryo morphology, growth, and development. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. 2nd ed. West Sussex: Wiley-Blackwell; 2011.
404. Griffin PG, Carnevale EM, Ginther OJ. Effects of the embryo on uterine morphology and function in mares. *Anim Reprod Sci*. 1993;31:311–329.
405. Leith GS, Ginther OJ. Characterization of intrauterine mobility of the early equine conceptus. *Theriogenology*. 1984;22: 401–408.
406. Stout TA, Meadows S, Allen WR. Stage-specific formation of the equine blastocyst capsule is instrumental to hatching and to embryonic survival in vivo. *Anim Reprod Sci*. 2005;87: 269–281.
407. Stout TA, Allen WR. Role of prostaglandins in intrauterine migration of the equine conceptus. *Reproduction*. 2001;121: 771–775.
408. Douglas RH, Ginther OJ. Concentration of prostaglandins F in uterine venous plasma of anesthetized mares during the estrous cycle and early pregnancy. *Prostaglandins*. 1976;11: 251–260.
409. Starbuck GR, Stout TAE, Lamming GE, et al. Endometrial oxytocin receptor and uterine prostaglandin secretion in mares during the oestrous cycle and early pregnancy. *J Reprod Fertil*. 1998;113:173–179.
410. Tannus RJ, Thun R. Influence of endometrial cysts on conception rate of mares. *J Vet Med Ser a-Zentralbl Fur Vet Reihe a-Physiol Pathol Clin Med*. 1995;42:275–283.
411. van Ittersum AR. The electrosurgical treatment of endometrial cysts in the mare. *Tijdschr Diergeneeskd*. 1999;124: 630–633.
412. Aurich C, Budik S. Early pregnancy in the horse revisited - does exception prove the rule? *J Anim Sci Biotechnol*. 2015;6:50.
413. Wilsher S, Allen WR. Uterine influences on embryogenesis and early placentation in the horse revealed by transfer of day 10 embryos to day 3 recipient mares. *Reproduction*. 2009;137:583–593.
414. Ginther OJ. Local versus systemic utero-ovarian relationships in farm animals. *Acta Vet Scand*. 1981;77:103–115.
415. Ginther OJ. Comparative anatomy of utero-ovarian vasculature. *Vet Scope*. 1976;20:3–17.
416. Ealy AD, Eroh ML, Sharp 3rd DC. Prostaglandin H synthase Type 2 is differentially expressed in endometrium based on pregnancy status in pony mares and responds to oxytocin and conceptus secretions in explant culture. *Anim Reprod Sci*. 2010;117:99–105.
417. Boerboom D, Brown KA, Vaillancourt D, et al. Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. *Biol Reprod*. 2004;70: 391–399.
418. Bae SE, Watson ED. A light microscopic and ultrastructural study on the presence and location of oxytocin in the equine endometrium. *Theriogenology*. 2003;60:909–921.
419. Silva LA, Ginther OJ. An early endometrial vascular indicator of completed orientation of the embryo and the role of dorsal endometrial encroachment in mares. *Biol Reprod*. 2006;74:337–343.
420. Silva LA, Gastal EL, Beg MA, et al. Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. *Biol Reprod*. 2005;72:755–761.

421. Ginther OJ. Fixation and orientation of the early equine conceptus. *Theriogenology*. 1983;19:613–623.
422. Feo JC. Contralateral implantation in mares mated during postpartum oestrus. *Vet Rec*. 1980;106:368.
423. Griffin PG, Ginther OJ. Uterine morphology and function in postpartum mares. *J Equine Vet Sci*. 1991;11:330–339.
424. Gilbert RO, Marlow CH. A field study of patterns of unobserved foetal loss as determined by rectal palpation in foaling, barren and maiden thoroughbred mares. *Equine Vet J*. 1992;24:184–186.
425. Steven DH, Samuel CA. Anatomy of the placental barrier in the mare. *J Reprod Fertil Suppl*. 1975;23:579–582.
426. Samuel CA, Allen WR, Steven DH. Ultrastructural development of the equine placenta. *J Reprod Fertil Suppl*. 1975;23: 575–578.
427. Samuel CA, Allen WR, Steven DH. Studies on the equine placenta II. Ultrastructure of the placental barrier. *J Reprod Fertil*. 1976;48:257–264.
428. Samuel CA, Allen WR, Steven DH. Studies on the equine placenta. III. Ultrastructure of the uterine glands and the overlying trophoblast. *J Reprod Fertil*. 1977;51:433–437.
429. Whitehead AE, Chenier TS, Foster RA. Placental characteristics of Standardbred mares. *Proc Am Assoc Equine Pract*. 2005;51:215–220.
430. Wooding FB, Fowden AL. Nutrient transfer across the equine placenta: correlation of structure and function. *Equine Vet J*. 2006;38:175–183.
431. Whitwell KE, Jeffcott LB. Morphological studies on the fetal membranes of the normal singleton foal at term. *Res Vet Sci*. 1975;19:44–55.
432. Vandeplassche M, Spincemaille J, Bouters R. Aetiology, pathogenesis and treatment of retained placenta in the mare. *Equine Vet J*. 1971;3:144–147.
433. Canisso IF, Rodriguez JS, Sanz MG, et al. A clinical approach to the diagnosis and treatment of retained fetal membranes with an emphasis placed on the critically ill mare. *J Equine Vet Sci*. 2013;33:570–579.
434. Sibbons P. The role of stereology in the study of placental transfer between fetal foal and mare. *Equine Vet J*. 2006;38:106–107.
435. Gerstenberg C, Allen WR, Stewart F. Cell proliferation patterns during development of the equine placenta. *J Reprod Fertil*. 1999;117:143–152.
436. Allen WR, Gower S, Wilsher S. Immunohistochemical localization of vascular endothelial growth factor (VEGF) and its two receptors (Flt-I and KDR) in the endometrium and placenta of the mare during the oestrous cycle and pregnancy. *Reprod Domest Anim*. 2007;42:516–526.
437. Wilsher S, Allen WR. The effects of maternal age and parity on placental and fetal development in the mare. *Equine Vet J*. 2003;35:476–483.
438. Abd-Elnaeim MM, Leiser R, Wilsher S, et al. Structural and haemovascular aspects of placental growth throughout gestation in young and aged mares. *Placenta*. 2006;27: 1103–1113.
439. Cottrill CM, Jefferslo J, Ousey JC, et al. The placenta as a determinant of fetal well-being in normal and abnormal equine pregnancies. *J Reprod Fertil*. 1991;44:591–601.
440. Rossdale PD, Ousey JC. Fetal programming for athletic performance in the horse: potential effects of IUGR. *Equine Vet Educ*. 2002;14:98–111.
441. Whitwell KE. Investigations into fetal and neonatal losses in the horse. *Vet Clin North Am Large Anim Pract*. 1980;2: 313–331.
442. Jeffcott LB, Whitwell KE. Twinning as a cause of fetal and neonatal loss in Thoroughbred mares. *Comp pathol*. 1973;83: 91–106.
443. Schoon D, Schoon HA, Klug E. Angiosis in the equine endometrium: pathogenesis and clinical correlations. *Pferdeheilkunde*. 1999;15:541–546.
444. Ferreira JC, Canesin HS, Ignacio FS, et al. Effect of age and endometrial degenerative changes on uterine blood flow during early gestation in mares. *Theriogenology*. 2015;84:1123–1130.
445. Tischner M, Klimczak M. The development of Polish ponies born after embryo transfer to large recipients. *Equine Vet J Suppl*. 1989;8:62–63.
446. Allen WR, Wilsher S, Stewart F, et al. The influence of maternal size on placental, fetal and postnatal growth in the horse. II. Endocrinology of pregnancy. *J Endocrinol*. 2002;172:237–246.
447. Peugnet P, Wimel L, Duchamp G, et al. Enhanced or reduced fetal growth induced by embryo transfer into smaller or larger breeds alters post-natal growth and metabolism in pre-weaning horses. *PLoS One*. 2014;9:e102044.
448. Ousey JC, Rossdale PD, Fowden AL, et al. Effects of manipulating intrauterine growth on postnatal adrenocortical development and other parameters of maturity in neonatal foals. *Equine Vet J*. 2004;36:616–621.
449. Allen WR, Wilsher S, Turnbull C, et al. Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. *Reproduction*. 2002;123:445–453.
450. Wilsher S, Allen WR. Effects of a *Streptococcus equi* infection-mediated nutritional insult during mid-gestation in primiparous Thoroughbred fillies. Part 1: placental and fetal development. *Equine Vet J*. 2006;38:549–557.
451. Conley AJ. Review of the reproductive endocrinology of the pregnant and parturient mare. *Theriogenology*. 2016;86:355–365.
452. Ferreira-Dias GM, Mateus L. The equine cyclic corpus luteum: microvascularization, luteal cells characterization and function. *Pferdeheilkunde*. 2003;19:585588.
453. Canisso IF, Gallacher K, Gilbert MA, et al. Preovulatory progestogen treatment in mares fails to delay ovulation. *Vet J*. 2013;197:324–328.
454. Betteridge KJ, Waelchli RO, Raeside JI, et al. Changes accompanying spontaneous embryonic loss in nine mares during the first month of pregnancy. *J Equine Vet Sci*. 2014;34:195–197.
455. Canisso IF, Beltaire KA, Bedford-Guaus SJ. Premature luteal regression in a pregnant mare and subsequent pregnancy maintenance with the use of oral altrenogest. *Equine Vet J*. 2013;45:97–100.
456. Allen WR, Hamilton DW, Moor RM. The origin of equine endometrial cups. II. Invasion of the endometrium by trophoblast. *Anat Rec*. 1973;177:485–501.
457. Allen WR, Moor RM. The origin of the equine endometrial cups. I. Production of PMSG by fetal trophoblast cells. *J Reprod Fertil*. 1972;29:313–316.
458. Enders AC, Liu IK. Lodgement of the equine blastocyst in the uterus from fixation through endometrial cup formation. *J Reprod Fertil Suppl*. 1991;44:427–438.
459. Enders AC, Liu IK. Trophoblast-uterine interactions during equine chorionic girdle cell maturation, migration, and transformation. *Am J Anat*. 1991;192:366–381.
460. Stewart F, Lennard SN, Allen WR. Mechanisms controlling formation of the equine chorionic circle. *Biol Reprod Mono*. 1995;1:151–159.
461. Antczak DF, Allen WR. Maternal immunological recognition of pregnancy in equids. *J Reprod Fertil Suppl*. 1989;37:69–78.
462. Lunn P, Vagnoni KE, Ginther OJ. The equine immune response to endometrial cups. *J Reprod Immunol*. 1997;34:203–216.
463. Antczak DF, Allen WR. Invasive trophoblast in the genus Equus. *Ann Immunol (Paris)*. 1984;135D:325–331.
464. Clegg MT, Boda JM, Cole HH. The endometrial cups and allantochorionic pouches in the mare with emphasis on the source of equine gonadotrophin. *Endocrinology*. 1954;54:448–463.
465. Stewart F, Allen WR, Moor RM. Pregnant mare serum gonadotrophin: ratio of follicle-stimulating hormone and luteinizing hormone activities measured by radioreceptor assay. *J Endocrinol*. 1976;71:471–482.

466. Evans MJ, Irvine CH. Serum concentrations of FSH, LH and progesterone during the oestrous cycle and early pregnancy in the mare. *J Reprod Fertil Suppl*. 1975;23:193–200.
467. Zavy MT, Vernon MW, Sharp 3rd DC, et al. Endocrine aspects of early pregnancy in pony mares: a comparison of uterine luminal and peripheral plasma levels of steroids during the estrous cycle and early pregnancy. *Endocrinology*. 1984;115: 214–219.
468. Daels PF, Albrecht BA, Mohammed HO. Equine chorionic gonadotropin regulates luteal steroidogenesis in pregnant mares. *Biology of Reproduction*. 1998;59:1062–1068.
469. Daels PF, Chang GC, Hansen B, et al. Testosterone secretion during early pregnancy in mares. *Theriogenology*. 1996;45:1211–1219.
470. Daels PF, Demoraes JJ, Stabenfeldt GH, et al. The corpus luteum: source of estrogen during early pregnancy in the mare. *J Reprod Fertil*. 1991;44:501–508.
471. Walt ML, Stabenfeldt GH, Hughes JP, et al. Development of the equine ovary and ovulation fossa. *J Reprod Fertil Suppl*. 1979;27:471–477.
472. Canisso IF, Ball BA, Esteller-Vico A, et al. Changes in maternal androgens and oestrogens in mares with experimentally-induced ascending placentitis. *Equine Vet J*. 2017;49: 244–249.
473. Pashen RL. Maternal and fetal endocrinology during late pregnancy and parturition in the mare. *Equine Vet J*. 1984;16: 233–238.
474. Scholtz EL, Krishnan S, Ball BA, et al. Pregnancy without progesterone in horses defines a second endogenous biopotent progesterone receptor agonist, 5α-dihydroprogesterone. *Proc Natl Acad Sci U S A*. 2014;111:3365–3370.
475. Holtan DW, Nett TM, Estergreen VL. Plasma progestins in pregnant, postpartum and cycling mares. *J Anim Sci*. 1975;40:251–260.
476. Rossdale PD, Ousey JC, Cottrill CM, et al. Effects of placental pathology on maternal plasma progestogen and mammary secretion calcium concentrations and on neonatal adrenocortical function in the horse. *J Reprod Fertil Suppl*. 1991;44: 579–590.
477. Rossdale PD, McGladdery AJ, Ousey JC, et al. Increase in plasma progestogen concentrations in the mare after foetal injection with CRH, ACTH or betamethasone in late gestation. *Equine Vet J*. 1992;24:347–350.
478. Liggins GC, Fairclough RJ, Grieves SA, et al. Parturition in the sheep. *Ciba Found Symp*. 1977;47:5–30.
479. Haluska GJ, Currie WB. Variation in plasma concentrations of oestradiol-17 β and their relationship to those of progesterone, 13,14-dihydro-15-keto-prostaglandin F-2α and oxytocin across pregnancy and at parturition in pony mares. *J Reprod Fertil*. 1988;84:635–646.
480. Rossdale PD, Ousey JC, Chavatte P. Readiness for birth: an endocrinological duet between fetal foal and mare. *Equine Vet J Suppl*. 1997;24:96–99.
481. Silver M. Prenatal maturation, the timing of birth and how it may be regulated in domestic animals. *Exp Physiol*. 1990;75:285–307.
482. Liggins GC. Adrenocortical-related maturational events in the fetus. *Am J Obstet Gynecol*. 1976;126:931–941.
483. Brendemuehl JP, Williams MA, Boosinger TR, et al. Plasma progestogen, triiodothyronine, and cortisol concentrations in postdate gestation foals exposed in utero to the tall fescue endophyte Acremonium coenophialum. *Biol Reprod Mono*. 1995;1:53–59.
484. Lovell JD, Stabenfeldt GH, Hughes JP, et al. Endocrine patterns of the mare at term. *J Reprod Fertil Suppl*. 1975;23: 449–456.
485. Cudd TA, LeBlanc M, Silver M, et al. Ontogeny and ultradian rhythms of adrenocorticotropin and cortisol in the late-gestation fetal horse. *J Endocrinol*. 1995;144:271–283.
486. Liggins GC, Thorburn GD. Role of the fetal pituitary-adrenal system and placenta in the initiation of parturition. In: Lamming GE, ed. *Marshall's Physiology of Reproduction*. London: Chapman & Hall; 1993.
487. Chavatte P, Rossdale PD, Tait AD. 11β-Hydroxysteroid dehydrogenase (11BHSD) in equine placenta. *Proc Am Assoc Equine Pract*. 1995;41:264–265.
488. Ousey JC, Kolling M, Kindahl H, et al. Maternal dexamethasone treatment in late gestation induces precocious fetal maturation and delivery in healthy Thoroughbred mares. *Equine Vet J*. 2011;43:424–429.
489. Fowden AL, Silver M. Comparative development of the pituitary-adrenal axis in the fetal foal and lamb. *Reprod Domest Anim*. 1995;30:170–177.
490. Pashen RL, Allen WR. The role of the fetal gonads and placenta in steroid production, maintenance of pregnancy and parturition in the mare. *J Reprod Fertil Suppl*. 1979;27:499–509.
491. O'Donnell LJ, Sheerin BR, Hendry JM, et al. 24-hour secretion patterns of plasma oestradiol 17β in pony mares in late gestation. *Reprod Domest Anim*. 2003;38:233–235.
492. Bain AM, Howey WP. Observations on the time of foaling in thoroughbred mares in Australia. *J Reprod Fertil Suppl*. 1975;23:545–546.
493. Haluska GJ, Lowe JE, Currie WB. Electromyographic properties of the myometrium correlated with the endocrinology of the pre-partum and post-partum periods and parturition in pony mares. *J Reprod Fertil Suppl*. 1987;35:553–564.
494. Bonafos LD, Carnevale EM, Smith CA, et al. Development of uterine tone in nonbred and pregnant mares. *Theriogenology*. 1994;42:1247–1255.
495. Cross DT, Ginther OJ. Uterine contractions in nonpregnant and early pregnant mares and jennies as determined by ultrasonography. *J Anim Sci*. 1988;66:250–254.
496. Griffin PG, Ginther OJ. Uterine contractile activity in mares during the estrous cycle and early pregnancy. *Theriogenology*. 1990;34:47–56.
497. Kastelic JP, Adams GP, Ginther OJ. Role of progesterone in mobility, fixation, orientation, and survival of the equine embryonic vesicle. *Theriogenology*. 1987;27:655–663.
498. Daels PF, Besognet B, Hansen B, et al. Effect of progesterone on prostaglandin F2α secretion and outcome of pregnancy during cloprostenol-induced abortion in mares. *Am J Vet Res*. 1996;57:1331–1337.
499. Csapo A. Progesterone block. *Am J Anat*. 1956;98:273–291.
500. Fowden AL, Ousey JC, Forhead AJ, et al. Uteroplacental production of 5 α-pregnane-3,20-dione (5 α DHP) in pregnant mares. *Theriogenology*. 2002;58:821–824.
501. Behrendt-Adam CY, Adams MH, Simpson KS, et al. Oxytocin-neurophysin I mRNA abundance in equine uterine endometrium. *Domestic Animal Endocrinology*. 1999;16:183–192.
502. Ousey JC, Freestone N, Fowden AL, et al. The effects of oxytocin and progestogens on myometrial contractility in vitro during equine pregnancy. *J Reprod Fertil Suppl*. 2000;56:681–691.
503. Fowden AL, Silver M. Effects of inhibiting 3 β-hydroxysteroid dehydrogenase on plasma progesterone and other steroids in the pregnant mare near term. *J Reprod Fertil Suppl*. 1987;35:539–545.
504. Dudan FE, Figueroa JP, Frank DA, et al. Frequency distribution and daily rhythm of uterine electromyographic epochs of different duration in pony mares in late gestation. *J Reprod Fertil Suppl*. 1987;35:725–727.
505. McGlothlin JA, Lester GD, Hansen PJ, et al. Alteration in uterine contractility in mares with experimentally induced placentitis. *Reproduction*. 2004;127:57–66.
506. Vivrette SL, Kindahl H, Munro CJ, et al. Oxytocin release and its relationship to dihydro-15-keto PGF2α and arginine vasopressin release during parturition and to suckling in postpartum mares. *J Reprod Fertil*. 2000;119:347–357.

507. Camillo F, Marmorini P, Romagnoli S, et al. Clinical studies on daily low dose oxytocin in mares at term. *Equine Vet J*. 2000;32:307–310.
508. Eroksuz H, Eroksuz Y, Ozer H, et al. Equine goiter associated with excess dietary iodine. *Vet Hum Toxicol*. 2004;46: 147–149.
509. Prichard JT, Voss JL. Fetal ankylosis in horses associated with hybrid Sudan pasture. *J Am Vet Med Assoc*. 1967;150:871–873.
510. Schutte JG, van den Ingh TS. Microphthalmia, brachygnathia superior, and palatocheiloschisis in a foal associated with griseofulvin administration to the mare during early pregnancy. *Vet Q*. 1997;19:58–60.
511. Toribio RE, Bain FT, Mrad DR, et al. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc*. 1998;212:697–701.
512. Ginther OJ. *Ultrasonic Imaging and Animal Reproduction: book 2* Horses. Cross Plains: Equiservices; 1995.
513. Ginther OJ. *Ultrasonic Imaging and Animal Reproduction: book 1* Fundamentals. Cross Plains: Equiservices; 1995.
514. Stabenfeldt GH, Daels PF, Munro CJ, et al. An oestrogen conjugate enzyme immunoassay for monitoring pregnancy in the mare: limitations of the assay between days 40 and 70 of gestation. *J Reprod Fertil Suppl*. 1991;44:37–44.
515. Henderson K, Stevens S, Bailey C, et al. Comparison of the merits of measuring equine chorionic gonadotrophin (eCG) and blood and faecal concentrations of oestrone sulphate for determining the pregnancy status of miniature horses. *Reprod Fertil Dev*. 1998;10:441–444.
516. Bruck I, Lehn-Jensen H, Yde G. Spontaneous multiple ovulation and development of multiple embryonic vesicles in a mare. *Equine Vet J Suppl*. 1997:63–68.
517. Mancill SS, Blodgett G, Arnott RJ, et al. Description and genetic analysis of three sets of monozygotic twins resulting from transfers of single embryos to recipient mares. *J Am Vet Med Assoc*. 2011;238:1040–1043.
518. Meadows SJ, Binns MM, Newcombe JR, et al. Identical triplets in a thoroughbred mare. *Equine Vet J*. 1995;27:394–397.
519. Short RV. Monozygotic triplets in the mare. *Equine Vet J*. 1995;27:321.
520. Deskur S. Twinning in thoroughbred mares in Poland. *Theriogenology*. 1985;23:711–718.
521. Ginther OJ. Effect of reproductive status on twinning and on side of ovulation and embryo attachment in mares. *Theriogenology*. 1983;20:383–395.
522. Merkt H, Jochle W. Abortions and twin pregnancies in Thoroughbreds: rate of occurrence, treatments and prevention. *J Equine Vet Sci*. 1993;13:690–694.
523. Morel MC, Newcombe JR, Swindlehurst JC. The effect of age on multiple ovulation rates, multiple pregnancy rates and embryonic vesicle diameter in the mare. *Theriogenology*. 2005;63:2482–2493.
524. Newcombe JR. Incidence of multiple ovulation and multiple pregnancy in mares. *Vet Rec*. 1995;137:121–123.
525. Ginther OJ. Twin embryos in mares. I: from ovulation to fixation. *Equine Vet J*. 1989;21:166–170.
526. Allen WR, Brown L, Wright M, et al. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. *Equine Vet J*. 2007;39:438–445.
527. Hemberg E, Lundeheim N, Einarsson S. Reproductive performance of thoroughbred mares in Sweden. *Reprod Domest Anim*. 2004;39:81–85.
528. Ginther OJ, Douglas RH, Lawrence JR. Twinning in mares: a survey of veterinarians and analyses of theriogenology records. *Theriogenology*. 1982;18:333–347.
529. Wolfsdorf KE. Management of postfixation twins in mares. *Vet Clin North Am Equine Pract*. 2006;22:713–725.
530. Ginther OJ. The twinning problem: from breeding to day 16. *Proc Am Assoc Equine Pract*. 1983;29:11–26.
531. Pascoe DR, Pascoe RR, Hughes J, et al. Comparison of two techniques and three hormone therapies for management of twin conceptuses by manual embryonic reduction. *J Reprod Fertil Suppl*. 1987;35:701–702.
532. Macpherson ML, Reimer JM. Twin reduction in the mare: current options. *Anim Reprod Sci*. 2000;60-61:233–244.
533. Veronesi MC, Faustini M, Villani M, et al. Plasma concentrations of 15-ketodihydro-PGF(2α), cortisol and progesterone during manual twin reduction in thoroughbred mares. *J Vet Med A Physiol Pathol Clin Med*. 2005;52:411–415.
534. Ginther OJ. Postfixation embryo reduction in unilateral and bilateral twins in mares. *Theriogenology*. 1984;22:213–223.
535. Ginther OJ. Twin embryos in mares. II: post fixation embryo reduction. *Equine Vet J*. 1989;21:171–174.
536. Ginther OJ. Twinning in mares: a review of recent studies. *J Equine Vet Sci*. 1982;2:127–135.
537. Ginther OJ. The nature of embryo reduction in mares with twin conceptuses: deprivation hypothesis. *Am J Vet Res*. 1989;50:45–53.
538. Watson ED, Nikolaopoulos E, Lawler DF. Survival and normal development of an embryo after prostaglandin treatment. *Equine Veterinary Education*. 1997;9:283–285.
539. Cuervo-Arango J, Aguilar JJ, Vettorazzi ML, et al. eCG concentrations, luteal structures, return to cyclicity, and postabortion fertility in embryo transfer recipient mares. *Theriogenology*. 2015;84:1003–1013.
540. Mari G, Iacono E, Merlo B, et al. Reduction of twin pregnancy in the mare by transvaginal ultrasound-guided aspiration. *Reprod Domest Anim*. 2004;39:434–437.
541. Journee SL, de Ruijter-Villani M, Hendriks WK, et al. Efficacy of transvaginal ultrasound-guided twin reduction in the mare by embryonic or fetal stabbing compared with yolk sac or allantoic fluid aspiration. *Theriogenology*. 2013;80:346–349.
542. Rantanen NW, McKinnon AO. *Equine Diagnostic Ultrasonography*. Baltimore: Williams & Wilkins; 1998.
543. Allen WR. Luteal deficiency and embryo mortality in the mare. *Reprod Domest Anim*. 2001;36:121–131.
544. Yang YJ, Cho GJ. Factors concerning early embryonic death in thoroughbred mares in south Korea. *J Vet Med Sci*. 2007;69:787–792.
545. Ball BA, Little TV, Hillman RB, et al. Pregnancy rates at days 2 and 14 and estimated embryonic loss rates prior to day 14 in normal and subfertile mares. *Theriogenology*. 1986;26:611–619.
546. Brinsko SP, Ball BA, Miller PG, et al. In-vitro development of day-2 embryos obtained from young, fertile mares and aged, subfertile mares. *J Reprod Fertil*. 1994;102:371–378.
547. Carnevale EM, Griffin PG, Ginther OJ. Age-associated subfertility before entry of embryos into the uterus in mares. *Equine Vet J*. 1993;25:31–35.
548. Woods GL, Baker CB, Baldwin JL, et al. Early pregnancy loss in brood mares. *J Reprod Fertil Suppl*. 1987;35:455–459.
549. Woods GL, J.A W, Vanderwall DK, et al. Selective oviductal transport and fertilization rate of equine embryos. *Proc Am Assoc Equine Pract*. 1991;37:197–201.
550. Vanderwall D. *Early embryonic loss in the mare: current perspectives*. San Antonio: Society for Theriogenology; 2000.
551. Carnevale EM, Uson M, Bozzola JJ, et al. Comparison of oocytes from young and old mares with light and electron microscopy. *Theriogenology*. 1999;51:299–299.
552. Carnevale EM, Ginther OJ. Defective oocytes as a cause of subfertility in old mares. *Biol Reprod Monogr*. 1995;1:209–214.
553. Ball BA, Little TV, Weber JA, et al. Survival of day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. *J Reprod Fertil*. 1989;85:187–194.
554. Vogelsang SG, Vogelsang MM. Influence of donor parity and age on the success of commercial equine embryo transfer. *Equine Vet J Suppl*. 1989;8:71–72.

555. Woods GL, Hillman RB, Schlafer DH. Recovery and evaluation of embryos from normal and infertile mares. *Cornell Vet*. 1986;76:386–394.
556. Ball BA, Hillman RB, Woods GL. Survival of equine embryos transferred to normal and subfertile mares. *Theriogenology*. 1987;28:167–174.
557. Squires EL, Imel KJ, Iuliano MF, et al. Factors affecting reproductive efficiency in an equine embryo transfer programme. *J Reprod Fertil Suppl*. 1982;32:409–414.
558. Morris LH, Allen WR. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. *Equine Vet J*. 2002;34:51–60.
559. Janosi S, Huszenicza G, Kulcsar M, et al. Endocrine and reproductive consequences of certain endotoxin-mediated diseases in farm mammals: a review. *Acta Veterinaria Hungarica*. 1998;46:71–84.
560. Daels PF, Stabenfeldt GH, Hughes JP, et al. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. *Am J Vet Res*. 1991;52:282–288.
561. Daels PF, Starr M, Kindahl H, et al. Effect of *Salmonella typhimurium* endotoxin on PGF-2 α release and fetal death in the mare. *J Reprod Fertil Suppl*. 1987;35:485–492.
562. Daels PF, Mohammed HO, Odensvik K, et al. Effect of flunixin meglumine on endogenous prostaglandin F2 α secretion during cloprostenol-induced abortion in mares. *Am J Vet Res*. 1995;56:1603–1610.
563. Daels PF, Besognet B, Hansen B, et al. Efficacy of treatments to prevent abortion in pregnant mares at risk. *Proc Am Assoc Equine Pract*. 1994;40:31–32.
564. Ousey JC. Peripartal endocrinology in the mare and foetus. *Reprod Domest Anim*. 2004;39:222–231.
565. LeBlanc MM. Equine perinatology: what we know and what we need to know. *Anim Reprod Sci*. 1996;42:189–196.
566. Santschi EM, Leblanc MM. Fetal and placental conditions that cause high-risk pregnancy in mares. *Compend Contin Educ Pract Vet*. 1995;17:710–721.
567. Daels PF, Stabenfeldt GH, Hughes JP, et al. Effects of flunixin meglumine on endotoxin-induced prostaglandin F2 α secretion during early pregnancy in mares. *Am J Vet Res*. 1991;52:276–281.
568. Slone DE. Treatment of pregnant mares with colic: practical considerations and concerns. *Compend Contin Educ Pract Vet*. 1993;15:117–120.
569. White NA. *The Equine Acute Abdomen*. Philadelphia: Lea & Febiger; 1990.
570. Boening KJ, Leendertse IP. Review of 115 cases of colic in the pregnant mare. *Equine Vet J*. 1993;25:518–521.
571. Santschi EM, Slone DE. Maternal conditions that cause high-risk pregnancy in mares. *Compend Contin Educ Pract Vet*. 1994;16:1481-&.
572. Santschi EM, Slone DE, Gronwall R, et al. Types of colic and frequency of postcolic abortion in pregnant mares: 105 cases (1984-1988). *J Am Vet Med Assoc*. 1991;199:374–377.
573. Dwyer RM, Garber LP, Traub-Dargatz JL, et al. Case-control study of factors associated with excessive proportions of early fetal losses associated with mare reproductive loss syndrome in central Kentucky during 2001. *J Am Vet Med Assoc*. 2003;222:613–619.
574. Choate BA, Rieske LK. Life history and age-specific mortality of eastern tent caterpillar (Lepidoptera : lasiocampidae). *Ann Entomol Soc Am*. 2005;98:496–502.
575. Morehead JP, Blanchard TL, Thompson JA, et al. Evaluation of early fetal losses on four equine farms in central Kentucky: 73 cases (2001). *J Am Vet Med Assoc*. 2002;220:1828–1830.
576. Sebastian M, Gantz MG, Tobin T, et al. The mare reproductive loss syndrome and the eastern tent caterpillar: a toxicokinetic/statistical analysis with clinical, epidemiologic, and mechanistic implications. *Vet Ther*. 2003;4:324–339.
577. Swerczek TW. Saprotrophic fungi and bacteria and commensal bacteria that infect frost-damaged pastures may be contributing to gut microbial overgrowth and lesions associated with the mare reproductive loss syndrome. *J Equine Vet Sci*. 2002;22:234–237.
578. Taylor JR. Theory of ammonia toxicity as the mechanism of abortion in the mare reproductive loss syndrome. *J Equine Vet Sci*. 2002;22:237–239.
579. Cohen ND, Carey VJ, Donahue JG, et al. Case-control study of late-term abortions associated with mare reproductive loss syndrome in central Kentucky. *J Am Vet Med Assoc*. 2003;222:199–209.
580. Cohen ND, Carey VJ, Donahue JG, et al. Descriptive epidemiology of late-term abortions associated with the mare reproductive loss syndrome in central Kentucky. *J Vet Diagn Invest*. 2003;15:295–297.
581. Cohen ND, Donahue JG, Carey VJ, et al. Case-control study of early-term abortions (early fetal losses) associated with mare reproductive loss syndrome in central Kentucky. *J Am Vet Med Assoc*. 2003;222:210–217.
582. Cohen ND, Carey VJ, Donahue JG, et al. Temporality of early-term abortions associated with mare reproductive loss syndrome in horses. *Am J Vet Res*. 2005;66:1792–1797.
583. Potter DA, Foss L, Baumler RE, et al. Managing Eastern tent caterpillars *Malacosoma americanum* (F) on horse farms to reduce risk of mare reproductive loss syndrome. *Pest Manag Sci*. 2005;61:3–15.
584. Thompson JA, Brown 2nd SE, Riddle WT, et al. Use of a Bayesian risk-mapping technique to estimate spatial risks for mare reproductive loss syndrome in Kentucky. *Am J Vet Res*. 2005;66:17–20.
585. Seahorn JL, Slovis NM, Reimer JM, et al. Case-control study of factors associated with fibrinous pericarditis among horses in central Kentucky during spring 2001. *J Am Vet Med Assoc*. 2003;223:832–838.
586. Bernard WV, LeBlanc MM, Webb BA, et al. Evaluation of early fetal loss induced by gavage with eastern tent caterpillars in pregnant mares. *J Am Vet Med Assoc*. 2004;225:717–721.
587. Webb BA, Barney WE, Dahlman DL, et al. Eastern tent caterpillars (Malacosoma americanum) cause mare reproductive loss syndrome. *J Insect Physiol*. 2004;50:185–193.
588. McDowell KJ. MRLS update. *Equine Dis Q*. 2008;17:5.
589. Donahue JM, Sells SF, Bolin DC. Classification of *Actinobacillus* spp. isolates from horses involved in mare reproductive loss syndrome. *Am J Vet Res*. 2006;67:1426–1432.
590. Cawdell-Smith AJ, Todhunter KH, Anderson ST, et al. Equine amnionitis and fetal loss: mare abortion following experimental exposure to Processionary caterpillars (*Ochrogaster lunifer*). *Equine Vet J*. 2012;44:282–288.
591. Hong CB, Donahue JM, Giles Jr RC, et al. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. *J Vet Diagn Invest*. 1993;5:560–566.
592. Smith KC, Blunden AS, Whitwell KE, et al. A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. *Equine Vet J*. 2003;35:496–501.
593. Bucca S. Diagnosis of the compromised equine pregnancy. *Vet Clin North Am Equine Pract*. 2006;22:749–761.
594. Canisso IF, Ball BA, Erol E, et al. Comprehensive review on equine placentitis. *Proc Am Assoc Equine Pract*. 2015;61:490–509.
595. Lyle SK. Immunology of infective preterm delivery in the mare. *Equine Vet J*. 2014;46:661–668.
596. Donahue JM, Williams NM. Emergent causes of placentitis and abortion. *Vet Clin North Am Equine Pract*. 2000;16:443–456, viii.
597. Bolin DC, Donahue JM, Vickers ML, et al. Equine abortion and premature birth associated with *Cellulosimicrobium cellulans* infection. *J Vet Diagn Invest*. 2004;16:333–336.

598. Canisso IF, Ball BA, Erol E, et al. Attempts to induce nocardioform placentitis (*Crossiela equi*) experimentally in mares. *Equine Vet J*. 2015;47:91–95.
599. Hong CB, Donahue JM, Giles Jr RC, et al. Etiology and pathology of equine placentitis. *J Vet Diagn Invest*. 1993;5:56–63.
600. Erol E, Jackson CB, Steinman M, et al. A diagnostic evaluation of real-time PCR, fluorescent antibody and microscopic agglutination tests in cases of equine leptospiral abortion. *Equine Vet J*. 2015;47:171–174.
601. Newman D, Donahue M. Equine leptospirosis. *J Equine Vet Sci*. 2007;27:424–425.
602. Szeredi L, Haake DA. Immunohistochemical identification and pathologic findings in natural cases of equine abortion caused by leptospiral infection. *Vet Pathol*. 2006;43:755–761.
603. Donahue JM, Smith BJ, Donahoe JK, et al. Prevalence and serovars of leptospira involved in equine abortions in central Kentucky during the 1990 foaling season. *J Vet Diagn Invest*. 1992;4:279–284.
604. Timoney JF, Kalimuthusamy N, Velineni S, et al. A unique genotype of *Leptospira interrogans* serovar *Pomona* type *kennewicki* is associated with equine abortion. *Vet Microbiol*. 2011;150:349–353.
605. Bernard WV, Bolin C, Riddle T, et al. Leptospirosis on a central Kentucky horse farm: preventive measures following a case of abortion. *Proc Am Assoc Equine Pract*. 1990;36:335–336.
606. Patterson-Kane JC, Donahue JM, Harrison LR. Placentitis, fetal pneumonia, and abortion due to *Rhodococcus equi* infection in a Thoroughbred. *J Vet Diagn Invest*. 2002;14:157–159.
607. Tortschanoff M, Aurich C, Rosengarten R, et al. Phase and size variable surface-exposed proteins in equine genital mycoplasmas. *Vet Microbiol*. 2005;110:301–306.
608. Bolon B, Buergelt CD, Cooley AJ. Abortion in two foals associated with *Nocardia* infection. *Vet Pathol*. 1989;26:277–278.
609. Bryant UK. Equine placentitis: common causes and newly emerging pathogens. *Equine Dis Q*. 2008;17:4.
610. Johnson AK, Roberts JF, Hagan A, et al. Infection of an equine placenta with a novel mycobacterial species leading to abortion. *J Vet Diagn Invest*. 2012;24:785–790.
611. Duarte PC, Conrad PA, Barr BC, et al. Risk of transplacental transmission of *Sarcocystis neurona* and *Neospora hughesi* in California horses. *J Parasitol*. 2004;90:1345–1351.
612. Locatelli-Dittrich R, Dittrich JR, Richartz RR, et al. Investigation of *Neospora* sp. and *Toxoplasma gondii* antibodies in mares and in precolostral foals from Parana State, Southern Brazil. *Vet Parasitol*. 2006;135:215–221.
613. McDole MG, Gay JM. Seroprevalence of antibodies against *Neospora caninum* in diagnostic equine serum samples and their possible association with fetal loss. *Vet Parasitol*. 2002;105:257–260.
614. Pitel PH, Romand S, Pronost S, et al. Investigation of Neospora sp. antibodies in aborted mares from Normandy, France. *Vet Parasitol*. 2003;118:1–6.
615. Villalobos EM, Ueno TE, de Souza SL, et al. Association between the presence of serum antibodies against Neospora spp. and fetal loss in equines. *Vet Parasitol*. 2006;142:372–375.
616. Dubey JP. Neosporosis in cattle. *Vet Clin North Am Food Anim Pract*. 2005;21:473–483.
617. Kligler EB, Shkap V, Baneth G, et al. Seroprevalence of *Neospora* spp. among asymptomatic horses, aborted mares and horses demonstrating neurological signs in Israel. *Vet Parasitol*. 2007;148:109–113.
618. Balasuriya UB. Equine viral arteritis. *Vet Clin North Am Equine Pract*. 2014;30:543–560.
619. Balasuriya UB, Evermann JF, Hedges JF, et al. Serologic and molecular characterization of an abortigenic strain of equine arteritis virus isolated from infective frozen semen and an aborted equine fetus. *J Am Vet Med Assoc*. 1998;213:1586–1589, 1570.
620. Glaser AL, de Vries AA, Rottier PJ, et al. Equine arteritis virus: a review of clinical features and management aspects. *Vet Q*. 1996;18:95–99.
621. Metcalf ES. The role of international transport of equine semen on disease transmission. *Anim Reprod Sci*. 2001;68:229–237.
622. Szeredi L, Hornyak A, Palfi V, et al. Study on the epidemiology of equine arteritis virus infection with different diagnostic techniques by investigating 96 cases of equine abortion in Hungary. *Vet Microbiol*. 2005;108:235–242.
623. Timoney PJ, McCollum WH. Equine viral arteritis: further characterization of the carrier state in stallions. *J Reprod Fertil Suppl*. 2000;56:3–11.
624. MacLachlan NJ, Balasuriya UBR, Rossitto PV, et al. Fatal experimental equine arteritis virus infection of a pregnant mare: immunohistochemical staining of viral antigens. *J Vet Diagn Invest*. 1996;8:367–374.
625. Paweska JT. Effect of the South African asinine-94 strain of equine arteritis virus (EAV) in pregnant donkey mares and duration of maternal immunity in foals. *Onderstepoort J Vet Res*. 1997;64:147–152.
626. Timoney PJ, McCollum WH. Equine viral arteritis. *Can Vet J*. 1987;28:693–695.
627. Wada R, Fukunaga Y, Kanemaru T, et al. Histopathological and immunofluorescent studies on transplacental infection in experimentally induced abortion by equine arteritis virus. *J Vet Med Ser B-Infect Dis Vet Public Health*. 1996;43:65–74.
628. Leon A, Fortier G, Fortier C, et al. Detection of equine herpesviruses in aborted foetuses by consensus PCR. *Vet Microbiol*. 2008;126:20–29.
629. Vickers ML. Equine herpes virus abortions. *Equine Dis Q*. 2001;10:3–4.
630. Kydd JH, Townsend HG, Hannant D. The equine immune response to equine herpesvirus-1: the virus and its vaccines. *Vet Immunol Immunopathol*. 2006;111:15–30.
631. Kydd JH, Wattrang E, Hannant D. Pre-infection frequencies of equine herpesvirus-1 specific, cytotoxic T lymphocytes correlate with protection against abortion following experimental infection of pregnant mares. *Vet Immunol Immunopathol*. 2003;96:207–217.
632. Gilkerson JR, Whalley JM, Drummer HE, et al. Epidemiological studies of equine herpesvirus 1 (EHV-1) in Thoroughbred foals: a review of studies conducted in the Hunter Valley of New South Wales between 1995 and 1997. *Vet Microbiol*. 1999;68:15–25.
633. Smith KC, Whitwell KE, Blunden AS, et al. Equine herpesvirus-1 abortion: atypical cases with lesions largely or wholly restricted to the placenta. *Equine Vet J*. 2004;36:79–82.
634. Smith KC, Whitwell KE, Mumford JA, et al. Virulence of the V592 isolate of equid herpesvirus-1 in ponies. *J Comp Pathol*. 2000;122:288–297.
635. Smith DJ, Hamblin AS, Edington N. Infection of endothelial cells with equine herpesvirus-1 (EHV-1) occurs where there is activation of putative adhesion molecules: a mechanism for transfer of virus. *Equine Vet J*. 2001;33:138–142.
636. Smith KC, McGladdery AJ, Binns MM, et al. Use of transabdominal ultrasound-guided amniocentesis for detection of equid herpesvirus 1-induced fetal infection in utero. *Am J Vet Res*. 1997;58:997–1002.
637. Smith KC, Mumford JA, Lakhani K. A comparison of equid herpesvirus-1 (EHV-1) vascular lesions in the early versus late pregnant equine uterus. *J Comp Pathol*. 1996;114:231–247.
638. Drummer HE, Reynolds A, Studdert MJ, et al. Application of an equine herpesvirus 1 (EHV1) type-specific ELISA to the management of an outbreak of EHV1 abortion. *Vet Rec*. 1995;136:579–581.
639. van Maanen C, Willink DL, Smeenk LAJ, et al. An equine herpesvirus 1 (EHV1) abortion storm at a riding school. *Veterinary Quarterly*. 2000;22:83–87.

640. Ostlund EN. The equine herpesviruses. *Vet Clin N Am-Equine Pract*. 1993;9:283–294.
641. Slater J. Equine herpesviruses. In: Sellon DC, Long MT, eds. *Equine Infectious Diseases*. 2nd ed. St. Louis: Elsevier; 2014.
642. Tashjian RJ. Transmission and clinical evaluation of an equine infectious anemia herd and their offspring over a 13-year period. *J Am Vet Med Assoc*. 1984;184:282–288.
643. Vest DJ, Cohen ND, Berezowski CJ, et al. Evaluation of administration of West Nile virus vaccine to pregnant broodmares. *J Am Vet Med Assoc*. 2004;225:1894–1897.
644. Hendry JM, Lester GD, Hansen PJ, et al. Patterns of uterine myoelectrical activity in reproductively normal mares in late gestation and in mares with experimentally induced ascending placentitis. *Theriogenology*. 2002;58:853–855.
645. LeBlanc MM, Giguere S, Brauer K, et al. Premature delivery in ascending placentitis is associated with increased expression of placental cytokines and allantoic fluid prostaglandins E-2 and F-2 α. *Theriogenology*. 2002;58:841–844.
646. Stawicki RJ, Ruebel H, Hansen PJ, et al. Endocrinological findings in an experimental model of ascending placentitis in the mare. *Theriogenology*. 2002;58:849–852.
647. Macpherson ML. Treatment strategies for mares with placentitis. *Theriogenology*. 2005;64:528–534.
648. Mays MBC, LeBlanc MM, Paccamonti D. Route of fetal infection in a model of ascending placentitis. *Theriogenology*. 2002;58:791–792.
649. Graczyk J, Macpherson ML, Pozor M, et al. Treatment efficacy of trimethoprim sulfamethoxazole and pentoxifylline in equine placentitis. *Anim Reprod Sci*. 2006;94:434–435.
650. Rebello S, Macpherson M, Murchie T, et al. The detection of placental drug transfer in equine allantoic fluid - Abstracts. *Theriogenology*. 2005;64:776–777.
651. Rebello SA, Macpherson ML, Murchie TA, et al. Placental transfer of trimethoprim sulfamethoxazole and pentoxifylline in pony mares. *Anim Reprod Sci*. 2006;94:432–433.
652. Macpherson ML, Giguere S, Hatzel JN, et al. Disposition of desfuroylceftiofur acetamide in serum, placental tissue, fetal fluids, and fetal tissues after administration of ceftiofur crystalline free acid (CCFA) to pony mares with placentitis. *J Vet Pharmacol Ther*. 2013;36:59–67.
653. Santschi EM, Papich MG. Pharmacokinetics of gentamicin in mares in late pregnancy and early lactation. *J Vet Pharmacol Ther*. 2000;23:359–363.
654. Murchie TA, Macpherson ML, LeBlanc MM, et al. Continuous monitoring of penicillin G and gentamicin in allantoic fluid of pregnant pony mares by in vivo microdialysis. *Equine Vet J*. 2006;38:520–525.
655. Garfield RE, Kannan MS, Daniel EE. Gap junction formation in myometrium: control by estrogens, progesterone, and prostaglandins. *Am J Physiol*. 1980;238:C81–C89.
656. Macpherson ML. Diagnosis and treatment of equine placentitis. *Vet Clin North Am Equine Pract*. 2006;22:763–776.
657. Ousey J, Rossdale PD, Palmer L, et al. Effects of progesterone administration to mares during late gestation. *Theriogenology*. 2002;58:793–795.
658. Chavatte PM, Rossdale PD, Tait AD. Modulation of 3-β-Hydroxysteroid Dehydrogenase (3-β-Hsd) Activity in the Equine Placenta by Pregnenolone and Progesterone Metabolites. *Equine Vet J*. 1995;27:342–347.
659. Zent WW, Williams NM, Donahue JM. Placentitis in Central Kentucky broodmares. *Pferdeheilkunde*. 1999;15:630–632.
660. Bostedt H. The use of a β 2-mimetic agent (clenbuterol) in equine pregnancy disorders and obstetrics. *Tierarztl Prax*. 1988;16:57–59.
661. Card CE, Wood MR. Effects of acute administration of clenbuterol on uterine tone and equine fetal and maternal heart rates. *Biol Reprod Mono*. 1995;1:7–11.
662. Palmer E, Chavatte-Palmer P, Duchamp G, et al. Lack of effect of clenbuterol for delaying parturition in late pregnant mares. *Theriogenology*. 2002;58:797–799.
663. Bailey CS, Heitzman JM, Buchanan CN, et al. B-mode and Doppler ultrasonography in pony mares with experimentally induced ascending placentitis. *Equine Vet J*. 2012;44:88–94.
664. Bailey CS, Sper RB, Schewmaker JL, et al. Uterine artery blood flow remains unchanged in pregnant mares in response to short-term administration of pentoxifylline. *Theriogenology*. 2012;77:430–436.
665. Wolc A, Bresinska A, Szwaczkowski T. Genetic and permanent environmental variability of twinning in Thoroughbred horses estimated via three threshold models. *J Anim Breed Genet*. 2006;123:186–190.
666. Ginther OJ, Griffin PG. Natural outcome and ultrasonic identification of equine fetal twins. *Theriogenology*. 1994;41:1193–1199.
667. Cassar TIY, Fallon LH, Martinez EH, et al. Segmental ossification of involuted yolk sacs in equine umbilical cords. *Anim Reprod Sci*. 2006;94:439–442.
668. Williams NM, Donahue JM, Bolin DC. Equine placental pathology: kentucky perspective. In: Powell DG, Furry D, Hale G, eds. *Workshop on the Equine Placenta*. Lexington: University of Kentucky; 2003:88–92.
669. Schlafer DH. The umbilical cord: lifeline to the outside world: structure, function, and pathology of the equine umbilical cord. In: Powell DG, Furry D, Hale G, eds. *Workshop on the equine Placenta*. Lexington: University of Kentucky; 2003.
670. Frazer GS. Umbilical cord compromise as a cause of abortion. *Equine Veterinary Education*. 2007;19:535–537.
671. Whitwell KE. Equine placental pathology: the Newmarket perspective. In: Powell DG, Furry D,Hale G, eds. *Workshop on the Equine Placenta*. Lexington: University of Kentucky; 2003.
672. Williams NM. Umbilical cord torsion. *Equine Dis Q*. 2002;10:3–4.
673. Ginther OJ. Equine physical utero-fetal interactions: a challenge and a wonder for the practitioner. *J Equine Vet Sci*. 1994;14:313–318.
674. Ginther OJ, Griffin PG. Equine fetal kinetics: presentation and location. *Theriogenology*. 1993;40:1–11.
675. Ginther OJ, Williams D, Curran S. Equine fetal kinetics: entry and retention of fetal hind limbs in a uterine horn. *Theriogenology*. 1994;41:795–807.
676. Ginther OJ. Equine fetal kinetics: allantoic-fluid shifts and uterine-horn closures. *Theriogenology*. 1993;40:241–256.
677. Vandeplassche M, Lauwers H. The twisted umbilical cord: an expression of kinesis of the equine fetus. *Anim Reprod Sci*. 1986;10:163–175.
678. Ricketts SW, Barrelet A, Whitwell KE. A review of the causes of abortion in UK mares and means of diagnosis used in an equine studfarm practice in Newmarket. *Pferdeheilkunde*. 2001;17:589–592.
679. Whitwell KE. Morphology and pathology of the equine umbilical cord. *J Reprod Fertil Suppl*. 1975;23:599–603.
680. Snider TA. Umbilical cord torsion and coiling as a cause of dystocia and intrauterine foal loss. *Equine Veterinary Education*. 2007;19:532–534.
681. Holder RD. Equine fetal sexing. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. Philadelphia: Saunders; 2002.
682. Curran S, Ginther OJ. Ultrasonic fetal gender diagnoses during months 5 to 11 in mares. *Theriogenology*. 1993;40:1127–1135.
683. Renaudin CD, Gillis CL, Tarantal AF. Transabdominal ultrasonographic determination of fetal gender in the horse during mid-gestation. *Equine Vet J*. 1999;31:483–487.
684. Ryan PL, Christiansen DL, Hopper RM, et al. Evaluation of systemic relaxin blood profiles in horses as a means of assessing placental function in high-risk pregnancies and responsiveness to therapeutic strategies. *Ann N Y Acad Sci*. 2009;1160:169–178.
685. Ryan PL, Vaala WE, Bagnell CA. Evidence that equine relaxin is a good indicator of placental insufficiency in the mare. *Proc Am Assoc Equine Pract*. 1998;44:62–63.

686. Santschi EM, Leblanc MM, Weston PG. Progestogen, estrone sulfate and cortisol concentrations in pregnant mares during medical and surgical disease. *J Reprod Fertil*. 1991:627–634.
687. Riddle T. Preparation of the mare for normal parturition. *Proc Am Assoc Equine Pract*. 2003;49:1–5.
688. Ousey JC, Houghton E, Grainger L, et al. Progestogen profiles during the last trimester of gestation in Thoroughbred mares with normal or compromised pregnancies. *Theriogenology*. 2005;63:1844–1856.
689. van Niekerk CH, Morgenthal JC. Fetal loss and the effect of stress on plasma progestogen levels in pregnant Thoroughbred mares. *J Reprod Fertil Suppl*. 1982;32:453–457.
690. Houghton E, Holtan D, Grainger L, et al. Plasma progestogen concentrations in the normal and dysmature newborn foal. *J Reprod Fertil Suppl*. 1991;44:609–617.
691. Rossdale PD, Ousey JC, McGladdery AJ, et al. A retrospective study of increased plasma progestogen concentrations in compromised neonatal foals. *Reprod Fertil Dev*. 1995;7:567–575.
692. Hoffmann B, Gentz F, Failing K. Investigations into the course of progesterone, oestrogen and eCG concentrations during normal and impaired pregnancy in the mare. *Reprod Domest Anim*. 1996;31:717–723.
693. Ousey J, McGladdery A. Clinical diagnosis and treatment of problems in the late pregnant mare. *In Pract*. 2000;22:200–207.
694. Bucca S, Fogarty U, Collins A, et al. Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. *Theriogenology*. 2005;64:542–557.
695. Kelleman AA, Luznar SL, Lester GD, et al. Evaluation of transrectal ultrasonographic combined thickness of the uterus and placenta (CTUP) in a model of induced ascending placentitis in late gestation in the pony mare. *Theriogenology*. 2002;58:845–848.
696. Renaudin CD, Liu IKM, Troedsson MHT, et al. Transrectal ultrasonographic diagnosis of ascending placentitis in the mare: a report of two cases. *Equine Vet Educ*. 1999;11:69–74.
697. Troedsson MHT. Transrectal ultrasonography of the placenta in normal mares and in mares with pending abortion: a field study. *Proc Am Assoc Equine Pract*. 1997;43:256–258.
698. Morris S, Kelleman AA, Stawicki RJ, et al. Transrectal ultrasonography and plasma progestin profiles identifies feto-placental compromise in mares with experimentally induced placentitis. *Theriogenology*. 2007;67:681–691.
699. Williams NM, Donahue JM. Nocardioform placentitis. *Equine Dis Q*. 2000;9:5–6.
700. Williams NM, Donahue JM. Placentitis in mares. *Equine Dis Q*. 1998;6:4.
701. Pipers FS, Adams-Brendemuehl CS. Techniques and applications of transabdominal ultrasonography in the pregnant mare. *J Am Vet Med Assoc*. 1984;185:766–771.
702. Reef VB, Vaala WE, Worth LT, et al. Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile. *Equine Vet J*. 1996;28:200–208.
703. Reef VB, Vaala WE, Worth LT, et al. Ultrasonographic evaluation of the fetus and intrauterine environment in healthy mares during late gestation. *Vet Radiol Ultrasound*. 1995;36:533–541.
704. Reef VB. *Equine Diagnostic Ultrasound*. Philadelphia: Saunders; 1998.
705. Reef VB, Vaala WE, Worth LT, et al. Transcutaneous ultrasonographic assessment of fetal well-being during late gestation: a preliminary report on the development of an equine biophysical profile. *Proc Am Assoc Equine Pract*. 1996;42:152–153.
706. Luukkanen L, Katila T, Koskinen E. Some effects of multiple administration of detomidine during the last trimester of equine pregnancy. *Equine Vet J*. 1997;29:400–402.
707. Pipers FS, Zent W, Holder R, et al. Ultrasonography as an adjunct to pregnancy assessments in the mare. *J Am Vet Med Assoc*. 1984;184:328–334.
708. Colles CM, Parks RD, May CJ. Foetal echocardiography in the mare. *Equine Vet J*. 1978;10:32–37.
709. Holmes JR, Darke PGG. Foetal electrocardiography in the mare. *Vet Rec*. 1968;82:651–655.
710. Renaudin CD, Gillis CL, Tarantal AF, et al. Evaluation of equine fetal growth from day 100 of gestation to parturition by ultrasonography. *J Reprod Fertil Suppl*. 2000;56:651–660.
711. Turner RM, McDonnell SM, Feit EM, et al. Real-time ultrasound measure of the fetal eye (vitreous body) for prediction of parturition date in small ponies. *Theriogenology*. 2006;66:331–337.
712. Barber JA, Troedsson MH. Mummified fetus in a mare. *J Am Vet Med Assoc*. 1996;208:1438–1440.
713. Burns TE, Card CE. Fetal maceration and retention of fetal bones in a mare. *J Am Vet Med Assoc*. 2000;217:878–880, 845.
714. Frazer GS, Embertson R, Perkins NR. Complications of late gestation in the mare. *Equine Vet Educ*. 1997;9:306–311.
715. Ross J, Palmer JE, Wilkins PA. Body wall tears during late pregnancy in mares: 13 cases (1995-2006). *J Am Vet Med Assoc*. 2008;232:257–261.
716. Vandeplassche M, Bouters R, Spincemaille J, et al. Dropsy of the fetal sacs in mares: induced and spontaneous abortion. *Vet Rec*. 1976;99:67–69.
717. Koterba AM, Haibel GK, Grimmet JB. Respiratory distress in a premature foal secondary to hydrops allantois and placentitis. *Compend Contin Educ Pract Vet*. 1983;5:S121–S125.
718. Waelchli RO, Ehrensperger F. 2 related cases of cerebellar abnormality in equine fetuses associated with hydrops of fetal membranes. *Vet Rec*. 1988;123:513–514.
719. Stich KL, Blanchard TL. Hydrallantois in mares. *Compend Contin Educ Pract Vet*. 2003;25:71–75.
720. Christensen BW, Troedsson MHT, Murchie TA, et al. Management of hydrops amnion in a mare resulting in birth of a live foal. *JAVMA-J Am Vet Med Assoc*. 2006;228:1228–1233.
721. Honnas CM, Spensley MS, Laverty S, et al. Hydramnios causing uterine rupture in a mare. *J Am Vet Med Assoc*. 1988;193:334–336.
722. Macpherson ML, Chaffin MK, Carroll GL, et al. Three methods of oxytocin-induced parturition and their effects of foals. *J Am Vet Med Assoc*. 1997;210:799–803.
723. Bain AM, Wolfsdorf KE. Placental hydrops. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. 5th ed. St. Louis: Elsevier; 2003:301–302.
724. Hendriks WK, Stout TAE, van der Weijden GC. Spinal cord trauma in a recently foaled Friesian mare as a complication of ventral abdominal rupture. *Equine Vet Educ*. 2007;19:247–250.
725. Hanson RR, Todhunter RJ. Herniation of the abdominal wall in pregnant mares. *J Am Vet Med Assoc*. 1986;189:790–793.
726. Frazer GS, Perkins NR, Blanchard TL, et al. Prevalence of fetal maldispositions in equine referral hospital dystocias. *Equine Vet J*. 1997;29:111–116.
727. Vandeplassche M, Spincemaille J, Bouters R, et al. Some aspects of equine obstetrics. *Equine Vet J*. 1972;4:105–109.
728. Chaney KP, Holcombe SJ, LeBlanc MM, et al. The effect of uterine torsion on mare and foal survival: a retrospective study, 1985–2005. *Equine Vet J*. 2007;39:33–36.
729. Spoormakers TJ, Graat EA, ter Braake F, et al. Mare and foal survival and subsequent fertility of mares treated for uterine torsion. *Equine Vet J*. 2016;48:172–175.
730. Vandeplassche M. Selected topics in equine obstetrics. *Proc Am Assoc Equine Pract*. 1992;38:623–628.
731. Ruffin DC, Schumacher J, Comer JS. Uterine torsion associated with small intestinal incarceration in a mare at 126 days of gestation. *J Am Vet Med Assoc*. 1995;207:329–330.
732. Barber SM. Complications of chronic uterine torsion in a mare. *Can Vet J*. 1995;36:102–103.
733. Frazer GS, Perkins NR, Constable PD. Bovine uterine torsion: 164 hospital referral cases. *Theriogenology*. 1996;46:739–758.

734. Pascoe JR, Meagher DM, Wheat JD. Surgical management of uterine torsion in the mare: a review of 26 cases. *J Am Vet Med Assoc*. 1981;179:351–354.
735. Pascoe JR, Pascoe RR. Displacements, malpositions, and miscellaneous injuries of the mare's urogenital tract. *Vet Clin North Am Equine Pract*. 1988;4:439–450.
736. Perkins NR, Robertson JT, Colon LA. Uterine torsion and uterine tear in a mare. *J Am Vet Med Assoc*. 1992;201:92–94.
737. Ragle CA, Southwood LL, Galuppo LD, et al. Laparoscopic diagnosis of ischemic necrosis of the descending colon after rectal prolapse and rupture of the mesocolon in two postpartum mares. *J Am Vet Med Assoc*. 1997;210:1646–1648.
738. Bowen JM, Gaboury C, Bousquet D. Non-surgical correction of a uterine torsion in the mare. *Vet Rec*. 1976;99:495–496.
739. Wichtel JJ, Reinertson EL, Clark TL. Nonsurgical treatment of uterine torsion in seven mares. *J Am Vet Med Assoc*. 1988;193:337–338.
740. Adams SB. Rupture of the prepubic tendon in the mare. *Equine Pract*. 1979;1:17–19.
741. Doyle AJ, Freeman DE, Sauberli DS, et al. Clinical signs and treatment of chronic uterine torsion in two mares. *J Am Vet Med Assoc*. 2002;220:349–353, 323.
742. Frazer GS. Differential diagnosis for vaginal haemorrhage in the mare. *Equine Vet Educ*. 2005;17:153–155.
743. Prell M, Ellerbrock RE, Canisso IF. Hemorrhagic vulvar discharge caused by vestibule trauma in a nonpregnant Thoroughbred mare. *J Equine Vet Sci*. 2016;43:44–47.
744. White RA, Gerring EL, Jackson PG, et al. Persistent vaginal haemorrhage in five mares caused by varicose veins of the vaginal wall. *Vet Rec*. 1984;115:263–264.
745. DeLuca C, Dascanio JJ, Berry DB. Nd : YAG laser treatment of a vestibulovaginal varicosity in a 15-year-old pregnant mare. *J Equine Vet Sci*. 2007;27:217–220.
746. Schumacher J, Schumacher J, Schmitz D. Macroscopic haematuria of horses. *Equine Vet Educ*. 2002;14:201–210.
747. Laverty S, Pascoe JR, Ling GV, et al. Urolithiasis in 68 horses. *Vet Surg*. 1992;21:56–62.
748. DeBowes RM, Nyrop KA, Boulton CH. Cystic calculi in the horse. *Compend Contin Educ Pract Vet*. 1984;6:S268–S276.
749. Fischer Jr AT, Spier S, Carlson GP, et al. Neoplasia of the equine urinary bladder as a cause of hematuria. *J Am Vet Med Assoc*. 1985;186:1294–1296.
750. Brendemuehl JP. *Fescue and agalactia: pathophysiology, diagnosis and management*. San Antonio: Society for Theriogenology; 2000:25–32.
751. Cross DL, Redmond LM, Strickland JR. Equine fescue toxicosis: signs and solutions. *J Anim Sci*. 1995;73:899–908.
752. Putnam MR, Bransby DI, Schumacher J, et al. Effects of the fungal endophyte *Acremonium coenophialum* in fescue on pregnant mares and foal viability. *Am J Vet Res*. 1991;52:2071–2074.
753. Boosinger TR, Brendemuehl JP, Schumacher J, et al. Effects of short-term exposure to and removal from the fescue endophyte *Acremonium coehophialum* on pregnant mare and foal viability. *Biol Reprod Mono*. 1994;1:61–67.
754. McCann JS, Caudle AB, Thompson FN, et al. Influence of endophyte-infected tall fescue on serum prolactin and progesterone in gravid mares. *J Anim Sci*. 1992;70:217–223.
755. Ireland FA, Loch WE, Worthy K, et al. Effects of bromocriptine and perphenazine on prolactin and progesterone concentrations in pregnant pony mares during late gestation. *J Reprod Fertil*. 1991;92:179–186.
756. Ryan PL, Bennett-Wimbush K, Vaala WE, et al. Systemic relaxin in pregnant pony mares grazed on endophyte-infected fescue: effects of fluphenazine treatment. *Theriogenology*. 2001;56:471–483.
757. Cross DL, Anas K, Bridges WC, et al. Clinical effects of domperidone on fescue toxicosis in pregnant mares. *Proc Am Assoc Equine Pract*. 1999;45:203–206.
758. Evans TJ, Youngquist RS, Loch WE, et al. A comparison of the relative efficacies of domperidone and reserpine in treating equine "fescue toxicosis". *Proc Am Assoc Equine Pract*. 1999;45:207–209.
759. Redmond LM, Cross DL, Strickland JR, et al. Efficacy of domperidone and sulpiride as treatments for fescue toxicosis in horses. *Am J Vet Res*. 1994;55:722–729.
760. Paccamonti DL. Milk electrolytes and induction of parturition. *Pferdeheilkunde*. 2001;17:616–618.
761. Valera M, Blesa F, Dos Santos R, et al. Genetic study of gestation length in Andalusian and Arabian mares. *Anim Reprod Sci*. 2006;95:75–96.
762. Hodge SL, Kreider JL, Potter GD, et al. Influence of photoperiod on the pregnant and postpartum mare. *Am J Vet Res*. 1982;43:1752–1755.
763. Sevinga M, Barkema HW, Stryhn H, et al. Retained placenta in Friesian mares: incidence, and potential risk factors with special emphasis on gestational length. *Theriogenology*. 2004;61:851–859.
764. Heidler B, Aurich JE, Pohl W, et al. Body weight of mares and foals, estrous cycles and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. *Theriogenology*. 2004;61:883–893.
765. Marteniuk JV, Carleton CL, Lloyd JW, et al. Association of sex of fetus, sire, month of conception, or year of foaling with duration of gestation in standardbred mares. *J Am Vet Med Assoc*. 1998;212:1743–1745.
766. Davies Morel MC, Newcombe JR, Holland SJ. Factors affecting gestation length in the Thoroughbred mare. *Anim Reprod Sci*. 2002;74:175–185.
767. Ousey JC, Dudan F, Rossdale PD. Preliminary studies of mammary secretions in the mare to assess foetal readiness for birth. *Equine Vet J*. 1984;16:259–263.
768. Rook JS, Braselton WE, Nachreiner RF, et al. Multi-element assay of mammary secretions and sera from periparturient mares by inductively coupled argon plasma emission spectroscopy. *Am J Vet Res*. 1997;58:376–378.
769. Ley WB, Bowen JM, Purswell BJ, et al. The sensitivity, specificity and predictive value of measuring calcium carbonate in mares' prepartum mammary secretions. *Theriogenology*. 1993;40:189–198.
770. Macpherson ML. *Induction of parturition*. San Antonio: Society for Theriogenology; 2000:51–58.
771. Ousey JC, Delclaux M, Rossdale PD. Evaluation of three strip tests for measuring electrolytes in mares' pre-partum mammary secretions and for predicting parturition. *Equine Vet J*. 1989;21:196–200.
772. Camillo F, Cela M, Grassi F, et al. Day-time management of the foaling mare: use of a rapid mammary Ca++ determination followed by a low dose of oxytocin. *Int Congress Anim Reprod*. 1992:883–885.
773. Cash RS, Ousey JC, Rossdale PD. Rapid strip test method to assist management of foaling mares. *Equine Vet J*. 1985;17:61–62.
774. Brook D. Evaluation of a new test kit for estimating the foaling time in the mare. *Equine Pract*. 1987;9:34–36.
775. Newcombe JR, Nout YS. Apparent effect of management on the hour of parturition in mares. *Vet Rec*. 1998;142:221–222.
776. Canisso IF, Ball BA, Troedsson MH, et al. Decreasing pH of mammary gland secretions is associated with parturition and is correlated with electrolyte concentrations in prefoaling mares. *Vet Rec*. 2013;173:218.
777. Ellerbrock RE, Canisso IF. How to interpret pH profiles from mammary gland secretions in mares. *Proc Am Assoc Equine Pract*. 2016;62:187–192.
778. Korosue K, Murase H, Sato F, et al. Comparison of pH and refractometry index with calcium concentrations in preparturient mammary gland secretions of mares. *J Am Vet Med Assoc*. 2013;242:242–248.

779. Alm CC, Sullivan JJ, First NL. The effect of a corticosteroid (dexamethasone), progesterone, oestrogen and prostaglandin F2α on gestation length in normal and ovariectomized mares. *J Reprod Fertil Suppl*. 1975;23:637–640.
780. Hillman RB. Induction of parturition in mares. *J Reprod Fertil Suppl*. 1975;23:641–644.
781. Jeffcott LB, Rossdale PD. A critical review of current methods for induction of parturition in the mare. *Equine Vet J*. 1977;9:208–215.
782. Purvis AD. The induction of labor in mares as a routine breeding farm procedure. *Proc Am Assoc Equine Pract*. 1977;23: 145–160.
783. Ousey JC, Kolling M, Allen WR. The effects of maternal dexamethasone treatment on gestation length and foal maturation in Thoroughbred mares. *Anim Reprod Sci*. 2006;94:436–438.
784. Alm CC, Sullivan JJ, First NL. Induction of premature parturition by parenteral administration of dexamethasone in the mare. *J Am Vet Med Assoc*. 1974;165:721–722.
785. First NL, Alm CC. Dexamethasone–induced parturition in pony mares. *J Anim Sci*. 1977;44:1072–1075.
786. Rossdale PD, Pashen RL, Jeffcott LB. The use of synthetic prostaglandin analogue (fluprostenol) to induce foaling. *J Reprod Fertil Suppl*. 1979;27:521–529.
787. Pashen RL. Oxytocin: the induction agent of choice in the mare? *J Reprod Fertil Suppl*. 1982;32:645.
788. Villani M, Romano G. Induction of parturition with daily low-dose oxytocin injections in pregnant mares at term: clinical applications and limitations. *Reprod Domest Anim*. 2008;43:481–483.
789. van Niekerk FE, van Niekerk CH. The effect of dietary protein on reproduction in the mare. II. Growth of foals, body mass of mares and serum protein concentration of mares during the anovulatory, transitional and pregnant periods. *J S Afr Vet Assoc*. 1997;68:81–85.
790. van Niekerk FE, van Niekerk CH. The effect of dietary protein on reproduction in the mare. VII. Embryonic development, early embryonic death, foetal losses and their relationship with serum progestogen. *J S Afr Vet Assoc*. 1998;69:150–155.
791. van Niekerk FE, van Niekerk CH. The effect of dietary protein on reproduction in the mare. VI. Serum progestogen concentrations during pregnancy. *J S Afr Vet Assoc*. 1998;69:143–149.
792. van Niekerk FE, van Niekerk CH. The effect of dietary protein on reproduction in the mare. V. Endocrine changes and conception during the early postpartum period. *J S Afr Vet Assoc*. 1998;69:81–88.
793. Gee EK, Firth EC, Morel PC, et al. Articular / epiphyseal osteochondrosis in Thoroughbred foals at 5 months of age: influences of growth of the foal and prenatal copper supplementation of the dam. *N Z Vet J*. 2005;53:448–456.
794. Practitioners AAoE. *Vaccination guidelines*. Lexington, KY: American Association of Equine Practitioners; 2017.
795. Embertson RM, Bernard WV, Hance SR, et al. Hospital approach to dystocia in the mare. *Proc Am Assoc Equine Pract*. 1995;41:13–14.
796. Frazer GS, Perkins NR, Embertson RM. Normal parturition and evaluation of the mare in dystocia. *Equine Vet Educ*. 1999;11:41–46.
797. Vandeplassche MM. The pathogenesis of dystocia and fetal malformation in the horse. *J Reprod Fertil Suppl*. 1987;35: 547–552.
798. Jeffcott LB, Rossdale PD. A radiographic study of the fetus in late pregnancy and during foaling. *J Reprod Fertil Suppl*. 1979:563–569.
799. Ginther OJ, Williams D. On-the-farm incidence and nature of equine dystocias. *J Equine Vet Sci*. 1996;16:159–164.
800. McKinnon AO, Voss JL. *Equine Reproduction*. Philadelphia: Lea & Febiger; 1993.
801. LeBlanc MM, Norman WM. Sedation and anaesthesia of the mare during obstetrical manipulations. *Proc Am Assoc Equine Pract*. 1992;38:619–622.
802. Frazer GS, Perkins NR, Embertson RM. Correction of equine dystocia. *Equine Vet Educ*. 1999;11:48–53.
803. Walton A, Hammond J. The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proc R Soc B*. 1938;125:311–335.
804. Grubb TL, Riebold TW, Huber MJ. Comparison of lidocaine, xylazine, and xylazine/lidocaine for caudal epidural analgesia in horses. *J Am Vet Med Assoc*. 1992;201:1187–1190.
805. Norton JL, Dallap BL, Johnston JK, et al. Retrospective study of dystocia in mares at a referral hospital. *Equine Vet J*. 2007;39:37–41.
806. Taylor PM. Anaesthesia for pregnant animals. *Equine Vet J Suppl*. 1997:1–6.
807. Embertson RM. The indications and surgical techniques for caesarean section in the mare. *Equine Vet Educ*. 1992;4:31–36.
808. Byron CR, Embertson RM, Bernard WV, et al. Dystocia in a referral hospital setting: approach and results. *Equine Vet J*. 2003;35:82–85.
809. Maaskant A, de Bruijn CM, Schutrups AH, et al. Dystocia in Friesian mares: prevalence, causes and outcome following caesarean section. *Equine Vet Educ*. 2010;22:190–195.
810. Fischer AT, Phillips TN. Surgical repair of a ruptured uterus in five mares. *Equine Vet J*. 1986;18:153–155.
811. Rossdale PD. Differential diagnosis of postparturient hemorrhage in the mare. *Equine Vet Educ*. 1994;6:135–136.
812. Hooper RN, Blanchard TL, Taylor TS, et al. Identifying and treating uterine prolapse and invagination of the uterine horn. *Vet Med*. 1993;88:60–65.
813. Frazer GS. Fetotomy technique in the mare. *Equine Vet Educ*. 2001;13:195–203.
814. Frazer GS. Review of the use of fetotomy to resolve dystocia in the mare. *Proc Am Assoc Equine Pract*. 1997;43:262–268.
815. Bierschwal CJ, deBois C. *The Technique of Fetotomy in Large Animals*. Bonner Springs: VM Publishing; 1972.
816. Carluccio A, Contri A, Tosi U, et al. Survival rate and short-term fertility rate associated with the use of fetotomy for resolution of dystocia in mares: 72 cases (1991-2005). *J Am Vet Med Assoc*. 2007;230:1502–1505.
817. Nimmo MR, Slone Jr DE, Hughes FE, et al. Fertility and complications after fetotomy in 20 brood mares (2001-2006). *Vet Surg*. 2007;36:771–774.
818. Schambourg MA, Laverty S, Mullim S, et al. Thoracic trauma in foals: post mortem findings. *Equine Vet J*. 2003;35:78–81.
819. Baldwin JL, Cooper WL, Vanderwall DK. Dystocia due to anterior presentation with unilateral or bilateral hip flexion posture ("dog-sitting" presentation) in the mare: incidence, management and outcomes. *Proc Am Assoc Equine Pract*. 1991;37:229–241.
820. Aanes WA. Surgical management of foaling injuries. *Vet Clin North Am Equine Pract*. 1988;4:417–438.
821. Auer JA, Stick JA. *Equine Surgery*. 3rd ed. St. Louis: Saunders; 2006.
822. Hodder AD, Ball BA. Theriogenology question of the month. Fetal hydrocephalus. *J Am Vet Med Assoc*. 2008;232:211–213.
823. Hausberger M, Henry S, Larose C, et al. First suckling: a crucial event for mother-young attachment? an experimental study in horses (*Equus caballus*). *J Comp Psychol*. 2007;121:109–112.
824. Gunduz MC, Kasikci G, Kaya HH. The effect of oxytocin and PGF2α on the uterine involution and pregnancy rates in postpartum Arabian mares. *Anim Reprod Sci*. 2008;104:257–263.
825. Dart AJ, Pascoe JR, Snyder JR. Mesenteric tears of the descending (small) colon as a postpartum complication in two mares. *J Am Vet Med Assoc*. 1991;199:1612–1615.
826. Dwyer R. Postpartum deaths of mares. *Equine Dis Q*. 1993;2:5.
827. Livesey MA, Keller SD. Segmental ischemic necrosis following mesocolic rupture in postparturient mares. *Compend Contin Educ Pract Vet*. 1986;8. 763-&.
828. Getty R. *Sisson and Grossman's The Anatomy of the Domestic Animals*. 5th ed. Philadelphia: Saunders; 1975.

829. Lofstedt R. Haemorrhage associated with pregnancy and parturition. *Equine Vet Educ*. 1994;6:138–141.
830. Pascoe RR. Rupture of the utero-ovarian or middle uterine artery in the mare at or near parturition. *Vet Rec*. 1979;104:77.
831. Rooney JR. Internal hemorrhage related to gestation in the mare. *Cornell Vet*. 1964;54:11–17.
832. Arnold CE, Payne M, Thompson JA, et al. Periparturient hemorrhage in mares: 73 cases (1998-2005). *J Am Vet Med Assoc*. 2008;232:1345–1351.
833. Stowe HD. Effects of age and impending parturition upon serum copper of thoroughbred mares. *J Nutr*. 1968;95:179–183.
834. Shideler RK, Squires EL, Trotter G, et al. Uterine hematoma in a mare. *J Equine Vet Sci*. 1990;10:187-&.
835. Wenzel JGW, Caudle AB, White NA. Treating for uterine intramural hematoma in a horse. *Vet Med*. 1985;80:6669.
836. Pycock JF. Uterine haematoma in 2 mares. *Equine Vet Educ*. 1994;6:132–134.
837. Causey R, Ruksznis D, Miles R. Field management of equine uterine prolapse in a Thoroughbred mare. *Equine Vet Educ*. 2007;19:254–259.
838. Mogg TD, Hart J, Wearn J. Postpartum hemoperitoneum and septic peritonitis in a Thoroughbred mare. *Vet Clin North Am Equine Pract*. 2006;22:61–71.
839. Dascanio JJ, Ball BA, Hendrickson DA. Uterine tear without a corresponding placental lesion in a mare. *J Am Vet Med Assoc*. 1993;202:419–420.
840. Hooper RN, Schumacher J, Taylor TS, et al. Diagnosing and treating uterine ruptures in mares. *Vet Med*. 1993;88:263–270.
841. Hassel DM, Ragle CA. Laparoscopic diagnosis and conservative treatment of uterine tear in a mare. *J Am Vet Med Assoc*. 1994;205:1531–1533.
842. Blanchard TL, Bierschwal CJ, Youngquist RS, et al. Sequelae to percutaneous fetotomy in the mare. *J Am Vet Med Assoc*. 1983;182:1127.
843. Vandeplassche M, Bouters R, Spincemaille J, et al. Observations on involution and puerperal endometritis in mares. *Ir Vet J*. 1983;37:126–132.
844. Provencher R, Threlfall WR, Murdick PW, et al. Retained fetal membranes in the mare: a retrospective study. *Can Vet J*. 1988;29:903–910.
845. Sevinga M, Barkema HW, Hesselink JW. Serum calcium and magnesium concentrations and the use of a calcium-magnesium-borogluconate solution in the treatment of Friesian mares with retained placenta. *Theriogenology*. 2002;57:941–947.
846. Welle MM, Audige L, Belz JP. The equine endometrial mast cell during the puerperal period: evaluation of mast cell numbers and types in comparison to other inflammatory changes. *Vet Pathol*. 1997;34:23–30.
847. Sevinga M, Vrijenhoek T, Hesselinks JW, et al. Effect of inbreeding on the incidence of retained placenta in Friesian horses. *J Anim Sci*. 2004;82:982–986.
848. Blanchard TL, Varner DD. Therapy for retained placenta in the mare. *Vet Med*. 1993;88:55–59.
849. Sertich PL. Periparturient emergencies. *Vet Clin North Am Equine Pract*. 1994;10:19–36.
850. Blanchard TL, Elmore RG, Kinden DA, et al. Effect of intrauterine infusion of Escherichia coli endotoxin in postpartum pony mares. *Am J Vet Res*. 1985;46:2157–2162.
851. Burns SJ, Judge NG, Martin JE, et al. Management of retained placenta in mares. *Proc Am Assoc Equine Pract*. 1977;23:381–390.
852. Martin KL, Hoffman RM, Kronfeld DS, et al. Calcium decreases and parathyroid hormone increases in serum of periparturient mares. *J Anim Sci*. 1996;74:834–839.
853. Sevinga M, Hesselink JW, Barkema HW. Reproductive performance of Friesian mares after retained placenta and manual removal of the placenta. *Theriogenology*. 2002;57:923–930.
854. Haffner JC, Fecteau KA, Held JP, et al. Equine retained placenta: technique for and tolerance to umbilical artery injections of collagenase. *Theriogenology*. 1998;49:711–716.
855. Fecteau KA, Haffner JC, Eiler H. The potential of collagenase as a new therapy for separation of human retained placenta: hydrolytic potency on human, equine and bovine placentae. *Placenta*. 1998;19:379–383.
856. Blanchard TL, Varner DD, Scrutchfield WL, et al. Management of dystocia in mares: retained placenta, metritis, and laminitis. *Compend Contin Educ Pract Vet*. 1990;12:563–569.
857. Lock TF. Antibiotic distribution in tissues of the reproductive tract of the mare. *J Am Vet Med Assoc*. 1981;179. 255–255.
858. Meijer M, Macpherson ML, Dijkman R. How to use umbilical vessel water infusion to treat retained fetal membranes in mares. *Proc Am Assoc Equine Pract*. 2015;61:478–484.
859. Turner TA, Fessler JF. Rectal prolapse in the horse. *J Am Vet Med Assoc*. 1980;177:1028–1032.
860. Jacobs KA, Barber SM, Leach DH. Disruption of the blood supply to the small colon following rectal prolapse and small colon intussusception in a mare. *Can Vet J*. 1982;23:132–134.
861. Blythman WG. Rectal prolapse in a foaling mare. *Vet Rec*. 1988;122:471–472.
862. Hance SR, Embertson RM. Colopexy in broodmares: 44 cases (1986-1990). *J Am Vet Med Assoc*. 1992;201:782–787.
863. Embertson RM, Cook G, Hance SR, et al. Large colon volvulus: surgical treatment in 204 horses (1986-1995). *Proc Am Assoc Equine Pract*. 1996;42:254–255.
864. Dart AJ, Pascoe JR. Mesenteric tear of the distal jejunum as a periparturient complication in a mare. *Aust Vet J*. 1994;71:427–428.
865. Edwards GB. A review of 38 cases of small colon obstruction in the horse. *Equine Vet J Suppl*. 1992;13:42–50.
866. Dart AJ, Hodgson DR, Snyder JR. Caecal disease in equids. *Aust Vet J*. 1997;75:552–557.
867. Donelan E, Sloss V. Two cases of rupture of the large intestine in the mare associated with unassisted parturition. *Aust Vet J*. 1972;48:413–414.
868. Voss JL. Rupture of the cecum and ventral colon of mares during parturition. *J Am Vet Med Assoc*. 1969;155:745–747.
869. Platt H. Caecal rupture in parturient mares. *J Comp Pathol*. 1983;93:343–346.
870. Auer DE, Wilson RG, Groenendyk S, et al. Diaphragmatic rupture in a mare at parturition. *Equine Vet J*. 1985;17:331–333.
871. Bristol DG. Diaphragmatic hernias in horses and cattle. *Comp Cont Educ Pract Vet*. 1986;8:S407–S412.
872. Hance SR, Clem MF, Debowes RM, et al. Intraabdominal hernias in horses. *Comp Cont Educ Pract Vet*. 1991;13:293299.
873. Hartzband LE, Kerr DV, Morris EA. Ultrasonographic diagnosis of diaphragmatic rupture in a horse. *Vet Radiol*. 1990;31:42–44.
874. Tulleners EP, Richardson DW, Reid BV. Vaginal evisceration of the small intestine in three mares. *J Am Vet Med Assoc*. 1985;186:385–387.
875. Singh P, Bugalia NS. Surgical management of a third degree perineal laceration and eversion of the bladder in a mare. *Vet Rec*. 2001;148:786–787.
876. Mansmann R, McAllister E, Pratt P. *Equine Medicine and Surgery*. Santa Barbara, CA: American Veterinary Publications; 1981.
877. Nyrop KA, Debowes RM, Cox JH, et al. Rupture of the urinary bladder in 2 postparturient mares. *Comp Cont Educ Pract Vet*. 1984;6:S510–S513.
878. Jones PA, Sertich PS, Johnston JK. Uroperitoneum associated with ruptured urinary bladder in a postpartum mare. *Aust Vet J*. 1996;74:354–358.
879. Rodgerson DH, Spirito MA, Thorpe PE, et al. Standing surgical repair of cystorrhexis in two mares. *Vet Surg*. 1999;28:113–116.
880. Kasikci G, Horoz H, Alkan S, et al. A modified surgical technique for repairing third-degree perineal lacerations in mares. *Acta Vet Hung*. 2005;53:257–264.

881. Kay AT, Spirito MA, Rodgerson DH, et al. Surgical technique to repair grade IV rectal tears in post-parturient mares. *Vet Surg*. 2008;37:345–349.
882. Welland LM. Transmural rectal intestinal evisceration associated with parturition in a primiparous mare. *Can Vet J*. 2003;44:740–742.
883. Squires EL, Wilson JM, Kato H, et al. A pregnancy after intracytoplasmic sperm injection into equine oocyte matured in vitro. *Theriogenology*. 1996;45:306.
884. Cochran R, Meintjes M, Reggio B, et al. Live foals produced from sperm-injected oocytes derived from pregnant mares. *J Equine Vet Sci*. 1998;18:736–740.
885. McKinnon AO, Lacham-Kaplan O, Trounson AO. Pregnancies produced from fertile and infertile stallions by intracytoplasmic sperm injection (ICSI) of single frozen-thawed spermatozoa into in vivo matured mare oocytes. *J Reprod Fertil Suppl*. 2000;56:513–517.
886. Vogelsang MM, Kraemer DC, Bowen MJ, et al. Recovery of equine follicular oocytes by surgical and nonsurgical techniques. *Theriogenology*. 1986;25:208.
887. Hinrichs K, Kenney RM. A colpotomy procedure to increase oocyte recovery rates on aspiration of equine preovulatory follicles. *Theriogenology*. 1987;27:237–237.
888. Hinrichs K, Kenney DF, Kenney RM. Aspiration of oocytes from mature and immature preovulatory follicles in the mare. *Theriogenology*. 1990;34:107–112.
889. Palmer E, Duchamp G, Bezard J, et al. Recovery of follicular fluid and oocytes of mares by nonsurgical puncture of the preovulatory follicle. *Theriogenology*. 1986;25:178.
890. Cook NL, Squires EL, Ray BS, et al. Transvaginal ultrasound-guided follicular aspiration of equine oocytes. *Equine Vet J*. 1993;25:71–74.
891. Carnevale EM, Ginther OJ. Use of a linear ultrasonic transducer for the transvaginal aspiration and transfer of oocytes in the mare. *J Equine Vet Sci*. 1993;13:331–333.
892. Carnevale EM, Squires EL, Maclellan LJ, et al. Use of oocyte transfer in a commercial breeding program for mares with reproductive abnormalities. *J Am Vet Med Assoc*. 2001;218:87–91, 37.
893. McKinnon AO, Carnevale EM, Squires EL, et al. Heterogeneous and xenogeneous fertilization of in vivo matured equine oocytes. *J Equine Vet Sci*. 1993;8:143–147.
894. Hinrichs K, Provost PJ, Torello EM. Treatments resulting in pregnancy in nonovulating, hormone-treated oocyte recipient mares. *Theriogenology*. 2000;54:1285–1293.
895. Carnevale EM, Bergfelt DR, Ginther OJ. Follicular activity and concentrations of FSH and LH associated with senescence in mares. *Anim Reprod Sci*. 1994;35:231–246.
896. Scott MA, Liu IKM, Overstreet JW. Sperm transport to the oviducts: abnormalities and their clinical implications. *Proc Am Assoc Equine Pract*. 1995;41:1–2.
897. Tsutsumi Y, Suzuki H, Takeda T, et al. Evidence of the origin of the gelatinous masses in the oviducts of mares. *J Reprod Fertil*. 1979;57:287–290.
898. Hinrichs K, Betschart RW, McCue PM, et al. Effect of time of follicle aspiration on pregnancy rate after oocyte transfer in the mare. *J Reprod Fertil Suppl*. 2000;56:493–498.
899. Carnevale EM, Maclellan LJ, da Silva MAC, et al. Comparison of culture and insemination techniques for equine oocyte transfer. *Theriogenology*. 2000;54:981–987.
900. Coutinho da Silva MA, Carnevale EM, Maclellan LJ. Injection of blood into preovulatory follicles of equine oocyte transfer recipients does not prevent fertilization of the recipient's oocyte. *Theriogenology*. 2002;57:538.
901. Carnevale EM, Alvarenga MA, Squires EL, et al. *Use of noncycling mares as recipients for oocyte transfer and GIFT*. Nashville, TN: Annual Conference of the Society for Theriogenology; 1999:44.
902. Carnevale EM, Checura CH, Coutinho da Silva MA, et al. Use of deslorelin acetate to suppress follicular activity in mares used as recipients for oocyte transfer. *Theriogenology*. 2001;55:358.
903. Squires EL, Seidel GE. Collection and transfer of equine embryos In: 08 ARaBLBN, ed. Fort Collins, CO: Colorado State University, 1995.
904. Carnevale EM, Maclellan LJ, da Silva MAC, et al. Equine sperm-oocyte interaction: results after intraoviductal and intrauterine inseminations of recipients for oocyte transfer. *Anim Reprod Sci*. 2001;68:305–314.
905. Scott TJ, Carnevale EM, Maclellan LJ, et al. Embryo development rates after transfer of oocytes matured in vivo, in vitro, or within oviducts of mares. *Theriogenology*. 2001;55:705–715.
906. Maclellan LJ, Carnevale EM, da Silva MAC, et al. Pregnancies from vitrified equine oocytes collected from super-stimulated and non-stimulated mares. *Theriogenology*. 2002;58:911–919.
907. Palmer E, Bezard J, Magistrini M, et al. In vitro fertilization in the horse. A retrospective study. *J Reprod Fertil Suppl*. 1991;44:375–384.
908. Franz LC, Squires EL, O'Donovan MK, et al. Collection and in vitro maturation of equine oocytes from estrus, diestrus and pregnant mares. *J Equine Vet Sci*. 2001;21:26–32.
909. Bezard J. In vitro fertilization in the mare. In: *International Scientific Conference on biotechnics in Horse Reproduction*. Crakow, Poland; 1992:12.
910. Colleoni S, Barbacini S, Necchi D, et al. Application of ovum pick-up, intracytoplasmic sperm injection and embryo culture in equine practice. *Proc Am Assoc Equine Pract*. 2007;53:554–559.
911. Carnevale EM, Maclellan LJ, da Silva MAC, et al. Pregnancies attained after collection and transfer of oocytes from ovaries of five euthanatized mares. *J Am Vet Med Assoc*. 2003;222:60–62.
912. Carnevale EM, Coutinho da Silva MA, Preis KA, et al. Establishment of pregnancies from oocytes collected from the ovaries of euthanized mares. *Proc Am Assoc Equine Pract*. 2004;50:531–533.
913. Yamamoto Y, Oguri N, Tsutsumi Y, et al. Experiments in the freezing and storage of equine embryos. *J Reprod Fertil Suppl*. 1982;32:399–403.
914. Seidel Jr GE. Cryopreservation of equine embryos. *Vet Clin North Am Equine Pract*. 1996;12:85–99.
915. Slade NP, Takeda T, Squires EL, et al. A new procedure for the cryopreservation of equine embryos. *Theriogenology*. 1985;24:45–58.
916. Lascombes FA, Pashen RL. Results from embryo freezing and post ovulation breeding in a commercial embryo transfer programme. *Havemeyer Found Monogr Ser No 3: Equine Embryo Transfer*; 2000.
917. Eldridge-Panuska WD, di Brienza VC, Seidel Jr GE, et al. Establishment of pregnancies after serial dilution or direct transfer by vitrified equine embryos. *Theriogenology*. 2005;63:1308–1319.
918. Carnevale EM, Eldridge-Panuska WD, Caracciolo di Brienza V. How to collect and vitrify equine embryos for direct transfer. *Proc Am Assoc Equine Pract*. 2004;50:402–405.
919. Hudson J, McCue PM, Carnevale EM, et al. The effects of cooling and vitrification of embryos from mares treated with equine follicle-stimulating hormone on pregnancy rates after nonsurgical transfer. *J Equine Vet Sci*. 2006;26:51–54.
920. Choi YH, Hinrichs K. Vitrification of in vitro-produced and in vivo-recovered equine blastocysts in a clinical program. *Theriogenology*. 2017;87:48–54.
921. Hochi S, Fujimoto T, Choi YH, et al. Cryopreservation of equine oocytes by 2-step freezing. *Theriogenology*. 1994;42:1085–1094.
922. Vatja G. Vitrification of oocytes and embryos of domestic animals. *Anim Reprod Sci*. 2000;60–61:357.
923. Householder DD, Pickett BW, Voss JL, et al. Effect of extender, number of spermatozoa and HCG on equine fertility. *J Equine Vet Sci*. 1981;1:9–13.

924. Katila T, Sankari S, Makela O. Transport of spermatozoa in the reproductive tracts of mares. *J Reprod Fertil Suppl*. 2000;56:571–578.
925. Rigby S, Derczo S, Brinsko SP, et al. Oviductal sperm numbers following proximal uterine horn or uterine body insemination. *Proc Am Assoc Equine Pract*. 2000;46:332–334.
926. Senger PL, Becker WC, Davidge ST, et al. Influence of cornual insemination on conception rates in dairy cattle. *J Anim Sci*. 1988;66:3010–3016.
927. Seidel GE, Allen CH, Johnson LA, et al. Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa. *Theriogenology*. 1997;48:1255–1264.
928. Brinsko SP, Rigby SL, Lindsey AC, et al. Pregnancy rates in mares following hysteroscopic or transrectally-guided insemination with low sperm numbers at the utero-tubal papilla. *Theriogenology*. 2003;59:1001–1009.
929. Buchanan BR, Seidel GE, McCue PM, et al. Insemination of mares with low numbers of either unsexed or sexed spermatozoa. *Theriogenology*. 2000;53:1333–1344.
930. Woods J, Rigby S, Brinsko S, et al. Effect of intrauterine treatment with prostaglandin E-2 prior to insemination of mares in the uterine horn or body. *Theriogenology*. 2000;53:1827–1836.
931. Morris LHA, Hunter RHF, Allen WR. Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares. *J Reprod Fertil*. 2000;118:95–100.
932. Lindsey AC, Bruemmer JE, Squires EL. Low dose insemination of mares using non-sorted and sex-sorted sperm. *Anim Reprod Sci*. 2001;68:279–289.
933. Leao KM, Alvarenga MA, Puolli-Filho JN. Hysteroscopic insemination in mares with low sperm number. *Theriogenology*. 2002;57:381.
934. Morris LHA, Allen WR. Hysteroscopic uterotubal insemination of mares with low numbers of spermatozoa. *Anim Reprod Sci*. 2001;68:330.
935. Alvarenga MA, Leao KM. Hysteroscopic insemination of mares with low number of frozen thawed spermatozoa selected by percoll gradient. *Theriogenology*. 2002;58:651–653.
936. Alvarenga MA, Trinque CC, Lima MM. Utilization of hysteroscopy for the application of stallion frozen semen in commercial programs. *REv Bras Reprod Anim*. 2001;25:361.
937. Coutinho da Silva MA, Carnevale EM, Maclellan LJ. Embryo development rates after oocyte transfer comparing intrauterine or intraoviductal insemination and fresh or frozen semen in mares. *Theriogenology*. 2001;55:359.
938. Coutinho da Silva MA, Carnevale EM, Maclellan LJ. Oocyte transfer in mares with intrauterine or intraoviductal insemination using fresh, cooled, and frozen stallion semen. *Theriogenology*. 2004;61:705.
939. Amann R. Functional anatomy of the adult male. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. West Sussex: Wiley-Blackwell; 2011.
940. Swierstra EE, Gebauer MR, Pickett BW. Reproductive physiology of the stallion. I. Spermatogenesis and testis composition. *J Reprod Fertil*. 1974;40:113–123.
941. Johnson L, Thompson Jr DL, Varner DD. Role of Sertoli cell number and function on regulation of spermatogenesis. *Anim Reprod Sci*. 2008;105:23–51.
942. Johnson L, Thompson Jr DL. Seasonal variation in the total volume of Leydig cells in stallions is explained by variation in cell number rather than cell size. *Biol Reprod*. 1986;35:971–979.
943. Whaley PD, Chaudhary J, Cupp A, et al. Role of specific response elements of the c-fos promoter and involvement of intermediate transcription factor(s) in the induction of Sertoli cell differentiation (transferrin promoter activation) by the testicular paracrine factor PModS. *Endocrinology*. 1995;136:3046–3053.
944. Johnson L, Tatum ME. Temporal appearance of seasonal changes in numbers of Sertoli cells, Leydig cells, and germ cells in stallions. *Biol Reprod*. 1989;40:994–999.
945. Knobil E, Neill JD. *The Physiology of Reproduction*. New York: Raven Press; 1994.
946. Goyal HO. Morphology of the bovine epididymis. *Am J Anat*. 1985;172:155–172.
947. Amann RP. Function of the epididymis in bulls and rams. *J Reprod Fertil Suppl*. 1987;34:115–131.
948. Crabo B. Studies on the Composition of Epididymal Content in Bulls and Boars. *Acta Vet Scand*. 1965;22(suppl 5):1–94.
949. Little TV, Holyoak GR. Reproductive anatomy and physiology of the stallion. *Vet Clin North Am Equine Pract*. 1992;8:1–29.
950. Gebauer MR, Pickett BW, Faulkner LC, et al. Reproductive physiology of the stallion. VII. Chemical characteristics of seminal plasma and spermatozoa. *J Anim Sci*. 1976;43:626–632.
951. Lindholmer C. The importance of seminal plasma for human sperm motility. *Biol Reprod*. 1974;10:533–542.
952. Aurich JE, Kuhne A, Hoppe H, et al. Seminal plasma affects membrane integrity and motility of equine spermatozoa after cryopreservation. *Theriogenology*. 1996;46:791–797.
953. Pozor AM, McDonnell SM. Ultrasound evaluation of stallion accessory sex glands. *Annu Meeting Soc Theriogenology*. 1996.
954. Johnson L, Amann RP, Pickett BW. Maturation of equine epididymal spermatozoa. *Am J Vet Res*. 1980;41:1190–1196.
955. DiNapoli L, Capel B. SRY and the standoff in sex determination. *Mol Endocrinol*. 2008;22:1–9.
956. Sekido R, Lovell-Badge R. Sex determination and SRY: down to a wink and a nudge? *Trends Genet*. 2009;25:19–29.
957. Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature*. 2008;453:930–934.
958. Svingen T, Koopman P. Building the mammalian testis: origins, differentiation, and assembly of the component cell populations. *Genes Dev*. 2013;27:2409–2426.
959. Eggers S, Ohnesorg T, Sinclair A. Genetic regulation of mammalian gonad development. *Nat Rev Endocrinol*. 2014;10:673–683.
960. Kim Y, Kobayashi A, Sekido R, et al. Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol*. 2006;4:e187.
961. Colvin JS, Green RP, Schmahl J, et al. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. *Cell*. 2001;104:875–889.
962. Kim Y, Bingham N, Sekido R, et al. Fibroblast growth factor receptor 2 regulates proliferation and Sertoli differentiation during male sex determination. *Proc Natl Acad Sci U S A*. 2007;104:16558–16563.
963. Jameson SA, Lin YT, Capel B. Testis development requires the repression of Wnt4 by Fgf signaling. *Dev Biol*. 2012;370:24–32.
964. Wilhelm D, Martinson F, Bradford S, et al. Sertoli cell differentiation is induced both cell-autonomously and through prostaglandin signaling during mammalian sex determination. *Dev Biol*. 2005;287:111–124.
965. Levy JB, Husmann DA. The hormonal control of testicular descent. *J Androl*. 1995;16:459–463.
966. Johnson AD, Gomes WR, VanDemark NL. *The Testis*. New York: Academic Press; 1970.
967. Claes AN, Ball BA, Almeida J, et al. Detection of serum anti-Mullerian hormone concentrations as a method for diagnosis of cryptorchidism in the horse. *Proc Am Assoc Equine Pract*. 2011;57:56.
968. Claes A, Ball BA, Almeida J, et al. Serum anti-Mullerian hormone concentrations in stallions: developmental changes, seasonal variation, and differences between intact stallions, cryptorchid stallions, and geldings. *Theriogenology*. 2013;79:1229–1235.
969. Johnson L, Varner DD, Thompson Jr DL. Effect of age and season on the establishment of spermatogenesis in the horse. *J Reprod Fertil Suppl*. 1991;44:87–97.

970. Samper JC. *Equine Breeding Management and Artificial Insemination*. 2nd ed. St. Louis: Saunders; 2009.
971. Amann RP. The cycle of the seminiferous epithelium in humans: a need to revisit? *J Androl*. 2008;29:469–487.
972. Cupps PT. *Reprod Domest Anim*. 5th ed. New York: Academic Press; 1991.
973. Pickett BW, Voss JL. Management of shuttle stallions for maximum reproductive efficiency - Part 1. *J Equine Vet Sci*. 1998;18:212–224. 226-227.
974. Pickett BW, Voss JL, Clay CM. Management of shuttle stallions for maximum reproductive efficiency - Part 2. *J Equine Vet Sci*. 1998;18:280–287.
975. Clay CM, Squires EL, Amann RP, et al. Influences of season and artificial photoperiod on stallions - luteinizing hormone, follicle stimulating hormone, and testosterone. *J Anim Sci*. 1988;66:1246–1255.
976. Burns PJ, Jawad MJ, Weld JM, et al. Effects of season, age and increased photoperiod on reproductive hormone concentrations and testicular diameters in thoroughbred stallions. *J Equine Vet Sci*. 1987;4:202–208.
977. Clay CM, Squires EL, Amann RP, et al. Influences of season and artificial photoperiod on stallions - testicular size, seminal characteristics, and sexual behavior. *J Anim Sci*. 1987;64:517–525.
978. McDonnell SM. Normal and abnormal sexual behavior. *Vet Clin N Am-Equine Pract*. 1992;8:71–89.
979. Love CC. The stallion breeding soundness evaluation: revisited. *Soc Theriogenology Annu Conf*; 2011.
980. Love CC, Garcia MC, Riera FR, et al. Evaluation of measures taken by ultrasonography and caliper to estimate testicular volume and predict daily sperm output in the stallion. *J Reprod Fertil*. 1991:99–105.
981. Blanchard TL, Brinsko SP, Love CC, et al. How to use testicular measurements for first-season subfertility insurance considerations in Thoroughbred stallions. *Proc Am Assoc Equine Pract*. 2008;54:374–379.
982. Love CC. Ultrasonographic evaluation of the testis, epididymis, and spermatic cord of the stallion. *Vet Clin North Am Equine Pract*. 1992;8:167–182.
983. Pozor M. Diagnostic applications of ultrasonography to stallion's reproductive tract. *Theriogenology*. 2005;64:505–509.
984. Pozor MA. Evaluation of testicular vasculature in stallions. *Clin Tech Equine Pract*. 2007;6:271–277.
985. Pozor MA, McDonnell SM. Color Doppler ultrasound evaluation of testicular blood flow in stallions. *Theriogenology*. 2004;61:799–810.
986. Little TV, Woods GL. Ultrasonography of accessory sex glands in the stallion. *J Reprod Fertil Suppl*. 1987;35:87–94.
987. Sullins KE, Traub-Dargatz JL. Endoscopic anatomy of the equine urinary tract. *Comp Contin Educ Pract Vet*. 1984;6:S663–S668.
988. Tibary A. Endoscopy of the reproductive tract in the stallion. In: Samper JC, Pycock JF, McKinnon AO, eds. *Current Therapy in Equine Reproduction*. St. Louis: Elsevier; 2007: 214–219.
989. Mancill SS. Clinical and sub-clinical seminal vesiculitis in the stallion. *Equine Vet Educ*. 2010;22:220–222.
990. Tischner M, Kosiniak K. Techniques for collection and storage of stallion semen with minimal secondary contamination. *Acta Vet Scand*. 1992;88:83–90.
991. McDonnell SM. Ejaculation: physiology and dysfunction. *Vet Clin N Am-Equine Pract*. 1992;8:57–70.
992. McDonnell SM, Odian MJ. Imipramine and xylazine-induced ex copula ejaculation in stallions. *Theriogenology*. 1994;41:1005–1010.
993. Turner RMO, Mcdonnell SM, Hawkins JF. Use of pharmacologically induced ejaculation to obtain semen from a stallion with a fractured radius. *J Am Vet Med Assoc*. 1995;206:1906–1908.
994. Blanchard TL, Varner DD, Brinsko SP, et al. Azoospermia in stallions: determining the cause. *Compend Contin Educ Vet*. 2012;34:E2.
995. McDonnell SM, Garcia MC, Kenney RM, et al. Imipramine-induced erection, masturbation, and ejaculation in male horses. *Pharmacol Biochem Behav*. 1987;27:187–191.
996. Jarrige R, Martin-Rosset W. *Le Cheval. Reproduction, Selkection, Alimentation*. Paris: INRA; 1984.
997. Hansen C, Vermeiden T, Vermeiden JP, et al. Comparison of FACSCount AF system, Improved Neubauer hemocytometer, Corning 254 photometer, SpermVision, UltiMate and NucleoCounter SP-100 for determination of sperm concentration of boar semen. *Theriogenology*. 2006;66:2188–2194.
998. Amann RP. Computerized evaluation of stallion spermatozoa. *Proc Am Assoc Equine Pract*. 1987;32:453–477.
999. Pickett BW, Voss JL, Bowen RA, et al. Seminal characteristics and total scrotal width (TSW) of normal and abnormal stallions. *Proc Am Assoc Equine Pract*. 1987;33:487–518.
1000. USDA-APHIS. *Contagious equine metritis*. Riverdale, MD: USDA-APHIS; 2014.
1001. Raudsepp T, Mccue ME, Das PJ, et al. Genome-wide association study implicates testis-sperm specific FKBP6 as a susceptibility locus for impaired acrosome reaction in stallions. *PloS Genetics*. 2012;8:e1003139.
1002. Harris MA, Anderson C, Webel S. Effect of feeding an omega-3 rich supplement on the fatty acid composition and motion characteristics of stallion spermatozoa. *19th Equine Science Society Symposium*; 2005.
1003. Gibb Z, Lambourne SR, Aitken RJ. The paradoxical relationship between stallion fertility and oxidative stress. *Biol Reprod*. 2014;91:77.
1004. Halliwell B, Gutteridge J. *Free Radicals in Biology and Medicine*. 5th ed. New York: Oxford University Press; 2015.
1005. Taylor MJ, Evans JW, Householder DD, et al. Reproductive parameters of breeding stallions in response to a moderate physical conditioning program. *Proc Fifteenth Equine Nutr Physiol Symp*. 1997:104–109.
1006. AAoE Practitioners. *AAEP guidelines for breeding a mare to an equine arteritis virus-shedding stallion*. Lexington, KY: AAEP; 2017.
1007. Kenney RM, Cooper WL. Therapeutic use of a phantom for semen collection from a stallion. *J Am Vet Med Assoc*. 1974;165:706–707.
1008. Varner DD. Handling the breeding stallion. *Proc Am Assoc Equine Pract*. 2005;51:498–505.
1009. McDonnell SM. Reproductive behavior of stallions and mares: comparison of free-running and domestic in-hand breeding. *Anim Reprod Sci*. 2000;60:211–219.
1010. Love CC. Semen collection techniques. *Vet Clin N Am-Equine Pract*. 1992;8:111–128.
1011. Love CC, Riera FL, Oristaglio RM. Sperm occluded (plugged) ampullae in the stallion. *Soc Theriogenology*. 1992:117–125.
1012. McDonnell SM. Practical review of self-mutilation in horses. *Anim Reprod Sci*. 2008;107:219–228.
1013. Luescher UA, McKeown DB, Dean H. A cross-sectional study on compulsive behaviour (stable vices) in horses. *Equine Vet J Suppl*. 1998;27:14–18.
1014. McDonnell SM, Turner RM, Diehl NK. Modifying unruly breeding behavior in stallions. *Compend Contin Educ Vet*. 1995;17:411–417.
1015. Ocal G. Current concepts in disorders of sexual development. *J Clin Res Pediatr Endocrinol*. 2011;3:105–114.
1016. Ono M, Harley VR. Disorders of sex development: new genes, new concepts. *Nat Rev Endocrinol*. 2013;9:79–91.
1017. Frandson RD, Epling GP, Davis RW. A case report: arrested testicular development in the horse. *J Am Vet Med Assoc*. 1960;137:255–257.
1018. Estrada A, Samper JC, Lillich JD, et al. Azoospermia associated with bilateral segmental aplasia of the ductus deferens in a stallion. *J Am Vet Med Assoc*. 2003;222:1740–1742, 1707.
1019. Sinclair AH, Berta P, Palmer MS, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*. 1990;346:240–244.

1020. Blanchard TL, Woods JA, Brinsko SP. Theriogenology question of the month. Azoospermia attributable to bilateral epididymal hypoplasia. *J Am Vet Med Assoc*. 2000;217:825–826.
1021. Pearson H, Weaver BMQ. Priapism after sedation, neuroleptoanalgesia and anesthesia in the horse. *Equine Vet J*. 1978;10:85–90.
1022. Klug E, Deegen E, Lazarz B, et al. Effect of adrenergic neurotransmitters upon the ejaculatory process in the stallion. *J Reprod Fertil Suppl*. 1982;32:31–34.
1023. Brinsko SP, Blanchard TL, Varner DD. How to treat paraphimosis. *Proc Am Assoc Equine Pract*. 2007;53:580–582.
1024. Koch C, O'Brien T, Livesey MA. How to construct and apply a penile repulsion device (probang) to manage paraphimosis. *Proc Am Assoc Equine Pract*. 2009;55:338–341.
1025. Sharrock AG. Reversal of drug-induced priapism in a gelding by medication. *Aust Vet J*. 1982;58:39–40.
1026. Lucke JN, Sansom J. Penile erection in the horse after acepromazine. *Vet Rec*. 1979;105:21–22.
1027. Elce YA. The aetiopathogenesis of squamous cell carcinomas in horses. Where are we? *Equine Veterinary Education*. 2009;21:17–18.
1028. Smith MA, Levine DG, Getman LM, et al. Vulvar squamous cell carcinoma in situ within viral papillomas in an aged Quarter Horse mare. *Equine Vet Educ*. 2009;21:11–16.
1029. Chambers G, Ellsmore VA, O'Brien PM, et al. Association of bovine papillomavirus with the equine sarcoid. *J Gen Virol*. 2003;84:1055–1062.
1030. Hoyumpa AH, McIntosh AL, Varner DD, et al. Normal bacterial flora of equine semen: antibacterial effects of amikacin, penicillin, and an amikacin-penicillin combination in seminal extender. *Int Congress Anim Reprod*. 1992:1427–1429.
1031. Bowen JM, Tobin N, Simpson RB, et al. Effects of washing on the bacterial flora of the stallion's penis. *J Reprod Fertil Suppl*. 1982;32:41–45.
1032. Blanchard TL, Bretzlaff KN. Identifying, treating and preventing scrotal skin disorders of large animals. *Vet Med*. 1990;85:290–294.
1033. McKinnon AO. Some selected diseases of the breeding stallion. Rio Cuarto: Congreso Argentino de Reproduccion Equina 3; 2013:53–63.
1034. Brinsko SP. Neoplasia of the male reproductive tract. *Vet Clin North Am Equine Pract*. 1998;14:517–533.
1035. Theon AP. Intralesional and topical chemotherapy and immunotherapy. *Vet Clin North Am Equine Pract*. 1998;14:659–671, viii.
1036. Earnshaw RE. Polyorchidism. *Can J Comp Med Vet Sci*. 1959;23:66.
1037. Perkins NR, Frazer GS, Threlfall WR. Testicular prosthesis in a Quarterhorse stallion: a case report. *Theriogenology*. 1996;45:535–540.
1038. Stickle RL, Fessler JF. Retrospective study of 350 cases of equine cryptorchidism. *J Am Vet Med Assoc*. 1978;172:343–346.
1039. Cox JE, Edwards GB, Neal PA. An analysis of 500 cases of equine cryptorchidism. *Equine Vet J*. 1979;11:113–116.
1040. Claes A, Ball BA, Corbin CJ, et al. Anti-Mullerian hormone as a diagnostic marker for equine cryptorchidism in three cases with equivocal testosterone concentrations. *J Equine Vet Sci*. 2014;34:442–445.
1041. Murase H, Saito S, Amaya T, et al. Anti-Mullerian hormone as an indicator of hemi-castrated unilateral cryptorchid horses. *J Equine Sci*. 2015;26:15–20.
1042. Brendemuehl JP. Effects of repeated hCG administration on serum testosterone and testicular descent in prepubertal thoroughbred colts with cryptorchid testicles. *Proc Am Assoc Equine Pract*. 2008;52:381–383.
1043. Stout TAE. Modulating reproductive activity in stallions: a review. *Anim Reprod Sci*. 2005;89:93–103.
1044. Roser JE. Regulation of testicular function in the stallion: an intricate network of endocrine, paracrine and autocrine systems. *Anim Reprod Sci*. 2008;107:179–196.
1045. Turner RMO. Pathogenesis, diagnosis, and management of testicular degeneration in stallions. *Clin Tech Equine Pract*. 2007;6:278–284.
1046. Boyle M. Immune related infertility in the stallion? *Equine Vet J*. 1990;22:67–69.
1047. Pugh RCB. *Pathology of the Testis*. Oxford: Blackwell; 1976.
1048. McEntee M. *Reproductive Pathology of Domestic Animals*. San Diego: Academic Press; 2012.
1049. Smith JA. The occurrence of larvae of *Strongylus edentatus* in the testicles of stallions. *Vet Rec*. 1973;93:604–606.
1050. Tung KSK. Pathogenesis of autoimmune orchitis. *Annu Meeting Soc Theriogenology*; 1991.
1051. Kaufman DG, Nagler HM. Specific nonsurgical therapy in male infertility. *Urol Clin North Am*. 1987;14:489–498.
1052. Thompson Jr DL, Pickett BW, Squires EL, et al. Sexual behavior, seminal pH and accessory sex gland weights in geldings administered testosterone and(or) estradiol-17 β. *J Anim Sci*. 1980;51:1358–1366.
1053. Held JP, Prater P, Toal RL, et al. Sperm granuloma in a stallion. *J Am Vet Med Assoc*. 1989;194:267–268.
1054. Schneider RK, Milne DW, Kohn CW. Acquired inguinal hernia in the horse: a review of 27 cases. *J Am Vet Med Assoc*. 1982;180:317–320.
1055. Gerona GR, Sikes JD. Effects of elevated scrotum temperature on spermatogenesis and semen characteristics. *J Dairy Sci*. 1970;53:659.
1056. Threlfall WR, Carleton CL, Robertson J, et al. Recurrent torsion of the spermatic cord and scrotal testis in a stallion. *J Am Vet Med Assoc*. 1990;196:1641–1643.
1057. Evers JL, Collins JA. Assessment of efficacy of varicocele repair for male subfertility: a systematic review. *Lancet*. 2003;361:1849–1852.
1058. Razi M, Malekinejad H. Varicocele-induced infertility in animal models. *Int J Fertil Steril*. 2015;9:141–149.
1059. Knobbe M, Levine D, Habecker P, et al. Prostatic masses in geldings: two cases. *J Equine Vet Sci*. 2012;32:628–633.
1060. Brinsko SP. Retrograde ejaculation in a stallion. *J Am Vet Med Assoc*. 2001;218:551–553.
1061. Moller G, Azevedo LR, Trein CR, et al. Effects of hemospermia on seminal quality. *Anim Reprod Sci*. 2005;89:264–267.
1062. Pearson LK, Campbell AJ, Tibary A. How to diagnose and treat hemospermia: a review and case series. *Proc Am Assoc Equine Pract*. 2013;59:40–50.
1063. Sullins KE, Bertone JJ, Voss JL, et al. Treatment of hemospermia in stallions: a discussion of 18 cases. *Compend Contin Educ Pract Vet*. 1988;10:13961403.
1064. Erdman MM, Creekmore LH, Fox PE, et al. Diagnostic and epidemiologic analysis of the 2008-2010 investigation of a multi-year outbreak of contagious equine metritis in the United States. *Preventive Veterinary Medicine*. 2011;101:219–228.
1065. Barrandeguy M, Vissani A, Olguin C, et al. Experimental reactivation of equine herpesvirus-3 following corticosteroid treatment. *Equine Vet J*. 2008;40:593–595.
1066. Cerny KL, Little TV, Scoggin CF, et al. Presence of bacteria on the external genitalia of healthy stallions and its transmission to the mare at the time of breeding by live cover. *J Equine Vet Sci*. 2014;34:369–374.

CHAPTER 20

Disorders of Foals

Harold C. McKenzie III

EQUINE NEONATOLOGY

Tremendous advances have been made in the medical care of newborn foals over the past 50 years, thanks to the pioneering efforts of many individuals and research groups and the increasing sophistication of veterinary medicine as a whole. Although the field of human neonatology has provided critically important guidance regarding the physiology and pathophysiology of the neonatal foal, and will continue to do so, fundamental insights have been achieved into the unique characteristics of this special population. The foundations of equine neonatology were built on the earliest descriptions of specific conditions in foals by clinician researchers, such as septicemia, joint ill, and hemolytic icterus,[1-4] as well as the "dummy foal" syndrome and the respiratory distress syndrome (RDS) associated with primary surfactant deficiency.[5,6] Significant strides were also being made in the understanding of the physiology of the equine fetus and neonate, in large part because of the efforts of a group of researchers working in the UK.[5-23] As the knowledge base grew dramatically,[24-29] there was increasing interest in providing improved care for the equine neonate, in large part because of the substantial investments of time, energy, and equity involved in the lengthy process of conception, gestation, parturition, and juvenile development. Owners were very interested in optimizing foal survival, and this interest, along with the increasing capability for providing effective critical care for foals, led to the development of neonatal intensive care units (ICUs) worldwide in the 1980s. Since that time there has been a steady increase in our understanding and ability to successfully treat and manage the critically ill foal. The result has been a dramatic improvement not only in the likelihood of survival but also the ability of surviving foals to mature into sound, healthy individuals that are able to perform their intended function.[30] Our knowledge base in equine neonatology continues to grow, and the goal of this chapter is to provide an overview of the current knowledge in this field as well as to provide some insights derived from human medicine.

FETAL ASSESSMENT, MONITORING, AND MANAGEMENT

The efforts of owners, managers, and veterinarians to maximize equine reproductive success extend far beyond successful conception. Close attention must be paid to a variety of influences before, during, and after pregnancy. These influences include numerous maternal, placental, and fetal risk factors. By assessing these variables and monitoring for changes during the course of pregnancy, it may be possible to implement medical therapies to minimize some risks, or at least to ensure that management strategies are in place to reduce the risk of problems during or after delivery.

Regarding maternal risk factors one must begin with a detailed assessment of the mare's reproductive history including endometrial health before breeding; problems during conception; vaccination history; possibility of twinning; previous gestational length; number of previous foals; the number of successful deliveries; history of and types of dystocia; and the occurrence of any problems with previous foals after birth, such as neonatal isoerythrolysis.[31] In addition, a number of maternal factors during pregnancy should be assessed, including maternal under- or overnutrition, maternal illnesses (especially colic or other severe stressors), and endocrine or metabolic maternal abnormalities. Physical abnormalities, such as abdominal wall muscular tears or prepubic tendon rupture, represent serious risks to both the dam and the fetus. Uterine torsion represents a life-threatening situation for the mare and the fetus, and if correction is successful the fetus must be regarded at high risk for the duration of the pregnancy.

Although it can be very difficult to appreciate placental risk factors, this is a critical issue given the complete dependence of the fetus on the dam for energy and oxygen supplies. Conditions such as endometriosis or twinning can reduce the available surface area for placental attachment, resulting in impaired transfer of nutrients and waste products between the fetus and the dam and potentially causing intrauterine growth retardation (IUGR). Placental dysfunction can result from abnormalities such as placental detachment, placentitis, placental edema, hydrops, and vascular inflammation or injury.[31] Exposure of the mare to any possible infectious causes of abortion or maternal ingestion of endophyte-infested fescue also can represent serious risk factors to the fetus.[32,33] Although there are often no outward signs of placental abnormalities, the development of excessive abdominal distention, vulvar discharge, or premature udder development may suggest their presence. A number of fetal factors can complicate pregnancy as well, including fetal malformations (congenital or developmental), fetal malpositioning, twinning, exposure to infectious agents, and neonatal isoerythrolysis.

If any potential risk factors are identified then the pregnancy should be characterized as high risk, requiring assessment of

the mare, the placenta, and the fetus. Evaluation of the mare should include a thorough physical examination, paying particular attention to the external genitalia and the udder. The abdomen should be evaluated for signs of excessive enlargement, ventral edema, or a pendulous appearance. Evidence of systemic illness (fever, tachycardia, tachypnea, abnormal mucous membranes, etc.) will serve as an indication for additional diagnostic evaluation to ascertain the focus and severity of the inflammation. A complete blood count and serum chemistry profile may be helpful in that regard. Evaluation of the reproductive tract should include a rectal examination, if feasible, as well as a vaginal examination and transrectal and transabdominal ultrasound examinations. These will allow for detection of abnormalities such as cervical discharge, uterine torsion, hydrops, and prepubic tendon rupture.

Ultrasound examination will provide the opportunity to thoroughly evaluate the placenta to assess placental attachment, placental thickness, and the echogenicity of the fetal fluids.[34-36] The transabdominal approach allows for prolonged periods of examination and allows for a thorough assessment of the uterus, placenta, and fetus.[37] After 4 months' gestation both transabdominal and transrectal ultrasonographic examinations should be performed to ensure thorough assessment. After 6 months of gestation transrectal ultrasonography can also be used to regularly monitor variables such as fetal heart rate, eye diameter, and fetal activity.[38] Although transrectal ultrasound examination does not allow visualization of all fetal structures, it is very useful during late gestation, because the caudal uterine body is the most common site for ascending placentitis to develop.[35] Measurement of the combined thickness of the uterus and placenta (CTUP) is a useful indicator of placental abnormalities, but this assessment should only be performed in areas of the placenta not in contact with the fetus. Although the CTUP can be measured transabdominally, this approach is more challenging because of the variations in the location measured as well as the potential for fetal contact compressing the placenta.[39] It is preferred to measure the CTUP transrectally at a site near the cervicoplacental junction, at which a branch of the uterine can be visualized between the uterus and the bladder, using the average of several measurements.[36] Normal measurements of the CTUP have been reported to be 0.6 cm at 7 months' gestation, less than 0.8 cm at 9 months, less than 1.0 cm at 10 months, and up to 1.0 to 1.2 cm at the end of gestation.[36,40] It is important to remember that even though an increased CTUP may suggest placental abnormalities it does not represent a definitive finding. There are reports of increased CTUP measurements that were not associated with placental pathology, and conversely ascending placentitis is not always associated with an increase in CTUP.[35,36,41] Although there may also be breed and parity influences on CTUP measurements, the 1.2-cm upper limit represents a reasonable cutoff for most Light Horse and Warmblood breeds.[42] Placental detachment may be associated with accumulation of exudate between the placental and uterine surfaces, which is typically hyperechoic in ultrasonographic appearance.

In addition to CTUP there are a number of other ultrasonographic parameters that can be used for assessment and monitoring of the high-risk fetus. Fetal viability can be assessed using fetal heart rate and rhythm, as well as fetal activity patterns and fetal responses to stimulation. Fetal heart rates change throughout gestation, trending from a high of 135 ± 6 beats per minute at 91 to 120 days' gestation to 67 ± 12 beats per minute at 330 to 360 days' gestation.[43] Transient alterations in fetal heart rate may be observed but do not likely indicate fetal distress unless they persist. Persistent tachycardia or bradycardia may be an indication of impending fetal death.[44,45] Fetal heart rate should increase during periods of fetal activity and return to normal ranges thereafter. Fetal electrocardiography can be used for monitoring fetal heart rate and rhythm as well and was widely used several decades ago but has fallen out of favor because of the ready availability, ease of use, and diagnostic yield associated with ultrasonographic fetal assessment. A recent report described the use of telemetric electrocardiography equipment for monitoring fetal heart rate and fetal heart rate variability, and this tool may hold some promise for fetal assessment in the future.[46] A biophysical profile based on ultrasonographic fetal assessment has been developed for use in late gestation, and this includes fetal heart rate, fetal activity, fetal aortic diameter, CTUP, uteroplacental contact, and maximal allantoic fluid depth.[43,45,47]

Once the determination has been made that the pregnancy is high risk, and particularly if there are uteroplacental or fetal abnormalities indicative of the risk or presence of fetal compromise, the next challenge is if, when, and how to intervene appropriately. Because of the very late maturation of the equine fetus in the last 1 to 2 weeks of gestation, combined with the difficulty of determining fetal readiness for birth, it is rarely advisable to induce delivery. It is far better in most situations to attempt to support the fetus in utero as best as possible while awaiting natural delivery.[48] If there are complications that threaten the survival of the dam, then cesarean section or induction of parturition may be indicated, although the survival of the foal should be considered highly unlikely. The other situation in which intervention may be indicated is if the fetus is thought to be near term and the mare suffers from a condition such as severe colic that is endangering fetal survival, although the prognosis for foal survival again remains poor.

Medical management of the high-risk pregnancy has two goals: the first is treatment of the primary condition, if possible, and the second is treatment intended to support the fetus in utero and maintain the pregnancy. The primary condition most commonly encountered in the mare is placentitis, and specific treatment consists of antimicrobial therapy and antiinflammatory therapy. Antimicrobial therapy, although typically empiric in nature, should ideally rely on drugs that are capable of reaching therapeutic concentrations within the uterus. Trimethoprim-sulfamethoxazole, penicillin, and gentamicin have all been shown to reach appropriate levels within the allantoic fluid, but trimethoprim-sulfamethoxazole is most commonly used because of the ease of oral administration and low cost.[49,50] Ceftiofur crystalline-free acid (CCFA), although appealing from the perspective of ease of administration and expense, did not achieve therapeutic concentrations within the placenta or fetal fluids and was not effective in an experimental model of bacterial placentitis.[51]

Antiinflammatory therapy in most cases consists of flunixin meglumine and pentoxifylline, although other agents such as aspirin and corticosteroids have been investigated. Although the efficacy of antiinflammatory therapy in placentitis is uncertain, they are commonly used. Flunixin meglumine is the most common agent used, because it is a potent nonsteroidal antiinflammatory drug (NSAID), but it is not clear that it reaches therapeutic concentrations within the placenta or uterus.[52] Other antiinflammatory drugs have been of interest,

although one study did not find any benefit to the addition of an antiinflammatory (dexamethasone and aspirin) to trimethoprim-sulfamethoxazole therapy in experimentally induced bacterial placentitis.[53] In addition to the potential antiinflammatory effects of corticosteroids, their use has been of interest because of the widespread use of this treatment to accelerate fetal maturation in human patients.[54] The administration of corticosteroids to mares in late gestation was shown to accelerate fetal maturation in one experimental study, but there are no reports regarding the use of this therapeutic approach in clinical practice.[55]

Treatments that may be beneficial in supporting gestation include exogenous progestagens, tocolytics, and agents promoting oxygen delivery to the fetus. Progestagen supplementation may have a multitude of effects, including antiprostaglandin effects, but primarily it is thought to function as a tocolytic. This is typically accomplished using altrenogest, a synthetic progestin, although injectable progesterone is sometimes used.[43] Although the use of these agents has been somewhat controversial, altrenogest therapy has shown some indications of efficacy in reducing fetal losses from bacterial placentitis.[56-58] Clenbuterol, a β_2-sympathomimetic drug, has been shown to induce uterine relaxation during late gestation but is short-acting, with only 2 hours' duration of effect.[52] For this reason clenbuterol is most useful in the acute situation, such as in the management of dystocia while preparing for cesarean section or assisted delivery.[59]

Treatments that may improve fetal oxygen delivery in utero and during an assisted delivery include pentoxifylline and intranasal oxygen administration to the mare. In addition to its antiinflammatory effects, pentoxifylline has rheologic effects that may enhance oxygen delivery to the placenta by improving microcirculation.[60] Intranasal oxygen administration (10–15 L/min) to the pregnant mare will increase the partial pressure of oxygen and oxygen saturation in the arterial blood supplying the placenta and may improve oxygen delivery to the fetus.[30] Vitamin E has been used in the management of high-risk pregnancy as an antioxidant and is administered orally to the pregnant mare.[30]

At this time the combination of trimethoprim-sulfamethoxazole, flunixin meglumine, and pentoxifylline remain the most commonly used treatment protocol for the medical management of bacterial placentitis in the mare. Generally, when managing mares with high-risk pregnancies it is critical that a well-formed plan is developed for dealing not only with parturition but also the high-risk foal after delivery, if it survives. Ideally these mares will be housed in a facility that is able to provide 24-hour monitoring and that has a team available to deal with any eventualities appropriately and in a timely manner. All necessary equipment needs to be organized and readily available. Thorough discussions with owners and agents need to occur before any crises so that all involved know the priorities regarding what procedures are authorized and whether the mare's life or the foal's life is the focus of the teams' efforts.

EXAMINATION OF THE NEWBORN FOAL

When assessing an equine neonate, it is important to remember that the first minutes and hours after birth are a profound adaptive period and that the normal parameters used in assessment are continually changing during this time. All of the foal's body systems are transitioning from the protected intrauterine environment in which many of their functions were primarily performed by the mare via the placenta, to the external environment in which these systems assume full responsibility for maintaining homeostasis. The time required for this transition varies by body system, and the adaptations required of the cardiopulmonary system are the most time critical. At birth the lungs must expand and clear themselves of fluid for gas exchange to occur, and this process must be coordinated with a shift in blood flow into the pulmonary circulation. If all elements of this transition do not occur within a few minutes, the foal will not be able to survive in the external environment. The adaptations required of the nervous, musculoskeletal, endocrine, alimentary, and thermoregulatory systems are similarly critical but occur over a longer period of time following birth.

There are a number of endocrine changes occurring in the periparturient period, and these changes are critical in readying a number of body systems, such as the lungs, gastrointestinal tract, kidneys, and liver, for the functions they will be responsible for following birth.[61] The most critical of these appears to be the activation of the hypothalamic-pituitary-adrenal (HPA) axis. This process begins in the period starting approximately 5 days before birth, when the fetal cortisol concentration rapidly begins to rise.[17] This increase prepares the body for many of the processes that may have little or no role in utero but are critical for survival after birth, such as respiration, renal sodium conservation, and glucose metabolism.[62] Serum cortisol concentrations continue to rise in the first few hours after birth before decreasing to normal basal values by approximately 1 day of age in healthy foals.[63] Very premature foals (<320 days' gestation) have low cortisol concentrations at birth, and these do not increase appropriately in the first 2 hours of life.[64] In addition to changes in the HPA axis there are also increases in circulating catecholamines, insulin, and glucagon, as well as thyroid hormones (T_3, T_4) associated with the immediate postpartum period.[62]

The neurologic system of the equine fetus is fully developed and functional because the precocious nature of foals at birth requires the immediate ability to ambulate and function independently.[65] The fetus is maintained in a sleeping state in utero through a combination of inhibitory factors, which include physical factors such as warmth, cushioned tactile stimulation, and buoyancy, as well as chemical factors, which include high circulating and/or cerebral concentrations of adenosine, prostaglandin D_2, and the pregnane neurosteroids allopregnanolone and pregnanolone.[65,66] These inhibitory neurosteroids are synthesized from progesterone primarily within the placenta during late gestation, and following delivery their concentrations within the normal neonate decline rapidly, usually over the first 48 hours of life.[67,68] This loss of cerebral inhibition, in combination with potent stimulation arising from transiting the birth canal, the onset of breathing, and the onset of multiple external stimuli (light, cold, hard surfaces, unlimited space, gravity, etc.), lead to a rapid increase in activity and awareness in the neonate.[66]

As the foal passes through the birth canal the chest undergoes significant compression, which aids in shifting fluid out of the airways. Once the lungs begin to inflate the action of surfactant contributes to further clearance of airway fluid and the maintenance of alveolar stability and lung inflation. Before birth the fetal circulation functionally bypasses the pulmonary

circulation by shunting through the ductus arteriosus and the foramen ovale. Once the foal begins to breathe the resistance in the pulmonary vasculature dramatically decreases, allowing blood from the pulmonary artery to enter the pulmonary vasculature rather than passing through the ductus arteriosus into the aorta. As the pressure in the left side of the heart increases, the foramen ovale closes, preventing the shunting of blood from the right atrium to the left atrium. Complete closure of the ductus arteriosus may take several days, and it is not unusual to hear a "machinery"-type murmur associated with patency of the ductus arteriosus in foals during the first 1 to 3 days of life. It is rare for the foramen ovale to remain patent in foals.

The foal's initial respirations primarily occur in response to the rapid rise of carbon dioxide in the bloodstream following the loss of placental gas exchange. The loss of placentally derived prostaglandins that suppress breathing in utero likely contributes as well, along with the initiation of cold and tactile stimuli associated with the external environment.[69] Spontaneous breathing may begin as soon as the chest clears the birth canal and should begin within 1 minute of birth. The first few breaths may be quite exaggerated in nature because of high carbon dioxide levels and the degree of effort required for initial lung expansion. Following these initial breaths most foals will continue to exhibit elevated respiratory rates of 50 to 75 breaths per minute for the first 20 to 30 minutes of life. The respiratory rate will then decrease to around 30 to 40 breaths per minute, and this remains fairly stable for the remainder of the first 2 days of life. By 2 to 3 days of age most foals will exhibit respiratory rates of approximately 20 breaths per minute. Foals show a much more exaggerated respiratory pattern than adult horses, with both inspiration and expiration requiring active respiratory effort because of the foal's highly compliant thoracic wall.

Immediately following birth the foal will typically exhibit bradycardia, with heart rates of 60 to 80 beats per minute.[8] The heart rate rapidly increases, peaking at 150 to 175 beats per minute around 40 to 60 minutes after birth, with the highest heart rates often observed in association with the foal's first attempts to stand.[70] The heart rate then gradually decreases, with a resting rate of 120 to 150 beats per minute from 1 to 2 hours after birth followed by a rate of 100 to 120 beats per minute by 3 hours after birth. By 1 day of age the normal resting heart rate will be in the range of 80 to 100 beats per minute. Because of the relatively late maturation of the HPA axis in the fetal foal, the cardiovascular system at birth does not appear to be as fully developed in the horse compared with other species.[71] As a result the neonatal foal does not have well-developed baroreceptor responses and is at risk for hypotension, especially if premature.[71]

The mare will begin interacting with the foal shortly after parturition, using both vocal and tactile stimuli. The normal foal should be responsive to stimulation shortly after birth, and by 20 to 30 minutes of age the foal should respond to visual stimuli by appropriate ocular and head movements.[72] Within 40 minutes the foal should be responding to auditory stimuli with independent orientation of the ears in the direction of the stimulus.[72] Because of the risks associated with prolonged recumbency for foals born in a natural environment, there is a strong imperative for foals to be able to stand quickly and become ambulatory. The normal foal will move into sternal recumbency within a few minutes after birth. It will begin making efforts to stand within 30 minutes after birth, and should be able to stand unassisted within 60 to 120 minutes.[73] There can be breed differences in the time to stand, with pony foals standing in less than 60 minutes and larger breed foals, such as Thoroughbreds, typically requiring 30 to 60 minutes longer.[9] When first standing the foal will exhibit some degree of incoordination and dysmetria and will assume a braced position, with all limbs spread laterally, the forelimbs positioned forward, and the hindlimbs extended posteriorly.[72] Once standing the normal foal will rapidly gain coordination and will begin circling the mare and attempting bursts of speed within 1 to 2 hours.[74]

Some foals will display flexor tendon laxity at birth, primarily noted in the form of dropped fetlocks.[75] Most foals with flexor tendon laxity will only demonstrate mild to moderate abnormalities, typically consisting of rocking back onto the heels and caudal hoof wall, which leads to pastern hyperextension and upward flipping of the toe.[76] Affected foals usually recover rapidly, with increasing strength and activity over the first few days of life.[77] Rarely, foals may demonstrate more dramatic musculoskeletal abnormalities, such as a "windswept" conformation, with either the front or hindlimbs affected by matching angular limb deformities. One limb will have a valgus deformity, whereas the opposite limb will exhibit a matching varus deformity. Unless there are underlying bony abnormalities most of these foals will also self-correct with time and activity.

The normal foal will begin displaying suckling behavior shortly after birth, even while still recumbent, and should be able to nurse unassisted within 1 hour of standing. Foals requiring more than 3 hours to suckle are considered abnormal. Successful nursing presents several challenges to the newborn foal, beginning with the absolute requirement for successful standing and ambulation to access the udder. Very weak foals, or those suffering from musculoskeletal or neurologic problems, will have great difficulty in nursing for this reason. Once able to stand successfully, the foal must locate the udder, which likely requires a combination of visual and olfactory abilities on the part of the foal. The ingestion of milk is a fairly complex neurologic process, beginning with successful capture of the teat by the lips, followed by coordination of the tongue, pharynx, larynx, epiglottis, and esophagus to ensure that milk enters the gastrointestinal tract rather than the respiratory tract. In addition to the potential adverse impact of muscular weakness or neurologic dysfunction on this process there may be physical abnormalities, such as cleft palate or subepiglottic cysts that may interfere with the normal passage of milk.

The gastrointestinal tract must undergo a number of changes to accommodate and use ingested colostrum and milk. During the first 12 to 24 hours after birth the small intestine remains permeable to macromolecules, most importantly immunoglobulins, allowing for the absorption of ingested antibodies contained in colostrum. Absorption occurs by a number of mechanisms, including pinocytosis, lymphatic transport, and exocytosis. The period of maximal absorption is the first 12 hours after birth, with approximately 50% of ingested immunoglobulins able to be absorbed during that time period.[78] From 12 to 18 hours after birth the efficiency of absorption decreases substantially, with only 28% of ingested immunoglobulins able to be absorbed, and this rate of absorption is not adversely affected by the prior ingestion of macromolecules.[78] Although some absorption may be possible after 18 hours, the efficiency of absorption continues to decrease and absorption is essentially absent by 36 hours of age.

Following the loss of maternally derived glucose supplied via the placenta, the foal must take control of glucose homeostasis. This is particularly challenging because nutrient intake transitions from a continuous mode, in which the dam ensures a continuous, regulated supply of glucose at all times, to intermittent energy intake based on milk ingestion. The pancreas must assume responsibility for the maintenance of normoglycemia through the differential production of glucagon and insulin. These dramatic alterations in energy metabolism may not always occur smoothly, and unfortunately the foal possesses limited energy reserves in the form of glycogen and fat. The result is that hypoglycemia occurs over the first 1 to 2 hours of life even in the normal neonatal foal, and the sick foal is at risk of profound hypoglycemia if deprived of energy intake for even a few hours. The metabolic response of the foal is to increase production of glucagon, epinephrine, and cortisol and suppress insulin production, increasing circulating blood glucose concentrations. Glycogenolysis provides a ready source of glucose, but hepatic glycogen stores are minimal in the foal, so gluconeogenesis rapidly becomes the primary route of endogenous glucose production. Premature or dysmature foals often have impaired glucose regulation and are susceptible to pathologic hypoglycemia in the absence of appropriate dietary or parenteral supplementation.

At birth the foal transitions from the normally sterile intrauterine environment into an environment teeming with nonpathogenic and pathogenic organisms. Colonization of the various tissues exposed to the outside environment occurs rapidly, beginning during the birthing process. This phenomenon is particularly important in the gastrointestinal tract, in which microbial activity is critical for full functionality of the local immune system and digestion. Colonization occurs through environmental exposure and is likely facilitated by the coprophagic behavior routinely exhibited by foals. Colostral immunoglobulins and oligosaccharides may play a role in regulating the composition of the gastrointestinal flora by affecting the ability of certain bacteria to attach to the mucosal surface and by opsonizing certain bacteria, facilitating the mucosal immune response to these organisms.[79] By 2 to 4 weeks of age the microbial population has become similar in composition and complexity to that of the foal's dam, which indicates a transition to an adult pattern in readiness for the dietary transition from a milk-based diet to a roughage-based diet.[80,81]

Thermoregulation can be a challenge for the neonatal foal as it transitions from the uterine environment into what is typically a much colder external environment. This challenge is increased by the presence of an initially wet hair coat, which enhances thermal losses to the environment as well as a large surface area to body mass ratio and minimal stores of fat. The healthy foal has a high resting metabolic rate compared with the adult, and in the immediate neonatal period the metabolic rate will be increased at two to three times the basal rate, in association with high concentrations of T_3, T_4, and cortisol.[82] This high metabolic rate allows for substantial endogenous heat production to achieve and maintain normothermia. This metabolic heat production requires substantial inputs of energy in the form of fats and carbohydrates derived from colostrum and milk, reinforcing the critical need for early successful nursing in foals. Another important mechanism that foals rely on for heat production in the immediate neonatal period is shivering thermogenesis. This appears to be particularly important, because foals do not appear to use nonshivering thermogenesis because of minimal or nonexistent stores of brown fat.[83] Foals also use behavioral approaches to thermoregulation, ranging from maintenance of sternal recumbency to minimize heat losses to vigorous activity when attempting to stand and once ambulatory, which generates substantial additional heat.

PREMATURITY, DYSMATURITY, AND INTRAUTERINE GROWTH RETARDATION

Abnormalities of gestation are associated with substantial risk for affected foals, both as a primary syndrome and as a risk factor for a number of other syndromes. Unfortunately, these abnormalities of gestation, which have also been termed *unreadiness for birth*, can be difficult to characterize because of the highly variable duration of equine gestation.[84] In humans the generally accepted definition of prematurity is birth more than 21 days before full term. This concept has been applied to horses as well, with delivery before 320 days' gestation considered premature.[85] Unfortunately gestational duration in the horse is much less consistent than in humans, with reported gestational lengths resulting in live foals ranging from 286 to 380 days (mean 344 days) in Thoroughbred mares and from 313 to 370 days (mean 339 days) in Warmblood mares.[86-88] Pony breeds typically have similar gestational lengths, ranging from 337 to 343 days.[89] Although there are anecdotal reports of draft mares routinely having gestational lengths over 365 days in duration, one recent report suggests that median duration of gestation in heavy horses is only 5 days longer than in light breeds.[90] In addition to the influence of breed there are a number of other variables that impact the duration of gestation including fetal gender; fetal weight; maternal age and parity; and environmental factors such as month of conception or foaling, maternal nutrition, and climate.[88]

Because of the difficulty in defining a specific time point that would be indicative of fetal maturity, equine prematurity should be considered as much a physical and functional characterization as a temporal one. The foal's size and physical appearance are important characteristics related to prematurity. Small body size and low birth weight, along with a prominent rounded forehead, silky hair coat, entropion, and floppy ears, are all common findings in premature foals. Some affected foals may have a prominent reddish discoloration of the tongue, and the mucous membranes may appear somewhat pale. Flexor and periarticular laxity are also common, although some premature foals may exhibit carpal or fetlock contracture. Incomplete ossification of the cuboidal bones in the carpus and tarsus is also a common finding in premature foals, and these areas are at risk of crushing injury when affected foals stand and ambulate. This risk can be minimized by limiting the amount of time that these foals are allowed to stand and by minimizing their activity level.

From a functional perspective premature foals typically exhibit generalized weakness and have great difficulty in standing without assistance. Affected foals often have impaired thermoregulation and abnormal glucose metabolism, as well as impaired cardiovascular, pulmonary, gastrointestinal, and renal function. Impaired thermoregulation is likely caused by inadequate endocrine responses to the change from the uterine environment to the external environment. Abnormal glucose regulation is also indicative

of endocrine dysfunction, potentially caused by impaired insulin production or peripheral insulin insensitivity. Cardiovascular dysfunction most often presents as persistent hypotension that is poorly responsive to pressor therapy. Increased vascular permeability may be present as well, potentially resulting in fluid shifts from the vasculature to the interstitium. For these reasons fluid, inotrope, and pressor support should target the maintenance of perfusion, rather than a target blood pressure range. Failure to use this approach will put the patient at risk of fluid overload and other complications. Respiratory dysfunction may be caused by surfactant deficiency but is also frequently associated with decreased respiratory drive, weak muscles of respiration, a highly compliant chest wall, and poorly compliant lungs. Gastrointestinal dysfunction is typically caused by inadequate motility, which can result in distention, colic, and diarrhea. Renal dysfunction may manifest primarily as decreased urine output rather than azotemia, and care must be taken to avoid fluid overload in these patients.

Clinicopathologic assessment may aid in characterizing these foals. Leukopenia caused by neutropenia combined with lymphocytosis leads to a neutrophil:lymphocyte ratio (N:L ratio) of less than 1.0, as opposed to a normal ratio of greater than 2.0. This reversal of the N:L ratio is a commonly used defining characteristic of equine prematurity and is directly associated with impaired adrenocortical function.[91] This impairment of adrenal function happens because maturation of the HPA axis normally occurs in the last several days of gestation and continues in the first weeks, and perhaps even months, after birth.[64,92-95] As a result, premature foals exhibit low serum cortisol concentrations at birth, in combination with elevated serum adrenocorticotropic hormone (ACTH) concentrations.[92] The inability of premature foals to produce appropriate concentrations of cortisol is caused by an impaired adrenal response to endogenous ACTH, and they exhibit impaired response to exogenous ACTH as well. Assessment of the response to exogenous ACTH may be helpful in characterizing foals as premature, although there is a subset of clinically ill full-term foals that may also exhibit impaired responses to ACTH stimulation testing.[96-98] Although not specific for prematurity, other clinicopathologic changes may include arterial hypoxemia and hypercapnia; hypoglycemia; acidosis; and low-grade macrocytic, normochromic anemia.

Dysmaturity typically refers to foals that are delivered at what appears to be full term but exhibit the characteristics of premature foals discussed earlier. Dysmaturity is most common secondary to placental insufficiency resulting in IUGR.[99] Placental insufficiency can be caused by placental disease, placental separation, or twinning. Other causes of IUGR may include maternal undernutrition or systemic disease. Postmaturity should be distinguished from dysmaturity, and refers to foals born postterm. These foals have been in utero for an excessive length of time, most commonly secondary to maternal ingestion of fescue infected with the endophyte fungus *Acremonium coenophialum*.[32] Physical characteristics associated with postmaturity include a normal to high birth weight associated with a large frame size but with poor body condition. Affected foals may also display flexor contracture, a long hair coat, and fully erupted incisors. They may share a number of other functional characteristics with premature foals, including impaired thermoregulation and abnormal glucose metabolism, as well as impaired gastrointestinal and renal function.

The severity of the abnormalities associated with prematurity are to some extent associated with the duration of the predisposing disease process that led to premature delivery. Foals that have suffered prolonged in utero stress caused by placental disease may be surprisingly "ready for birth" because of stimulation of endogenous cortisol production and early maturation of the endocrine system.[100] In rare cases such foals may survive with aggressive medical intervention even if born as early as 286 days of gestational age, based on the author's personal experience. Foals that have not experienced in utero stress and that suffer from some acute onset condition resulting in premature delivery are much more likely to be "unready" for birth and will exhibit the most dramatic clinical abnormalities. These foals are unlikely to survive if born more than 2 weeks before their expected due date.

Most premature and dysmature foals will require medical intervention and supportive care to survive. These conditions affect all body systems, so thorough evaluation is indicated. Because of substantial crossover in the clinical presentation of these foals with that of the term, septic foal it can be difficult to discriminate among syndromes. Given that premature and dysmature foals are also at heightened risk of sepsis the clinician should reasonably assume that bacterial infection is present at presentation in most cases. Appropriate broad-spectrum antimicrobial therapy is indicated. Specific recommendations for the treatment and support of the critically ill foal will be discussed in detail in the relevant sections of this chapter.

The prognosis for survival of foals suffering from prematurity, postmaturity, and dysmaturity is good with appropriate care. Survival rates can be as high as 80% to 85%, and many of these foals will "catch up" with their peers and attain a normal adult size and normal function. It is important, however, for the owners of foals born profoundly premature (less than 300 days' gestation) to understand that if they survive to adulthood these foals will not likely reach a normal adult size and morphology, and they are unlikely to be able to function as athletes.

Prevention

The best approach for preventing abnormalities of gestation is close monitoring of the pregnancy to identify any conditions that may affect fetal viability or that may lead to premature delivery. Early treatment of placental infections with antimicrobials, antiinflammatories, and progesterone (altrenogest) may allow for prolongation of gestation and minimization of the abnormalities associated with premature delivery. Induction of parturition should be avoided if at all possible, but if it must be performed then care should be taken to avoid induction of delivery in mares unless the gestational length is known with great certainty. Removing mares from pastures containing endophyte-infected fescue, in combination with administration of dopamine receptor antagonists (domperidone), can decrease the risk of dysmaturity.

NEONATAL RESUSCITATION

Although the need to resuscitate a foal is uncommon, the critical and time-sensitive nature of this intervention requires that all those involved are appropriately trained in recognizing when and how to implement resuscitation efforts. It also requires that the appropriate equipment and drugs are organized and readily available before the event so that there are no delays in implementation. Cardiopulmonary arrest (CPA)

in foals most commonly begins with respiratory arrest, which subsequently leads to cardiac arrest caused by asphyxia, because primary cardiac disease is very rare in foals. This is a very different situation from that seen in most human patients, in which cardiac arrest is usually the primary initiating factor.[101] Because of this the protocol for resuscitation of foals differs substantially from the approach used in human patients. Establishment of a patent airway and the provision of ventilatory support is the initial priority in foals, rather than cardiac compressions (Box 20.1).

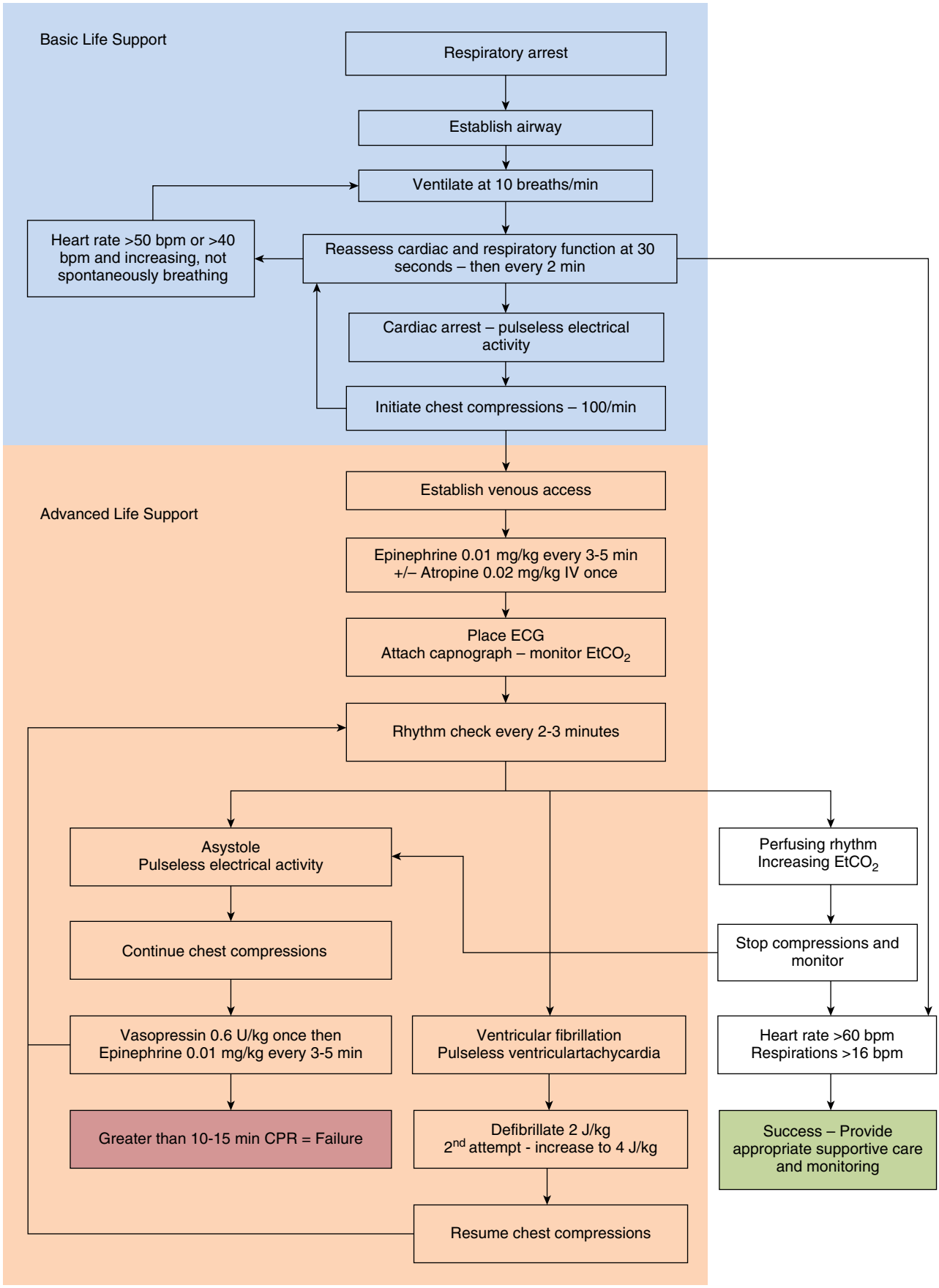
The first challenge is in recognizing which foals require resuscitation, because early intervention before cardiac arrest is associated with a much higher likelihood of successful resuscitation, potentially as high as 50%.[102] The situation in which foals are most likely to require resuscitation is parturition, because asphyxia can occur in utero or during dystocia, and the foal may have been in arrest for a prolonged period of time and will be unable to establish normal respirations without resuscitation.[30] The clinical signs that may indicate the need for resuscitation in the immediate postparturient period include absence of breathing, irregular gasping, respiratory rate less than 10 breaths per minute, heart rate less than 40 beats per minute, irregular or absent muscle flaccidity, or lack of response to tactile stimulation.[103] Other situations in which resuscitation may be required include primary lung disease, septic shock, hypovolemia, metabolic acidosis, hyperkalemia, hypoglycemia, vasovagal reflex, and hypothermia.[102,104] It is important to recognize that the prognosis for successful resuscitation and patient survival is much lower with these disease processes because of the severity of systemic illness and likelihood of multiple organ failure. Although it may be possible to resuscitate the patient, unless the underlying disease can be effectively addressed, the likelihood of survival is very low. Cardiac causes of CPA in foals may include secondary myocardial damage associated with severe hypoxia or sepsis, myocarditis, congenital cardiac defects, endocarditis with coronary artery embolism, and cardiac tamponade.[102,104]

Direct monitoring of the foal during delivery by veterinary staff is unlikely except in cases of dystocia and hospitalized high-risk pregnancies. When foaling is attended the assessment of the foal's respiratory and cardiovascular function should begin during delivery. If the foal is noted to be meconium stained the upper respiratory tract should be suctioned immediately, preferably while still in the birth canal and before the first breath. After delivery suctioning of the trachea may be required if the foal is heavily meconium stained or if meconium is detected in the upper respiratory tract. Mechanical suction is discouraged because it is often too aggressive and may actually worsen hypoxemia and potentiate bradycardia caused by vagal reflexes, and if used it should be limited to no more than 5 to 10 seconds of suction time. Following delivery the foal may gasp a few times but should establish a regular breathing pattern within 30 seconds. Persistent gasping, open-mouth breathing, or severe respiratory distress may be an indication of severe or total upper airway obstruction, such as bilateral choanal atresia, dorsal displacement of the soft palate, or stenotic nares. Tactile stimulation, such as vigorous drying with towels, may aid in stimulating the initiation of respiration. A rapid examination should be performed during this time to identify any congenital defects that would render resuscitation inappropriate or inhumane. If the foal fails to begin breathing spontaneously and regularly then an airway needs to be established immediately and basic life support (BLS) should be implemented. The best approach to establishing an airway is to pass a cuffed nasotracheal tube, which is facilitated by placing the foal's head in an extended position. Because of the urgency of the situation a maximum of two attempts to place a nasotracheal tube should be allowed, up to a maximum of 15 seconds. If nasotracheal intubation is unsuccessful then one should immediately proceed with orotracheal intubation or placement of a tracheotomy tube. If nasotracheal tubes are not available then mouth-to-nose ventilation can be used, because the foal is an obligate nasal breather. Care should be taken to occlude the opposite nostril manually to prevent air leakage from that site when ventilation is attempted, and the effectiveness of ventilation can be easily gauged by monitoring the chest rise during ventilation.[104]

After intubation ventilation should be provided with a self-inflating valved manual resuscitation device (Ambu Inc., Ballerup, Denmark) at 10 breaths per minute with a tidal volume of 10 mL/kg.[104] It is very difficult to limit breaths to this low rate, however, because of the stress and excitement of the resuscitation effort, which often leads to ventilation rates upward of 60 breaths per minute. Unfortunately excessive ventilation negatively affects cardiac return and coronary perfusion and has been associated with worsened outcomes.[105] Another challenge is associated with monitoring tidal volume, because there is a tremendous range of manual resuscitation bags available, all of which have different internal volumes and deliver variable amounts of air depending on how fully they are compressed. For this reason it is best to monitor ventilation by observing chest excursion during ventilation, keeping in mind that aggressive ventilation is contraindicated. Although somewhat controversial there may be some benefit to ventilating the newborn foal with 100% oxygen for a brief period of time (1 minute) immediately after delivery to encourage the reversal of persistent fetal circulation.[30,106] However, there is no indication for continued administration of supraphysiologic oxygen concentrations, because these may actually be harmful, and the delivery of room air is most appropriate in foals without underlying respiratory dysfunction.[104] Following 30 seconds of assisted ventilation discontinue ventilation, and observe the foal for spontaneous breathing while also assessing cardiovascular function by monitoring for a heartbeat. If a heart rate of 50 or greater (or between 40 and 50 but increasing)[104] is present but respirations are not, then resume ventilations, stopping every 2 minutes to monitor for spontaneous efforts or sooner if such efforts are obvious. As many as 90% of foals that require resuscitation at birth will respond to ventilatory support alone and require no additional therapy.[30]

If a heartbeat is absent or the rate is too low then immediately initiate thoracic compressions, because any delay may be very deleterious. No more than 10 seconds should be spent trying to assess cardiac function, and the risk associated with unnecessary thoracic compressions is less than that associated with the failure to provide compressions to an animal that needs them. Place the foal in lateral recumbency on a firm surface and quickly palpate for rib fractures before initiating compressions. If there are unilateral rib fractures position the foal with the affected side facing down, and if there are bilateral rib fractures place the side with more cranial rib fractures facing down.[104] The person providing compressions should position themselves to the dorsal aspect of the foal, with their knees against the spine, which will prevent the foal from shifting during compressions. Both hands should be used, with one closed in a fist and placed with the palm down over the

BOX 20.1 Cardiopulmonary Resuscitation Flowchart



Bpm, beats per minute; *$EtCO_2$*, end-tidal CO_2.

foal's heart, and the other hand placed on top of the closed fist. The arms should be kept straight, with the upper body weight being used to drive compressions. A rapid rate of 100 compressions per minute is advised.[102] The depth of compression is difficult to gauge, but "pushing hard" is recommended.[104] Chest compressions should be briefly stopped every 2 to 3 minutes to monitor for the presence of a heartbeat but should be resumed within 10 seconds. If there is only a single individual available to initiate resuscitation for a patient requiring chest compressions, then adjustments must be made. If the patient is not intubated, then it is recommended to provide 30 chest compressions to 2 ventilations, whereas for the intubated patient it is recommended to provide continuous chest compressions and no ventilatory efforts.[104] Although it is not feasible in single-rescuer resuscitation, if multiple individuals are participating in the resuscitation effort then an intravenous (IV) catheter should be placed while resuscitation efforts are ongoing to have IV access for administration of drugs or fluids.

If the foal is not responding to BLS then more aggressive measures (advanced life support) are indicated. These may include administration of pharmacologic agents and implementation of improved monitoring. Many drugs have been used empirically in foal resuscitation, but none has been specifically studied in the foal. The utility of many of these agents is debatable, either because they are directed toward primary cardiac dysfunction, which is rare in foals, or because their use in human cardiopulmonary resuscitation (CPR) has been discontinued. The drug that is most likely to be used is epinephrine, although this is the subject of ongoing debate in the human literature.[107] Epinephrine is administered at 0.01 mg/kg IV every 3 to 5 minutes. If IV access is not available then epinephrine can be administered intratracheally at 0.1 mg/kg, preferably diluted in a small volume (3–5 mL) of sterile water or saline.[104] Vasopressin has been used either in place of, or in combination with, epinephrine in CPR.[108] If used it should be given as a single dose of 0.6 U/kg IV following the first dose of epinephrine.[102] Doxapram has been commonly used in foal resuscitation as a respiratory stimulant, but it is contraindicated because it does not reverse secondary apnea and has been shown to decrease cerebral blood flow and increase cerebral oxygen consumption.[104,109] Atropine and glycopyrrolate have also been used in foal resuscitation, but as high vagal tone is an unlikely cause of bradycardia the benefit of these treatments is unclear. The administration of a single dose of atropine seems unlikely to be harmful, however, and if used atropine should be administered at 0.02 mg/kg IV. Corticosteroids, calcium gluconate, lidocaine, magnesium sulfate, and sodium bicarbonate, while potentially indicated in the treatment of primary cardiac arrest, are not recommended in routine resuscitation of neonatal foals at this time.[104]

Defibrillation

Defibrillation is indicated in cases of ventricular fibrillation and pulseless ventricular tachycardia. Access to appropriate equipment is often limited, however. The widespread availability of human automated electrical defibrillators may alter this situation, but at this time there are no published studies regarding their use in foals. When performing defibrillation the cardiac compressions and ventilation should continue until the moment of defibrillation and resumed immediately afterward. The initial charge should be 2 J/kg, which should be increased to 4 J/kg for subsequent attempts.[102] Defibrillation presents danger to the operator and anyone in contact with the foal, and operators should receive appropriate training before using a defibrillator.

Monitoring Resuscitation

Patient status during resuscitation is extremely dynamic, requiring constant reassessment, and it can be difficult to monitor for return of spontaneous circulation and spontaneous respiration without interfering with the process of resuscitation itself. Following initiation of resuscitation efforts cardiac and respiratory function should be reassessed at 2- to 3-minute intervals unless the foal shows obvious signs of response (Box 20.1). Monitoring via electrocardiography can be challenging, because electrical activity does not necessarily represent effective cardiac contractility, which is a phenomenon termed *pulseless electrical activity*. Respiratory efforts are much more readily observed and their effectiveness subjectively evaluated. One technique that can greatly facilitate monitoring during resuscitation is the monitoring of end-tidal CO_2 ($EtCO_2$), if capnography is available. The sensor is placed on the endotracheal tube and real-time monitoring is readily performed. Normal resting $EtCO_2$ in a healthy spontaneously breathing patient is approximately 40 mm Hg (35–45 mm Hg), whereas the CO_2 in room air is 0.3 mm Hg. During resuscitation a reasonable target for $EtCO_2$ is 10 mm Hg, because this indicates that an adequate degree of perfusion and ventilation is being achieved. If resuscitation is successful then a rapid increase in $EtCO_2$ toward the normal range will be observed, indicating that both perfusion and ventilation are improving. A decrease in $EtCO_2$ during resuscitation indicates inadequate ventilation and perfusion, requiring reevaluation of how resuscitation is being performed. If no response is detected after 10 to 15 minutes of resuscitation it is extremely unlikely that the patient will respond with further efforts.

Postresuscitation Support

Following successful resuscitation the patient should be considered at high risk because of the presence of any primary, initiating diseases and the likelihood of secondary hypoxic injury.[110] Appropriate medical care and monitoring are critical during this period of time. Foals should receive intranasal insufflation of humidified oxygen at 5 to 10 L/min until they have been demonstrated to be stable with adequate cardiopulmonary function. Fluid therapy will likely be of benefit in ensuring normovolemia and supporting cardiovascular function. Care should be taken to avoid overhydration, because these patients may be at risk of pulmonary edema. Pressor and inotrope therapy may be indicated if cardiovascular function is inadequate. Glucose support is important, especially in neonates, but care should be taken to avoid hyperglycemia. If resuscitation occurred in the field then serious consideration should be given to immediately referring the foal to a facility equipped to provide intensive care.

NEONATAL SEPSIS

The management of critically equine neonates is extremely challenging, but there have been tremendous advancements in the care of these foals over the past several decades. The gradual decline in mortality rates for hospitalized foals over the past 30 years, although likely because of a number of factors in addition to advances in treatment, serves as strong evidence of the progress that has been achieved. Reported survival rates

for hospitalized foals in North America have slowly trended upward from approximately 60% in the 1980s to around 70% in the 1990s and then 70% to 80% in the 2000s.[111-116] As encouraging as these numbers are it is important to note that the survival rates for foals suffering from sepsis are lower than those for all sick foals presenting to a hospital. In the 1980s it was reported that only 26% of septicemic foals survived, whereas a more recent study in 2006 reported that 57% of septic foals survived.[117,118] This is compared with reported survival rates of hospitalized sick, nonseptic foals of 75% to 95%.[118-122] The relatively poor prognosis for septic foals is caused by many factors, not least of which is the fact that sepsis represents a systemic inflammatory response to infection or injury and can rapidly progress to septic shock and death despite aggressive treatment. Traditionally, the term *septicemia* is used to refer to this process in the equine neonate and describes a systemic disease process involving the presence of pathogenic microorganisms and/or their toxins in the blood.[123,124] Historically the typical presentation of sepsis in foals was that of disseminated gram-negative bacterial infections; however, it has become clear that an identical syndrome may occur in patients with gram-positive or mixed bacterial infections, viral infections, trauma, hypovolemia, hemorrhage, and immunologic and drug reactions.[125-127]

Pathophysiology

The abnormalities associated with the clinical syndrome of sepsis result from a nonspecific innate inflammatory response, which has been termed the *systemic inflammatory response syndrome* (SIRS).[125] SIRS represents a common terminal phase of the inflammatory response characterized by malignant global activation of multiple proinflammatory pathways and is characterized by parameters that can be readily defined in clinical patients. These manifestations of disease are the same as those previously used to define sepsis, but the appreciation that many different stimuli can induce this response has resulted in sepsis being redefined as SIRS because of infection (Table 20.1).[128,129] The changes associated with SIRS can lead to shock, which is characterized by severe hypotension not responsive to IV fluid therapy (Box 20.2). Shock can result in hypoperfusion and organ dysfunction such that homeostasis cannot be maintained without intervention. This process is termed *multiple organ dysfunction syndrome* (MODS).[125] MODS represents a progressive syndrome with initial dysfunction seen in the cardiovascular system, followed by involvement of the respiratory, hepatic, gastrointestinal, renal, cardiac, and neurologic systems. These processes result in the development of refractory hypotension, lactic acidosis, and oliguria and may progress to death. SIRS criteria were proposed 15 years ago for the neonatal foal population and are based on human criteria using information regarding normal values in foals.[124,130] SIRS criteria have been used in a number of studies of clinically ill foals since that time and have been correlated with increases in both lactate concentration and mortality.[130,131] A recent report has proposed a revised set of SIRS criteria that are age dependent and based on insight from revised human pediatric SIRS criteria and that incorporate the changes in normal values that occur with increasing age in foals (Table 20.2).[132]

Inflammation represents the response of tissues to either injury or the presence of microorganisms. It serves a vital role because it enhances the movement of phagocytic cells and defensive molecules, such as immunoglobulin and complement, from the bloodstream to the site of infection or injury.

TABLE **20.1 Definitions of Terms Used to Describe Clinical Syndromes Associated with Systemic Inflammation**

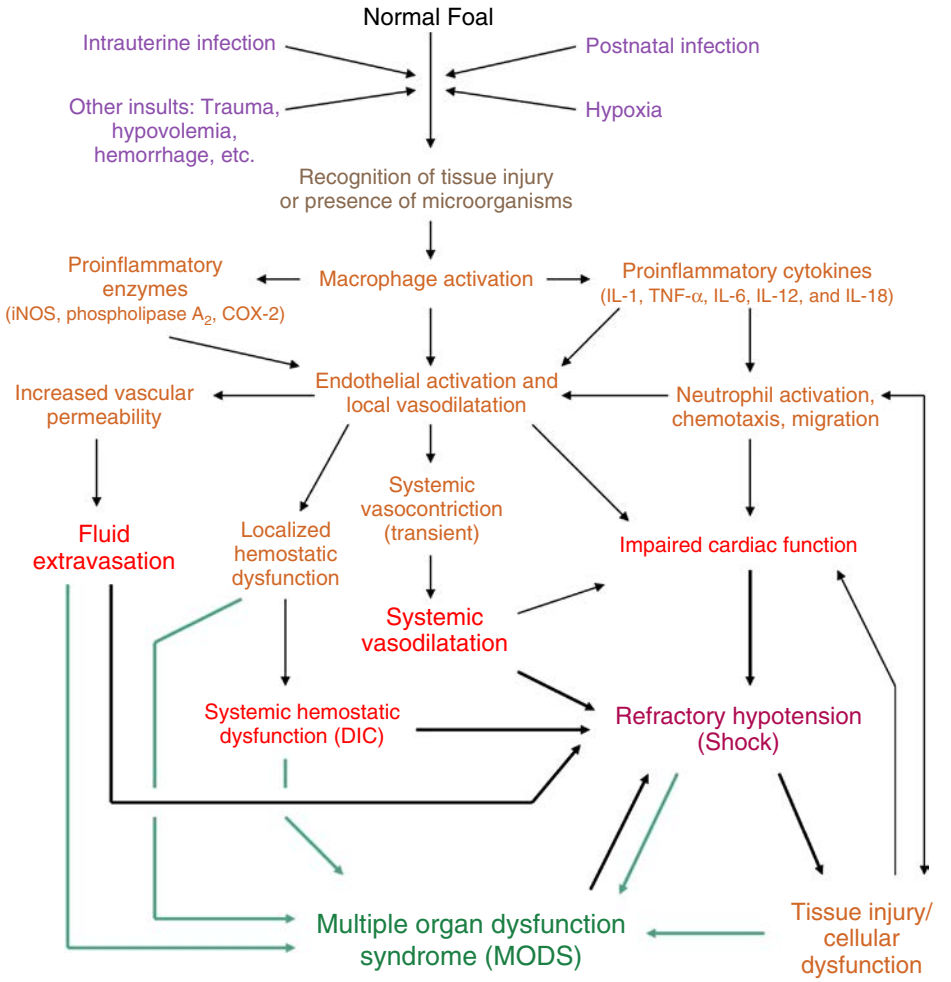
Infection	Inflammatory Response to the Presence of Microorganisms or the Invasion of Normally Sterile Host Tissue by Microorganisms
Bacteremia/septicemia	Presence of viable bacteria in the bloodstream
SIRS	Systemic response to an array of severe clinical insults
Shock	SIRS-induced hypotension refractory to fluid resuscitation in association with hypoperfusion
Sepsis	SIRS caused by infection
Severe sepsis	Sepsis associated with organ dysfunction, hypoperfusion, or hypotension
Septic shock	Sepsis-induced shock
MODS	Altered organ function in an acutely ill patient requiring intervention to maintain homeostasis

MODS, multiple organ dysfunction syndrome; *SIRS,* systemic inflammatory response syndrome.
Adapted from Nathens AB, Marshall, JC. Sepsis, SIRS, and MODS: What's in a name? *World J Surg*. 1996;20(4):386-391; Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest*. 1997;112(1):235-343.

The first step in this process is the recognition of tissue injury or microbial invasion. Trauma or microbial invasion results in tissue injury, and injured cells then release or produce damage-associated molecular patterns, and these mediators can initiate a nonspecific inflammatory response.[133] Alternatively, specific bacterial cell components, termed *pathogen-associated molecular patterns* (PAMPs), may be recognized by immune cells that produce inflammatory mediators, leading to the initiation of an inflammatory response and subsequent amplification of that response.[133,134] Some of the PAMPs that can be recognized by the immune system include lipopolysaccharide (LPS; endotoxin), exotoxins from gram-negative bacteria, peptidoglycan, lipoteichoic acids, enterotoxins, or superantigenic exotoxins from gram-positive bacteria.[124,135] The development of an inflammatory response is dependent on the production, primarily by the activated mononuclear phagocyte, of numerous inflammatory mediators, including proinflammatory cytokines (tumor necrosis factor-α [TNF-α,] interleukin [IL]-1, IL-6), proinflammatory enzymes (inducible nitric oxide synthase, phospholipase A_2, cyclooxygenase-2 [COX-2]) and adhesion molecules (selectins and intercellular adhesion molecules).[136] The transcription of many of the genes encoding for these mediators, or the enzymes that produce them, is dependent on the transcription activator nuclear factor-κB, and this molecule may be a potential target for intervention in SIRS.[137-139]

It is important to understand that although bacterial infection may be responsible for the initiation of an inflammatory response, the inflammatory process itself results solely from the production of endogenous mediators. The initial changes seen in an inflammatory response are primarily the result of local vasodilatation and increased vascular permeability

BOX 20.2 Pathophysiology of Shock in the Neonatal Foal



COX-2, cyclooxygenase-2; *DIC,* disseminated intravascular coagulation; *IL,* interleukin; *iNOS,* inducible nitric oxide synthase; *TNF,* tumor necrosis factor.

Adapted from McKenzie HC, Furr MO. Equine neonatal sepsis: the pathophysiology of severe inflammation and infection. *Comp Contin Educ Pract Vet.* 2001; 23:661-672.

TABLE **20.2 Proposed Systemic Inflammatory Response Syndrome Criteria for Foals That Require the Presence of at Least Three of the Following Criteria, One of Which Must Be Abnormal Temperature or Leukocyte Count**

Parameter	Newborn (Birth to 3 Days)	Neonate (4–14 Days)	Juvenile (15 Days to 6 Months)	Weanling (7 Months to 1 Year)
Fever or hypothermia	>102.6°F (39.2°C) or <99°F (37.2°C)	>102.6°F (39.2°C) or <99°F (37.2°C)	>102.6°F (39.2°C) or <99°F (37.2°C)	>102.6°F (39.2°C) or <99°F (37.2°C)
Tachycardia (beats/min)	>115	>120	>96>44	>60>20>12.5 or <4.0
Tachypnea (breaths/min)	>56	>56	>44	>20
Leukocytosis (×10^3), leukopenia, or >5% band neutrophils	>14.4 or <6.9	>12.5 or <4.0	>12.5 or <4.0	>12.5 or <4.0
Venous blood lactate (mmol/L)	>5.0	>2.5	>2.5	>2.5
Venous blood glucose (mg/dL)	<50	<50	<50	<50

From Wong DM, Wilkins PA. Defining the systemic inflammatory response syndrome in equine neonates. *Vet Clin North Am Equine Pract*. 2015;31(3): 463-381.

caused by the effects of vasoactive mediators released by the injured or infected cell. On their arrival at the site of tissue injury, neutrophils and macrophages phagocytose foreign material and injured or dead tissue cells and attempt to destroy the phagocytosed material. In addition, macrophages release a number of factors that augment the immune response, including the proinflammatory cytokines IL-1, TNF-α, IL-6, IL-12, and IL-18. These proinflammatory cytokines increase the production of secondary inflammatory mediators, including phospholipid derivatives (prostaglandins, thromboxane A_2, and leukotrienes), and reactive oxygen species (ROS), further increasing the activity of the inflammatory response. The systemic manifestations of inflammation/infection (fever, lethargy, malaise, loss of appetite, and cachexia) are primarily caused by TNF-α and IL-1. The cytokines IL-6, IL-1, and TNF-α initiate the acute phase response in which the liver increases production of acute-phase proteins (fibrinogen, serum amyloid A, plasminogen, complement, haptoglobin, ceruloplasmin, ferritin, C-reactive protein, etc.).[140] These substances are important in many phases of the response to inflammatory stimuli, including complement activation, coagulation, fibrinolysis, transport of substances within the bloodstream, inhibition of neutrophil proteases, and modulation of the inflammatory response.[141] The acute phase response is critical in inflammation, healing, and adaptation to noxious stimuli. Also included in the acute phase response is a counterregulatory antiinflammatory component that normally functions to minimize and resolve the inflammatory response to localized stimuli. This counterregulatory response consists of antiinflammatory mediators that inhibit macrophage activation (IL-4, IL-10, IL-13, adrenal corticosteroids, transforming growth factor-β and prostaglandin-E_2), antagonists to the receptors for proinflammatory cytokines (IL-1 receptor antagonists), and soluble receptors of proinflammatory cytokines (soluble IL-1 receptor type II and soluble TNF receptors).[142] The balance between these proinflammatory and antiinflammatory components is very important in determining the characteristics of the inflammatory response, because excessive activity of the antiinflammatory component may result in immunosuppression during or after a severe inflammatory response, which has been termed the *compensatory antiinflammatory response syndrome*.[125]

In moderation the changes associated with an inflammatory response are protective, resulting in the enhanced killing of microbes by antigen-specific and nonspecific mechanisms, generalized immune stimulation, and increased activity of the systems required for healing damaged tissue. SIRS, the excessive, malignant form of the inflammatory and acute-phase responses, is characterized by the systemic activity of numerous proinflammatory mediators. These mediators all represent components of the normal inflammatory response to a localized stimulus, but the systemic activity of these proinflammatory mediators may result in an excessive, and often detrimental, response. One of the first effects seen in SIRS is widespread endothelial activation, resulting in the increased production of vasoactive mediators and alteration of vascular homeostasis. Inflammatory cytokines are responsible for activation of the endothelium, and the activated cells produce inflammatory cytokines, as well as increased amounts of nitric oxide (NO), prostaglandins, and endothelin-1.[143] Activated endothelial cells retract from one another, increasing the size of the intercellular pores and allowing for increased vascular permeability, and increase their production of tissue factor and von Willebrand factor, resulting in localized thrombosis and platelet adherence.[144]

One of the earliest systemic effects is pulmonary vasoconstriction leading to pulmonary hypertension.[145] This phase is followed by systemic hypotension caused by decreased arterial tone resulting in decreased left ventricular afterload, combined with venous vasodilatation in the large-capacity vessels that decreases venous return and right ventricular preload. These effects can progress to the syndrome of hyperdynamic shock, with increases in heart rate and cardiac output developing as compensatory mechanisms to maintain tissue perfusion.[146] This compensatory response is impaired by the reduction in left ventricular preload resulting from decreased peripheral vascular resistance, combined with decreased cardiac contractility.[147] Changes occurring in the microvasculature further contribute to the impairment of tissue perfusion. Arteriolar vasoconstriction develops because of the impairment of the normal autoregulatory systems caused by inflammatory cytokines and endothelin-1, combined with the increased production of vasoconstrictive substances. Adherence of neutrophils to the endothelium and endothelial cell swelling further reduces blood flow. Accumulation of fibrin and aggregation of platelets and red blood cells secondary to activation of the clotting system results in occlusion of the vasculature, resulting in tissue hypoperfusion. Arteriovenous shunting occurs in some tissues, whereas increased vascular permeability results in leakage of intravascular fluid into the interstitial space, further contributing to hypotension and hypovolemia and contributing to the development of tissue edema. Progressive alteration of the microcirculation leading to failure may represent the "common final pathway" of SIRS-related injury contributing to or resulting in MODS.[148]

Activation of coagulation occurs primarily through the extrinsic pathway, and endothelial injury secondary to neutrophil degranulation results in increased platelet adhesion.[149] In the normal state the accumulation of the resulting excessive amounts of fibrin would be prevented by the action of plasmin, the primary mediator of fibrinolysis. In the presence of SIRS the fibrinolytic system is suppressed because of the increased plasma concentration of plasminogen-activator inhibitor type 1, the primary inhibitor of fibrinolysis.[150] The widespread activation of the clotting system combined with impairment of fibrinolysis and depression of the inhibitors of coagulation can result in a consumptive coagulopathy potentially leading to disseminated intravascular coagulation (DIC).[150,151] Several reports have demonstrated that clinicopathologic and histopathologic evidence of coagulopathy is common in septic foals, and these findings are associated with a worsened prognosis for survival.[152-156]

The progression of these processes affecting the cardiovascular system ultimately results in shock. Shock occurs when cardiovascular function is severely impaired to the point that hypotension cannot be corrected with IV fluid administration, requiring the use of inotropic and/or vasopressor agents.[157] Shock represents severe cardiovascular dysfunction associated with SIRS and is a primary component of MODS. The development of MODS is likely the result of cardiovascular dysfunction leading to tissue hypoperfusion combined with changes in cellular metabolism that result in impairment of oxygen delivery and uptake.[158] Tissue hypoxia is manifested by increased oxygen extraction ratios and metabolic acidosis caused by increased lactate production. Pulmonary dysfunction is manifested by refractory hypoxemia, potentially caused

by increased pulmonary vascular permeability, microthrombi formation, pulmonary epithelial injury, pulmonary edema, and impairment of surfactant production.[159] Renal dysfunction is manifested by the development of azotemia and oliguria, and this acute renal failure is likely caused by alterations in the distribution of intrarenal blood flow arising from microvascular alterations with or without systemic hypotension.[158] Gastrointestinal dysfunction is primarily manifested by the presence of ileus but may also result in loss of the normal barrier function of the gastrointestinal mucosa. Impairment of the mucosal barrier may further contribute to the pathogenesis of MODS caused by bacterial translocation or endotoxin absorption.[160] Hepatic dysfunction is manifested by the development of hyperbilirubinemia and in some cases increased serum activities of hepatic enzymes (sorbitol dehydrogenase [SDH] and aspartate aminotransferase [AST]).[158] Hepatic dysfunction may result from hypoperfusion but may be heightened by the production of inflammatory mediators by the hepatic Kupffer cells secondary to the actions of systemic mediators or stimuli derived from the gastrointestinal tract.[161] Dysfunction of the central nervous system (CNS) is frequently present and may manifest as depression, which can progress to septic encephalopathy with extensive neuronal injury.[162] The development of consumptive coagulopathy (DIC) could also be considered a component of MODS, rather than merely a pathophysiologic process contributing to the development of organ failure.

Risk Factors for Neonatal Sepsis

A number of factors have been identified that increase the likelihood of septicemia in equine neonates. These risk factors may include history of placentitis, prenatal vulvar discharge, dystocia, maternal illness, premature or delayed parturition, premature placental separation, induced parturition, partial or complete failure of transfer of passive immunity (FTPI), poor sanitary conditions, improper umbilical care, prolonged transport of the pregnant mare, and the presence of localized disease in the neonate (anterior uveitis, diarrhea, pneumonia, infectious arthritis, and open wounds).[163,164] Inadequate transfer of passive immunity (serum IgG concentration of <800 mg/dL) represents a critical risk factor for septicemia and death in foals, with the risk of infection and death increasing proportionally with decreasing IgG concentrations (see later section Immunologic Disorders).[165] Pathogenic organisms can infect the equine neonate by numerous routes, although in the intrauterine environment the fetus may be exposed to organisms that have invaded the placenta or that cross the placentochorial barrier and gain direct access to the foal's bloodstream. Bacteria associated with placental disease may enter the amniotic fluid and gain access to the respiratory and gastrointestinal tracts of the fetus. After birth, infection can occur following contamination of the umbilical stump, ingestion, inhalation, or secondary to wounds.[123,164]

Clinical Presentation and Identification of the Septic Neonate

The presenting signs associated with sepsis in the neonatal foal are often vague and nonspecific. Foals may exhibit depression, lethargy, and partial or complete absence of nursing behavior. Tachycardia and tachypnea are also common initial signs but are not consistently present. Signs of systemic inflammation may include abnormal mucous membranes, with a prolonged capillary refill time, and a dark red or injected appearance. Petechiation of the mucous membranes or the skin of the inner pinnae may be present. Fever is not consistently present, because neonatal foals may exhibit impaired thermoregulation, and hypothermia is not uncommon in this population. There may be additional signs of localized infection, such as diarrhea, joint effusion (with or without lameness), respiratory disease or distress, lameness, uveitis, seizures, subcutaneous abscesses, omphalitis, or patent urachus.[164] Additional diagnostic evaluation using ultrasonography may be useful in identifying the presence of pulmonary inflammation or infection, joint or physeal infection, and internal involvement of the umbilical remnants.

Clinicopathologic evaluation of the clinically ill foal can be helpful in supporting the clinical suspicion of sepsis. Septic neonatal foals have been demonstrated to exhibit lower white blood cell (WBC), neutrophil and lymphocyte counts, and higher band neutrophil and monocyte counts compared with healthy age-matched control foals.[152] Azotemia is commonly encountered in this population but may reflect placental insufficiency (spurious hypercreatininemia) rather than neonatal sepsis or renal insufficiency.[114,166] Azotemia typically resolves rapidly with standard treatment unless underlying renal disease or uroperitoneum is present.[166] Blood glucose abnormalities are common in septic foals, with hypoglycemia being the most common.[131] This is likely caused by decreased milk intake combined with limited endogenous energy stores but may also be associated with SIRS. Arterial blood gas evaluation is extremely useful and often reveals the presence of acidosis, which may be metabolic, respiratory, or mixed in nature. Hypoxemia and/or hypercapnia may also be identified, typically in more severely affected patients. The measurement of blood lactate concentration is increasingly performed because of the availability of handheld lactate meters, and hyperlactatemia is a common finding.[167-169] Blood lactate concentrations are consistently higher in normal neonatal foals than normal adult horses for the first 24 to 72 hours of life, with normal foals exhibiting values of up to 5.0 mmol/L in the first 24 hours,[20,64] with a recent report finding values as high as 10.2 mmol/L immediately after birth.[167] In that study lactate concentrations steadily decreased over the first 3 days of life, but the median concentration at 72 hours remained slightly increased relative to normal adult concentrations, at 1.7 mmol/L. Serial measurement of blood lactate may be useful in assessing the foal's response to therapy, because the failure to normalize lactate over time has been associated with a poor prognosis for survival.[170]

The most definitive test for antemortem identification of the septicemic equine neonate remains blood culture, which should be performed at presentation in any equine neonate with a clinical suspicion of sepsis, ideally before the administration of antimicrobial therapy. This test has been reported to have fairly poor sensitivity, with false negative results obtained in up to 37% of cases of fatal septicemia.[171] It is likely that the false negative rate would be much higher in less severely ill foals that recover with antimicrobial therapy. For this reason, it has been recommended that multiple samples be collected to maximize the sensitivity of blood culture, but the majority of isolates have been reported to be present in the first culture sample, and the need for immediate antimicrobial therapy usually results in only one sample being obtained. Although it is recommended that blood culture sampling should occur before the initiation of antimicrobial therapy, there is evidence

in human patients that a delay in the initiation of antimicrobial therapy of more than 3 hours from the onset of clinical signs is associated with increased morbidity and mortality, and current recommendations suggest a delay of no more than 1 hour.[172-175] In the referral setting most foals will have been exhibiting signs of sepsis for longer than that period of time, so early blood culture collection and initiation of antimicrobial therapy is critical. For this reason when a case is being referred from the field it is advisable to initiate antimicrobial therapy on the farm before referral if transport to the referral center is likely to require more than 30 minutes. Although this may decrease the likelihood of obtaining a positive blood culture, the benefit of early treatment likely outweighs the loss of diagnostic information. It is also important to obtain bacterial cultures from suspected areas of infection during the course of treatment because the blood culture may be falsely negative or nosocomial infection may have developed during the course of treatment. The appropriate samples are dependent on the system affected but could include transtracheal aspirate, blood, urine, synovial fluid, peritoneal fluid, cerebrospinal fluid (CSF), and umbilical remnants following surgical resection.

Although historically gram-positive organisms were most commonly associated with neonatal infections, over the past 30 years the most common bacteria isolated from infected foals have been gram-negative organisms (Table 20.3).[120,171,176-182] Gram-positive organisms isolated from infected foals are often present in mixed infections with gram-negative organisms, and streptococcal species are most common. Other organisms associated with severe systemic inflammation in the equine neonate include equine herpesvirus type 1 (EHV-1) and equine viral arteritis.[183,184] It is also possible that severe hypoxia leading to hypoxic ischemic encephalopathy (HIE; neonatal encephalopathy [NE], neonatal asphyxia, and dummy foal syndrome) represents a causative factor for induction of SIRS in the equine neonate.[185]

Rapid identification of the septic neonate is critically important in ensuring that appropriate treatment is administered and in determining the prognosis for survival. The identification of the septic neonate remains problematic, however, because neonates presenting with clinical abnormalities consistent with sepsis (SIRS) may be negative on blood culture with no evidence of a focal site of infection. Attempts to identify equine neonates with septicemia have included the development of a sepsis scoring system combining historical information, objective data, and subjective measures to derive a numerical representation of the patient's condition.[24,117] Using a cutoff of ≥12 the sensitivity and specificity of the modified version of this scoring system were reported to be 93% and 86%, respectively. Unfortunately the specificity and sensitivity may not always be this high; a subsequent study found that the modified sepsis score yielded sensitivity of 67%, a specificity of 76%, a positive predictive value (PPV) of 84%, and an NPV of 55%.[186] The authors of that report suggested that it might be that this scoring system must be adapted to each ICU in which it is applied to achieve reasonably high levels of sensitivity and specificity. In another study the modified sepsis scoring system failed to predict sepsis in 49% of bacteremic foals.[178] A more recent study investing the application of the modified sepsis scoring system in a large population of 1065 foals proposed a revised cutoff value of >7, resulting in a sensitivity of 84.4% and a specificity of 41.8%, compared with the original cutoff of ≥12, which had a sensitivity of 56.4% and specificity of 73.4%.[187]

TABLE **20.3 Organisms Isolated from Foals with Sepsis/ Septicemia with Frequency of Isolation**

	Organism	Pre-2000 Isolates	Post-2000 Isolates
Gram negative	*Escherichia coli*	30.6–56	22.4
	Klebsiella pneumoniae	3.7–12.9	0.8–5.7
	Actinobacillus spp.	8–30	6.5–12.4
	Enterobacter spp.	3.5–14	3.2–8.1
	Pseudomonas aeruginosa	2–4.7	2.9
	Citrobacter spp.	4.7	—
	Pasteurella spp.	3.7	—
	Salmonella spp.	2.8–5	3.4
	Serratia marcescens	2.8–3.7	—
	Acinetobacter spp.		5.6
Gram positive	β-Hemolytic streptococci	1.2–8.8	10.6
	Other streptococci	5.9–7.1	4.1
	Staphylococcus spp.	2.8–4.8	6.3–9.7
	Clostridium spp.	2.4–3.7	1.6
	Enterococcus spp.	4.6–14	12.0–12.1

Data from Brewer B, Koterba A. Bacterial isolates and susceptibility patterns in foals in a neonatal intensive care unit. *Comp Contin Educ Pract Vet*. 1990;12(12):1773-1781; Koterba AM, Brewer BD, Tarplee FA. Clinical and clinicopathological characteristics of the septicaemic neonatal foal: review of 38 cases. *Equine Vet J*. 1984;16(4):376-382; Stewart AJ, et al. *Actinobacillus* sp. bacteremia in foals: clinical signs and prognosis. *J Vet Intern Med*. 2002;16(4):464-471; Theelen MJ, et al. Temporal trends in prevalence of bacteria isolated from foals with sepsis: 1979–2010. *Equine Vet J*. 2014;46(2):169-173; Wilson WD, Madigan JE. Comparison of bacteriologic culture of blood and necropsy specimens for determining the cause of foal septicemia: 47 cases (1978–1987). *J Am Vet Med Assoc*. 1989;195(12):1759-1763; Russell CM, et al. Blood culture isolates and antimicrobial sensitivities from 427 critically ill neonatal foals. *Aust Vet J*. 2008;86(7):266-271.

Ultimately, no sepsis score can substitute for clinical judgment, and these scores should be used as a diagnostic aid in the identification of high-risk individuals, with full consideration given to their limitations.[163,188] There has been interest in the use of serum amyloid A (SAA) as an early and accurate biomarker of bacterial infection in neonatal foals, and an early report suggested a cutoff of >100 mg/L as being highly suggestive of infection.[189] A recent unpublished study by Jokisalo et al.[104] found that using the cutoff point of >100 mg/L yielded a sensitivity of 68.6% and specificity of 68.5%, and the NPV was 87%.[190] Unfortunately 11 of 36 foals considered to be septic in that study had SAA concentrations below 25 mg/L, and 9 of 36 septic foals had SAA concentrations less than 10 mg/L, which indicates that low SAA concentrations do not rule out the possibility of bacterial sepsis.[190] Other potential biomarkers of sepsis in foals, such as IL-1β, plasma C-reactive protein, and haptoglobin, have been investigated but do not appear to be strong or accurate predictors of sepsis.[191,192] Although abnormalities of blood glucose concentration and lactate concentration are common in septic foals, these biomarkers appear to be most useful in prognostication rather than identification of the septic neonate.[126,131,193]

Because of the difficulty of definitively identifying neonates with bacterial infection it is appropriate to make the assumption that all high-risk neonatal foals presenting with clinical illness are septic.[194] Aggressive, early antimicrobial therapy is indicated, and although there are some risks associated with antimicrobial therapy these are greatly outweighed by the risks associated with withholding antimicrobial therapy from a patient with sepsis secondary to bacterial infection. An initial antimicrobial regimen should provide broad-spectrum coverage including organisms commonly identified in the practice population or region. While a detailed discussion of antimicrobial therapy can be found in the antimicrobial section of this chapter, a reasonable initial approach is the use of penicillin or ampicillin in combination with an aminoglycoside, typically amikacin. If there are concerns regarding renal insufficiency then the aminoglycoside can be replaced by a third-generation cephalosporin. Monotherapy with a third- or fourth-generation cephalosporin may be a reasonable alternative to combination therapy, particularly in the severely dehydrated patient, but may not provide full coverage for gram-positive pathogens.

Management

Management of the septic neonate can be challenging because of the requirement for constant monitoring and the need for substantial nursing care. Fluid therapy is indicated in the resuscitation and stabilization of clinically ill foals and can initially take the form of intermittent 20-mL/kg boluses given over 10 to 30 minutes. This typically needs to be accompanied by appropriate maintenance fluid therapy (see later section Fluid Therapy in the Foal). The presence of fluid-refractory hypotension may require the use of inotropic and vasopressor therapies (see later section Inotrope and Vasopressor Therapy). Respiratory support is beneficial, and in most cases it consists of intranasal insufflation of humidified oxygen at 5 to 10 L/min using a nasal cannula. Respiratory stimulants (caffeine and doxapram) may be beneficial in foals with decreased respiratory drive. If these interventions are not adequate then mechanical ventilation may be necessary. Additional therapies may include the administration of hyperimmune plasma, either for supplementation of immunoglobulins, colloidal support, or the treatment of SIRS. Antiinflammatory therapies are sometimes used, primarily consisting of nonsteroidals, but these sometimes including corticosteroid therapy if critical illness–associated corticosteroid insufficiency (CIRCI) is suspected (see later sections Antiinflammatory and Analgesic Therapy and Endocrine Disorders). Care should be taken regarding the use of antiinflammatory therapies in critically ill foals, however, because of the risk of gastrointestinal ulceration or renal injury. Gastroprotectant therapy using proton pump inhibitors is generally not used in the first few days of life because of concerns regarding increased susceptibility to nosocomial infections, but sucralfate may be a reasonable option to those drugs (see later section Gastrointestinal Disorders). The use of low-molecular-weight heparin and/or the administration of fresh or fresh frozen plasma have been suggested to address coagulopathies in the critically ill foal.[190,195]

Nursing care is one of the most important aspects of treating septic foals. Foals should be kept warm and dry, which can be difficult in the recumbent patient because of frequent urination and defecation. The placement of a urinary catheter should be considered in these patients, even though it may present a risk regarding urinary tract infections, because it greatly facilitates their nursing care, decreases the risk of decubital ulceration, and provides a means of monitoring urine output. Recumbent foals should be kept in a sternal position as much as possible to minimize the development of atelectasis in the dependent lung, and they should be turned at no longer than 2-hour intervals. Feeding septic foals can be a challenge if gastrointestinal function is abnormal, and parenteral nutrition may be needed (see the later section Nutritional Support for the Foal). Blood glucose levels should be monitored frequently, especially in foals receiving parenteral nutritional support. The development of persistent hyperglycemia may require the institution of insulin therapy. If at all possible, foals should be weighed daily; this can be another means of monitoring for fluid retention.

Prognosis

The costs associated with the intensive care of affected foals are substantial, and for that reason it is important to strive to provide an accurate prognosis for survival as well as for long-term athletic function. Given the broad range of prognostic indicators that one can use to assess the odds for survival of a critically ill foal, it can be challenging to provide an accurate and timely prognosis for the owner of such a patient. Experienced clinicians are able to provide accurate predictions for survival, but given that there will always be variations in clinician experience level and performance there has been substantial interest in developing a scoring system that could be used prospectively to enhance prognostic accuracy. In 1992 Hoffman et al. performed a study of predictive variables for survival in hospitalized neonatal foals.[111] In that study they retrospectively identified two predictive variables, anion gap and the venous partial pressure of oxygen (Pvo_2), and when these were applied prospectively in a population of 48 foals their model showed a PPV for survival of 62% and an NPV of 100%. In 1997 Furr et al. performed a similar study, developing a predictive equation for outcome in a population of 99 hospitalized neonatal foals.[112] They identified several variables associated with nonsurvival, but their model ultimately retained a different set of predictive variables, which were heart rate, rectal temperature, and neutrophil count. This model yielded a PPV of 78% and an NPV of 78% in the retrospective population. When applied prospectively to two populations of hospitalized foals the model performed differently in the two populations, with a PPV of 93% and an NPV of 72% in one population as opposed to a PPV of 83% and an NPV of 44% in the other. This was likely caused by fundamental differences in the types of cases referred and the timing of presentation to the two different hospitals.

Rohrbach et al. performed a large, multicenter study to create a predictive model for the probability of hospital discharge in hospitalized foals less than 7 days of age.[196] The variables retained in their model were foal age, ability to stand, presence of a suckle reflex, WBC count, serum creatinine concentration, and anion gap. This model yielded a PPV of 95% and an NPV of 65% in the retrospective population. When applied prospectively the model demonstrated a PPV of 90% and an NPV of 46%. Rohrbach et al. also reported the accuracy of clinician prediction of outcome, which was 83% in the retrospective population, as opposed to 81% accuracy for the mathematical model; however, the predictions were not well correlated. Interestingly, by combining the clinician's initial prediction with the model output they were able to improve the accuracy of

TABLE **20.4 Survival Scoring System Proposed by Dembek et al.**[115]

Variable	Value	Points	Value	Points	Score
Cold extremities	No	2	Yes	0	
Prematurity (<320 days)	No	1	Yes	0	
>2 infection/inflammatory sites	No	1	Yes	0	
IgG (mg/dL)	<400	0	≥400	1	
Glucose (mg/dL)	<80	0	≥80	1	
White blood cells × $10^3/\mu L$	≤4	0	>4	1	

From Dembek KA, et al. Development of a likelihood of survival scoring system for hospitalized equine neonates using generalized boosted regression modeling. *PLoS One*. 2014;9(10):e109212.

TABLE **20.5 Probability of Survival Using Survival Scoring System of Dembek et al.**[115]

Total Score	Probability of Survival (%)
0	3
1	8
2	18
3	38
4	62
5	82
6	92
7	97

From Dembek KA, et al. Development of a likelihood of survival scoring system for hospitalized equine neonates using generalized boosted regression modeling. *PLoS One*. 2014;9(10):e109212.

the survival estimate by 12%.[196] Another multicenter study was performed by Dembek et al., in which they developed a survival scoring system for critically ill foals.[115] Their final model retained six variables, which were cold extremities, prematurity, ≥2 infection/inflammation sites, IgG concentration, glucose concentration, and WBC count. These variables were assigned scores depending on their respective values, and totaling of the individual scores yields the final survival score (Table 20.4). A score of 0 was associated with a 3% probability of survival, whereas the maximum score of 7 was associated with a 97% probability of survival (Table 20.5). They validated their foal survival score (FSS) prospectively using score cutoffs of ≥4 for foals predicted to survive and <4 for foals predicted to die, which resulted in values for a PPV of 91% and an NPV of 86%. Foals with an FSS of 4 to 5 were 24.2 more likely to survive than foals with a score <4, and foals with a score of 6 to 7 were 91 times more likely to survive.[115] Most recently, Giguère et al. reported a retrospective study examining factors associated with survival in a population of 1065 hospitalized foals over 26 years at one academic referral hospital.[116] The variables retained in their model included positive blood culture, neutropenia, hypothermia, bicarbonate, P_{CO_2}, presence of infectious orthopedic disorders, and sepsis score. The reported sensitivity of their model was 97%, and the specificity was 75%. Of particular interest was the increase in the odds of survival for foals born in the 1990s, which were 2.2 times greater compared with those born in the1980s, whereas foals born in the 2000s had a 3.4 times greater odds of survival compared with those born in the 1980s.[116]

NEUROLOGIC DISORDERS

Weakness and abnormal behavior are common presenting signs in clinically ill foals and may be associated with neurologic dysfunction; however, they may also be caused by a myriad of other conditions. For this reason thorough evaluation and assessment of abnormal foals is extremely important. This evaluation should include the collection of a thorough history (to include maternal health and reproductive history) and performance of a complete physical examination, which should include a detailed neurologic assessment. The first objective of neurologic assessment is to ascertain whether true neurologic dysfunction is present and, subsequently, to establish neuroanatomic localization of any lesions. This information is then used to direct further diagnostic and therapeutic efforts.

The first stage of the neurologic examination should be assessment of the foal's behavior and level of alertness, but when doing so it is important to remember the normal development and progression of foal behavior. In the first few hours after parturition foals exhibit fairly dramatic increases in consciousness, strength, and coordination, as well as awareness of and interaction with their environment. Thus it is important to understand at what point they should be along that continuum based on the time since birth. Most foals will be able to stand within 1 to 2 hours of birth, and it is typical for the initial efforts at standing to be poorly coordinated and at times unsuccessful. The initial stance will be base wide, with prominent swaying back and forth. However, once the foal stands successfully rapid improvement in ambulation should be noted and the foal will begin seeking the mare's udder. A suckle reflex should be present within minutes after birth, and foals will actively engage in suckling behavior even while recumbent and without access to the udder. Most foals suckle successfully within 1 to 3 hours after birth, but this can range from 0.5 to 7 hours. Nursing episodes should last from 1 to 5 minutes and are typically followed by periods of drowsiness, recumbency, and sleep.[197] Foals become increasingly coordinated and confident over the first few days of life and will avoid handlers if possible. Once restrained they typically struggle vigorously but will become limp when compressed along their long axis.[198] This relaxation response in response to squeezing is particularly pronounced in the first several hours after birth.[199]

The next stage of the examination is an assessment of the foal's conformation, posture, and gait. The head should be above the horizontal plane when foals are standing, and no head tilt or rotation should be noted.[197] Foals carry their heads with pronounced flexion of the atlantooccipital joint and tend to move their heads with jerky motions that may resemble cerebellar dysfunction in the adult.[198] The foal's gait should be evaluated by observing the foal in unrestrained, spontaneous movement, and this can be facilitated by leading the dam and observing the foal as it follows.[200] The neonatal foal may appear somewhat dysmetric compared with an adult, but this typically improves with exercise.[201] Foals typically have a somewhat exaggerated "bouncy" and hypermetric gait compared with an adult.[197]

Examination of the cranial nerves is similar to the adult, and begins by observation of facial expression, muscle tone, and symmetry. Touching the nasal septum and the inside of the ear canal, which should elicit avoidance behavior, will aid in the assessment of facial sensation. The flick reflexes should be evaluated by gently touching the tip of a hemostat to the pinna of the ear, the medial and lateral canthi of the eyes, and the commissure of the lips.[197] The eyes should be examined for orientation, pupillary size, and pupillary light reflexes. The globe typically shows a ventromedial deviation that will resolve by 1 month of age.[198] The pupil is somewhat round at birth, but direct and consensual pupillary light reflexes, although somewhat slow initially after birth, should be present in normal foals in the first day of life.[202] Dazzle reflexes should be present as well. A menace response is not present at birth but will develop within 1 to 2 weeks of life.[202] Even though a menace response is not present, most foals will show visual avoidance by moving their head away from a threatening gesture toward the eye.[197] The eyes should be assessed for the presence of nystagmus, and although lateral movements of the head will elicit normal physiologic nystagmus, spontaneous nystagmus with the head stationary is abnormal.[197] The suckle reflex and jaw tone should be assessed by inserting a gloved finger into the mouth. The foal should also be observed to nurse, paying close attention to swallowing movements in the pharyngeal region and observing for nasal regurgitation after nursing.

Testing the cervicofacial reflex along the neck and the cutaneous trunci reflex along the thorax will allow assessment of the long spinal reflexes. If these are abnormal, then segmental cutaneous sensation should be assessed. Further assessment of spinal reflexes is best accomplished after placing the foal into lateral recumbency, which also allows for better assessment of muscle tone in the limbs. There should be substantial extensor tone, but the limbs should be able to be flexed with persistent gentle pressure. The triceps, extensor carpi radialis, biceps, patellar, sciatic, and gastrocnemius reflexes should all be able to be reliably elicited and are more pronounced than in adults. The foal should respond to pressure on the sole of the hoof and flexion of the upper limb with an extensor response, known as the extensor thrust reflex.[197] A flexor or withdrawal reflex should be present in response to pinching of the skin of the distal limb, and most foals will exhibit a crossed extensor response by extending the opposite limb at the same time.[197] This crossed extensor reflex should be absent after the first 3 weeks of life.[200]

Although most foals with neurologic dysfunction will display generalized signs, such as abnormal behavior, seizures, depression, poor affinity for the mare, and loss of suckle, the findings of the neurologic examination can be used to assess the nature and severity of the dysfunction and to provide neuroanatomic localization of any lesions that may be present (Table 20.6).

Selected Disorders

Neonatal Encephalopathy and Hypoxic Ischemic Encephalopathy

NE in humans is a clinically defined syndrome of neonatal brain dysfunction, manifested by difficulty with the initiation and maintenance of respiration, depression of tone and reflexes, subnormal levels of consciousness, and frequently seizures.[203] NE represents a very broad categorization that encompasses any neonate with neurologic signs, regardless of etiology. This term has recently come into use in the equine literature as well.[65,185,197,204] HIE is a specific type of NE typically associated with adverse peripartum events that result in episodes of cerebral hypoxia and ischemia. A wide variety of terms have been used synonymously with HIE in reference to affected foals, including neonatal maladjustment syndrome, perinatal asphyxia syndrome, dummy foal, wanderer, and barker foal.[185,205] The clinical presentation associated with HIE in foals can range quite dramatically from mild behavioral abnormalities to severe neurologic abnormalities including central blindness, seizures, coma, and death.[206] Unfortunately these clinical signs are not specific for HIE, and diagnosis is typically made based on elimination of other potential etiologies. Indeed, the confusion associated with the nomenclature reflects the clinical challenges associated with determining the etiology of neurologic dysfunction in the neonatal foal, because it has long been recognized that many foals presenting with the clinical signs typically associated with HIE have no documented evidence of asphyxiation or a hypoxic-ischemic event.[65,207] The fact that many affected foals recover rapidly and demonstrate no neurologic sequelae, in contrast to the situation in human infants, further reinforces this dilemma.[65] There is evidence that elevations in neurosteroid concentrations may be associated with NE in some cases, particularly those with no discernible history of a hypoxic insult.[65,67,68,205,208,209] A novel "squeeze" technique has been reported primarily as an aid in foal restraint and, although only anecdotal, in time this technique may have some benefit in moderating excessive inhibitory neurosteroid concentrations and improving foal consciousness.[199]

With the introduction of the term *NE* in human medicine there has been an effort toward restricting the term *HIE* to the subset of cases of NE in which there is evidence of a hypoxic-ischemic cause, and that approach will be used here.[203] Multiple conditions have been associated with peripartum asphyxia and HIE in foals, including dystocia, induction of parturition, cesarean section, placentitis, premature placental separation, meconium aspiration, twinning, in utero infection, severe maternal illness or surgery, and postterm pregnancy.[185,210,211] Affected foals may appear normal at birth but will typically demonstrate neurologic dysfunction within the first 72 hours of life.[65] The clinical signs of HIE can be highly variable but may include behavioral changes (loss of affinity for the mare, inappropriate nursing behavior, loss of awareness of the environment, head-pressing, and abnormal vocalization), altered mentation (depression, stupor, somnolence, difficult to arouse, and coma), cranial nerve dysfunction (loss of suckle reflex, weak tongue tone, tongue protrusion, and dysphagia), and CNS dysfunction (hypotonia, tremors, hypertonia, proprioceptive deficits, central blindness, irregular respiratory patterns, opisthotonus, and seizures).[65,185,210-213] Two major categories of HIE-affected foals have been described, with Category 1 foals born normally but then developing signs within the first 48 hours of life, and Category 2 foals being abnormal from birth, usually in association with documented risk factors for HIE.[197] It is common to see multisystem involvement, because hypoxic injury will adversely affect most body systems. The gastrointestinal tract and renal system appear most susceptible, but the cardiovascular system, pulmonary system, and liver and endocrine systems may all be involved.

The pathophysiology of hypoxic injury is complex and may vary to some extent in different body systems, but the focus will be on the nervous system here. The initial insult of tissue

TABLE **20.6** **Neuroanatomic Localization Based on Results of the Neurologic Examination**

Evaluation	Pathways	Major Signs of Disorder
Behavior	Forebrain (primarily cerebrum)	Reduced affinity for dam, restlessness, head pressing, compulsive walking
Alertness	All of brain	Lethargy, stupor, semicoma, coma
Avoidance, nasal	Cranial nerve Va, pons, cerebral cortex	Facial hypoalgesia
Avoidance, visual	Cranial nerve II, thalamus, cerebral cortex	Blindness
Head position	Cranial nerve VIII, hindbrain	Head tilt, turn, body lean, walking in circles, ataxia, nystagmus
Eye position and movement	Cranial nerves III, IV, VI, and VIII, midbrain, hindbrain	Strabismus, nystagmus
Flick reflexes	Cranial nerves V and VII, pons, hindbrain	Facial paralysis, facial hypoalgesia, absent flick reflexes
Dazzle reflex	Cranial nerve II, subcortex, midbrain, cranial nerve VII	Absent dazzle reflex
Pupillary light reflex	Cranial nerve II, midbrain, cranial nerve III	Absent pupillary light reflex
Suckle	Cranial nerves V, VII, and XII, pons, hindbrain, cerebrum	Weak or absent suckle
Swallow	Cranial nerves IX and X, hindbrain	Flow of milk from the nose
Cervicofacial reflex	Cervical spinal nerves and spinal cord, cranial nerve, VII, (hindbrain)	Diminished cervicofacial reflex
Cutaneous trunci reflex	Thoracic spinal nerves and spinal cord, brachial plexus, lateral thoracic nerve	Diminished reflex caudal to spinal cord lesion
Cutaneous sensation	Peripheral nerves, spinal cord, cerebral cortex	Cutaneous hypoalgesia/anesthesia
Gait	Cranial nerve VIII, cerebellum, hindbrain, spinal cord, peripheral nerves	Ataxia
Limb strength	Spinal cord, peripheral nerves	Limb weakness at or caudal to the level of the lesion
Flexor reflex (pelvic), patella, sciatic reflexes	Spinal cord, peripheral nerves (L3-S2)	Pelvic limb weakness, diminished or absent reflexes
Flexor reflex (thoracic), biceps, triceps reflexes	Spinal cord, peripheral nerves (C6-T2)	Weakness of thoracic with or without pelvic limbs, diminished or absent reflexes
Anal/tail clamp reflex	Spinal cord, cauda equine, peripheral nerves (S2-Coccyx)	Reduced or absent anal/tail clamp reflex

From MacKay RJ. Neurologic disorders of neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:387-406, vii.

hypoxia initiates a cascade of deleterious events. First, there is a shift toward anaerobic metabolism that leads to depletion of high-energy phosphate (adenosine triphosphate [ATP]) reserves, accumulation of lactate, and a failure of cellular homeostasis.[204] Decreased activity of transcellular pumps that depend on ATP leads to intracellular accumulation of sodium, calcium, and water.[214] Membrane depolarization leads to release of the potent excitatory amino acid glutamate, which accumulates in the extracellular space. Glutamate then acts on the *N*-methyl-D-aspartate (NMDA) receptor, opening NMDA channels, potentiating calcium influx into the neurons, and contributing to neuronal injury.[215] This cascade of events results in the development and perpetuation of excitotoxicity, which ultimately leads to neuronal cell death and brain injury.[204,214] Hypoxia and subsequent reperfusion also initiate the increased production of ROS and NO, ultimately resulting in the process known as *reperfusion injury* and leading to further tissue damage via cell death and activation of apoptotic cascades.[204,214] Tissue injury initiates an inflammatory response, leading to increased local blood flow and vascular permeability, which may result in the development of edema. Inflammatory cell infiltration leads to further tissue injury and upregulation of ROS production.[185,216] Increased concentrations of neurosteroids have been documented in affected foals, but their role in the pathophysiology of HIE is unclear because they may actually have a neuroprotective effect.[65,66,68] In situations where the degree or duration of hypoxia is not overwhelming, a biphasic pattern of injury may be observed in which the initial acute phase of injury is stabilized, followed by an ongoing latent phase of injury that results in more severe clinical deterioration several hours later.[214]

The diagnosis of HIE is challenging because of the substantial overlap between the clinical signs associated with HIE and other causes of NE, as previously discussed. Unfortunately

there is no definitive test for HIE, so diagnosis is typically made based on historical information, clinical presentation, and the elimination of other potential causes. Additional diagnostics that are used in human infants include electroencephalography (EEG) as well as imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI).[204] An EEG is difficult to accomplish in the unsedated foal, and interpretation can be confounded by patient movement or sedation, rendering this modality of limited use in foals. Brainstem auditory evoked response testing is widely used in human neonatal ICUs to aid in the assessment of CNS function and the diagnosis of HIE, and this technique has been described and validated in foals.[217,218] A recent report described the MRI findings associated with a presumptive case of NE in a foal, and the increasing availability of MRI in equine referral centers may render this modality of utility in the future.[219] There has been tremendous interest in identifying biomarkers of HIE in human infants, and two potential biomarkers of HIE have been investigated in foals.[206,220] A recent study measured the plasma concentration of the phosphorylated axonal forms of neurofilament H (pNF-H) and ubiquitin C-terminal hydrolase 1 (UCHL1) and found that the diagnostic performance of UCHL1 was significantly higher than that of pNF-H, with the sensitivity and specificity of UCHL1 for diagnosis of neonatal HIE reported as 70% and 94%, respectively.[206] This test is not available for clinical use at this time, however.

Prevention of HIE is difficult because of the occult nature of the injury in many cases, but all efforts should be made to identify mares at risk of maternal or placental disease during pregnancy and to implement appropriate treatment and monitoring. Close observation of at-risk mares during parturition may allow for early intervention and assistance, if needed. Foals should be closely observed during and after parturition for signs of distress or neurologic dysfunction and appropriate supportive care or resuscitation provided.[185] Foals that exhibit seizure activity within the first 24 hours of hospitalization have been shown to have poorer outcomes than foals that do not exhibit seizure activity.[205] The prognosis for survival for foals with uncomplicated disease that survive the first 5 days of life and demonstrate neurologic improvement during that time appears to be good.[185]

Sepsis-Associated Encephalopathy

Brain dysfunction occurring as a result of severe systemic inflammation secondary to infection, but not involving overt CNS infection or other types of encephalopathies, has been termed *sepsis-associated encephalopathy* (SAE).[221] This syndrome has also been referred to as septic encephalopathy and sepsis-associated brain dysfunction.[222] SAE is thought to represent a neglected cause of neurologic dysfunction in human patients because of the difficulty in diagnosing a condition that has no precise clinical or biological markers, and the situation is likely similar in NE of foals.[223] In humans the clinical spectrum of SAE may include signs ranging from mild behavioral abnormalities and altered sleep states to severe disturbances of consciousness and even coma.[224] The pathophysiology of SAE appears to involve direct neuronal cell injury, mitochondrial and endothelial dysfunction, disturbances of neurotransmission, and derangements of neuronal calcium homeostasis.[223,224] Given the substantial overlap between the presentations of HIE and SAE, and the frequent involvement of sepsis in the critically ill foal population, SAE should be considered as a potential cause of NE in foals.

Seizure Disorders

Seizures are a common manifestation of neurologic dysfunction in human neonates and in foals.[65,225] Seizures in foals can be idiopathic, the result of primary CNS disorders, or secondary to numerous conditions involving other body systems or diffuse disease. Seizures are defined as abnormal, stereotyped, and paroxysmal alterations in neurologic function and may include behavioral changes, motor dysfunction, autonomic dysfunction, and loss of consciousness.[226] Seizure activity indicates neurologic dysfunction of the forebrain associated with abnormal electrical activity. Neonates appear to be at increased risk of seizure activity because of the relative excitability of the developing brain combined with the high risk of brain injury in the neonatal period.[227] Diagnosis of seizure activity is primarily based on clinical signs but can be challenging because of the subtle signs in some cases, the potential confounding effects of other conditions altering patient behavior, neurologic function, or level of consciousness. Subtle signs may include abnormal eye movements, tremors, excessive stretching, excessive extensor tone, hyperesthesia, and apneustic breathing, whereas overt signs of seizures typically include rapid nystagmus, paddling, hyperextension, and excessive movements of the mouth.[198] Unexplained physical trauma may represent evidence of unobserved seizure activity. Confirmation of seizure activity is achieved by EEG examination, but this can be challenging in foals. Specific treatment may not be required in individuals with very subtle or rare signs of seizure activity but is clearly indicated in patients with repeated, generalized seizure activity. Foals that exhibit seizure activity within the first 24 hours of hospitalization have been shown to have poorer outcomes than foals that do not exhibit seizure activity.[205]

Bacterial Meningitis

Bacterial meningitis is an uncommon condition but appears to be more frequent in foals than adults.[228] It has been reported to occur in 2.6% to 10% of foals suffering from sepsis and is associated with high mortality rates.[25,176,228,229] The most common clinical findings in foals with bacterial meningitis are lethargy, weakness, recumbency, decreased suckling reflex, abnormal pupillary light reflexes, hyperesthesia, cervical pain, fever, blindness, seizures, and coma.[228,230,231] Clinicopathologic findings may include FTPI, neutropenia or neutrophilia, and hyperfibrinogenemia and most often reflects systemic inflammation related to sepsis.[197,228,230,231] Definitive diagnosis is by means of CSF analysis, with neutrophilic pleocytosis (total nucleated cell count >7 cells/μL) and increased total protein (TP) concentration (TP >120 mg/dL) being present in most cases.[232,233] Cellular degeneration is frequently observed in the CSF, along with intracellular bacteria.[197,233] Meningitis is most commonly associated with the same types of bacteria associated with neonatal sepsis, and *Escherichia coli, Actinobacillus* spp., *Klebsiella* spp., *Streptococcus* spp., and *Salmonella* spp. are the most commonly reported.[228,233,234] Treatment depends on intensive antimicrobial therapy using drugs capable of achieving therapeutic concentrations within the CNS and is ideally guided by culture results.[235] Drugs such as penicillin, ceftiofur, and the aminoglycosides are unlikely to be effective because of poor penetration, so consideration should be given to other treatments such as ceftriaxone, cefepime, cefotaxime, imipenem, chloramphenicol, and rifampin (as an adjunct).[235] Because

much of the damage occurring in the CNS in meningitis is secondary to the inflammatory response, there is a rationale for using antiinflammatory therapy in these cases. The use of corticosteroids as adjunct therapy in human patients has been associated with a reduction in neurologic sequelae but did not alter mortality.[236,237] The prognosis for survival in foals with appropriate treatment is fair to poor, depending on the severity of the presenting signs.[228]

Trauma

Trauma should always be considered when evaluating the foal with acute onset of neurologic signs. Brain trauma may be associated with blunt force trauma in the region of the frontal or parietal bones or may be secondary to trauma to the poll or temporal region secondary to the foal flipping over backward.[197] Vertebral injury can occur as well and may be associated with the foal running into an immobile object. Outward signs of trauma may not be obvious, and imaging studies may be required for confirmation. Examination may reveal superficial abrasions, pain on palpation of affected areas, soft tissue swelling, crepitus, or bleeding from the nares or ears. Treatment is supportive in nature along with stabilization of bony injuries if possible. Antiinflammatory and antioxidant therapy may be of some benefit, but corticosteroid administration is not recommended. Appropriate antimicrobial therapy is indicated when there is the possibility that the CNS may have been contaminated.

Botulism

Botulism is a syndrome of flaccid paralysis caused by the neurotoxins produced by *Clostridium botulinum,* colloquially known as "shaker foal syndrome." Most equine cases are associated with toxin types B and C, although type A cases have been reported.[238] The syndrome in foals is thought to be toxicoinfectious in nature, in which the foal ingests spores from the environment that germinate in the gastrointestinal tract and spread toxin, as opposed to the more common form of "forage poisoning" associated with ingestion of a preformed toxin as seen in adults.[239] Botulism toxin acts presynaptically at the neuromuscular junction by inhibiting the release of the neurotransmitter acetylcholine. Severely affected foals may be found dead, but more commonly there is a sudden development of weakness, trembling, and dysphagia in a previously healthy foal.[238] Progressive deterioration is typically observed, even with appropriate treatment, and respiratory paralysis is the cause of death in most cases. Diagnosis is typically presumptive based on the history and clinical presentation because confirmation requires demonstration of spores and/or toxin in the feces of affected animals, which can be difficult. A mouse bioassay has been validated for the diagnosis of botulism in horses and foals.[240] This assay is highly specific in foals, with specificity and PPV reported as 100%, but it exhibits low sensitivity (53%) and NPV (52%).[240] Treatment is based on the administration of antitoxin and the provision of supportive care. Foals that become recumbent will likely die unless they receive ventilatory support in the form of mechanical ventilation, which may be required for as long as 2 weeks.[238] With early and appropriate care the prognosis for survival in cases of types B and C botulism is reported to be good.[238] Type A botulism has been associated with more severe clinical signs and a higher case fatality rate, but a recent report described the successful treatment of a foal affected by type A botulism.[241]

Tetanus

Tetanus is a syndrome of spastic paralysis caused by the neurotoxin tetanospasmin produced by *C. tetani.* Although rare because of the widespread use of tetanus toxoid vaccines, recent cases have been reported in foals.[242,243] This syndrome usually affects foals over 7 days of age and is associated with the development of an anaerobic site of infection, reportedly most often within the umbilical remnants.[244] Tetanospasmin acts to prevent the release of the inhibitory neurotransmitter GABA by spinal interneurons, leading to disinhibition of spinal motor neurons, resulting in excessive motor activity and spastic paralysis.[245] The primary clinical signs are rigidity, excessive autonomic activity, and episodic muscle spasms, which lead to trismus, facial spasm, third-eyelid protrusion, and dysphagia. Progression leads to recumbency, and death is usually caused by respiratory failure. Treatment of tetanus depends on the administration of tetanus antitoxin, supportive care, control of muscle spasms, and source control.[242,246] Control of muscle spasms is often accomplished using benzodiazepines, either administered as needed or by continuous rate infusion (CRI). Magnesium sulfate represents an affordable and viable option to benzodiazepines in humans and may be a reasonable option in foals as well.[197] Source control may involve surgical excision or drainage of the site of infection in combination with antimicrobial therapy. Penicillin has been the standard treatment, but metronidazole may also be used. The prognosis for survival is good in cases that do not progress to recumbency, but it is guarded to poor once recumbency occurs, although recent success has been reported.[242,243]

Metabolic Encephalopathies

There are a number of metabolic derangements that have the potential to adversely affect neurologic function and potentially cause neurologic injury in foals. These include hypoglycemia, hyponatremia and hypernatremia, hypocalcemia, hyperbilirubinemia, and hyperammonemia. Hypoglycemia is common in critically ill foals, and severe hypoglycemia is associated with nonsurvival to hospital discharge.[131] Persistent, severe hypoglycemia is associated with neuronal degeneration in both the central and peripheral nervous systems, but interestingly this phenomenon is only seen in human patients receiving insulin therapy or suffering from hyperinsulinemias.[247,248] Although short-term hypoglycemia may not cause permanent injury, it certainly can cause neurologic dysfunction and adversely affects other body systems and should therefore be addressed with dextrose supplementation.[249,250]

Sodium disorders are fairly common in critically ill foals, but they are not typically associated with neurologic dysfunction unless the derangements are very severe or there are dramatic, acute changes in the serum sodium concentration. These rapid changes in sodium concentration cause dramatic fluid shifts in the tissues of the CNS, with hyponatremia potentially being associated with the development of cerebral edema, whereas hypernatremia may cause an osmotic demyelination syndrome.[250] Hyponatremia is more common than hypernatremia, and neurologic signs are most typically associated with sodium concentrations less than 120 mEq/L.[249,251] The neurologic manifestations of hyponatremia may include an abnormal stance or gait, depression, blindness, or seizures.[252,253] Treatment of these disorders involves correction of the underlying whole body sodium/water imbalances, because hyponatremia represents a free-water excess and hypernatremia a free-water deficit.[254] Hyponatremia should be addressed

by administering fluids that are relatively hypertonic compared with the plasma osmolality, and although the literature suggests that initial correction should be rapid followed by slower correction, a more conservative approach may be appropriate in foals.[249] Hypernatremia should be addressed using fluids that are relatively hypotonic but should be gradual (<0.5 mEq/L/h).[249]

Hypocalcemia is often asymptomatic but can be associated with tetany and seizures.[254] Rapid decreases in the ionized calcium concentration appear more likely to be associated with clinical signs in critically ill foals.[250] A syndrome of hypocalcemic seizures has been described in young foals, typically from 2 to 5 weeks of age.[255,256] This condition is thought to be caused by primary hypoparathyroidism.[249,256]

Bilirubin is capable of crossing the blood-brain barrier, especially when plasma concentrations of bilirubin are very high and in neonates where the blood-brain barrier is more permeable. Bilirubin is neurotoxic and can cause neurologic dysfunction and irreversible brain damage, which is a clinical syndrome termed *kernicterus*.[257,258] The most important cause of clinical icterus in equine neonates is neonatal isoerythrolysis (NI). Treatment of NI is discussed elsewhere in this chapter, but primarily it consists of supportive care and blood transfusions. These treatments do not address the hyperbilirubinemia, which can only be reduced using therapeutic plasma exchange.[258]

Hyperammonemia has been associated with neurologic dysfunction in foals and adult horses, and the clinical signs associated with hyperammonemia include depression, blindness, aimless wandering, head pressing, ataxia, circling, recumbency, and death.[259-262] Hyperammonemia may develop secondary to hepatic insufficiency, portosystemic shunts, or increased gastrointestinal production (intestinal hyperammonemia).[259-262] A disorder suspected to represent hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, a genetic defect in the urea cycle resulting in persistent hyperammonemia, has been reported in Morgan foals.[263] Intestinal hyperammonemia is typically seen in association with clinical signs of gastrointestinal disease.[261]

Congenital Disorders

Hydrocephalus

Hydrocephalus is a condition resulting from impaired drainage of CSF caused by congenital defects or inflammation that results in the compression of the brain caused by increased intracranial pressure.[264,265] This condition is considered to be uniformly fatal and is rare in horses but may be more frequent in the Friesian breed.[265] Diagnosis is typically presumptive and confirmed on postmortem examination, but CT and MRI can be used for definitive antemortem diagnosis if necessary.[266]

Occipitoatlantoaxial Malformation

Foals born with occipitoatlantoaxial malformation typically exhibit paresis and ataxia of all four limbs, but in some cases foals may be born dead or comatose.[197] Foals may have a head tilt or abnormal head carriage, movement of the head may reveal a clicking sound, and neurologic deficits may range from inapparent to severe enough to result in quadriplegia.[197,267] The condition is familial in the Arabian breed but may occur in other breeds as well.[267] Diagnosis is typically confirmed radiographically, although contrast myelography, CT, or MRI may be useful in some cases.[268,269] Foals exhibiting clinical signs are typically euthanized because of the poor long-term prognosis.

Cerebellar Abiotrophy

Equine cerebellar abiotrophy is a neurodegenerative condition affecting the cerebellum, which results in ataxia, head tremors, and loss of equilibrium.[270,271] Cerebellar abiotrophy is relatively rare in horses, and, although it can occur in multiple breeds, it is most common in Arabian horses. It is inherited as an autosomal recessive trait, with clinical signs generally detectable between 4 weeks and 6 months of age.[270-272] Diagnosis is typically presumptive based on breed association and clinical presentation because definitive antemortem diagnosis is difficult.

Epilepsy

A syndrome of juvenile idiopathic epilepsy (JIE) has been described in Arabian foals of Egyptian lineage.[273,274] This syndrome is characterized by recurrent tonic-clonic seizures and resembles benign-familial neonatal convulsion syndrome in humans.[275] JIE exhibits an early onset of clinical signs in the first days to months of life but appears to be self-limiting and resolves by 1 to 2 years of age.[273,275]

Narcolepsy and Cataplexy

Narcolepsy in the horse is characterized by paroxysmal sleep attacks, and affected animals may remain standing, whereas cataplexy represents the complete loss of muscle tone combined with areflexia leading to recumbency.[276] Narcolepsy without cataplexy has been described in Thoroughbred, Warmblood, and Icelandic horses, and in some cases there may be a familial component.[276,277] Narcolepsy with cataplexy is very rare, but it has been described in horses.[276,278]

Treatment of Neonatal Encephalopathy

Because of the broad range of disease processes associated with the development of NE in foals, there is no single treatment strategy. This discussion will focus on treatments directed toward the prevention of CNS injury and normalization of nervous system function. Foals with NE are typically suffering from severe systemic inflammation as well, and specific treatment recommendations for supportive care; infection control; correction of metabolic disorders; and normalization of cardiovascular, pulmonary, gastrointestinal, and renal function in critically ill foals is covered in detail elsewhere in this chapter.

The first objective is stabilization of the patient systemically, because the restoration and maintenance of CNS perfusion is critical in ensuring adequate delivery of oxygen and glucose. IV fluid therapy is often required to correct hypovolemia and support cardiovascular function, but vasopressors and inotropes may be required to normalize blood pressure. It is important to avoid overhydration or hypertension, however, because these may potentiate CNS injury.[185,279] Normalization of blood glucose concentrations is important as well, but care must be taken to avoid hyperglycemia, because critically ill foals may be intolerant of dextrose infusions. Intranasal oxygen supplementation is recommended in weak or recumbent foals, or those with documented arterial hypoxemia, to support oxygen delivery, and blood transfusions may be needed in anemic patients for the same reason.

The second objective is to control seizure activity, if present. Specific treatment may not be required in individuals with very subtle or rare signs of seizure activity, but it is clearly

TABLE **20.7 Drugs Used for the Control of Seizures in Foals**

Drug	Dosage	Route	Frequency	Comments
Diazepam	0.1–0.4 mg/kg	IV	As needed	Short-term seizure control
Midazolam	0.04–0.1 mg/kg 0.02–0.06 mg/kg/h	IV IV	As needed CRI	Short-term seizure control For persistent seizures
Phenobarbital	2–10 mg/kg 3–10 mg/kg	IV PO	12 h 12 h	For persistent seizures Monitor serum concentrations
Pentobarbital	2–10 mg/kg	IV	Single dose	For status epilepticus—use caution
Phenytoin	1–5 mg/kg 1–5 mg/kg	IV, PO PO	4 h, up to 6 doses 12 h	—
Potassium bromide	10 mg/kg 60 mg/kg	PO PO	8 24 h	Monitor serum concentrations

CRI, Continuous rate infusion; *IV,* intravenously; *PO,* orally.
From Wilkins PA. Disorders of foals In: Reed SM, Bayly WM, Sellon DC, eds. *Equine internal medicine*. St. Louis, MO: Saunders Elsevier; 2010; Morresey PR. Neurological conditions and seizure management. *AAEP Focus on the First Year of Life*. 2014;29-35; Wong D, Wilkins PA, Bain FT, et al. Neonatal encephalopathy in foals. *Comp Contin Educ Pract Vet*. 2011;33:E5; Magdesian KG. Foals are not just mini horses. In: Cole C, Bentz B, Maxwell L, eds. *Equine pharmacology*. Oxford, UK: Wiley-Blackwell; 2015:99-117.

indicated in patients with repeated, generalized seizure activity. Benzodiazepines (diazepam and midazolam) represent the first-choice therapy for control of acute seizures in foals because of their rapid onset of action and minimal depressive effects (Table 20.7).[30,198] Bolus IV doses are used for the control of single seizure episodes, but CRI of midazolam can be used to control persistent seizures. Other options for the control of persistent seizures include phenobarbital, pentobarbital, and potassium bromide. Phenobarbital can cause substantial CNS depression when first administered but is generally well tolerated, even with prolonged use. Serum phenobarbital levels should be monitored when used long term to ensure that concentrations remain within the therapeutic range, which has been reported to be 5 to 30 μg/mL in foals.[280] Pentobarbital should only be used in cases with status epilepticus that cannot be controlled with other medications and is associated with substantial risk caused by profound respiratory and cardiovascular depression. Phenytoin has unpredictable kinetics and is not widely used but has been suggested for anticonvulsant use in foals. Potassium bromide may be useful for long-term maintenance in foals with epilepsy because it has fewer side effects than phenobarbital and is well tolerated. Reported effective plasma concentrations of bromide in other species are 70 to 240 mg/dL, but this has not been validated in foals.[281]

The next goal is to control ongoing CNS injury and prevent further damage. Hypothermia is considered the standard of care in the treatment of human neonates suffering from HIE. This involves cooling the patient to 92.3 to 95.0°F using whole body hypothermia, although selective head-cooling approaches can be used as well.[214] A recent systematic review confirmed that therapeutic hypothermia is beneficial in human infants suffering from HIE, because hypothermia reduced mortality without increasing long-term neurologic disability in survivors.[282] There are some complications associated with hypothermia, most notably overcooling, skin problems, altered drug metabolism, and an increased risk of seizure during rewarming.[283] The logistics of providing therapeutic hypothermia in foals, including the appropriate techniques of cooling, patient selection, and duration of cooling remain, is to be defined before clinical application of this approach in equine medicine.

A number of pharmacologic approaches to neuroprotection have been used empirically in foals with HIE, but their use has not been validated (Table 20.8). One of the most widely used drugs for decades has been dimethyl sulfoxide (DMSO), which has been used because of its purported free radical scavenging and antiinflammatory effects, which are perhaps reinforced by ease of administration and low cost.[284] DMSO has osmotic and diuretic effects, which may be helpful in reducing intracranial pressure, and reportedly blocks sodium channel activation, suppresses calcium influx and prevents glutamate excitotoxic cell death.[284] As promising as this appears, there is actually little to no scientific evidence of efficacy regarding DMSO treatment of HIE, and clinical consensus on its use is lacking.[30,211] Mannitol has been administered to reduce cerebral edema, but evidence of efficacy is also lacking for this treatment modality, and it is not commonly used.[185] Magnesium sulfate treatment has been of interest because of its NMDA-receptor antagonist effects, and magnesium may also stabilize cell membranes, inhibit free radical production, and reduce secondary CNS inflammation and associated injury.[285] The use of magnesium sulfate in foals with HIE has been reported, and treatment appears to be well tolerated, although excessive doses can cause muscular weakness and hyotension.[279] No data are available regarding efficacy in foals and, although there are some studies with encouraging results, this therapeutic approach currently remains preclinical in human infants with HIE.[285,286] Pentoxifylline, a phosphodiesterase inhibitor, has antiinflammatory and immune-modulating effects and may improve local tissue perfusion.[287] It has been suggested that pentoxifylline may inhibit TNF-α production in foals with NE.[185] Antioxidant administration may be of some benefit in addressing CNS inflammation, and vitamins C and E and thiamine (vitamin B_1) have all been administered to foals with HIE.[65] Allopurinol is an antioxidant that has shown promise in human infants, but it remains preclinical at this time.[288] Allopurinol administration has been suggested for foals with HIE, but there are no reports of its use.[185] A myriad of additional treatments have been investigated in human neonatal HIE, and some of those that appear promising include inhaled xenon as an antiexcitotoxic agent, melatonin as an antioxidant, erythropoietin as a growth factor, and stem cell therapy.[204,289,290]

RESPIRATORY DISORDERS

Lower respiratory tract disease is common in foals and accounts for substantial morbidity and mortality. Foals are at risk for the development of respiratory disease caused by

TABLE **20.8 Drugs Used for the Treatment of Central Nervous System Disorders**

Drug	Dosage	Route	Frequency	Comments
DMSO	0.1–1 g/kg as a 10% solution	IV	12–24	Hemolysis, dehydration, OSHA restrictions
Mannitol	0.25–2.0 g/kg as a 20% solution over 15–20 min	IV	12–24	Dehydration
Magnesium sulfate	0.05 mg/kg/h (loading) 0.025 mg/kg/h	IV	CRI	Can precipitate other fluids
Pentoxifylline	10 mg/ kg	PO	12	—
Gabapentin	3–5 mg/kg	PO	6–8	—
Allopurinol	44 mg/kg	PO	Once in first 4 h	—
Vitamin E	5000 IU	PO	24	—
Vitamin C	100 mg/kg	IV, PO	24	—
Thiamine	1–20 mg/kg	IV	Added to fluids	—

CRI, Continuous rate infusion; *DMSO,* dimethyl sulfoxide; *IV,* intravenously; *OSHA,* Occupational Safety and Health Administration; *PO,* orally.
From Wilkins PA. Perinatal asphyxia syndrome. In: Sprayberry KA, Robinson NE, eds. *Robinson's current therapy in equine medicine*. St. Louis, MO: Elsevier Saunders; 2015:732-736; Wong D, Wilkins PA, Bain FT, et al. Neonatal encephalopathy in foals. *Comp Contin Educ Pract Vet*. 2011;33:E5.

complex interactions between innate immunologic factors and a number of risk factors. Immunologic factors include possible FPTI, delayed endogenous immunoglobulin production, and potentially impaired immunologic responses. Risk factors in neonates include systemic sepsis, congenital abnormalities, meconium aspiration, milk aspiration, and birth trauma. Risk factors in older foals include the stresses of weaning, sales preparation, and transport, as well as confinement in crowded or dusty conditions that result in heavy exposure to potential respiratory pathogens.

Respiratory Distress

There are several syndromes associated with respiratory distress in the foal, and these syndromes can result from a variety of initiating causes. The defining characteristics change with age, caused by the normal maturation of pulmonary function in the newborn foal, and to some extent caused by the changing nature of the insults that result in respiratory distress. The finding of acute respiratory distress immediately after birth is likely associated with extrapulmonary disorders causing upper respiratory obstruction, such as bilateral choanal atresia, stenosis of the nares, severe laryngeal edema or collapse, dorsal displacement of the soft palate, subepiglottic cysts, or severe congenital pulmonary abnormalities.[30] Congenital cardiac anomalies, such as malpositioning of the great vessels, resulting in severe right-to-left shunts may also be involved.[30]

Acute Lung Injury and Acute Respiratory Distress Syndrome

When dealing with respiratory distress in foals beyond the immediate postpartum period the situation can become confusing because of the broad range of etiologies involved and the broad spectrum of the clinical syndromes. Although this has been appreciated in human and veterinary medicine for many years, it is only in the past decade that a consensus approach to this situation has been developed in veterinary medicine.[291] The terms *acute lung injury* (ALI) and acute RDS (ARDS) were originally developed in human medicine to refer to a syndrome of respiratory failure associated with noncardiogenic pulmonary edema, decreased pulmonary compliance, and ventilation/perfusion mismatching.[292,293] These syndromes are associated with an exaggerated and self-perpetuating inflammatory response, which results in severe tissue damage within the lungs. Protein-rich edema fluid accumulates within the alveoli and interstitium, resulting in impairment of gas exchange leading to hypoxemia.[294] ALI and ARDS are not primary disease syndromes and always occur secondary to other disease processes. Risk factors for ALI and ARDS in human patients include processes resulting in both direct pulmonary injury (pneumonia, aspiration of gastric contents, smoke inhalation, pulmonary contusion, and drowning) and indirect pulmonary injury (nonpulmonary sepsis, pancreatitis, multiple transfusions, and major trauma).[294]

Accurate assessment of respiratory function in the neonate requires arterial blood gas analysis, and the ready availability of portable blood gas analyzers has greatly improved access to this important diagnostic tool. Management of critically ill foals without this information is extremely difficult, because it is important in initial evaluation, formulation of an appropriate treatment plan, monitoring the response to treatment, and prognostication. Foals are most readily sampled in lateral recumbency using the lateral metatarsal artery and the brachial or transverse facial artery. Sampling should be performed quickly after placing the foal in lateral recumbency, and the patient should be returned to a sternal or standing position as soon as possible, because positioning in lateral recumbency has a substantial negative impact on arterial oxygenation (10–14 mm Hg). Diagnostically this impact can be addressed by using appropriate reference ranges derived from foals sampled in lateral recumbency (Table 20.9). The other major element that has to be factored in to interpretation of arterial blood gas data in neonatal foals is the changes in pulmonary function that occur over the course of the first several days of life, because newborn foals have a small degree of shunt fraction that renders them relatively hypoxemic and less responsive to oxygen supplementation than adults. This phenomenon is also addressed in Table 20.9.

The most common arterial blood gas abnormalities identified in neonatal foals are hypoxemia with normocapnia or hypocapnia and hypoxemia with hypercapnia. Hypoxemia can result from several mechanisms: hypoventilation, ventilation/perfusion mismatching, impaired diffusion, intrapulmonary or extrapulmonary (cardiac) shunts, and decreased

TABLE **20.9** **Normal Arterial Blood Gas Values for Foals**[a]

Gestational Age	Postnatal Age	Pa_{O_2}	Pa_{CO_2}	pH
Term	2 min	56.4 ± 2.3	54.1 ± 2.0	7.31 ± 0.02
	15 min	57.5 ± 3.6	50.4 ± 2.7	7.32 ± 0.03
	30 min	57.0 ± 1.8	51.5 ± 1.5	7.35 ± 0.01
	60 min	60.9 ± 2.7	47.3 ± 2.2	7.36 ± 0.01
	2 h	66.5 ± 2.3	47.7 ± 1.7	7.36 ± 0.01
	4 h	75.7 ± 4.9	45.0 ± 1.9	7.35 ± 0.02
	12 h	73.5 ± 3.0	44.3 ± 1.2	7.36 ± 0.02
	48 h	74.9 ± 3.3	46.1 ± 1.1	7.37 ± 0.01
	4 days	81.2 ± 3.1	45.8 ± 1.1	7.40 ± 0.01
	7 days	86.9 ± 2.2	46.7 ± 1.1	7.37 ± 0.01
Premature	0.5–11 hours	53.7 ± 1.5	55.3 ± 3.6	7.21 ± 0.05

Adapted from Wilkins PA: Disorders of foals. In: Reed SM, Bayly WM, Sellon DC, eds. *Equine Internal Medicine*. St. Louis, MO: Saunders Elsevier; 2010:1311-1363; Stewart JH, Rose RJ, Barko AM: Respiratory studies in foals from birth to seven days old. *Equine Vet J*. 1984;16:323-328; Rose RJ, Rossdale PD, Leadon DP: Blood gas and acid-base status in spontaneously delivered, term-induced and induced premature foals. *J Reprod Fertil Suppl*. 1982;32:521-528.

concentration of oxygen in the inspired air (high altitude or inappropriate ventilator setting). Weak, recumbent neonatal foals will frequently suffer from hypoventilation, whereas the presence of intrapulmonary inflammation or surfactant deficiency will cause substantial ventilation/perfusion mismatching with intrapulmonary shunting of blood. Severe inflammation or pulmonary edema can cause diffusion impairment, whereas extrapulmonary shunts are most commonly associated with persistent fetal circulation or cardiac anomalies. Foals suffering from pneumonia typically show profound hypoxemia initially, but the development of hypercapnia and respiratory acidosis indicates substantial worsening of pulmonary function and is associated with a poorer prognosis for survival.[111,295]

ALI in human patients is characterized by an acute onset, the presence of bilateral pulmonary infiltrates on thoracic radiographs, a decreased ratio of the partial pressure of oxygen in arterial blood (Pa_{O_2}) to the fraction of inspired oxygen (Fi_{O_2}) of ≤300 mm Hg, and a pulmonary arterial wedge pressure of less than 18 mm Hg or the absence of clinical evidence of left atrial hypertension.[292] ARDS represents a more severe degree of dysfunction and is characterized by the same criteria but with a lower Pa_{O_2}/Fi_{O_2} ratio of ≤200 mm Hg.[292] Similar terminology and definitions have been proposed for foals suffering from respiratory distress.[291] Multiple classifications have been defined, including neonatal equine RDS (NERDS), equine neonatal ALI (EqNALI) and equine neonatal ARDS (EqNARDS), and veterinary ALI (VetALI) and veterinary ARDS (VetARDS).[291] The diagnostic criteria for these syndromes are provided in tabular form (Boxes 20.3–20.5).

Although the term *NERDS* is new, the syndrome of severe, progressive respiratory distress in foals within the first 24 hours of life has been recognized for at least 50 years.[5] NERDS is very similar to infant RDS in human infants, which is caused by primary surfactant deficiency associated with premature delivery.[291] Surfactant has three primary roles within the lung: to prevent atelectasis at the end of expiration, to increase

BOX 20.3 **Definition of Neonatal Equine Respiratory Distress Syndrome**

Etiology: Primary surfactant deficiency caused by the failure of final fetal pulmonary surfactant metabolism maturation. Diagnosis requires meeting all criteria listed next:

1. Persistent hypoxemia in combination with progressive hypercapnia
   a. Hypoxemia defined as Pa_{O_2} <60 mm Hg with the foal breathing room air and in lateral recumbency
2. The presence of at least one of three appropriate risk factors:
   a. Very early gestational age: less than 290 days' gestational age or less than 88% of the average of the dam's previous gestation lengths
   b. Induction of parturition
   c. Caesarean section
3. Failure to develop normal immediate postpartum respiratory patterns: development/persistence of paradoxic breathing over the first several hours of life, persistent tachypnea
4. Ground glass appearance of lateral thoracic radiographs at ≤24 hours of age (standing or lateral recumbency)
5. Absence of evidence of fetal inflammation
   a. Normal white blood cell count, differential, and fibrinogen concentration for gestational age
6. Congenital cardiac disease ruled out as a cause of hypoxemia

Adapted from Wilkins PA, Otto CM, Baumgardner JE, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: the Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine. *J Vet Emerg Crit Care*. 2007;17:333-339.

pulmonary compliance, and to facilitate recruitment of collapsed airways.[296] Surfactant deficiency leads to progressive atelectasis and decreased pulmonary compliance, worsening of ventilation/perfusion mismatching caused by intrapulmonary and possibly extrapulmonary shunting of blood, hypoventilation, and increased work of breathing. If not addressed the end result is progressive hypoxia and hypercapnia and ultimately respiratory failure.[296]

Treatment of NERDS is challenging, because the primary interventions used in human infants are not readily available in equine medicine (exogenous surfactant) or are only available in tertiary care referral centers (mechanical ventilation). Given the benefits of surfactant therapy in human respiratory distress syndrome (RDS) patients,[297] this intervention is of great interest in affected foals, but the author's personal experience and the limited references to this therapy in the literature suggest that the effect of this therapy has a short duration and needs to be repeated frequently, making it difficult to justify the significant expense, if surfactant is even available.[298,299] Interestingly, one of the primary changes in the management of human neonates with RDS has been driven by the recognition that mechanical ventilation via endotracheal intubation, although life-saving in many cases, is also associated with the potential for serious acute complications and long-term pulmonary dysfunction.[300] The acute complications may include trauma to the upper respiratory tract associated with intubation, increased risk of lower respiratory tract infections, pulmonary barotrauma

BOX 20.4 Definition of Veterinary Acute Lung Injury and Acute Respiratory Distress Syndrome

Must meet at least one each of the first four criteria; criterion five is a recommended but optional measure.

1. Acute onset (<72 hours) of tachypnea and labored breathing at rest
2. Known risk factors
3. Evidence of pulmonary capillary leak without increased pulmonary capillary pressure[a] (any one or more of the following):
   a. Bilateral/diffuse infiltrates on thoracic radiographs (more than one quadrant per lobe)
   b. Bilateral dependent density gradient on CT
   c. Proteinaceous fluid within the conducting airways
   d. Increased extravascular lung water
4. Evidence of inefficient gas exchange (any one or more of the following):
   a. Hypoxemia without PEEP or CPAP and known Fio_2
      i. Pao_2/Fio_2 ratio
      ii. ≤300 mm Hg for VetALI
      iii. ≤200 mm Hg for VetARDS
      iv. Increased alveolar-arterial oxygen gradient
      v. Venous admixture (noncardiac shunt)
   b. Increased "dead-space" ventilation
5. Evidence of diffuse pulmonary inflammation
   a. Transtracheal wash/bronchoalveolar lavage sample neutrophilia
   b. Transtracheal wash/bronchoalveolar lavage biomarkers of inflammation
   c. Molecular imaging (PET)

[a]No evidence of cardiogenic edema (one or more of the following): PAOP <18 mm Hg (adult horse). No clinical or diagnostic evidence supporting left heart failure, including echocardiography.

CPAP, Continuous positive airway pressure; *CT,* computed tomography; *Fio_2,* fraction inspired oxygen; *PAOP,* pulmonary artery occlusion pressure; *PEEP,* positive end expiratory pressure; *PET,* positron emission tomography; *VetARDS,* veterinary acute respiratory distress syndrome; *VetALI,* veterinary acute lung injury.

From Wilkins PA, Otto CM, Baumgardner JE, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: the Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine. *J Vet Emerg Crit Care.* 2007;17:333-339.

BOX 20.5 Definition of Equine Neonatal Acute Lung Injury/ Respiratory Distress Syndrome

Postnatal Age	Normal Pao_2 (mm Hg)	Normal Pao_2/Fio_2 Ratio (mm Hg)	NALI Pao_2/Fio_2 Cutoff (mm Hg)	NARDS Pao_2/Fio_2 Cutoff (mm Hg)
60 min	60.9 ± 2.7	>300	<175	<115
12 h	73.5 ± 3.0	>350	<200	<140
24 h	67.6 ± 4.4	>350	<200	<140
48 h	74.9 ± 3.3	>350	<200	<140
4 days	81.2 ± 3.1	>400	<250	<160
7 days	90.0 ± 3.1	>430	<280	<190

This is for VetALI/VetARDS but adheres to the following age-dependent adaptation of the Pao_2/Fio_2 ratio in term foals in lateral recumbency breathing room air (Fio_2 = 0.21) based on age-dependent normal values for Pao_2 under similar conditions.

Fio_2, fraction inspired oxygen; *NALI,* neonatal acute lung injury; *NARDS,* neonatal acute respiratory distress syndromes; *VetALI,* veterinary acute lung injury; *VetARDS,* veterinary acute respiratory distress syndrome.

From Wilkins PA, Otto CM, Baumgardner JE, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: the Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine. *J Vet Emerg Crit Care.* 2007;17:333-339.

and volutrauma, air leak syndromes (pneumothorax), and hemodynamic complications caused by impaired venous return, whereas the primary long-term complication is bronchopulmonary dysplasia.[301] Because of these concerns noninvasive forms of respiratory support are widely used in human infants with RDS, which include all forms of respiratory support not delivered with an endotracheal tube.[302] The simplest and most readily implemented form of noninvasive respiratory support in foals is the intranasal administration of humidified oxygen via intranasal cannula. Although a single cannula is most often used, this may not provide sufficient oxygen flow rates, in which case a second cannula can be placed in the opposite nostril, and combined flow rates of up to 20 L/minute can be readily delivered.[303] A potential downside to bilateral cannula placement is that this may cause some degree of upper respiratory obstruction in small foals, potentially impairing exhalation and CO_2 elimination, although this effect was not detected when this technique was investigated in healthy foals.[303] If nasal cannulation is not possible or is not achieving adequate flow rates, then an intratracheal catheter can be used for oxygen insufflation, but this rarely represents anything more than a short-term solution.[304]

Although oxygen insufflation can be tremendously helpful, it is unlikely to be sufficient in foals with severe respiratory impairment secondary to surfactant deficiency caused by progressive alveolar collapse and worsening ventilation/perfusion mismatching. In the most severely affected cases mechanical ventilation will represent the only viable means of respiratory support, and this technique is discussed in detail later in this section. However, noninvasive respiratory support strategies that provide continuous positive distending pressure, such as continuous positive airway pressure (CPAP), are widely used in human neonates with RDS and have been shown to reduce respiratory failure and mortality.[305] There are a few older reports of noninvasive ventilatory support techniques for foals that used mechanical ventilators and either nasal prongs or face masks.[306,307] A recent report described the adaptation of a commercial human CPAP device and face mask to provide CPAP to foals.[308] The authors reported that although both CPAP and nonpressurized oxygen supplementation with a face mask normalized pretreatment hypoxemia, oxygen extraction and CO_2 elimination were highest with CPAP at a lower respiratory rate than nonpressurized oxygen administration. Both treatments were associated with the development of modest hypercarbia, but this was not associated with adverse effects. This treatment modality holds promise for foals suffering from NERDS, but further evaluation in affected foals is needed.

The syndromes of EqNALI and EqNARDS represent a subset of ALI and ARDS that occurs in foals in the first week of life. The criteria for EqNALI/EqNARDS differ from those for ALI/ARDS because of the changes that occur in the efficiency of pulmonary gas exchange as foals develop

during this time frame. Diagnosis of these syndromes is fundamentally similar to that of VetALI and VetARDS with the exception of the use of an age-specific scale of Pao_2/Fio_2 ratios based on blood gas values obtained from foals breathing room air and positioned in lateral recumbency, as is typically encountered in clinical practice. It is important to remember that EqNALI/EqNARDS is not a primary diagnosis, and affected foals may be suffering from a broad range of disease processes. There certainly may be overlap with NERDS, but bacterial sepsis and bacterial pneumonia will be the most common disease processes in foals of this age. Other potential etiologies include meconium aspiration, milk aspiration, viral pneumonia, fungal pneumonia, or thoracic trauma, all of which are covered in detail in the following sections.

Treatment of EqNALI/EqNARDS is primarily focused on addressing hypoxemia and hypercapnia while pursuing correction of the primary etiology where possible. Respiratory support may involve the provision of intranasal oxygen, CPAP, or mechanical ventilation, depending on the severity of pulmonary dysfunction. Because of the substantial inflammation present, there may be an indication for antiinflammatory therapy. Nonsteroidal antiinflammatories are unlikely to be sufficient, but there is evidence in adult humans with ARDS[309,310] and in older foals with VetARDS[311,312] that corticosteroid therapy may be beneficial. Although specific therapeutic protocols have not been defined, recent work has suggested that hydrocortisone administration at a dosage of 1 to 4 mg/kg per day IV divided into four to six doses may be appropriate for septic foals with CIRCI, but this approach has not been investigated in septic foals or foals with EqNALI/EqNARDS.[313-315] Because of the likelihood that bacterial infection may be involved in this age group, broad-spectrum antimicrobial therapy is also indicated along with judicious fluid therapy and supportive care.

The most definitive descriptions of acute respiratory distress in foals have been associated with bronchointerstitial pneumonia in foals 1 to 12 months of age.[311,312,316,317] Affected foals often appear clinically normal until they are found in acute respiratory distress or dead.[311] Some cases may have a history of previous respiratory disease associated with sudden clinical deterioration.[293] Physical examination finds affected foals depressed, lethargic, and inappetent, with severe respiratory distress characterized by tachycardia, tachypnea, and nostril flare. A paradoxic breathing pattern may be present, in which the thorax and abdomen move opposite from one another, rather than synchronously, during inspiration and expiration. Auscultation may reveal widespread abnormal sounds, with prominent wheezes and crackles, or lung sounds may be very quiet to absent with only large airway sounds detectable. Fever and injected mucous membranes are common as well. Care should be taken to auscult the heart thoroughly, which may be difficult in the presence of diffuse abnormal respiratory sounds, to rule out a cardiac cause of pulmonary edema. Clinicopathologic evaluation is important both for assessment of pulmonary function and identification of the primary underlying disease process. Arterial blood gas analysis is extremely helpful and should be performed before initiation of intranasal oxygen insufflation, if clinically appropriate. Complete blood count, fibrinogen concentration, and SAA concentration are all helpful in characterizing the severity of systemic inflammation, and very high WBC counts, fibrinogen, or SAA concentrations, although not definitive, may be suggestive of *Rhodococcus equi* infection.[318,319]

Thoracic imaging is extremely helpful in staging the degree of pulmonary inflammation and identifying underlying primary disease processes. Thoracic ultrasonography is readily performed and may provide useful information, such as the presence of severe atelectasis, pleural effusion, or pulmonary abscesses. Diffuse lung inflammation, such as occurs in ALI, will be seen as thickening of the visceral pleura and the appearance of echogenic projections arrayed perpendicular to the surface of the lung (comet-tail artifacts).[320] Thoracic radiographs provide much more detailed information regarding the severity and extent of pulmonary inflammation, and reasonable image quality can be obtained with stall-side digital radiographic equipment.[321] The radiographic abnormalities observed in affected foals may range from a diffuse interstitial pattern to a focal or diffuse coalescing alveolar pattern with multiple air bronchograms, and serial radiography can be very helpful in monitoring the progression of disease and response to treatment.[293,321] The presence of pulmonary abscesses on thoracic ultrasonographic examination or in thoracic radiographs is strongly suggestive of *R. equi* involvement but is not definitive.[318] CT imaging of the thorax for the evaluation of foals with pulmonary disease has been recently reported as well.[321,322] CT imaging provides much more detailed information regarding the nature and degree of pulmonary changes but is only available in a few specialty referral settings at this time.

Foals affected with VetALI/VetARDS most commonly suffer from a primary bacterial pneumonia, but other causes such as viral pneumonia are possible. A transtracheal aspirate should be performed unless the patient is too unstable to undergo the procedure; this sample can provide important cytologic evidence regarding the type and severity of pulmonary inflammation and the presence of extracellular and intracellular bacteria and should also be submitted for bacterial culture and sensitivity. Neutrophilic inflammation is typically present, and the detection of intracellular gram-positive coccobacilli within pulmonary macrophages is strong evidence of the presence of *R. equi.*[318] Polymerase chain reaction (PCR) testing of tracheal aspirates may be helpful in confirming rhodococcal involvement.[323] Treatment of ALI/ARDS-affected foals should focus on addressing the hypoxemia, treating the primary underlying disease process, controlling the excessive inflammatory response, and providing supportive care. Intranasal oxygen insufflation, as described previously, should be initiated as soon as possible in foals suffering from respiratory distress and represents the primary means of respiratory support in these older foals. Antimicrobial therapy should target the most likely bacterial pathogens based on the overall assessment. In younger foals in which sepsis may be playing a primary role, broad-spectrum antimicrobial therapy is recommended. In older foals empiric treatment should address the possible involvement of *R. equi.* Aggressive systemic antiinflammatory therapy using potent corticosteroids, such as dexamethasone and methylprednisolone sodium succinate, has been shown to be the most important intervention in effecting survival of affected foals.[311,312,324] Corticosteroid therapy should be gradually tapered as the clinical condition improves, rather than rapidly withdrawn. Prolonged therapy has been shown to be beneficial in human ARDS patients.[310] Bronchodilator therapy has been used in affected foals because of the severity of

respiratory distress, but bronchoconstriction is not a prominent feature of ARDS, and bronchodilator administration may actually worsen the ventilation/perfusion mismatching and result in clinical deterioration or even death.[293] If used, bronchodilator therapy should only be administered after nasal insufflation of oxygen has been instituted, and the clinician should be prepared to address sudden changes in clinical status. The use of the inhalational route for the administration of corticosteroids and bronchodilators in patients suffering from severe pulmonary dysfunction with substantial ventilation/perfusion mismatching is often unrewarding because of poor pulmonary penetration and delivery, so the systemic route is recommended. The prognosis for survival in affected foals is poor to guarded, with early and aggressive corticosteroid and antimicrobial therapy offering the best chance of survival.[324]

Persistent Pulmonary Hypertension

Persistent pulmonary hypertension of the newborn (PPHN), also known as persistent fetal circulation or reversion to fetal circulation, is a syndrome characterized by sustained increases in pulmonary vascular resistance. Increased pulmonary vascular resistance, possibly combined with decreased right ventricular function, results in right-to-left shunting through the ductus arteriosus and/or foramen ovale.[325] PPHN can be idiopathic, but in human infants it is more commonly associated with pulmonary disease, such as meconium aspiration, perinatal asphyxia, surfactant deficiency, and pneumonia.[326] In foals PPHN may also develop secondary to systemic sepsis, hypoxemia, or acidosis.[327,328] The pathophysiology of PPHN is complex but fundamentally represents dysfunction of the factors that regulate pulmonary vascular tone. PPHN should be suspected in any neonatal foal exhibiting progressive or refractory hypercapnic hypoxemia. Care should be taken to rule out the presence of congenital cardiac anomalies that may be causing extrapulmonary shunting of blood and other causes of hypoxemic respiratory failure, such as sepsis or infectious pneumonia. PPHN should be suspected in cases of hypoxemia that do not show a significant increase in Pao_2 following the institution of intranasal oxygen therapy. Definitive diagnosis can be challenging, but ultrasonography can be very helpful in ruling out congenital cardiac anomalies and detecting primary pulmonary disease.

Treatment of PPHN is challenging but depends on addressing any predisposing or underlying factors while addressing the severe hypoxia. Foals that do not respond well to nasal insufflation of oxygen will require intubation and mechanical ventilation with 100% oxygen.[30] This can be useful both in addressing the hypoxia and in potentially stimulating pulmonary vascular relaxation.[327,328] Following a brief period of hyperoxia, it is recommended to taper the inhaled oxygen concentration to target a Pao_2 of 60 to 100 mm Hg.[325] Therapeutic agents that directly address the pulmonary vasoconstriction are widely used in human neonates, but inhaled nitric oxide (iNO) is the only approved pulmonary vasodilator for human use. The successful use of iNO therapy in foals has been reported,[327] but unfortunately it is impractical in most settings because it requires mechanical ventilation along with specialized equipment for introducing the NO gas into the ventilator circuit.[328] Sildenafil (Viagra, Pfizer Inc., New York), a phosphodiesterase type 5 inhibitor, has been used in humans and is reported to be effective.[326,329] There are anecdotal reports of the use of sildenafil in foals with PPHN, at a dosage rate of 0.5 to 2.5 mg/kg PO, up to every 4 hours, starting at the lower end of the dosage range to avoid systemic hypotension.[330] Although supported by only one case report in humans, the use of pentoxifylline, a nonselective phosphodiesterase inhibitor, as an adjunctive therapy may be worthy of consideration in foals because of availability, low cost, and an accepted safety profile.[331,332]

Congenital Upper Respiratory Tract Disorders

There are a number of congenital upper respiratory tract disorders in foals, although none is common. These can include wry nose, choanal atresia, cleft palate, nasopharyngeal cysts, and subepiglottic cysts.[333] Wry nose involves lateral and rotational deviations in the structure of the nose; in moderate to severe cases it will cause significant upper airway obstruction.[334] Surgical repair is possible, but this must be regarded as a salvage procedure, because the prognosis for athletic function is poor.[334] Choanal atresia is rare in foals but involves unilateral or bilateral soft tissue narrowing or complete obstruction of the caudal aspect of the nasal passages.[335] Foals born with bilateral complete obstruction will die without rapid intervention to establish an airway because of the obligate nasal breathing nature of equines.[333] Foals with unilateral choanal atresia often remain undiagnosed until later in life.[336] Diagnosis is most often made using endoscopy, but CT examinations have been useful in some cases.[336] Surgical correction is feasible, but the prognosis for performance remains guarded because of the persistent airway narrowing in many cases. Cleft palate deformities are rare and are reported in only 0.1% to 0.8% of foals.[337] Most affected foals will show clinical signs of dysphagia when nursing, including coughing or nasal milk drainage, but this is inconsistent.[338] Aspiration pneumonia is a common complication. The primary site of involvement is the soft palate, and the defect can range from a small, focal site to essentially the absence of the entire soft palate. Diagnosis is most readily made endoscopically, although surgical correction is theoretically possible; the approach is difficult and the outcome uncertain, especially with large defects.[337] There is one recent report of successful transoral endoscopically assisted repair in a foal.[339] Subepiglottic cysts are also rare in foals, but when present they can interfere with epiglottal placement, resulting in persistent palate displacement and severe dysphagia and aspiration pneumonia.[333,340] Diagnosis is primarily by endoscopic examination, although visualization may be difficult if the cyst is positioned below the palate. Radiographs or CT may be helpful in detecting these lesions. Treatment is by cyst removal, either via laryngotomy or by nasal or oral endoscopic approaches.[333]

Meconium Aspiration

Meconium aspiration syndrome (MAS) is defined as respiratory distress in a foal born through amniotic fluid stained with meconium in which the symptoms cannot be explained by other etiologies. Meconium staining can occur before, during, or immediately after parturition, and meconium passage in utero is thought to primarily occur in response to fetal hypoxia leading to fetal stress. Fetal hypoxia also may result in fetal gasping, facilitating introduction of meconium into the respiratory tract while in utero. Meconium aspiration may also occur in association with the first few breaths after birth. The presence of meconium-stained amniotic fluid does not guarantee that MAS will occur, however, because only

2% to 9% of human infants born through meconium-stained amniotic fluid develop MAS.[341] When meconium contamination of the lower respiratory tract occurs there are a number of pathophysiologic mechanisms leading to pulmonary dysfunction, including mechanical airway obstruction, regional air trapping, surfactant inactivation, surfactant displacement, chemical pneumonitis and alveolitis, and persistent pulmonary hypertension.[327,341]

If meconium aspiration is suspected then aspiration of the material from the nasal passages and pharynx can be performed, even while the foal is in the birth canal, if the birth is attended. If more severe contamination is suspected then nasotracheal intubation followed by aseptic suctioning is suggested, although care must be taken to avoid contamination of the lower respiratory tract or overly zealous suction. Intranasal oxygen supplementation is recommended in affected foals, but mechanical ventilation will be required in more severely affected cases and is associated with a worsened prognosis. Mechanical ventilation is challenging in these cases because of the degree of pulmonary inflammation and airway compromise as well as the severity of ventilation/perfusion mismatching and strategies to minimize barotrauma, such as CPAP ventilation and synchronized intermittent mandatory ventilation (SIMV), which may be helpful.[342,343] Surfactant administration and lung lavage with dilute surfactant appear to be effective in human neonates with MAS but are not routinely used in foals.[344] Antiinflammatory therapy may be beneficial because of the substantial pulmonary inflammation associated with MAS. Pentoxifylline has shown some promise in animal models, appears safe in foals, and is easily administered.[345,346] Corticosteroids, either inhaled or systemically administered, are commonly used in human infants with MAS.[347,348] Although meconium aspiration is not associated with primary bacterial pneumonia, affected foals are at risk of secondary bacterial infection, and broad-spectrum antimicrobial therapy is recommended.

Milk Aspiration

Neonatal foals may aspirate milk into the lower respiratory tract secondary to a number of conditions, although this is most common secondary to generalized weakness, a poor suckle reflex, or dysphagia related to prematurity or NE.[349] This risk may be exacerbated by attempts at bottle feeding foals affected with these problems. Other potential causes of aspiration include physical abnormalities such as subepiglottic cysts, cleft palate, megaesophagus, esophageal stenosis, esophageal compression by vascular anomalies, or persistent dorsal displacement of the soft palate.[342,349,350] Functional abnormalities of the pharynx, larynx, and esophagus resulting in aspiration have been associated with pharyngeal dysfunction of unknown etiology, botulism, and hyperkalemic periodic paralysis.[342,349,351] Incorrect placement of nasoesophageal feeding tubes has also been associated with direct instillation of milk into the lungs, leading to aspiration pneumonia. Detection of milk aspiration can be difficult because some affected foals may not cough or show nasal milk regurgitation. Diagnosis is based on historic information that may reveal episodes of nasal milk regurgitation, physical examination findings demonstrating abnormal lower respiratory sounds, and clinicopathologic evidence of systemic inflammation (inflammatory leukogram and hyperfibrinogenemia or increased SAA) and pulmonary dysfunction (arterial hypoxemia).[342] Endoscopic examination of the upper respiratory tract may reveal structural abnormalities of the pharynx, larynx, or esophagus suggestive of aspiration, or pharyngeal dysfunction may be observed. Thoracic radiographs typically reveal an alveolar pattern with or without air bronchograms in the caudoventral lung, but this may be localized to the perihilar region.[321] It is important to pursue the identification and correction of the cause of aspiration, if possible, to prevent further aspiration. Placement of a nasoesophageal feeding tube will allow for the safe delivery of milk pending correction of the underlying disorder. Broad-spectrum antimicrobial therapy is indicated to address the bacterial pneumonia accompanying milk aspiration, and successful treatment may require several weeks of treatment.

Transient Tachypnea

Idiopathic or transient tachypnea has been reported in Clydesdale, Thoroughbred, and Arabian foals and is generally seen during hot, humid weather conditions.[352] This condition is believed to be the result of immature or dysfunctional thermoregulatory mechanisms.[327] Affected foals are usually normal at birth and of a normal gestational age, but they develop hyperthermia and tachypnea at variable times following birth.[342] It is critical that other potential causes of respiratory disease are ruled out before making the diagnosis of idiopathic tachypnea, because the consequences of withholding antimicrobial treatment could be severe. Treatment is primarily directed at controlling the hyperthermia and may include body clipping; alcohol baths; and movement to a cool, shady environment.[342] In most foals the condition is self-limiting and will resolve within a few days to a few weeks.

Rib Fractures, Pneumothorax, and Hemothorax

Rib fractures are a fairly common problem, having been reported in 3% to 5% of the general population of neonatal foals and as many as 30% of foals presenting to a neonatal ICU.[353] Fractured ribs can cause a number of traumatic insults to the thoracic viscera, including pulmonary contusions and lacerations of the lungs, major arteries, heart, or diaphragm. Pneumothorax, hemothorax, and diaphragmatic herniation may all occur as a result of these traumatic insults, and myocardial injury is typically fatal. Rib fractures can be single, but are often multiple, most often affecting adjacent ribs on one side of the chest. The most common site of injury is at the costochondral junction or immediately dorsal to it.[354] Flail chest, or paradoxic thoracic wall motion, may occur when multiple ribs are fractured, and the affected region will move inward during inspiration and outward during expiration, counter to the movements of the intact portions of the thoracic wall. Rib fractures are commonly found on physical examination by palpation of the fracture itself or by the detection of crepitus at the site of the fracture. Auscultation may reveal grinding or "clicking" sounds in the area of the fracture as well. Confirmation of the diagnosis is best accomplished ultrasonographically because this modality is much more sensitive than radiography for this purpose.[355] Ultrasonography may also be used to document the presence of air, blood, or abdominal viscera within the thoracic cavity, although radiography may be helpful in this evaluation. Treatment depends on the structures involved and the severity of the complications observed. Most minimally displaced rib fractures, particularly those involving the costochondral junction, can be managed conservatively with stall rest and avoidance of pressure on the affected area when the foal is handled. Mild to moderate pneumothorax may not

require intervention, but if substantial air is presented within the pleural cavity it will cause respiratory distress and should be evacuated. Placement of an indwelling thoracic catheter will facilitate ongoing drainage. If multiple ribs are fractured, and particularly if sharp bony projections are exposed and threatening internal injury, then surgical repair may be indicated.[356] Hemothorax can be life-threatening because of pulmonary compression and/or severe blood loss anemia, and treatment should focus on addressing the primary cause of hemorrhage and patient stabilization and support.[357]

Viral Infections

Viral pneumonia is uncommon in neonatal foals but has been associated with EHV-1, EHV-4, equine influenza virus (EIV), equine arteritis virus (EAV), and equine adenovirus (EAdV). EHV-1 infections in neonatal foals are severe and typically fatal, despite aggressive treatment. One of the challenges associated with these cases is the difficulty of ascertaining EHV-1 involvement, because the presenting signs are very similar to neonatal sepsis. Foals may be delivered somewhat early and with little or no warning of impending parturition, and the placenta is usually grossly normal.[328] Farm outbreaks have been reported.[184,358,359] Clinical signs are of cardiovascular and respiratory insufficiency, and the mucous membranes are severely congested and icteric. Clinicopathologic abnormalities include severe leukopenia with neutropenia, toxic neutrophil morphology, and lymphopenia. The combination of severe leukopenia and icterus may be suggestive of EHV-1 infection.[183] Bone marrow examination will reveal depletion of the myeloid cell lines. Many affected foals will display dilation of the retinal vasculature with reddish discoloration of the optic disk. Diagnostic confirmation is most readily accomplished by PCR testing of nasal secretions or whole blood. Treatment of affected foals with acyclovir has been attempted, and this appeared to have some efficacy in less severely affected foals.[184] Given the superior bioavailability of valacyclovir in adult horses, this drug may represent a more suitable choice for use in EHV-1–infected foals.[328,360] EHV-4 appears to be a rare cause of neonatal disease and is more commonly associated with abortion or stillbirth, but it has been associated with neonatal mortality caused by multisystemic disease.[361,362]

Infections with EIV in neonatal foals are not common, particularly in endemic areas in which mares are routinely vaccinated and foals are typically protected by maternal colostral antibodies.[363-366] Outbreaks have been reported when EIV has been introduced into naïve populations.[364,366] EIV infection in susceptible foals is associated with severe bronchointerstitial pneumonia leading to respiratory distress.[364] Antemortem diagnosis is by PCR of nasal secretions. There is no specific treatment for EIV infection.

Neonatal infections with EAV are associated with severe interstitial pneumonia, which has been reported to be uniformly fatal.[367-369] Both solitary cases and farm outbreaks have been reported.[367,368,370-372] The clinical signs associated with neonatal EAV infection are severe and primarily respiratory and initially include edema, weakness, and depression, ultimately progressing to terminal respiratory distress, although acute death has also been reported.[368,372] Gastrointestinal involvement has been reported in some cases but is not consistently present.[367,369] Clinicopathologic abnormalities are not specific to EAV infection but often include leukopenia and thrombocytopenia.[368] There is no specific antiviral treatment for EAV, and despite attempts at aggressive medical therapy there are no reports of successful treatment of neonatal EAV infections. Diagnostic confirmation of EAV involvement is most readily achieved by PCR testing of blood, respiratory secretions, or tissues (lung, kidney, and spleen).[372]

EAdV infections are rare in immunocompetent foals, and the primary concern is in immunocompromised foals. The best described syndrome associated with EAdV infections is in Arabian foals with severe combined immunodeficiency syndrome, although a similar syndrome may occur in Fell pony foals with an unidentified immunodeficiency.[373-375] Two distinct EAdVs have been identified, with EAdV1 being ubiquitous and readily isolated from normal and diseased foals and horses, whereas EAdV2 is only isolated from foals with upper respiratory tract disease and diarrhea.[373,376,377] Experimental infection of healthy foals with EAdV has been associated with pneumonia regardless of breed.[378,379] PCR testing for EAdV1 and EAdV2 is available, but care must be taken with interpretation because EAdV1 is frequently detected in normal animals. Confirmation of diagnosis may require histopathologic evidence of EAdV infection or virus isolation.[373]

Viral respiratory infections are more common in older foals than in neonatal foals, and respiratory disease in this age group has been associated with EHV-1, EHV-4, EIV, and EAV.[380] The clinical signs associated with EHV and EIV infections in this age group are very similar and are consistent with those reported in adult horses. Clinical signs typically include a dry cough and fever, although a mucopurulent nasal discharge may be present as well. The presence of such a discharge may be suggestive of secondary bacterial involvement, however. EIV and EHV infections in older foals are typically much less severe than in neonates and ultimately self-limiting. A rare, fatal pulmonary vasculotropic form of EHV-1 infection has also been described in young horses.[381] EAV infections typically show similar respiratory signs and fever, but affected individuals often exhibit substantial ventral and limb edema secondary to vasculitis.[369]

The potential role of the gammaherpesviruses, EHV-2 and EHV-5, in foal pneumonia is intriguing but currently unclear. Both of these viruses are ubiquitous in equine populations worldwide, and foals become infected within the first several months of life.[382] Infection in foals is generally considered to occur without clinical signs, but EHV-2 infection has been associated with mild to severe chronic pharyngitis of foals following natural and experimental infection.[383] Other signs of upper respiratory tract disease, such as lymphadenopathy, mild pyrexia, and rhinitis may be associated with natural infections.[384-387] Some intriguing evidence exists that EHV-2 may play a role in potentiating lower respiratory bacterial infections; there are reports that use of an EHV-2 vaccine protected foals from *R. equi* pneumonia.[388,389] Natural infection of foals with EHV-2 has been associated with fever and immunopathologic changes similar to those observed in human adolescents suffering from infectious mononucleosis following infection with Epstein-Barr virus.[390] In addition, the EHV-2 genome contains an IL-10 homolog as well as encoding for several other proteins that may have immunomodulatory effects that could predispose a patient to bacterial infections.[382] The case for a pathogenic role for EHV-5 is stronger, because this virus has been convincingly associated with the syndrome of equine multinodular pulmonary fibrosis.[391-394]

Fungal Diseases

Although fungal pneumonia is very rare in neonatal foals, there are a few conditions to be aware of in this population. In utero infection with *Histoplasma capsulatum* has been associated with placentitis, abortion, and the birth of infected foals.[395,396] Affected foals have multisystemic disease, including granulomatous pneumonia.[395] Antemortem diagnosis is challenging but may be facilitated by tracheal aspirate or bronchoalveolar lavage cytology, in which characteristic yeastlike organisms (3- to 5-μm diameter) may be detected within the macrophages.[342] Serology is useful as well. The mare and foal should be positive for anti-*Histoplasma* antibodies, which can be detected using an agar gel immunodiffusion test.[328] Although successful treatment of infected adults has been reported using amphotericin B, there are no reports of successful treatment in neonatal foals.[328]

Although *Candida* spp. infections are a major problem in human intensive care patients, they appear to be an infrequent problem in foals. Systemic candidiasis has been reported in foals, but superficial infections of the mucous membranes (thrush) appear to be more common.[397-400] The presence of superficial infections may be related to the development of systemic infections, so these lesions should be taken seriously and treatment implemented in a timely manner. This concern is reinforced by the fact that the clinical signs associated with systemic candidiasis are indistinguishable from those seen in bacterial sepsis.[400] Confirmation of the diagnosis of systemic candidiasis is by blood culture of the organism. Successful treatment has been reported with IV amphotericin B and/or oral fluconazole, with fluconazole preferred.[400]

Parasitic Pneumonia

Following ingestion of the larvated eggs of *Parascaris equorum* the infective larvae emerge in the intestinal lumen and migrate through the liver and lungs before being coughed up and reingested, returning to the small intestine in which they mature into adults.[401] Passage through the lungs is associated with substantial inflammation, and infected foals may present with clinical signs of lower respiratory disease when the larvae are migrating through the lungs.[402] Infestation is ultimately self-limiting, and the pulmonary signs rarely require medical treatment. Because of concerns regarding possible colic secondary to intestinal obstruction by the adult parasites, it is recommended that foals suspected of parasitic pneumonia be dewormed. Ivermectin has been recommended traditionally, but the widespread emergence of macrocyclic lactone–resistant strains has led to the recommendation that benzimidazole or pyrimidine anthelmintics should be used.[401] Resistance has been demonstrated in these classes as well, unfortunately, requiring individuals to be monitored with fecal egg counts to ensure efficacy of anthelmintic therapy.[403]

Bacterial Infections

Neonatal Foals

Bacterial pneumonia in neonates is most often associated with hematogenous spread secondary to bacteremia, but it may also occur secondary to infection in utero or meconium or milk aspiration. The bacteria associated with neonatal pneumonia are typically the same as those involved in neonatal sepsis, and gram-negative bacteria are the organisms most commonly involved. Gram-positive infections appear to be increasing in prevalence, however, and mixed infections occur as well.[404] The most common isolate is *E. coli,* with *Klebsiella* spp., *Actinobacillus* spp., *Pasteurella* spp. *Salmonella* spp., *Streptococcus* spp., *Enterococcus* spp., and *Staphylococcus* spp. also occurring with some frequency.[25,171,176,178,180,181] Successful treatment depends on early and effective support of respiratory function with correction of hypoxemia, control of systemic and pulmonary inflammation, and appropriate antimicrobial therapy. Unfortunately antimicrobial selection is almost always empiric initially, but collection of blood culture samples before initiation of antimicrobial therapy is encouraged to provide confirmation of bacterial involvement and to guide future antimicrobial therapy on the basis of antimicrobial sensitivity patterns. Transtracheal aspirates are not commonly performed in neonatal foals because they can be technically challenging and may worsen the foal's respiratory distress. They can be very useful diagnostically, especially when the respiratory infection is secondary to aspiration.[404] Because of the possibility of polymicrobial and mixed infections, broad-spectrum therapy is recommended initially. This often consists of a β-lactam (penicillin) in combination with an aminoglycoside, but a third-generation cephalosporin, such as ceftiofur, may represent a better choice because of its superior pulmonary penetration.[328]

Older Foals

Pneumonia is common in foals from 1 to 6 months of age and is the most common cause of death in this age group.[405] The most common etiologic agent of bacterial pneumonia in these older foals is *Streptococcus equi* subsp. *zooepidemicus,* which is a normal upper respiratory commensal organism. This age group of foals may be at risk for the development of respiratory infections caused by decaying levels of maternal immunoglobulins in conjunction with delayed endogenous immunoglobulin production and possibly impaired immunologic responses to pathogen exposure. Additional risk factors in older foals include the stresses of weaning and sales preparation and transport, as well as confinement in crowded or dusty conditions that result in heavy exposure to potential respiratory pathogens. Secondary infection with gram-negative organisms is not uncommon, and foals in which this occurs may exhibit a poor response to treatment or deteriorate clinically in the face of treatment. Viral infections such as EHV-1, EHV-4, and EIA may cause pulmonary inflammation and injury, whereas other viral infections such as EHV-2 may directly impair pulmonary immune responses, facilitating the development of secondary bacterial pneumonia.

The second most common agent of pneumonia in this age group is *R. equi,* which is a facultative, intracellular gram-positive coccobacillus. This organism was first isolated from foals with pyogranulomatous pneumonia in Sweden in 1923 and was originally named *Corynebacterium equi.*[406] Although it has been known as *R. equi* for several decades, the taxonomic nomenclature for this organism is a matter of ongoing debate, and the alternative names *Prescottella equi* and *Prescottia equi* have been recently proposed.[407,408] The conventional terminology *R. equi* will be used here. *R. equi* is considered to be a ubiquitous bacterium with a worldwide distribution,[409] although a recent study suggests that it may not be present in Iceland.[410] All isolates capable of causing disease in foals carry a plasmid encoding a virulence-associated protein (VapA). Although VapA is required for virulence, it alone is not sufficient, and other plasmid-encoded genes also influence virulence.[349] Rhodococcal pneumonia is occasionally seen as a

sporadic disease but is more common on endemic farms, on which there is often substantial variation in the incidence of disease on an annual basis.[411] On endemic farms as many as one third of the foals are affected with clinical disease, and up to 50% of affected foals may die.[412] The most likely route of infection in foals is by inhalation, and increased airborne concentrations of virulent *R. equi* are positively associated with the development of pneumonia.[413,414] High stocking densities of mares and foals have also been associated with an increased incidence of rhodococcal pneumonia.[415,416] Higher concentrations of *R. equi* have been detected in stalls and barns, as opposed to pastures and paddocks,[417,418] and this could lend support to the suggestion that dusty conditions are associated with an increased incidence of rhodococcal infection.[419]

R. equi infections are rare in adult horses unless they are suffering from some form of immunodeficiency. Given the ubiquitous nature of *R. equi* in the environment, this suggests that foals are in some way uniquely susceptible to infection by this organism. Several studies have investigated the immune responses of foals, but the results have been inconclusive and there is no clear evidence of an age-related immunodeficiency that renders them susceptible.[420,421] Recent studies have investigated the potential for genetic predispositions to rhodococcal infections, with some intriguing findings. Polymorphisms in the transferrin and *SLC11A1* genes have been associated with rhodococcal pneumonia in Thoroughbred and Arabian foals, respectively.[422,423] More recently a genetic region on chromosome 26 that contains the *TRPM2* gene, which is associated with neutrophil function, was found to be positively associated with rhodococcal pneumonia, and foals with a single nucleotide polymorphism in this region were three to four times more likely to be clinically affected than their herdmates.[424]

Because of the occult nature of this disease, there has been tremendous interest in identifying infected foals as early as possible so that treatment can be implemented early in the disease process. To this end a number of screening programs for early detection of infected foals have been investigated, including serial monitoring of physical examinations, WBC concentration, fibrinogen assays, SAA concentration, and quantitative fecal VapA PCR testing, but none has proven to be clinically effective.[349,425] Serology has been unrewarding as well,[426] although there are recent reports that VapA-specific serum IgG(T) measurement may be useful in identifying infected foals, although this requires further validation in the field setting.[410,427] Ultrasonographic screening programs were developed, and these demonstrated that large numbers of foals had pulmonary lesions in the absence of clinical signs.[428] Because of the widespread implementation of these ultrasonographic screening protocols on endemic farms, subclinical *R. equi* infections have become the most common form of this disease.[429] This approach has also resulted in the treatment of many foals in which the pulmonary lesions would likely have resolved spontaneously, contributing to the development of macrolide resistance.[430,431]

Clinically evident rhodococcal pneumonia is often insidious, ultimately presenting with signs consistent with lower respiratory tract infection, and fever, lethargy, coughing, tachypnea, and dyspnea are common clinical signs. Dyspnea can be severe, with nostril flaring and prominent abdominal expiratory effort. Although relatively rare, some foals may present with a subacute, severe form of respiratory disease, consistent with ARDS, and severely affected foals may be found dead.[429] Auscultation typically reveals diffuse crackles and wheezes, with prominent large airway sounds (rattles) caused by the accumulation of exudate in the cranial thoracic region. Rebreathing examination is typically not necessary because of the prominence of the abnormal lung sounds, and it is contraindicated in animals in respiratory distress. It is important to perform a thorough examination in affected foals, because extrapulmonary disorders are common.[432] The most common of these are diarrhea, ulcerative enterotyphlocolitis, presumed immunomediated synovitis, intraabdominal lymphadenitis, or abscessation and uveitis.[432] Arterial blood gas analysis is extremely helpful in assessing the degree of pulmonary dysfunction and should be performed before initiation of intranasal oxygen insufflation if clinically appropriate. Complete blood count, fibrinogen concentration, and SAA concentration are all helpful in characterizing the severity of systemic inflammation, and very high WBC counts, fibrinogen, or SAA concentrations, although not definitive, may be suggestive of *R. equi* infection.[318,319] Serial monitoring of these values can be helpful in assessing the response to treatment and in determining the duration of therapy.

Thoracic imaging is extremely helpful in staging the degree of pulmonary inflammation and supporting the diagnosis of rhodococcal involvement, and serial imaging studies can be very helpful in monitoring the progression of disease and response to treatment. Thoracic ultrasonography is readily performed and is very effective for the detection of peripheral pulmonary consolidation or abscesses, but it is unable to detect axial lesions unless peripheral lesions are present.[349] Despite these limitations ultrasonography is the most widely used imaging technique in evaluating foals suspected to have rhodococcal pneumonia because of the combination of ready availability, ease of performance, and diagnostic utility. Abdominal ultrasonography should also be performed in foals with suspected rhodococcal pneumonia because of the frequency of abdominal extrapulmonary disorders. Thoracic radiographs provide more detailed information regarding the severity and extent of pulmonary disease, however, and should be considered in more severely affected cases, particularly given that reasonable image quality can be obtained with stall-side digital radiographic equipment.[321] The radiographic abnormalities observed in affected foals may include a diffuse interstitial or alveolar pattern, tracheobronchial lymphadenopathy, intrapulmonary abscesses, and pleural effusion. A scoring system has been developed to assess the severity of alveolar pattern, interstitial pattern, tracheobronchial lymphadenopathy, pleural effusion, and the number of nodular opacities and cavitary lesions in affected foals.[433] Foals with higher median scores (≥15) were less likely to survive (odds ratio [OR] 6.15, 95% confidence interval [CI]: 1.35–28.2) than foals with a lower score, with only the severity of the alveolar pattern and number of cavitary lesions being associated with decreased survival. Care should be taken to avoid overinterpretation of imaging studies, especially thoracic ultrasonography, because many of the lesions detected may resolve without therapeutic intervention. A recent study on an endemic farm found that 80% of foals developed ultrasonographic evidence of pulmonary consolidation or abscess formation, but only 21% developed clinically apparent *R. equi* pneumonia.[434] As a result of this concern a thoracic ultrasonographic scoring system has been used in several prospective studies of rhodococcal pneumonia therapies in an effort to more effectively identify foals that require treatment.[431,435-438] This scoring

system defines a pulmonary abscess as a focal hypoechoic area with a diameter of ≥1.0 cm, and the number of abscesses identified is recorded along with the diameter of each abscess. The diameter of each individual abscess is totaled to generate a total abscess score in centimeters, with foals that have a total score less than 8 cm or 10 cm typically not receiving treatment.

Following the completion of any imaging studies a transtracheal aspirate should be performed unless the patient is too unstable to undergo the procedure; this sample can provide important cytologic evidence regarding the type and severity of pulmonary inflammation and presence of extracellular and intracellular bacteria. This sample should also be submitted for bacterial culture and sensitivity. Profound neutrophilic inflammation with degranulation of neutrophils is typically present, and the detection of intracellular gram-positive coccobacilli within pulmonary macrophages is strong evidence of the presence of *R. equi*.[318] PCR testing of tracheal aspirates may also be helpful in confirming rhodococcal involvement, because the culture is frequently negative even when intracellular bacteria are seen on cytologic examination.[323] Quantitative PCR testing of feces was recently investigated on an endemic farm and was found to be useful in diagnosing *R. equi* in foals with clinical signs of pneumonia and, although these results are preliminary, this technique may prove useful in situations where tracheal aspirates cannot be collected.[439]

Treatment of foals with rhodococcal pneumonia should focus on addressing respiratory distress, if present, and treatment of the infection. Oxygen insufflation should be provided, if possible, to animals with dyspnea. Nonsteroidal antiinflammatory therapy may be helpful in controlling the fever, and affected animals should be kept in a cool, shaded area if possible because of the risk of exacerbation of physiologic hyperthermia. Antimicrobial therapy of rhodococcal pneumonia has been based on the combination of a macrolide and rifampin for over 30 years, which is an approach that was validated by a recent ACVIM consensus statement.[440] Although erythromycin was initially the primary macrolide used, it has been supplanted by azithromycin and clarithromycin for a variety of reasons, including less frequent administration, superior drug distribution, and pharmacokinetics as well as evidence of superior efficacy in a retrospective study.[420,441,442] There has been interest in the use of gamithromycin for the treatment of *R. equi* infections in foals because the pharmacokinetics support once-weekly intramuscular administration.[443] A recent study reported that gamithromycin therapy was noninferior to the combination of azithromycin and rifampin, although they also reported that 58% of foals treated with gamithromycin showed adverse reactions consisting of either colic or hindlimb lameness following drug administration, which is likely caused by tissue irritation from the drug.[435] The potential efficacy of the macrolide tulathromycin has been investigated, but despite early reports of efficacy[437] it has unacceptable pharmacokinetics and has subsequently been demonstrated to be ineffective.[436]

Rifampin has been used in combination with macrolide therapy on the basis of in vitro evidence of synergistic effects with erythromycin and limited evidence of enhanced treatment efficacy.[440] A recent study compared the efficacy of an azithromycin-rifampin combination to azithromycin alone and found that both treatments were superior to placebo, but there was no difference in efficacy between treatments.[436] Despite this evidence of efficacy the combination has been called into question because of the interference of rifampin with macrolide absorption after coadministration.[444,445] It is still recommended to use rifampin in combination with a macrolide to aid in minimizing the development of resistant strains, because these combinations have been shown to have lower mutant prevention concentrations than any of the individual macrolides or rifampin alone.[440,446] Reports of the emergence of macrolide-resistant and rifampin-resistant strains of *R. equi,* especially on endemic farms in which large numbers of foals have received treatment for subclinical pulmonary lesions identified on survey ultrasonographic examination, reinforce the need for this approach.[430,447,448] Indeed, one can reasonably question the need to treat foals that are not suffering from more severe forms of the clinical disease; recent studies examining treatment efficacy have also reported that 78% to 88% of foals in the untreated control groups recovered without intervention.[431,435] The prognosis for survival and athletic function in foals with *R. equi* pneumonia are good, at 60% and 54%, respectively.[349] Unfortunately the odds of survival are approximately sevenfold lower in foals infected with macrolide-resistant strains.[448]

There has been tremendous interest in reducing the incidence of rhodococcal infections on endemic farms for many years, but no highly effective approaches have been identified. The mainstay of prevention on many farms has been the administration of hyperimmune plasma, despite some conflicting evidence regarding efficacy, and widespread acceptance that even in the best-case scenario protection is incomplete.[449-453] Despite the expense, difficulty of administration, and potential for complications, the administration of hyperimmune plasma will likely continue to be used in prevention efforts until better approaches become available. Chemoprophylactic strategies have been investigated using azithromycin and gallium maltolate, with azithromycin yielding conflicting results and gallium showing no evidence of efficacy.[438,454,455] The prophylactic use of macrolides should be discouraged, however, because of the emergence of resistant strains on farms in which large numbers of foals receive these drugs.[430] Ultimately vaccination represents the ideal prevention strategy, but despite intensive investigation no effective vaccine is yet available.[420,456]

Respiratory Therapeutics

Bronchodilators

In foals suffering from respiratory dysfunction or respiratory distress there may be some utility in using bronchodilators to increase the airway diameter, decreasing the work of breathing and improving ventilation. The clinical utility of this approach is limited, however, because bronchoconstriction does not appear to play a prominent role in ARDS.[457] The other factor limiting the utility of bronchodilator therapy is the risk that the resulting increase in ventilation may actually worsen the degree of ventilation/perfusion mismatching caused by increased ventilation of areas of the lung that are poorly perfused. To determine whether bronchodilator therapy may be beneficial, one can perform a bronchodilator response test under close clinical monitoring. Because of the risk of worsening hypoxemia, it is best if the foal is already receiving oxygen by nasal insufflation, but at the very least oxygen should be readily available in case of clinical deterioration. Administer a single dose of a short-acting bronchodilator, preferably by inhalation rather than systemically, and then monitor the response using clinical assessment and ideally with repeated arterial blood gas analysis.[324] If the patient responds favorably,

TABLE **20.10 Inhaled Drugs Used for Treating the Respiratory Tract**

Class	Drug	Dose	Route	Frequency (h)
Bronchodilator	Albuterol	90–180 μg (young foals) 360 μg (weanlings)	MDI, Neb	2–6
	Ipratropium bromide	18–36 μg	MDI, Neb	8–12
	Ipratropium bromide with albuterol	18–36 μg ipratropium, 90–180 μg albuterol	MDI, Neb	8–12
Aerosolized antimicrobial	Ceftiofur	2.2 mg/kg	Neb	24
	Gentamicin	2.2 mg/kg	Neb	24

MDI, Metered dose inhaler; *Neb,* nebulization.
Adapted from Wilkins PA, Lascola KM: Update on interstitial pneumonia. *Vet Clin North Am Equine Pract*. 2015;31:137-157; Mckenzie HC: Treating foal pneumonia. *Comp Equine*. 2006;47-53.

with decreased respiratory effort and improved arterial oxygenation, then it may be reasonable to continue bronchodilator therapy. Regarding the choice of bronchodilator, the methylxanthines (aminophylline and theophylline), although readily available, are not recommended because of their systemic effects and narrow therapeutic index.[458] Generally, the use of the aerosol route is preferred; this targeted application allows for the use of smaller dosages, reducing the risk of systemic toxicity. β_2-Adrenergic agonists (albuterol and clenbuterol) are easily administered and may have additional benefits including enhancement of mucociliary clearance. Albuterol is inexpensive and the metered dose inhaler (MDI) formulation is most often used (Table 20.10). This drug has a fairly short duration of action of 1 to 2 hours. The anticholinergic bronchodilator ipratropium bromide (Atrovent, Boehringer Ingelheim) has a longer duration of action than albuterol (6–8 hours) and can be used alone or in combination with a β_2-agonist (Combivent, Boehringer Ingelheim). Ipratropium is only available as an aerosol preparation, either as an MDI or a solution for nebulization.

Aerosolized Antimicrobials

The targeted administration of antimicrobial drugs to the respiratory tract is of interest because it achieves very high local drug concentrations, which may allow for a more rapid response while minimizing the risk of systemic toxicity.[459] Although controlled studies in horses are lacking, there is evidence in human medicine that the use of aerosolized antimicrobials as an adjunct to systemic therapy is well tolerated and may improve outcomes in certain subsets of patients.[460,461] Although a number of antimicrobial drugs have been administered via nebulization empirically in the clinical setting, only a few drugs have been investigated for aerosolized administration in horses, including ceftiofur, cefquinome, gentamicin, and marbofloxacin.[459,462-466] Aerosol administration of these drugs to horses has been shown to achieve high respiratory drug concentrations, is well tolerated, and appears safe. Anecdotally, the two most widely used inhaled antimicrobials in horses are ceftiofur and gentamicin[458] (Table 20.10). A variety of devices have been used to aerosolize these drugs, including jet and ultrasonic nebulizers, but the recently developed vibrating mesh nebulizers yield the most uniform and appropriately sized aerosols with a wide variety of drugs. The availability of a vibrating mesh nebulizer that is integrated into a face mask device specifically designed for horses (Flexineb, Nortev, Galway, Ireland) has greatly facilitated the ability to routinely deliver aerosolized medications in the clinical or field setting.

Mechanical Ventilation

The goals of mechanical ventilatory support are to achieve and maintain adequate pulmonary gas exchange, reduce the work of breathing, and minimize patient discomfort and distress.[467,468] Mechanical ventilation provides pressure and volume support to ensure that adequate minute ventilation is achieved and also provides control over the composition of the inspired gases to provide sufficient oxygen to support arterial oxygenation. Patients requiring mechanical ventilation typically are suffering from arterial hypercapnia and/or arterial hypoxemia. Treatment of hypercapnia, defined as a $Paco_2$ of greater than 60 mm Hg, is fundamentally to increase the patient's alveolar ventilation by increasing the rate and/or depth of breathing. For initial therapy some clinicians will use respiratory stimulants to increase the patient's ventilatory efforts and try to avoid the need for mechanical ventilation, but if respiratory stimulant therapy is unsuccessful at resolving the hypercapnia, or if the hypercapnia is more severe ($Paco_2$ > 70 mm Hg) one should consider instituting mechanical ventilation. Early intervention is preferable, because there is no benefit to the patient from prolonged hypercapnia and the duration of the requirement for mechanical ventilation is usually shorter with early intervention.

Treatment of hypoxemia, defined here as a Pao_2 of less than 60 mm Hg, is initially by increasing the fraction of inspired oxygen (Fio_2) through the provision of supplemental oxygen by intranasal insufflation, but this will be inadequate in patients with significant hypoventilation or ventilation/perfusion mismatching. Mechanical ventilation allows for much higher Fio_2 than can be achieved with insufflation, up to 100%, dramatically increasing alveolar oxygen tension. Mechanical ventilation is also useful in terms of increasing minute ventilation through controlled respiratory rate and volume and in recruitment of alveoli for participation in gas exchange. An additional benefit is the decrease in the effort required for respiration, which can be dramatically increased in the face of pulmonary disease, which allows for a substantial decrease in the patient's energy and oxygen needs. This can also ease patient distress and increase patient comfort.

In equine patients the provision of mechanical ventilatory support is indicated in only a few basic situations. The first of these is the patient lacking normal respiratory drive, such as the obtunded or comatose neonatal foal, or the patient with impaired ventilatory effort, such as a patient with botulism. These types of patients are fairly easy to ventilate because the lungs are not diseased and pulmonary function is relatively normal. The prognosis for survival of these foals is excellent if

mechanical ventilation is instituted early.[469,470] A fundamentally different situation is present in the patient with severe pulmonary disease resulting in respiratory failure, such as a foal with severe sepsis, ARDS, meconium aspiration, or severe pneumonia. In these patients the presence of pulmonary disease results in profound alterations in pulmonary function, primarily in the form of increased pulmonary resistance and decreased dynamic compliance, making these patients much more challenging to ventilate. The prognosis for survival in this population of foals is much more guarded. The decision to initiate mechanical ventilation can be an agonizing one because of concerns regarding prognosis, client cost, potential complications, and the intimidation factor associated with managing the ventilator. Unfortunately this can result in delays in the initiation of therapy that dramatically decrease the likelihood of a successful outcome. The development of some familiarity with the process of mechanical ventilation on the part of the neonatal intensive care team often results in a willingness to use mechanical ventilation earlier. Training sessions that provide familiarity with ventilator setup and initial settings are extremely useful, and so is a pictorial guide to setting up a circuit and attaching it to the patient.

PPV functions by generating a positive pressure within the patient's airways, which is used to overcome the resistance to airflow arising from the airways themselves, the lungs, and the thoracic wall. Invasive PPV relies on intubation for the establishment of an airway for gas exchange and aids in preventing the aspiration of upper respiratory secretions or refluxed stomach contents. Intubation is best achieved in the conscious patient by the nasal route, and this is easiest to do with the head fully extended. Nasotracheal tubes in the range of 7 to 10 mm outside diameter will accommodate most horse foals, and these tubes should be cuffed. The largest tube that can be passed through the upper respiratory tract without trauma should be used. Care should be taken to ensure that the tip of the tube is located in the cervical portion of the trachea, because it is possible to insert the tube too deeply, resulting in placement within a mainstem bronchus and ventilation of only one lung. After final positioning of the tube the cuff should be inflated with only enough pressure to maintain a seal during PPV, because excessive cuff pressure can cause tracheal injury and necrosis. The tube should be anchored to the patient's head to prevent inadvertent removal resulting from patient movement, and this can be done by tying a section of umbilical tape to the hub of the tube and anchoring the ends of the tape to an elastic tape bandage placed around the muzzle or to a soft cotton halter around the patient's head. Endotracheal tubes should be changed at least every 12 to 24 hours, because respiratory secretions and exudate will accumulate within the lumen of the tube, impeding gas flow and potentially causing obstruction of the tube. The presence of large amounts of pulmonary exudate may require even more frequent changing of the tube.

The ventilator gases must be appropriately pretreated before delivery to the patient to ensure that the gases are humidified and warmed and avoid injury to the respiratory mucosa. This can be achieved with an active humidifier, which is typically a component of the mechanical ventilator, and these devices are very effective in humidifying and warming the large volumes of gases involved. Unfortunately the gases tend to cool as they pass through the tubing from the ventilator to the patient, resulting in substantial condensation (rain out) within the circuit that must be periodically drained. This effect is accentuated if the hospital environmental temperature is low. Alternatively a passive humidifier can be used, which consists of a heat–moisture exchange (HME) filter device placed in between the ventilator circuit and the patient.[469] These devices are effective with small foals but may not be adequate for foals over 70 kg and may need to be supplemented with a cold active humidifier in the ventilator circuit.[469] The primary limitation to HME filters is that they tend to clog with airway discharge, which can result in obstruction and failure of mechanical ventilation.[470,471] For these reasons the author typically uses HME filters only for short periods of time, such as when first initiating mechanical ventilation, and relies on active humidification for the duration of ventilation.

The settings on the ventilator should be established before attaching the patient to the ventilator to avoid inadvertent overinflation of the lungs. Although there are several different ventilator modes to select from, on most ventilators there are some basic settings that are fairly universal including Fio_2, tidal volume, peak flow, and breath rate. Fio_2 should be set to the minimum level required to maintain an adequate Pao_2 but no lower than atmospheric O_2 (0.21% or 21%). The tidal volume is the volume of gas delivered with each machine-controlled breath, and this should be set such that the peak inspiratory pressure (PIP) does not exceed 25 to 35 cm H_2O to avoid trauma to the pulmonary tissues. Peak flow is the maximal gas flow rate delivered by the ventilator during the inspiratory phase of ventilation. Excessive peak flow rates will result in overly rapid inflation of the lungs and high peak airway pressures. Low peak flow rates will result in an overly long inspiratory phase, resulting in a loss of synchrony with the ventilator, especially at high respiratory rates. Reasonable initial settings for these parameters are an Fio_2 of 0.5 (0.3–1.0), a tidal volume of 5 mL/kg (up to 8 mL/kg), a peak flow of 70 L/min (60–80 L/min) and a breath rate of 20 to 30 breaths/min.[469] These values may require adjustment once ventilation is instituted, and the response to ventilation should be closely monitored. Additional variables that may be controlled with the ventilator are positive end-expiratory pressure (PEEP), which should be set at 3 to 5 cm H_2O, trigger sensitivity (−2 cm H_2O), pressure support (8–10 cm H_2O), and inspiration:expiration ratio (I:E ratio = 1:2).

When considering mechanical ventilation one encounters a bewildering array of modes of ventilation, but the ideal mode is one that maintains consistent and adequate tidal volume and minute ventilation at moderate airway pressures, is synchronized to the patient's respiratory efforts, responds to patient demands, and allows for the lowest possible work of breathing.[468] This discussion will be limited to the most commonly used modes of mechanical ventilation. SIMV is a modification of assist-control ventilation that still delivers a set minimum rate of mandatory breaths, which are rigidly controlled; however, the ventilator attempts to synchronize these breaths with the patient's inspiratory efforts if possible. The primary advantage of this mode is that it is well tolerated by the patient because of the synchronization of mandatory breaths with patient effort. In addition, SIMV ensures a minimum breath rate in patients with variable respiratory efforts or that are exhibiting periods of apnea. The limitation of SIMV is that the spontaneous breaths are not supported by the ventilator and require a large degree of patient inspiratory effort. Pressure support ventilation (PSV) allows the patient complete control over all aspects of the ventilatory cycle except for the pressure limit. In this mode each patient breath is supported by a preset assist pressure, which is stopped when the flow rate decreases below a set fraction of peak flow at end inspiration. This allows

the patient to determine the size of the breath and the inspiratory flow rate, substantially decreasing the work of breathing. The risk of PSV is that there is no mandatory minimal breath rate guaranteed by the ventilator. The limitations of SIMV and PSV can be overcome by using SIMV with pressure support. SIMV provides a guaranteed minimal breath rate, and the PS provides pressure support of the patient-initiated breaths to overcome the inherent resistance of the ventilator circuit and endotracheal tube. CPAP is used to provide for a positive airway pressure throughout the ventilatory cycle. The pressure provided is typically the clinician-selected PEEP level. CPAP is often combined with PS to aid in overcoming the resistance of the circuit and decreasing the work of breathing.

It is generally desirable when ventilating a patient to not only provide for the minimal ventilatory needs of the patient but to use the ventilator to recruit poorly ventilated regions of the lung back into participating in gas exchange. The most commonly utilized recruitment maneuver is PEEP, which functions to maintain positive pressure within the ventilatory circuit during exhalation and between breaths to prevent alveolar collapse that might result during periods of negative pressure. Over time the presence of PEEP will keep open the alveoli that are "recruited" during the inspiratory cycle, and this allows for gradual improvement in the functional capacity of the lungs. This can result in substantial increases in Pao_2 and may allow for a decrease in the Fio_2.[472] By preventing cyclical alveolar collapse and reopening PEEP, the amount of shear stress-induced pulmonary injury may also decreased.[472] The use of PEEP is not always benign, however, because it can cause compression of the intrathoracic venous system and impair cardiac return with detrimental effects on cardiac output. When first initiating mechanical ventilation it is reasonable to use PEEP in most patients, especially at moderate settings, because the benefits appear to outweigh the potential negative effects.

The provision of mechanical ventilation is a very dynamic process requiring substantial involvement of the clinician in the supervision of ventilator setup and adjustment. Appropriate monitoring is critical to effective mechanical ventilation, and this cannot be overemphasized. The two most important aspects of monitoring are the assessment of pulmonary mechanics and pulmonary function. When considering the assessment of pulmonary mechanics one is really analyzing the interaction of the mechanical ventilator and the patient, and the three most important parameters are the driving pressure, the tidal volume, and the tidal airflow. The primary parameters of ventilator function and ventilator/patient interaction are the PIP and tidal volume (V_T). These parameters are interrelated, because increasing pressures will be associated with increasing tidal volume in normal lungs within normal physiologic limits. In ventilated patients, especially those with substantial pulmonary disease, one will often find that this relationship is abnormal, with normal pressures resulting in inadequate tidal volumes, or excessively high pressures being required to achieve a desired V_T. Continuous monitoring of PIP is extremely important, because sudden decreases may indicate a circuit leak, ventilator failure, inadequate gas supply, or a leak in the endotracheal tube cuff.[469] Increases in PIP may indicate obstruction of the endotracheal tube caused by kinking or accumulation of exudate, bronchospasm, or pneumothorax.[469]

The other critical aspect of monitoring involves monitoring pulmonary function, which fundamentally consists of the evaluation of pulmonary ventilation, in the form of elimination of CO_2, and pulmonary oxygenation, in the form of arterial oxygen tension. Assessment of pulmonary oxygenation relies primarily on the measured Pao_2, because the Fio_2 is known. In the patient with normal pulmonary function there should be a linear increase in Pao_2 with increasing Fio_2, but this relationship breaks down in the presence of pulmonary disease caused by ventilation/perfusion mismatching. The presence of intrapulmonary shunts, resulting from the perfusion of poorly ventilated regions of the lungs, will cause the Pao_2 to be lower than expected. A reasonable target for Pao_2 is 80 to 100 mm Hg or slightly higher, because values above 150 mm Hg are not beneficial and are an indication to decrease the Fio_2. The goal is to use the Fio_2 closest to room air (0.21) that achieves the desired Pao_2, and Fio_2 values above 50% are associated with oxygen toxicity to the respiratory mucosa. Additional information can be derived from knowledge of the exhaled $EtCO_2$ concentration, which is measured using capnography. The $EtCO_2$ is representative of the $Paco_2$ in patients with normal pulmonary function, usually with a value 2 to 5 mm Hg less than the $Paco_2$.[469] Decreases in $EtCO_2$ indicate decreased pulmonary perfusion secondary to decreases in cardiac output or increases in pulmonary vascular resistance.[469]

An important aspect of clinical monitoring is to check the endotracheal tube for exudate accumulation when the tube is changed, which should be done at least daily (more often if copious exudate is present). An increase in the amount of exudate within the tube, or a change in the character of the exudate to a more purulent appearance, may be an early indication of ventilator-associated pneumonia (VAP). A hemorrhagic appearance to the tube exudate may suggest ventilator-associated lung injury (VILI) or worsening of pulmonary inflammation caused by other insults.

The administration of mechanical ventilation is inherently unnatural and unphysiologic and has the potential to disrupt the normal functioning of many body systems. The provision of PPV, especially in the form of excess PEEP, has the potential to substantially decrease venous return, leading to impaired cardiac output. This is a serious concern, especially in the critical patient where cardiac output is often already impaired. The presence of an endotracheal tube has a number of potential adverse effects. First, the tube causes an increase in airway resistance because of its long narrow lumen and increases the work of breathing associated with spontaneous respirations. Second, the presence of the endotracheal tube results in a bypass of the normal protective functions of the upper respiratory tract and can allow access to the respiratory tract for pathogenic organisms. Infections arising by this route are termed *VAP*, and the likelihood of VAP developing is increased with prolonged duration of mechanical ventilation. The presence of inflammation secondary to the process of mechanical ventilation itself will also increase the likelihood of VAP, as will impairment of the patient's immune function associated with systemic illness. The organisms involved in VAP are often nosocomial in nature, which can be associated with a pattern of increased resistance to antimicrobials, complicating treatment of the condition. Aerosolized ceftazidime has been shown to be effective in the prevention of VAP in human patients, while also attenuating the proinflammatory response in the lung.[473] The author has subjectively observed a decreased rate of VAP in mechanically ventilated foals when aerosolized amikacin is nebulized into the ventilator circuit starting within 24 hours of the initiation of mechanical ventilation.

Ventilator-induced lung injury (VILI) should be considered to be inevitable, and the clinician's goal is fundamentally

to minimize the severity of this effect. There are three fundamental types of VILIs: barotrauma, volutrauma, and atelectotrauma.[474] In patients with poor pulmonary compliance and areas of pulmonary atelectasis, high positive inspiratory pressure may be required to achieve adequate ventilation, and this causes overdistention of the ventilated regions of the lung (local volutrauma).[475] Volutrauma results from exceeding the normal physiologic functional residual capacity of the ventilated regions of the lung, resulting in the development of pulmonary edema and initiation/amplification of the local inflammatory response. PEEP is somewhat protective in that it appears to slow the development of pulmonary edema unless it too is excessive, at which point it contributes to overinflation and causes further harm.[476] Atelectotrauma is caused by the repeated opening and closing of lung units during tidal ventilation and essentially represents a syndrome of low-volume injury resulting in the initiation of an inflammatory response.[474] By appreciating that the lungs are susceptible to both high- and low-volume injury it is apparent that optimal mechanical ventilation is achieved within a fairly narrow range of tidal volumes and that this range may vary dramatically from patient to patient.

The discontinuation of mechanical ventilation *(weaning)* can be the most challenging part of the entire process, and in human patients this phase may constitute as much as 50% of the time the patient is being mechanically ventilated.[477] Arguably the process or weaning begins as soon as the patient is placed on the ventilator, because the clinician constantly strives to identify the minimum level of support required to maintain adequate ventilation and oxygenation. By minimizing the degree of support, one can accelerate the patient's recovery of strength and stamina in the muscles of respiration (or minimize its loss) while also increasing the clinician's ability to identify when the patient no longer requires ventilatory support. Gradually decreasing the level of pressure support provided and increasing the trigger sensitivity level can accomplish progressive challenge of the patient's ability to ventilate on its own. Ultimately, however, the determination of readiness to be removed from the ventilator is a subjective one, and the question can only be answered by an extubation challenge. This is preferable to simply removing the patient from the ventilator while leaving the endotracheal tube in place, because the tube causes significant resistance to airflow and increases the patient's work of breathing. Intranasal oxygen insufflation should be provided immediately following tube removal in most cases, unless the patient has been maintaining normal oxygenation at an Fio_2 of 0.21 while on the ventilator. Care must also be taken so that one is fully prepared to immediately reintubate the patient if the challenge is not successful and mechanical ventilation must be reinstituted. The primary endpoints of the challenge are respiratory rate, respiratory effort, and arterial blood gas evaluation, and some patients may fail within minutes while others fail over several hours. Several challenges may be required over a period of hours to days before one is certain that the patient is able be maintained without ventilatory support.

GASTROINTESTINAL DISORDERS

Colic

Colic is a common presenting complaint in foals.[478,479] Unfortunately the evaluation of foals with colic can be frustrating because of the common clinical presentation associated with acute abdominal pain, despite the many possible underlying etiologies. Foals are typically more demonstrative of abdominal pain than adult horses, and signs of colic in foals may range from very nonspecific signs such as tachycardia, tachypnea, anorexia, agitation, and bruxism to more classic signs such as abdominal distention, recumbency, rolling, and lying in dorsal recumbency.[480] Causes of colic in neonatal foals can include meconium impaction, infectious and noninfectious enterocolitis, dysmotility associated with other disease processes, small intestinal strangulating obstructions, congenital abnormalities, intussusceptions, hernias, gastric or duodenal ulceration, aerophagia, foreign body obstructions, lactose intolerance, hypoxic injury, and ovarian or testicular torsion.[480] Causes of colic in older foals are similar but also include conditions such as ascarid impactions, displacements, pyloric or duodenal strictures, abdominal adhesions, and nonstrangulating obstructions such as fecaliths. Nonintestinal pain may cause clinical signs indistinguishable from true colic in foals, and this may result from pain in the thorax, liver, or urogenital tract; distention associated with uroperitoneum; or nongastrointestinal inflammation such as peritonitis.

Examination of foals with colic is somewhat facilitated by their small size, which allows for repositioning and external abdominal palpation, as well as thorough ultrasonographic examination. The inability to perform a rectal examination beyond digital palpation can be a frustrating limitation, however. Just as in adults the initial goals of colic evaluation are stabilization of the patient and differentiation of cases requiring surgical versus medical management. Although most cases of colic in foals can be successfully managed medically, there are cases in which prompt surgical intervention will be required, and small intestinal strangulating obstruction is the most common.[479] The presence of diarrhea is usually an indication that medical management will be sufficient, but this may be present in association with a surgical problem. Unrelenting pain that is poorly controlled with analgesics is a strong indication of a surgical problem, just as in adults. Severe intestinal distention on ultrasonographic examination, particularly caused by fluid or mixed fluid and gas rather than gas alone, may also suggest the presence of a surgical lesion.

The most common cause of intestinal obstruction in neonatal foals is meconium impaction.[480,481] Meconium is a mixture of glandular secretions, mucus, bile, digested amniotic fluid, and epithelial cells and should normally be expelled within the first few hours of life following the foal's first feedings. Colostrum feeding may be helpful in the expulsion of meconium because of the laxative effects of colostrum, but the lack of colostrum intake in a recent study had no effect on meconium release compared with foals fed milk replacer.[482] If the meconium is not passed by 12 hours of age it will likely cause intestinal obstruction, typically at the level of the small colon or the pelvic inlet.[481,483] Progressive intestinal and abdominal distention will develop over the next several hours secondary to gas accumulation orad to the obstruction. Identification of the mass of meconium may be possible on manual palpation of the abdomen, but ultrasonography is a more sensitive and specific means of identifying meconium obstructions. In some cases retrograde contrast radiography may be helpful in confirming the presence and level of the obstruction and in ruling out other causes of obstruction such as congenital defects. Administration of an enema can be useful both as a therapeutic intervention and a diagnostic test and is indicated in all cases of suspected meconium impaction. Sodium phosphate

enemas are widely used because of their ready availability in a premixed human preparation that is easily administered. Care must be taken to avoid hyperphosphatemia caused by excessive administration, however, and for this reason this type of enema should be administered no more than twice in the first 24 hours of life. Soapy water enemas given by gravity flow have also been frequently used, but because of the likelihood of rectal irritation and limited efficacy they should not be used repeatedly.

The most effective type of enema for the treatment of meconium impaction is the acetylcysteine retention enema.[481] These enemas can be prepared using commercially available acetylcysteine (*N*-acetyl-L-cysteine powder, Sigma-Aldrich, St. Louis, MO) by adding 8 g of acetylcysteine to a solution of 20 g of baking soda in 200 mL of water. Commercially prepared acetylcysteine enemas for foals are also available (http://www.scahealth.com/e-z-pass-foal-enema-kit.html). The foal is placed in lateral recumbency and restrained and sedated, if necessary. A 30-Fr Foley catheter with a 30-mL balloon is placed 2.5 to 5 cm into the rectum, and the balloon is filled, taking care not to overinflate. The acetylcysteine solution is infused using gravity flow to a volume of 100 to 200 mL and left within the rectum for 30 to 45 minutes.[481] Because this type of enema does not seem to be highly irritating, this procedure can be repeated as many as three times, usually at 12- to 24-hour intervals. Additional treatments that are helpful in managing affected foals include IV fluid therapy to address any deficits and ongoing maintenance needs, as well as analgesic therapy. Butorphanol administration may be sufficient to control discomfort in many foals, but individuals with more pain may require flunixin administration to control their pain while medical management is pursued. Cases refractory to medical management may require surgical correction of the meconium impaction, but this is uncommon since the introduction of acetylcysteine enemas.[481]

Roundworm *(P. equorum)* infestation is a common condition among foals, with a reported prevalence around 40%.[484] Most foals will not show evidence of infestation, but the clinical signs associated with the presence of this parasite may include lethargy, anorexia, decreased weight gain, coughing, hypoproteinemia, nasal discharge, colic from gastrointestinal impaction, and occasionally rupture or perforation of the small intestine.[485,486] The colic signs result from acute small intestinal obstruction caused by a large burden of the parasite, and in most cases the colic is associated with recent administration of anthelmintic medications but may be associated with a stressor such as transportation or weaning.[485] Horses with impaction of the small intestine caused by *P. equorum* commonly suffer from secondary intestinal abnormalities caused by luminal obstruction, including small intestinal volvulus or intussusception, and surgical intervention is typically required to prevent intestinal rupture.[486,487] As a result of the increasing resistance of *P. equorum* to commonly used anthelmintics, especially the macrocyclic lactones, it seems likely that the prevalence of this condition will increase.[403,488-490]

Although congenital defects of the gastrointestinal tract are rare, they must be ruled out when evaluating the neonatal foal with colic because of the similarities in clinical presentation with meconium impaction. The most common congenital defects are atresia of the colon, rectum, or anus. Affected foals typically present within the first 2 to 48 hours of life and exhibit signs of acute, progressive colic and abdominal distention.[338] In addition to signs of colic (tachycardia, tachypnea, recumbency, rolling, and anorexia), tenesmus may be noted. No fecal material will have been passed, and there will be no meconium staining noted in enema fluids that have been administered. Atresia ani is readily diagnosed on visual examination and digital palpation, whereas rectal and colonic lesions are more challenging to identify.[491] Ultrasonography may be useful in demonstrating distention orad to the site of the lesion but rarely is able to characterize the lesion. Retrograde contrast radiography can be helpful in further characterizing the lesion and differentiating atresia from meconium impaction. Colonoscopy may aid in diagnosing lesions in the terminal colon and rectum. This procedure can be performed under standing sedation, and the use of *n*-butyl scopolamine bromide (Buscopan, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) has been shown to aid visualization.[492] Ultimately surgical exploration may be the only way a definitive diagnosis of atresia coli can be made. Surgical correction of atresia ani is straightforward and typically successful, although anal sphincter function may not be normal.[491] Atresia ani may be accompanied by penile malformations (hypospadias) that will also require surgical correction.[493] Surgical resection and anastomosis has been attempted in some cases of atresia coli in which the lesion was accessible, but outcomes have been poor.[491] This is likely caused by additional underlying neurologic and motility disorders and means that this condition should be considered typically fatal.

Congenital aganglionosis, or overo lethal white syndrome, is a syndrome that affects white American Paint Horse foals born from overo-overo matings, although it has been reported in one foal with one solid color Quarter Horse parent.[494] This syndrome occurs when foals receive one copy of the mutated endothelin receptor B gene (*EDNRB,* overo lethal white gene) from each parent.[495,496] Affected foals suffer from aganglionosis of the distal small intestine and large intestine, which results in a lack of intestinal motility leading to colic.[494] Recent work suggests that the extrinsic innervation may be affected as well.[497] Not all foals born from overo-overo matings will be affected, so care should be taken to ensure that the foal is not simply suffering from meconium impaction before making a potentially terminal decision. A mutagenically separated PCR was recently developed for the detection of the *EDNRB* genotype in horses.[498]

Diarrhea

Diarrhea is a common problem in foals, with substantial morbidity and mortality.[405] It is associated with a number of potential pathophysiologic mechanisms and many etiologies, making diagnosis and management challenging. Syndromes involving noninfectious etiologies as well as bacterial, viral, protozoal, and parasitic etiologies are all reported in foals. Although many of the conditions associated with diarrhea can affect foals of any age, some conditions, such as necrotizing enterocolitis (NEC) and asphyxia-associated enteric dysfunction, are primarily seen in neonates, whereas others, such as proliferative enteropathy, are seen in older, weanling age foals.

Foal heat diarrhea is a mild, self-limiting condition seen in foals from 5 to 15 days of age, and is temporally associated with the occurrence of the mare's first heat following parturition. This temporal association has implied causation, but this is not the case. Foals raised on milk replacer also exhibit diarrhea during this time frame, and analysis of the composition of mares' milk has not revealed any changes that might precipitate the development of foal diarrhea.[499] Affected foals are

clinically normal with the exception of diarrhea and remain bright with good appetites. It appears more likely that changes occurring within the gastrointestinal tract in relation to solid feed ingestion and development of the fecal microbiota are responsible.[500,501] Additionally there may be hypersecretion of the small intestinal mucosa that is inadequately compensated for by immature colonic absorption that contributes to foal heat diarrhea.[502] Diarrhea in a foal of this age should not be taken lightly, however, because rapid clinical progression is possible. Any sign of systemic illness, dullness, or inappetence should prompt rapid assessment for a more severe condition than foal heat diarrhea that may warrant medical intervention.

A common cause of diarrhea in hospitalized neonatal foals is perinatal asphyxia-associated gastrointestinal dysfunction. Affected foals typically have associated risk factors such as dystocia, delivery by cesarean section, umbilical problems during delivery, or some cause of inadequate oxygenation immediately postpartum.[503] These foals may exhibit dysmotilities leading to ileus, gastroduodenal reflux, intolerance of enteral feeding, abdominal distention, colic, and diarrhea.[503] Evidence of perinatal asphyxia syndrome may be observed in other body systems, especially the neurologic system. Affected foals should be considered at risk for developing sepsis, and a similar presentation may be observed in foals suffering from sepsis, likely caused by the presence of severe systemic inflammation. For these reasons appropriate broad-spectrum antimicrobial therapy is indicated in these cases.

Another condition affecting hospitalized neonatal foals is NEC. This syndrome is the most frequent and lethal disorder affecting preterm human infants and is associated with disruption of the intestinal barrier leading to intestinal necrosis, multiorgan failure, and death.[504] NEC has been sporadically reported in foals for many years, but it appears likely that it represents an underdiagnosed condition.[505-511] A broad variety of infectious agents, including not only bacteria but also viruses and fungal species, have been associated with NEC in humans.[512] Although most of the cases reported in foals have been linked to the presence of a variety of pathogenic bacteria, the cause of NEC remains the subject of much discussion in human literature, and it is not clear if it is primarily an infectious condition. It appears likely that the pathophysiology is truly multifactorial, involving a complex interplay between intestinal immaturity, hemodynamic instability, inflammation, genetic factors, formula feeding, and dysbiosis.[513-515] The clinical presentation may be similar to that described for the asphyxia-associated disorders mentioned previously, and certainly those foals may be considered at risk for NEC. In a recent report foals diagnosed with NEC presented most commonly with colic or diarrhea, but these signs were absent in the majority of affected foals.[511] Other abnormalities observed in association with NEC included prematurity, gastric reflux, abdominal distention, bloody feces, and pneumonia.[511] Interestingly, the authors of that report found no evidence of an association of *C. difficile, C. perfringens,* or *Salmonella* species with NEC.

The diagnosis of NEC is made through radiographic or ultrasonographic evidence of intramural gas in the intestinal wall (pneumatosis intestinalis) or surgical or postmortem evidence of gastrointestinal necrosis.[511] Although as many as 80% of affected human neonates are reported to survive, the presence of NEC is associated with a poor prognosis for survival in foals.[511,513] Treatment of foals with NEC will be as for any critically ill foal and will likely include IV fluid therapy and broad-spectrum antimicrobial therapy. Because of concerns that enteral feeding may precipitate or worsen NEC, it has been recommended that enteral feeding should be avoided, which is generally recommended in the foal with colic, ileus, or reflux. Parenteral nutrition will be required until enteral feeding can be gradually reintroduced. Human studies have suggested that a minimal enteral nutrition approach, rather than complete withdrawal of enteral feeding, using less than 20 mL/kg per day for the first several days, then gradually advancing to normal intake levels over several days, appears to be well tolerated and safe.[516,517] Human studies have shown that breast milk is protective against NEC in low-birth-weight infants, and for this reason it makes sense to use the mare's milk for feeding the foal if possible.[503,518] Metronidazole therapy may be indicated because of the reported association of NEC with clostridial infection in foals, although a recent human report found that metronidazole therapy may not prevent clinical deterioration in cases of NEC.[519]

Other causes of noninfectious diarrhea can include dietary intolerance, particularly when milk replacers are fed rather than mare's milk.[503,520] This can be the result of products used in the milk replacers that are poorly digested by foals, such as maltodextrins, corn syrup, oligosaccharides, or glucose polymers.[520] Gastrointestinal irritation secondary to ingestion of sand, grit, or dirt may also result in diarrhea caused by mechanical irritation of the mucosa.[521]

Infectious Causes of Diarrhea

The most common cause of infectious diarrhea in foals is rotavirus, which is detected in the feces of foals with diarrhea in 20% to 77% of cases.[522-524] Equine coronavirus has been associated with clinical disease in adult horses[525] and has been detected in the feces of diarrheic foals, but the role of this virus in foal disease is unclear.[524,526-528] EAdV2 has also been detected in the feces of foals with diarrhea, but the actual involvement of adenovirus in the development of disease is unclear, especially in the immunocompetent individual.[529-532] There are older reports of parvovirus-like particles identified in foals with diarrhea, but no further evidence has emerged regarding the potential pathogenic role of such organisms in the foal.[533,534]

The pathogenesis of rotaviral diarrhea is multifactorial, and this organism is frequently detected in combination with other potential gastrointestinal pathogens. Rotavirus infects the epithelial tips of the villi of the duodenum, jejunum, and ileum, but it does not affect the crypt epithelium.[523] The virus replicates within the villous epithelium and is released following cell lysis, which results in destruction of the infected cell and desquamation of the villous tips. This leads to a loss of the normal absorptive capacity of the villi, but the secretory crypt epithelium is unharmed, resulting in malabsorption that likely contributes to the development of diarrhea. Villous injury also results in the decreased production of disaccharidases, particularly lactase, which may impair the digestion of lactose and contribute to the development of diarrhea by osmotic mechanisms. Other factors contributing to the development of rotaviral diarrhea may be the activity of viral enterotoxins, inhibition of sodium-glucose cotransport, dysregulation of calcium homeostasis, and activation of the enteric nervous system.[523]

Clinically rotaviral diarrhea often occurs in large, comingled groups of mares and foals, and affected foals are typically from 5 to 35 days of age.[535] Foals initially exhibit anorexia and

depression, which quickly progresses to acute, profuse watery diarrhea. Affected foals may become rapidly dehydrated and frequently develop electrolyte abnormalities and metabolic acidosis. Although the morbidity associated with rotavirus infections is high, which is caused by the highly contagious nature of the virus, the prognosis for survival is good. Treatment is generally supportive and symptomatic, but IV fluid therapy is often needed to effectively address the fluid and electrolyte deficits. Balanced electrolyte replacement solutions are most effective given the frequent involvement of hyponatremia and hypochloremia. Severely affected foals may also benefit from a brief period of enteral rest (1–2 days), which necessitates the provision of parenteral nutrition until feeding can be reintroduced. Supplementation with lactase enzymes (Lactaid, McNeil Nutritionals, LLC, Ft. Washington, PA) at 9000 U (one tablet) PO every 3 to 8 hours may be helpful in improving milk digestion in foals that remain on the mare, or when nursing is reintroduced.[503] The diagnosis of rotaviral diarrhea is by detection of viral particles using electron microscopy, virus isolation, enzyme-linked immunosorbent assay (ELISA), immunochromatography tests, and real-time reverse-transcriptase PCR (RT-PCR). Of these the immunochromatography tests and RT-PCR are the most rapid and simplest to perform and provide high sensitivity and specificity.[523,536] Control of rotaviral diarrheal outbreaks can be challenging, especially in crowded environments because of the highly contagious nature of the virus, its persistence in the environment, and the resistance to disinfectants.[523] Prevention can be facilitated by the use of maternal vaccination, which has been associated with a reduction in the frequency and severity of rotaviral diarrhea on endemic farms, and a conditionally licensed commercial vaccine is available for provisional use (equine rotavirus vaccine, Zoetis, Kalamazoo, MI).

The primary bacterial agents of concern in foal diarrhea are *C. difficile* and *C. perfringens*. Discerning the relationship between these organisms and clinical disease has been challenging because either or both organisms can be identified in normal animals as well as diseased animals, but both organisms are isolated significantly more frequently from foals with enterocolitis than from healthy foals.[506,509,522,537-540] *C. difficile*–associated diarrhea (CDAD) is commonly associated with a history of antimicrobial therapy and likely represents the primary agent of antimicrobial-associated diarrhea (AAD) in foals.[541] Spontaneous cases of CDAD can occur without exposure to antimicrobials, however, both sporadically and in outbreaks.[509,537,542,543] Additional risk factors that may predispose foals to CDAD include hospitalization, stress, dietary alterations or starvation, transportation, nasogastric intubation, and surgical or medical treatment.[544] Clinically CDAD can be quite variable both in terms of the clinical signs and the severity of the disease. The primary clinical sign of CDAD is diarrhea, which may be watery or bloody, typically accompanied by signs of systemic inflammation and hypovolemia (hyperemic mucous membranes, prolonged capillary refill time, pyrexia, tachycardia, and tachypnea) and occasionally by abdominal distention and colic.[545] Unfortunately these signs are nonspecific, and although CDAD may be suspected on the basis of history and clinical presentation, diagnostic testing is required for confirmation of the diagnosis. Because normal animals may carry the organism and these often represent nonpathogenic strains, culture is not useful for diagnosis of CDAD. Virulence of *C. difficile* depends on the elaboration of toxin A (TcdA) and toxin B (TcdB), and there is evidence to suggest that both toxins are important in the development of clinical disease.[546,547] An additional toxin, termed *C. difficile transferase* (CDT), appears to play a role in virulence as well; CDT-producing strains have been associated with increased mortality in human patients.[547] Confirmation of a diagnosis of CDAD is based on the detection of TcdA and/or TcdB in the feces of the affected individual, and this is most often performed using a commercial ELISA (*C. difficile* Tox A/B II, Techlab, Inc., Blacksburg, VA) or RT-PCR.[524,541]

C. perfringens has also been associated with enterocolitis in foals, and this syndrome is typically quite severe in nature, with high mortality rates.[508] Both type A and type C *C. perfringens* have been implicated in these cases, but the more severe form of the disease is typically associated with type C, and affected foals do not typically respond to treatment.[508] *C. perfringens* produces four major toxins, but the β-toxin appears to be the primary one responsible for intestinal injury.[509] A novel β-pore-forming toxin, NetF, has recently been described and has been associated with severe NEC in dogs and foals.[548,549] Risk factors may include birth on dirt, sand, or gravel and housing in stalls or dry lots in the first few days of life. Clinical signs are similar to CDAD, but because of enterotoxemia affected foals may show more pronounced signs of systemic inflammation and shock. Hemorrhagic diarrhea has been reported in some cases but may be transient in nature. Diagnosis of *C. perfringens* enterocolitis can be challenging, because the organism may be present in the feces of healthy foals. A positive fecal culture in combination with appropriate signalment and clinical presentation may be supportive, however, because healthy foals will generally shed very low numbers of the organism. Fecal Gram stain may also be supportive if it demonstrates large numbers of large gram-positive rods or spores. Commercial assays are available for detection of fecal enterotoxin, but they lack sensitivity.

Treatment of CDAD and *C. perfringens* enterocolitis is primarily supportive in nature, similar to other causes of enterocolitis. Broad-spectrum antimicrobial coverage is indicated, even in situations in which AAD is suspected, because of the risk of bacteremia secondary to bowel wall inflammation and injury. Metronidazole therapy represents the primary specific therapy for clostridial infections, but metronidazole-resistant strains of *C. difficile* have been reported.[550] A recent pharmacokinetic study suggests that the metronidazole dosing regimens used in foals should be revised to 10 mg/kg orally every 12 hours for newborn foals and 15 mg/kg orally every 12 hours for the 10- to 12-day-old foal.[551] After that age the current recommendation of 15 mg/kg orally every 8 hours remains unchanged. Additional therapies that may be of some benefit in CDAD include enterally administered adsorbents, such as di-tri-octahedral smectite.[552] Probiotic therapy is of interest because of the positive results reported in human patients, but the limited studies do not support the efficacy of probiotics in foal diarrhea at this time.[553,554]

A number of other bacteria have been associated with diarrhea in foals, including *Salmonella* spp., *E. coli, Enterococcus* spp., *Aeromonas* spp., and *Bacteroides fragilis,* although causation can be difficult to establish.[531,555-558] Given that diarrhea is a common clinical abnormality associated with sepsis in foals, it is important to be aware that bacterial involvement may be present, even if not in the form of a primary enteric pathogen. One retrospective study found that of foals less than 30 days of age presenting with diarrhea, 50% were bacteremic at admission, further reinforcing this concern.[120] Blood cultures

should be collected routinely at admission in this population, not only to confirm bacteremia but also to yield information regarding antimicrobial sensitivities.

R. equi, although primarily a respiratory pathogen, commonly exhibits extrapulmonary involvement and has been associated with enteric disease, primarily in older foals.[558] Diarrhea, ulcerative enterotyphlocolitis, abdominal abscessation, mesenteric lymphadenitis, and peritonitis have all been reported.[432] In one study 33% of foals with *R. equi* infections were reported to have diarrhea, and in half of those foals diarrhea only began following the initiation of treatment for the primary infection.[432]

Equine proliferative enteropathy (EPE) is an infectious disease affecting young horses, primarily foals from 2 to 8 months in age.[559,560] EPE is caused by the obligate intracellular bacterium *Lawsonia intracellularis.* This disease causes dramatic structural changes in the mucosal epithelium of the small intestine caused by proliferation of the crypt epithelial cells. These alterations lead to a protein-losing enteropathy that can result in variable degrees of hypoalbuminemia, hypoproteinemia, ventral edema, lethargy, diarrhea, fever, weight loss, and colic.[561,562] Diagnosis of proliferative enteropathy is by a combination of clinical signs (ventral edema), clinicopathologic abnormalities (hypoalbuminemia), abdominal ultrasonographic examination, immunoperoxidase monolayer assay serology, and fecal PCR testing for *Lawsonia* DNA.[563] Treatment is best accomplished using lipophilic antimicrobials administered for 3 weeks, such as tetracyclines (oxytetracycline, doxycycline, or minocycline), macrolides (azithromycin and clarithromycin), or chloramphenicol.[564] Rifampin has been used in combination with macrolides but does not appear necessary to achieve a positive clinical response. Regarding prevention, unfortunately passively acquired antibodies have been demonstrated to have no effect on the occurrence of subclinical or clinical EPE.[565] Promising results regarding prevention have been seen with the intrarectal administration of a commercial avirulent porcine vaccine against *L. intracellularis,* which has been demonstrated to result in complete protection against EPE and reduces fecal shedding.[566] The prognosis for survival with appropriate treatment is good, but affected animals often exhibit a decrease in growth rate and as a result may be at a slight disadvantage relative to their peers for several months.[560] Recent reports have detailed a more severe form of EPE that is associated with fatal outcomes, but these cases appear to be unusual.[567,568]

The pathogenic role of *Cryptosporidium* spp. in foal diarrhea has been the subject of debate because normal foals have been shown to shed the organism, but there have been several studies that implicate this organism in both sporadic and outbreak situations.[569-572] Although the most commonly identified species has been *C. parvum,* another *Cryptosporidium* (the horse genotype) has been identified in horses, humans, and a calf.[573,574] Affected foals usually do not exhibit substantial systemic inflammation, and the disease is typically self-limiting. Profuse watery diarrhea may necessitate IV fluid therapy in addition to routine supportive therapy. This disease has clear zoonotic potential, so appropriate biosecurity precautions are indicated to protect in-contact individuals. Indeed, a recent report detailed an outbreak of human cryptosporidiosis in veterinary students associated with exposure to infected foals in an equine perinatology unit.[575] Diagnosis has traditionally relied on the identification of oocysts in the feces using acid-fast staining techniques, but RT-PCR testing can be performed, and rapid and easily performed immunofluorescence assays are commercially available (Xpect Giardia/Cryptosporidium Test, Thermo Scientific, Waltham, MA; Merifluor Cryptosporidium/Giardia, Meridian Bioscience, Inc., Cincinnati, OH).

Strongyloides westeri is a nematode primarily transmitted to foals by the transmammary route. Although this organism has been suspected as a cause of diarrhea in young foals, it appears likely that clinical disease would only be present when the parasite is present in very high numbers.[503,569] Control is typically by means of ivermectin administration to the mare shortly after delivery, but the prevalence of this parasite appears to be increasing, perhaps related to the decrease in ivermectin administration to foals because of concerns for ivermectin resistance in *P. equorum.*[576]

Gastric Ulceration

Gastric ulceration has been frequently reported in foals, with prevalence ranging from 22% to 57%.[577-580] The clinical signs associated with gastric ulceration in foals are often nonspecific and may include diarrhea, colic, rolling, restlessness, lying in dorsal recumbency, excessive salivation, bruxism, and anorexia.[581] For this reason diagnosis should not be made on clinical grounds alone but should rely on gastroscopic examination. In addition to confirming the presence of gastric ulceration, gastroscopic examination allows for thorough characterization of the site(s) involved and the severity of the lesions present. Noninvasive techniques for diagnosing gastric ulceration in foals are of interest but are not well validated and would not provide detailed information regarding the extent and severity of gastric ulceration.

The greatest challenge associated with the diagnosis and treatment of gastric ulceration in foals is the fact that many of the animals with endoscopically evident ulceration do not show any clinical signs suggesting that the ulcers are causing dysfunction or discomfort.[580] Therefore the clinical significance of gastric ulceration in some foals, and the need for treatment, is unclear. Despite this fact there has been substantial concern related to the risk of intestinal perforation secondary to the progression of clinically silent gastric ulcers, because this condition is fatal when it occurs.[582] For this reason prophylactic antiulcer therapy has often been administered to clinically ill foals or those receiving nonsteroidal therapies. The efficacy of these prophylactic approaches has not been confirmed in clinically ill foals, however, and the response of sick foals to acid-suppressive therapy is less consistent than in healthy foals.[583] Indeed, two large retrospective studies reported that the prevalence of gastric ulceration on postmortem examination of hospitalized foals was not related to whether or not prophylactic treatments were administered.[578,584] Unfortunately prophylactic acid suppression may be associated with some risks, and human studies have documented an increased risk of pneumonia and CDAD in non-ICU hospitalized patients treated with acid-suppressive drugs.[585,586] Proton-pump inhibitors (PPIs) are more potent inhibitors of acid suppression than histamine 2-receptor antagonists (H2RAs), and for this reason PPIs appear to be associated with a greater risk of these complications.[587,588] In human ICU patients there does not appear to be an increased risk of pneumonia or differences in overall mortality associated with acid suppression, but the use of PPIs is associated with an increased risk of CDAD.[586] A recent multicenter study found that the use of acid-suppressive therapy in hospitalized foals was associated with an increased risk of

undifferentiated diarrhea but not of CDAD.[589] Together these concerns suggest that such use should be avoided and that treatment be limited to those foals with documented disease requiring treatment.

The pathophysiology of gastric ulceration in the foal is likely complex and multifactorial and may differ between the squamous and glandular mucosal regions of the stomach. Within the stomach there is a constant interplay between protective factors and aggressive factors. The protective factors maintain a healthy gastric mucosa by promotion of mucosal blood flow, mucus and bicarbonate production, prostaglandin E2 production, promotion of epithelial growth factors, epithelial cell restitution, gastric afferent innervation, and gastroduodenal motility. The aggressive factors, which include gastric acid, bile salts, pepsin, and numerous enzymes, act to induce and potentiate gastric epithelial injury. As gastric acid production is highly variable in neonatal foals and the intragastric environment is often alkaline,[583] it seems likely that ulceration results most commonly from suppression of the protective factors rather than from excess acid production. Perinatal hypoxia, systemic illness, or the administration of nonsteroidal antiinflammatory medications may lead to gastric mucosal hypoxia or ischemia, impairing the local protective factors and predisposing the patient to mucosal ulceration. Ulceration may occur in the squamous mucosa, the glandular mucosa, or both, and may extend to involve the duodenum and/or the esophagus. The clinical syndromes associated with ulceration include subclinical, clinically significant ulceration, perforating ulceration, and gastroduodenal ulcer syndrome (GDUS). This scheme should perhaps be expanded to include gastroesophageal ulceration, although this is most commonly associated with reflux esophagitis secondary to GDUS-induced delayed gastric emptying. The classification of ulceration as subclinical or clinical may be challenging and cannot be based solely on the number and severity of lesions identified on gastroscopic evaluation. Instead, it must involve consideration of the history and clinical signs associated with those findings. The diagnosis of perforation may also be difficult and cannot be based on gastroscopic assessment alone because of the sometimes benign surface appearance of these lesions. For this reason abdominal ultrasonography and abdominocentesis are important in confirming the presence of perforation. The diagnosis of GDUS is based on gastroscopic evaluation combined with the presence of one or more of the signs of gastric ulceration listed previously, although signs of gastric-outflow obstruction, such as ptyalism and bruxism, are typically more common with this diagnosis.

GDUS is most commonly seen in foals 2 to 6 months of age and is often associated with an unthrifty, pot-bellied appearance and foals being small relative to their peers.[590] Ultrasonographic examination is indicated in foals suspected to be suffering from GDUS because it allows for noninvasive assessment for gastric and duodenal distention. When gastric distention is present a nasogastric tube should be passed to extract the fluid and facilitate gastroscopic evaluation. Gastroscopic abnormalities commonly observed with GDUS include esophagitis, gastric squamous and glandular ulceration, pyloric ulceration, and pyloric stricture. Examination of the duodenum is usually not possible because of the presence of pyloric stricture. Treatment of affected foals is challenging and requires intensive management. Frequent gastric decompression is required, and parenteral nutrition is typically necessary because of the inability of the foal to eat. Supportive care including IV fluids, antimicrobials, and analgesics is also indicated. Acid-suppressive therapy is required, and this is usually accompanied by gastroprotectants and prokinetics. Although medical management may be helpful in resolving delayed gastric emptying, many affected foals will have severe fibrosis and constriction of the pylorus and duodenum, necessitating surgical correction. The goal of surgical correction is to bypass the pylorus and proximal duodenum, which typically involves gastrojejunostomy. Surgery is challenging, and success likely depends on early intervention, surgical expertise, and appropriate postoperative care.[590] Reported surgical outcomes have improved over time, with the most recent report demonstrating 100% survival to hospital discharge and 50% long-term survival.[591]

The cornerstones of treatment of gastric ulceration in foals are acid-suppressive drugs, primarily the H2RA drugs cimetidine and ranitidine and the PPI omeprazole (Table 20.11). The acid-suppressive effects of the H2RAs are variable and less dramatic than those seen with PPIs, but these drugs may suppress acid production sufficiently to facilitate ulcer healing.[592] Because of the more consistent and pronounced acid-suppressive effects of the PPI omeprazole, this drug has become the mainstay of gastric ulcer therapy in horses.[593] Dosing for foals can be challenging, because there is no labeled form specifically designed for use in foals, but the product marketed for use in adults can be measured out on a volume basis and administered orally at a body weight–appropriate dosage to foals. An IV formulation of omeprazole is not readily available for administration to foals that cannot take medications orally, but the IV pharmacokinetics of the PPI pantoprazole have been determined in foals.[594] Other treatments include

TABLE **20.11** **Drugs Used in the Prophylaxis and Treatment of Gastric Disorders in Foals**

Drug Class	Drug	Dosage (mg/kg)	Route	Frequency (h)
H2RA	Ranitidine	6.6	PO	8
		1.5–2.0	IV	6
PPI	Omeprazole—treatment	4	PO	24
	Omeprazole—prevention	1	PO	24
	Pantoprazole	1.5	IV	24
Mucosal protectant	Sucralfate	20–80	PO	6–12
Prostaglandin E1 analog	Misoprostol	2–5 μg/kg	PO	8–12
Antacids	Aluminum/magnesium hydroxide	120–240 mL	PO	4–8

H2RA, Histamine 2-receptor antagonists; *IV,* intravenously; *PO,* orally; *PPI,* proton pump inhibitor.

mucosal adherents, such as sucralfate, and oral antacids, such as aluminum hydroxide and magnesium. Sucralfate dissociates in the acidic environment of the stomach to sucrose octasulfate and aluminum hydroxide. Sucrose octasulfate binds to the ulcerated mucosa and decreases tissue exposure to hydrochloric acid and may also interfere with the activity of peptic enzymes.[595] Sucralfate appears safe and is well tolerated and likely represents a safer approach for ulcer prevention in hospitalized neonatal foals compared with the use of a PPI. The prostaglandin E1 analog misoprostol has been used in an effort to enhance ulcer healing by increasing epithelial mucous secretion, bicarbonate secretion, and mucosal blood flow.[596] Misoprostol may also enhance epithelial repair via closure of tight junctions following epithelial restitution of denuded regions of the mucosa.[597]

UROGENITAL DISORDERS

Abnormalities involving the urogenital tract are common in foals and can be congenital or acquired in nature. Acquired conditions may occur secondary to infectious, toxic, traumatic, or iatrogenic causes. The presence of urogenital disorders may represent the primary disease process or may occur secondary to other disease processes. In some cases the presenting signs may be directly associated with the urogenital disorder, but often these cases present with nonspecific signs such as depression and poor body condition. Clinicopathologic evaluation may be normal, but cases involving impaired renal function or urine retention are typically associated with azotemia and electrolyte abnormalities. Azotemia most often presents as an elevation in serum creatinine concentration, but with more chronic or insidious conditions an elevation in blood urea nitrogen (BUN) may be more prominent. Serum electrolyte abnormalities may include hyponatremia, hypochloremia, and hyperkalemia. Metabolic acidosis is also common. Urinalysis in normal foals will demonstrate hyposthenuria, because they produce dilute urine with a specific gravity 1.010 or less. Foals with renal dysfunction may have hyposthenuric, isosthenuric (specific gravity of 1.010), or concentrated, hypersthenuric urine (specific gravity >1.010), depending on the nature of dysfunction. Transient proteinuria is typical at birth, but this should resolve in the first few days of life in normal foals, and casts should not be present.

Congenital Disorders

Congenital urinary tract disorders are quite rare in foals, but of these renal dysplasia or hypoplasia is the most commonly reported.[598-604] No familial or breed predisposition has been demonstrated for this condition in horses. In utero exposure to therapeutic drugs or toxins may cause renal dysplasia, and there are reports of renal dysplasia in foals born to mares treated for equine protozoal myeloencephalitis with pyrimethamine, trimethoprim, sulfonamides, folic acid, and vitamin E.[605] Renal dysplasia or hypoplasia may represent an incidental finding in a foal or young adult horse presented for evaluation of other problems. Azotemia is typically not present unless renal function has been impaired by at least 65% to 75%, because of the substantial reserves of renal function that are normally present. Cases with bilateral renal involvement, urine retention, or suffering from acute-on-chronic disease are more likely to be associated with clinical and clinicopathologic findings consistent with renal insufficiency. Patients with unilateral involvement often remain asymptomatic, whereas treatment of renal dysplasia or hypoplasia is usually unrewarding if bilateral renal involvement is present.

Pollakiuria, dysuria, stranguria, and incontinence may be observed with renal dysplasia/hypoplasia, but these are more commonly associated with conditions such as ectopic ureters.[606-608] Ectopic ureters may be associated with urine scald caused by urinary incontinence. Surgical intervention, while often challenging, is the only option for addressing cases of ectopic ureter, with relocation of the ureteral opening being the primary intervention. Hematuria is uncommonly associated with congenital urogenital disorders but may be present in association with renal arteriovenous malformations.[609] Congenital abnormalities of the bladder have been suspected in some cases of uroperitoneum,[610,611] but evidence of a true congenital origin is lacking in horses or other species.[612] Umbilical hernias may be present at birth but are usually small and will resolve without intervention. Larger hernias, typically over 3 cm in size, may require surgical repair, but there is no rush to perform this procedure as long as the hernia remains easily reducible. Inguinal hernias may also be present at birth but are also typically small and easily reducible. Conservative medical management consisting of frequent, repeated reduction, or bandaging to maintain reduction, is often successful in resolving small hernias. Larger inguinal hernias may allow for substantial herniation of the small intestine and often require surgical repair.

Uroperitoneum

Accumulations of urine within the peritoneal cavity represent one of the most common urogenital conditions in young foals, involving as many as 2.5% of hospitalized neonates.[613] Most cases are observed within the first few days of life and are associated with bladder wall rupture.[614,615] The most common site of bladder wall failure is the dorsal wall, and it is believed that most cases occur during parturition. The proposed etiology is that the fetus has a very full bladder, which ruptures under the intense pressures occurring during transit through the birth canal. Obstruction of urine outflow via the urachus during delivery may contribute to high intravesicular pressures. This theory is supported by anecdotal reports of bladder distention identified on fetal ultrasonographic examination in foals that subsequently developed bladder rupture after birth. There may be some sex predilection for bladder rupture, with colts being overrepresented in cases presenting shortly after birth, but in cases developing later there does not seem to be a difference between the sexes.[612] Not all cases of uroperitoneum are associated with primary bladder rupture, however, because ureteral tears and urachal rupture can also result in uroperitoneum. Although ureteral tears initially result in urine accumulation in the retroperitoneal space, this typically progresses to uroperitoneum after several days. Urachal rupture may be associated with infection and necrosis of the umbilical remnant, and for this reason these foals may present slightly later than foals with bladder rupture.

Diagnosis of uroperitoneum is made on the basis of history, physical examination, clinical pathology, and ultrasound examination. The clinical signs associated with uroperitoneum may vary based on the site of leakage, but affected foals most often present with depression and weakness and signs of hypovolemia. It can be difficult to differentiate these foals from those suffering from neonatal sepsis, and indeed many affected foals may be suffering from sepsis in addition to uroperitoneum.[613] The rate at which clinical signs develop is often

associated with the size of the defect and the associated rate of urine leakage into the peritoneal space, with small defects in the bladder or retroperitoneal ureteral leakage often not becoming clinically obvious for several days to a week post foaling. Some foals with uroperitoneum will present with ventral edema, although this is most dramatic in foals with umbilical trauma and associated subcutaneous urine leakage. Foals with retroperitoneal urine accumulation may present with stranguria before the development of uroperitoneum, and foals with urethral defects most often demonstrate substantial subcutaneous edema in the perineal region. Some foals with uroperitoneum will present with abdominal distention or abdominal discomfort but will otherwise be in good physical condition. Palpation or ballottement of the abdomen may aid in detecting fluid accumulation within the peritoneal cavity.

The classic clinicopathologic presentation of the foal with uroperitoneum is that of azotemia, with increases in serum creatinine and (less reliably) BUN. Azotemia is typically accompanied by hyperkalemia, hyponatremia, hypochloremia, and metabolic acidosis. Hyperkalemia is caused by the reabsorption of substantial amounts of potassium, whereas hyponatremia and hypochloremia are caused by reabsorption of water from the urine within the abdomen. These changes are likely more pronounced because of the milk-based diet of foals; milk contains relatively high concentrations of potassium (25 mEq/L) and is relatively low in sodium (12 mEq/L).[616] Hyperkalemia is potentially life-threatening because of the possibility of fatal bradyarrhythmias. An electrocardiogram (ECG) should be performed in all foals with uroperitoneum or hyperkalemia of any cause. Typical ECG findings in hyperkalemia will progress from peaked T waves and a shortened QT interval to lengthening PR interval and loss of P waves, followed by widening of the QRS complex, and potentially leading to cardiac arrest and death.[617] Other potential cardiac sequelae associated with hyperkalemia include third-degree atrioventricular block, ventricular fibrillation, and ventricular premature contractions. This classic pattern of electrolyte abnormalities is not typically observed, however, in hospitalized foals that develop uroperitoneum while being treated with IV fluid therapy with balanced electrolyte solutions.[612,613]

Ultrasonographic examination is the most useful ancillary diagnostic test in the evaluation of uroperitoneum. Not only can one confirm the presence of free fluid within the peritoneal space, but one can also assess the degree of fluid accumulation and investigate possible sites of urine leakage. The free fluid within the abdomen is typically hypoechoic to mildly hyperechoic, and numerous loops of small intestine or other intraabdominal structures may be suspended in the free fluid contained within the abdomen. The dorsal bladder wall is the most common site of bladder rupture, and this area should be thoroughly investigated because in some cases the free margins of the defect can be readily observed. It is important to remember that there may be more than one site of urine leakage, however, either from the bladder itself or potentially involving the urachus, ureters, or urethra. Although ultrasound examination is typically less useful when evaluating ureteral or urethral defects, it is important to perform a complete examination of the urinary tract to detect any defects in the kidneys or ureters that might be missed if only the bladder and urachus are examined. Contrast radiography may be required to identify sites of leakage other than the bladder or urachus.

Definitive confirmation of uroperitoneum requires abdominocentesis. Creatinine diffuses poorly across the semipermeable peritoneal membrane, so the creatinine concentration in the peritoneal fluid will be at least twofold higher than the creatinine concentration in the serum. Abdominocentesis is both a diagnostically important technique and a therapeutic technique, because it allows for the drainage of accumulated urine from the peritoneal cavity. Removal of this fluid will aid in controlling hyperkalemia, and it will minimize further potassium absorption from the peritoneal cavity. Drainage will also decrease the pressure being exerted on the diaphragm and thoracic cavity, aiding in the resolution of any tachypnea, dyspnea, or respiratory distress. Abdominocentesis should be performed with a teat cannula or stainless bitch catheter or with an indwelling type of catheter such as a Foley or mushroom-tip peritoneal catheter. This approach minimizes the risk of trauma to the abdominal viscera and allows for safe manipulation and maintenance of the catheter while the abdomen is being drained. Care should be taken to administer appropriate fluid therapy before and during the drainage of the abdomen to avoid hypovolemia resulting from the removal of this third-space fluid. Drainage should be continued until surgical repair is performed, because premature discontinuation will simply allow for reaccumulation of urine within the abdomen.

Although medical management has been described, the treatment of uroperitoneum is primarily surgical because the site of urine leakage must be identified and addressed by surgical repair or removal. Correction of hypovolemia and electrolyte disturbances before surgery is important in minimizing the risk of anesthetic complications. The most critical of these disturbances is hyperkalemia because of the risk of cardiac disturbances. Hyperkalemia is addressed by the administration of IV fluids that do not contain potassium, and isotonic (0.9%) or hypotonic (0.45%) saline solutions are most commonly used. Dextrose is typically added to the fluids at a rate of 5%, and this will aid in lowering serum potassium concentrations by encouraging movement of potassium from the extracellular to the intracellular compartment, but in severe cases the administration of exogenous insulin (0.1 U/kg) may be necessary to substantially reduce serum potassium concentrations. Sodium bicarbonate therapy may aid in lowering serum potassium concentrations and may be helpful in addressing the concurrent hyponatremic metabolic acidosis.[617] Administration of calcium gluconate may also be helpful in decreasing the negative effects of hyperkalemia.[617] Recent reports have suggested that the frequency of anesthetic complications during surgical correction of uroperitoneum is less common than previously reported, perhaps because of less severe electrolyte disturbances, better preoperative stabilization, or the use of safer inhalant anesthetics (primarily isoflurane).[612]

The prognosis for survival in foals with uncomplicated uroperitoneum secondary to bladder rupture is good, whereas the prognosis for foals suffering from ureteral or urethral disorders is less favorable. Delays in the identification of affected foals and the presence of concurrent disease, especially sepsis, will result in worsening of the prognosis for survival.[612,613]

Umbilical Disorders

The umbilicus represents the primary channel for exchange of nutrients and oxygen between the dam and the fetus and the primary outlet for fetal urine. The umbilical cord consists of two arteries carrying poorly oxygenated blood away from the fetal heart and one vein carrying relatively better oxygenated blood from the placenta toward the fetal heart and the

urachus, which connects the fetal urinary bladder to the allantoic cavity.[618] Following second-stage parturition the umbilicus will narrow and separate at a point 2 to 3 cm from the body wall, typically within about 10 minutes if the mare and foal are undisturbed.[619] A small amount of neonatal blood loss may occur normally following separation of the cord, but it should be of minimal volume and should cease within a few minutes. If the mare stands too soon, then premature separation of the umbilical cord or umbilical cord damage may occur. Subcutaneous rupture of the urachus can result in substantial accumulation of urine in the tissues of the abdominal wall, which will likely require surgical resection of the umbilical remnants. Following separation from the placenta the umbilical structures immediately become vestigial, and in the normal foal the umbilical remnants will atrophy over the first few months of life.

One of the most common umbilical disorders is patent urachus, in which the urachal remnant is open and urine drains to the external environment. Although this may occur immediately following cord separation, it is more typically seen several days after birth and is most often associated with umbilical infections. Systemic illness and the associated stress of handling involved in the management of sick foals may also predispose the patient to the resumption of patency. Patent urachus may present simply as moistness of the external umbilical remnant or may be associated with a streaming flow of urine. Urine scald of the abdominal wall and the medial aspect of the hindlimbs may occur secondarily. It is important that an ultrasound examination be performed in these cases to determine whether there are internal abnormalities, such as umbilical remnant infections, associated with a patent urachus. Conservative management consisting of topical cleansing of the external umbilicus and close monitoring for the development of infection is often sufficient, although complete resolution may take days to weeks in some cases. If any infectious component is suspected then appropriate systemic antimicrobial therapy is indicated. Chemical cautery using silver nitrate sticks is often used and may accelerate closure of the urachal remnant, especially in early, mild cases. Excessive use of silver nitrate may result in local tissue necrosis, however, and may precipitate the development of infection. If conservative management fails then surgical excision of the umbilical remnants is indicated, but this is rarely a problem that needs to be addressed urgently, especially in a foal suffering from systemic illness or other disease processes.

Umbilical remnant infections are also common and can take several forms. Affected foals may appear clinically normal or they may exhibit signs of systemic inflammation (fever, leukocytosis, hyperfibrinogenemia, and elevated SAA). Infection of the external umbilical structures will result in local abscess formation, which presents as swelling, inflammation, and often discharge from the area of the external umbilicus, but it can become more locally invasive, involving the body wall. External umbilical abscesses can often be treated with the establishment of drainage combined with appropriate systemic antimicrobial therapy. Internal umbilical remnant infections can occur in all three of the distinct umbilical structures: the urachus (infection of the urachus is called *urachitis*), the umbilical arteries (infection of the umbilical arteries is called *omphaloarteritis*), and the umbilical vein (infection of the umbilical vein is called *omphalophlebitis*). It has traditionally been thought that umbilical remnant infections occurred secondary to external contamination of the umbilicus, with ascending migration of bacteria. This is likely true in some cases, and for this reason it is important to practice appropriate routine cleaning and disinfection of the external remnant. Excessive cleansing or treatment with caustic agents may actually increase the risk of ascending infection, so moderation is key. It is very possible that bacteremia occurs secondary to umbilical infections, resulting in seeding of other sites. Infection of remote structures, especially synovial structures and growth plates, has been frequently associated with the presence of umbilical remnant infections. Although the umbilical structures may represent the primary site of infection, it is also possible that some umbilical remnant infections actually develop secondary to systemic illness and bacteremia, rather than secondary to external contamination.

Ultrasonographic examination of the umbilical remnants is well described and can be performed readily with either a linear or curved probe using a frequency of 5 to 7.5 MHz, although higher quality imaging can be obtained with a linear 10- to 12-MHz probe.[620] All four umbilical structures (urachus, vein, and paired arteries) can be readily imaged in the external remnant as it courses out of the body wall. Internally the paired umbilical arteries are found ventral and lateral to the urachus and bladder, and they course caudally and deep toward their origins from the iliac arteries. They are thick-walled structures and should have a diameter of less than 13 mm.[621] Although the arteries may still be observed to pulsate in very young foals, they should contract down and be immobile by 24 hours of age. The lumen may be filled with clotted blood in some cases. The urachus extends from the apex of the bladder outward to the umbilical stump, and the lumen is typically collapsed and difficult to appreciate. A transverse view of the urachus and umbilical arteries taken at the apex of the bladder should normally measure less than 25 mm in total diameter.[621] The umbilical vein courses cranially from the site of the external umbilicus to the liver and is located along the midline very close to the abdominal wall. The vein is a thin-walled structure and may contain anechoic fluid within its lumen. The vein should measure less than 5 to 10 mm in diameter.[621] Infection involving these structures will typically manifest as filling of the lumen with material of variable echogenicity, thickening of the wall, and an increase in the overall size of the structure.[622,623] Thorough assessment is important, because multiple structures may be involved in the infectious process.

Treatment of umbilical remnant infections can be medical or surgical in nature. Medical management is generally preferred, and treatment with appropriate broad-spectrum antimicrobials is often successful in resolving the infection. Prolonged treatment of several weeks' duration and ongoing ultrasonographic monitoring is required to ensure complete resolution. In cases suffering from a combination of umbilical remnant infection and infections in other sites the clinician may feel a sense of urgency to surgically remove the umbilical remnants, but the stress of general anesthesia and abdominal surgery may be counterproductive. Stabilization of the patient and the institution of antimicrobial therapy before surgery may be beneficial in these cases. Broad-spectrum antimicrobial therapy is indicated, preferably using oral medications because of the need for prolonged therapy in most cases. The presence of gas within the internal structures on ultrasonographic examination or a fetid order represents an indication for the addition of metronidazole to the treatment regimen. In cases refractory to medical therapy, surgical excision of the affected

internal umbilical remnants is indicated. Thorough ultrasonographic examination preoperatively is helpful in developing the surgical plan and may reveal areas of infection deep in the umbilical arteries or vein that may not be able to be surgically removed. Continued medical management may be indicated in such a situation, but marsupialization of the umbilical vein or arteries can be performed to allow for external drainage and possible lavage of the infected structures.[624-626]

Acute Kidney Injury and Acute Renal Failure

Acute kidney injury can occur in foals secondary to systemic disease, toxin exposure, or postrenal obstruction. The most common of these etiologies is systemic disease, which has previously been termed *vasomotor nephropathy*, although the actual pathophysiology involved in these cases is not well understood.[627] It appears likely that there is a complex interaction among systemic cardiovascular responses, local vasomotor tone, systemic and local inflammatory mediators, microvascular dysfunction, and local renal tissue responses that result in decreased renal function. This decrease in renal function may represent a self-protective response by the kidneys in which decreased energy and oxygen demands within the kidney may help to improve tissue survival and recovery.[628] The most common toxic causes of renal injury in foals are iatrogenic in nature—namely, aminoglycoside antimicrobials and oxytetracycline (administered as a treatment for tendon contracture).[629] Nonsteroidal-associated renal injury is relatively uncommon in foals and is likely because these drugs are not commonly used in foals at this time, although this may change with increasing use of COX-2–specific NSAIDs in foals. Other causes of toxin-induced renal injury are hemoglobin released because of NI and myoglobin released because of myopathies, most commonly white muscle disease. Postrenal obstruction is very rare in foals and is primarily associated with congenital abnormalities.

Diagnosis of acute kidney injury is complicated by the poor sensitivity of the commonly used biomarkers of renal function—namely, serum creatinine and BUN. Because of the substantial renal reserve, it is likely that substantial injury has occurred by the time changes in these markers are detected clinically. BUN is so insensitive that it is of limited use in evaluation of acute kidney injury in foals, but one should be concerned when substantial simultaneous increases in BUN and serum creatinine are observed. Serum creatinine, despite its poor sensitivity, remains the best biomarker for renal function in foals. Although there is tremendous interest in identifying more sensitive biomarkers of renal function, none has yet been characterized in foals. Interpretation of serum creatinine concentrations in neonatal foals is complicated by spurious hypercreatininemia, which can be caused by placental insufficiency or neonatal hypoxia.[166] Although foals presenting with spurious hypercreatininemia had similar elevations of serum creatinine concentration compared with foals presenting with acute renal failure, serum creatinine concentrations in foals with spurious hypercreatininemia typically decrease by 50% within 24 hours of initiation of treatment and normalize by 72 hours.[166] The most effective way of improving the sensitivity of serum creatinine is to become more critical about evaluation of changes in the serum concentration. Even though the values may remain within the published normal range, an elevation of as little as 0.3 mg/dL above baseline may be indicative of decreased renal function. Because serum creatinine may be elevated from prerenal causes at presentation, it is important to evaluate the serum creatinine concentration frequently to assess the response to therapy and detect upward trends as early as possible.

Given the limitations of serum creatinine concentration as a marker of renal function, and more specifically glomerular filtration rate (GFR), it may be helpful in some cases to obtain a more accurate assessment of GFR. The exogenous marker iohexol has been validated in foals, but iohexol assays are not readily available.[630] Endogenous creatinine clearance is actually fairly simple to perform in foals with an indwelling urinary catheter and urine collection system. This involves obtaining a baseline serum sample for creatinine determination and simultaneous emptying of the urine collection bag, followed by a timed urine collection over at least several hours, but ideally over 24 hours. At the end of that time a second serum sample is obtained for serum creatinine measurement. The volume of urine collected is accurately measured, and a representative sample of urine is collected for creatinine determination. The endogenous creatinine clearance is calculated using the formula[631]:

$$\text{endogenous creatinine clearance (mL/min/kg)} = (\text{urine [creatinine]/plasma [creatinine]}) \times (\text{urine output [mL]/time [minutes]})/\text{body weight [kg]}$$

Reported values for endogenous creatinine clearance in normal foals are 1.78 to 2.17 mL/min per kilogram.[630,632,633]

Urinalysis can be helpful in detecting acute kidney injury, but interpretation can be challenging because of temporal changes in the composition of neonatal urine and the influences of fluid therapy and other treatments on urine constituents. Urine specific gravity is normally hyposthenuric (<1.010) in foals that are well hydrated and should increase appropriately (>1.012) in foals that are dehydrated. Isosthenuria (specific gravity of 1.010) may indicate a loss of renal function, but serial determination may be required to confirm the inability of the kidneys to dilute or concentrate the urine with changes in hydration status. Proteinuria is normally present in the first 24 hours of life but should then be absent unless there is renal injury. If the urine is alkaline, then mild spurious proteinuria (1+) may be observed on dipstick examination. Sick foals often will have acidic urine, making this less of an issue of interpretation than in adults. Examination for enzymuria has been advocated as an early indicator of renal injury in foals, primarily regarding urine γ-glutamyl transferase (GGT). This is assessed using the GGT-to-creatinine ratio (urine GGT/urine creatinine × 100), which corrects for dilutional effects. Elevations of the urine GGT:creatinine ratio above 25 are suggestive of proximal tubular injury, but this test does not have good specificity. Unfortunately elevations of the GGT:creatinine ratio are to be expected in foals receiving systemic aminoglycoside therapy, and the magnitude of the increase is not well correlated with the degree of renal injury.[634]

Treatment of acute renal failure is primarily supportive in nature. All efforts should be made to remove exposure to the potential initiating cause of acute kidney injury, if possible. Fluid therapy is typically indicated, both to correct any existing dehydration or hypovolemia and to induce diuresis. Carefully monitoring of urine output is indicated to be sure that the patient is not oliguric or anuric, and placement of a urinary catheter and urine collection system is extremely helpful in this regard. This may be difficult to achieve in the ambulatory

patient but is readily accomplished in recumbent foals. Urine output in a normal 4-day-old foal is approximately 6 mL/kg per hour, but this value should increase in response to fluid therapy.[632] Monitoring of "ins" versus "outs" should be routinely performed, with the goal of at least two thirds of fluid intake being excreted. If this is not achieved, then fluid overload is likely to develop. If urine output is inadequate after restoration of normovolemia and correction of systemic hypotension, then diuretic therapy is indicated. Furosemide appears safe and is well tolerated. In addition to its loop diuretic effects, furosemide may actually lessen renal metabolic demands by suppression of tubuloglomerular feedback. Recommended dosing of furosemide ranges from 0.5 to 1.0 mg/kg IV q 8–12 h to 1 to 2 mg/kg every 30 to 120 minutes over 6 hours.[185,635] CRIs have also been used in foals based on dosing regimens developed for adult horses, starting with a loading dose of 0.12 mg/kg IV, followed by 0.12 mg/kg per hour CRI.[636] If furosemide is ineffective, then osmotic diuretics can be used. DMSO is commonly administered to foals suspected of suffering from NE, and in addition to its free radical scavenging effects it does have some osmotic diuretic effects. Mannitol can be used as a more potent osmotic diuretic, at a dosage of 0.5 to 1.0 g/kg IV as a 20% solution over 20 minutes.[185] If medical therapy is not effective, then renal replacement therapy (dialysis) can be considered. Hemodialysis is the gold standard therapy and has been performed in foals, but it is rarely available for equine patients because of the expense and lack of access to the appropriate equipment.[629] Although typically less effective than hemodialysis, peritoneal dialysis and thoracic dialysis have been performed in foals and are much more feasible in most clinical settings.[635]

ENDOCRINE DISORDERS

The critically ill foal undergoes severe physiologic stress, and the body responds to this stress with a complex, integrated series of neuroendocrine and humoral adaptations that are directed toward support of critical hemodynamic, immune, and metabolic functions. The HPA axis, sympathetic-adrenomedullary axis, hypothalamic-pituitary-thyroid (HPT) axis, and the somatotropic axis are all of critical importance in this response.

Hypothalamic-Pituitary-Adrenal Axis Function and Dysfunction

Activation of the HPA axis leads to increased secretion of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus. CRH is the primary stimulus for the production and secretion of ACTH by the anterior pituitary, whereas AVP acts synergistically with CRH to increase ACTH secretion. ACTH is secreted into the systemic circulation and interacts with the cells of the zona fasciculata of the adrenal glands, which subsequently produce cortisol. Cortisol is not stored in the adrenal glands; it is released into the systemic circulation immediately after it is produced. Only the unbound, or "free," cortisol is biologically active, and in the normal adult state the vast majority of the cortisol in circulation is bound to cortisol binding globulin (CBG), with much smaller amounts bound to albumin.[637] Foals have been shown to have a much greater free cortisol fraction (58 ± 8 at birth to 33 ± 6 at 7 days of age) than adult horses (7 ± 3) or human infants (32% at birth to 19% by 3 months of age).[638] Increases in the free cortisol fraction can be associated with localized or systemic inflammation, because bound cortisol can be released from CBG by the neutrophil elastase.[639] The concentration of active cortisol is also regulated at the tissue level by the action of two isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD1 primarily generates active cortisol from inactive cortisone, whereas 11β-HSD2 primarily converts active cortisol to inactive cortisone.[640] Unbound cortisol reaches the tissues by diffusion and enters the cell, in which it binds to the intracellular glucocorticoid receptor (GR). The cortisol–GR complex then translocates to the nucleus in which it is able to exert both genomic (regulation of gene transcription) and nongenomic (protein–protein) effects.[309] In critical illness cortisol is important in supporting cardiovascular function, regulating immune responses, and increasing the availability of glucose as an energy source.

A variety of approaches has been used to assess the function of the HPA axis in both health and disease. These include the determination of neutrophil:lymphocyte ratios, basal serum cortisol concentrations, basal serum ACTH concentrations, ACTH/cortisol ratios, and ACTH stimulation tests that measure the ability of the adrenal glands to produce cortisol (delta cortisol). There are a number of challenges associated with HPA axis assessment, however.[641] The first is that the activation and response of the HPA axis are highly dynamic, with rapid changes in a short period of time and substantial variation even on an hour-to-hour basis. The second challenge is that measurement of total serum cortisol concentrations does not reflect the biologically active free portion, and determination of the active, free cortisol fraction is technically challenging, time-consuming, and not readily available in the clinical setting. The third is that the concentration of cortisol in the circulation provides no information related to the concentration of cortisol at the tissue or intracellular levels, in which it is active. The fourth challenge is that ACTH stimulation testing, either high or low dose, may not represent an accurate assessment of HPA axis function in critical illness. Finally, there may be substantial variation among cortisol assays, rendering it extremely difficult to compare the results of studies using different assays[114] and calling into question the use of specific values of serum cortisol as indicators of HPA axis function or dysfunction.

The maturation of HPA axis function in the equine fetus occurs very late in gestation, in the last few days before birth, and continues for the first several weeks of life.[64,92,93,642] As a result, foals delivered prematurely often suffer from inadequate HPA axis function, manifested by inappropriately low serum cortisol concentrations despite increased serum ACTH concentrations. These findings are indicative of a decreased response to ACTH at the level of the adrenal gland, and this is supported by the fact that these premature foals also show impaired responses to ACTH stimulation testing.[64,91,92] The neutrophil:lymphocyte ratio was noted by Rossdale et al. to be an easily determined surrogate marker for basal cortisol concentration in foals, with a ratio of <1.0 suggestive of impaired HPA axis function in premature foals, whereas a ratio of >2.0 was associated with adequate HPA function in term foals.[92] Normal term foals also show some evidence of incomplete HPA axis development, however, because they exhibit impaired responses to endogenous ACTH and both low- and high-dose ACTH stimulation tests, compared with adult horses.[64,642] Following birth the serum cortisol and

ACTH concentrations peak in the first hour of life and then gradually decline over 6 to 12 hours to levels lower than normally seen in adult horses.[638,643]

The correlation between HPA axis dysfunction and equine prematurity led to an early interest in this area of equine medicine, but little work was done in this area until the past decade. This renewal of interest was primarily the result of extensive work done in humans regarding the role of the HPA axis in critical illness.[644] The concept of relative adrenocortical insufficiency (RAI) was developed and defined as an inadequate cortisol response for the degree of illness.[645] RAI is considered to be a transient, reversible condition and has been associated with exaggerated proinflammatory responses in conditions such as sepsis, ARDS, and severe trauma. Potential mechanisms of RAI include suppression of the HPA axis at the level of the hypothalamus, the pituitary, or the adrenal gland.[646] RAI has been associated in a number of studies with an increase in the risk of death in critically ill human patients.[647] Subsequent studies using corticosteroid supplementation demonstrated a trend toward decreased mortality and improved vasopressor responsiveness, which further supported the concept of RAI in critical illness.[648-650] The diagnosis of RAI remained challenging, however, because of the wide range of diagnostic criteria used in different studies. The most widely accepted criteria was a random total serum cortisol of <10 mg/dL or a delta cortisol of <9 mg/dL following ACTH stimulation.[651]

Several studies have been performed in healthy foals to characterize normal HPA axis function in this population.[95,642,652] Substantial age-related changes in basal cortisol concentrations were found, and normal age-based ranges were reported. Unfortunately the rapid changes over time and the high variability of a single cortisol measurement mean that the application of basal cortisol concentration as a clinical marker of RAI cannot be recommended at this time.[315] The response to both low- and high-dose ACTH stimulation testing has also been examined, and although the dosages of ACTH varied among some studies there was a consistent pattern of age-related differences in the response to ACTH, with the most profound responses observed immediately after birth.[95,642,653] Age-related reference ranges for both low-dose and paired low- and high-dose ACTH stimulation testing have been reported.[95,642]

Several studies have been performed in clinically ill foals to determine whether RAI occurs in the septic foal. The first report of transient adrenal insufficiency in a septic foal was published in 1998, and the foal in that report had a low basal serum cortisol concentration and an inadequate response to high-dose ACTH stimulation testing.[654] Gold et al. reported that septic foals had higher mean cortisol and ACTH concentrations than normal foals, and the septic foals also had higher ACTH/cortisol ratios than normal foals.[655] Castagnetti et al. reported that nonsurviving septic foals had higher ACTH and ACTH/cortisol ratios at admission than healthy foals or surviving septic foals.[656] Interestingly, Wong et al. reported no difference in baseline cortisol or ACTH concentrations or ACTH/cortisol ratios between normal and clinically ill foals.[96] A small number of foals in that study did exhibit low baseline cortisol and ACTH concentrations or inadequate responses to ACTH stimulation suggestive of RAI, however. Hurcombe et al. reported that ACTH, cortisol, and AVP concentrations were increased in septic foals, compared with sick, nonseptic foals and normal foals.[121] They also found that ACTH, cortisol, and AVP concentrations were higher in sick, nonseptic foals than in healthy foals. Some septic foals in that study exhibited low or normal serum cortisol concentrations despite an increased ACTH concentration, which was considered to be suggestive of RAI.[657] Hart et al. examined a population of hospitalized foals and an age-matched population of healthy foals.[97] They found that 46% of hospitalized foals had an inappropriately low baseline cortisol concentration, and 52% had an inadequate response to high-dose ACTH stimulation. A significant correlation was observed between an inadequate response to high-dose ACTH stimulation and both shock and MODS in hospitalized foals as well as a correlation with decreased survival in a subset of septic foals. Armengou et al. reported that nonsurviving septic foals have higher cortisol concentrations and ACTH/cortisol ratios than surviving foals.[114] Although there are no reports examining the role of peripheral tissue corticosteroid resistance in the development of CIRCI in foals, a recent study performed in adult horses with SIRS found that decreased GR binding affinity was associated with nonsurvival and a trend toward an increased ACTH:cortisol ratio.[658] Investigation of the role of peripheral tissue corticosteroid resistance in foals may be warranted.

The variability seen in these equine studies mirrors the challenges observed in human medicine in applying basal cortisol concentrations and the results of high- and low-dose ACTH stimulation testing in the diagnosis of RAI. The use of baseline cortisol concentrations and ACTH stimulation testing is no longer recommended in human critical care patients.[659-661] Similarly, there is currently no consensus regarding the appropriate diagnostic features of this condition in foals.[662] Because of these difficulties and the increasing appreciation that it is not possible to determine the actual activity of cortisol at the tissue level, a new term was developed in human medicine: CIRCI. CIRCI is inadequate cellular corticosteroid activity for the severity of the patient's illness.[309] The current recommendations in human medicine regarding the diagnosis of CIRCI are based on clinical criteria rather than diagnostic testing. Patients with hypotension refractory to fluid replacement and vasopressor therapy should be suspected of suffering from CIRCI, and they should be treated with hydrocortisone. The positive response to hydrocortisone therapy is potentially supportive of a diagnosis of CIRCI.[309,660]

There is very little information available regarding the treatment of foals that may be suffering from CIRCI. The results of multiple studies in human patients have demonstrated that low-dose corticosteroid therapy in septic shock is associated with improved hemodynamics and a decreased requirement for vasopressor therapy, but it is not clear that there is a survival benefit associated with this intervention.[663] Based on the experience in human medicine, it appears reasonable to reserve corticosteroid therapy for those foals in which CIRCI is suspected and that demonstrate fluid-refractory, vasopressor-resistant hypotension.[190,662,664,665] The potential role of corticosteroid replacement therapy may be supported by the recent study regarding factors associated with nonsurvival in foals with NE. This study reported that persistent hypotension requiring vasopressor and/or inotrope therapy was significantly associated with nonsurvival.[666] Recommendations for corticosteroid therapy in foals are by necessity based primarily on the recommendations derived from human medicine, because no specific therapeutic protocols have been investigated in the foal.[667] Current human recommendations are for the use of physiologic dosages of hydrocortisone. This approach most closely mimics normal corticosteroid

dynamics rather than using high supraphysiologic dosages or long-acting drugs like prednisolone or dexamethasone.[315] A 3.5-day course of tapering dosages of hydrocortisone (1.3 mg/kg/day divided q 4 h IV) in healthy 2- to 6-day-old foals was reported to potentially have beneficial antiinflammatory effects without any apparent adverse effect on innate immunity.[313] The daily endogenous production of cortisol and the pharmacokinetics of hydrocortisone have been studied in neonatal foals, and it was determined that an appropriate dosage of hydrocortisone for foals suspected to have CIRCI might be in the range of 1 to 3 mg/kg per day.[314] This is slightly lower than the 2- to 4-mg/kg-per-day dosage (divided into two to four IV boluses per day) currently recommended in human medicine,[309] and it has since been suggested that a reasonable hydrocortisone dosage for foals may be 1 to 4 mg/kg per day divided into four to six IV boluses per day.[315] The appropriate duration of therapy is unknown in foals, but in humans it has been shown that early withdrawal of hydrocortisone therapy is deleterious, and prolonged treatment (7–10 days) with subsequent tapering of dosages is recommended.[646,668]

Hypothalamic-Pituitary-Thyroid Axis Function and Dysfunction

The production of thyroid hormones is regulated by the hypothalamus, which releases thyrotropin-releasing hormone (TRH) into the pituitary portal system. TRH then stimulates the thyrotropes of the anterior pituitary to release thyroid-stimulating hormone (TSH) into the systemic circulation. TSH regulates the synthesis and release of thyroid hormones by the thyroid gland. The thyroid hormones are stored within the thyroid gland as the precursor thyroglobulin, which is cleaved to produce triiodothyronine (T_3) and thyroxine (T_4) under the influence of TSH. T_3 and T_4 then enter the systemic circulation. In the circulation the thyroid hormones are bound to thyroid binding globulin and other proteins, including albumin. T_4 has minimal activity and essentially serves as a prohormone. It is converted to T_3, the primary active form of thyroid hormone, at the tissue level. Only the unbound, free thyroid hormones can be transported into the cell, in which they bind to thyroid hormone receptors in the nucleus and subsequently influence gene transcription. The effects of thyroid hormones are extensive but primarily involve increasing metabolic rate, enhancing cardiovascular function, and thermogenesis.[643]

The assessment of HPT function relies primarily on the measurement of TSH and/or the measurement of basal thyroid hormone concentrations (T_3, T_4, free T_3, free T_4, and reverse T_3). This approach is complicated by the age-related changes in these hormone concentrations that occur in newborn foals, as well as the myriad of influences of other nonthyroidal factors. The measurement of circulating concentrations is also poorly reflective of the activity of these hormones at the tissue level. Stimulation testing is difficult because of the lack of ready availability of TRH or TSH and is not practical in the clinical setting. In newborn foals thyroid hormone concentrations are substantially higher at birth than in adult horses.[669-674] These elevated concentrations gradually decrease to adult levels over several months' time. Only a few studies have measured TSH in neonatal foals, and they found that the concentrations were not elevated; instead, they were similar to the levels seen in adult horses.[674,675] This suggests that the HPT axis is different in foals, perhaps because of increased activity of TSH or increased receptor sensitivity to TSH. This alteration in the HPT axis may account for the rapid growth of foals in the first few months of life and their high neonatal thermogenic capacity.[669,674]

Dysfunction of the HPT axis has been described in neonatal foals and is caused by congenital hypothyroidism, prematurity, and systemic illness. A syndrome of congenital hypothyroidism and dysmaturity syndrome was first described in neonatal foals in Western Canada in 1981.[676,677] This syndrome is associated with thyroid hyperplasia (goiter), increased gestational length, dysmaturity, and numerous musculoskeletal abnormalities, including flexural limb deformities, rupture of the common digital extensor tendon, incomplete ossification of the cuboidal bones, mandibular prognathism, and incomplete closure of the abdominal wall.[676,678-684] This syndrome is also suspected to be associated with abortion in some cases.[685] Thyroid hormone levels in affected foals may be low or normal, but the response to TSH stimulation is consistently decreased.[686,687] Although the etiology is unknown, this syndrome is suspected to be associated with exposure to some causative agent, and epidemiologic investigations have suggested increased dietary nitrate intake or exposure to mustard plants could be involved.[643,678,682] Other potential risk factors could include inadequate iodine or selenium intake.[682,688] The prognosis for survival in affected foals is poor, and those that do survive are likely to suffer a variety of orthopedic abnormalities that render them unsound.[680] Congenital hypothyroidism has also been reported in foals whose dams suffered dietary iodine deficiency or excess, and affected foals typically exhibit prominent goiter.[689-691] In utero exposure of foals to endophyte *(A. coenophialum)*-infected fescue has been associated with clinical signs of hypothyroidism after birth and with decreased circulating concentrations of T_3.[32,692]

Premature foals exhibit hypothyroidism because they have substantially lower thyroid hormone concentrations compared with healthy, full-term foals.[82,671] This may be analogous to the syndrome of transient hypothyroxinemia of prematurity that has been described in premature human infants.[693,694] One report described that both premature and term hospitalized foals had lower T_3 concentrations compared with healthy term foals, whereas the premature foals had lower T_4 concentrations than either group of term foals.[674] The presence of systemic illness is also associated with hypothyroidism in both premature and term foals, and this has been termed the *sick euthyroid syndrome* or the *nonthyroidal illness syndrome* (NTIS). NTIS is well described in humans, dogs, and cats, and there is increasing evidence that it occurs in adult horses and foals.[671-674,695,696] NTIS in sick foals is characterized primarily by low T_3 concentrations.[674] The mechanisms underlying NTIS are thought to be associated with the proinflammatory state associated with illness, but they may also be related to decreased feed intake, because fasting induces NTIS in healthy humans.[697,698] It has been proposed that this phenomenon may represent an appropriate and beneficial response to critical illness, at least in the acute stages, and may not require any therapeutic intervention beyond addressing the primary disease.[697,699]

Dysfunction of the Somatotropic Axis and Energy Regulation

As previously discussed the maturation of the endocrine system of the foal is incomplete at birth, and development

continues for weeks to months after parturition. This is also the case for the systems regulating energy metabolism in foals.[700] These systems are very complex, involving neuroendocrine (cortisol, vasopressin, and thyroid hormone), somatotropic (growth hormone [GH] and insulin-like growth factor-1 [IGF-1]), sympathoadrenal (epinephrine and norepinephrine), and pancreatic (insulin and glucagon) responses, as well as signals derived from fat stores (leptin and adiponectin) and the gastrointestinal tract (ghrelin).[701] The somatotropic axis is stimulated by hypothalamic release of growth hormone-releasing hormone and inhibited by the release of somatostatin.[702] The somatotropic cells of the anterior pituitary respond to these signals by increasing or decreasing the release of GH. The effects of GH are primarily mediated by IGF-1, which is produced by the liver and has similar effects on insulin when regulating blood glucose concentrations by decreasing hepatic gluconeogenesis and stimulating the uptake, utilization, and storage of glucose.[703] The secretion of GH is stimulated by ghrelin and under negative feedback control of IGF-1 and leptin.

The sympathoadrenal response is stimulated by physiologic stress and hypoglycemia, leading to the release of epinephrine from the adrenal gland.[704] The metabolic effect of epinephrine is to increase energy substrate availability by inhibiting pancreatic insulin secretion and increasing glucagon secretion, interfering with the effects of insulin on the tissues, stimulating hepatic glycogenolysis, and increasing lipolysis. The pancreatic hormones insulin and glucagon are critical mediators of blood glucose concentrations, with insulin being produced by β cells in response to hyperglycemia and glucagon produced by α cells in response to hypoglycemia. Insulin has a multitude of effects, primarily focused on lowering blood glucose concentrations, and these include increasing glucose uptake in insulin-dependent tissues such as muscle and adipose tissue, increasing glycogen synthesis, increasing fat synthesis, and decreasing gluconeogenesis and lipolysis. Glucagon acts in opposition to insulin, primarily functioning to increase blood glucose concentrations by increasing glycogenolysis and gluconeogenesis, as well as by increasing lipolysis. The adipokine leptin is involved in the control of appetite, with increases seen following ingestion of a meal and with increasing total body fat, whereas adiponectin is negatively correlated with total body fat and has some activities similar to glucagon. Ghrelin mainly acts to increase feed intake and to stimulate GH secretion.

Hypoglycemia is a common finding in critically ill equine neonates, perhaps because of limited endogenous energy stores and decreased enteral intake.[131,705,706] Care should be taken in interpreting blood glucose concentrations in neonatal foals, however, because they typically have relatively low blood glucose concentrations (50–60% of maternal blood glucose) in the immediate postpartum period, especially before their first nursing.[707] Hypoglycemia has been repeatedly associated with an increased risk of nonsurvival in critically ill foals, although different diagnostic criteria have been used to define hypoglycemia.[118,131,708,709] In the study reported by Hollis et al. there was an association between the severity of hypoglycemia and the risk of nonsurvival, with each increase of 18 mg/dL from a cutoff of 50 mg/dL being associated with an increase of the odds of survival to discharge of 3.4 times (95% CI 2.1–5.4).[131] In one study the percentage of survivors was lower among foals with blood glucose values ≤120 mg/dL (31%) compared with foals with blood glucose concentrations >120 mg/dL (55.6%).[708] A second study, however, found no clinically relevant pattern of alteration in blood glucose concentration when comparing septic and sick, nonseptic neonatal foals to healthy controls.[114] Septic foals had slightly lower median blood glucose concentrations than controls, but this remained within the normal reference range. The treatment of hypoglycemia in sick foals is of critical importance when it is documented and should be addressed by the immediate provision of nutritional support, either enterally and/or parenterally, as is appropriate to the individual case (see the later section Nutritional Support for the Foal).

Hyperglycemia is common in critically ill foals as well, with one study reporting that 36.5% of foals demonstrated hyperglycemia at admission, which was similar to the rate of hypoglycemia (34.4%).[131] That study reported that extreme hyperglycemia (>180 mg/dL) was associated with an increased risk of nonsurvival, which is similar to the situation reported in human neonates and adult horses with acute abdominal disease.[710-713] Interestingly, Barsnick et al. reported different findings, because the septic foals with the highest blood glucose concentrations (up to 329 mg/dL) in their study all survived.[709] Insulin resistance may play a role in the development of hyperglycemia, although there are many other factors that may contribute to the development of hyperglycemia, as discussed previously. Increased insulin concentrations have been associated with mortality in critically ill foals, which might be an indication of insulin resistance in the most severely affected individuals.[709] Insulin resistance does not appear to be a common feature of critical illness in foals, however, because both sick nonseptic and septic foals have exhibited normal insulin sensitivity in the studies that have investigated this phenomenon.[114,709]

There has been tremendous interest and debate in human medicine regarding therapeutic interventions to control hyperglycemia in the critically ill. Tight regulation of blood glucose concentrations (between 80 and 110 mg/dL) was initially advocated in human medicine,[714] but this approach has been shown to be deleterious because of frequent hypoglycemia resulting from insulin administration. A more permissive approach is now recommended, in which blood glucose concentrations are maintained between 145 to 180 mg/dL.[715] This approach attempts to avoid the requirement for aggressive insulin therapy, minimizing hypoglycemic episodes, while also recognizing that stress hyperglycemia is a normal physiologic response that is important for maximizing energy delivery to noninsulin-dependent tissues, such as the brain, intestine, red blood cells, and kidney.[716,717] The primary goal should be the avoidance of severe hypoglycemia that exceeds the renal threshold, which in foals is likely to be above 180 mg/dL.[718] Insulin therapy may be required to accomplish this goal, and various regimens have been described, including intermittent bolus administration or continuous rate IV infusions[30,643,718-720] (see the later section Nutritional Support for the Foal). The institution of insulin therapy is not to be taken lightly, because it requires frequent monitoring of blood glucose concentrations and rapid intervention to avoid hypoglycemia until a stable blood glucose level is achieved, which may take several hours of fine-tuning.[720]

Other indicators of alterations of energy regulation that have been investigated in foals include triglycerides (TG), nonesterified fatty acids (NEFAs), GH, IGF-1, leptin, and glucagon. Although our understanding of the role of these factors in equine neonatal critical illness is still developing, these studies

give valuable insight into the complexities involved and provide some indication of the potential benefits of assaying these variables. Barsnick et al. reported that septic foals had higher glucagon and TG concentrations than healthy foals but found no difference in leptin concentrations between groups.[709] Lower leptin concentrations were associated with mortality in that study, however. In a later study Barsnick et al. found that septic foals had higher ghrelin, GH, and TG concentrations and lower IGF-1 and glucose concentrations compared with healthy foals, and these findings are likely related to inanition and a negative energy balance.[721] Sick, nonseptic foals in that study had higher GH and TG and lower IGF-1 concentrations than were observed in healthy foals. The GH:IGF-1 ratio was higher in septic and sick, nonseptic foals than healthy foals and was highest in nonsurvivors. This finding was indicative of somatotropic axis resistance in clinically ill foals.[721] Panzani et al. reported that IGF-1 concentrations did not differ between healthy and sick foals but increased over time in all groups, whereas NEFA concentrations were higher in sick foals at some time points.[672] Armengou et al. also reported that NEFA concentrations were higher in sick foals than in healthy foals but found no differences in TG concentrations between groups.[114] Although direct intervention in the somatotropic axis does not appear to be indicated, [722] these findings support the importance of correcting the negative energy balance that exists in many critically ill foals.

IMMUNOLOGIC DISORDERS

Failure of Transfer of Passive Immunity

The most common type of immunodeficiency disorder in horses is FTPI, which is the failure of the foal to ingest or absorb adequate immunoglobulin from colostrum.[723,724] This is critically important because foals are essentially agammaglobulinemic at birth from the inability of macromolecules to transfer across the diffuse epitheliochorial equine placenta from the dam to the fetus.[725] FTPI is regarded as the most important risk factor for the development of infection and death for foals within the first month of life, and the incidence of FTPI has been estimated at 3% to 24%.[726] FTPI can occur by several mechanisms, including failure of the foal to ingest adequate volumes of colostrum in the early neonatal period, loss of colostrum before ingestion because of premature lactation, low colostral immunoglobulin concentration, and inadequate absorption of colostrum by the neonatal gastrointestinal tract.[725,727] The timing of colostrum ingestion is critically important, because the ability of the neonatal gastrointestinal tract to absorb macromolecules is maximal at birth but rapidly declines over the first 24 hours of life.

The diagnosis of FTPI cannot be made on clinical grounds and requires determination of the foal's serum IgG concentration within the first 18 to 24 hours of life. The gold standard assay for the quantification of immunoglobulin quantification is the single radial immunodiffusion assay, but this test requires a prolonged incubation period, rendering it of little utility in the clinical setting. A number of types of rapid screening tests are available for estimating foal IgG concentrations, including glutaraldehyde coagulation, zinc sulfate turbidity, turbidimetric immunoassay, latex agglutination, and enzyme immunoassay tests, as well as serum globulin or TP concentrations.[727-735] The ultimate decision regarding which test to use is based on the consideration of each test's accuracy, time requirement, cost, and ease of implementation.[733] Several studies have indicated that serum globulin concentrations can be used to estimate the IgG concentration, but it appears that the cutoff points must be established in each individual clinical laboratory because of the variations in testing methodology.[733,736,737] The enzyme immunoassay test is widely used because of its acceptable diagnostic performance as a screening test (high sensitivity), combined with simple performance and rapid results.[733] The interpretation of the results of IgG testing has been the subject of tremendous interest over the past 40 years and can be a point of confusion because of the different diagnostic criteria and different test methodologies used over time. A recent report supported the traditional cutoff value of >800 mg/dL as indicative of adequate transfer of passive immunity (ATPI) and found that in a large population of hospitalized foals values <800 mg/dL were proportionally associated with mortality.[165] This study also reported a regression equation that could be used to estimate serum IgG concentration from serum TP, albumin, and globulin concentrations with good diagnostic performance (IgG = −241.4 × [albumin] + 462 × [globulin] + 222.8 × [TP] − 370.3).[165] This estimation may be helpful in situations where specific testing for serum IgG concentration is not readily available.

Treatment of FTPI depends to some extent on the clinical status of the foal and the foal's environment. If the foal is known or suspected to have ingested inadequate amounts of colostrum, then early enteral administration of maternal or banked colostrum may be effective; however, such treatment should not be assumed to result in ATPI, and monitoring of serum IgG is still indicated.[738] A clinically normal foal examined in the home farm environment may not require treatment if the IgG value is between 400 and 800 mg/dL (partial FTPI), whereas a value of less than 400 mg/dL (complete FTPI) represents grounds for treatment. If the foal shows any signs of clinical illness or is being examined in a hospital environment, then any IgG value of less than 800 mg/dL is an indication for treatment. Treatment consists of providing IgG supplementation, which most often is accomplished by IV administration of equine plasma, because test results are typically not available until after the time period in which enteral supplementation would likely be effective. The dosage for IV plasma administration is based on the severity of the foal's deficit, but it is not precise because of the tremendous variability in IgG content in commercial equine plasma.[724] In clinical practice the administration of 1 L of plasma can reasonably be expected to provide an approximately 200-mg/dL increase in serum IgG.[739] Unless plasma with a higher IgG concentration is used, it can be difficult to administer enough plasma to completely normalize serum IgG in severely affected foals because of the risk of volume overload as well as the expense of treatment, so the maximum initial dosage of plasma is typically 2 L.[740] It is important to monitor the effect of treatment by repeated measurement of serum IgG to ensure that further treatment is not required, especially in severely affected clinically ill foals that may have ongoing catabolism of IgG associated with their systemic illness.[740]

Neonatal Isoerythrolysis

NI is the most common cause of icterus in newborn foals. It is an immunogenetic disorder in which the foal's red blood cells are destroyed by preformed maternal anti–red blood cell antibodies ingested in the colostrum. These maternal antibodies are produced in response to exposure of the mare to "foreign"

red blood cell antigens by several possible routes, including placental pathology allowing exposure to fetal red blood cells during gestation, exposure to foal red blood cells during parturition, or blood transfusions administered to the mare.[741] Blood group incompatibilities between the mare and the foal are caused by the foal inheriting a blood group antigen from the sire that the mare does not possess. These incompatibilities are likely commonly caused by the multitude of equine blood group antigens, with an estimated incidence of 14%, yet the reported incidence of NI is only 1% in Thoroughbreds and 2% in Standardbreds.[122,742,743] This is because the majority of blood group antigens are not strongly immunogenic under these conditions of exposure. Some blood group factors are strongly immunogenic, and factor Aa in the A system and factor Qa in the Q system have historically been the factors associated with the majority of cases of NI.[742] More recent reports have documented the involvement of other alloantigens, perhaps as a result of an enhanced ability to detect these other factors or because of differences in the breed composition of the local horse population.[119,744] The incidence of NI in mules is much higher than in horses, with estimates as high as 10% because of the presence in these foals of a donkey-specific red blood cell antigen ("donkey factor") that horses lack.[745,746]

Foals affected with NI are typically born clinically normal and begin to show clinical signs within 2 to 5 days of birth, although intervals of as long as 12 days have been reported.[119] The clinical signs of NI result from anemia and tissue hypoxia and include progressive lethargy, weakness, tachypnea, and tachycardia. Affected foals will initially have pale mucous membranes but subsequently develop icterus, although in peracute cases foals may die before exhibiting icterus.[741] As the disease progresses foals will become dyspneic, and seizure-like activity may occur, potentially caused by kernicterus in cases with severe hyperbilirubinemia. Pigmenturia may be present, which is caused by severe hemolysis. Affected foals are anemic, although the magnitude of the anemia may be masked by concurrent hemoconcentration at presentation.[119] Although most foals will present with hematocrits between 10% and 20%, the anemia can be severe, with hematocrits as low as 5% reported.[119,741] Thrombocytopenia may be present as well, and this finding appears to be more common in mule foals.[741,747] Venous blood gas analysis reveals decreased venous oxygen concentration and saturation caused by increased oxygen extraction at the tissue level. There is metabolic acidosis caused by lactic acidosis, which is caused by anaerobic tissue metabolism, resulting in increased lactate production. Hyperbilirubinemia, primarily caused by increased indirect bilirubin concentrations, is a consistent feature, although increased direct bilirubin concentration is seen in some affected animals, potentially caused by hepatic injury secondary to hypoxia or iron toxicity associated with bilirubin accumulation.[119,122] Increased SDH concentrations have also been associated with severe anemia (hematocrit <11%) in foals with NI, further supporting that hepatocellular injury occurs in severely affected foals.[119] Hemoglobinemia and hemoglobinuria can be pronounced, and pigment nephropathy resulting in acute renal failure can occur in severely affected cases.

Diagnosis is often made based on the signalment, history, and clinical signs, but confirmation is important because there are other causes of hemolytic anemia in neonatal foals, including DIC associated with sepsis, bacteria-induced hemolysis, and iatrogenic causes such as incompatible transfusion of blood or plasma.[119] A definitive diagnosis is made by demonstrating the presence of antibodies in the mare's colostrum or serum directed against the foal's red blood cell antigens. This can be done using agglutination or lytic tests, with lytic tests regarded as more reliable.[743] The hemolytic crossmatch of mare serum with foal red blood cells using exogenous complement is considered the test of choice. Identification of the specific red blood cell factors involved can also be accomplished by testing panels of red cells that represent all the known blood groups.[741] Despite their limitations, agglutination tests are commonly used because they are more readily performed and provide more rapid results. A crossmatch of mare serum with foal red blood cells without the addition of exogenous complement has agglutination rather than hemolysis as the endpoint and is less sensitive than the hemolytic crossmatch. The direct antiglobulin (Coombs) test can be used to demonstrate the presence of antibodies on the surface of the foal's red blood cells, but this test can lack sensitivity. The most readily performed test is the jaundiced foal agglutination (JFA) test, which is simple and provides rapid results. The JFA test is performed using red blood cells from the foal and colostrum from the mare, with agglutination as the endpoint. Unfortunately the JFA test can lack both sensitivity and specificity and is most useful in prevention of NI rather than diagnosis of an affected foal.

The treatment for NI will be dictated by the severity of the anemia and the associated clinical signs. Moderately affected foals primarily require supportive care, exercise restriction, and close monitoring. Stress should be avoided in handling the foal. If the foal presents at less than 24 hours of age then it should be withheld from nursing until the risk of further antibody ingestion has passed. This time interval can be difficult to assess accurately, but it should not exceed 24 hours because of waning maternal antibody content in the milk and decreased gastrointestinal absorption in the foal. Serial monitoring of the JFA test can be used, if desired, to assess the decline of maternal alloantibodies in the milk and confirm that the introduction of nursing should be safe.[748] The foal will need to be provided with nutritional support during this time period, which is most readily performed using an indwelling feeding tube. IgG supplementation with colostrum from another mare or with frozen plasma will be necessary to ensure adequate passive transfer. Despite some concerns in the literature, withholding colostrum does not appear to have any deleterious effects on gastrointestinal development in foals.[749] IV fluid therapy is helpful in supporting cardiovascular function and tissue perfusion as well as ensuring diuresis, minimizing the potential nephrotoxic effects of hemoglobin. Fluid therapy should be administered conservatively, however, to avoid hemodilution, particularly in more severely affected foals. Plasma exchange has been successfully used to treat foals affected with severe hyperbilirubinemia secondary to NI.[258]

Further treatment is indicated if the affected foal shows evidence of shock caused by tissue hypoxia, which becomes more likely in foals with profoundly low hematocrits (<10–12%), but it should not be based on the hematocrit reading alone. The decision to treat in these cases may be supported by findings of persistent or progressive tachycardia and tachypnea and demonstration of decreased mixed venous oxygen tension.[750,751] Treatment in these foals is directed at the restoration of the oxygen-carrying capacity of the blood and improving tissue oxygen delivery. Intranasal oxygen insufflation is readily performed but may be of limited benefit because oxygen saturation is typically not adversely affected. The only other way to improve oxygen

delivery is by transfusion of red blood cells that will not be damaged by the maternal alloantibodies. The transfused red blood cells will likely only persist in the foal's circulation for a few days, but the goal of the transfusion is to provide support while the foal produces sufficient red blood cells to support itself. In the ideal situation a known Aa and Qa negative "universal" donor could be used, but these are very difficult to identify and not commonly available. In most cases the mare represents the most logical donor, and once her red blood cells have been washed to remove the alloantibodies present in her serum they can be transfused to the foal. The most effective way to wash the maternal red blood cells is by centrifugation, but this is often not readily available. Serial gravity sedimentations of anticoagulated maternal red blood cells using sterile isotonic saline to remove and discard the supernatant is readily performed and reasonably effective, but it does require a substantial period of time (several hours) during which the foal's clinical status may deteriorate. There are reports of the administration of polymerized bovine hemoglobin (Oxyglobin, Biopure Corporation, Cambridge, MA) as an alternative form of bolstering oxygen transport capacity. This is appealing because the product is shelf-stable and requires no delay in administration.[119,750,752] These reports provide some conflicting evidence regarding the potential safety of this approach because the administration of bovine hemoglobin was associated with poor outcomes in some instances. It is unclear if this is the result of the product administered or a result of case selection, in which the most severely affected foals were likely selected to receive this therapy.[752] When blood transfusions are used the volume of blood to be administered to the foal should be kept to a minimum; administration of greater than 4 L of blood is associated with the development of hepatic failure (OR 19.5).[122] Reinforcing this concern is the finding that the administration of each additional unit of blood was shown to increase the odds of nonsurvival 8.4 times.[122]

The most effective treatment for NI is prevention. Blood typing of the mare can be performed before breeding to fully characterize the mare's blood group, and if the mare lacks factors Aa and/or Qa she would be considered at risk for becoming sensitized in the future.[741] Testing to identify the specific alloantibodies produced by a mare that has produced an NI-affected foal can be used in combination with blood typing of the sire to predict future risk.[753] This is rarely done, however, and it is simpler to perform the JFA test at the time the foal is born and before the foal is allowed to nurse. When performed in this manner the JFA test is reasonably diagnostic, and a negative result supports allowing the foal to nurse.[748,754] In the absence of JFA test results, any foal born to a mare that has had a previous foal affected by NI should be considered at high risk, and the foal should be muzzled at birth and not allowed to nurse for the first 24 hours of life. Although clinically affected foals have likely received adequate passive transfer of immunoglobulins, any foals that are deprived of maternal colostrum as a preventive measure must receive immunoglobulins in the form of pooled colostrum administered enterally or fresh frozen plasma administered intravenously.

Neonatal Alloimmune Thrombocytopenia and Neutropenia

Neonatal alloimmune thrombocytopenia (NAIT) and neonatal alloimmune neutropenia (NAN) appear to be rare in the equine, but they have been reported in horse and mule foals.[747,755-759] A syndrome of ulcerative dermatitis, thrombocytopenia, and neutropenia has also been described in foals.[760] It is possible that the incidence of these conditions is underappreciated, however, because neutropenia and thrombocytopenia are common findings in critically ill patients secondary to a number of other mechanisms.[761] The pathophysiology of NAIT and NAN is very similar to that of NI: affected foals ingest maternal alloantibodies directed against antigens on platelets or neutrophils, resulting in their destruction or removal from circulation. Diagnosis of these conditions is based on the demonstration of persistent thrombocytopenia or neutropenia in the absence of other disease processes that might cause these abnormalities, such as bacterial sepsis. Foals affected by NAIT may appear clinically normal or they may exhibit petechial hemorrhages or prolonged bleeding from venipuncture sites. Foals with NAN are typically clinically normal, but they are predisposed to secondary bacterial infections that may lead them to exhibit clinical signs of local or systemic inflammation. Confirmation of the clinical diagnosis requires the demonstration of antibodies bound to the patient's platelets (NAIT) or neutrophils (NAN). Direct fluorescent antibody tests, ELISA, immunoradiometric tests, and flow cytometry have been used to confirm the diagnosis.[747,755-759,762]

Treatment of NAIT is supportive in most cases, although whole blood or platelet-rich plasma transfusions can be administered to foals exhibiting clinical bleeding diathesis.[763] Supportive care is indicated in foals with NAN, although recombinant human granulocyte colony-stimulating factor can be administered in an effort to increase endogenous neutrophil production.[758,759] Foals affected by NAIT and especially NAN appear to be at increased risk for secondary bacterial infections, so appropriate broad-spectrum antimicrobial therapy is indicated. Both conditions are ultimately self-limiting because the offending alloantibodies are consumed and eliminated from circulation. The use of immunosuppressive therapy has been proposed but does not appear to be indicated in these conditions.

HEPATIC DISORDERS

Hepatic disorders are uncommon in foals, but there are a wide range of possible etiologies. True hepatic failure is rare, and hepatic dysfunction is the most common disorder. Detection and diagnosis of hepatic disease in foals are challenging because the clinical signs are nonspecific and often related to a primary disease process. These nonspecific signs can include anorexia, depression, fever, weight loss, and abdominal pain. Icterus may be present but is not specific for hepatic dysfunction, and it may be secondary to anorexia, intravascular or extravascular hemolysis, sepsis, or severe systemic illness. With more severe hepatic involvement one may observe any or all of the following: CNS signs, bleeding disorders, edema, ascites, diarrhea, or dermatitis. A definitive diagnosis requires clinicopathologic examination consisting of serum enzyme activities and conjugated and unconjugated bilirubin at a minimum but potentially including serum bile acids, serum ammonia, and prothrombin time. Additional biochemical abnormalities that may be supportive of, but not specific for, hepatic disease include hypoglycemia, metabolic acidosis, low BUN, and polycythemia. Ultrasonographic examination also can be very helpful because it allows for the assessment of the hepatic parenchyma and may allow for the detection of structural abnormalities. Hepatic biopsy, although rarely performed

TABLE **20.12 Biochemical Parameters in Foals over the First Month of Life**

Biochemical Parameters	Units	1 Day	7 Days	1 Month
Alkaline phosphatase	IU/L	<2670	<1170	<866
γ-Glutamyl transferase	IU/L	<33	<98	<44
Sorbitol dehydrogenase	IU/L	<21	<18	<6
Aspartate aminotransferase	IU/L	<340	<620	<440
Glutamate dehydrogenase	IU/L	<27.5	<17	—
Total bilirubin	mg/dL	<4.5	<3.3	<1.7
Direct bilirubin	mg/dL	<0.35	<0.7	<0.6
Ammonia	μg/dL	—	<60	—
Bile acids	μmol/L	<82	<30	<17

Adapted from Axon JE, Palmer JE. Clinical pathology of the foal. *Vet Clin North Am Equine Pract*. 2008;24:357-385, vii; Divers TJ, Byars TD: Hepatic disease. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine Reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:409-415; Barton MH, LeRoy BE. Serum bile acids concentrations in healthy and clinically ill neonatal foals. *J Vet Intern Med*. 2007;21:508-513; Armengou L, Jose-Cunilleras E, Rios J, et al. Metabolic and endocrine profiles in sick neonatal foals are related to survival. *J Vet Intern Med*. 2013;27:567-575.

in foals, may be useful in diagnosis, treatment planning, and prognostication.

The interpretation of the biochemistry profile can be challenging in young foals because the activities of some hepatic enzymes and other parameters are substantially different from those normally seen in adult horses (Table 20.12). Serum alkaline phosphatase (ALP) activity shows the most striking elevations compared with adults, with values as much as 10-fold greater in the first week of life. This is primarily caused by bone metabolism, which rapidly declines over the first month of life and then gradually decreases to the adult range by 6 months of age.[707,764,765] Serum GGT activity transiently increases during the second week of life and then returns to the normal adult range by 1 month of age.[766,767] AST concentrations increase slightly after the first week of life but then remain similar to adult values.[707,764] SDH and glutamate dehydrogenase (GLDH) values do not differ significantly with age.[707,768] Serum bile acid concentrations are also increased at birth but gradually decrease to normal adult ranges over the first 6 weeks of life.[769] Neonatal hyperbilirubinemia is well documented in foals and is primarily associated with increased total bilirubin concentration.[707] This elevation gradually resolves, and values are typically within normal adult ranges after the first week of life. Direct bilirubin concentrations are low at birth but increase to normal adult ranges after the first few days of life.[769] The serum enzymes that are liver specific—namely, SDH, GLDH, and GGT—are of greatest utility in evaluating the foal with hepatic dysfunction compared with the nonspecific enzymes ALP and AST. GGT is liver and biliary tract specific, whereas SDH and GLDH are of hepatocellular origin.

Acquired Disorders

Tyzzer's Disease

Tyzzer's disease is an enterohepatic syndrome affecting foals from 7 to 42 days of age that is associated with the rapid onset of septic shock and death, usually within 2 to 48 hours from the onset of clinical signs.[770,771] The primary manifestation of this disease is a peracute bacterial hepatitis. This disease is well described in foals, with numerous cases documented in the United States and other countries over the past 40 years.[772-778] The causative agent of Tyzzer's disease is *C. piliforme,* a soil- and manure-borne gram-negative, pleomorphic, motile, spore-forming, rod-shaped obligate intracellular bacterium.[779] The epidemiology is poorly understood, but as the disease does not affect adult horses it is likely that adult carriers contaminate the environment with subsequent fecal-oral transmission.[770] Risk factors for this disease include seasonality, with a 7.2 times greater risk between March 13 and April 13.[770] Foals born to young mares (<6 years of age) and nonresident mares are also at increased risk, possibly because of lack of previous exposure leading to poor colostral antibody production resulting in inadequate passive immunity in their foals.[770]

Tyzzer's disease often affects otherwise healthy foals, and affected individuals, especially younger foals, may simply be found dead. In foals presenting alive the clinical signs are often nonspecific, including lethargy, anorexia, dehydration, icterus, fever, colic, diarrhea, and seizures followed by the rapid onset of recumbency, weakness, coma, and death.[771,776] Antemortem diagnosis is difficult and is typically based on the signalment, history, clinical signs, and clinicopathologic findings. PCR testing may be useful for early and specific diagnosis,[771] but the results may not be available within a useful time frame because of the rapid progression of disease. Clinicopathologic examination typically demonstrates severe leukopenia with a left shift; thrombocytopenia; marked hypoglycemia; severe metabolic acidosis; elevated serum fibrinogen; and elevated total, indirect, and direct bilirubin concentrations.[771,779] The hepatocellular enzymes AST and SDH are markedly increased, but GGT and ALP are typically within normal limits.[768,771] Ultrasonographic examination of the liver reveals marked hepatomegaly with increased echogenicity of the hepatic parenchyma, or possibly an enhanced vascular pattern.[623,771] Treatment is extremely difficult because of the severe, acute nature of the disease, and the prognosis should be regarded as grave. There are, however, reports of successful treatment of three suspected and one confirmed case of the disease.[770,771,780] Treatment consists of antimicrobial therapy with penicillin or ampicillin in combination with an aminoglycoside, as well as fluid therapy, correction of electrolyte and acid-base disorders, parenteral nutritional support, and appropriate supportive care.[771,780]

Bacterial Hepatitis

Bacteria other than *C. piliforme* can cause hepatic disease, and are commonly associated with severe systemic inflammation and bacteremia, but these cases do not typically present with primary hepatic disease. These foals typically present as septic, critically ill foals, and the finding of hepatic involvement is secondary.[781] *Actinobacillus* spp. and *S. equi* subsp. *zooepidemicus* have been reported to be involved in acute bacterial hepatitis of young foals.[768,781] The spirochete *Bartonella henselae* was recently associated with severe suppurative cholangiohepatitis in a foal, which was successfully treated with trimethoprim-sulfamethoxazole and rifampin along with *S*-adenosylmethionine and pentoxifylline for their potential antifibrotic and antioxidant effects.[782] Leptospirosis has been associated with jaundice and death in a 10-day-old foal,[783] and *Leptospira interrogans* serovar Pomona appears to be a rare cause of hepatic disease in neonatal foals.[784] Ascending cholangiohepatitis and bile duct obstruction have been associated

with duodenal stenosis resulting from severe gastroduodenal ulcer disease (GDUD).[785-787] Effective surgical bypass procedures have been described for foals suffering from delayed gastric emptying associated with GDUD, but the prognosis for foals with hepatic involvement appears to be guarded.[591,787]

Equine Herpesvirus 1

EHV-1 infection of the near-term fetus can result in the delivery of a nonviable foal suffering from hepatic, respiratory, and/or gastrointestinal disease.[358,788] Most affected foals will be born premature and will be severely icteric on initial examination. Clinicopathologic examination typically demonstrates severe leukopenia caused by neutropenia and lymphopenia, which may be more severe than seen in septic foals.[183] Hyperbilirubinemia may be present, although the magnitude of hyperbilirubinemia may be less than one might expect based on the profound icterus observed clinically.[183,184] No increase is usually observed in the serum activity of the hepatic enzymes, which is surprising given the severity of the hepatic necrosis detected on postmortem examination.[183] Although hepatic involvement is prominent in most affected foals, death is usually caused by overwhelming respiratory disease within 1 to 5 days of birth.[184] Treatment with acyclovir was associated with an improvement in survival in one report, although valacyclovir may represent a superior choice given the superior bioavailability of that drug in adult horses after oral administration.[184,789]

Hepatic Lipidosis

Hepatic lipidosis can occur secondary to hyperlipemia in neonatal foals, but this appears to be most common in Miniature breeds.[790-792] Hypertriglyceridemia (>500 mg/dL) typically develops as a consequence of a negative energy balance resulting from inanition or anorexia caused by some other primary disease process and, if unresolved may progress to hyperlipemia, with characteristic grossly lipemic serum.[709,793] Fat accumulation in the liver can proceed rapidly, resulting in hepatic lipidosis. Hepatic enzyme activities may be increased, but this is not consistently observed.[790,791] Clinical signs of hepatic lipidosis may be referable to the primary disease process but can include severe depression, seizures, blindness, and ventral edema.[768] Treatment should be directed toward resolving any primary disease process and correction of the negative energy balance with parenteral nutrition. Insulin therapy is often critical in facilitating the resolution of the hyperlipemia and allowing for the administration of adequate amounts of parenteral nutrition.

Neonatal Isoerythrolysis

NI is the most common cause of hyperbilirubinemia in foals, and hepatic disease may develop because of hepatocellular injury resulting from excessive bilirubin accumulation, anemic hypoxia, and/or iron toxicosis.[119,122] Total and direct bilirubin, AST, ALP, and SDH activities may be increased in foals with NI. Foals with the lowest presenting packed cell volumes tend to have the highest SDH values, suggesting that hepatic injury was present at the time of presentation.[119] One study reported that foals receiving multiple blood transfusions (4 L or more) were 19.5 times more likely to develop hepatic failure than those receiving smaller volumes of blood products.[122] It is thought that multiple blood transfusions may cause iron overload leading to iron toxicity.[122,794] The administration of deferoxamine mesylate, an iron chelator, has been shown to decrease hepatic iron accumulation in healthy foals receiving blood transfusions.[794] Although the use of this drug in foals with NI has not been reported, the results of this study suggest that the administration of deferoxamine to foals with NI starting before blood administration may be beneficial in decreasing iron-associated hepatic injury.[784,794]

Iron Toxicity

The oral administration of iron fumarate is a very well-described cause of hepatic injury in foals. The greatest risk of toxicity is associated with iron administration before the foals first suckle, presumably caused by the presence of protective factors in colostrum or milk that decrease the hepatotoxicity of iron when administered after nursing has begun.[795-797] When iron is administered at birth and before colostrum ingestion, clinical signs will typically manifest within 2 to 5 days and are primarily associated with hepatoencephalopathy. Seizures, profound depression, head pressing, ataxia, and abnormal behavior are common. Icterus is usually present at the time of onset of neurologic signs. Clinicopathologic examination typically reveals elevated bilirubin, GGT, ALP, and SDH, as well as increased ammonia concentrations.

Inherited or Congenital Disorders

Glycogen Storage Disease Type IV

Glycogen storage disease type IV, a fatal recessive inherited deficiency of the glycogen branching enzyme, has been described in Quarter Horse foals.[798] The clinical signs associated with this disease are variable, ranging from stillbirth to progressive weakness, transient flexural limb deformities, persistent recumbency, seizures, and respiratory or cardiac failure.[798] Affected foals are leukopenic and typically have elevated AST and GGT activities, in addition to elevated creatine kinase (CK) caused by muscle pathology. The genetic lesion is a single base nonsense mutation in the glycogen branching enzyme encoded by the GBE1 gene.[799,800] One study reported that approximately 2.5% of fetal and early neonatal deaths in Quarter Horse–related breeds were homozygous for the mutant GBE1 allele, suggesting that this syndrome has a clinically relevant impact on the reproductive efficiency of these breeds.[801]

Persistent Hyperammonemia

Persistent hyperammonemia associated with encephalopathy has been described in Morgan foals 4 to 7 months of age.[263,802] Affected foals exhibit acute onset of clinical signs shortly after weaning, which can include coma, blindness, and seizures. Hepatic enzymes may be mildly elevated, but hepatic pathology is modest. Blood ammonia levels are extremely high in affected foals (300–600 μmol/L), and this syndrome is thought to be related to an inherited disorder of hepatic ammonia metabolism and possibly other amino acids.[803] The metabolic defect may resemble HHH syndrome in humans.[263] Although some foals may survive the first encephalopathic crisis, the syndrome is considered to be fatal and may end with a terminal hemolytic crisis.[768]

Congenital Fibrosis

A syndrome of congenital hepatic fibrosis has been described in the Swiss Franches-Montagnes and Swiss Frieberger breeds.[804,805] The onset of clinical signs is sudden, and affected foals are jaundiced and encephalopathic, with abdominal

distention and colic. Fever and tachypnea are also common. Leukocytosis caused by neutrophilia and increased GGT, ALP, and serum bile acid values are reported. Histopathology demonstrates diffuse bridging portal fibrosis with numerous small and irregular bile ducts, which are sometimes cystic.[805]

Portosystemic Shunts

Portosystemic shunts are an infrequent congenital anomaly in foals that allow for shunting of blood directly from the portal circulation to the systemic circulation.[806-808] The shunts can be located intra- or extrahepatic and may be single or multiple in number.[807] Clinical signs of encephalopathy often do not develop until foals are 6 to 12 weeks of age. The diagnosis is based on the signalment, particularly age of onset, and presence of encephalopathic signs with normal serum hepatic enzyme activities but marked elevations of serum ammonia. Confirmation of the diagnosis may be possible using hepatic ultrasonography, or by a "bubblegram" study involving the injection of 10 mL of agitated saline into the spleen while monitoring for the rapid appearance of bubbles via simultaneous echocardiography.[808] CT, positive contrast portography, and transrectal portoscintigraphy may also be useful in confirmation of the diagnosis.[784] Successful surgical repair using banding techniques that provide progressive occlusion has been described.[807,808]

MUSCULAR DISORDERS

Polysaccharide Storage Myopathy

Polysaccharide storage myopathy (PSSM) was first described in Quarter Horses and related breeds, such as Paint and Appaloosa horses, but is now recognized in some draft, Warmblood, and light horse breeds as well.[809-813] PSSM is characterized by abnormal glycogen accumulation in skeletal muscle associated with muscle damage following exertional exercise. This syndrome has been associated with an incompletely penetrant autosomal dominant mutation in the *GYS1* gene, which encodes the skeletal muscle glycogen synthase enzyme.[814] This mutation has been identified in more than 30 horse breeds, although at highly variable frequencies, with Quarter Horses and Belgians having the highest reported prevalences.[814-819] PSSM is rarely reported in foals because it remains subclinical, especially if foals are stall confined.[820] The only indication of the presence of the syndrome may be increased serum CK activity, unless the foals undergo a period of exertional activity or are affected by concurrent disease. Typically the disease is not recognized until affected individuals are mature enough to be put into training or work.[820] The clinical signs may range from muscle soreness and gait abnormalities in Warmbloods to progressive weakness and muscle atrophy in draft breeds, whereas Quarter Horses may suffer from acute exertional rhabdomyolysis.[815,821] Diagnosis in adults depends on muscle biopsy, but this may not be diagnostically useful in foals, and genetic testing is preferred.[822] Daily pasture exercise and appropriate feeding of a balanced, low-starch, fat-supplemented diet to both the foal and its dam will likely be beneficial in management of affected foals.[822]

Glycogen Branching Enzyme Deficiency

Glycogen branching enzyme deficiency represents an important cause of stillbirth and early neonatal death in the Quarter Horse and Paint breeds.[798,801,822] The syndrome is caused by a mutation in the *Gbe1* gene and results in severe impairment of glucose homeostasis.[800] The clinical presentation is variable, with foals born alive that are weak and hypothermic. Although they often respond positively when assisted to nurse or bottle-feed, intermittent hypoglycemia and seizures may develop unless they nurse regularly.[823] Other clinical signs can include fever, tachypnea, and tachycardia, and because of this very nonspecific spectrum of clinical signs it can be difficult to differentiate these foals from those suffering from neonatal sepsis.[824] Respiratory failure and sudden death can occur, even with only mild to moderate exercise.[823] Clinicopathologic evaluation may reveal leukopenia; intermittent hypoglycemia; and persistent elevations of CK, AST, and GGT activities.[822] Despite supportive care the condition is ultimately fatal, with most foals succumbing by 6 to 8 weeks and no reports of survival beyond 18 weeks of age.[822,823] A genetic test is available for diagnosis of this disease.[801]

Nutritional Myodegeneration (White Muscle Disease)

Nutritional myodegeneration (NMD) is a noninflammatory, degenerative disease primarily seen in foals less than 30 days of age. It affects skeletal as well as cardiac muscle and is associated with low serum selenium and vitamin E concentrations.[825-829] The primary deficiency of vitamin E and selenium appears to be in the mare, and for this reason NMD is most commonly seen in areas with selenium-deficient soils, forages, and grains.[830] These areas include the northeastern, northwestern, mid-Atlantic, and Great Lakes regions of the United States. The clinical presentation of NMD can be acute or subacute. The subacute form is associated with muscular weakness, whereas the acute form is associated with rapidly progressive weakness leading to recumbency and death within a few days.[831] Dysphagia is a common finding in the subacute form and is related to involvement of the muscles involved in prehension, mastication, and swallowing. It may contribute to the development of FTPI and aspiration pneumonia.[828] Severely affected foals typically have involvement of the myocardium and the respiratory musculature, contributing to cardiovascular and respiratory failure and leading to death.[822] Diagnosis of NMD is based on clinical signs in combination with increased muscle enzyme activities (CK and AST), decreased blood selenium, decreased glutathione peroxidase activity, and response to vitamin E and selenium treatment.[828,830] Profound electrolyte abnormalities may be present, including hyperkalemia, hyponatremia, hypochloremia, and hypocalcemia, with hyperkalemia potentially contributing to cardiac arrest in severely affected foals. Myoglobinuria may be present, and pigment nephropathy can lead to the development of acute renal failure. Muscle biopsies may be supportive of NMD, but a more definitive antemortem diagnosis requires assessment of vitamin E and selenium concentration or glutathione peroxidase activity.[828] Postmortem examination consistently reveals bilaterally symmetric myodegeneration with a dry appearance and pale discoloration of affected muscles. Treatment requires supportive care, IV fluid therapy for correction of hypovolemia, electrolyte derangements, metabolic acidosis, and diuresis. Dysphagic foals will require nutritional support, either by indwelling nasogastric feeding tube or parenterally. Vitamin E and selenium supplementation is essential in the treatment of affected foals (Table 20.13). The injectable form of selenium also contains vitamin E but at an insufficient concentration, requiring that foals also receive injectable or

TABLE **20.13 Treatments Drugs Used for Nutritional Myodegeneration**

Drug	Dosage	Route	Frequency (h)
Vitamin E (preferably in the form of DL-alpha-tocopheryl acetate)	2–6 IU/kg	PO	24
Selenium	0.055–0.067 mg/kg	IM (deep)	Once
	1 mg total dose	PO	24

IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

oral vitamin E. Although the initial prognosis is guarded, foals that regain the ability to stand have a more favorable prognosis.[823] Prevention of NMD can be aided by ensuring that mares have adequate dietary vitamin E and selenium intake and by prophylactic parenteral administration of vitamin E and selenium to newborn foals in endemic areas.

FOAL ANTIMICROBIAL THERAPY

The presence of documented focal or systemic bacterial infection, such as septic arthritis, pneumonia, enterocolitis, meningitis, or umbilical remnant infections, represents a clear indication for the use of antimicrobial therapy in the foal. Unfortunately the clinical situation is not always so straightforward. Because of the risk of septicemia in any compromised neonatal foal, it is often better to make the assumption that bacterial infection is present rather than risk allowing the patient to deteriorate further while awaiting confirmation of the diagnosis.[194] In those situations in which bacterial infection is likely or suspected, aggressive therapy that includes antimicrobials is indicated. Although there are some risks associated with antimicrobial therapy, primarily AAD, these risks are greatly outweighed by the risks associated with withholding antimicrobial therapy from a patient with sepsis secondary to bacterial infection. Early appropriate empiric antimicrobial therapy has been shown to reduce mortality in human patients suffering from septic shock,[832] and although published evidence is lacking, clinical experience in neonatal foals supports this conclusion as well.[833]

When implementing antimicrobial therapy one must consider some basic pharmacologic concepts. The first concept is whether the antimicrobial selected is appropriate for the likely target of the therapy. To understand this, one must have a concept of the drug's mechanism of action and how that relates to the different classes of bacteria and their inherent susceptibilities. Second, how is the drug to be delivered, and is that route of delivery likely to achieve therapeutic concentrations of the drug at the affected site? There are key factors that profoundly affect our ability to effectively deliver antimicrobials to some sites of infection, and failure to take these into account will preclude effective treatment. The physical and pharmacologic characteristics of the different antimicrobials and their interactions with various tissues will also have a significant influence on the efficiency and efficacy of the treatment of bacterial infections. Consideration should also be given to the route of administration and frequency of dosing, particularly if treatment is to be administered by lay personnel or owners.

Achieving therapeutic concentrations at the site of infection can be challenging, especially when the drug has to cross an epithelial barrier (CNS, eye, lung, biliary, etc.). Drugs that are highly lipid soluble tend to distribute more fully to the tissues, whereas water-soluble drugs tend to distribute primarily within the extracellular water and penetrate epithelia poorly. The presence of inflammation will allow for increased tissue permeability of epithelial barriers; however, the resolution of inflammation allows for epithelial restitution. This may limit the ability of water-soluble drugs to maintain therapeutic respiratory concentrations as healing progresses, and recurrent infections may result.

The pharmacodynamic characteristics of the different antimicrobials also impact decisions regarding the route and frequency of administration. The two fundamental classes of drugs are the time-dependent and the concentration-dependent antimicrobials. Efficacy of time-dependent antimicrobials, such as the β-lactams, require that the concentration at the site of infection be maintained above the minimum inhibitory concentration (MIC) for as much of the treatment interval as possible. Time-dependent drugs benefit from intramuscular and oral administration because slower absorption yields lower serum concentrations, but therapeutic concentrations are typically maintained for longer periods of time. The use of CRIs for delivery of time-dependent drugs can yield optimal pharmacokinetics and is feasible in the hospital environment using electronic infusion pumps. The efficacy of concentration-dependent drugs requires high peak serum concentrations for maximal efficacy (greater than 10 times MIC for the aminoglycosides) but does not require high concentrations throughout the treatment interval. These compounds benefit from IV or topical administration to achieve high peak concentrations, with prolonged treatment intervals.

It is also important to remember that foals differ from adult horses in a number of ways that may impact the choice of antimicrobial therapy. First, foals have substantially greater total body water content than adult horses, typically requiring that the dosage of water-soluble drugs be increased. For example, doses of aminoglycosides are often increased as much as 100% in foals, compared with dosing in adults, to compensate for this increase in the volume of distribution. Also, neonatal foals do not have complete maturation of their hepatic and renal systems, especially during the first week of life, which may result in impaired elimination of certain drugs metabolized or eliminated by those routes. Different types of toxicities associated with antimicrobial use in foals must also be considered—for example, the risk of arthropathy associated with the administration of fluoroquinolones (because their rapidly growing cartilage tissue).

There are some advantages in designing foal antimicrobial therapy. Most orally administered antimicrobials are more effectively absorbed in foals compared with adults, which allows for wider use of this route. In addition, foals appear to be at reduced risk of AAD, which is likely because they have not yet fully transitioned to hindgut fermentation. Last, some antimicrobials used in in foals are cost-prohibitive in adult horses because of the total dose required; hence, the cost for foals is much less.

Therapeutic Targets

The most common isolates encountered will vary somewhat based on the specific site of infection and the predisposing factors associated with the infection. However, when looking at

retrospective studies of isolates from bacteremic neonatal foals some patterns become apparent. Over the past 30 years the most common bacteria isolated from blood cultures of infected foals have been gram-negative organisms.[25,123,171] Gram-positive organisms isolated from infected foals represented less than 20% of isolates and were typically present in mixed infections with gram-negative organisms.[25,123] Although some of the variance in the patterns of bacteria isolated from foals may result from regional differences, it does appear that more recently an increase in the percentage of gram-positive isolates has occurred.[177] Several studies have reported increased numbers of gram-positive isolates ranging from 30% to 43% of the total isolates.[180,181] This temporal change in patterns of isolates highlights the need to consider the likely pathogens present in the formulation of the initial empiric antimicrobial plan. Based on the most recent reports, *E. coli* remains the most common isolate followed by *Enterococcus* spp., *Actinobacillus* spp., β-hemolytic *Streptococci, Staphylococcus* spp., and *Enterobacter* spp.[180,834] Unfortunately there have also been temporal changes in the patterns of antimicrobial susceptibility observed in the bacteria isolated from bacteremic foals. Although there may be some debate as to the driving force behind these changing patterns of antimicrobial sensitivity, there is no doubt that the phenomenon is real. Some earlier reports mentioned the presence of a few isolates that were resistant to multiple antimicrobials,[120,177] but recent reports have demonstrated substantial numbers of multidrug-resistant (MDR) organisms (up to 38% of isolates).[180,182] Definitions vary somewhat among references, but generally speaking, MDR organisms display resistance to three or more of the core antimicrobials in a sensitivity panel. Recent studies in human medicine have characterized the most common and problematic of the MDR organisms as the ESKAPE group, which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *Enterobacter* spp.[835] This group of pathogens is responsible for the majority of nosocomial infections in human hospitals, and all routinely "escape" most currently used antimicrobial drugs.[836] The increasing prevalence of MDR organisms will require that antimicrobials are used judiciously and appropriately to limit the further spread of these organisms. The risk associated with these organisms also reinforces the need to perform appropriate culture sampling (blood, synovial fluid, CSF, body cavity effusions, etc.) to be sure of the pathogens involved and tailor antimicrobial therapy appropriately.

Antimicrobials

β-Lactams

β-Lactam antibiotics bind to penicillin binding proteins, interfering with bacterial cell wall synthesis. This leads to the formation of defective cell walls that are osmotically unstable, and cell death usually results from cell lysis. These drugs include the penicillins, synthetic penicillins, cephalosporins, and carbapenems. Modifications of the basic penicillin or cephalosporin molecule confer differences in antimicrobial activity, as seen with the synthetic penicillins and third-generation cephalosporins, which exhibit an increased gram-negative spectrum. Increased stability against β-lactamases may also be achieved, such as seen in the carbapenems. β-Lactam drugs are time-dependent antimicrobials and must be present in the tissues at concentrations greater than the MIC throughout most, preferably all, of the dosage interval to be effective.

Penicillin G is the most commonly used β-lactam antimicrobial in equine medicine, and it exhibits good efficacy against many gram-positive organisms and anaerobes, but it has a limited gram-negative spectrum. Penicillin G exhibits good synergy with aminoglycoside antimicrobials, making it a useful drug in a combination therapy approach. Procaine penicillin is administered intramuscularly, whereas the aqueous solutions of sodium or potassium penicillin are administered intravenously (Table 20.14). Procaine penicillin G is readily available and inexpensive, but the requirement for repeated intramuscular injections makes it less desirable for use in neonatal foals because of their minimal muscle mass. Potassium or sodium penicillins are substantially more expensive than procaine penicillin on a daily cost basis but are commonly used in hospitalized foals with ready venous access. There are reports of administering potassium or sodium penicillin as an IV CRI, which can provide favorable pharmacokinetics with these time-dependent compounds.[833] Toxicity is low for the penicillins and is primarily associated with adverse reactions to the inadvertent IV or intraarterial injection of the intramuscular preparation, procaine penicillin G, which likely represents adverse reactions to procaine rather than penicillin, although anaphylactic reactions have been reported.[837]

Ampicillin and amoxicillin are synthetic penicillins that exhibit better activity against gram-negative bacteria because of improved outer cell wall penetration. They do suffer some loss of efficacy compared with penicillin G regarding susceptible gram-positive bacteria. Ampicillin is widely used as an alternative to penicillin in the treatment of neonatal sepsis, typically in combination with amikacin.[177,194,834,838] Other β-lactam drugs that are occasionally used in foals include the antipseudomonal penicillins and the carbapenems. These drugs exhibit a superior gram-negative spectrum of action and are primarily used in cases where aminoglycosides cannot be administered or in the treatment of pathogens resistant to aminoglycosides. The use of these drugs should be restricted to cases with documented infections involving susceptible MDR organisms. The most commonly used antipseudomonal penicillin is ticarcillin, which is typically combined with clavulanic acid.[839,840] Clavulanic acid causes irreversible time-dependent inactivation of many of the β-lactamases, enhancing the activity of ticarcillin against organisms producing these enzymes. Imipenem, a carbapenem antimicrobial, has been used in foals for the treatment of MDR infections. Imipenem is used in combination with cilastatin, which slows renal elimination of imipenem and allows for a decreased frequency of dosing.[841]

Cephalosporins

Cephalosporins are traditionally grouped into "generations" according to their order of discovery and their spectrum of action. First-generation drugs exhibit a primarily gram-positive spectrum, whereas subsequent generations have an increasing gram-negative spectrum and corresponding decreases in the gram-positive spectrum. Fourth-generation drugs are considered to be truly broad spectrum. The primary cephalosporin administered to horses is ceftiofur, which is a third-generation drug.[842] It exhibits a fairly broad spectrum with an emphasis toward gram-negative coverage. Because of this spectrum of activity ceftiofur has often been used as the sole therapy in the initial treatment of foals. The relative safety of ceftiofur in foals has fostered this approach, because it allows the clinician to avoid using combined therapy including aminoglycosides. Unfortunately some recent studies have documented frequent

TABLE **20.14 Antimicrobial Dosages for Use in Foals**

Drug	Dosage	Route	Frequency (hours)
Acyclovir	16–20 mg/kg	PO	8
Amikacin	25–30 mg/kg (neonates) 20–25 mg/kg (2–4 weeks) 7–14.5 mg/kg (weanlings)	IV	24
Amikacin	8 mg/kg	Aerosol	24
Ampicillin sodium	10–20 mg/kg	IV, IM	8
Amoxicillin trihydrate	10–20 mg/kg 20–30 mg/kg	IM PO	8 6–8
Azithromycin	10 mg/kg	PO	24 h for 5 days, then every 48 h
Cefazolin	25 mg/kg	IM	6–8
Cefepime	11 mg/kg	IV, IM	8
Cefpodoxime proxetil	10 mg/kg	PO	6–12
Ceftiofur sodium	5 mg/kg	IV	12
	2.2 mg/kg	IM	12–24
	1.5 mg/kg/h	IV	CRI
	2.2 mg/kg	Aerosol	24
Ceftiofur crystalline-free acid	6.6 mg/kg	IM	96 (two doses)
Cefotaxime	50–100 mg/kg	IV	6
Cephalexin	30 mg/kg	PO	8
Ceftriaxone	25 mg/kg	IV	12
Chloramphenicol	50 mg/kg	PO	6
Clarithromycin	7.5 mg/kg	PO	12
Doxycycline	10 mg/kg	PO	12
Erythromycin	25 mg/kg	PO	6–8
Fluconazole	8 mg/kg (loading) 4 mg/kg (maintenance)	PO PO	Once 12
Gentamicin	11–13 mg/kg (neonates) 6.6 mg/kg (weanlings)	IV, IM	24
Gentamicin	2.2 mg/kg as a 50-mg/mL solution	Aerosol	24
Imipenem/cilastatin	5–10 mg/kg 10–20 mg/kg	IM IV	12 6
Metronidazole	10 mg/kg (neonates) 15 mg/kg (10–12 day old) 15 mg/kg (over 2 weeks)	PO PO PO	12 12 8
Minocycline	4 mg/kg	PO	12
Penicillin G	20,000 IU	IM (procaine penicillin) IV (K or Na penicillin)	12 (IM) 6 (IV)
Rifampin	5–10 mg/kg	PO	12–24
Sulfadiazine-trimethoprim	30 mg/kg	PO	12
Ticarcillin	44 mg/kg	IV	6
Valacyclovir	27 mg/kg (loading) 18 mg/kg (maintenance)	PO PO	8 12

CRI, Continuous rate infusion; *IM,* intramuscularly; *IV,* intravenously; *PO,* orally.

resistance to ceftiofur in organisms isolated from foals, and there does appear to be a trend toward increasing resistance over time, especially among gram-negative enteric bacteria.[180,834] The use of ceftiofur sodium as a CRI has also been investigated and appears to yield suitable pharmacokinetics.[843] Ceftiofur is also available in a sustained-release form, which contains CCFA. Subcutaneous administration of CCFA yields appropriate pharmacokinetics for highly susceptible bacteria in foals.[844] Self-limiting diarrhea was reported in 66% of the foals treated with CCFA, but this does not appear to be a clinical problem associated with this drug. Repeated subcutaneous dosing of CCFA (13.2 mg/kg) at 48-hour intervals for four doses has been reported to maintain appropriate therapeutic concentrations in foals.[845] Other cephalosporins may have utility in foals, such as cefquinome, ceftriaxone, cefpodoxime proxetil, cefotaxime, ceftazidime, and cefepime. Cefquinome

is a fourth-generation cephalosporin that is labeled in Europe for the treatment of foal septicemia caused by *E. coli,* but it is not available in the United States at this time.[846] Cefquinome is dosed at 1 mg/kg IV or intramuscularly every 12 hours, for very susceptible pathogens, but a higher dose of 4.5 mg/kg IV every 12 hours is indicated when dealing with pathogens with higher MIC values.[847] Cefpodoxime proxetil is a third-generation drug that is administered orally, but unfortunately the expense of this drug renders it of limited utility in most cases.[833] Cefepime is a fourth-generation drug that can be useful in the management of MDR infections.[848]

Aminoglycosides

The aminoglycosides continue to constitute a major component of the equine clinician's armamentarium, despite their well-described potential for nephrotoxicity. The primary reasons for this are that the aminoglycosides are bactericidal, exhibit primarily a gram-negative spectrum, and demonstrate good synergy with β-lactam antimicrobials.[634] The bacterial penetration of aminoglycosides is in part an oxygen-dependent transport process and partially accomplished by passive diffusion. This dependence on oxygen-driven transport renders anaerobes resistant to the aminoglycosides. The role of passive diffusion leads to a dependence on high tissue concentrations of the drug to achieve high intracellular concentrations. Routes of administration and/or dosage schedules associated with high peak concentrations at the site of infection, such as extended-interval dosing, are associated with improved clinical responses.[849] Prolonged treatment intervals decrease the risk of nephrotoxicity while still demonstrating clinical efficacy caused by the prolongation of bacterial killing, even after the aminoglycoside concentration drops below the MIC for the targeted organism. This phenomenon is termed the *postantibiotic effect.* The use of extended interval dosing also has been demonstrated to lessen the development of both acquired and adaptive antimicrobial resistance, which are caused by the high peak concentrations achieved and the period of time during which the drug is below the MIC.[634]

Neonatal foals have reduced rates of drug clearance, which necessitates prolonged treatment intervals, but their clearance increases to adult levels within the first few weeks of life.[634] They also have greater volumes of distribution, compared with adults, which necessitates substantially higher dosages to achieve the desired peak serum concentrations. A recent study examining the pharmacokinetics of gentamicin in foals indicated that gentamicin should be dosed at 12 mg/kg IV every 36 hours in foals less than 2 weeks of age, whereas a lower dose of 6.6 mg/kg IV every 24 hours was estimated to be adequate in foals 2 weeks of age or older.[850] Because of the unpredictability of aminoglycoside pharmacokinetics in sick foals, it is recommended that therapeutic drug monitoring be performed to ensure that both an adequate peak serum concentration is achieved as well as appropriate trough concentrations. Peak serum concentrations are typically monitored at 30 minutes after administration of the IV dose, and trough concentrations are monitored immediately before the subsequent dose. This approach is effective but may lack sensitivity in detecting impaired clearance of the drug. In addition, because of the timing of the sample collection the results of the assay will not be available until after the administration of the next dose, which may delay adjustment of the dosage or interval, if indicated. An alternative approach is to obtain serum samples at 30 minutes and 8 hours after drug administration, which can allow for estimation of drug clearance in a more timely manner, allowing for more rapid adjustment of the therapeutic protocol.[634]

Potentiated Sulfonamides

Potentiated sulfa compounds are very common in equine medicine because of their broad spectrum, oral route of administration, and affordability. These drugs exhibit time-dependent pharmacodynamics, and their effects are considered to be bactericidal. The potentiated sulfas distribute well to the tissues. Toxicity is most commonly associated with disturbance of gastrointestinal flora associated with the development of colitis. Although these drugs are not appropriate for initial therapy of septic foals, when organisms are documented to be susceptible they can be used quite effectively.

Tetracyclines

Tetracyclines are bacteriostatic and broad spectrum, possessing activity against a wide variety of bacteria and protozoa as well as *Mycoplasma* and *Rickettsia.* These compounds are time dependent in nature. The most commonly used compounds are oxytetracycline and minocycline. Toxicity has been reported from the disturbance of gastrointestinal flora in adults, but this appears to be rare in foals. Because the tetracyclines are primarily eliminated via the urinary tract, there is the risk for toxicity in animals with renal insufficiency. Very large dosages of oxytetracycline are administered to foals suffering from tendon contracture, and repeated or injudicious administration has been associated with nephrotoxicity.[629] Rapid IV injection of oxytetracycline has been associated with collapse in horses, perhaps caused by chelation of calcium in the blood. For this reason oxytetracycline is administered at 6 to 8 mg/kg slowly IV twice daily, usually diluted in 0.5 to 1 L of normal saline and administered over 30 minutes. Doxycycline is no longer widely used because of decreased availability and increased expense. Minocycline has come into widespread use in horses because of superior pharmacokinetics compared with doxycycline and a much more reasonable cost of treatment. Although no pharmacokinetic studies have been performed in foals, minocycline is empirically administered at adult dosages.[851]

Macrolides

Macrolides (erythromycin, azithromycin, and clarithromycin) are considered to be bacteriostatic; have a broad spectrum of action; and accumulate in tissues, particularly the lungs.[852] Their primary application is for the treatment of rhodococcal pneumonia in foals. Macrolides are eliminated by hepatic metabolism and can affect the pharmacokinetics of other drugs metabolized by the P450 system. Erythromycin administration in foals with rhodococcal pneumonia has been associated with frequent hyperthermia and diarrhea, and this drug is no longer used for this purpose in most areas of the United States. Azithromycin and clarithromycin are the most commonly used macrolides in foals at this time. Rifampin therapy is typically administered in conjunction with the macrolides in the treatment of rhodococcal pneumonia because of its potential synergistic effects. The combination of clarithromycin-rifampin has been shown to be more effective than erythromycin-rifampin or azithromycin-rifampin in the treatment of rhodococcal pneumonia.[441] Despite decades of clinical experience supporting the efficacy of the combination of macrolides and rifampin, there is recent evidence that coadministration of rifampin with macrolides substantially inhibits

macrolide absorption.[444,445] Current recommendations support the continued use of macrolide-rifampin combinations in the treatment of rhodococcal pneumonia in foals.[349] There has been recent interest in the use of the macrolide gamithromycin in foals because this drug exhibits favorable pharmacokinetics in foals following once-weekly intramuscular injections. It also has been shown to be noninferior to the combination of azithromycin and rifampin in the treatment of rhodococcal pneumonia in foals.[435,443]

Rifamycins

Rifampin is a macrocyclic antibiotic and is used primarily in foals as an adjunct therapy to the macrolides for treatment of rhodococcal pneumonia. It can be combined with other antibiotics for treatment of abscesses, but it should never be used alone because of the risk of rapidly developing antimicrobial resistance. In addition to using rifampin in the treatment of rhodococcal infections, it has been reported to be effective for the treatment of staphylococcal infections.

Chloramphenicol

Chloramphenicol is widely distributed to the tissues and has a wide spectrum of action including both gram-positive and gram-negative bacteria, as well as *Rickettsia, Mycoplasma,* and anaerobes. The half-life of chloramphenicol is short, and it is a time-dependent drug, requiring frequent dosing (6-hour intervals).[853] Human health concerns regarding possible chloramphenicol-associated nondose-dependent fatal aplastic anemia made it illegal to use this drug in food animals over the past 50 to 60 years. As a result of the minimal use of the drug there are low levels of resistance to chloramphenicol in many types of bacteria, which has created a potentially important role for chloramphenicol in the treatment of MDR organisms. Intriguingly, the usage of chloramphenicol has been proposed for human patients infected with MDR organisms, despite the risk of aplastic anemia.[854] Regardless, the potential risk of aplastic anemia to humans handling chloramphenicol necessitates thorough client education when dispensing this drug (avoid contact with aerosolized drug or direct contact with mucous membranes). Animal toxicity appears to be rare and is associated with reversible or irreversible bone marrow suppression. Florfenicol is closely related to chloramphenicol but has not been associated with aplastic anemia in humans and is labeled for use in food animals. Administration of florfenicol to adult horses has been associated with acute colitis and is contraindicated, but this has not been reported in foals. There are anecdotal reports of successful clinical use of florfenicol in foals at 24- to 48-hour dosing intervals; however, a recent pharmacokinetic study suggested that a shorter treatment interval of 10 hours may be appropriate.[833,855]

Fluoroquinolones

Fluoroquinolones have a relatively broad spectrum, with excellent activity against gram-negative organisms but minimal activity against streptococci. They are bactericidal and exhibit excellent tissue penetration. The fluoroquinolones exhibit peak concentration-dependent bactericidal effects and prolonged postantibiotic effects, which is similar to the aminoglycosides. As a result they can be given at relatively high doses at a decreased frequency. Enrofloxacin is the most widely used fluoroquinolone in horses, but its use is contraindicated in foals because of adverse effects on cartilage maturation.[856] Marbofloxacin has been used in foals and appears to be safe, although no controlled studies have been performed to confirm this clinical impression.[833,857]

Metronidazole

Metronidazole is a nitroimidazole antimicrobial that is highly effective against anaerobic organisms. Because of the limited anaerobic spectrum of most other antimicrobials used in foals, metronidazole is commonly used when anaerobic involvement is suspected or confirmed in foals. A recent pharmacokinetic study found that there were age-dependent influences on metronidazole kinetics in foals and suggested a range of dosages over the course of the first 2 weeks of life.[551]

Aerosol Administration

Aerosol administration of antimicrobials has been demonstrated to achieve high concentrations of antimicrobials at the respiratory mucosal surface while minimizing the development of systemic side effects. Other potential advantages to delivering medications to the lower respiratory tract by aerosolization include a decrease in the total dose administered, avoidance of systemic side effects, and a rapid onset of action.[463] The administration of antimicrobials by aerosolization does have limitations, however, including potential problems with drug delivery and pulmonary irritation, as well as the expense of the required equipment and the time required for administration. Several antimicrobials have been investigated for aerosol administration in horses, including gentamicin, ceftiofur, cefquinome, and marbofloxacin. Both gentamicin and ceftiofur are well tolerated and can be administered as aerosols.[459,466]

FLUID THERAPY IN THE FOAL

One of the fundamental dilemmas facing a clinician when evaluating a sick foal is whether or not fluid therapy is indicated. To determine the answer to that question one must consider the variety of indications for fluid therapy. The most common indication is for the correction of volume depletion or dehydration. Other indications for fluid therapy can be the correction of specific electrolyte imbalances, the provision of colloidal support, correction of acid-base disorders, restoration of oxygen-carrying capacity, and provision of immunoglobulins. In many cases more than one of these indications is present, and one must be careful in assessing the patient because changes in clinical status over time may result in one or more new indications for fluid therapy arising, even as others may have been resolved.

The next question regarding fluid therapy is what type of fluid does this patient need. The choice of fluid composition will depend on multiple variables, which include the primary goal of fluid therapy as well as patient-specific, route-specific, and practical considerations. The clinician must then determine the most appropriate route for administration of fluid therapy in the individual patient, and this may also factor into the decision-making process regarding the types of fluids to be administered. The amount of fluid therapy to be administered must also be determined, primarily based on the patient's fluid deficit and need for maintenance and replacement of ongoing losses. The anticipated duration of therapy can be more difficult to assess in the formulation of a treatment plan, but this represents an important factor in deciding how fluids will be administered and the potential costs to the client for fluid therapy. Finally, the clinician must develop a plan for monitoring ongoing fluid therapy and for determining when fluid therapy is no longer required.

Patient Requirement for Fluid Therapy

The first challenge in patient evaluation is the accurate assessment of hydration status. The typical parameters used in assessing clinical hydration during a physical examination are mucous membrane color, capillary refill time, jugular refill time, pulse quality, the temperature of the extremities, mental status, and urine production. Fundamentally these represent indicators of perfusion, rather than hydration, which is an important distinction given that there are situations, such as cardiogenic or vasodilatory shock, in which a well-hydrated patient can present with extremely poor perfusion. The term *dehydration* actually refers to situations in which there is a total body deficit of pure water. The clinical signs most commonly used for the assessment of dehydration are increased skin turgor, tacky mucous membranes, and sunken eyes. Unfortunately skin turgor is difficult to assess in foals, and a recent study in adult horses indicated that it was also not a reliable estimator of clinical hydration status in that population.[858] Further insight into hydration status can be gained by the measurement of urine specific gravity, which in the properly hydrated foal should be in the range of 1.010 to 1.001. Values greater than 1.010 indicate that the foal is retaining water and is likely not adequately hydrated. Urine output is another useful parameter, but it can be hard to gauge in foals that are not continuously monitored. The normal foal should urinate at least once every 2 hours. More accurate assessment of urine output is only possible in foals that have a urinary catheter in place, allowing for urine collection and measurement. Urine output should be greater than approximately two thirds of the total fluid intake including all IV infusions and any enteral fluids or feeding. Hypotension, considered a mean arterial pressure (MAP) less than 60 mm Hg, may be an indication of hypovolemia but can also result from decreased cardiac output or excessive venous capacitance. If the hypotensive patient does not respond to volume replacement then additional therapy in the form of vasopressors and/or inotropes is indicated (see the later section Inotrope and Vasopressor Therapy).

When attempting to determine whether hypovolemia and/or dehydration are present it is helpful to consider how fluid is distributed within the body. Neonatal foals have substantially higher total body water (TBW) content than adult horses, at 75% and 67%, respectively.[859] TBW is present in two primary compartments, the intracellular fluid (ICF) and ECF, with the ECF subdivided into the interstitial fluid and the plasma volume. In neonatal foals the ECF and ICF each represent approximately half of the TBW, and the plasma volume represents approximately one fourth of the ECF.[859] TBW is primarily determined by the total body sodium content, which is closely related to the sodium concentration within the ECF compartment and regulated by the ingestion or elimination of sodium.[860] Decreases in the plasma sodium concentration (hyponatremia) are indicative of a relative excess of free water and/or decreases in total body sodium content. The kidneys will respond by eliminating water while retaining sodium, restoring normal sodium concentration and TBW content. Increases in the plasma sodium concentration represent a loss of free water and will stimulate thirst, leading to water ingestion and correction of the TBW deficit.

Types of Fluids

Having determined that the patient needs fluids, the next challenge is determining the types of fluids that should be administered. There are many choices of fluids, and they fall into three broad categories: crystalloids, colloids, and oxygen-carrying solutions. Crystalloid solutions contain electrolytes and nonelectrolytes (such as dextrose) that are capable of diffusing through the capillary wall into the interstitium, whereas colloid solutions contain larger molecules that do not readily diffuse and tend to remain within the vascular lumen. Oxygen-carrying solutions contain either red blood cells or other substances capable of delivering oxygen from the lungs to the tissues. In the majority of situations the most appropriate fluid will be a crystalloid solution. These solutions are classified based on their tonicity relative to normal plasma and are isotonic, hypertonic, or hypotonic.

Isotonic electrolyte-containing fluids will have little influence on the ICF volume but will expand the ECF volume, expanding the circulating blood volume. Because these fluids contain large amounts of sodium, a substantial amount of the fluid will be retained in the body unless the kidneys excrete the sodium and water together. Sodium overload can result in hypernatremia, which has been associated with increased mortality and severity of illness in human patients.[861] In addition to the substantial sodium content of the isotonic electrolyte solutions, there is typically a substantial chloride content as well. This is most pronounced with isotonic saline (0.9%), which contains 154 mEq/L of both sodium and chloride (Table 20.15). Although the sodium content is only slightly supraphysiologic, the chloride content is dramatically greater than that present normally in the plasma. In some patients renal elimination of chloride is impaired and is not adequate to deal with this high chloride load.[862] Excessive chloride administration is associated with the development of hyperchloremic metabolic acidosis, inflammation, hypotension, and adverse renal outcomes in human patients.[861] More physiologic fluids, such as lactated Ringer's solution (LRS), Plasma-Lyte A, and Normosol-R, contain lower concentrations of chloride more closely mimicking normal serum concentrations. Dextrose 5% in water is an exception to these rules, because it is an isotonic crystalloid fluid that does not contain any electrolytes and is primarily used to replace total body water deficits.

Hypertonic crystalloid fluids have higher electrolyte concentrations than plasma and have fewer indications for their use. The most commonly used hypertonic crystalloid is hypertonic saline, which is primarily used to expand the circulating blood volume. This effect results from the shift of fluid from the interstitial space into the circulation, but it is very transient because of the rapid diffusion of the sodium load from the circulation into the interstitium. The other commonly used hypertonic crystalloid is hypertonic sodium bicarbonate, which is typically administered to correct metabolic acidosis. By providing sodium without any accompanying chloride this solution directly addresses strong ion acidosis, such as hyponatremic metabolic acidosis. It is less useful in addressing other types of metabolic acidosis, such as lactic acidosis, and care must be taken when administering this solution to foals with depressed ventilation because it will increase carbon dioxide production and potentially worsen respiratory acidosis.

Hypotonic crystalloid solutions contain much less sodium and chloride than the isotonic replacement fluids. This can be very helpful, because the lower sodium load decreases the risk of water retention and edema formation, and the lower chloride load decreases the risk of hyperchloremic metabolic acidosis. For these reasons hypotonic crystalloid solutions are most useful for maintenance fluid therapy, in which there is less need for electrolyte replacement and more need for the

TABLE **20.15 Composition of Intravenous Fluid Solutions Commonly Administered to Foals**

	Fluid Types							
Components	Plasma	0.9% Saline	Lactated Ringer's Solution	Plasma-Lyte A/Normosol-R	Plasma-Lyte 56/ Normosol-M with 5% dextrose	5% Dextrose in Water	50% Dextrose in water	7.2% Saline
Na^+ (mEq/L)	140	154	131	140	40	—	—	1232
Cl^- (mEq/L)	104	154	111	98	40	—	—	1232
K^+ (mEq/L)	5	—	5.4	5	13	—	—	—
Mg^{2+} (mEq/L)	1	—	1	3	3	—	—	—
Ca^{2+} (mEq/L)	2.2	—	2	—	—	—	—	—
Dextrose (g/L)	—	—	—	—	50	50	500	—
Bicarbonate (mEq/L)	25	—	—	—	—	—	—	—
Lactate (mEq/L)	1	—	29	—	—	—	—	—
Gluconate (mEq/L)	—	—	—	23	—	—	—	—
Acetate (mEq/L)	—	—	—	27	16	—	—	—
Osmolarity (mOsm/L)	285	309	278	295	363	253	2525	—

provision of water. The use of resuscitation fluids, such as LRS and Normosol-R/Plasma-Lyte A, for maintenance therapy in foals has been associated with the development of hypernatremia and hyperchloremia, which reinforces this concern.[863] Because patients on maintenance fluid therapy often have reduced dietary intake, there is an increased need for potassium supplementation, and maintenance fluids have accordingly higher concentrations of potassium. There are concerns that the administration of hypotonic fluids could cause red blood cell lysis, and for this reason maintenance fluids often have dextrose added to bring the tonicity of the solution into a more appropriate physiologic range. Following administration the dextrose is metabolized and has no impact on fluid shifts within the body. The use of 5% dextrose in water (D5W) represents an alternative to hypotonic crystalloid fluids for maintenance therapy in foals; this solution will not contribute any sodium load because there are no electrolytes. This is problematic because there is an ongoing need for electrolytes caused by renal, gastrointestinal, and insensible losses. For this reason D5W should not be used alone and is typically combined with isotonic polyionic crystalloid solutions to provide for replacement of any electrolyte deficits as well as ongoing losses.

Colloids are fluids containing relatively large molecules less readily able to diffuse across the capillary membrane. This allows them to exert colloid oncotic pressure within the circulation, resulting in fluid shifts from the interstitium into the vasculature and expanding circulating blood volume. Colloids will also aid in retaining fluids that have been administered within the vasculature for a longer period of time than would be possible with crystalloids alone. The most commonly used colloid solution is fresh frozen equine plasma, which contains albumin as the primary colloid. Albumin, while the most physiologic colloid, also has a relatively small size and will diffuse into the interstitial fluid space in which it will then exert a colloidal effect, potentially exacerbating the development of edema. Blood is another natural colloid. In addition to the colloidal effect of the albumin that it contains, blood has the benefit of providing red blood cells to improve oxygen-carrying capacity and tissue oxygen delivery. Another solution with oxygen-carrying capacity is ultrapurified polymerized bovine hemoglobin. This product has colloidal effects, but they are not the primary reason that this product would be used. Polymerized hemoglobin has been used with apparent clinical efficacy in foals and horses at doses of 2 to 5 mL/kg.[750,752] This product is not currently available in the United States, but it is available in Europe (Oxyglobin, Dechra Veterinary Products, Shrewsbury, UK).

Synthetic colloids, such as hetastarch and pentastarch, are shelf-stable and do not require collection or thawing before administration, making them very useful in the critically ill patient. The synthetic colloids are typically less expensive than frozen equine plasma and also may provide a more long-lasting colloidal effect than albumin. There are concerns that synthetic colloids, especially hetastarch, may interfere with coagulation, especially in patients with severe systemic inflammation that may already be suffering from subclinical coagulative dysfunction. New-generation hetastarch preparations have lower molecular weight and lower degrees of molar substitution and may have improved safety profiles.[864] Of even greater concern is the recent evidence from human medicine that resuscitation with hetastarch solutions may be associated with an increased risk of acute kidney injury and mortality.[865] Although there are no studies addressing these concerns, in veterinary patients it is recommended that hetastarch solutions should only be used in patients with a demonstrated need for colloid therapy, and potentially in combination with equine plasma, rather than as the primary resuscitation fluid or the sole colloid. Hetastarch solutions should not be administered in doses of more than 5 to 10 mL/kg, and cumulative dosing should be restricted to no more than 20 mL/kg.

Route of Administration

The next challenge is determining the route by which fluids will be administered to the patient. The most physiologic route is the enteral route, but it can be easy to overlook the utility of this route, especially in hospitalized patients. Generally, if the gut is working it should be used for at least whatever component of the fluid therapy is appropriate. Enteral administration will be limited in both the volume that can be delivered and the rate at which the fluid will be absorbed, rendering it of limited utility in the critically ill patient, but it may be very useful in

delivering maintenance fluid therapy. Enteral therapy may also be less expensive and more practical in the field setting when treating the less severely compromised patient. IV administration will achieve the most rapid results because it allows for the rapid administration of large amounts of fluids and is the most commonly used route of administration in critically ill patients. IV access is associated with some risks, primarily catheter-associated complications such as thrombophlebitis and air embolus aspiration. Neither subcutaneous nor intraperitoneal fluid administration is well tolerated in equine patients, and these routes of administration are rarely used.

Practical considerations related to the ability to administer fluid therapy may impact all of these decisions. IV access can be challenging to establish in the sick neonatal foal, especially in a field setting. Although over-the-stylet catheters are commonly used, they can be difficult to place in hypotensive foals and are susceptible to kinking or displacement caused by foal movement and frequent handling. These catheters are best used for short-term fluid administration in normotensive foals. The most commonly used over-the-stylet catheters are made of Teflon, which can be more irritating to the vein than other materials, but polyurethane over-the-stylet catheters also are available (MILA International, Inc., Erlanger, KY; Arrow, Teleflex Inc., Wayne, PA). Over-the-wire catheters, although slightly more complex regarding placement, are easier to use in hypotensive patients and are easier to maintain for longer periods of time. These catheters are typically made of polyurethane, which is less thrombogenic than Teflon. Silicone over-the-wire catheters are the least thrombogenic and are rarely used in foals. Over-the-wire catheters are also available in longer lengths (20 cm) than over-the-stylet catheters (13 cm), which is helpful in critically ill foals because they can be placed almost as deep as a central venous line, potentially decreasing the risk of vascular irritation associated with delivery of hypertonic solutions or irritating medications. Multiple-lumen over-the-wire catheters are available that can be helpful in critically ill patients because they allow for parenteral nutrition or medications to be delivered through a separate catheter lumen than the one used for IV fluid administration. The downside to these multilumen catheters is primarily their large outer diameter, which can make them more challenging to place and may increase the risk of catheter site or vascular complications.

Enteral administration of fluids can be achieved using a nasogastric tube, but this approach is primarily useful for administration of a few doses of fluids, after which the requirement to repeatedly pass the tube may represent a very stressful intervention. Leaving the nasogastric tube in place is also problematic because of local irritation within the pharynx and interference with voluntary drinking and eating. If repeated enteral administration is anticipated, then serious consideration should be given to the placement of an indwelling enteral feeding tube (14 Fr × 125 cm, MILA International, Inc., Erlanger, KY). The small outer diameter of these tubes allows for ongoing voluntary intake of feed or water. Having one of these tubes in place makes it very simple to administer multiple small boluses of enteral fluids or milk with minimal patient stress. Further discussion of enteral feeding can be found in the section Nutritional Support for the Foal later in this chapter.

Dosage

The clinician must also determine the volume of fluids to be administered and the duration of fluid therapy. The factors that must be considered in determining the amount of fluids to administer are the volume needed for maintenance of normal bodily function, the magnitude of the existing fluid deficit, and the ongoing losses potentially associated with the disease process (diarrhea, reflux) as well as any insensible losses (sweat). When designing the fluid therapy plan it is important to consider the maintenance fluid plan as separate from the replacement fluid plan, which includes both replacement of existing deficits and ongoing losses. The types of fluids used for these purposes are typically different, and inadvertent use of maintenance fluids for replacement, or vice versa, can be deleterious to the patient. The calculation of maintenance fluid requirements in the foal can be approached in two ways. The traditional approach is based on the accepted value of 3 to 5 mL/kg per hour (75–120 mL/kg/day) as the maintenance fluid requirement. The primary problem with this approach is that it represents a very broad range of fluid rates and may result in fluid overload if not closely monitored. A second approach to the determination of maintenance fluid requirements has been proposed, based on the Holliday–Segar formula, which provides a "drier," more conservative rate of fluid administration. The calculation is as follows: for the first 10 kg of body weight, 100 mL/kg per day of fluids are administered, then 50 mL/kg per day for each kilogram from 11 to 20 kg body weight, and then 25 mL/kg per day for each kilogram of body weight >20 kg.[190,866] In a 50-kg foal this calculation yields a maintenance rate of 2250 mL/day, or 94 mL/h, which equates to 1.9 mL/kg per hour.[866] When formulating the fluid plan do not forget to incorporate other fluid sources that may be administered to the foal, which includes any enteral feedings, as well as fluids given as drug infusions or parenteral nutrition. It is easy to overlook these additional sources, yet they may constitute a significant proportion of the daily fluid intake.

The other consideration in the design of the maintenance fluid regimen is the ongoing need for electrolytes, particularly sodium and potassium. If isotonic replacement solutions are administered for maintenance purposes, then hypernatremia and hyperchloremia are likely complications.[863] Those solutions also contain inadequate amounts of potassium for maintenance. The use of hypotonic crystalloid maintenance solutions is, therefore, more appropriate. The recommended daily rate of sodium supplementation in foals is less than 3 mEq/kg, unless they have increased renal sodium losses.[866] Administration of a maintenance solution such as Plasma-Lyte 56 at 1.9 mL/kg per hour will provide approximately 2 mEq/kg per day of sodium. It is important to remember that the foal will likely be receiving other sources of sodium in the form of drug infusions and catheter flushes and in solutions such as parenteral nutrition, plasma, or synthetic colloids. Regarding potassium, the daily maintenance requirements are quite substantial in foals that are not nursing because milk contains high levels of potassium. Supplementation of potassium at 1 to 3 mEq/kg per day is a reasonable starting point. Hypotonic maintenance solutions, such as Plasma-Lyte 56, contain substantially more potassium than replacement solutions (13 mEq/L vs. 5 mEq/L, respectively), but this is still insufficient for the neonatal foal, and supplementation will be required. The addition of potassium chloride at 20 to 40 mEq/L to a hypotonic maintenance solution is a reasonable starting point for supplementation. Care should be taken not to administer a fluid with that amount of potassium supplementation at a high rate, however, because administration of potassium at a rate greater than 0.5 mEq/kg per hour may result in cardiac complications caused by hyperkalemia.

TABLE **20.16** Clinical Assessment of Hydration Status

Degree of Dehydration	Skin Tenting	Capillary Refill Time	Mucous Membranes
<5%	1–3 sec	<2 sec	Slightly tacky
5–10%	3–5 sec	2–3 sec	Tacky
>10%	>5 sec	>3 sec	Dry

TABLE **20.17** Clinical and Physiologic Parameters Useful in the Assessment and Monitoring of Hydration Status

Parameters	Overhydrated	Ideal	Underhydrated
Heart rate	—	80–120	>140
Respiratory rate/effort	Upward trend	<56	—
Hematocrit	20	35–45	>45 (increasing)
Total protein (g/dL)	<3	5–8	>8
Urine output (mL/kg/h)	>2	1–2	<0.5
Urine specific gravity	—	1.005–1.010	>1.012
Plasma lactate (mmol/L)	—	<2	>5 (neonate) >2.5 (over 4 days)
Peripheral edema	Obvious signs	May be seen in some foals	—
Central venous pressure (cm H_2O)	>8–12	3–8	Negative to 5
BUN/creatinine	—	Normal	Increasing

BUN, Blood urea nitrogen.

Using the clinical perfusion indicators mentioned previously (mucous membrane color, capillary refill time, jugular refill time, heart rate, and pulse quality) one can derive a rough estimate of the degree of clinical dehydration (Table 20.16). This approach yields estimates of dehydration ranging from 5% to >10%, and these values can be used when calculating the estimated fluid deficit. For example, a 50-kg foal with a clinical estimate of 5% dehydration will require 2.5 L of fluids (50 kg × 0.05 = 2.5 kg of fluid) to replace their deficit. Clinicopathologic testing can aid in refining the estimate of the fluid deficit, with variables such as packed cell volume, total plasma protein concentration, urine specific gravity, BUN concentration, serum creatinine concentration, and serum lactate concentration all potentially providing useful insight (Table 20.17). The packed cell volume will be increased with hypovolemia but may show less marked elevations with dehydration. Total protein concentration will typically increase with hypovolemia and dehydration as well, but this marker may lack sensitivity in patients with hypoalbuminemia and/or hypoglobulinemia. Urine specific gravity is a fairly sensitive marker of dehydration and is very clinically useful. BUN and creatinine concentrations are indicators of hypovolemia and decreased perfusion rather than dehydration. Both BUN and creatinine are relatively insensitive markers of decreased perfusion, especially BUN, and only begin to increase with substantial decreases in GFR. Serum lactate concentration may be a more sensitive marker of perfusion but can also be elevated in response to impaired tissue oxygen utilization or decreased hepatic lactate clearance. Measurement of central venous pressure (CVP) can be very useful in assessing the foal's fluid status. This technique is somewhat technically challenging but is feasible with limited equipment. If the foal has been catheterized with an over-the-wire 20-cm catheter, then the placement of the catheter tip will be fairly central, and this catheter can be used for the determination of an approximate CVP. With the foal in lateral recumbency all ingoing fluid therapy is stopped while the measurement is taken. A water manometer or electronic pressure transducer can be used, and the zero position on the manometer should be set at approximately the height of the base of the heart. The manometer is primed with sterile heparinized saline and then opened to the IV catheter. Slight fluctuations of pressure should be observed with respirations, and the measurement taken is the approximate mean of this range.

Once a determination of the existing fluid deficit has been made, a deficit fluid replacement plan can be formulated. Use of an isotonic replacement fluid (LRS, Normosol-R, and Plasma-Lyte A) is appropriate for this purpose, as previously discussed; remember that deficit therapy is given in addition to the ongoing maintenance fluid support described earlier. Rather than simply dividing up the calculated fluid deficit and administering it over a set period of time (i.e., 2.5 L over 12 hours), it is more useful to administer boluses of replacement fluids at 20 mL/kg (e.g., 1 L to a 50-kg foal) over 10 to 30 minutes and then reassess the patient's status and determine whether further boluses are required. Some foals may require more than replacement of their calculated deficit, but care should be taken not to exceed a 60-mL/kg total dose unless there are compelling clinical reasons that this is indicated, such as ongoing losses. Excessive administration of bolus fluid therapy has been associated with worsened outcomes in human patients.[867,868] If greater than 60 to 80 mL/kg of fluid replacement is required and the patient remains hypotensive, then they should be considered nonresponsive to fluid therapy, and the initiation of inotrope and/or vasopressor therapy should be seriously considered (see the next section Inotrope and Vasopressor Therapy). Reassessment of the patient's hydration status and response to fluid therapy involves the use of all the parameters discussed earlier, with serial measurements being critical to this process.

Replacement therapy is intended to address the patient's ongoing fluid losses, and determination of the magnitude of these losses is more art than science because they are difficult to measure. In many clinical situations the foal does not have a urinary catheter in place, so assessment of renal losses is entirely empiric based on frequency of urination and perceived volume. The assessment of gastrointestinal losses is similar in nature, whereas the losses caused by sweating are extremely difficult to estimate. The fluids used for replacement of losses should be isotonic balanced replacement solutions, because the fluids lost by these routes will be accompanied by electrolyte losses as well. If foals appear to be urinating and defecating normally and are not obviously sweating, then additional replacement fluids are not indicated. In those foals with increased losses associated with these systems one can add intermittent boluses of 10 to 20 mL/kg of replacement-type fluids to address the estimated loss. Monitoring of ongoing fluid therapy is very important and requires frequent assessment to

ensure that the goals for fluid therapy in the individual patient are being achieved and to avoid the development of adverse outcomes such as fluid overload. Repeated assessment of the clinical indicators of hydration status and perfusion is readily performed and extremely valuable in monitoring fluid therapy because normalization of these parameters is evidence of therapeutic efficacy.

INOTROPE AND VASOPRESSOR THERAPY

Hemodynamic factors, such as volume depletion, low cardiac output, or inappropriate vasodilation, lead to systemic hypotension, which may result in organ hypoperfusion through reductions in perfusion pressure with potentially deleterious consequences for the gastrointestinal and renal systems.[869] Tissue perfusion can be supported by the provision of IV fluids to address hypovolemia and improve venous return to the heart, providing adequate preload to support cardiac output, and fluid repletion is always the first intervention to be used in affected foals. Although many foals suffering from hypotension will respond well to IV fluid administration, a subset of these cases will be nonresponsive to fluids and will require additional blood pressure support. Treatment with inotropes and vasopressors may be indicated in these cases to increase cardiac contractility and afterload, respectively. Administration of these agents requires that the foal be in a hospital environment with close monitoring of hemodynamic function and the availability of electronic infusion pumps that can provide accurate CRIs. Blood pressure monitoring is absolutely essential, and this can be accomplished by direct (arterial catheter) or indirect (oscillometric tail cuff) means. In most situations the indirect approach will be used because the placement and maintenance of arterial catheters in foals can be extremely challenging. Although the data obtained from direct measurement are superior, the technical challenges render this approach impractical. Indirect techniques are not ideal because they can be affected by tail cuff size and placement and can be inaccurate, but they provide acceptable accuracy for clinical use, particularly in the monitoring of trends in MAP over time.[870,871]

It can be challenging to determine the point at which a patient requires additional blood pressure support. In human patients, exceeding the critical lower level for MAP, which is regarded as 60 to 65 mm Hg, is vital to maintain cerebral, coronary, and renal blood flow.[872] A major goal in the management of critically ill foals, therefore, is to maintain the MAP above 60 mm Hg, although this level does not necessarily indicate adequate tissue perfusion.[130,873,874] There is no apparent advantage to achieving higher MAP, and doing so may actually be deleterious because of altered distribution of blood flow resulting in decreased perfusion of some tissues.[718,872] It is critical to use other indicators of tissue perfusion, including heart rate, mentation, central venous oxygen tension, urine output, acid-base status, and trends in blood lactate concentration, because it has been suggested that neonatal foals may have a different physiologic response to hypotension than adult horses.[873] When implementing inotrope or vasopressor therapy it is advised to start at the lowest end of the dosage range (Table 20.18) and to monitor the response to therapy before increasing the dosage because it is impossible to predict the response of an individual patient. The short half-life of these agents means that the response to therapy can be assessed quickly, often in as little as 10 to 15 minutes, reinforcing the need for continual patient reevaluation.

TABLE **20.18 Dosages of Inotrope and Vasopressor Agents**

Agent	Dosage Range
Dopamine	3–20 μg/kg/min
Dobutamine	1–20 μg/kg/min
Norepinephrine	0.1–2 μg/kg/min
Vasopressin	0.1–2 mU/kg/min
Epinephrine	0.2–2 μg/kg/min

Adapted from Palmer J. Update on the management of neonatal sepsis in horses. *Vet Clin North Am Equine Pract*. 2014;30:317-336, vii; Dickey EJ, McKenzie H 3rd, Johnson A, et al. Use of pressor therapy in 34 hypotensive critically ill neonatal foals. *Aust Vet J*. 2010;88:472-477; Tennent-Brown, BS, Seahorn, JL. Inotrope and vasopressor therapy. In: Southwood LL, Wilkins PA, eds. *Equine Emergency and Critical Care Medicine*. Boca Raton, FL: CRC Press; 2015:675-684.

TABLE **20.19 Target Sites of Action of Inotropes and Vasopressors**

Agent	α_1	β_1	β_2	Dopaminergic	Vasopressin-1
Dopamine	+++	++++	++	+++++	0
Dobutamine	+	+++++	+++	0	0
Norepinephrine	+++++	+++	++	0	0
Vasopressin	0	0	0	0	+++++
Epinephrine	+++++	++++	+++	0	0
Phenylephrine	+++++	0	0	0	0

0 = no significant affinity; + through +++++ = minimal to maximal affinity.
Adapted from Pollard S, Edwin SB, Alaniz C. Vasopressor and inotropic management of patients with septic shock. *P T*. 2015;40:438-450.

Dobutamine is a positive inotrope commonly used to treat hypotension in neonatal foals, and it often represents the first-line drug of choice[30,875] (Table 20.19). The β-adrenoceptor agonist increases myocardial contractility, and therefore cardiac output, via its action on β_1-receptors. Concurrent stimulation of β_2-receptors may also produce peripheral and splanchnic vasodilation. This vasodilatory effect may be unproductive because it can result in decreases in systemic vascular resistance and MAP, which means that dobutamine may not be the ideal choice as a single agent in the treatment of fluid-refractory hypotension.[876] For this reason dobutamine is normally used in combination with a vasopressor in human patients.[877] The positive benefit of the vasodilatory effect of dobutamine is that it may improve splanchnic perfusion when used concurrently with vasopressors.[876] By increasing myocardial contractility dobutamine also increases myocardial oxygen demand, which can be problematic in the patient with already impaired oxygen delivery. The other concern with dobutamine is that because of its stimulation of β_1-receptors it can cause tachycardia and arrhythmias, especially at higher doses.[877]

Dopamine was traditionally used as a first-line agent in human and veterinary medicine because it exhibits dose-dependent stimulation of a wide variety of receptors, with dopaminergic activity at low doses, β_1 and β_2 activity at moderate doses, and α_1 activity at high doses. Through the stimulation of dopaminergic receptors low-dose dopamine increases renal and splanchnic perfusion, although this has not

proven to be beneficial in preventing organ failure in human patients.[878] At higher doses dopamine increases cardiac contractility, vasoconstriction, and heart rate, all of which tend to increase MAP.[877] Unfortunately the vasopressor response to dopamine is less pronounced and less consistent than that achieved with norepinephrine.[879] When the inotropic effects of dopamine were compared with dobutamine in hypotensive human neonates, dopamine was less effective in restoring blood flow.[880] At high doses (greater than 20 μg/kg/min) dopamine can cause pulmonary venous vasoconstriction and reduced splanchnic perfusion.[30] For all of these reasons dopamine is now much less commonly used in human or equine critical care.

Norepinephrine is primarily an α-adrenergic receptor agonist, but it also has some limited β_1 and β_2 effects. Norepinephrine induces arterial and venous vasoconstriction, resulting in increases in MAP, effective circulating blood volume, and venous return and preload, with minimal increase of heart rate or stroke volume.[881] Despite historic concerns regarding potential splanchnic hypoperfusion, norepinephrine is now regarded as the first-line pressor agent in hypotensive human patients.[877] Current human guidelines recommended the administration of norepinephrine as a sole agent initially, with dobutamine added if hypoperfusion persists despite adequate MAP.[175] Norepinephrine has been reported to be effective in increasing MAP and urine output in hypotensive foals that were nonresponsive to both fluid administration and dobutamine therapy.[130] Another study compared the effects of norepinephrine alone and the combination of norepinephrine and dobutamine with a control treatment of saline infusion.[882] Both norepinephrine and norepinephrine-dobutamine increased arterial blood pressure and systemic vascular resistance and decreased heart rate and cardiac index compared with saline, but no effect was observed on renal function. Norepinephrine-dobutamine did result in higher arterial pressures than norepinephrine alone. This combination therapy approach is now commonly used for the treatment of foals with refractory hypotension.

In recent years in human medicine, AVP (antidiuretic hormone) has gained popularity in managing vasodilatory shock and cardiac arrest despite concerns regarding potential splanchnic hypoperfusion at higher dosages.[883] This concern regarding splanchnic hypoperfusion deserves consideration because this effect was observed at high doses of vasopressin in a study that examined the effects of dobutamine, norepinephrine, and vasopressin in healthy anesthetized foals with induced hypotension.[875] Although vasopressin has a myriad of effects involving numerous receptors and pathways, it controls blood pressure primarily by vasoconstriction via its actions on the vasopressin-1 receptor.[884] Vasopressin has stronger vasoconstrictive effects on large arterioles than norepinephrine, which may explain its effectiveness even in patients refractory to standard catecholamine therapy.[885] Vasopressin also has been shown to have a positive interaction with hydrocortisone therapy in the treatment of refractory shock in humans.[175] When used as a treatment for vasodilatory shock vasopressin has been shown to decrease heart rate, improve overall hemodynamics, and lead to a reduction in inotrope requirement.[886] A recent human meta-analysis determined that vasopressin therapy in human patients in septic shock was safe, useful in weaning patients off of catecholamines, and associated with decreased mortality.[887] The consensus in human medicine appears to be that vasopressin is a reasonable second-line choice as a vasopressor in patients not exhibiting a positive response to norepinephrine therapy.[877]

There are a few anecdotal reports of the use of vasopressin as a second-line vasopressor in equine critical care, with one reference, by Collins et al., indicating that vasopressin has begun to be used as the first-line therapy for foals with fluid-refractory hypotension.[30,718,888] At this time there is only one published report regarding vasopressin therapy in clinically ill foals.[889] In that report the effects of norepinephrine or vasopressin on cardiovascular responses and fluid balance were compared in a group of 34 foals with hypotension refractory to both fluid therapy and dobutamine. Eighteen foals were treated with vasopressin, whereas 16 foals received norepinephrine. Six of the foals in the vasopressin group had failed to respond to norepinephrine therapy, which was withdrawn before initiation of vasopressin therapy. The severity of illness was pronounced and similar between groups, with a mean lactate of 10.5 ± 4.4 mmol/L in the vasopressin group and 9.6 ± 4.6 mmol/L in the norepinephrine group. The two groups also had similar sepsis scores at admission, with a mean of 15.9 ± 6.1 in the vasopressin group and 16.1 ± 4.5 in the norepinephrine group. Vasopressin administration was associated with a significant increase in MAP and in urine output as well as a significant decrease in heart rate, whereas norepinephrine administration was associated with a significant increase in MAP. The overall survival rate in this study was only 38%, likely reflecting the severe systemic disease present in this population of foals evidenced by their admission sepsis scores and lactate concentrations, and was consistent with a previous report from the same institution, in which the survival rate for foals presenting with lactate concentrations greater than 6 mmol/L was reported to be only 40%.[126] The authors of this report also describe increased use of vasopressin as a first-line therapy for foals with refractory hypotension.[889]

Other pressor agents include epinephrine and phenylephrine, both of which are strong adrenergic receptor agonists and can have negative effects on splanchnic and renal perfusion.[890] Epinephrine is a catecholamine with potent activity at β-adrenergic and α-adrenergic receptors. Epinephrine increases MAP by increasing cardiac output and vascular tone, and in human patients it is regarded as the first alternative to the combination of norepinephrine and dobutamine.[877] However, epinephrine has been associated with hyperglycemia, hypokalemia, lipolysis, tachycardia, decreased splanchnic perfusion, increased lactate concentration, and increased platelet aggregation.[30,877] Phenylephrine has potent α_1 activity but has no cardiac effects, mediating its effects through the constriction of the peripheral arterial vasculature.[877] Phenylephrine is primarily of utility in patients with arrhythmias resulting from dobutamine and/or norepinephrine administration and has little application in equine neonatal critical care.

Although not a vasopressor per se, low-dose hydrocortisone therapy has been shown to improve the response to vasopressors and shorten the duration of vasopressor therapy in human shock patients.[891,892] The mechanisms responsible for this effect are unclear, but do not appear to be associated with primary corticosteroid insufficiency. Rather, it appears that corticosteroid therapy directly results in improved vasopressor responsiveness of peripheral vessels, because hydrocortisone raises blood volume, increases vascular tone, and enhances endothelial reactivity to vasopressors.[893] A thorough discussion of hydrocortisone replacement therapy can be found in the previous section Endocrine Disorders.

ANTIINFLAMMATORY AND ANALGESIC THERAPY

The indications for antiinflammatory therapy in the foal are numerous. Most often these compounds are used to regulate fever and local inflammation, reducing patient discomfort, but there are situations in which they are required for the control of systemic inflammation as well. The primary class of antiinflammatories used in foals is NSAIDs, which consists of drugs inhibiting arachidonic acid synthesis via COX inhibition. These drugs typically exhibit antipyretic, antiinflammatory, and analgesic effects.[894] There are also several drugs in the NSAID group that have effects on pathways other than arachidonic acid metabolism. The second class of antiinflammatory drugs is the corticosteroids (steroidal antiinflammatory drugs [SAIDs]). These drugs have potent dose-dependent antiinflammatory and immunosuppressive effects, but typically have little antipyretic or analgesic activity.[665] In situations where the primary goal is analgesia one can use NSAIDs, but opioid drugs provide analgesia without the concerns regarding gastrointestinal and renal toxicity associated with many NSAIDs. Although not commonly used in very young foals, alternative analgesics such as ketamine and lidocaine may be beneficial in managing older foals with severe, refractory pain.

The NSAIDs include nonspecific COX inhibitors such as phenylbutazone and flunixin meglumine, more selective COX-2 inhibitors such as ketoprofen, and COX-2–specific drugs like meloxicam and firocoxib. Nonspecific COX inhibition can be associated with side effects including gastrointestinal ulceration and renal injury.[895] Phenylbutazone has been associated experimentally and clinically with toxicity in foals[895,896] and appears to have a narrow therapeutic index in this population. In addition, it can be difficult to administer appropriate dosages of phenylbutazone to foals because of their small size and the composition of the forms of phenylbutazone that are available. For all of these reasons phenylbutazone is rarely used in foals. Flunixin meglumine, however, when given parenterally at appropriate dosages, has been demonstrated to be safe in foals, even when administered for several weeks,[897,898] and this is supported by clinical experience. Flunixin meglumine is primarily used in foals with severe systemic inflammation, severe discomfort, or persistent fevers. It is important to note that the metabolism and elimination of NSAIDs can be impaired in neonatal foals, potentially requiring increases in dosing or prolongation of the dosing interval.[899-901] For these reasons it is recommended that the nonspecific COX inhibitors be used with caution in the clinically ill neonatal foal, particularly in foals with severe systemic inflammation, hypovolemia, or the potential for gastric ulceration.

In theory, drugs like ketoprofen and carprofen should be safer for use in foals because they are more COX-2 selective than phenylbutazone and flunixin. Although there is no peer-reviewed evidence to support the claim of reduced toxicity, both of these drugs have been used in foals with apparent safety.[902,903] The pharmacokinetics of ketoprofen has been studied in foals, and the dosage and treatment intervals recommended in that report are both greater than those for adult horses[899] (Table 20.20). Ibuprofen has also been studied in foals and was found to be safe when administered for up to 6 days.[904] There are no published reports of the pharmacokinetics of carprofen in foals.

The mostly highly COX-2–specific NSAIDs that have been studied in horses and foals are meloxicam and firocoxib, with firocoxib demonstrating the greatest degree of COX-2 specificity. Firocoxib has favorable pharmacokinetics and is reported to be safe in foals when administered by the oral or IV routes.[905,906] Firocoxib has been demonstrated to be effective for musculoskeletal and visceral pain in horses,[907,908] but the analgesic efficacy of firocoxib has not been assessed in foals. Anecdotally it seems that firocoxib may be more effective for musculoskeletal pain than for visceral pain, but this has not been scientifically evaluated. Meloxicam is marketed for equine use in Europe but is currently only labeled for small-animal use in the United States. Meloxicam pharmacokinetics and safety have been evaluated in foals,[909] and no evidence of gastrointestinal or renal toxicity was detected. Interestingly, meloxicam was eliminated more rapidly in foals than in adult horses, which is quite different from what has been found with other NSAIDs. For that reason the authors of that study recommended that meloxicam be dosed every 12 hours in foals, rather than 24 hours.[909]

In addition to the COX inhibitors there are several antiinflammatory drugs that have been used in foals and may have some application. Dipyrone (metamizole) has been widely used in horses as an analgesic, antipyretic, and antispasmodic drug and clinically appears to be safe when used as a single dose in foals with abdominal pain. Acetaminophen has been used very little in horses because of concerns about the potential for hepatotoxicity, but recent reports suggest that this drug may be both safe and effective as an analgesic.[910] Although dipyrone and acetaminophen have some COX inhibitory effects, that pathway is not considered to be primarily responsible for the drugs' therapeutic effects.[911,912] The current hypothesis is that these drugs act primarily through other pathways, including the serotonergic, opioid, and cannabinoid systems.[913,914] Dipyrone or acetaminophen are sometimes combined with more traditional NSAIDs to achieve enhanced analgesic effects,[915] but there are limited reports regarding this approach in the horse.[912]

In addition to the NSAIDs there are other drugs that may exhibit antiinflammatory effects, such as pentoxifylline and DMSO. Pentoxifylline is a phosphodiesterase inhibitor that has been shown to have wide-ranging antiinflammatory effects and is frequently used in adult horses suffering from severe systemic inflammation.[916] Although the pharmacokinetics of pentoxifylline are not known in foals, it has been used in foals at adult dosages.[185] DMSO is a free radical scavenger that is widely used in horses and foals suffering from severe localized or systemic inflammation, despite a relative lack of evidence of efficacy.[917] When administered intravenously at an appropriate dilution DMSO appears to be safe and is frequently administered to foals suspected to be suffering from NE.[185] Although not a traditional antiinflammatory, the antimediator polymyxin B is sometimes used in foals with SIRS because it has the capability of binding circulating endotoxin and inhibiting the upregulation of the inflammatory response.[917,918] A recent study performed in healthy foals with experimentally induced endotoxemia found that administration of polymyxin B at 6000 U/kg IV every 8 hours attenuated some of the clinical and clinicopathologic effects of endotoxemia.[919] Polymyxin B is potentially nephrotoxic, however, so caution is indicated when considering the utilization of this therapy in the critically ill, hypovolemic foal.

The administration of SAIDs to foals is not frequently indicated and should be restricted to clinical syndromes in

TABLE **20.20 Dosages of Antiinflammatory and Analgesic Medications Used in Foals**

Drug	Dosage	Route	Frequency (h)
Aspirin	10–100 mg/kg	PO	24
Butorphanol	0.1 mg/kg (up to 8 weeks of age)	IV, IM	PRN
Carprofen	0.7 mg/kg (adults)	PO	24
Dexamethasone	0.01–0.02 mg/kg 0.05–0.2 mg/kg	IM, IV, PO	24–48
Dimethyl sulfoxide	0.5–1.0 mg/kg as a 10% solution over 30–60 min	IV	12–24
Firocoxib	0.1 mg/kg 0.09 mg/kg	PO IV	24
Flunixin	0.25–0.5 mg/kg 1.0 mg/kg	IV, PO	8 12–24
Hydrocortisone	0.17–0.67 mg/kg (total dose 1–4 mg/kg/day)	IV	4–6
Ibuprofen	25 mg/kg	PO	12
Ketamine	0.4–1.2 mg/kg/h	IV	CRI
Ketoprofen	2.2 mg/kg 3.3 mg/kg (<24 h)	IV	24
Lidocaine	1.3 mg/kg loading 0.05 mg/kg/min CRI	IV	CRI
Meloxicam	0.6 mg/kg	PO	12
Methylprednisolone sodium succinate	1 mg/kg	IM, IV (slow)	12–24
Phenylbutazone	1.1–2.2 mg/kg	IV, PO	12–24
Polymyxin B	6000 U/kg in saline	IV	8
Prednisolone sodium succinate	0.25–2.5 mg/kg 0.8–5.0 mg/kg	IV	6, PRN
Prednisolone	1–2.2 mg/kg	PO	12–24
Pentoxifylline	10 mg/kg (adults)	PO	12
Tramadol	3 mg/kg	IV	Not defined

CRI, Continuous rate infusion; *IM,* intramuscularly; *IV,* intravenously; *PO,* orally; *PRN,* as needed.

which overwhelming localized or systemic inflammation must be controlled. In foals with interstitial pneumonia, ALI, or ARDS, SAID therapy is critical in the downregulation of pulmonary inflammation and restoration of pulmonary function and represents the primary pharmacologic therapy for these conditions.[324] This syndrome is discussed in detail in the previous section Respiratory Disorders. Severely ill foals can suffer from CIRCI, which is associated with poor clinical outcomes.[667] This syndrome is discussed in detail in the previous section Immunologic Disorders. Cortisol replacement therapy using small doses of hydrocortisone administered frequently may provide antiinflammatory and physiologic benefits in CIRCI-affected foals and does not appear to interfere with the functioning of the innate immune system.[313,315]

If the primary goal of therapy is analgesia independent of antiinflammatory effects there are a number of alternative therapies available. For short-term analgesia the α_2-adrenergic agonists, such as xylazine and detomidine, are commonly used because they are very effective in controlling severe pain. The limitations of the α_2 drugs are profound sedation and decreases in gastrointestinal motility.[920] Alternatively, one could consider the use of an opioid agonist or agonist/antagonist. Butorphanol, an opioid agonist/antagonist, is the most commonly used drug because it is readily available and safe to administer. The limitations of butorphanol are primarily that it causes substantial sedation and only lasts for a short period of time when given as a bolus. Administering butorphanol as a CRI allows for a much more stable analgesic effect and may lessen the sedative effects. Opioid agonists, such as tramadol and fentanyl, are rarely used in foals but could have utility in managing chronic, severe musculoskeletal pain.[921,922] Lidocaine is a local anesthetic, but when administered systemically as a CRI it can provide effective analgesia, especially for visceral pain. It is not associated with sedative effects but can cause neurologic signs if given at an excessive rate. In a recent study lidocaine administration to foals achieved lower systemic concentrations than in adult horses but appeared to be safe and clinically effective.[923] Ketamine is a dissociative agent most commonly used for induction and/or maintenance of general anesthesia, but it has been reported to have analgesic effects when administered at lower doses as a CRI.[920]

NUTRITIONAL SUPPORT OF THE FOAL

Developing an appropriate nutritional plan when treating a sick foal can be challenging because one must ensure that the foal has adequate energy and nutrients for basal metabolism and immune function and ideally for growth as well. This should be a straightforward process in which the number of calories required by the foal is calculated, followed by a determination of the volume of the appropriate nutrient-containing solution and the administration of this nutritional source by

the appropriate route. Unfortunately the energy requirements for sick foals are not well understood and will vary among patients. Additionally, the ability of the foal to appropriately metabolize the nutrients provided is not guaranteed because both age and degree of illness impact the foal's ability to produce metabolic hormones as well as the ability of the tissues to respond to hormonal stimulation. Another difficulty arises because the energy content of mare's milk cannot be easily determined, and the exact formulation of substitutes such as artificial milk replacer is not always known. Ultimately the burden falls on the clinician to formulate the best plan possible with the information at hand and to ensure that the foal is closely monitored. This will ensure that the goals of the nutritional plan are met while the risk of complications is minimized.

The foal's nutritional requirements and dietary composition change substantially during the transition from neonate to weanling, requiring careful consideration of the foal's stage of growth when formulating a nutritional plan. At birth the foal transitions from a continuous supply of nutrients provided by the dam via the placenta to intermittent absorption of ingested nutrients. At the same time the metabolism of the neonate is no longer able to depend on the maternal glucose concentration to maintain normoglycemia, and the pancreas assumes responsibility for regulating glucose homeostasis. These dramatic alterations in energy metabolism do not always occur smoothly, and the foal possesses limited energy reserves in the form of glycogen and fat. The result is that hypoglycemia occurs frequently in even the normal neonatal foal, and clinically ill foals are at risk of profound hypoglycemia if deprived of energy intake for even a few hours.

The caloric requirements of the normal foal are sizable; they need to support not only their high basal metabolic needs but also maintain a rate of growth of as much as 2.5% of body weight per day in the neonatal period. This means that the neonates caloric requirement is as great as 150 kilocalories per kg body weight per day (kcal/kg/day) but decreases gradually to around 120 kcal/kg per day at 3 weeks of age and then to 80 to 100 kcal/kg per day by 1 to 2 months of age.[924,925] A more recent study has suggested that actual energy requirements of foals from 2 to 6 months of age may be 10% to 20% less than these values.[926] Because these measurements are given in terms of foal body weight, it is important to realize that as the energy requirement per kilogram is decreasing the foal's body weight is increasing; thus, the total caloric requirement increases with age. The initial energy source for the foal is mare's milk, which has substantially greater lactose content than cow's milk, with lower milk fat content. On a dry matter basis, mare's milk averages about 64%, 22%, and 13% sugar, protein, and fat, respectively, compared with 38%, 26%, and 30% sugar, protein, and fat, respectively, for cow's milk. Mare's milk therefore derives most of its energy content from carbohydrates, and an appropriate endogenous production of insulin by the pancreatic β cells is required for the foal to metabolize and utilize these carbohydrates appropriately.

Maturation of pancreatic β-cell function occurs very late in gestation in the fetal foal and is dependent on the normal rise in fetal circulating cortisol concentration, which occurs in the final days of gestation.[927] This preparturient cortisol rise is critical for many aspects of readying the foal for birth, both in terms of endocrine function as well as respiratory and cardiovascular function. Following birth there is a gradual maturation of the endocrine response to ingested carbohydrates. It has been shown that normal newborn pony foals demonstrated impaired glucose clearance following the administration of exogenous glucose on the first day of life, suggesting a degree of insulin resistance.[928] By 10 days of age foals demonstrate increased rates of glucose clearance, but this response remains lower than that seen in normal adult equines. This gradual maturation may be an appropriate response to changes in the composition of mare's milk, because colostrum contains little lactose, and in the volume of milk ingested, which is less on the first day of life than on subsequent days.[924,928]

Starting as early as the second day of life foals will begin ingesting small amounts of hay, grass, and grain while at the same time they are ingesting maternal feces, which likely provides the initial microbial flora required to support digestion of these feeds. It is unlikely that grain and roughage are thoroughly digested until several weeks of age, at which point the foal begins the gradual transition from a milk-based diet to a forage-based diet. The amount of milk produced by the mare peaks at around 2 months of lactation and then begins a steady decline, which continues until the time of weaning, necessitating that the foal begin relying on ingestion of solid food for an increasing proportion of its nutritional requirements. At the same time, the foal's hindgut function is increasing and is likely fully functional by around 3 to 4 months of age. By the age of 6 months the foal is receiving less than 30% of the total nutritional requirement in the form of milk, which allows for a fairly easy dietary transition when weaning occurs. As the foal's hindgut function increases, there is a corollary shift in the primary energy substrate from carbohydrates absorbed in the small intestine to volatile fatty acids absorbed from the large intestine.

When evaluating the sick neonatal foal one must always remember that the disease processes at work in the foal may have had their origins in utero. Maternal illness, maternal malnutrition, maternal toxin exposure, placentitis, and placental insufficiency all have the potential to profoundly influence the development and maturation of fetal metabolism. Studies investigating the role of a "restricted" uterine environment on fetal development have demonstrated lifelong impairment of growth and development in affected foals.[929] Conversely, the provision of a "luxurious" in utero environment can lead to enhanced growth rates out to 3 years of age.[929] The influence of maternal diet is important, because a diet high in soluble carbohydrates fed to the mare in late gestation contributed to a decrease in insulin sensitivity of the foals at 160 days of age.[930] It is clear that there is a potential for lifelong effects on metabolic function secondary to this "prenatal programming" effect, potentially contributing to the development of metabolic disease later in life.[931] This prenatal programming may affect the neonatal foal's ability to appropriately metabolize nutrients in the clinical setting, with foals from a compromised placental environment potentially exhibiting insulin resistance and carbohydrate intolerance.

As previously discussed the late gestation rise in fetal cortisol is critical in the final maturation of energy metabolism, and many foals delivered prematurely fail to undergo this rise in fetal cortisol concentration; therefore, they are unable to respond normally to the changes in metabolism that occur after birth. Hypoglycemia, complicated by decreased endogenous energy reserves and the impairment of nursing caused by concurrent weakness, depression, and/or difficulty standing, is a common problem in these foals. Following the successful delivery of nutrients by the enteral or parenteral routes

these foals are likely to be intolerant of carbohydrates, which is caused by impaired endogenous insulin production, and they may suffer profound hyperglycemia. Foals suffering from systemic inflammation, such as that associated with septicemia, may also exhibit hyperglycemia caused by insulin resistance and carbohydrate intolerance. Management of these foals may require lipid-containing parenteral nutrition solutions and/or the administration of exogenous insulin to achieve adequate caloric input.

Attempting to determine the true caloric needs of the clinically ill foal is one of the greatest challenges in designing a nutritional strategy. Historically it was believed that critical illness created a "hypermetabolic" situation in which the patient had increased energy needs caused by increased tissue energy consumption. The energy requirements of the sick foal do not appear to be as great as once was thought,[932] however, because there is a reduction in the overall metabolic rate from a decrease in activity level in combination with a temporary reduction in growth rate. Indirect calorimetry testing of clinically ill neonatal foals has shown that their resting energy requirement is only 45 to 50 kcal/kg per day, which is one third of the energy requirement for growing, active, normal foals.[933,934] As these foals recover, their energy requirements gradually increase to approximately 65 to 70 kcal/kg per day, which is similar to the energy requirements of age-matched control foals.[934]

When managing the critically ill neonatal foal it may be preferable to pursue a hypocaloric approach in which one endeavors to prevent the foal from entering a severely catabolic state while accepting that all of the nutritional needs of the patient may not be met.[935,936] This approach addresses the fact that aggressive nutritional support can result in overfeeding, the risks of which may easily outweigh the possible benefits of providing nutritional support. Excessive carbohydrate administration will lead to an increased generation of carbon dioxide and can worsen hypercapnia in foals with compromised respiratory function. Excessive carbohydrate delivery will also cause hyperglycemia, which is considered to be a proinflammatory stimulus and has been associated with worsening of outcome in human critical illness.[937,938] Overfeeding of protein will result in increased protein catabolism and can result in the potentiation and/or development of azotemia.[938] The excessive administration of lipids may result in hypertriglyceridemia.[719] In contrast to the risks of overfeeding, there is little evidence in human patients that short-term (several days) hypocaloric nutritional support results in worsened outcomes compared with regimens designed to meet the patient's metabolic needs.[935,936] There is some evidence to suggest that this approach, especially regarding the maintenance of appropriate control of blood glucose levels, is associated with decreased rates of complications and improved outcomes.[937]

The first step in the development of a nutritional plan involves selection of the route of nutrient delivery. Providing nutritional support by the enteral route is generally preferred for two reasons. First, this is the most natural and physiologically sound means of nutrient delivery. Second, the intestinal mucosa is partially dependent on the products of digestion for energy and nutrients. A thorough evaluation of gastrointestinal function is needed before the institution of enteral nutritional support. This will include abdominal auscultation, checking for gastric reflux, and possibly abdominal radiographs and ultrasonographic examination for the evaluation of bowel dimensions and motility. Foals with evidence of gastrointestinal dysfunction, such as gastric reflux, bowel distention, increased bowel wall thickness, and ileus, are unlikely to tolerate enteral feeding. A conservative approach to enteral feeding is also indicated for premature or immature foals in which there may be incomplete development of the gastrointestinal tract. Foals with perinatal asphyxia syndrome may be intolerant of enteral feeding caused by ileus and dysfunction as a result of intestinal ischemic injury.

Mare's milk is the preferred substrate for enteral feeding. It is highly digestible and obviously provides the correct balance of nutrients for normal growth and development. Commercial mare's milk replacers can be used, but it should be recognized that these products are bovine in origin and have lower digestibility compared with mare's milk. This increases the risk of intestinal dysfunction associated with enteral feeding. Semiskimmed (2% fat) cow's milk to which 20 g/L dextrose (equivalent to 40 mL of 50% dextrose solution per liter) has been added can be used if mare's milk or mare's milk replacer is unavailable. Foals that are unable to nurse the mare may be fed through a bottle, bowl, or nasogastric feeding tube. Many sick, recumbent foals have a weak and/or uncoordinated suckle reflex, and therefore, milk should be administered through a feeding tube.

Use of a small-bore, indwelling tube and feeding of small volumes at frequent intervals (e.g., every 20 minutes) is preferred over repeated passage of a nasogastric tube at 1- to 2-hour intervals. Large-bolus feedings may overwhelm digestive capacity, and repeated passage of a stomach tube is an unnecessary stress on the foal. Another advantage of small-bore indwelling tubes is that they do not interfere with voluntary feed and water intake. Therefore the tube may be left in place as the foal is transitioned to feeding from the mare. The feeding tube should be inserted with the foal in sternal recumbency, and correct placement within the esophagus should be confirmed by radiography or endoscopy. The tube may be fastened to the external nares by sutures. At each feeding, it is important to check that the tube is still in place and that there is no reflux. The foal should be in sternal recumbency or standing when it is fed. Milk should be administered by gravity flow followed by a small amount of clean water to flush the tube. The tube should be capped between feedings to prevent aspiration of air. Feeding tubes should be replaced every 1 to 2 days to reduce risk of gastrointestinal tract infection.

A suggested initial rate of milk delivery is 2 to 3 mL/kg body weight per hour, or 100 to 150 mL/h for a 50-kg foal (Table 20.21). This will provide 2.4 to 3.6 L of milk to a 50-kg foal during the first 24 hours of enteral support. Dextrose-containing fluids can be administered IV to provide additional calories during the transition to an adequate level of enteral feeding. The feeding rate can be gradually increased over the next 2 to 3 days (e.g., increase to 4–5 mL/kg/h on day 1 and then to 6–8 mL/kg/h on day 3), which represents a total daily intake of 10% to 15% of body weight. Simultaneously, IV caloric support (dextrose) can be gradually withdrawn. This feeding level will likely meet the resting energy requirements of hospitalized foals. Depending on the rate of clinical improvement and the length of hospitalization, it may be possible to increase the volume of feeding to 20% to 22% body weight per day, which approximates the milk intake of healthy neonatal foals. Clinical monitoring should include frequent assessments of gastrointestinal function, including gastric reflux, intestinal sounds, abdominal distention, and quantity and quality of feces. Gastric reflux, bloating, colic, diarrhea,

TABLE **20.21 Feeding Recommendations for Neonatal and Growing Foals**

Foal Age (Days)	Energy Requirement	Volume of Mare's Milk or Milk Replacer	Percentage of Body Weight Fed
0–1	50–150 kcal/kg/day	2–3 mL/kg/h	5–7%
2–3	100–150 kcal/kg/day	4–5 mL/kg/h	10–12%
4–7	150 kcal/kg/day	6–8 mL/kg/h	14–20%
8–30	120 kcal/kg/day	9–10 mL/kg/h	22%
30 to weaning	80–100 kcal/kg/day	Gradually decreasing and replaced with solid feed	—

or constipation can indicate intolerance to enteral feeding and the need for adjustments to the feeding program. This may involve a decrease in the volume or frequency of enteral feedings. In some situations there may be concerns regarding the ability of the foal's gastrointestinal tract to digest and absorb lactose, as in rotaviral infections during which production of lactase by the enterocytes is likely impaired. In these situations lactase enzyme can be added to the milk or milk replacer before feeding at the rate of 9000 U (one tablet) per feeding. Additional volumes of enteral fluid may be required in foals with diarrhea, and a simple balanced isotonic enteral solution can be formulated by adding 5.6 g of table salt (NaCl), 0.6 g of Lite Salt (50% NaCl, 50% KCl), and 3.4 g of baking soda ($NaHCO_3$) per liter of water.[939]

It is generally preferred to support foals via the enteral route,[940,941] both because this is the most natural and physiologically desirable route and because the epithelial cells lining the intestine are partially dependent on the products of digestion for energy and nutrients. Unfortunately there are a variety of situations in which a foal may be unable to receive enteral nutrition or is unable to tolerate the volume of enteral nutrition required to support basal metabolism and growth. These range from the critically ill neonate with gastrointestinal complications to the suckling foal with severe enterocolitis. The rapid institution of parenteral nutrition can aid in preventing the development of protein/calorie malnutrition and substantial energy deficits. The limitations of parenteral nutritional support are primarily caused by the expense of this therapy and the risk of secondary complications. These complications may include hyperglycemia, hypertriglyceridemia, thrombophlebitis, and an increased risk of bloodstream infections.

The primary goal of parenteral nutrition, as with any type of nutritional support, is to ensure that the patient is supplied with adequate calories to support basal metabolism at a minimum and ideally to provide additional support to allow for ongoing growth. A reasonable initial goal for parenteral nutrition administration in the foal is 30 to 40 kcal/kg per day. Although this level of caloric support does not fully meet the theoretic energy requirements of the healthy neonate, it comes close to meeting the resting energy requirement in hospitalized foals.[924,933] In this situation parenteral nutrition is used purely as a temporary support to prevent the foal from entering into a severely catabolic state, in which protein catabolism would increase and use amino acids for energy production.[720,924,942] Failure to provide adequate nutritional support may also have a substantial negative influence on the immune response.[942-944] Short-term parenteral supplementation (less than 24 hours) may consist of IV carbohydrate solutions and does not require the patient to receive a balanced nutritional source consisting of carbohydrates, amino acids, and lipids, but if parenteral nutrition is expected to be administered for a longer period then a more complete formula should be used.

Carbohydrate-containing solutions represent the simplest means of providing IV caloric support to foals. A solution containing 5% dextrose can be used, and there are several options available including D5W, LRS with 5% dextrose, 0.45% saline with 5% dextrose, Normosol-M with 5% dextrose (Hospira, Lake Forest, IL), and Plasma-Lyte 56 with 5% dextrose (Baxter Healthcare Corp., Deerfield, IL). Fluids containing dextrose should not be used for initial fluid resuscitation because this will almost certainly result in the delivery of excessive amounts of dextrose to a foal with any degree of dehydration, resulting in profound hyperglycemia. Following initial fluid resuscitation, solutions containing electrolytes as well as dextrose (0.45% saline with 5% dextrose, Normosol-M with 5% dextrose, and Plasma-Lyte 56 with 5% dextrose) may be used as the primary fluids for maintenance therapy in foals with minimal ongoing fluid losses. D5W is not an ideal choice as a maintenance solution because of the absence of electrolytes and is primarily useful in providing free water to patients suffering from hyperosmolar conditions. The caloric content of a 5% dextrose solution is 0.17 kcal/mL, so an infusion rate of 10 mL/kg per hour would be required to deliver approximately 40 kcal/kg per day (0.17 kcal/kg/h × 24 h/day = 41 kcal/kg/day). This rate of infusion is over twice that considered to be a maintenance rate for a neonatal foal. In addition, care must always be taken when adjusting the infusion rates of 5% dextrose-containing solutions in response to changes in the patient's fluid status to ensure that excessive amounts of dextrose are not infused, especially in premature or very sick foals that are likely to be poorly tolerant of dextrose infusions.

Alternatively, a 50% dextrose solution can be delivered without further dilution using an infusion pump, as long as additional isotonic fluids are being administered concurrently to provide dilution and avoid endothelial injury caused by the hypertonic nature of this solution. Use of 50% dextrose solution should be avoided if an infusion pump is not available because it is very easy to inadvertently administer an excessive amount of dextrose, leading to hyperglycemia. The caloric content of 50% dextrose solution is 1.7 kcal/mL, so an infusion rate of 1 mL/kg per hour of this solution will deliver approximately 40 kcal/kg per day (1.7 kcal/kg/h × 24 h/day = 41 kcal/kg/day). This low rate of infusion means that the primary fluid needs of the patient can be met with a dextrose-free isotonic electrolyte-containing fluid, the infusion rate of which can be altered in response to changes in patient fluid status without concerns related to the requirements of the nutritional plan. At the end of the first 24 hours of treatment the fluid therapy plan and nutritional plan should be revisited to determine whether the patient can begin to rely on enteral fluid and nutritional intake or if continued parenteral therapy is required. Because dextrose-containing fluids are a very incomplete nutritional source, they should not be used as the primary nutritional source for more than 24 hours. Continued

parenteral nutritional support will require the formulation of a more complete solution that provides amino acids and possibly lipids.

One important aspect of providing longer term (over 24 hours) parenteral nutrition to foals is the inclusion of a protein source. The metabolic response to injury and sepsis is to increase protein degradation in muscle tissue. This catabolic response can be reduced by supplying a source of nitrogen or by increasing energy intake. The recommended ratio for nonprotein calories to nitrogen is 100 to 200 nonprotein calories per gram of nitrogen.[945] The inclusion of lipids in the parenteral nutrition formulation allows for the provision of a larger number of calories per unit volume compared with solutions containing only dextrose. Another advantage of lipid emulsions is that they are isotonic, so they moderate the hypertonicity of the parenteral nutrition formulation and potentially decrease the risk of thrombophlebitis. Unfortunately, formulating parenteral nutrition solutions with lipids increases the cost of the solution and may increase the risk of complications.[946] Hyperlipidemia can occur in association with lipid administration to foals but does not appear to result in adverse effects.[719,946] Lipid emulsions are prone to contamination and promote bacterial growth. Because of these risks the IV lines through which lipid-containing solutions are administered should be changed daily, substantially increasing client costs. In a recent report the use of lipid-containing parenteral nutrition solutions allowed for the provision of 40 to 92 kcal/kg per day (mean = 63 kcal/kg/day) to foals, as opposed to only 25 to 66 kcal/kg per day (mean = 41 kcal/kg/day) with a dextrose-based solution.[719]

There are two basic approaches to the formulation of parenteral nutrition to foals. The first approach involves the exact determination of the anticipated metabolic needs of the patient, followed by the development of a formulation that will meet all of these needs in a fairly precise manner, using a mixture of dextrose, amino acids, and lipids. This approach is fairly complex and is best performed using a computerized spreadsheet to aid in performing the various calculations. The second approach is more practical and consists of using two basic parenteral nutrition formulas (Table 20.22). One of these solutions is intended for short-term use and consists only of 50% dextrose and 8.5% amino acid solutions (Solution I). The second solution incorporates a lipid energy source and is preferred for long-term administration or for administration to foals that are poorly tolerant of infused dextrose (Solution II). Solution I is formulated using 2000 mL of 50% dextrose (Dextrose 50%, Baxter Healthcare Corp., Clintec Nutrition Division, Deerfield, IL) and 2000 mL of 8.5% amino acids (Travasol 8.5%, Baxter Healthcare Corp., Clintec Nutrition Division, Deerfield, IL), whereas Solution II is formulated with 1500 mL of 50% dextrose, 500 mL of 20% lipids (Intralipid 20%, Baxter Healthcare Corp., Clintec Nutrition Division, Deerfield, IL), and 2000 mL of 8.5% amino acids.[719] The caloric density of these solutions is 1.02 kcal/mL for Solution I and 1.08 kcal/mL for Solution II. The ratio of nonprotein calories to nitrogen is 125 nonprotein calories per gram of nitrogen (NPC/gN) for Solution I and 131 NPC/gN for Solution II. An easy-to-use, shelf-stable multichamber bag preparation identical to Solution I is manufactured for human use, and it is cost-effective and practical for use in foals (Clinimix 4.25/25 sulfite-free [4.25% amino acid in 25% dextrose] injection, Baxter Healthcare Corp., Clintec Nutrition Division, Deerfield, IL).

TABLE **20.22 Formulation of Parenteral Nutrition Solutions**

Formula	Composition	Caloric Density (kcal/mL)	Nonprotein Calories/gN
1	1500 mL 50% dextrose, 1500 mL 8.5% amino acids	1.02	125
2	1500 mL 50% dextrose, 500 mL 20% lipids, 2000 mL 8.5% amino acids	1.08	131

An electronic infusion pump should always be used when administering parenteral nutrition solutions, because the rate must be tightly controlled and adjustments to the infusion rate must be made easily and accurately. Excessive rates of administration can easily induce profound hyperglycemia, which has been shown in other species to be associated with severe complications and increased risk of death.[947] The solutions used for parenteral nutrition are all hypertonic and can cause injury to the vascular endothelium, increasing the risk of thrombophlebitis. For this reason it is recommended that parenteral nutrition solutions be administered through a 20-cm-long polyurethane long-term catheter placed in the jugular vein, because this provides a "central" line in most foals. The use of a multiple-lumen catheter allows for one lumen to be dedicated to infusion of the parenteral nutrition solution, minimizing the risks of contamination. Catheter management is extremely important when foals are receiving parenteral nutrition, and the catheter site and vein should be monitored at least twice daily for heat, swelling, or exudation. Increased resistance to fluid flow in the catheter may be an indication of thrombosis deeper within the vasculature and will often necessitate the placement of a catheter in an alternative site, such as the opposite jugular vein, a cephalic vein, or a lateral thoracic vein.

All components of parenteral nutrition solutions must be mixed in a sterile manner before administration. The bag containing the final parenteral nutrition composition should be covered with a brown plastic bag during administration to protect it from light, which can degrade the amino acids within the solution. The rate of infusion (in mL/h) is calculated based on the desired kcal/kg per day to be administered. A reasonable initial goal is 40 to 60 kcal/kg per day. The initial infusion rate of parenteral nutrition solutions should be 25% of the calculated final rate, and the rate should be gradually increased every 1 to 3 hours following monitoring of the blood glucose concentration to ensure that hyperglycemia (blood glucose >150 mg/dL) is not present. If the patient tolerates parenteral nutrition well and maintains blood glucose concentrations at or near normal levels, then consideration can be given to increasing the parenteral nutrition administration rate to a maximum of 50 to 60 kcal/kg per day.[948] When parenteral nutrition is to be discontinued it is recommended that the infusion rate gradually be reduced, decreasing the infusion rate in 25% to 50% increments every 4 to 6 hours while gradually introducing enteral feeding. It is important that blood glucose monitoring is continued during this weaning process to detect or prevent the development of hypoglycemia.

The foal must be frequently monitored, especially during the initial phase of parenteral nutrition therapy. This monitoring should include a general physical examination, with close

attention to neurologic status and respiratory function. Rectal temperature should also be closely monitored because fever is a common early manifestation of systemic infection. Blood glucose concentrations should be frequently monitored, initially on an hourly basis until the patient has stabilized with the appropriate rate of parenteral nutrition infusion, followed by monitoring every 3 to 6 hours for the first day of therapy. The frequency of blood glucose monitoring is dependent on the stability of the patient and may need to be more frequent in the critically ill, but it may not need to be monitored beyond every 12 hours in the stable patient. Monitoring of urine output and urine glucose concentration may aid in the detection of hyperglycemia. Although the actual renal threshold for glucose is not well described in foals, glucosuria and diuresis are typically seen when blood glucose levels exceed 180 mg/dL. Additional clinicopathologic monitoring should consist of daily complete blood counts and serum chemistry profiles in the critical case, whereas these can be performed every 48 to 72 hours in more stable patients. Serum electrolytes should be monitored at least twice daily. Particular attention should be paid to serum potassium concentrations because they can decrease rapidly, especially in foals receiving insulin therapy. Urine output should be monitored continuously, in combination with intermittent monitoring of urine glucose concentration, because of the risk of hyperglycemia-induced diuresis and glucosuria. Ideally body weight should be assessed on a daily basis to ensure that the foal is at least maintaining its body weight while on parenteral nutrition. Foals receiving parenteral nutrition solutions containing lipids should be monitored for the development of hypertriglyceridemia.[719,949]

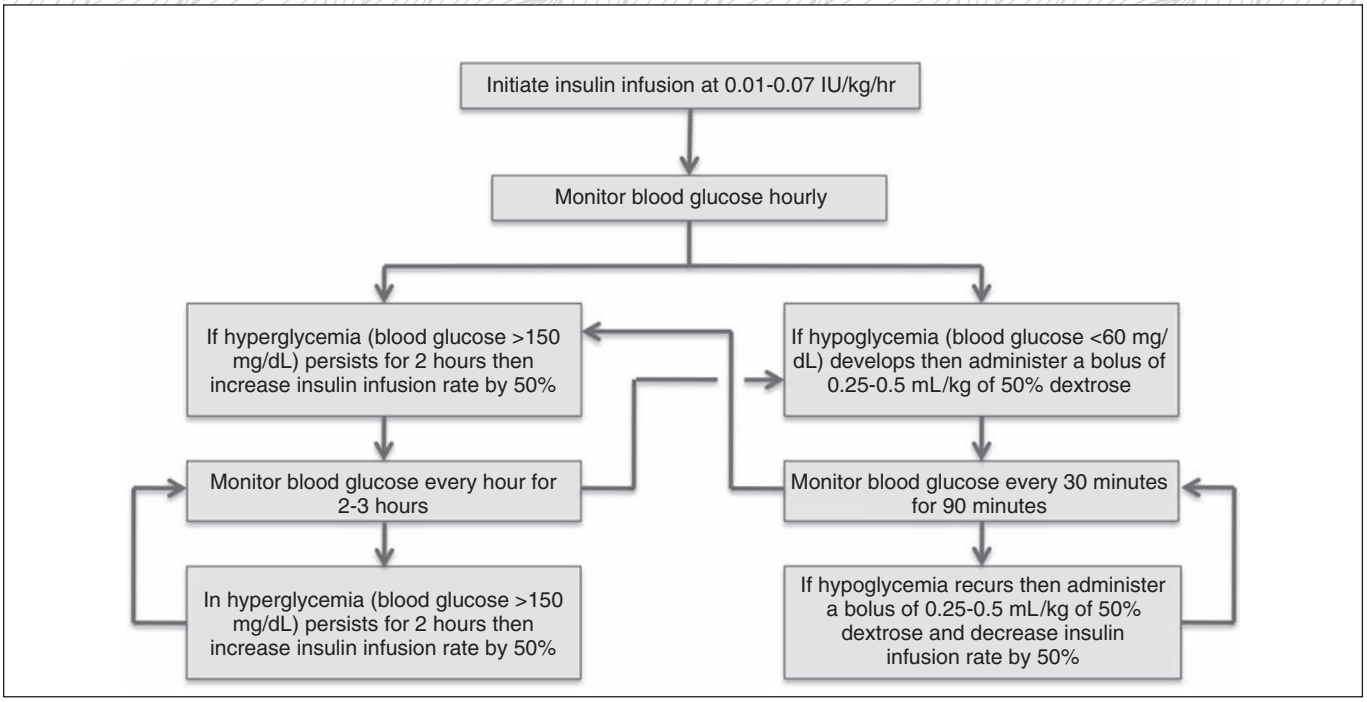
Insulin Therapy

The critically ill foal will often demonstrate carbohydrate intolerance, and this can make it very difficult to achieve even a conservative rate of administration of IV nutrition. This situation can be addressed by the use of a lipid-containing parenteral nutrition solution, but if this is ineffective then the only alternative is administration of exogenous insulin. The administration of insulin to the neonatal foal is not to be undertaken lightly because this therapy places additional demands on both the clinician and nursing staff to ensure that profound hypoglycemia does not occur. Intermittent dosing of subcutaneous insulin may offer some advantages in terms of simplicity of administration, expense, and moderation of effects, but this route of administration does not allow for changes in dosage over the short term. One recommended dosage for subcutaneous insulin in foals is 0.1 to 0.5 IU of regular insulin every 12 hours.[950] A retrospective report of foal parenteral nutrition described subcutaneous insulin dosage rates 0.02 to 0.1 IU/kg given every 6 to 24 hours.[949] This approach is not recommended because the risks of hypoglycemia and hyperglycemia are greater than with CRIs, and it can be much more challenging to achieve homeostasis in terms of blood glucose concentrations when using intermittent bolus insulin therapy.

The use of CRI for the administration of insulin allows for a fairly rapid onset of action while providing a simple and timely means of adjustment of the dosage. Because of the gradual saturation of the cellular insulin receptors, the maximal effect of CRI insulin is not typically seen until roughly 90 minutes after initiation of the infusion. The response to the alteration of the rate of infusion occurs over a similar time frame, so one should take care to avoid altering the rate of infusion of parenteral nutrition solutions too soon after changing the rate of insulin infusion. An initial insulin infusion rate of 0.07 IU/kg per hour of regular insulin has been reported to be well tolerated and may represent a reasonable starting point in foals intolerant of parenteral nutrition.[720,951] This dosage was derived from a retrospective study of foals treated with parenteral nutrition, which reported initial insulin doses ranging from 0.014 to 0.2 IU/kg per hour, with a mean of 0.065 IU/kg per hour.[719] Interestingly, in that study the final insulin dose ranges remained very similar, from 0.015 to 0.2 IU/kg per hour, with a mean of 0.07 IU/kg per hour. Some advocate starting at lower dosages in foals, such as 0.01 IU/kg per hour.[952] This conservative approach is very safe, with less risk of hypoglycemia than the higher dosage rate, but may require a longer period of time before the dose is titrated to a high enough level to control hyperglycemia. Even lower dosage rates of insulin have been reported, with a recent retrospective report describing insulin infusions at dosages of 0.0016 to 0.018 IU/kg per hour.[949]

Therefore an initial insulin infusion rate of 0.01 to 0.07 IU/kg per hour represents a reasonable starting point. When "fine-tuning" insulin therapy it is best to avoid simultaneous alterations in both the insulin infusion rate and the parenteral nutrition infusion rate, because this can lead to a "roller-coaster ride" in which the blood glucose concentration rises and falls wildly because of the delay in the body's response to these changes[720] (Box 20.6). Blood glucose monitoring should be performed at least hourly for the first 2 to 3 hours after initiation of the insulin CRI, and if hyperglycemia (blood glucose >150 mg/dL) is persistent beyond the first 2 hours of insulin therapy, then the insulin infusion rate may be increased by 50%, followed by hourly blood glucose monitoring for a further 2 to 3 hours. This procedure for increasing the insulin infusion rate may be repeated if hyperglycemia persists. Conversely, if hypoglycemia (blood glucose <60 mg/dL) is noted then a bolus of 0.25 to 0.5 mL/kg of 50% dextrose solution should be administered intravenously over 3 to 5 minutes. The blood glucose level should then be reassessed every 30 minutes for at least 90 minutes ensure that hypoglycemia does not recur. If hypoglycemia does recur, then a second bolus of dextrose is administered and the insulin infusion rate is decreased by 50%. Close monitoring will then be required for a further 60 to 90 minutes to ensure that hypoglycemia does not recur and that hyperglycemia does not develop. Further changes to the insulin infusion rate are not usually necessary once a steady state has been achieved in which the blood glucose level is stable and the desired rate of parenteral nutrition administration has been achieved. Patient reassessment is indicated if one finds that the foal has become even more insulin resistant (requiring additional insulin administration to avoid hyperglycemia) as there may be an overall deterioration in the patient's condition accompanied by increasing systemic inflammation. When insulin therapy is to be discontinued the insulin administration rate should be gradually titrated downward in parallel with the parenteral nutrition rate, but there can be a substantial lag between changes in the insulin infusion rate and the patient's response to this change. Some time should be allowed to account for this lag. It is critical that foals being weaned from insulin infusions receive some form of enteral nutrition during this time period. It may be wise to wean them from parenteral nutrition and insulin over longer periods of time (24–36 hours) than would be required for a foal receiving parenteral nutrition alone to prevent glucose derangements.

BOX 20.6 Protocol for the Monitoring and Regulation of Insulin When Administered as a Continuous Rate Infusion to Foals



Adapted from McKenzie HC 3rd, Geor RJ. Feeding management of sick neonatal foals. *Vet Clin North Am Equine Pract*. 2009;25:109-119, vii.

REFERENCES

1. Schofield FW. Sulfamethazine in the treatment of joint ill in the foal. *Can J Comp Med Vet Sci*. 1948;12:305.
2. Doll ER, Hull FE. Observations of hemolytic icterus of newborn foals. *Cornell Vet*. 1951;41:14–35.
3. Doll ER, Mc GW. Septicemic infections of newborn foals. *Vet Med*. 1951;46:123–127.
4. Platt H. Septicaemia in the foal. A review of 61 cases. *Br Vet J*. 1973;129:221–229.
5. Rossdale PD, Pattle RE, Mahaffey LW. Respiratory distress in a newborn foal with failure to form lung lining film. *Nature*. 1967;215:1498–1499.
6. Rossdale PD. Clinical studies on 4 newborn thoroughbred foals suffering from convulsions with special reference to blood gas chemistry and pulmonary ventilation. *Res Vet Sci*. 1969;10:279–291.
7. Mahaffey LW, Rossdale PD. A convulsive syndrome in newborn foals resembling pulmonary syndrome in the newborn infant. *Lancet*. 1959;1:1223–1225.
8. Rossdale PD. Clinical studies on the newborn thoroughbred foal. II. Heart rate, auscultation and electrocardiogram. *Br Vet J*. 1967;123:521–532.
9. Rossdale PD. Clinical studies on the newborn thoroughbred foal. I. Perinatal behaviour. *Br Vet J*. 1967;123:470–481.
10. Rossdale PD. Blood gas tensions and pH values in the normal thoroughbred foal at birth and in the following 42h. *Biol Neonat*. 1968;13:18–25.
11. Rossdale PD. Abnormal perinatal behaviour in the thoroughbred horse. *Br Vet J*. 1968;124:540–553.
12. Rossdale PD. PH and Pco2 of equine amniotic fluid at the time of birth. *Biol Neonat*. 1968;12:378–380.
13. Rossdale PD. Measurements of pulmonary ventilation in normal newborn thoroughbred foals during the first three days of life. *Br Vet J*. 1969;125:157–161.
14. Rossdale PD. The adaptive processes of the newborn foal. *Vet Rec*. 1970;87:37–38.
15. Rossdale PD. Some parameters of respiratory function in normal and abnormal newborn foals with special reference to levels of paO_2 during air and oxygen inhalation. *Res Vet Sci*. 1970;11:270–276.
16. Rossdale PD. Modern concepts of neonatal disease in foals. *Equine Vet J*. 1972;4:117–128.
17. Rossdale P, Silver M, Comline RS, et al. Plasma cortisol in the foal during the late fetal and early neonatal period. *Res Vet Sci*. 1973;15:395–397.
18. Arvidson G, Astedt B, Ekelund L, et al. Surfactant studies in the fetal and neonatal foal. *J Reprod Fertil Suppl*. 1975: 663–665.
19. Johnson P, Rossdale PD. Preliminary observations on cranial cardiovascular changes during asphyxia in the newborn foal. *J Reprod Fertil Suppl*. 1975:695–699.
20. Kitchen H, Rossdale PD. Metabolic profiles of newborn foals. *J Reprod Fertil Suppl*. 1975:705–707.
21. Palmer AC, Rossdale PD. Neuropathology of the convulsive foal syndrome. *J Reprod Fertil Suppl*. 1975:691–694.
22. Pattle RE, Rossdale PD, Schock C, et al. The development of the lung and its surfactant in the foal and in other species. *J Reprod Fertil Suppl*. 1975:651–657.
23. Nathanielsz PW, Rossdale PD, Silver M, et al. Studies on fetal, neonatal and maternal cortisol metabolism in the mare. *J Reprod Fertil Suppl*. 1975:625–630.

24. Brewer BD, Koterba AM, Carter RL, et al. Comparison of empirically developed sepsis score with a computer generated and weighted scoring system for the identification of sepsis in the equine neonate. *Equine Vet J*. 1988;20:23–24.
25. Koterba AM, Brewer BD, Tarplee FA. Clinical and clinicopathological characteristics of the septicaemic neonatal foal: review of 38 cases. *Equine Vet J*. 1984;16:376–382.
26. Kosch PC, Koterba AM, Coons TJ, et al. Developments in management of the newborn foal in respiratory distress 1: evaluation. *Equine Vet J*. 1984;16:312–318.
27. Koterba AM, Drummond WH. Nutritional support of the foal during intensive care. *Vet Clin North Am Equine Pract*. 1985;1:35–40.
28. Koterba AM, Drummond WH, Kosch P. Intensive care of the neonatal foal. *Vet Clin North Am Equine Pract*. 1985;1:3–34.
29. Koterba AM, Kosch PC. Respiratory mechanics and breathing pattern in the neonatal foal. *J Reprod Fertil Suppl*. 1987;35:575–585.
30. Wilkins PA. Disorders of foals. In: Reed SM, Bayly WM, Sellon DC, eds. *Equine internal medicine*. St. Louis: Saunders Elsevier; 2010:1311–1363.
31. Bucca S. Diagnosis of the compromised equine pregnancy. *Vet Clin North Am Equine Pract*. 2006;22:749–761.
32. Putnam MR, Bransby DI, Schumacher J, et al. Effects of the fungal endophyte *Acremonium coenophialum* in fescue on pregnant mares and foal viability. *Am J Vet Res*. 1991;52:2071–2074.
33. Blodgett DJ. Fescue toxicosis. *Vet Clin North Am Equine Pract*. 2001;17:567–577.
34. Bailey CS, Heitzman JM, Buchanan CN, et al. B-mode and Doppler ultrasonography in pony mares with experimentally induced ascending placentitis. *Equine Vet J Suppl*. 2012:88–94.
35. Morris S, Kelleman AA, Stawicki RJ, et al. Transrectal ultrasonography and plasma progestin profiles identifies fetoplacental compromise in mares with experimentally induced placentitis. *Theriogenology*. 2007;67:681–691.
36. Renaudin CD, Troedsson MH, Gillis CL, et al. Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. *Theriogenology*. 1997;47:559–573.
37. Baska-Vincze B, Baska F, Szenci O. Transabdominal ultrasonographic evaluation of fetal well-being in the late-term mare and cow. *Acta Vet Hung*. 2014;62:439–451.
38. Murase H, Endo Y, Tsuchiya T, et al. Ultrasonographic evaluation of equine fetal growth throughout gestation in normal mares using a convex transducer. *J Vet Med Sci*. 2014;76:947–953.
39. Bucca S, Fogarty U, Collins A, et al. Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. *Theriogenology*. 2005;64:542–557.
40. LeBlanc MM. Identification and treatment of the compromised equine fetus: a clinical perspective. *Equine Vet J Suppl*. 1997:100–103.
41. Bucca S. Equine fetal gender determination from mid- to advanced-gestation by ultrasound. *Theriogenology*. 2005;64:568–571.
42. Hendriks WK, Colenbrander B, van der Weijden GC, et al. Maternal age and parity influence ultrasonographic measurements of fetal growth in Dutch Warmblood mares. *Anim Reprod Sci*. 2009;115:110–123.
43. Bucca S. Ultrasonographic monitoring of the fetus. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2011:39–54.
44. Parkes RD, Colles CM. Fetal electrocardiography in the mare as a practical aid to diagnosing singleton and twin pregnancy. *Vet Rec*. 1977;100:25–26.
45. Reef VB, Vaala WE, Worth PL, et al. Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile. *Eq Vet J*. 1996;28:200–208.
46. Baska-Vincze B, Baska F, Szenci O. Fetal heart rate and fetal heart rate variability in Lipizzaner broodmares. *Acta Vet Hung*. 2015;63:89–99.
47. Adams-Brendemuehl C, Pipers FS. Antepartum evaluations of the equine fetus. *J Reprod Fertil Suppl*. 1987;35:565–573.
48. Feijó LS, Curcio BR, Haetinger C, et al. Maturidade de potros nascidos de éguas com placentite (Maturity of foals from the mares with placentitis). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 2014;66:1662–1670.
49. Murchie TA, Macpherson ML, LeBlanc MM, et al. Continuous monitoring of penicillin G and gentamicin in allantoic fluid of pregnant pony mares by in vivo microdialysis. *Equine Vet J*. 2006;38:520–525.
50. Rebello SA, Macpherson ML, Murchie TA, et al. Placental transfer of trimethoprim sulfamethoxazole and pentoxifylline in pony mares. *Anim Reprod Sci*. 2006;94:432–433.
51. Macpherson ML, Giguere S, Hatzel JN, et al. Disposition of desfuroylceftiofur acetamide in serum, placental tissue, fetal fluids, and fetal tissues after administration of ceftiofur crystalline free acid (CCFA) to pony mares with placentitis. *J Vet Pharmacol Ther*. 2013;36:59–67.
52. Cummins C, Carrington S, Fitzpatrick E, et al. Ascending placentitis in the mare: a review. *Ir Vet J*. 2008;61:307–313.
53. Christiansen DL, Moulton K, Hopper RM, et al. Evidence-based medicine approach to develop efficacious therapies for late-gestation mares presenting with uterine infections using an experimentally-induced placentitis model. *Anim Reprod Sci*. 2010;121:S345–S346.
54. Msan AK, Usta IM, Mirza FG, et al. Use of antenatal corticosteroids in the management of preterm delivery. *Am J Perinatol*. 2015;32:417–426.
55. Ousey JC, Kolling M, Kindahl H, et al. Maternal dexamethasone treatment in late gestation induces precocious fetal maturation and delivery in healthy Thoroughbred mares. *Equine Vet J*. 2011;43:424–429.
56. Bailey CS, Macpherson ML, Pozor MA, et al. Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. *Theriogenology*. 2010;74:402–412.
57. Macpherson ML, Bailey CS. A clinical approach to managing the mare with placentitis. *Theriogenology*. 2008;70:435–440.
58. Macpherson ML. Treatment strategies for mares with placentitis. *Theriogenology*. 2005;64:528–534.
59. Gastal MO, Gastal EL, Torres CA, et al. Effect of oxytocin, prostaglandin F2 alpha, and clenbuterol on uterine dynamics in mares. *Theriogenology*. 1998;50:521–534.
60. Geor RJ, Weiss DJ, Burris SM, et al. Effects of furosemide and pentoxifylline on blood flow properties in horses. *Am J Vet Res*. 1992;53:2043–2049.
61. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc Nutr Soc*. 1998;57:113–122.
62. Fowden AL, Forhead AJ, Ousey JC. Endocrine adaptations in the foal over the perinatal period. *Equine Vet J Suppl*. 2012:130–139.
63. Silver M. Prenatal maturation, the timing of birth and how it may be regulated in domestic animals. *Exp Physiol*. 1990;75:285–307.
64. Silver M, Ousey JC, Dudan FE, et al. Studies on equine prematurity 2: post natal adrenocortical activity in relation to plasma adrenocorticotrophic hormone and catecholamine levels in term and premature foals. *Equine Vet J*. 1984;16:278–286.
65. Tennent-Brown BS, Morrice AV, Reed S. The equine neonatal central nervous system: development and diseases. *Vet Clin North Am Equine Pract*. 2015;31:587–600.
66. Diesch TJ, Mellor DJ. Birth transitions: pathophysiology, the onset of consciousness and possible implications for neonatal maladjustment syndrome in the foal. *Equine Vet J*. 2013;45:656–660.

67. Houghton E, Holtan D, Grainger L, et al. Plasma progestagen concentrations in the normal and dysmature newborn foal. *J Reprod Fertil Suppl*. 1991;44:609–617.
68. Aleman M, Pickles KJ, Conley AJ, et al. Abnormal plasma neuroactive progestagen derivatives in ill, neonatal foals presented to the neonatal intensive care unit. *Equine Vet J*. 2013;45:661–665.
69. Hillman NH, Kallapur SG, Jobe AH. Physiology of transition from intrauterine to extrauterine life. *Clin Perinatol*. 2012;39:769–783.
70. Machida N, Yasuda J, Too K. Auscultatory and phonocardiographic studies on the cardiovascular system of the newborn Thoroughbred foal. *Jpn J Vet Res*. 1987;35:235–250.
71. O'Connor SJ, Gardner DS, Ousey JC, et al. Development of baroreflex and endocrine responses to hypotensive stress in newborn foals and lambs. *Pflugers Arch*. 2005;450:298–306.
72. Waring GH. *Horse behavior*. 2nd ed. Norwich, NY: Noyes Publications/William Andrew Publishing; 2003.
73. Campitelli S, Carenzi C, Verga M. Factors which influence parturition in the mare and development of the foal. *Appl Anim Ethology*. 1982;9:7–14.
74. Grogan EH, McDonnell SM. Mare and foal bonding and problems. *Clin Tech Equine Pract*. 2005;4:228–237.
75. Kidd JA, Barr ARS. Flexural deformities in foals. *Eq Vet Educ*. 2002;14:311–321.
76. Trumble TN. Orthopedic disorders in neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:357–385. vi.
77. Embertson RM. Congenital abnormalities of tendons and ligaments. *Vet Clin North Am Equine Pract*. 1994;10:351–364.
78. Raidal SL, McTaggart C, Penhale J. Effect of withholding macromolecules on the duration of intestinal permeability to colostral IgG in foals. *Aust Vet J*. 2005;83:78–81.
79. Vendrig JC, Fink-Gremmels J. Intestinal barrier function in neonatal foals: options for improvement. *Vet J*. 2012;193:32–37.
80. Earing JE, Durig AC, Gellin GL, et al. Bacterial colonization of the equine gut: comparison of mare and foal pairs by PCR-DGGE. *Adv Microbiol*. 2012;2:79–86.
81. Kuhl J, Winterhoff N, Wulf M, et al. Changes in faecal bacteria and metabolic parameters in foals during the first six weeks of life. *Vet Microbiol*. 2011;151:321–328.
82. Silver M, Fowden AL, Knox J, et al. Relationship between circulating tri-iodothyronine and cortisol in the perinatal period in the foal. *J Reprod Fertil Suppl*. 1991;44:619–626.
83. Ousey JC. Physiology and metabolism in the newborn foal with reference to orphaned or sick foals. In: Miraglia N, Martin-Rosset W, eds. *Nutrition and feeding of the broodmare*. The Netherlands: Wageningen Academic Publishers; 2006:416.
84. Leadon DP, Jeffcott LB, Rossdale PD. Behavior and viability of the premature neonatal foal after induced parturition. *Am J Vet Res*. 1986;47:1870–1873.
85. Rossdale PD. Clinical view of disturbances in equine foetal maturation. *Equine Vet J Suppl*. 1993:3–7.
86. Sato K, Miyaki M, Sugiyama K, et al. An analytical study of the duration of gestation in horses. *Jap J Zoo Tech Sci*. 1973;44:375–379.
87. Kuhl J, Stock KF, Wulf M, et al. Maternal lineage of warmblood mares contributes to variation of gestation length and bias of foal sex ratio. *PLoS One*. 2015;10. e0139358.
88. Davies Morel MC, Newcombe JR, Holland SJ. Factors affecting gestation length in the Thoroughbred mare. *Anim Reprod Sci*. 2002;74:175–185.
89. Bos H, van der May GJW. Length of gestation periods of horses and ponies belonging to different breeds. *Livest Prod Sci*. 1980;7:181–187.
90. Aoki T, Yamakawa K, Ishii M. Factors affecting gestation length in heavy draft mares. *J Eq Vet Sci*. 2013;33:437–440.
91. Rossdale PD, Silver M, Ellis L, et al. Response of the adrenal cortex to tetracosactrin (ACTH1-24) in the premature and full-term foal. *J Reprod Fertil Suppl*. 1982;32:545–553.
92. Rossdale PD, Ousey JC, Silver M, et al. Studies on equine prematurity 6: guidelines for assessment of foal maturity. *Equine Vet J*. 1984;16:300–302.
93. Silver M, Fowden AL. Prepartum adrenocortical maturation in the fetal foal: responses to ACTH. *J Endocrinol*. 1994;142:417–425.
94. Ousey JC, Rossdale PD, Fowden AL, et al. Effects of manipulating intrauterine growth on post natal adrenocortical development and other parameters of maturity in neonatal foals. *Equine Vet J*. 2004;36:616–621.
95. Wong DM, Vo DT, Alcott CJ, et al. Adrenocorticotropic hormone stimulation tests in healthy foals from birth to 12 weeks of age. *Can J Vet Res*. 2009;73:65–72.
96. Wong DM, Vo DT, Alcott CJ, et al. Baseline plasma cortisol and ACTH concentrations and response to low-dose ACTH stimulation testing in ill foals. *J Am Vet Med Assoc*. 2009;234:126–132.
97. Hart KA, Slovis NM, Barton MH. Hypothalamic-pituitary-adrenal axis dysfunction in hospitalized neonatal foals. *J Vet Intern Med*. 2009;23:901–912.
98. Castagnetti C, Rametta M, Tudor Popeia R, et al. Plasma levels of ACTH and cortisol in normal and critically-ill neonatal foals. *Vet Res Commun*. 2008;32(suppl 1):S127–S129.
99. Han VK. Pathophysiology, cellular and molecular mechanisms of foetal growth retardation. *Equine Vet J Suppl*. 1993:12–16.
100. Lyle SK. Immunology of infective preterm delivery in the mare. *Equine Vet J*. 2014;46:661–668.
101. Varvarousis D, Varvarousi G, Iacovidou N, et al. The pathophysiologies of asphyxial vs dysrhythmic cardiac arrest: implications for resuscitation and post-event management. *Am J Emerg Med*. 2015;33:1297–1304.
102. Palmer JE. Neonatal foal resuscitation. *Vet Clin North Am Equine Pract*. 2007;23:159–182.
103. Slovis NM. Field techniques for resuscitation of foals. *AAEP Focus on the first year of life proceedings*. 2014:6–9.
104. Jokisalo JM, Corley KT. in the neonatal foal: has RECOVER changed our approach? *Vet Clin North Am Equine Pract*. 2014;30:301–316. vii.
105. Aufderheide TP, Lurie KG. Death by hyperventilation: a common and life-threatening problem during cardiopulmonary resuscitation. *Crit Care Med*. 2004;32:S345–S351.
106. van Vonderen JJ, Narayen NE, Walther FJ, et al. The administration of 100% oxygen and respiratory drive in very preterm infants at birth. *PLoS One*. 2013;8. e76898.
107. Atiksawedparit P, Rattanasiri S, McEvoy M, et al. Effects of prehospital adrenaline administration on out-of-hospital cardiac arrest outcomes: a systematic review and meta-analysis. *Crit Care*. 2014;18:463.
108. Wenzel V, Lindner KH. Vasopressin combined with epinephrine during cardiac resuscitation: a solution for the future? *Crit Care*. 2006;10:125.
109. Plunkett SJ, McMichael M. Cardiopulmonary resuscitation in small animal medicine: an update. *J Vet Intern Med*. 2008;22:9–25.
110. Corley KT, Axon JE. Resuscitation and emergency management for neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:431–455. vii.
111. Hoffman AM, Staempfli HR, Willan A. Prognostic variables for survival of neonatal foals under intensive care. *J Vet Intern Med*. 1992;6:89–95.
112. Furr M, Tinker MK, Edens L. Prognosis for neonatal foals in an intensive care unit. *J Vet Intern Med*. 1997;11:183–188.
113. Borchers A, Wilkins PA, Marsh PM, et al. Association of admission L-lactate concentration in hospitalised equine neonates with presenting complaint, periparturient events, clinical diagnosis and outcome: a prospective multicentre study. *Equine Vet J Suppl*. 2012:57–63.
114. Armengou L, Jose-Cunilleras E, Rios J, et al. Metabolic and endocrine profiles in sick neonatal foals are related to survival. *J Vet Intern Med*. 2013;27:567–575.

115. Dembek KA, Hurcombe SD, Frazer ML, et al. Development of a likelihood of survival scoring system for hospitalized equine neonates using generalized boosted regression modeling. *PLoS One*. 2014;9. e109212.
116. Giguère S, Weber EJ, Sanchez LC. Factors associated with outcome and gradual improvement in survival over time in 1065 equine neonates admitted to an intensive care unit. *Equine Vet J*. 2015.
117. Brewer BD, Koterba AM. Development of a scoring system for the early diagnosis of equine neonatal sepsis. *Equine Vet J*. 1988;20:18–22.
118. Peek SF, Semrad S, McGuirk SM, et al. Prognostic value of clinicopathologic variables obtained at admission and effect of antiendotoxin plasma on survival in septic and critically ill foals. *J Vet Intern Med*. 2006;20:569–574.
119. Boyle AG, Magdesian KG, Ruby RE. Neonatal isoerythrolysis in horse foals and a mule foal: 18 cases (1988-2003). *J Am Vet Med Assoc*. 2005;227:1276–1283.
120. Hollis AR, Wilkins PA, Palmer JE, et al. Bacteremia in equine neonatal diarrhea: a retrospective study (1990-2007). *J Vet Intern Med*. 2008;22:1203–1209.
121. Hurcombe SD, Toribio RE, Slovis N, et al. Blood arginine vasopressin, adrenocorticotropin hormone, and cortisol concentrations at admission in septic and critically ill foals and their association with survival. *J Vet Intern Med*. 2008;22:639–647.
122. Polkes AC, Giguere S, Lester GD, et al. Factors associated with outcome in foals with neonatal isoerythrolysis (72 cases, 1988-2003). *J Vet Intern Med*. 2008;22:1216–1222.
123. Paradis MR. Update on neonatal septicemia. *Vet Clin North Am Equine Pract*. 1994;10:109–135.
124. McKenzie HC, Furr MO. Equine neonatal sepsis: the pathophysiology of severe inflammation and infection. *Compend Contin Educ Vet*. 2001;23:661–672.
125. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. *Chest*. 1992;101: 1481–1483.
126. Corley KT, Donaldson LL, Furr MO. Arterial lactate concentration, hospital survival, sepsis and SIRS in critically ill neonatal foals. *Equine Vet J*. 2005;37:53–59.
127. Taylor S. A review of equine sepsis. *Equine Veterinary Education*. 2015;27:99–109.
128. Nathens AB, Marshall JC. Sepsis, SIRS, and MODS: what's in a name? *World J Surg*. 1996;20:386–391.
129. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest*. 1997;112:235–243.
130. Corley K, Amoroso L, McKenzie HC, et al. Initial experience with norepinephrine in neonatal foals. *Vet Emerg Crit Care*. 2000;10:267–277.
131. Hollis AR, Furr MO, Magdesian KG, et al. Blood glucose concentrations in critically ill neonatal foals. *J Vet Intern Med*. 2008;22:1223–1227.
132. Wong DM, Wilkins PA. Defining the systemic inflammatory response syndrome in equine neonates. *Vet Clin North Am Equine Pract*. 2015;31:463–481.
133. Wiersinga WJ, Leopold SJ, Cranendonk DR, et al. Host innate immune responses to sepsis. *Virulence*. 2014;5:36–44.
134. de Jong HK, van der Poll T, Wiersinga WJ. The systemic pro-inflammatory response in sepsis. *J Innate Immun*. 2010;2:422–430.
135. Werners AH, Bryant CE. Pattern recognition receptors in equine endotoxaemia and sepsis. *Equine Vet J*. 2012;44: 490–498.
136. Adrie C, Pinsky MR. The inflammatory balance in human sepsis. *Intensive Care Med*. 2000;26:364–375.
137. Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. *Am J Physiol Lung Cell Mol Physiol*. 2006;290:L622–L645.
138. Auphan N, DiDonato JA, Rosette C, et al. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science*. 1995;270:286–290.
139. Bohrer H, Nawroth PP. Nuclear factor kappaB—a new therapeutic approach? *Intensive Care Med*. 1998;24:1129–1130.
140. Samols D, Agrawal A, Kushner I. Acute phase proteins. In: Oppenheim JJ, Feldman M, eds. *Cytokine Reference Online*. London, UK: Academic Press; 2002:1–16.
141. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448–454.
142. Karima R, Matsumoto S, Higashi H, et al. The molecular pathogenesis of endotoxic shock and organ failure. *Mol Med Today*. 1999;5:123–132.
143. Morris DD. Endotoxemia in horses. A review of cellular and humoral mediators involved in its pathogenesis. *J Vet Intern Med*. 1991;5:167–181.
144. Wort SJ, Evans TW. The role of the endothelium in modulating vascular control in sepsis and related conditions. *Br Med Bull*. 1999;55:30–48.
145. Lavoie JP, Madigan JE, Cullor JS, et al. Haemodynamic, pathological, haematological and behavioural changes during endotoxin infusion in equine neonates. *Equine Vet J*. 1990;22:23–29.
146. Liepert D, Rosenthal M, Thomas S. Shock: a comparative physiologic approach to mechanisms and therapy. Seminars in Anesthesia. *Periop Med and Pain*. 1999;18:1–14.
147. Astiz ME, Rackow EC. Septic shock. *Lancet*. 1998;351:1501–1505.
148. Sheagren JN. Mechanism-oriented therapy for multiple systems organ failure. *Crit Care Clin*. 1989;5:393–409.
149. Weiss DJ, Rashid J. The sepsis-coagulant axis: a review. *J Vet Intern Med*. 1998;12:317–324.
150. Levi M. Diagnosis and treatment of disseminated intravascular coagulation. *Int J Lab Hematol*. 2014;36:228–236.
151. Moore JN, Barton MH. An update on endotoxaemia Part 1: mechanisms and pathways. *Equine Vet Educ*. 1998;10: 300–306.
152. Barton MH, Morris DD, Norton N, et al. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. *J Vet Intern Med*. 1998;12:26–35.
153. Bentz AI, Palmer JE, Dallap BL, et al. Prospective evaluation of coagulation in critically ill neonatal foals. *J Vet Intern Med*. 2009;23:161–167.
154. Dallap Schaer BL, Epstein K. Coagulopathy of the critically ill equine patient. *J Vet Emerg Crit Care (San Antonio)*. 2009;19: 53–65.
155. Cotovio M, Monreal L, Armengou L, et al. Fibrin deposits and organ failure in newborn foals with severe septicemia. *J Vet Intern Med*. 2008;22:1403–1410.
156. Armengou L, Monreal L, Tarancon I, et al. Plasma D-dimer concentration in sick newborn foals. *J Vet Intern Med*. 2008;22:411–417.
157. Roy MF. Sepsis in adults and foals. *Vet Clin North Am Equine Pract*. 2004;20:41–61.
158. Osterbur K, Mann FA, Kuroki K, et al. Multiple organ dysfunction syndrome in humans and animals. *J Vet Intern Med*. 2014;28:1141–1151.
159. Standiford TJ, Ward PA. Therapeutic targeting of acute lung injury and acute respiratory distress syndrome. *Transl Res*. 2015;167:183–191.
160. Sertaridou E, Papaioannou V, Kolios G, et al. Gut failure in critical care: old school versus new school. *Ann Gastroenterol*. 2015;28:309–322.
161. Jarrar D, Chaudry IH, Wang P. Organ dysfunction following hemorrhage and sepsis: mechanisms and therapeutic approaches (Review). *Int J Mol Med*. 1999;4:575–583.
162. Streck EL, Comim CM, Barichello T, et al. The septic brain. *Neurochem Res*. 2008;33:2171–2177.
163. Brewer B. Neonatal infection. In: Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Lea and Febiger; 1990:296–317.
164. Sanchez LC. Equine neonatal sepsis. *Vet Clin North Am Equine Pract*. 2005;21:273–293. v.

165. Liepman RS, Dembek KA, Slovis NM, et al. Validation of IgG cut-off values and their association with survival in neonatal foals. *Equine Vet J*. 2015;47:526–530.
166. Chaney KP, Holcombe SJ, Schott 2nd HC, et al. Spurious hypercreatininemia: 28 neonatal foals (2000-2008). *J Vet Emerg Crit Care (San Antonio)*. 2010;20:244–249.
167. Castagnetti C, Pirrone A, Mariella J, et al. Venous blood lactate evaluation in equine neonatal intensive care. *Theriogenology*. 2010;73:343–357.
168. Tennent-Brown B. Blood lactate measurement and interpretation in critically ill equine adults and neonates. *Vet Clin North Am Equine Pract*. 2014;30:399–413. viii.
169. Sheahan BJ, Wilkins PA, Lascola KM, et al. The area under the curve of l-lactate in neonatal foals from birth to 14 days of age. *J Vet Emerg Crit Care (San Antonio)*. 2015;26:305–309.
170. Borchers A, Wilkins PA, Marsh PM, et al. Sequential L-lactate concentration in hospitalised equine neonates: a prospective multicentre study. *Equine Vet J Suppl*. 2013:2–7.
171. Wilson WD, Madigan JE. Comparison of bacteriologic culture of blood and necropsy specimens for determining the cause of foal septicemia: 47 cases (1978-1987). *J Am Vet Med Assoc*. 1989;195:1759–1763.
172. Barie PS, Hydo LJ, Shou J, et al. Influence of antibiotic therapy on mortality of critical surgical illness caused or complicated by infection. *Surg Infect (Larchmt)*. 2005;6:41–54.
173. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34:1589–1596.
174. Castellanos-Ortega A, Suberviola B, Garcia-Astudillo LA, et al. Impact of the Surviving Sepsis Campaign protocols on hospital length of stay and mortality in septic shock patients: results of a three-year follow-up quasi-experimental study. *Crit Care Med*. 2010;38:1036–1043.
175. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med*. 2013;41:580–637.
176. Brewer B, Koterba A. Bacterial isolates and susceptibility patterns in foals in a neonatal intensive care unit. *Compend Contin Educ Pract Vet*. 1990;12:1773–1781.
177. Marsh PS, Palmer JE. Bacterial isolates from blood and their susceptibility patterns in critically ill foals: 543 cases (1991-1998). *J Am Vet Med Assoc*. 2001;218:1608–1610.
178. Stewart AJ, Hinchcliff KW, Saville WJ, et al. *Actinobacillus* sp. bacteremia in foals: clinical signs and prognosis. *J Vet Intern Med*. 2002;16:464–471.
179. Corley KT, Pearce G, Magdesian KG, et al. Bacteraemia in neonatal foals: clinicopathological differences between Gram-positive and Gram-negative infections, and single organism and mixed infections. *Equine Vet J*. 2007;39:84–89.
180. Russell CM, Axon JE, Blishen A, et al. Blood culture isolates and antimicrobial sensitivities from 427 critically ill neonatal foals. *Aust Vet J*. 2008;86:266–271.
181. Theelen MJ, Wilson WD, Edman JM, et al. Temporal trends in prevalence of bacteria isolated from foals with sepsis: 1979-2010. *Equine Vet J*. 2014;46:169–173.
182. Toombs-Ruane LJ, Riley CB, Rosanowski SM, et al. Antimicrobial sensitivity of bacteria isolated from neonatal foal samples in New Zealand (2004 to 2013). *Eq Vet J*. 2015;47:3.
183. Perkins G, Ainsworth DM, Erb HN, et al. Clinical, haematological and biochemical findings in foals with neonatal equine herpesvirus-1 infection compared with septic and premature foals. *Equine Vet J*. 1999;31:422–426.
184. Murray MJ, del Piero F, Jeffrey SC, et al. Neonatal equine herpesvirus type 1 infection on a thoroughbred breeding farm. *J Vet Intern Med*. 1998;12:36–41.
185. Wong D, Wilkins PA, Bain FT, et al. Neonatal encephalopathy in foals. *Compend Contin Educ Vet*. 2011;33. E5.
186. Corley KTT, Furr MO. Evaluation of a score designed to predict sepsis in foals. *J Vet Emerg Crit Care*. 2003;13:149–155.
187. Weber EJ, Sanchez LC, Giguere S. Re-evaluation of the sepsis score in equine neonates. *Equine Vet J*. 2015;47:275–278.
188. van Nieuwenhoven EJ, Lefering R, Neugebauer E, et al. Clinical relevance of sepsis scores. *Langenbecks Arch Surg*. 1998;383:11–14.
189. Stoneham SJ, Palmer L, Cash R, et al. Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Vet J*. 2001;33:599–603.
190. Dunkel B, Corley KTT. Pathophysiology, diagnosis and treatment of neonatal sepsis. *Equine Veterinary Education*. 2015;27:92–98.
191. Castagnetti C, Mariella J, Pirrone A, et al. Expression of interleukin-1beta, interleukin-8, and interferon-gamma in blood samples obtained from healthy and sick neonatal foals. *Am J Vet Res*. 2012;73:1418–1427.
192. Zabrecky KA, Slovis NM, Constable PD, et al. Plasma C-reactive protein and haptoglobin concentrations in critically ill neonatal foals. *J Vet Intern Med*. 2015;29:673–677.
193. Wilkins PA, Sheahan BJ, Vander Werf KA, et al. Preliminary investigation of the area under the L-lactate concentration-time curve (LACArea) in critically ill equine neonates. *J Vet Intern Med*. 2015;29:659–662.
194. Wichtel ME, Buys E, DeLuca J, et al. Pharmacologic considerations in the treatment of neonatal septicemia and its complications. *Vet Clin North Am Equine Pract*. 1999;15:725–746.
195. Armengou L, Monreal L, Delgado MA, et al. Low-molecular-weight heparin dosage in newborn foals. *J Vet Intern Med*. 2010;24:1190–1195.
196. Rohrbach BW, Buchanan BR, Drake JM, et al. Use of a multivariable model to estimate the probability of discharge in hospitalized foals that are 7 days of age or less. *J Am Vet Med Assoc*. 2006;228:1748–1756.
197. MacKay RJ. Neurologic disorders of neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:387–406. vii.
198. Morresey PR. Neurological conditions and seizure management. *AAEP Focus on the first year of life*. Proceedings 2014:29–35.
199. Toth B, Aleman M, Brosnan RJ, et al. Evaluation of squeeze-induced somnolence in neonatal foals. *Am J Vet Res*. 2012;73:1881–1889.
200. Adams R, Mayhew IG. Neurological examination of newborn foals. *Equine Vet J*. 1984;16:306–312.
201. Adams R, Mayhew IG. Neurologic diseases. *Vet Clin North Am Equine Pract*. 1985;1:209–234.
202. Enzerink E. The menace response and pupillary light reflex in neonatal foals. *Equine Vet J*. 1998;30:546–548.
203. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*. 2010;86:329–338.
204. Dickey EJ, Long SN, Hunt RW. Hypoxic ischemic encephalopathy—what can we learn from humans? *J Vet Intern Med*. 2011;25:1231–1240.
205. Gold JR. Perinatal asphyxia syndrome. *Equine Veterinary Education*. 2015.
206. Ringger NC, Giguere S, Morresey PR, et al. Biomarkers of brain injury in foals with hypoxic-ischemic encephalopathy. *J Vet Intern Med*. 2011;25:132–137.
207. Vaala WE. Peripartum asphyxia. *Vet Clin North Am Equine Pract*. 1994;10:187–218.
208. Holtan DW, Houghton E, Silver M, et al. Plasma progestagens in the mare, fetus and newborn foal. *J Reprod Fertil Suppl*. 1991;44:517–528.
209. Rossdale PD, Ousey JC, McGladdery AJ, et al. A retrospective study of increased plasma progestagen concentrations in compromised neonatal foals. *Reprod Fertil Dev*. 1995;7:567–575.

210. Green S. Current perspectives on equine neonatal maladjustment syndrome. *Comp Cont Educ Pract Vet*. 1993;15:1550–1552.
211. Furr MO. Perinatal asphyxia in foals. *Comp Cont Educ Pract Vet*. 1996;18:1342–1351.
212. Bernard WV, Reimer JM, Cudd T, et al. Historical factors, clinicopathologic findings, clinical features, and outcome of equine neonates presenting with or developing signs of central nervous system disease. *41st AAEP Convention*. 1995:221–224.
213. Vaala W. Peripartum asphyxia syndrome in foals. *45th AAEP Convention*. 1999:247–253.
214. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. *JAMA Pediatr*. 2015;169:397–403.
215. Johnston MV. Excitotoxicity in neonatal hypoxia. *Ment Retard Dev Disabil Res Rev*. 2001;7:229–234.
216. Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. *Nat Rev Neurol*. 2015;11:192–208.
217. Aleman M, Madigan JE, Williams DC, et al. Brainstem auditory evoked responses in an equine patient population. Part II: foals. *J Vet Intern Med*. 2014;28:1318–1324.
218. Lecoq L, Gains M, Blond L, et al. Brainstem auditory evoked responses in foals: reference values, effect of age, rate of acoustic stimulation, and neurologic deficits. *J Vet Intern Med*. 2015;29:362–367.
219. Wong DM, Jeffery N, Hepworth-Warren KL, et al. Magnetic resonance imaging of presumptive neonatal encephalopathy in a foal. *Equine Veterinary Education*. 2016.
220. Lv H, Wang Q, Wu S, et al. Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. *Clin Chim Acta*. 2015;450:282–297.
221. Zhang QH, Sheng ZY, Yao YM. Septic encephalopathy: when cytokines interact with acetylcholine in the brain. *Mil Med Res*. 2014;1:20.
222. Hosokawa K, Gaspard N, Su F, et al. Clinical neurophysiological assessment of sepsis-associated brain dysfunction: a systematic review. *Crit Care*. 2014;18:674.
223. Chaudhry N, Duggal AK. Sepsis associated encephalopathy. *Adv Med*. 2014;2014:762320.
224. Oddo M, Taccone FS. How to monitor the brain in septic patients? *Minerva Anestesiol*. 2015;81:776–788.
225. Tekgul H, Gauvreau K, Soul J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. *Pediatrics*. 2006;117:1270–1280.
226. Shetty J. Neonatal seizures in hypoxic-ischaemic encephalopathy—risks and benefits of anticonvulsant therapy. *Dev Med Child Neurol*. 2015;57(suppl 3):40–43.
227. Glass HC. Neonatal seizures: advances in mechanisms and management. *Clin Perinatol*. 2014;41:177–190.
228. Viu J, Monreal L, Jose-Cunilleras E, et al. Clinical findings in 10 foals with bacterial meningoencephalitis. *Equine Vet J Suppl*. 2012:100–104.
229. Sanchez LC, Giguere S, Lester GD. Factors associated with survival of neonatal foals with bacteremia and racing performance of surviving Thoroughbreds: 423 cases (1982-2007). *J Am Vet Med Assoc*. 2008;233:1446–1452.
230. Moore BR. Bacterial meningitis in foals. *Comp Cont Educ Pract Vet*. 1995;17:1417–1420.
231. Pellegrini-Masini A, Livesey LC. Meningitis and encephalomyelitis in horses. *Vet Clin North Am Equine Pract*. 2006;22:553–589. x.
232. Corley K, Stephen J. *The equine hospital manual*. 1st ed. Oxford: Blackwell; 2008.
233. Furr MO. Bacterial infections of the central nervous system. In: Furr MO, Reed S, eds. *Equine neurology*. Oxford: Wiley-Blackwell; 2008:187–194.
234. Seino K. Central nervous system infections. In: Sellon D, Long M, eds. *Equine infectious diseases*. 1st ed. Oxford: Elsevier; 2007:46–57.
235. Mitchell E, Furr MO, McKenzie HC. Antimicrobial therapy for bacterial meningitis. *Equine Veterinary Education*. 2007;19:316–323.
236. Ogunlesi TA, Odigwe CC, Oladapo OT. Adjuvant corticosteroids for reducing death in neonatal bacterial meningitis. *Cochrane Database Syst Rev*. 2015;11. CD010435.
237. Brouwer MC, McIntyre P, Prasad K, et al. Corticosteroids for acute bacterial meningitis. *Cochrane Database Syst Rev*. 2015;9. CD004405.
238. Wilkins PA, Palmer JE. Botulism in foals less than 6 months of age: 30 cases (1989-2002). *J Vet Intern Med*. 2003;17:702–707.
239. Stratford CH, Mayhew IG, Hudson NPH. Equine botulism: a clinical approach to diagnosis and management. *Equine Veterinary Education*. 2014;26:441–448.
240. Johnson AL, McAdams-Gallagher SC, Aceto H. Accuracy of a mouse bioassay for the diagnosis of botulism in horses. *J Vet Intern Med*. 2016;30:1293–1299.
241. Prutton JS, Magdesian KG, Plummer A, et al. Survival of a foal with type A botulism. *J Vet Intern Med*. 2016;30:675–678.
242. Mykkanen AK, Hyytiainen HK, McGowan CM. Generalised tetanus in a 2-week-old foal: use of physiotherapy to aid recovery. *Aust Vet J*. 2011;89:447–451.
243. Simonen T, Järvimaa TA. Foals recovery from tetanus. *Finnish Vet J*. 2001;12:696–700.
244. MacKay RJ. Tetanus. In: Sellon DC, Long MT, eds. *Equine infectious disease*. St. Louis: Saunders; 2007:376–380.
245. Cook TM, Protheroe RT, Handel JM. Tetanus: a review of the literature. *Br J Anaesth*. 2001;87:477–487.
246. Rodrigo C, Fernando D, Rajapakse S. Pharmacological management of tetanus: an evidence-based review. *Crit Care*. 2014;18:217.
247. Auer RN. Hypoglycemic brain damage. In: McCandless DW, ed. *Metabolic encephalopathy*. New York: Springer; 2009.
248. Mohseni S. Neurologic damage in hypoglycemia. *Handb Clin Neurol*. 2014;126:513–532.
249. Divers TJ. Metabolic causes of encephalopathy in horses. *Vet Clin North Am Equine Pract*. 2011;27:589–596.
250. Johnson AL, Gilsenan WF, Palmer JE. Metabolic encephalopathies in foals – pay attention to the serum biochemistry panel! *Equine Veterinary Education*. 2012;24:233–235.
251. Hardefeldt LY. Hyponatraemic encephalopathy in azotaemic neonatal foals: four cases. *Aust Vet J*. 2014;92:488–491.
252. Arroyo LG, Vengust M, Dobson H, et al. Suspected transient pseudohypoaldosteronism in a 10-day-old quarter horse foal. *Can Vet J*. 2008;49:494–498.
253. Lakritz J, Madigan J, Carlson GP. Hypovolemic hyponatremia and signs of neurologic disease associated with diarrhea in a foal. *J Am Vet Med Assoc*. 1992;200:1114–1116.
254. Espay AJ. Neurologic complications of electrolyte disturbances and acid-base balance. *Handb Clin Neurol*. 2014;119:365–382.
255. Beyer MJ, Freestone JF, Reimer JM, et al. Idiopathic hypocalcemia in foals. *J Vet Intern Med*. 1997;11:356–360.
256. Schwarz B, van den Hoven R. Seizures in an Arabian foal due to suspected prolonged transient neonatal hypoparathyroidism. *Equine Veterinary Education*. 2012;24:225–232.
257. Loynachan AT, Williams NM, Freestone JF. Kernicterus in a neonatal foal. *J Vet Diagn Invest*. 2007;19:209–212.
258. Broux B, Lefere L, Deprez P, et al. Plasma exchange as a treatment for hyperbilirubinemia in 2 foals with neonatal isoerythrolysis. *J Vet Intern Med*. 2015;29:736–738.
259. Stickle JE, McKnight CA, Williams KJ, et al. Diarrhea and hyperammonemia in a horse with progressive neurologic signs. *Vet Clin Pathol*. 2006;35:250–253.
260. Dunkel B, Chaney KP, Dallap-Schaer BL, et al. Putative intestinal hyperammonaemia in horses: 36 cases. *Equine Vet J*. 2011;43:133–140.
261. Unt VE, McSloy A, Kerbyson N, et al. Gastrointestinal hyperammonaemia in a 35-day-old Warmblood-cross filly. *Equine Veterinary Education*. 2012;24:387–391.

262. Ness SL, Kennedy LA, Slovis NM. Hyperammonemic encephalopathy associated with portal vein thrombosis in a thoroughbred foal. *J Vet Intern Med*. 2013;27:382–386.
263. McConnico RS, Duckett WM, Wood PA. Persistent hyperammonemia in two related Morgan weanlings. *J Vet Intern Med*. 1997;11:264–266.
264. Ojala M, Ala-Huikku J. Inheritance of hydrocephalus in horses. *Equine Vet J*. 1992;24:140–143.
265. Sipma KD, Cornillie P, Saulez MN, et al. Phenotypic characteristics of hydrocephalus in stillborn Friesian foals. *Vet Pathol*. 2013;50:1037–1042.
266. Oey L, Muller JM, von Klopmann T, et al. Diagnosis of internal and external hydrocephalus in a warmblood foal using magnetic resonance imaging. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2011;39:41–45.
267. Mayhew IG, Watson AG, Heissan JA. Congenital occipitoatlantoaxial malformations in the horse. *Equine Vet J*. 1978;10: 103–113.
268. Rosenstein DS, Schott 2nd HC, Stickle RL. Imaging diagnosis—occipitoatlantoaxial malformation in a miniature horse foal. *Vet Radiol Ultrasound*. 2000;41:218–219.
269. Viu J, Armengou L, Jose-Cunilleras E, et al. Cranioencephalic malformation with atlanto-occipital luxation in an Andalusian neonate foal. *J Vet Intern Med*. 2010;24:639–642.
270. Dungworth DL, Fowler ME. Cerebellar hypoplasia and degeneration in a foal. *Cornell Vet*. 1966;56:17–24.
271. Brault LS, Penedo MC. The frequency of the equine cerebellar abiotrophy mutation in non-Arabian horse breeds. *Equine Vet J*. 2011;43:727–731.
272. DeBowes RM, Leipold HW, Turner-Beatty M. Cerebellar abiotrophy. *Vet Clin North Am Equine Pract*. 1987;3:345–352.
273. Aleman M, Gray LC, Williams DC, et al. Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985-2005). *J Vet Intern Med*. 2006;20:1443–1449.
274. Mittel L. Seizures in the horse. *Vet Clin North Am Equine Pract*. 1987;3:323–332.
275. Lichter-Peled A, Polani S, Stanyon R, et al. Role of KCNQ2 and KCNQ3 genes in juvenile idiopathic epilepsy in Arabian foals. *Vet J*. 2013;196:57–63.
276. Bathen-Nothen A, Heider C, Fernandez AJ, et al. Hypocretin measurement in an Icelandic foal with narcolepsy. *J Vet Intern Med*. 2009;23:1299–1302.
277. Ludvikova E, Nishino S, Sakai N, et al. Familial narcolepsy in the Lipizzaner horse: a report of three fillies born to the same sire. *Vet Q*. 2012;32:99–102.
278. Mayhew IG. *Large animal neurology*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2008.
279. Wilkins PA. Perinatal asphyxia syndrome. In: Sprayberry KA, Robinson NE, eds. *Robinson's current therapy in equine medicine*. St. Louis: Elsevier Saunders; 2015:732–736.
280. Magdesian KG. Intensive care medicine. In: Higgins AJ, Snyder JR, eds. *The equine manual*. Philadelphia: W. B. Saunders Co; 2006:1255–1326.
281. Magdesian KG. Foals are not just mini horses. In: Cole C, Bentz B, Maxwell L, eds. *Equine pharmacology*. Oxford, UK: Wiley-Blackwell; 2015:99–117.
282. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2013;1. CD003311.
283. Sarkar S, Barks J. Management of neonatal morbidities during hypothermia treatment. *Semin Fetal Neonatal Med*. 2015;20: 97–102.
284. Jacob SW, de la Torre JC. Pharmacology of dimethyl sulfoxide in cardiac and CNS damage. *Pharmacol Rep*. 2009;61:225–235.
285. Galinsky R, Bennet L, Groenendaal F, et al. Magnesium is not consistently neuroprotective for perinatal hypoxia-ischemia in term-equivalent models in preclinical studies: a systematic review. *Dev Neurosci*. 2014;36:73–82.
286. Bhat MA, Charoo BA, Bhat JI, et al. Magnesium sulfate in severe perinatal asphyxia: a randomized, placebo-controlled trial. *Pediatrics*. 2009;123:e764–e769.
287. Liska DA, Akucewich LH, Marsella R, et al. Pharmacokinetics of pentoxifylline and its 5-hydroxyhexyl metabolite after oral and intravenous administration of pentoxifylline to healthy adult horses. *Am J Vet Res*. 2006;67:1621–1627.
288. Chaudhari T, McGuire W. Allopurinol for preventing mortality and morbidity in newborn infants with hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2012;7. CD006817.
289. Juul SE, Ferriero DM. Pharmacologic neuroprotective strategies in neonatal brain injury. *Clin Perinatol*. 2014;41:119–131.
290. Wu Q, Chen W, Sinha B, et al. Neuroprotective agents for neonatal hypoxic-ischemic brain injury. *Drug Discov Today*. 2015;20:1372–1381.
291. Wilkins PA, Otto CM, Baumgardner JE, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: The Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine. *J Vet Emerg Crit Care*. 2007;17:333–339.
292. Bernard GR, Artigas A, Brigham KL, et al. Report of the American-European Consensus conference on acute respiratory distress syndrome: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Consensus Committee. *J Crit Care*. 1994;9:72–81.
293. Dunkel B. Acute lung injury and acute respiratory distress syndrome in foals. *Clin Tech Equine Pract*. 2006;5:127–133.
294. Han S, Mallampalli RK. The acute respiratory distress syndrome: from mechanism to translation. *J Immunol*. 2015;194: 855–860.
295. Bedenice D, Heuwieser W, Solano M, et al. Risk factors and prognostic variables for survival of foals with radiographic evidence of pulmonary disease. *J Vet Intern Med*. 2003;17: 868–875.
296. Holme N, Chetcuti P. The pathophysiology of respiratory distress syndrome in neonates. *Pediatr and Child Health*. 2012; 22:507–512.
297. Subiramanian S, Sweet DG. Management of neonatal respiratory distress syndrome. *Pediatr and Child Health*. 2012;22: 518–522.
298. Freeman KP, Cline JM, Simmons R, et al. Recognition of bronchopulmonary dysplasia in a newborn foal. *Equine Vet J*. 1989;21:292–296.
299. Wilkins PA, Seahorn T. Acute respiratory distress syndrome. *Vet Clin North Am Equine Pract*. 2004;20:253–273.
300. Bancalari E, Claure N. Non-invasive ventilation of the preterm infant. *Early Hum Dev*. 2008;84:815–819.
301. Abubakar KM. Complications of mechanical ventilation. In: Elzouki AY, Harfi HA, Nazer HM, et al., eds. *Textbook of clinical pediatrics*. 2nd ed. Berlin: Springer; 2012:251–256.
302. Deis JN, Abramo TJ, Crawley L. Noninvasive respiratory support. *Pediatr Emerg Care*. 2008;24:331–338. quiz 339.
303. Wong DM, Alcott CJ, Wang C, et al. Physiologic effects of nasopharyngeal administration of supplemental oxygen at various flow rates in healthy neonatal foals. *Am J Vet Res*. 2010;71: 1081–1088.
304. Hoffman AM, Viel L. A percutaneous transtracheal catheter system for improved oxygenation in foals with respiratory distress. *Equine Vet J*. 1992;24:239–241.
305. Ho JJ, Subramaniam P, Davis PG. Continuous distending pressure for respiratory distress in preterm infants. *Cochrane Database Syst Rev*. 2015;7. CD002271.
306. Hoffman AM, Kupcinskas RL, Paradis MR. Comparison of alveolar ventilation, oxygenation, pressure support, and respiratory system resistance in response to noninvasive versus conventional mechanical ventilation in foals. *Am J Vet Res*. 1997;58:1463–1467.

307. Koterba AM, Haibel GK, Grimmet JB. Respiratory distress in a premature foal secondary to hydrops allantois and placentitis. *Compend Contin Educ Pract Vet*. 1983;5:S121–S125.
308. McKean R, Raidal SL, Nielsen SG, et al. Novel continuous positive airway pressure system for respiratory support of foals. *61st AAEP Convention*. 2015:47–48.
309. Marik PE, Pastores SM, Annane D, et al. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med*. 2008;36:1937–1949.
310. Meduri GU, Bridges L, Shih MC, et al. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. *Intensive Care Med*. 2015;42:829–840.
311. Lakritz J, Wilson WD, Berry CR, et al. Bronchointerstitial pneumonia and respiratory distress in young horses: clinical, clinicopathologic, radiographic, and pathological findings in 23 cases (1984-1989). *J Vet Intern Med*. 1993;7:277–288.
312. Dunkel B, Dolente B, Boston RC. Acute lung injury/acute respiratory distress syndrome in 15 foals. *Equine Vet J*. 2005;37:435–440.
313. Hart KA, Barton MH, Vandenplas ML, et al. Effects of low-dose hydrocortisone therapy on immune function in neonatal horses. *Pediatr Res*. 2011;70:72–77.
314. Hart KA, Dirikolu L, Ferguson DC, et al. Daily endogenous cortisol production and hydrocortisone pharmacokinetics in adult horses and neonatal foals. *Am J Vet Res*. 2012;73:68–75.
315. Hart KA. Review of critical illness-related corticosteroid insufficiency in the neonatal foal. *AAEP Focus on the first year of life*. 2014:51–56.
316. Buergelt CD, Hines SA, Cantor G, et al. A retrospective study of proliferative interstitial lung disease of horses in Florida. *Vet Pathol*. 1986;23:750–756.
317. Prescott JF, Wilcock BP, Carman PS, et al. Sporadic, severe bronchointerstitial pneumonia of foals. *Can Vet J*. 1991;32:421–425.
318. Leclere M, Magdesian KG, Kass PH, et al. Comparison of the clinical, microbiological, radiological and haematological features of foals with pneumonia caused by *Rhodococcus equi* and other bacteria. *Vet J*. 2011;187:109–112.
319. Passamonti F, Vardi DM, Stefanetti V, et al. *Rhodococcus equi* pneumonia in foals: an assessment of the early diagnostic value of serum amyloid A and plasma fibrinogen concentrations in equine clinical practice. *Vet J*. 2015;203:211–218.
320. Sprayberry KA. Ultrasonographic examination of the equine neonate: thorax and abdomen. *Vet Clin North Am Equine Pract*. 2015;31:515–543.
321. Lascola KM, Joslyn S. Diagnostic imaging of the lower respiratory tract in neonatal foals: radiography and computed tomography. *Vet Clin North Am Equine Pract*. 2015;31:497–514.
322. Schliewert EC, Lascola KM, O'Brien RT, et al. Comparison of radiographic and computed tomographic images of the lungs in healthy neonatal foals. *Am J Vet Res*. 2015;76:42–52.
323. Muscatello G. *Rhodococcus equi* pneumonia in the foal—part 2: diagnostics, treatment and disease management. *Vet J*. 2012;192:27–33.
324. Wilkins PA, Lascola KM. Update on interstitial pneumonia. *Vet Clin North Am Equine Pract*. 2015;31:137–157.
325. Jain A, McNamara PJ. Persistent pulmonary hypertension of the newborn: advances in diagnosis and treatment. *Semin Fetal Neonatal Med*. 2015;20:262–271.
326. Nair J, Lakshminrusimha S. Update on PPHN: mechanisms and treatment. *Semin Perinatol*. 2014;38:78–91.
327. Wilkins PA. Lower respiratory problems of the neonate. *Vet Clin North Am Equine Pract*. 2003;19:19–33. v.
328. Lester GD. Respiratory disease specific to the neonate. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:586–591.
329. Shah PS, Ohlsson A. Sildenafil for pulmonary hypertension in neonates. *Cochrane Database Syst Rev*. 2011. CD005494.
330. Corley KT, Jokisalo JM. Evaluation of the compromised neonatal foal. In: Robinson NE, ed. *Robinson's current therapy in equine medicine*. 7th ed. St. Louis: Elsevier Saunders; 2015:717–721.
331. Lauterbach R. Pentoxifylline treatment of persistent pulmonary hypertension of newborn. *Eur J Pediatr*. 1993;152:460.
332. De Luca D, Piastra M, Tosi F, et al. Pharmacological therapies for pediatric and neonatal ALI/ARDS: an evidence-based review. *Curr Drug Targets*. 2012;13:906–916.
333. Wilkins PA. Congenital abnormalities of the respiratory system. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2011:609–614.
334. Robertson JT. Surgical correction of wry nose in newborn foals. *Equine Veterinary Education*. 2010;22:462–466.
335. Aylor MK, Campbell ML, Goring RL, et al. Congenital bilateral choanal atresia in a Standardbred foal. *Equine Vet J*. 1984;16:396–398.
336. Bienert-Zeit A, Ohnesorge B. Congenital bilateral choanal stenosis in a warmblood foal. *Vet Rec*. 2011;169:232b.
337. Barakzai SZ, Fraser BS, Dixon PM. Congenital defects of the soft palate in 15 mature horses. *Equine Vet J*. 2014;46:185–188.
338. Ryan CA, Sanchez LC. Nondiarrheal disorders of the gastrointestinal tract in neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:313–332. vi.
339. Krause HR, Koene M, Rustemeyer J. Transoral endoscopically assisted closure of cleft palate in foals. *Plast Reconstr Surg*. 2008;122:166e–167e.
340. Stick JA, Boles C. Subepiglottic cyst in three foals. *J Am Vet Med Assoc*. 1980;177:62–64.
341. Swarnam K, Soraisham AS, Sivanandan S. Advances in the management of meconium aspiration syndrome. *Int J Pediatr*. 2012;2012:359571.
342. Vaala W. Disorders of respiration. In: Smith BP, ed. *Large animal internal medicine*. 5th ed. St. Louis: Elsevier Mosby; 2015:266–270.
343. Wu R, Tian ZF, Zheng GF, et al. Treatment of neonates with meconium aspiration syndrome by proportional assist ventilation and synchronized intermittent mandatory ventilation: a comparison study. *Minerva Pediatr*. 2015;68:262–268.
344. El Shahed AI, Dargaville PA, Ohlsson A, et al. Surfactant for meconium aspiration syndrome in term and late preterm infants. *Cochrane Database Syst Rev*. 2014;12. CD002054.
345. Harris E, Schulzke SM, Patole SK. Pentoxifylline in preterm neonates: a systematic review. *Paediatr Drugs*. 2010;12:301–311.
346. Korhonen K, Kiuru A, Svedstrom E, et al. Pentoxifylline reduces regional inflammatory and ventilatory disturbances in meconium-exposed piglet lungs. *Pediatr Res*. 2004;56:901–906.
347. Garg N, Choudhary M, Sharma D, et al. The role of early inhaled budesonide therapy in meconium aspiration in term newborns: a randomized control study. *J Matern Fetal Neonatal Med*. 2016;29:36–40.
348. Mokra D, Mokry J. Glucocorticoids in the treatment of neonatal meconium aspiration syndrome. *Eur J Pediatr*. 2011;170:1495–1505.
349. Reuss SM, Cohen ND. Update on bacterial pneumonia in the foal and weanling. *Vet Clin North Am Equine Pract*. 2015;31:121–135.
350. Berlin D, Shaabon K, Peery D. Congenital oesophageal stricture in an Arabian filly treated by balloon dilation. *Equine Veterinary Education*. 2015;27:230–236.
351. Holcombe SJ, Hurcombe SD, Barr BS, et al. Dysphagia associated with presumed pharyngeal dysfunction in 16 neonatal foals. *Equine Vet J Suppl*. 2012:105–108.
352. Barr BS. Respiratory disorders. In: Bernard WV, Barr BS, eds. *Equine pediatric medicine*. London: Manson Publishing Ltd; 2012:169–202.

353. Wilkins PA. Other disorders of breathing. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2011:597–600.
354. Schambourg MA, Laverty S, Mullim S, et al. Thoracic trauma in foals: post mortem findings. *Equine Vet J*. 2003;35:78–81.
355. Jean D, Picandet V, Macieira S, et al. Detection of rib trauma in newborn foals in an equine critical care unit: a comparison of ultrasonography, radiography and physical examination. *Equine Vet J*. 2007;39:158–163.
356. Downs C, Rodgerson D. The use of nylon cable ties to repair rib fractures in neonatal foals. *Can Vet J*. 2011;52:307–309.
357. Groover ES, Wooldridge AA. Equine haemothorax. *Equine Veterinary Education*. 2013;25:536–541.
358. Hartley WJ, Dixon RJ. An outbreak of foal perinatal mortality due to equid herpesvirus type 1: pathological observations. *Equine Vet J*. 1979;11:215–218.
359. McCartan CG, Russell MM, Wood JL, et al. Clinical, serological and virological characteristics of an outbreak of paresis and neonatal foal disease due to equine herpesvirus-1 on a stud farm. *Vet Rec*. 1995;136:7–12.
360. Maxwell LK, Bentz BG, Bourne DW, et al. Pharmacokinetics of valacyclovir in the adult horse. *J Vet Pharmacol Ther*. 2008;31:312–320.
361. Smith KC, Blunden AS, Whitwell KE, et al. A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. *Equine Vet J*. 2003;35:496–501.
362. O'Keefe JS, Alley MR, Jones D, et al. Neonatal mortality due to equid herpesvirus 4 (EHV-4) in a foal. *Aust Vet J*. 1995;72:353–354.
363. Britton AP, Robinson JH. Isolation of influenza A virus from a 7-day-old foal with bronchointerstitial pneumonia. *Can Vet J*. 2002;43:55–56.
364. Patterson-Kane JC, Carrick JB, Axon JE, et al. The pathology of bronchointerstitial pneumonia in young foals associated with the first outbreak of equine influenza in Australia. *Equine Vet J*. 2008;40:199–203.
365. Peek SF, Landolt G, Karasin AI, et al. Acute respiratory distress syndrome and fatal interstitial pneumonia associated with equine influenza in a neonatal foal. *J Vet Intern Med*. 2004;18:132–134.
366. Miller WMC. Equine influenza. Further observations on the "coughing" outbreak. *Vet Rec*. 1965;77:455–456.
367. Vaala WE, Hamir AN, Dubovi EJ, et al. Fatal, congenitally acquired infection with equine arteritis virus in a neonatal thoroughbred. *Equine Vet J*. 1992;24:155–158.
368. Del Piero F, Wilkins PA, Lopez JW, et al. Equine viral arteritis in newborn foals: clinical, pathological, serological, microbiological and immunohistochemical observations. *Equine Vet J*. 1997;29:178–185.
369. Bell SA, Balasuriya UBR, MacLachlan NJ. Equine viral arteritis. *Clinical Techniques in Equine Practice*. 2006;5:233–238.
370. Doll ER, Kr E, Bryans JT. An outbreak of abortion caused by the equine arteritis virus. *Cornell Vet*. 1957;47:69–75.
371. Golnick W, Michalska Z, Michalak T. Natural equine viral arteritis in foals. *Schweiz Arch Tierheilkd*. 1981;123:523–533.
372. Szeredi L, Hornyak A, Denes B, et al. Equine viral arteritis in a newborn foal: parallel detection of the virus by immunohistochemistry, polymerase chain reaction and virus isolation. *J Vet Med B Infect Dis Vet Public Health*. 2003;50:270–274.
373. Bell SA, Leclere M, Gardner IA, et al. Equine adenovirus 1 infection of hospitalised and healthy foals and horses. *Equine Vet J*. 2006;38:379–381.
374. Richards AJ, Kelly DF, Knottenbelt DC, et al. Anaemia, diarrhoea and opportunistic infections in Fell ponies. *Equine Vet J*. 2000;32:386–391.
375. Studdert MJ. Primary, severe, combined immunodeficiency disease of Arabian foals. *Aust Vet J*. 1978;54:411–417.
376. Studdert MJ, Blackney MH. Isolation of an adenovirus antigenically distinct from equine adenovirus type 1 from diarrheic foal feces. *Am J Vet Res*. 1982;43:543–544.
377. Horner GW, Hunter R. Isolation of two serotypes of equine adenovirus from horses in New Zealand. *N Z Vet J*. 1982;30:62–64.
378. Gleeson LJ, Studdert MJ, Sullivan ND. Pathogenicity and immunologic studies of equine adenovirus in specific-pathogen-free foals. *Am J Vet Res*. 1978;39:1636–1642.
379. McChesney AE, England JJ, Whiteman CE, et al. Experimental transmission of equine adenovirus in Arabian and non-Arabian foals. *Am J Vet Res*. 1974;35:1015–1023.
380. Dunowska M, Wilks CR, Studdert MJ, et al. Equine respiratory viruses in foals in New Zealand. *N Z Vet J*. 2002;50:140–147.
381. Del Piero F, Wilkins PA, Timoney PJ, et al. Fatal nonneurological EHV-1 infection in a yearling filly. *Vet Pathol*. 2000;37:672–676.
382. Hartley CA, Dynon KJ, Mekuria ZH, et al. Equine gammaherpesviruses: perfect parasites? *Vet Microbiol*. 2013;167:86–92.
383. Blakeslee Jr JR, Olsen RG, McAllister ES, et al. Evidence of respiratory tract infection induced by equine herpesvirus, type 2, in the horse. *Can J Microbiol*. 1975;21:1940–1946.
384. Borchers K, Wolfinger U, Ludwig H, et al. Virological and molecular biological investigations into equine herpes virus type 2 (EHV-2) experimental infections. *Virus Res*. 1998;55:101–106.
385. Dunowska M, Howe L, Hanlon D, et al. Kinetics of Equid herpesvirus type 2 infections in a group of Thoroughbred foals. *Vet Microbiol*. 2011;152:176–180.
386. Fu ZF, Robinson AJ, Horner GW, et al. Respiratory disease in foals and the epizootiology of equine herpesvirus type 2 infection. *N Z Vet J*. 1986;34:152–155.
387. Murray MJ, Eichorn ES, Dubovi EJ, et al. Equine herpesvirus type 2: prevalence and seroepidemiology in foals. *Equine Vet J*. 1996;28:432–436.
388. Nordengrahn A, Rusvai M, Merza M, et al. Equine herpesvirus type 2 (EHV-2) as a predisposing factor for *Rhodococcus equi* pneumonia in foals: prevention of the bifactorial disease with EHV-2 immunostimulating complexes. *Vet Microbiol*. 1996;51:55–68.
389. Varga J, Fodor L, Rusvai M, et al. Prevention of *Rhodococcus equi* pneumonia of foals using two different inactivated vaccines. *Vet Microbiol*. 1997;56:205–212.
390. Brault SA, Blanchard MT, Gardner IA, et al. The immune response of foals to natural infection with equid herpesvirus-2 and its association with febrile illness. *Vet Immunol Immunopathol*. 2010;137:136–141.
391. Pusterla N, Magdesian KG, Mapes SM, et al. Assessment of quantitative PCR for equine herpesvirus-5 in blood, nasal secretions and bronchoalveolar lavage fluid for the laboratory diagnosis of equine multinodular pulmonary fibrosis. *Equine Vet J*. 2015.
392. Schwarz B, Klang A, Bezdekova B, et al. Equine multinodular pulmonary fibrosis (EMPF): five case reports. *Acta Vet Hung*. 2013;61:319–332.
393. Marenzoni ML, Passamonti F, Lepri E, et al. Quantification of Equid herpesvirus 5 DNA in clinical and necropsy specimens collected from a horse with equine multinodular pulmonary fibrosis. *J Vet Diagn Invest*. 2011;23:802–806.
394. Wong DM, Belgrave RL, Williams KJ, et al. Multinodular pulmonary fibrosis in five horses. *J Am Vet Med Assoc*. 2008;232:898–905.
395. Rezabek GB, Donahue JM, Giles RC, et al. Histoplasmosis in horses. *J Comp Pathol*. 1993;109:47–55.
396. Szeredi L, Tenk M, Janosi S, et al. A survey of equine abortion and perinatal foal losses in Hungary during a three-year period (1998-2000). *Acta Vet Hung*. 2008;56:353–367.
397. Gross TL, Mayhew IG. Gastroesophageal ulceration and candidiasis in foals. *J Am Vet Med Assoc*. 1983;182:1370–1373.
398. McClure JJ, Addison JD, Miller RI. Immunodeficiency manifested by oral candidiasis and bacterial septicemia in foals. *J Am Vet Med Assoc*. 1985;186:1195–1197.
399. Hutchison JM. Candidiasis in foals. *J Am Vet Med Assoc*. 1994;205:1114–1115.

400. Reilly LK, Palmer JE. Systemic candidiasis in four foals. *J Am Vet Med Assoc*. 1994;205:464–466.
401. Reinemeyer CR. Diagnosis and control of anthelmintic-resistant *Parascaris equorum*. *Parasit Vectors*. 2009;2(suppl 2):S8.
402. Srihakim S, Swerczek TW. Pathologic changes and pathogenesis of *Parascaris equorum* infection in parasite-free pony foals. *Am J Vet Res*. 1978;39:1155–1160.
403. von Samson-Himmelstjerna G. Anthelmintic resistance in equine parasites - detection, potential clinical relevance and implications for control. *Vet Parasitol*. 2012;185:2–8.
404. Leguillette R. Bacterial and viral respiratory disease of the neonate. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackewll; 2011:601–608.
405. Cohen ND. Causes of and farm management factors associated with disease and death in foals. *J Am Vet Med Assoc*. 1994;204:1644–1651.
406. Magnusson H. Spezifische infecktiose pneumonie beim fohlen. Ein neuer eitererreger beim pferd. *Arch Wiss Prakti Tierheilk*. 1923;50:22–38.
407. Jones AL, Sutcliffe IC, Goodfellow M. Proposal to replace the illegitimate genus name Prescottia Jones et al. 2013 with the genus name Prescottella gen. nov. and to replace the illegitimate combination Prescottia equi Jones, et al. 2013 with Prescottella equi comb. nov. *Antonie Van Leeuwenhoek*. 2013;103: 1405–1407.
408. Garrity GM. Conservation of *Rhodococcus equi* (Magnusson 1923) Goodfellow and Alderson 1977 and rejection of *Corynebacterium hoagii* (Morse 1912) Eberson 1918. *Int J Syst Evol Microbiol*. 2014;64:311–312.
409. Takai S. Epidemiology of *Rhodococcus equi* infections: a review. *Vet Microbiol*. 1997;56:167–176.
410. Sanz MG, Oliveira AF, Loynachan A, et al. Validation and evaluation of VapA-specific IgG and IgG subclass enzyme-linked immunosorbent assays (ELISAs) to identify foals with *Rhodococcus equi* pneumonia. *Equine Vet J*. 2016;48:103–108.
411. Chaffin MK, Cohen ND, Martens RJ, et al. Foal-related risk factors associated with development of *Rhodococcus equi* pneumonia on farms with endemic infection. *J Am Vet Med Assoc*. 2003;223:1791–1799.
412. Muscatello G, Anderson GA, Gilkerson JR, et al. Associations between the ecology of virulent *Rhodococcus equi* and the epidemiology of *R. equi* pneumonia on Australian thoroughbred farms. *Appl Environ Microbiol*. 2006;72:6152–6160.
413. Kuskie KR, Smith JL, Wang N, et al. Effects of location for collection of air samples on a farm and time of day of sample collection on airborne concentrations of virulent *Rhodococcus equi* at two horse breeding farms. *Am J Vet Res*. 2011;72:73–79.
414. Cohen ND, Chaffin MK, Kuskie KR, et al. Association of perinatal exposure to airborne *Rhodococcus equi* with risk of pneumonia caused by *R equi* in foals. *Am J Vet Res*. 2013;74:102–109.
415. Chaffin MK, Cohen ND, Martens RJ. Evaluation of equine breeding farm management and preventative health practices as risk factors for development of *Rhodococcus equi* pneumonia in foals. *J Am Vet Med Assoc*. 2003;222:476–485.
416. Cohen ND, Carter CN, Scott HM, et al. Association of soil concentrations of *Rhodococcus equi* and incidence of pneumonia attributable to *Rhodococcus equi* in foals on farms in central Kentucky. *Am J Vet Res*. 2008;69:385–395.
417. Muscatello G, Gerbaud S, Kennedy C, et al. Comparison of concentrations of *Rhodococcus equi* and virulent *R. equi* in air of stables and paddocks on horse breeding farms in a temperate climate. *Equine Vet J*. 2006;38:263–265.
418. Cohen ND, Kuskie KR, Smith JL, et al. Association of airborne concentration of virulent *Rhodococcus equi* with location (stall versus paddock) and month (January through June) on 30 horse breeding farms in central Kentucky. *Am J Vet Res*. 2012;73:1603–1609.
419. Giguere S, Prescott JF. Clinical manifestations, diagnosis, treatment, and prevention of *Rhodococcus equi* infections in foals. *Vet Microbiol*. 1997;56:313–334.
420. Cohen ND. *Rhodococcus equi* foal pneumonia. *Vet Clin North Am Equine Pract*. 2014;30:609–622.
421. Perkins GA, Wagner B. The development of equine immunity: current knowledge on immunology in the young horse. *Equine Vet J*. 2015;47:267–274.
422. Mousel MR, Harrison L, Donahue JM, et al. *Rhodococcus equi* and genetic susceptibility: assessing transferrin genotypes from paraffin-embedded tissues. *J Vet Diagn Invest*. 2003;15:470–472.
423. Halbert ND, Cohen ND, Slovis NM, et al. Variations in equid SLC11A1 (NRAMP1) genes and associations with *Rhodococcus equi* pneumonia in horses. *J Vet Intern Med*. 2006;20:974–979.
424. McQueen CM, Doan R, Dindot SV, et al. Identification of genomic loci associated with *Rhodococcus equi* susceptibility in foals. *PLoS One*. 2014;9. e98710.
425. Madrigal RG, Shaw SD, Witkowski LA, et al. Use of serial quantitative PCR of the vapA gene of *Rhodococcus equi* in feces for early detection of *R. equi* pneumonia in foals. *J Vet Intern Med*. 2016;30:664–670.
426. Giguere S, Hernandez J, Gaskin J, et al. Performance of five serological assays for diagnosis of *Rhodococcus equi* pneumonia in foals. *Clin Diagn Lab Immunol*. 2003;10:241–245.
427. Sanz MG, Villarino N, Ferreira-Oliveira A, et al. VapA-specific IgG and IgG subclasses responses after natural infection and experimental challenge of foals with *Rhodococcus equi*. *Vet Immunol Immunopathol*. 2015;164:10–15.
428. McCracken JL, Slovis NM. Use of thoracic ultrasound for the prevention of *Rhodococcus equi* pneumonia on endemic farms. *55th Annual Convention AAEP*. 2009:38–44.
429. Giguere S, Cohen ND, Chaffin MK, et al. *Rhodococcus equi*: clinical manifestations, virulence, and immunity. *J Vet Intern Med*. 2011;25:1221–1230.
430. Burton AJ, Giguere S, Sturgill TL, et al. Macrolide- and rifampin-resistant *Rhodococcus equi* on a horse breeding farm, Kentucky. *USA*. *Emerg Infect Dis*. 2013;19:282–285.
431. Venner M, Astheimer K, Lammer M, et al. Efficacy of mass antimicrobial treatment of foals with subclinical pulmonary abscesses associated with *Rhodococcus equi*. *J Vet Intern Med*. 2013;27:171–176.
432. Reuss SM, Chaffin MK, Cohen ND. Extrapulmonary disorders associated with *Rhodococcus equi* infection in foals: 150 cases (1987-2007). *J Am Vet Med Assoc*. 2009;235:855–863.
433. Giguere S, Roberts GD. Association between radiographic pattern and outcome in foals with pneumonia caused by *Rhodococcus equi*. *Vet Radiol Ultrasound*. 2012;53:601–604.
434. Chaffin MK, Cohen ND, Blodgett GP, et al. Evaluation of ultrasonographic screening parameters for predicting subsequent onset of clinically apparent *Rhodococcus equi* pneumonia in foals. *59th Convention AAEP*. 2013:267–268.
435. Hildebrand F, Venner M, Giguere S. Efficacy of gamithromycin for the treatment of foals with mild to moderate bronchopneumonia. *J Vet Intern Med*. 2015;29:333–338.
436. Venner M, Credner N, Lammer M, et al. Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with *Rhodococcus equi*. *Vet Rec*. 2013;173:397.
437. Venner M, Kerth R, Klug E. Evaluation of tulathromycin in the treatment of pulmonary abscesses in foals. *Vet J*. 2007;174:418–421.
438. Venner M, Reinhold B, Beyerbach M, et al. Efficacy of azithromycin in preventing pulmonary abscesses in foals. *Vet J*. 2009;179:301–303.
439. Shaw SD, Cohen ND, Chaffin MK, et al. Estimating the sensitivity and specificity of real-time quantitative PCR of fecal samples for diagnosis of *rhodococcus equi* pneumonia in foals. *J Vet Intern Med*. 2015;29:1712–1717.

440. Giguere S, Cohen ND, Chaffin MK, et al. Diagnosis, treatment, control, and prevention of infections caused by *Rhodococcus equi* in foals. *J Vet Intern Med*. 2011;25:1209–1220.
441. Giguere S, Jacks S, Roberts GD, et al. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med*. 2004;18:568–573.
442. Suarez-Mier G, Giguere S, Lee EA. Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals. *J Vet Pharmacol Ther*. 2007;30:109–115.
443. Berghaus LJ, Giguere S, Sturgill TL, et al. Plasma pharmacokinetics, pulmonary distribution, and in vitro activity of gamithromycin in foals. *J Vet Pharmacol Ther*. 2012;35:59–66.
444. Peters J, Block W, Oswald S, et al. Oral absorption of clarithromycin is nearly abolished by chronic comedication of rifampicin in foals. *Drug Metab Dispos*. 2011;39:1643–1649.
445. Peters J, Eggers K, Oswald S, et al. Clarithromycin is absorbed by an intestinal uptake mechanism that is sensitive to major inhibition by rifampicin: results of a short-term drug interaction study in foals. *Drug Metab Dispos*. 2012;40:522–528.
446. Berghaus LJ, Giguere S, Guldbech K. Mutant prevention concentration and mutant selection window for 10 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol*. 2013;166:670–675.
447. Takai S, Takeda K, Nakano Y, et al. Emergence of rifampin-resistant *Rhodococcus equi* in an infected foal. *J Clin Microbiol*. 1997;35:1904–1908.
448. Giguere S, Lee E, Williams E, et al. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R equi*. *J Am Vet Med Assoc*. 2010;237:74–81.
449. Giguere S, Gaskin JM, Miller C, et al. Evaluation of a commercially available hyperimmune plasma product for prevention of naturally acquired pneumonia caused by *Rhodococcus equi* in foals. *J Am Vet Med Assoc*. 2002;220:59–63.
450. Higuchi T, Arakawa T, Hashikura S, et al. Effect of prophylactic administration of hyperimmune plasma to prevent *Rhodococcus equi* infection on foals from endemically affected farms. *Zentralbl Veterinarmed B*. 1999;46:641–648.
451. Hurley JR, Begg AP. Failure of hyperimmune plasma to prevent pneumonia caused by *Rhodococcus equi* in foals. *Aust Vet J*. 1995;72:418–420.
452. Madigan JE, Hietala S, Muller N. Protection against naturally acquired *Rhodococcus equi* pneumonia in foals by administration of hyperimmune plasma. *J Reprod Fertil Suppl*. 1991;44:571–578.
453. Martens RJ, Martens JG, Fiske RA, et al. *Rhodococcus equi* foal pneumonia: protective effects of immune plasma in experimentally infected foals. *Equine Vet J*. 1989;21:249–255.
454. Chaffin MK, Cohen ND, Martens RJ. Chemoprophylactic effects of azithromycin against *Rhodococcus equi*-induced pneumonia among foals at equine breeding farms with endemic infections. *J Am Vet Med Assoc*. 2008;232:1035–1047.
455. Chaffin MK, Cohen ND, Martens RJ, et al. Evaluation of the efficacy of gallium maltolate for chemoprophylaxis against pneumonia caused by *Rhodococcus equi* infection in foals. *Am J Vet Res*. 2011;72:945–957.
456. Giles C, Vanniasinkam T, Ndi S, et al. *Rhodococcus equi (Prescottella equi)* vaccines: the future of vaccine development. *Equine Vet J*. 2015;47:510–518.
457. Roch A, Hraiech S, Dizier S, et al. Pharmacological interventions in acute respiratory distress syndrome. *Ann Intensive Care*. 2013;3:20.
458. Mckenzie HC. Treating foal pneumonia. *Compend Equine*. 2006:47–53.
459. McKenzie 3rd HC, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days. *Am J Vet Res*. 2004;65:173–178.
460. Ioannidou E, Siempos II, Falagas ME. Administration of antimicrobials via the respiratory tract for the treatment of patients with nosocomial pneumonia: a meta-analysis. *J Antimicrob Chemother*. 2007;60:1216–1226.
461. Kollef MH, Hamilton CW, Montgomery AB. Aerosolized antibiotics: do they add to the treatment of pneumonia? *Curr Opin Infect Dis*. 2013;26:538–544.
462. McKenzie 3rd HC, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration of gentamicin to horses. *Am J Vet Res*. 2000;61:1185–1190.
463. McKenzie HC. Characterization of antimicrobial aerosols for administration to horses. *Vet Ther*. 2003;4:110–119.
464. Art T, de Moffarts B, Bedoret D, et al. Pulmonary function and antimicrobial concentration after marbofloxacin inhalation in horses. *Vet Rec*. 2007;161:348–350.
465. Winther L, Baptiste KE, Friis C. Antimicrobial disposition in pulmonary epithelial lining fluid of horses, part III. Cefquinome. *J Vet Pharmacol Ther*. 2011;34:482–486.
466. Fultz L, Giguere S, Berghaus LJ, et al. Pulmonary pharmacokinetics of desfuroylceftiofur acetamide after nebulisation or intramuscular administration of ceftiofur sodium to weanling foals. *Equine Vet J*. 2015;47:473–477.
467. McKenzie HC. Monitoring and treating the respiratory system. In: Corley KTT, Stephen J, eds. *The equine hospital manual*. Oxford: Blackwell; 2008:457–483.
468. Donn SM, Sinha SK. Newer techniques of mechanical ventilation: an overview. *Semin Neonatol*. 2002;7:401–407.
469. Palmer JE. Ventilatory support of the critically ill foal. *Vet Clin North Am Equine Pract*. 2005;21:457–486. vii-viii.
470. Wilkins PA, Palmer JE. Mechanical ventilation in foals with botulism: 9 cases (1989-2002). *J Vet Intern Med*. 2003;17:708–712.
471. Morgan-Hughes NJ, Mills GH, Northwood D. Air flow resistance of three heat and moisture exchanging filter designs under wet conditions: implications for patient safety. *Br J Anaesth*. 2001;87:289–291.
472. Tobin MJ. Mechanical ventilation. *N Engl J Med*. 1994;330:1056–1061.
473. Wood GC, Boucher BA, Croce MA, et al. Aerosolized ceftazidime for prevention of ventilator-associated pneumonia and drug effects on the proinflammatory response in critically ill trauma patients. *Pharmacotherapy*. 2002;22:972–982.
474. Attar MA, Donn SM. Mechanisms of ventilator-induced lung injury in premature infants. *Semin Neonatol*. 2002;7:353–360.
475. Tobin MJ. Advances in mechanical ventilation. *N Engl J Med*. 2001;344:1986–1996.
476. Dreyfuss D, Saumon G. Role of tidal volume, FRC, and end-inspiratory volume in the development of pulmonary edema following mechanical ventilation. *Am Rev Respir Dis*. 1993;148:1194–1203.
477. MacIntyre NR. Respiratory mechanics in the patient who is weaning from the ventilator. *Respir Care*. 2005;50:275–286. discussion 275–286.
478. Furr M. Evaluation of colic in the neonatal foal. In: Robinson NE, ed. *Current therapy in equine medicine*. 4th ed. Philadelphia: WB Saunders; 1997:627–631.
479. Mackinnon MC, Southwood LL, Burke MJ, et al. Colic in equine neonates: 137 cases (2000-2010). *J Am Vet Med Assoc*. 2013;243:1586–1595.
480. Palmer JE. Colic and diaphragmatic hernias in neonatal foals. *Equine Veterinary Education*. 2012;24:340–342.
481. Pusterla N, Magdesian KG, Maleski K, et al. Retrospective evaluation of the use of acetylcysteine enemas in the treatment of meconium retention in foals: 44 cases (1987-2002). *Equine Veterinary Education*. 2010;16:133–136.
482. Palm F, Nagel C, Bruckmaier RM, et al. Clinical parameters, intestinal function, and IGF1 concentrations in colostrum-deprived and colostrum-fed newborn pony foals. *Theriogenology*. 2013;80:1045–1051.

483. Knottenbelt DC, Holdstock N, Madigan JE. *Equine neonatology: medicine and surgery*. Edinburgh: Saunders; 2004.
484. Lyons ET, Tolliver SC, Collins SS. Field studies on endoparasites of Thoroughbred foals on seven farms in central Kentucky in 2004. *Parasitol Res*. 2006;98:496–500.
485. Southwood LL, Baxter GM, Bennet DG, et al. Ascarid impaction in young horses. *Comp Cont Educ Pract Vet*. 1998;20:100–106.
486. Cribb NC, Cote NM, Boure LP, et al. Acute small intestinal obstruction associated with *Parascaris equorum* infection in young horses: 25 cases (1985–2004). *N Z Vet J*. 2006;54:338–343.
487. Tatz AJ, Segev G, Steinman A, et al. Surgical treatment for acute small intestinal obstruction caused by *Parascaris equorum* infection in 15 horses (2002-2011). *Equine Vet J Suppl*. 2012:111–114.
488. Lyons ET, Tolliver SC, Ionita M, et al. Evaluation of parasiticidal activity of fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate in horse foals with emphasis on ascarids *(Parascaris equorum)* in field studies on five farms in Central Kentucky in 2007. *Parasitol Res*. 2008;103:287–291.
489. Reinemeyer CR. Anthelmintic resistance in non-strongylid parasites of horses. *Vet Parasitol*. 2012;185:9–15.
490. Armstrong SK, Woodgate RG, Gough S, et al. The efficacy of ivermectin, pyrantel and fenbendazole against *Parascaris equorum* infection in foals on farms in Australia. *Vet Parasitol*. 2014;205:575–580.
491. Epstein KL. Congenital causes of gastrointestinal disease. *Equine Veterinary Education*. 2014;26:345–346.
492. Hunter B, Belgrave RL. Atresia coli in a foal: diagnosis made with colonoscopy aided by N-butylscopolammonium bromide. *Equine Veterinary Education*. 2010;22:429–433.
493. Nelson BB, Ferris RA, McCue PM, et al. Surgical management of atresia ani and perineal hypospadias in a miniature donkey foal. *Equine Veterinary Education*. 2015;27:525–529.
494. Lightbody T. Foal with Overo lethal white syndrome born to a registered quarter horse mare. *Can Vet J*. 2002;43:715–717.
495. Metallinos DL, Bowling AT, Rine J. A missense mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome: an equine version of Hirschsprung disease. *Mamm Genome*. 1998;9:426–431.
496. Santschi EM, Purdy AK, Valberg SJ, et al. Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. *Mamm Genome*. 1998;9:306–309.
497. Giancola F, Gentilini F, Romagnoli N, et al. Extrinsic innervation of ileum and pelvic flexure of foals with ileocolonic aganglionosis. *Cell Tissue Res*. 2016;366:13–22.
498. Ayala-Valdovinos MA, Galindo-Garcia J, Sanchez-Chipres D, et al. New test for endothelin receptor type B (EDNRB) mutation genotyping in horses. *Mol Cell Probes*. 2016;30:182–184.
499. Johnston RH, Kamstra LD, Kohler PH. Mares' milk composition as related to "foal heat" scours. *J Anim Sci*. 1970;31:549–553.
500. Kuhl J, Winterhoff N, Wulf M, et al. Changes in fecal bacteria and occurrence of diarrhea during the first 6 weeks of life in foals born to mares supplemented with oral β-carotene. *Animal Reproduction Science*. 2010;121:371–373.
501. Sgorbini M, Nardoni S, Mancianti F, et al. Foal-heat diarrhea is not caused by the presence of yeasts in gastrointestinal tract of foals. *Journal of Equine Veterinary Science*. 2008;28:145–148.
502. Masri MD, Merritt AM, Gronwall R, et al. Faecal composition in foal heat diarrhoea. *Equine Vet J*. 1986;18:301–306.
503. Magdesian KG. Neonatal foal diarrhea. *Vet Clin North Am Equine Pract*. 2005;21:295–312. vi.
504. Good M, Sodhi CP, Hackam DJ. Evidence-based feeding strategies before and after the development of necrotizing enterocolitis. *Expert Rev Clin Immunol*. 2014;10:875–884.
505. Yager JA. The pathogenesis of *Rhodococcus equi* pneumonia in foals. *Vet Microbiol*. 1987;14:225–232.
506. Jones RL, Adney WS, Alexander AF, et al. Hemorrhagic necrotizing enterocolitis associated with *Clostridium difficile* infection in four foals. *J Am Vet Med Assoc*. 1988;193:76–79.
507. Cudd T, Pauly TH. Necrotizing enterocolitis in two equine neonates. *Comp Cont Educ Pract Vet*. 1987;9:88–89.
508. East LM, Savage CJ, Traub-Dargatz JL, et al. Enterocolitis associated with *Clostridium perfringens* infection in neonatal foals: 54 cases (1988-1997). *J Am Vet Med Assoc*. 1998;212:1751–1756.
509. Uzal FA, Diab SS, Blanchard P, et al. *Clostridium perfringens* type C and *Clostridium difficile* co-infection in foals. *Vet Microbiol*. 2012;156:395–402.
510. Warner SL, Boggs J, Lee JK, et al. Clinical, pathological, and genetic characterization of *Listeria monocytogenes* causing sepsis and necrotizing typhlocolitis and hepatitis in a foal. *J Vet Diagn Invest*. 2012;24:581–586.
511. de Solis CN, Palmer JE, Boston RC, et al. The importance of ultrasonographic pneumatosis intestinalis in equine neonatal gastrointestinal disease. *Equine Vet J Suppl*. 2012:64–68.
512. Grishin A, Papillon S, Bell B, et al. The role of the intestinal microbiota in the pathogenesis of necrotizing enterocolitis. *Semin Pediatr Surg*. 2013;22:69–75.
513. Neu J. Necrotizing enterocolitis: the mystery goes on. *Neonatology*. 2014;106:289–295.
514. Stewart CJ, Cummings SP. Gut bacteria and necrotizing enterocolitis: cause or effect? *Trends Microbiol*. 2015;23:332–333.
515. Lim JC, Golden JM, Ford HR. Pathogenesis of neonatal necrotizing enterocolitis. *Pediatr Surg Int*. 2015;31:509–518.
516. Hay Jr WW. Strategies for feeding the preterm infant. *Neonatology*. 2008;94:245–254.
517. Ramani M, Ambalavanan N. Feeding practices and necrotizing enterocolitis. *Clin Perinatol*. 2013;40:1–10.
518. Chowning R, Radmacher P, Lewis S, et al. A retrospective analysis of the effect of human milk on prevention of necrotizing enterocolitis and postnatal growth. *J Perinatol*. 2016;36:221–224.
519. Luo LJ, Li X, Yang KD, et al. Broad-spectrum antibiotic plus metronidazole may not prevent the deterioration of necrotizing enterocolitis from stage II to III in full-term and near-term infants: a propensity score-matched cohort study. *Medicine (Baltimore)*. 2015;94. e1862.
520. Paradis MR. Nutritonal support: enteral and parenteral. *Clin Tech Eq Pract*. 2003;2:87–95.
521. Ramey DW, Reinertson EL. Sand-induced diarrhea in a foal. *J Am Vet Med Assoc*. 1984;185:537–538.
522. Frederick J, Giguere S, Sanchez LC. Infectious agents detected in the feces of diarrheic foals: a retrospective study of 233 cases (2003-2008). *J Vet Intern Med*. 2009;23:1254–1260.
523. Bailey KE, Gilkerson JR, Browning GF. Equine rotaviruses—current understanding and continuing challenges. *Vet Microbiol*. 2013;167:135–144.
524. Slovis NM, Elam J, Estrada M, et al. Infectious agents associated with diarrhoea in neonatal foals in central Kentucky: a comprehensive molecular study. *Equine Vet J*. 2014;46:311–316.
525. Fielding CL, Higgins JK, Higgins JC, et al. Disease associated with equine coronavirus infection and high case fatality rate. *J Vet Intern Med*. 2015;29:307–310.
526. Traub-Dargatz JL, Gay CC, Evermann JF, et al. Epidemiologic survey of diarrhea in foals. *J Am Vet Med Assoc*. 1988;192:1553–1556.
527. Guy JS, Breslin JJ, Breuhaus B, et al. Characterization of a coronavirus isolated from a diarrheic foal. *J Clin Microbiol*. 2000;38:4523–4526.
528. Navas de Solis C, Foreman JH. Transient diabetes mellitus in a neonatal Thoroughbred foal. *J Vet Emerg Crit Care (San Antonio)*. 2010;20:611–615.
529. Corrier DE, Montgomery D, Scutchfield WL. Adenovirus in the intestinal epithelium of a foal with prolonged diarrhea. *Vet Pathol*. 1982;19:564–567.
530. Mair TS, Taylor FG, Harbour DA, et al. Concurrent cryptosporidium and coronavirus infections in an Arabian foal with combined immunodeficiency syndrome. *Vet Rec*. 1990;126:127–130.

531. Browning GF, Chalmers RM, Snodgrass DR, et al. The prevalence of enteric pathogens in diarrhoeic thoroughbred foals in Britain and Ireland. *Equine Vet J*. 1991;23:405–409.
532. Davis E, Rush BR, Cox J, et al. Neonatal enterocolitis associated with coronavirus infection in a foal: a case report. *J Vet Diagn Invest*. 2000;12:153–156.
533. Baker JC, Ames TR. Total parenteral nutritional therapy of a foal with diarrhoea from which parvovirus-like particles were identified. *Equine Vet J*. 1987;19:342–344.
534. Biermann U, Herbst W, Krauss H, et al. [Electron microscopic detection rate of enteral viruses in diarrhea of dogs, cats, calves, swine and foals in the year 1988—electron microscopic study results]. *Berl Munch Tierarztl Wochenschr*. 1989;102:412–414.
535. Conner ME, Darlington RW. Rotavirus infection in foals. *Am J Vet Res*. 1980;41:1699–1703.
536. Mino S, Kern A, Barrandeguy M, et al. Comparison of two commercial kits and an in-house ELISA for the detection of equine rotavirus in foal feces. *J Virol Methods*. 2015;222:1–10.
537. Jones RL, Adney WS, Shideler RK. Isolation of *Clostridium difficile* and detection of cytotoxin in the feces of diarrheic foals in the absence of antimicrobial treatment. *J Clin Microbiol*. 1987;25:1225–1227.
538. Traub-Dargatz JL, Jones RL. *Clostridia*-associated enterocolitis in adult horses and foals. *Vet Clin North Am Equine Pract*. 1993;9:411–421.
539. Weese JS, Staempfli HR, Prescott JF. A prospective study of the roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in equine diarrhoea. *Equine Vet J*. 2001;33:403–409.
540. Medina-Torres CE, Weese JS, Staempfli HR. Prevalence of *Clostridium difficile* in horses. *Vet Microbiol*. 2011;152:212–215.
541. Diab SS, Songer G, Uzal FA. *Clostridium difficile* infection in horses: a review. *Vet Microbiol*. 2013;167:42–49.
542. Magdesian KG, Hirsh DC, Jang SS, et al. Characterization of *Clostridium difficile* isolates from foals with diarrhea: 28 cases (1993-1997). *J Am Vet Med Assoc*. 2002;220:67–73.
543. Diab SS, Rodriguez-Bertos A, Uzal FA. Pathology and diagnostic criteria of *Clostridium difficile* enteric infection in horses. *Vet Pathol*. 2013;50:1028–1036.
544. Baverud V, Gunnarsson A, Karlsson M, et al. Antimicrobial susceptibility of equine and environmental isolates of *Clostridium difficile*. *Microb Drug Resist*. 2004;10:57–63.
545. Weese JS, Toxopeus L, Arroyo L. *Clostridium difficile* associated diarrhoea in horses within the community: predictors, clinical presentation and outcome. *Equine Vet J*. 2006;38:185–188.
546. Kuehne SA, Cartman ST, Minton NP. Both, toxin A and toxin B, are important in *Clostridium difficile* infection. *Gut Microbes*. 2011;2:252–255.
547. Vedantam G, Clark A, Chu M, et al. *Clostridium difficile* infection: toxins and non-toxin virulence factors, and their contributions to disease establishment and host response. *Gut Microbes*. 2012;3:121–134.
548. Mehdizadeh Gohari I, Parreira VR, Timoney JF, et al. NetF-positive *Clostridium perfringens* in neonatal foal necrotising enteritis in Kentucky. *Vet Rec*. 2016;178:216.
549. Mehdizadeh Gohari I, Kropinski AM, Weese SJ, et al. Plasmid characterization and chromosome analysis of two netF+ clostridium perfringens isolates associated with foal and canine necrotizing enteritis. *PLoS One*. 2016;11. e0148344.
550. Jang SS, Hansen LM, Breher JE, et al. Antimicrobial susceptibilities of equine isolates of *Clostridium difficile* and molecular characterization of metronidazole-resistant strains. *Clin Infect Dis*. 1997;25(suppl 2):S266–S267.
551. Swain EA, Magdesian KG, Kass PH, et al. Pharmacokinetics of metronidazole in foals: influence of age within the neonatal period. *J Vet Pharmacol Ther*. 2015;38:227–234.
552. Weese JS, Cote NM, deGannes RV. Evaluation of in vitro properties of di-tri-octahedral smectite on clostridial toxins and growth. *Equine Vet J*. 2003;35:638–641.
553. John J, Roediger K, Schroedl W, et al. Development of intestinal microflora and occurrence of diarrhoea in sucking foals: effects of *Bacillus cereus* var. *toyoi* supplementation. *BMC Vet Res*. 2015;11:355.
554. Schoster A, Staempfli HR, Abrahams M, et al. Effect of a probiotic on prevention of diarrhea and *Clostridium difficile* and *Clostridium perfringens* shedding in foals. *J Vet Intern Med*. 2015;29:925–931.
555. Myers LL, Shoop DS, Byars TD. Diarrhea associated with enterotoxigenic *Bacteroides fragilis* in foals. *Am J Vet Res*. 1987;48:1565–1567.
556. Walker RL, Madigan JE, Hird DW, et al. An outbreak of equine neonatal salmonellosis. *J Vet Diagn Invest*. 1991;3:223–227.
557. Holland RE, Sriranganathan N, DuPont L. Isolation of enterotoxigenic *Escherichia coli* from a foal with diarrhea. *J Am Vet Med Assoc*. 1989;194:389–391.
558. Mallicote M, House AM, Sanchez LC. A review of foal diarrhoea from birth to weaning. *Equine Veterinary Education*. 2012;24:206–214.
559. McGurrin MK, Vengust M, Arroyo LG, et al. An outbreak of *Lawsonia intracellularis* infection in a standardbred herd in Ontario. *Can Vet J*. 2007;48:927–930.
560. Frazer ML. *Lawsonia intracellularis* infection in horses: 2005-2007. *J Vet Intern Med*. 2008;22:1243–1248.
561. Lavoie JP, Drolet R, Parsons D, et al. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine Vet J*. 2000;32:418–425.
562. Pusterla N, Gebhart C. Equine proliferative enteropathy caused by *Lawsonia intracellularis*. *Equine Veterinary Education*. 2009;21:415–419.
563. Pusterla N, Gebhart C. *Lawsonia intracellularis* infection and proliferative enteropathy in foals. *Vet Microbiol*. 2013;167:34–41.
564. Atherton RP, McKenzie HC. Alternative antimicrobial agents in the treatment of proliferative enteropathy in horses. *J Eq Vet Sci*. 2006;26:535–541.
565. Page AE, Stills Jr HF, Horohov DW. The effect of passively acquired antibodies on *Lawsonia intracellularis* infection and immunity in the horse. *Equine Vet J*. 2015;47:655–661.
566. Pusterla N, Vannucci FA, Mapes SM, et al. Efficacy of an avirulent live vaccine against *Lawsonia intracellularis* in the prevention of proliferative enteropathy in experimentally infected weanling foals. *Am J Vet Res*. 2012;73:741–746.
567. Arroyo LG, Ter Woort F, Baird JD, et al. *Lawsonia intracellularis*-associated ulcerative and necro-hemorrhagic enteritis in 5 weanling foals. *Can Vet J*. 2013;54:853–858.
568. Page AE, Fallon LH, Bryant UK, et al. Acute deterioration and death with necrotizing enteritis associated with *Lawsonia intracellularis* in 4 weanling horses. *J Vet Intern Med*. 2012;26:1476–1480.
569. Netherwood T, Wood JL, Townsend HG, et al. Foal diarrhoea between 1991 and 1994 in the United Kingdom associated with *Clostridium perfringens,* rotavirus, *Strongyloides westeri* and *Cryptosporidium* spp. *Epidemiol Infect*. 1996;117:375–383.
570. Cole DJ, Cohen ND, Snowden K, et al. Prevalence of and risk factors for fecal shedding of *Cryptosporidium parvum* oocysts in horses. *J Am Vet Med Assoc*. 1998;213:1296–1302.
571. Grinberg A, Oliver L, Learmonth JJ, et al. Identification of *Cryptosporidium parvum* "cattle" genotype from a severe outbreak of neonatal foal diarrhoea. *Vet Rec*. 2003;153:628–631.
572. Grinberg A, Pomroy WE, Carslake HB, et al. A study of neonatal cryptosporidiosis of foals in New Zealand. *N Z Vet J*. 2009;57:284–289.
573. Ryan U, Xiao L, Read C, et al. Identification of novel *Cryptosporidium* genotypes from the Czech Republic. *Appl Environ Microbiol*. 2003;69:4302–4307.
574. Burton AJ, Nydam DV, Dearen TK, et al. The prevalence of *Cryptosporidium,* and identification of the *Cryptosporidium* horse genotype in foals in New York State. *Vet Parasitol*. 2010;174:139–144.

575. Galuppi R, Piva S, Castagnetti C, et al. *Cryptosporidium parvum:* from foal to veterinary students. *Vet Parasitol*. 2016;219:53–56.
576. Lyons ET, Tolliver SC. Prevalence of patent *Strongyloides westeri* infections in Thoroughbred foals in 2014. *Parasitol Res*. 2014;113:4163–4164.
577. Murray MJ, Grodinsky C, Cowles RR, et al. Endoscopic evaluation of changes in gastric lesions of Thoroughbred foals. *J Am Vet Med Assoc*. 1990;196:1623–1627.
578. Elfenbein JR, Sanchez LC. Prevalence of gastric and duodenal ulceration in 691 nonsurviving foals (1995-2006). *Equine Vet J Suppl*. 2012:76–79.
579. Okai K, Taharaguchi S, Orita Y, et al. Comparative endoscopic evaluation of normal and ulcerated gastric mucosae in thoroughbred foals. *J Vet Med Sci*. 2015.
580. Murray MJ, Murray CM, Sweeney HJ, et al. Prevalence of gastric lesions in foals without signs of gastric disease: an endoscopic survey. *Equine Vet J*. 1990;22:6–8.
581. Murray MJ. Gastroduodenal ulceration in foals. *Equine Vet Educ*. 1999;11:199–207.
582. Becht JL, Byars TD. Gastroduodenal ulceration in foals. *Equine Vet J*. 1986;18:307–312.
583. Sanchez LC, Lester GD, Merritt AM. Intragastric pH in critically ill neonatal foals and the effect of ranitidine. *J Am Vet Med Assoc*. 2001;218:907–911.
584. Barr BS, Wilkins PA, Del Piero F, et al. Is prophylaxis for gastric ulcers necessary in critically ill equine neonates? A retrospective study of necropsy cases 1989-1999. *18th American College of Veterinary Internal Medicine Forum*. 2000:705.
585. Herzig SJ, Howell MD, Ngo LH, et al. Acid-suppressive medication use and the risk for hospital-acquired pneumonia. *JAMA*. 2009;301:2120–2128.
586. Buendgens L, Bruensing J, Matthes M, et al. Administration of proton pump inhibitors in critically ill medical patients is associated with increased risk of developing *Clostridium difficile*-associated diarrhea. *J Crit Care*. 2014;29(696):e611–e695.
587. Alhazzani W, Alenezi F, Jaeschke RZ, et al. Proton pump inhibitors versus histamine 2 receptor antagonists for stress ulcer prophylaxis in critically ill patients: a systematic review and meta-analysis. *Crit Care Med*. 2013;41:693–705.
588. MacLaren R, Reynolds PM, Allen RR. Histamine-2 receptor antagonists vs proton pump inhibitors on gastrointestinal tract hemorrhage and infectious complications in the intensive care unit. *JAMA Intern Med*. 2014;174:564–574.
589. Furr M, Cohen ND, Axon JE, et al. Treatment with histamine-type 2 receptor antagonists and omeprazole increases the risk of diarrhoea in neonatal foals treated in intensive care units. *Equine Vet J Suppl*. 2012:80–86.
590. Sprayberry KA. Gastroduodenal ulcer syndrome in foals. In: Robinson NE, Sprayberry KA, eds. *Robinson's current therapy in equine medicine*. St. Louis: Saunders Elsevier; 2014: 753–757.
591. Coleman MC, Slovis NM, Hunt RJ. Long-term prognosis of gastrojejunostomy in foals with gastric outflow obstruction: 16 cases (2001-2006). *Equine Vet J*. 2009;41:653–657.
592. Sanchez LC, Lester GD, Merritt AM. Effect of ranitidine on intragastric pH in clinically normal neonatal foals. *J Am Vet Med Assoc*. 1998;212:1407–1412.
593. Sanchez LC, Murray MJ, Merritt AM. Effect of omeprazole paste on intragastric pH in clinically normal neonatal foals. *Am J Vet Res*. 2004;65:1039–1041.
594. Ryan CA, Sanchez LC, Giguere S, et al. Pharmacokinetics and pharmacodynamics of pantoprazole in clinically normal neonatal foals. *Equine Vet J*. 2005;37:336–341.
595. Geor RJ, Petrie L, Papich MG, et al. The protective effects of sucralfate and ranitidine in foals experimentally intoxicated with phenylbutazone. *Can J Vet Res*. 1989;53:231–238.
596. Sangiah S, MacAllister CC, Amouzadeh HR. Effects of misoprostol and omeprazole on basal gastric pH and free acid content in horses. *Res Vet Sci*. 1989;47:350–354.
597. Blikslager AT. Misoprostol: is it safety or a lack of understanding that prevents its more frequent usage? *Equine Veterinary Journal*. 2013;45. 8–8.
598. Andrews FM, Rosol TJ, Kohn CW, et al. Bilateral renal hypoplasia in four young horses. *J Am Vet Med Assoc*. 1986;189:209–212.
599. Brown CM, Parks AH, Mullaney TP, et al. Bilateral renal dysplasia and hypoplasia in a foal with an imperforate anus. *Vet Rec*. 1988;122:91–92.
600. Zicker SC, Marty GD, Carlson GP, et al. Bilateral renal dysplasia with nephron hypoplasia in a foal. *J Am Vet Med Assoc*. 1990;196:2001–2005.
601. Jones SL, Langer DL, Sterner-Kock A, et al. Renal dysplasia and benign ureteropelvic polyps associated with hydronephrosis in a foal. *J Am Vet Med Assoc*. 1994;204:1230–1234.
602. Gull T, Schmitz DG, Bahr A, et al. Renal hypoplasia and dysplasia in an American miniature foal. *Vet Rec*. 2001;149:199–203.
603. Naylor RJ, Mair TS, Last R. Severe hyperkalaemia associated with renal dysplasia in a 2-day-old foal. *Equine Veterinary Education*. 2009;21:358–363.
604. Gilday RA, Wojnarowicz C, Tryon KA, et al. Bilateral renal dysplasia, hydronephrosis, and hydroureter in a septic neonatal foal. *Can Vet J*. 2015;56:257–260.
605. Toribio RE, Bain FT, Mrad DR, et al. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc*. 1998;212:697–701.
606. Ordidge RM. Urinary incontinence due to unilateral ureteral ectopia in a foal. *Vet Rec*. 1976;98:384.
607. Houlton JE, Wright IM, Matic S, et al. Urinary incontinence in a shire foal due to ureteral ectopia. *Equine Vet J*. 1987;19:244–247.
608. Schott HC. Ectopic ureter - a leaky problem no matter how you look at it! *Equine Veterinary Education*. 2011;23:603–605.
609. Schott 2nd HC, Barbee DD, Hines MT, et al. Clinical vignette. Renal arteriovenous malformation in a quarter horse foal. *J Vet Intern Med*. 1996;10:204–206.
610. Pascoe RR. Repair of a defect in the bladder of a foal. *Aust Vet J*. 1971;47:343–344.
611. Wellington JK. Bladder defects in newborn foals. *Aust Vet J*. 1972;48:426.
612. Dunkel B, Palmer JE, Olson KN, et al. Uroperitoneum in 32 foals: influence of intravenous fluid therapy, infection, and sepsis. *J Vet Intern Med*. 2005;19:889–893.
613. Kablack KA, Embertson RM, Bernard WV, et al. Uroperitoneum in the hospitalised equine neonate: retrospective study of 31 cases, 1988-1997. *Equine Vet J*. 2000;32:505–508.
614. Behr MJ, Hackett RP, Bentinck-Smith J, et al. Metabolic abnormalities associated with rupture of the urinary bladder in neonatal foals. *J Am Vet Med Assoc*. 1981;178:263–266.
615. Richardson DW, Kohn CW. Uroperitoneum in the foal. *J Am Vet Med Assoc*. 1983;182:267–271.
616. Vaala W. Manifestations and management of disease in foals: distended and/or painful abdomen. In: Lester G, ed. *Large animal internal medicine*. 5th. ed. St. Louis: Elsevier Mosby; 2015:273–278.
617. Love EJ. Anaesthesia in foals with uroperitoneum. *Equine Veterinary Education*. 2011;23:508–511.
618. Morresey PR. Umbilical problems. *AAEP Focus on the First Year of Life*. 2014:18–21.
619. Morresey PR. The placenta. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. Ames: Wiley-Blackwell; 2011:84–95.
620. Magri M. Ultrasonography of umbilical structures. In: Kidd JA, Lu KG, Frazer ML, eds. *Atlas of equine ultrasonography*. Oxford, UK: John Wiley & Sons, Ltd; 2014.
621. Reef VB. *Equine diagnostic ultrasound*. Philadelphia: WB Saunders; 1998.
622. McAuliffe SB. Abdominal ultrasonography of the foal. *Clinical Techniques in Equine Practice*. 2004;3:308–316.
623. Porter MB, Ramirez S. Equine neonatal thoracic and abdominal ultrasonography. *Vet Clin North Am Equine Pract*. 2005;21:407–429. vii.

624. Steiner A, Lischer CJ, Oertle C. Marsupialization of umbilical vein abscesses with involvement of the liver in 13 calves. *Vet Surg*. 1993;22:184–189.
625. Edwards 3rd RB, Fubini SL. A one-stage marsupialization procedure for management of infected umbilical vein remnants in calves and foals. *Vet Surg*. 1995;24:32–35.
626. Lopez MJ, Markel MD. Umbilical artery marsupialization in a calf. *Can Vet J*. 1996;37:170–171.
627. Toth-Heyn P, Drukker A, Guignard JP. The stressed neonatal kidney: from pathophysiology to clinical management of neonatal vasomotor nephropathy. *Pediatr Nephrol*. 2000;14:227–239.
628. Gomez H, Ince C, De Backer D, et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. *Shock*. 2014;41:3–11.
629. Vivrette S, Cowgill LD, Pascoe J, et al. Hemodialysis for treatment of oxytetracycline-induced acute renal failure in a neonatal foal. *J Am Vet Med Assoc*. 1993;203:105–107.
630. Gonda KC, Wilcke JR, Crisman MV, et al. Evaluation of iohexol clearance used to estimate glomerular filtration rate in clinically normal foals. *Am J Vet Res*. 2003;64:1486–1490.
631. Corley K, Hollis AR. Monitoring and treating the urogenital system. In: Corley K, Stephen J, eds. *The equine hospital manual*. Chichester, UK: Blackwell Publishing; 2008:736.
632. Brewer BD, Clement SF, Lotz WS, et al. A comparison of inulin, para-aminohippuric acid, and endogenous creatinine clearances as measures of renal function in neonatal foals. *J Vet Intern Med*. 1990;4:301–305.
633. Holdstock NB, Ousey JC, Rossdale PD. Glomerular filtration rate, effective renal plasma flow, blood pressure and pulse rate in the equine neonate during the first 10 days post partum. *Equine Vet J*. 1998;30:335–343.
634. McKenzie HC, Furr MO. Aminoglycoside antibiotics in neonatal foals. *Compend Contin Educ Vet*. 2003;25:457–469.
635. Divers T, Perkins G. Urinary and hepatic disorders in neonatal foals. *Clin Tech Eq Pract*. 2003;2:67–78.
636. Johansson AM, Gardner SY, Levine JF, et al. Furosemide continuous rate infusion in the horse: evaluation of enhanced efficacy and reduced side effects. *J Vet Intern Med*. 2003;17:887–895.
637. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. *Endocrinol Metab Clin North Am*. 2005;34:293–313. viii.
638. Hart KA, Barton MH, Ferguson DC, et al. Serum free cortisol fraction in healthy and septic neonatal foals. *J Vet Intern Med*. 2011;25:345–355.
639. Perogamvros I, Ray DW, Trainer PJ. Regulation of cortisol bioavailability—effects on hormone measurement and action. *Nat Rev Endocrinol*. 2012;8:717–727.
640. Cooper MS, Stewart PM. 11Beta-hydroxysteroid dehydrogenase type 1 and its role in the hypothalamus-pituitary-adrenal axis, metabolic syndrome, and inflammation. *J Clin Endocrinol Metab*. 2009;94:4645–4654.
641. Venkatesh B, Cohen J, Cooper M. Ten false beliefs about cortisol in critically ill patients. *Intensive Care Med*. 2015;41:1817–1819.
642. Hart KA, Heusner GL, Norton NA, et al. Hypothalamic-pituitary-adrenal axis assessment in healthy term neonatal foals utilizing a paired low dose/high dose ACTH stimulation test. *J Vet Intern Med*. 2009;23:344–351.
643. Hines MT. Endocrine abnormalities. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:524–532.
644. Annane D, Sebille V, Troche G, et al. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA*. 2000;283:1038–1045.
645. Beishuizen A, Thijs LG. Relative adrenal failure in intensive care: an identifiable problem requiring treatment? *Best Pract Res Clin Endocrinol Metab*. 2001;15:513–531.
646. Marik PE. Critical illness-related corticosteroid insufficiency. *Chest*. 2009;135:181–193.
647. Venkatesh B, Cohen J. Adrenocortical (dys)function in septic shock - a sick euadrenal state. *Best Pract Res Clin Endocrinol Metab*. 2011;25:719–733.
648. Briegel J, Kellermann W, Forst H, et al. Low-dose hydrocortisone infusion attenuates the systemic inflammatory response syndrome. The Phospholipase A2 Study Group. *Clin Investig*. 1994;72:782–787.
649. Bollaert PE, Charpentier C, Levy B, et al. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. *Crit Care Med*. 1998;26:645–650.
650. Annane D, Sebille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA*. 2002;288:862–871.
651. Annane D, Maxime V, Ibrahim F, et al. Diagnosis of adrenal insufficiency in severe sepsis and septic shock. *Am J Respir Crit Care Med*. 2006;174:1319–1326.
652. Hart KA, Ferguson DC, Heusner GL, et al. Synthetic adrenocorticotropic hormone stimulation tests in healthy neonatal foals. *J Vet Intern Med*. 2007;21:314–321.
653. Stewart AJ, Wright JC, Behrend EN, et al. Validation of a low-dose adrenocorticotropic hormone stimulation test in healthy neonatal foals. *J Am Vet Med Assoc*. 2013;243:399–405.
654. Couetil LL, Hoffman AM. Adrenal insufficiency in a neonatal foal. *J Am Vet Med Assoc*. 1998;212:1594–1596.
655. Gold JR, Divers TJ, Barton MH, et al. Plasma adrenocorticotropin, cortisol, and adrenocorticotropin/cortisol ratios in septic and normal-term foals. *J Vet Intern Med*. 2007;21:791–796.
656. Castagnetti C, Veronesi MC. Prognostic factors in the sick neonatal foal. *Vet Res Commun*. 2008;32(suppl 1):S87–S91.
657. de Jong MF, Molenaar N, Beishuizen A, et al. Diminished adrenal sensitivity to endogenous and exogenous adrenocorticotropic hormone in critical illness: a prospective cohort study. *Crit Care*. 2015;19:1.
658. Hoffman CJ, McKenzie 3rd HC, Furr MO, et al. Glucocorticoid receptor density and binding affinity in healthy horses and horses with systemic inflammatory response syndrome. *J Vet Intern Med*. 2015;29:626–635.
659. Baker CF, Barks JD, Engmann C, et al. Hydrocortisone administration for the treatment of refractory hypotension in critically ill newborns. *J Perinatol*. 2008;28:412–419.
660. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med*. 2013;39:165–228.
661. Venkatesh B, Cohen J. The utility of the corticotropin test to diagnose adrenal insufficiency in critical illness: an update. *Clin Endocrinol (Oxf)*. 2015;83:289–297.
662. Creedon JM. Controversies surrounding critical illness-related corticosteroid insufficiency in animals. *J Vet Emerg Crit Care (San Antonio)*. 2015;25:107–112.
663. Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med*. 2012;185:133–139.
664. Fielding CL, Magdesian KG. Sepsis and septic shock in the equine neonate. *Vet Clin North Am Equine Pract*. 2015;31:483–496.
665. Castagnetti C, Mariella J. Anti-inflammatory drugs in equine neonatal medicine. Part II: corticosteroids. *Journal of Equine Veterinary Science*. 2015;35:547–554.
666. Lyle-Dugas J, Giguere S, Mallicote MF, et al. Factors associated with outcome in 94 hospitalised foals diagnosed with neonatal encephalopathy. *Equine Vet J*. 2016.
667. Hart KA, Barton MH. Adrenocortical insufficiency in horses and foals. *Vet Clin North Am Equine Pract*. 2011;27:19–34.
668. Marik PE. Glucocorticoids in sepsis: dissecting facts from fiction. *Crit Care*. 2011;15:158.
669. Irvine CH, Evans MJ. Postnatal changes in total and free thyroxine and triiodothyronine in foal serum. *J Reprod Fertil Suppl*. 1975:709–715.

670. Chen CL, Riley AM. Serum thyroxine and triiodothyronine concentrations in neonatal foals and mature horses. *Am J Vet Res*. 1981;42:1415–1417.
671. Himler M, Hurcombe SD, Griffin A, et al. Presumptive nonthyroidal illness syndrome in critically ill foals. *Equine Vet J Suppl*. 2012:43–47.
672. Panzani S, Comin A, Galeati G, et al. How type of parturition and health status influence hormonal and metabolic profiles in newborn foals. *Theriogenology*. 2012;77:1167–1177.
673. Pirrone A, Panzani S, Govoni N, et al. Thyroid hormone concentrations in foals affected by perinatal asphyxia syndrome. *Theriogenology*. 2013;80:624–629.
674. Breuhaus BA. Thyroid function and dysfunction in term and premature equine neonates. *J Vet Intern Med*. 2014;28: 1301–1309.
675. Berg EL, McNamara DL, Keisler DH. Endocrine profiles of periparturient mares and their foals. *J Anim Sci*. 2007;85: 1660–1668.
676. Doige CE, McLaughlin BG. Hyperplastic goitre in newborn foals in Western Canada. *Can Vet J*. 1981;22:42–45.
677. McLaughlin BG, Doige CE. Congenital musculosketal lesions and hyperplastic goitre in foals. *Can Vet J*. 1981;22:130–133.
678. Allen AL, Doige CE, Fretz PB, et al. Hyperplasia of the thyroid gland and concurrent musculoskeletal deformities in western Canadian foals: reexamination of a previously described syndrome. *Can Vet J*. 1994;35:31–38.
679. Breuhaus BA. Disorders of the equine thyroid gland. *Vet Clin North Am Equine Pract*. 2011;27:115–128.
680. Koikkalainen K, Knuuttila A, Karikoski N, et al. Congenital hypothyroidism and dysmaturity syndrome in foals: first reported cases in Europe. *Equine Veterinary Education*. 2014;26:181–189.
681. Kreplin C, Allen A. Alberta. Congenital hypothyroidism in foals in Alberta. *Can Vet J*. 1991;32:751.
682. Allen AL, Townsend HG, Doige CE, et al. A case-control study of the congenital hypothyroidism and dysmaturity syndrome of foals. *Can Vet J*. 1996;37:349–351, 354-348.
683. Allen A. Congenital hypothyroidism in foals. *Can Vet J*. 2001;42:418.
684. Gawrylash SK. Thyroid hyperplasia and musculoskeletal deformity in a standardbred filly in Ontario. *Can Vet J*. 2004;45:424–426.
685. Allen AL. Hyperplasia of the thyroid gland and musculoskeletal deformities in two equine abortuses. *Can Vet J*. 1995;36:234–236.
686. McLaughlin BG, Doige CE, McLaughlin PS. Thyroid hormone levels in foals with congenital musculoskeletal lesions. *Can Vet J*. 1986;27:264–267.
687. Hines MT, Gay C, Talcott C. Congenital hypothyroidism and dysmaturity syndrome of foals: diagnosis and possible risk factors. *15th ACVIM Forum*. 1997:363–364.
688. Hines MT, Doles J, Gay C, et al. Thyroid hyperplasia and musculoskeletal deformity in neonatal foals. *14th ACVIM Forum*. 1996:544–545.
689. Drew B, Barber WP, Williams DG. The effect of excess dietary iodine on pregnant mares and foals. *Vet Rec*. 1975;97:93–95.
690. Irvine CH. Hypothyroidism in the foal. *Equine Vet J*. 1984;16: 302–306.
691. Eroksuz H, Eroksuz Y, Ozer H, et al. Equine goiter associated with excess dietary iodine. *Vet Hum Toxicol*. 2004;46:147–149.
692. Boosinger TR, Brendemuehl JP, Bransby DL, et al. Prolonged gestation, decreased triiodothyronine concentration, and thyroid gland histomorphologic features in newborn foals of mares grazing *Acremonion coenophialum*-infected fescue. *Am J Vet Res*. 1995;56:66–69.
693. Fisher DA. Thyroid function in premature infants. The hypothyroxinemia of prematurity. *Clin Perinatol*. 1998;25:999–1014. viii.
694. Williams FL, Visser TJ, Hume R. Transient hypothyroxinaemia in preterm infants. *Early Hum Dev*. 2006;82:797–802.
695. Hilderbran AC, Breuhaus BA, Refsal KR. Nonthyroidal illness syndrome in adult horses. *J Vet Intern Med*. 2014;28:609–617.
696. Breuhaus BA, Refsal KR, Beyerlein SL. Measurement of free thyroxine concentration in horses by equilibrium dialysis. *J Vet Intern Med*. 2006;20:371–376.
697. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. *Thyroid*. 2014;24:1456–1465.
698. de Vries EM, Fliers E, Boelen A. The molecular basis of the nonthyroidal illness syndrome. *J Endocrinol*. 2015;225:R67–R81.
699. DeGroot LJ. The non-thyroidal illness syndrome. In: DeGroot LJ, Beck-Peccoz P, Chrousos G, eds. *Endotext (Internet)*. South Dartmouth, MA: MDText.com, Inc.; 2015.
700. Barsnick RJ, Toribio RE. Endocrinology of the equine neonate energy metabolism in health and critical illness. *Vet Clin North Am Equine Pract*. 2011;27:49–58.
701. Barsnick R. *Hormones of energy metabolism in critically Ill foals: insulin, glucagon, leptin, adiponectin, ghrelin and growth hormone.* Graduate School of The Ohio State University. Columbus, OH: The Ohio State University; 2010:59.
702. Renaville R, Hammadi M, Portetelle D. Role of the somatotropic axis in the mammalian metabolism. *Domest Anim Endocrinol*. 2002;23:351–360.
703. Simpson HL, Jackson NC, Shojaee-Moradie F, et al. Insulin-like growth factor I has a direct effect on glucose and protein metabolism, but no effect on lipid metabolism in type 1 diabetes. *J Clin Endocrinol Metab*. 2004;89:425–432.
704. Silver M, Fowden AL. Sympathoadrenal and other endocrine and metabolic responses to hypoglycaemia in the fetal foal during late gestation. *Exp Physiol*. 1995;80:651–662.
705. Stammers JP, Hull D, Leadon DP, et al. Maternal and umbilical venous plasma lipid concentrations at delivery in the mare. *Equine Vet J*. 1991;23:119–122.
706. Fowden AL, Mundy L, Ousey JC, et al. Tissue glycogen and glucose 6-phosphatase levels in fetal and newborn foals. *J Reprod Fertil Suppl*. 1991;44:537–542.
707. Axon JE, Palmer JE. Clinical pathology of the foal. *Vet Clin North Am Equine Pract*. 2008;24:357–385. vii.
708. Gayle JM, Cohen ND, Chaffin MK. Factors associated with survival in septicemic foals: 65 cases (1988-1995). *J Vet Intern Med*. 1998;12:140–146.
709. Barsnick RJ, Hurcombe SD, Smith PA, et al. Insulin, glucagon, and leptin in critically ill foals. *J Vet Intern Med*. 2011;25: 123–131.
710. Wintergerst KA, Buckingham B, Gandrud L, et al. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. *Pediatrics*. 2006;118:173–179.
711. Sung J, Bochicchio GV, Joshi M, et al. Admission hyperglycemia is predictive of outcome in critically ill trauma patients. *J Trauma*. 2005;59:80–83.
712. Hassel DM, Hill AE, Rorabeck RA. Association between hyperglycemia and survival in 228 horses with acute gastrointestinal disease. *J Vet Intern Med*. 2009;23:1261–1265.
713. Hollis AR, Boston RC, Corley KT. Blood glucose in horses with acute abdominal disease. *J Vet Intern Med*. 2007;21: 1099–1103.
714. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med*. 2001;345: 1359–1367.
715. Investigators N-SS, Finfer S, Chittock DR, et al. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med*. 2009;360:1283–1297.
716. Boonen E, Van den Berghe G. Endocrine responses to critical illness: novel insights and therapeutic implications. *J Clin Endocrinol Metab*. 2014;99:1569–1582.
717. Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response! *Crit Care*. 2013;17:305.
718. Palmer J. Update on the management of neonatal sepsis in horses. *Vet Clin North Am Equine Pract*. 2014;30:317–336. vii.

719. Krause JB, McKenzie 3rd HC. Parenteral nutrition in foals: a retrospective study of 45 cases (2000—2004). *Equine Vet J*. 2007;39:74–78.
720. McKenzie 3rd HC, Geor RJ. Feeding management of sick neonatal foals. *Vet Clin North Am Equine Pract*. 2009;25:109–119. vii.
721. Barsnick RJ, Hurcombe SD, Dembek K, et al. Somatotropic axis resistance and ghrelin in critically ill foals. *Equine Vet J*. 2014;46:45–49.
722. Mesotten D, Van den Berghe G. Changes within the growth hormone/insulin-like growth factor I/IGF binding protein axis during critical illness. *Endocrinol Metab Clin North Am*. 2006;35:793–805. ix-x.
723. Sellon DC. Secondary immunodeficiencies of horses. *Vet Clin North Am Equine Pract*. 2000;16:117–130.
724. Giguere S, Polkes AC. Immunologic disorders in neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:241–272. v.
725. Jeffcott LB. Some practical aspects of the transfer of passive immunity to newborn foals. *Equine Vet J*. 1974;6:109–115.
726. Crisman MV, Scarratt WK. Immunodeficiency disorders in horses. *Vet Clin North Am Equine Pract*. 2008;24:299–310. vi.
727. Clabough DL, Conboy HS, Roberts MC. Comparison of four screening techniques for the diagnosis of equine neonatal hypogammaglobulinemia. *J Am Vet Med Assoc*. 1989;194: 1717–1720.
728. Rumbaugh GE, Ardans AA, Ginno D, et al. Measurement of neonatal equine immunoglobulins for assessment of colostral immunoglobulin transfer: comparison of single radial immunodiffusion with the zinc sulfate turbidity test, serum electrophoresis, refractometry for total serum protein, and the sodium sulfite precipitation test. *J Am Vet Med Assoc*. 1978;172:321–325.
729. Kent JE, Blackmore DJ. Measurement of IgG in equine blood by immunoturbidimetry and latex agglutination. *Equine Vet J*. 1985;17:125–129.
730. Beetson SA, Hilbert BJ, Mills JN. The use of the glutaraldehyde coagulation test for detection of hypogammaglobulinaemia in neonatal foals. *Aust Vet J*. 1985;62:279–281.
731. Saikku A, Koskinen E, Sandholm M. Detection of hypogammaglobulinaemia in neonatal foals using the glutaraldehyde coagulation test. *Zentralbl Veterinarmed B*. 1989;36:168–174.
732. Pusterla N, Pusterla JB, Spier SJ, et al. Evaluation of the SNAP foal IgG test for the semiquantitative measurement of immunoglobulin G in foals. *Vet Rec*. 2002;151:258–260.
733. Metzger N, Hinchcliff KW, Hardy J, et al. Usefulness of a commercial equine IgG test and serum protein concentration as indicators of failure of transfer of passive immunity in hospitalized foals. *J Vet Intern Med*. 2006;20:382–387.
734. McCue PM. Evaluation of a turbidimetric immunoassay for measurement of plasma IgG concentration in foals. *Am J Vet Res*. 2007;68:1005–1009.
735. Wong DM, Giguere S, Wendel MA. Evaluation of a point-of-care portable analyzer for measurement of plasma immunoglobulin G, total protein, and albumin concentrations in ill neonatal foals. *J Am Vet Med Assoc*. 2013;242:812–819.
736. Tscheschlok L, Venner M, Howard J. Comparison of IgG concentrations by radial immunodiffusion, electrophoretic gamma globulin concentrations and total globulins in neonatal foals. *Equine Vet J*. 2016.
737. Fouche N, Graubner C, Howard J. Correlation between serum total globulins and gamma globulins and their use to diagnose failure of passive transfer in foals. *Vet J*. 2014;202:384–386.
738. Nath LC, Anderson GA, Savage CJ, et al. Use of stored equine colostrum for the treatment of foals perceived to be at risk for failure of transfer of passive immunity. *J Am Vet Med Assoc*. 2010;236:1085–1090.
739. LeBlanc MM. Immunologic considerations. In: Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Lea & Febiger; 1990:280.
740. Vivrette SL. Assesmment and modification of passive transfer. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:346–352.
741. Johnson JR. Neonatal isoerythrolysis. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2011:353–360.
742. Bailey E. Prevalence of anti-red blood cel antibodies in the serum and colostrum of mares and its relationship to neonatal isoerythrolysis. *Am J Vet Res*. 1982;43:1917–1921.
743. Becht JL. Neonatal isoerythrolysis in the foal. 1. Background, blood group antigens, and pathogenesis. *Comp Cont Educ Pract Vet*. 1985;5:S591–S596.
744. de Graaf-Roelfsema E, van der Kolk JH, Boerma S, et al. Non-specific haemolytic alloantibody causing equine neonatal isoerythrolysis. *Vet Rec*. 2007;161:202–204.
745. Brumpt E. Considerations defavorables a l'hypothese de l'etiologie parasitaire de la jaunisse des muletons: jaundice in the newly born mule foals not caused by babesia infections. *Ann Parasitol Hum Comp*. 1947;22:5–10.
746. McClure JJ, Koch C, Traub-Dargatz J. Characterization of a red blood cell antigen in donkeys and mules associated with neonatal isoerythrolysis. *Anim Genet*. 1994;25:119–120.
747. Traub-Dargatz JL, McClure JJ, Koch C, et al. Neonatal isoerythrolysis in mule foals. *J Am Vet Med Assoc*. 1995;206:67–70.
748. Uner AG, Kaya G, Oltu A. Prevention of predicted neonatal isoerythrolysis with jaundice foal agglutination test in a newborn foal. *Turkish Journal of Veterinary & Animal Sciences*. 2012;36:734–736.
749. Palm F, Kuhl J, Walter I, et al. Colostrum withdrawal is without effect on duodenal development in newborn foals. *Journal of Equine Veterinary Science*. 2015;35:584–590.
750. Perkins GA, Divers TJ. Polymerized hemoglobin therapy in a foal with neonatal isoerythrolysis. *J Vet Emerg Crit Care*. 2001;11:141–146.
751. Sprayberry KA. Neonatal transfusion medicine: the use of blood, plasma, oxygen-carrying solutions, and adjunctive therapies in foals. *Clin Tech Eq Pract*. 2003;2:31–41.
752. Hollis AR, Corley KTT. Initial experience of ultrapurified bovine haemoglobin use in horses. *Equine Veterinary Education*. 2011;23:562–568.
753. Tocci LJ, Ewing PJ. Increasing patient safety in veterinary transfusion medicine: an overview of pretransfusion testing. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:66–73.
754. Blackmer JM, Paccamonti D, Eilts B, et al. Strategies for preventing neonatal isoerythrolysis. *Compendium on Continuing Education for the Practicing Veterinarian*. 2002;24:562.
755. Buechner-Maxwell V, Scott MA, Godber L, et al. Neonatal alloimmune thrombocytopenia in a quarter horse foal. *J Vet Intern Med*. 1997;11:304–308.
756. Ramirez S, Gaunt SD, McClure JJ, et al. Detection and effects on platelet function of anti-platelet antibody in mule foals with experimentally induced neonatal alloimmune thrombocytopenia. *J Vet Intern Med*. 1999;13:534–539.
757. Sockett DC, Traub-Dargatz J, Weiser MG. Immune-mediated hemolytic anemia and thrombocytopenia in a foal. *J Am Vet Med Assoc*. 1987;190:308–310.
758. Davis EG, Rush B, Bain F, et al. Neonatal neutropenia in an Arabian foal. *Equine Vet J*. 2003;35:517–520.
759. Wong DM, Alcott CJ, Clark SK, et al. Alloimmune neonatal neutropenia and neonatal isoerythrolysis in a Thoroughbred colt. *J Vet Diagn Invest*. 2012;24:219–226.
760. Perkins GA, Miller WH, Divers TJ, et al. Ulcerative dermatitis, thrombocytopenia, and neutropenia in neonatal foals. *J Vet Intern Med*. 2005;19:211–216.
761. Agueda S, Rocha G, Ferreira F, et al. Neonatal alloimmune neutropenia: still a diagnostic and therapeutical challenge. *J Pediatr Hematol Oncol*. 2012;34:497–499.
762. Sellon DC. Thrombocytopenia in horses. *Eq Vet Educ*. 1998;10: 133–139.

763. Dunkel B. Platelet transfusion in thrombocytopenic horses. *Equine Veterinary Education*. 2013;25:359–362.
764. Bauer JE, Harvey JW, Asquith RL, et al. Clinical chemistry reference values of foals during the first year of life. *Equine Vet J*. 1984;16:361–363.
765. Hank AM, Hoffmann WE, Sanecki RK, et al. Quantitative determination of equine alkaline phosphatase isoenzymes in foal and adult serum. *J Vet Intern Med*. 1993;7:20–24.
766. Patterson WH, Brown CM. Increase of serum gamma-glutamyltransferase in neonatal Standardbred foals. *Am J Vet Res*. 1986;47:2461–2463.
767. Bauer JE, Asquith RL, Kivipelto J. Serum biochemical indicators of liver function in neonatal foals. *Am J Vet Res*. 1989;50:2037–2041.
768. Divers TJ, Byars TD. Hepatic disease. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:409–415.
769. Barton MH, LeRoy BE. Serum bile acids concentrations in healthy and clinically ill neonatal foals. *J Vet Intern Med*. 2007;21:508–513.
770. Fosgate GT, Hird DW, Read DH, et al. Risk factors for *Clostridium piliforme* infection in foals. *J Am Vet Med Assoc*. 2002;220:785–790.
771. Borchers A, Magdesian KG, Halland S, et al. Successful treatment and polymerase chain reaction (PCR) confirmation of Tyzzer's disease in a foal and clinical and pathologic characteristics of 6 additional foals (1986-2005). *J Vet Intern Med*. 2006;20:1212–1218.
772. Swerczek TW, Crowe MW, Prickett ME, et al. Focal bacterial hepatitis in foals: preliminary report. *Mod Vet Pract*. 1973;54:66–67.
773. Whitwell KE. Four cases of Tyzzer's disease in foals in England. *Equine Vet J*. 1976;8:118–122.
774. Yates WD, Hayes MA, Finell GR, et al. Tyzzer's disease in foals in western Canada. *Can Vet J*. 1980;21:63.
775. Carrigan MJ, Pedrana RG, McKibbin AW. Tyzzer's disease in foals. *Aust Vet J*. 1984;61:199–200.
776. Scarratt WK, Saunders GK, Welker FH, et al. *Bacillus piliformis* infection (Tyzzer's disease) in two Virginia foals. *Eq Vet Sci*. 1985;5:135–138.
777. Shirakawa T, Maruyama K, Nakamura N, et al. Tyzzer's disease in a foal. *Nihon Juigaku Zasshi*. 1989;51:444–446.
778. St Denis KA, Waddell-Parks N, Belanger M. Tyzzer's disease in an 11-day-old foal. *Can Vet J*. 2000;41:491–492.
779. Swerczek TW. Tyzzer's disease in foals: retrospective studies from 1969 to 2010. *Can Vet J*. 2013;54:876–880.
780. Peek SF, Byars TD, Rueve E. Neonatal hepatic failure in a Thoroughbred foal: successful treatment of a case of presumptive Tyzzer's disease. *Equine Vet Educ*. 1994;6:307–309.
781. Haggett EF, Magdesian KG, Kass PH. Clinical implications of high liver enzyme activities in hospitalized neonatal foals. *J Am Vet Med Assoc*. 2011;239:661–667.
782. Setlakwe EL, Sweeney R, Engiles JB, et al. Identification of *Bartonella henselae* in the liver of a thoroughbred foal with severe suppurative cholangiohepatitis. *J Vet Intern Med*. 2014;28:1341–1345.
783. Hodgin EC, Miller DA, Lozano F. *Leptospira* abortion in horses. *J Vet Diagn Invest*. 1989;1:283–287.
784. Davis JL. Liver disease in foals. In: Smith BP, ed. *Large animal internal medicine*. St. Louis: Elsevier Mosby; 2015:851–853.
785. Orsini JA, Donawick WJ. Hepaticojejunostomy for treatment of common hepatic duct obstructions associated with duodenal stenosis in two foals. *Vet Surg*. 1989;18:34–38.
786. Buote M. Cholangiohepatitis and pancreatitis secondary to severe gastroduodenal ulceration in a foal. *Can Vet J*. 2003;44:746–748.
787. Zedler ST, Embertson RM, Bernard WV, et al. Surgical treatment of gastric outflow obstruction in 40 foals. *Vet Surg*. 2009;38:623–630.
788. Bryans JT, Swerczek TW, Dr W, et al. Neonatal foal disease associated with perinatal infection by equine herpesvirus 1. *J Eq Med Surg*. 1977;1:20–26.
789. Garre B, Shebany K, Gryspeerdt A, et al. Pharmacokinetics of acyclovir after intravenous infusion of acyclovir and after oral administration of acyclovir and its prodrug valacyclovir in healthy adult horses. *Antimicrob Agents Chemother*. 2007;51:4308–4314.
790. Hughes KJ, Hodgson DR, Dart AJ. Hyperlipaemia in a 7-week-old miniature pony foal. *Aust Vet J*. 2002;80:350–351.
791. Tan RH, Hughes KJ, Hodgson DR. Hyperlipaemia, neonatal isoerythrolysis and hepatocellular necrosis in a 3-day-old Thoroughbred foal. *Aust Vet J*. 2005;83:740–741.
792. Mogg TD, Palmer JE. Hyperlipidemia, hyperlipemia, and hepatic lipidosis in American miniature horses: 23 cases (1990-1994). *J Am Vet Med Assoc*. 1995;207:604–607.
793. Dunkel B, McKenzie 3rd HC. Severe hypertriglyceridaemia in clinically ill horses: diagnosis, treatment and outcome. *Equine Vet J*. 2003;35:590–595.
794. Elfenbein JR, Giguere S, Meyer SK, et al. The effects of deferoxamine mesylate on iron elimination after blood transfusion in neonatal foals. *J Vet Intern Med*. 2010;24:1475–1482.
795. Divers TJ, Warner A, Vaala WE, et al. Toxic hepatic failure in newborn foals. *J Am Vet Med Assoc*. 1983;183:1407–1413.
796. Acland HM, Mann PC, Robertson JL, et al. Toxic hepatopathy in neonatal foals. *Vet Pathol*. 1984;21:3–9.
797. Mullaney TP, Brown CM. Iron toxicity in neonatal foals. *Equine Vet J*. 1988;20:119–124.
798. Valberg SJ, Ward TL, Rush B, et al. Glycogen branching enzyme deficiency in quarter horse foals. *J Vet Intern Med*. 2001;15:572–580.
799. Ward TL, Valberg SJ, Lear TL, et al. Genetic mapping of GBE1 and its association with glycogen storage disease IV in American Quarter horses. *Cytogenet Genome Res*. 2003;102:201–206.
800. Ward TL, Valberg SJ, Adelson DL, et al. Glycogen branching enzyme (GBE1) mutation causing equine glycogen storage disease IV. *Mamm Genome*. 2004;15:570–577.
801. Wagner ML, Valberg SJ, Ames EG, et al. Allele frequency and likely impact of the glycogen branching enzyme deficiency gene in Quarter Horse and Paint Horse populations. *J Vet Intern Med*. 2006;20:1207–1211.
802. Divers TJ, Tennant BC, Murray MJ, et al. Unusual cases of liver disease in Morgan foals. *Gastroint Viewpnt*. 1994;2:6.
803. Divers TJ. The equine liver in health and disease. *61st Annual Convention AAEP*. 2015:66–103.
804. Haechler S, Van den Ingh TS, Rogivue C, et al. Congenital hepatic fibrosis and cystic bile duct formation in Swiss Freiberger horses. *Vet Pathol*. 2000;37:669–671.
805. Drogemuller M, Jagannathan V, Welle MM, et al. Congenital hepatic fibrosis in the Franches-Montagnes horse is associated with the polycystic kidney and hepatic disease 1 (PKHD1) gene. *PLoS One*. 2014;9. e110125.
806. Buonanno AM, Carlson GP, Kantrowitz B. Clinical and diagnostic features of portosystemic shunt in a foal. *J Am Vet Med Assoc*. 1988;192:387–389.
807. Fortier LA, Fubini SL, Flanders JA, et al. The diagnosis and surgical correction of congenital portosystemic vascular anomalies in two calves and two foals. *Vet Surg*. 1996;25:154–160.
808. Hug SA, Guerrero TG, Makara M, et al. Diagnosis and surgical cellophane banding of an intrahepatic congenital portosystemic shunt in a foal. *J Vet Intern Med*. 2012;26:171–177.
809. Valberg SJ, Cardinet 3rd GH, Carlson GP, et al. Polysaccharide storage myopathy associated with recurrent exertional rhabdomyolysis in horses. *Neuromuscul Disord*. 1992;2:351–359.
810. Firshman AM, Baird JD, Valberg SJ. Prevalences and clinical signs of polysaccharide storage myopathy and shivers in Belgian draft horses. *J Am Vet Med Assoc*. 2005;227:1958–1964.
811. McCue ME, Ribeiro WP, Valberg SJ. Prevalence of polysaccharide storage myopathy in horses with neuromuscular disorders. *Equine Vet J Suppl*. 2006:340–344.

812. Valentine BA, Credille KM, Lavoie JP, et al. Severe polysaccharide storage myopathy in Belgian and Percheron draught horses. *Equine Vet J*. 1997;29:220–225.
813. Valentine BA, McDonough SP, Chang YF, et al. Polysaccharide storage myopathy in Morgan, Arabian, and Standardbred related horses and Welsh-cross ponies. *Vet Pathol*. 2000;37:193–196.
814. Naylor RJ. Polysaccharide storage myopathy – the story so far. *Equine Veterinary Education*. 2015;27:414–419.
815. McCue ME, Valberg SJ, Miller MB, et al. Glycogen synthase (GYS1) mutation causes a novel skeletal muscle glycogenosis. *Genomics*. 2008;91:458–466.
816. McCue ME, Anderson SM, Valberg SJ, et al. Estimated prevalence of the type 1 polysaccharide storage myopathy mutation in selected North American and European breeds. *Anim Genet*. 2010;41(suppl 2):145–149.
817. McCoy AM, Schaefer R, Petersen JL, et al. Evidence of positive selection for a glycogen synthase (GYS1) mutation in domestic horse populations. *J Hered*. 2014;105:163–172.
818. Baird JD, Valberg SJ, Anderson SM, et al. Presence of the glycogen synthase 1 (GYS1) mutation causing type 1 polysaccharide storage myopathy in continental European draught horse breeds. *Vet Rec*. 2010;167:781–784.
819. Stanley RL, McCue ME, Valberg SJ, et al. A glycogen synthase 1 mutation associated with equine polysaccharide storage myopathy and exertional rhabdomyolysis occurs in a variety of UK breeds. *Equine Vet J*. 2009;41:597–601.
820. De La Corte FD, Valberg SJ, MacLeay JM, et al. Developmental onset of polysaccharide storage myopathy in 4 Quarter Horse foals. *J Vet Intern Med*. 2002;16:581–587.
821. Corley KTT. Metabolic disorders in foals. *Equine Veterinary Education*. 2012;24:392–395.
822. McKenzie EC, MacLeay JM. Muscle disorders. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. 2nd ed. West Sussex, UK: Wiley-Blackwell; 2011:463–468.
823. Valberg SJ. A review of the diagnosis and treatment of rhabdomyolysis in foals. *48th Annual AAEP Convention*. 2002:117–121.
824. Sponseller BT, Valberg SJ, Ward TL, et al. Muscular weakness and recumbency in a Quarter Horse colt due to glycogen branching enzyme deficiency. *Equine Veterinary Education*. 2003;15:182–187.
825. Gabbedy BJ, Richards RB. White muscle disease in a foal. *Aust Vet J*. 1970;46:111–112.
826. Hamir AN. White muscle disease of a foal. *Aust Vet J*. 1982;59:57–58.
827. Higuchi T, Ichijo S, Osame S, et al. Studies on serum selenium and tocopherol in white muscle disease of foal. *Nihon Juigaku Zasshi*. 1989;51:52–59.
828. Katz L, O'Dwyer S, Pollock P. Nutritional muscular dystrophy in a four-day-old Connemara foal. *Ir Vet J*. 2009;62:119–124.
829. Streeter RM, Divers TJ, Mittel L, et al. Selenium deficiency associations with gender, breed, serum vitamin E and creatine kinase, clinical signs and diagnoses in horses of different age groups: a retrospective examination 1996-2011. *Equine Vet J Suppl*. 2012:31–35.
830. Dill SG, Rebhun WC. White muscle disease in foals. *Comp Cont Educ Pract Vet*. 1985;7:S627–S635.
831. Lofstedt J. White muscle disease of foals. *Vet Clin North Am Equine Pract*. 1997;13:169–185.
832. Liang SY, Kumar A. Empiric antimicrobial therapy in severe sepsis and septic shock: optimizing pathogen clearance. *Curr Infect Dis Rep*. 2015;17:493.
833. Corley KTT, Hollis AR. Antimicrobial therapy in neonatal foals. *Equine Veterinary Education*. 2009;21:436–448.
834. Theelen MJ, Wilson WD, Edman JM, et al. Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979-2010. *Equine Vet J*. 2014;46:161–168.
835. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis*. 2008;197:1079–1081.
836. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis*. 2009;48:1–12.
837. Olsen L, Ingvast-Larsson C, Brostrom H, et al. Clinical signs and etiology of adverse reactions to procaine benzylpenicillin and sodium/potassium benzylpenicillin in horses. *J Vet Pharmacol Ther*. 2007;30:201–207.
838. Henson S, Barton M. *Bacterial isolates and antibiotic sensitivity patterns from septicemic neonatal foals: a 15 year retrospective study (1986–2000)*. Dorothy R Havemeyer Foundation Neonatal Septicemia Workshop III. Talloires, France: Dorothy R Havemeyer Foundation; 2001.
839. Wilson WD, Spensley MS, Baggot JD, et al. Pharmacokinetics and bioavailability of ticarcillin and clavulanate in foals after intravenous and intramuscular administration. *J Vet Pharmacol Ther*. 1991;14:78–89.
840. Sweeney RW, Beech J, Simmons RD. Pharmacokinetics of intravenously and intramuscularly administered ticarcillin and clavulanic acid in foals. *Am J Vet Res*. 1988;49:23–26.
841. Orsini JA, Moate PJ, Boston RC, et al. Pharmacokinetics of imipenem-cilastatin following intravenous administration in healthy adult horses. *J Vet Pharmacol Ther*. 2005;28:355–361.
842. Meyer S, Giguere S, Rodriguez R, et al. Pharmacokinetics of intravenous ceftiofur sodium and concentration in body fluids of foals. *J Vet Pharmacol Ther*. 2009;32:309–316.
843. Wearn JM, Davis JL, Hodgson DR, et al. Pharmacokinetics of a continuous rate infusion of ceftiofur sodium in normal foals. *J Vet Pharmacol Ther*. 2013;36:99–101.
844. Hall TL, Tell LA, Wetzlich SE, et al. Pharmacokinetics of ceftiofur sodium and ceftiofur crystalline free acid in neonatal foals. *J Vet Pharmacol Ther*. 2011;34:403–409.
845. Pusterla N, Hall TL, Wetzlich SE, et al. Pharmacokinetic parameters for single- and multi-dose regimens for subcutaneous administration of a high-dose ceftiofur crystalline-free acid to neonatal foals. *J Vet Pharmacol Ther*. 2016.
846. Rohdich N, Zschiesche E, Heckeroth A, et al. Treatment of septicaemia and severe bacterial infections in foals with a new cefquinome formulation: a field study. *Dtsch Tierarztl Wochenschr*. 2009;116:316–320.
847. Smiet E, Haritova A, Heil BA, et al. Comparing the pharmacokinetics of a fourth generation cephalosporin in three different age groups of New Forest ponies. *Equine Vet J Suppl*. 2012:52–56.
848. Gardner SY, Papich MG. Comparison of cefepime pharmacokinetics in neonatal foals and adult dogs. *J Vet Pharmacol Ther*. 2001;24:187–192.
849. Kashuba AD, Nafziger AN, Drusano GL, et al. Optimizing aminoglycoside therapy for nosocomial pneumonia caused by gram-negative bacteria. *Antimicrob Agents Chemother*. 1999;43:623–629.
850. Burton AJ, Giguere S, Warner L, et al. Effect of age on the pharmacokinetics of a single daily dose of gentamicin sulfate in healthy foals. *Equine Vet J*. 2013;45:507–511.
851. Schnabel LV, Papich MG, Divers TJ, et al. Pharmacokinetics and distribution of minocycline in mature horses after oral administration of multiple doses and comparison with minimum inhibitory concentrations. *Equine Vet J*. 2012;44:453–458.
852. Villarino N, Martin-Jimenez T. Pharmacokinetics of macrolides in foals. *J Vet Pharmacol Ther*. 2013;36:1–13.
853. Brumbaugh GW, Martens RJ, Knight HD, et al. Pharmacokinetics of chloramphenicol in the neonatal horse. *J Vet Pharmacol Ther*. 1983;6:219–227.
854. Civljak R, Giannella M, Di Bella S, et al. Could chloramphenicol be used against ESKAPE pathogens? A review of in vitro data in the literature from the 21st century. *Expert Rev Anti Infect Ther*. 2014;12:249–264.
855. Tohamy MA, Radi AM. Bioavailability and pharmacokinetics of florfenicol in healthy foals. *J Egypt Soc Pharmacol Exp Ther*. 2008;29:529–538.

856. Vivrette S, Bostian A, Bermingham E, et al. Quinolone induced arthropathy in neonatal foals. *47th Annual American Association of Equine Practitioners Convention*. 2001:376–377.
857. Tohamy MA, El-Gendy AAM. Some pharmacokinetic aspects and bioavailability of marbofloxacin in foals. *Beni-Suef University Journal of Basic and Applied Sciences*. 2013;2: 46–50.
858. Pritchard JC, Burn CC, Barr AR, et al. Validity of indicators of dehydration in working horses: a longitudinal study of changes in skin tent duration, mucous membrane dryness and drinking behaviour. *Equine Vet J*. 2008;40:558–564.
859. Fielding CL, Magdesian KG, Edman JE. Determination of body water compartments in neonatal foals by use of indicator dilution techniques and multifrequency bioelectrical impedance analysis. *Am J Vet Res*. 2011;72:1390–1396.
860. Mange K, Matsuura D, Cizman B, et al. Language guiding therapy: the case of dehydration versus volume depletion. *Ann Intern Med*. 1997;127:848–853.
861. Besen BA, Gobatto AL, Melro LM, et al. Fluid and electrolyte overload in critically ill patients: an overview. *World J Crit Care Med*. 2015;4:116–129.
862. Kumar G, Walker E, Stephens R. Intravenous fluid therapy. *Trends in Anaesthesia and Critical Care*. 2014;4:55–59.
863. Buchanan BR, Sommardahl CS, Rohrbach BW, et al. Effect of a 24-hour infusion of an isotonic electrolyte replacement fluid on the renal clearance of electrolytes in healthy neonatal foals. *J Am Vet Med Assoc*. 2005;227:1123–1129.
864. Myburgh JA. Fluid resuscitation in acute medicine: what is the current situation? *J Intern Med*. 2015;277:58–68.
865. Perel P, Roberts I, Ker K. Colloids versus crystalloids for fluid resuscitation in critically ill patients. *Cochrane Database Syst Rev*. 2013;2. CD000567.
866. Palmer JE. Fluid therapy in the neonate: not your mother's fluid space. *Vet Clin North Am Equine Pract*. 2004;20:63–75.
867. Maitland K, George EC, Evans JA, et al. Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. *BMC Med*. 2013;11:68.
868. Polderman KH, Varon J. Do not drown the patient: appropriate fluid management in critical illness. *Am J Emerg Med*. 2015;33:448–450.
869. Divers TJ, Whitlock RH, Byars TD, et al. Acute renal failure in six horses resulting from haemodynamic causes. *Equine Vet J*. 1987;19:178–184.
870. Nout YS, Corley KTT, Donaldson LL, et al. Indirect oscillometric and direct blood pressure measurements in anesthetized and conscious neonatal foals. *Journal of Veterinary Emergency and Critical Care*. 2002;12:75–80.
871. Giguere S, Knowles Jr HA, Valverde A, et al. Accuracy of indirect measurement of blood pressure in neonatal foals. *J Vet Intern Med*. 2005;19:571–576.
872. LeDoux D, Astiz ME, Carpati CM, et al. Effects of perfusion pressure on tissue perfusion in septic shock. *Crit Care Med*. 2000;28:2729–2732.
873. Corley KT. Monitoring and treating hemodynamic disturbances in critically ill neonatal foals. Part 1: haemodynamic monitoring. *Eq Vet Educ*. 2002;14:270–277.
874. Kato R, Pinsky MR. Personalizing blood pressure management in septic shock. *Ann Intensive Care*. 2015;5:41.
875. Valverde A, Giguere S, Sanchez LC, et al. Effects of dobutamine, norepinephrine, and vasopressin on cardiovascular function in anesthetized neonatal foals with induced hypotension. *Am J Vet Res*. 2006;67:1730–1737.
876. Corley KT. Inotropes and vasopressors in adults and foals. *Vet Clin North Am Equine Pract*. 2004;20:77–106.
877. Pollard S, Edwin SB, Alaniz C. Vasopressor and inotropic management of patients with septic shock. *P T*. 2015;40:438–450.
878. Van den Berghe G, de Zegher F. Anterior pituitary function during critical illness and dopamine treatment. *Crit Care Med*. 1996;24:1580–1590.
879. Meadows D, Edwards JD, Wilkins RG, et al. Reversal of intractable septic shock with norepinephrine therapy. *Crit Care Med*. 1988;16:663–666.
880. Osborn DA, Paradisis M, Evans N. The effect of inotropes on morbidity and mortality in preterm infants with low systemic or organ blood flow. *Cochrane Database Syst Rev*. 2007. CD005090.
881. Herget-Rosenthal S, Saner F, Chawla LS. Approach to hemodynamic shock and vasopressors. *Clin J Am Soc Nephrol*. 2008;3:546–553.
882. Hollis AR, Ousey JC, Palmer L, et al. Effects of norepinephrine and a combined norepinephrine and dobutamine infusion on systemic hemodynamics and indices of renal function in normotensive neonatal thoroughbred foals. *J Vet Intern Med*. 2006;20:1437–1442.
883. Russell JA. Bench-to-bedside review: vasopressin in the management of septic shock. *Crit Care*. 2011;15:226.
884. Mutlu GM, Factor P. Role of vasopressin in the management of septic shock. *Intensive Care Med*. 2004;30:1276–1291.
885. Friesenecker BE, Tsai AG, Martini J, et al. Arteriolar vasoconstrictive response: comparing the effects of arginine vasopressin and norepinephrine. *Crit Care*. 2006;10. R75.
886. Jerath N, Frndova H, McCrindle BW, et al. Clinical impact of vasopressin infusion on hemodynamics, liver and renal function in pediatric patients. *Intensive Care Med*. 2008;34:1274–1280.
887. Serpa Neto A, Nassar AP, Cardoso SO, et al. Vasopressin and terlipressin in adult vasodilatory shock: a systematic review and meta-analysis of nine randomized controlled trials. *Crit Care*. 2012;16. R154.
888. Collins NM, Axon JE, Palmer JE. Shock. In: McKinnon AO, Squires EL, Vaala WE, et al., eds. *Equine reproduction*. West Sussex, UK: Wiley-Blackwell; 2011:136–146.
889. Dickey EJ, McKenzie 3rd H, Johnson A, et al. Use of pressor therapy in 34 hypotensive critically ill neonatal foals. *Aust Vet J*. 2010;88:472–477.
890. De Backer D, Creteur J, Silva E, et al. Effects of dopamine, norepinephrine, and epinephrine on the splanchnic circulation in septic shock: which is best? *Crit Care Med*. 2003;31:1659–1667.
891. Gordon AC, Mason AJ, Perkins GD, et al. The interaction of vasopressin and corticosteroids in septic shock: a pilot randomized controlled trial. *Crit Care Med*. 2014;42:1325–1333.
892. Venet F, Plassais J, Textoris J, et al. Low-dose hydrocortisone reduces norepinephrine duration in severe burn patients: a randomized clinical trial. *Crit Care*. 2015;19:21.
893. Hoen S, Mazoit JX, Asehnoune K, et al. Hydrocortisone increases the sensitivity to alpha1-adrenoceptor stimulation in humans following hemorrhagic shock. *Crit Care Med*. 2005;33:2737–2743.
894. Castagnetti C, Mariella J. Anti-inflammatory drugs in equine neonatal medicine. Part I: nonsteroidal anti-inflammatory drugs. *Journal of Equine Veterinary Science*. 2015;35:475–480.
895. Traub JL, Gallina AM, Grant BD, et al. Phenylbutazone toxicosis in the foal. *Am J Vet Res*. 1983;44:1410–1418.
896. Leveille R, Miyabayashi T, Weisbrode SE, et al. Ultrasonographic renal changes associated with phenylbutazone administration in three foals. *Can Vet J*. 1996;37:235–236.
897. Carrick JB, Papich MG, Middleton DM, et al. Clinical and pathological effects of flunixin meglumine administration to neonatal foals. *Can J Vet Res*. 1989;53:195–201.
898. Traub-Dargatz JL, Bertone JJ, Gould DH, et al. Chronic flunixin meglumine therapy in foals. *Am J Vet Res*. 1988;49:7–12.
899. Wilcke JR, Crisman MV, Scarratt WK, et al. Pharmacokinetics of ketoprofen in healthy foals less than twenty-four hours old. *Am J Vet Res*. 1998;59:290–292.
900. Crisman MV, Wilcke JR, Sams RA. Pharmacokinetics of flunixin meglumine in healthy foals less than twenty-four hours old. *Am J Vet Res*. 1996;57:1759–1761.
901. Wilcke JR, Crisman MV, Sams RA, et al. Pharmacokinetics of phenylbutazone in neonatal foals. *Am J Vet Res*. 1993;54: 2064–2067.

902. Barr BS. Pharmacology. In: Bernard WV, Barr BS, eds. *Equine pediatric medicine*. London: Manson Publishing Ltd; 2012: 322–346.
903. Magdesian KG. Neonatology. In: Orsini JA, Divers TJ, eds. *Equine emergencies: treatment and procedures*. 4th ed. St. Louis: Elsevier Saunders; 2014:528–564.
904. Breuhaus BA, DeGraves FJ, Honore EK, et al. Pharmacokinetics of ibuprofen after intravenous and oral administration and assessment of safety of administration to healthy foals. *Am J Vet Res*. 1999;60:1066–1073.
905. Hovanessian N, Davis JL, McKenzie 3rd HC, et al. Pharmacokinetics and safety of firocoxib after oral administration of repeated consecutive doses to neonatal foals. *J Vet Pharmacol Ther*. 2014;37:243–251.
906. Wilson KE, Davis JL, Crisman MV, et al. Pharmacokinetics of firocoxib after intravenous administration of multiple consecutive doses in neonatal foals. *J Vet Pharmacol Ther*. 2016. In press.
907. Cook VL, Meyer CT, Campbell NB, et al. Effect of firocoxib or flunixin meglumine on recovery of ischemic-injured equine jejunum. *Am J Vet Res*. 2009;70:992–1000.
908. Doucet MY, Bertone AL, Hendrickson D, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc*. 2008;232:91–97.
909. Raidal SL, Edwards S, Pippia J, et al. Pharmacokinetics and safety of oral administration of meloxicam to foals. *J Vet Intern Med*. 2013;27:300–307.
910. West E, Bardell D, Morgan R, et al. Use of acetaminophen (paracetamol) as a short-term adjunctive analgesic in a laminitic pony. *Vet Anaesth Analg*. 2011;38:521–522.
911. Rezende RM, Franca DS, Menezes GB, et al. Different mechanisms underlie the analgesic actions of paracetamol and dipyrone in a rat model of inflammatory pain. *Br J Pharmacol*. 2008;153:760–768.
912. Foreman JH, Foreman C, Bergstrom B. Medical alternatives to conventional cyclooxygenase inhibitors for treatment of acute foot pain in a reversible lameness model in horses. *J Vet Intern Med*. 2015;29:1280.
913. Graham GG, Davies MJ, Day RO, et al. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology*. 2013;21:201–232.
914. Jasiecka A, Maślanka T, Jaroszewski JJ. Pharmacological characteristics of metamizole. *Polish Journal of Veterinary Sciences*. 2014;17:207–214.
915. Merry AF, Gibbs RD, Edwards J, et al. Combined acetaminophen and ibuprofen for pain relief after oral surgery in adults: a randomized controlled trial. *Br J Anaesth*. 2010;104:80–88.
916. Fugler LA, Eades SC, Moore RM, et al. Plasma matrix metalloproteinase activity in horses after intravenous infusion of lipopolysaccharide and treatment with matrix metalloproteinase inhibitors. *Am J Vet Res*. 2013;74:473–480.
917. Sykes BW, Furr MO. Equine endotoxaemia—a state-of-the-art review of therapy. *Aust Vet J*. 2005;83:45–50.
918. Barton MH, Parviainen A, Norton N. Polymyxin B protects horses against induced endotoxaemia in vivo. *Equine Vet J*. 2004;36:397–401.
919. Wong DM, Sponseller BA, Alcott CJ, et al. Effects of intravenous administration of polymyxin B in neonatal foals with experimental endotoxemia. *J Am Vet Med Assoc*. 2013;243:874–881.
920. Elfenbein JR, Robertson SA, MacKay RJ, et al. Systemic and anti-nociceptive effects of prolonged lidocaine, ketamine, and butorphanol infusions alone and in combination in healthy horses. *BMC Vet Res*. 2014;10(suppl 1):S6.
921. Knych HK, Steffey EP, Casbeer HC, et al. Disposition, behavioural and physiological effects of escalating doses of intravenously administered fentanyl to young foals. *Equine Vet J*. 2015;47:592–598.
922. Knych HK, Steffey EP, White AM, et al. Effects of age on the pharmacokinetics of tramadol and its active metabolite, O-desmethyltramadol, following intravenous administration to foals. *Equine Vet J*. 2014:1–7.
923. Ohmes C. *The disposition of lidocaine during a 6-hour intravenous infusion to young foals*. Department of Clinical Sciences, College of Veterinary Medicine. Manhattan, KS: Kansas State University; 2014:67.
924. Ousey JC, Holdstock NB, Rossdale PD, et al. How much energy do sick neonatal foals require compared with healthy foals? *Pferdeheilkunde*. 1996;12:231–237.
925. Ousey JC. Thermoregulation and the energy requirement of the newborn foal, with reference to prematurity. *Equine Vet J Suppl*. 1997:104–108.
926. Mack JK, Remler HP, Senckenberg E, et al. Influence of the amount of concentrate feeding on concentrate intake and development of body weight and growth parameters of suckling foals from birth until the 6th month of life. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2012;40:150–156.
927. Fowden AL, Gardner DS, Ousey JC, et al. Maturation of pancreatic beta-cell function in the fetal horse during late gestation. *J Endocrinol*. 2005;186:467–473.
928. Holdstock NB, Allen VL, Bloomfield MR, et al. Development of insulin and proinsulin secretion in newborn pony foals. *J Endocrinol*. 2004;181:469–476.
929. Allen WR, Wilsher S, Tiplady C, et al. The influence of maternal size on pre- and postnatal growth in the horse: III Postnatal growth. *Reproduction*. 2004;127:67–77.
930. George LA, Staniar WB, Treiber KH, et al. Insulin sensitivity and glucose dynamics during pre-weaning foal development and in response to maternal diet composition. *Domest Anim Endocrinol*. 2009;37:23–29.
931. Forhead AJ, Ousey JC, Allen WR, et al. Postnatal insulin secretion and sensitivity after manipulation of fetal growth by embryo transfer in the horse. *J Endocrinol*. 2004;181:459–467.
932. Ousey J. Total parenteral nutrition in the young foal. *Equine Vet Educ*. 1994;6:316–317.
933. Paradis MR. Caloric needs of the sick foal: determined by the use of indirect calorimetry. *Dorothy Havemeyer Foundation Neonatal Septicemia Workshop (III)*. 2001:1–5.
934. Jose-Cunilleras E, Viu J, Corradini I, et al. Energy expenditure of critically ill neonatal foals. *Equine Vet J Suppl*. 2012:48–51.
935. Boitano M. Hypocaloric feeding of the critically ill. *Nutr Clin Pract*. 2006;21:617–622.
936. Marik PE, Hooper MH. Normocaloric versus hypocaloric feeding on the outcomes of ICU patients: a systematic review and meta-analysis. *Intensive Care Med*. 2016;42:316–323.
937. Dandona P, Mohanty P, Chaudhuri A, et al. Insulin infusion in acute illness. *J Clin Invest*. 2005;115:2069–2072.
938. Klein CJ, Stanek GS, Wiles 3rd CE. Overfeeding macronutrients to critically ill adults: metabolic complications. *J Am Diet Assoc*. 1998;98:795–806.
939. Lopes MAF, Hepburn RJ, McKenzie HC, et al. Enteral fluid therapy for horses. *Comp Cont Educ Pract Vet*. 2003;25: 390–397.
940. Ousey JC, Prandi S, Zimmer J, et al. Effects of various feeding regimens on the energy balance of equine neonates. *Am J Vet Res*. 1997;58:1243–1251.
941. Settle CS, Vaala WE. Management of the critically ill foal: initial respiratory, fluid and nutritional support. *Equine Vet Educ*. 1991;3:49–54.
942. Furr MO. Intravenous nutrition in horses: clinical applications. *20th Annual ACVIM Forum*. 2002:186–187.
943. Lopes MA, White 2nd NA. Parenteral nutrition for horses with gastrointestinal disease: a retrospective study of 79 cases. *Equine Vet J*. 2002;34:250–257.
944. Naylor JM, Kenyon SJ. Effect of total caloric deprivation on host defense in the horse. *Res Vet Sci*. 1981;31:369–372.

945. Hansen TO. Nutritional support: parenteral feeding. In: Koterba AM, Drummond WH, Kosch PC, eds. *Equine clinical neonatology*. Philadelphia: Lea and Febiger; 1990:747–762.
946. Hansen TO. Parenteral nutrition in foals. *32nd Annu Conv AAEP*. 1986:153–156.
947. Hays SP, Smith EO, Sunehag AL. Hyperglycemia is a risk factor for early death and morbidity in extremely low birth-weight infants. *Pediatrics*. 2006;118:1811–1818.
948. Tillotson K, Traub-Dagartz JL, Morgan PK. Partial parenteral nutrition in equine neonatal clostridial enterocolitis. *Comp Cont Educ Pract Vet*. 2002;24:964–969.
949. Myers CJ, Magdesian KG, Kass PH, et al. Parenteral nutrition in neonatal foals: clinical description, complications and outcome in 53 foals (1995-2005). *Vet J*. 2009;181:137–144.
950. Stratton-Phelps M. Nutritional management of the hospitalised horse. In: Corley KTT, Stephen J, eds. *The equine hospital manual*. Oxford: Blackwell; 2008:261–311.
951. Han JH, McKenzie HC, McCutcheon LJ, et al. Glucose and insulin dynamics associated with continuous rate infusion of dextrose solution or dextrose solution and insulin in healthy and endotoxin-exposed horses. *Am J Vet Res*. 2011;72:522–529.
952. Buechner-Maxwell VA. Nutritional support for neonatal foals. *Vet Clin North Am Equine Pract*. 2005;21:487–510. viii.

CHAPTER 21

Toxicologic Problems

Patricia Talcott

It is the dose that makes the toxin. In theory, almost anything that a horse could encounter in its daily regimen could act as a poison. In reality, that is not the case. There are a finite number of organic and inorganic substances that could pose a risk to horses given their typical environment, and these substances are what will be focused on in this chapter. Many toxins target specific organ systems, so the individual toxins are divided up by the major system affected. However, it should be recognized that many toxins target multiple organ systems; therefore clinical signs of illness can reflect changes occurring in more than one system. Very rarely will the clinical signs associated with toxicity be pathognomonic for a certain toxic substance. And the clinician needs to be aware that nontoxic differentials (e.g., infectious, inflammatory, nutrition, tumor) should be considered when presented with a patient where there is a concern that a toxicosis has occurred. Excellent history taking is essential in helping to confirm a poisoning, because many toxins, such as plant toxins, are not routinely tested for by veterinary diagnostic laboratories. Common toxicities seen in horses include plant toxins, cyanobacteria (i.e., blue-green algae), pesticides, and feed additives.

Toxicoses Related to the Gastrointestinal Tract

A wide range of toxic agents are capable of inducing signs of gastrointestinal disease, considering that the majority of toxicoses confirmed in horses is a result of oral exposures. Clinical signs of illness vary from mild to peracute and life threatening. Determination that a toxicosis is the cause of a problem such as diarrhea or colic sometimes is difficult, time consuming, and relatively costly.

PLANTS

Oak (*Quercus* spp.)

Clinical Signs

Oak blossoms, buds, leaves, stems, and acorns can be toxic to most animals. Most reports of toxicosis in livestock involve cattle, with a rare occurrence in the horse. Clinical signs attributed to acorn toxicosis in horses are acute onset of severe abdominal pain, rectal straining, hemorrhagic diarrhea, and pronounced intestinal borborygmi. Acorn parts may be apparent in feces. Occasionally, horses are found dead; other reported signs are hemoglobinuria and elevated heart and respiratory rates.

Pathophysiology

The toxicity of oak is attributed to a group of structurally similar compounds called *gallotannins* and their metabolites. Digallic acid is the major active metabolite produced by oak tannins. After bacterial fermentation digallic acid is converted to gallic acid and pyrogallol, both of which are considered toxic.[1] Pyrogallol and gallic acid are toxic to renal tubules and result in acute tubular necrosis, anuria, electrolyte abnormalities, and uremia.[1,2] Pyrogallol is also responsible for causing hemorrhagic gastroenteritis, subcutaneous hemorrhage, and hemolysis. Tannic acid itself is thought to result in increased vascular permeability, hemorrhage, and subsequent fluid loss into body spaces.[1]

Diagnosis

The diagnosis of oak toxicity is based on compatible clinical signs and a history of potential exposure to the tree. Under adequate forage conditions horses would seem to find large quantities of oak leaves and acorns distasteful; therefore most horses with oak trees in their environment do not develop toxicity. Toxicosis is more likely to result when abnormal conditions coupled with environmental factors (e.g., lack of access to normal forage or feed) result in large numbers of oak leaves, buds, and acorns becoming accessible to horses.

Laboratory findings compatible with oak toxicosis include dehydration or hemoconcentration to varying degrees, azotemia, hyperphosphatemia, hypocalcemia, and hypoproteinemia. Abnormal urine findings might include occult blood, proteinuria, and casts. An increase in gallic acid equivalent content in urine also has been used to support exposure to oak trees.[1] However, oak trees are not the only plant that can contain these tannins, and normal and toxic ranges of gallic acid in urine have not been established in livestock.

Necropsy findings suggestive of oak toxicosis include pericardial, thoracic, and peritoneal effusion; gastrointestinal and mesenteric edema; and pale and swollen kidneys that may bulge on cut surface. The intestinal tract may contain large quantities of oak and acorn parts, and colonic ulceration has been reported.[1]

Specific Treatment and Management

No specific antidote for oak toxicity is available. Animals should be allowed no further access to oak. Treatment of the acutely affected animal aims to maintain fluid and acid-base balance and to correct any electrolyte abnormalities. Fluids given intravenously to promote diuresis are the basic therapy. This therapy should be supplemented with calcium, bicarbonate, and other electrolytes, as necessary. The clinician should attempt evacuation of the intestinal tract using mineral oil or another suitable laxative.

The prognosis for affected horses is guarded. A paucity of information exists regarding mortality rates in affected horses, but death caused by ingestion of acorns has been reported.[1]

Castor Bean *(Ricinus communis)*

Clinical Signs

Castor beans contain ricin, a protein phytotoxin that acts as a potent proteolytic enzyme with significant antigenic qualities.[2] A latent period ranging from hours to several days usually occurs before the onset of clinical signs in affected horses. Poisonings with castor beans is rare in horses, and intoxication most likely occurs when the bean or plant material inadvertently is mixed into the feed source. To cause problems, the bean must be broken apart by chewing; beans swallowed intact are known to pass through the gastrointestinal tract uneventfully, at least in dogs.

The most commonly reported clinical signs of castor bean toxicosis described in the literature are varying degrees of abdominal pain, diarrhea, depression, incoordination, profuse sweating, and increased body temperature. Muscle twitching, convulsions, and prominent cardiac contractions occasionally are observed. If the horse absorbs enough ricin, signs of shock and anaphylaxis predominate.[2,3] Death may ensue as soon as 24 to 36 hours after ingestion.

Ricin is reported to be toxic to horses. One reference cites a ricin dosage of 0.1 µg/kg as a lethal dose,[2] and a second source indicates that 25 g of castor beans is lethal.[3] A published report in 1945 describes seven deaths attributed to castor bean toxicosis from a stable of 48 horses in London in 1931.[4] The exact number of affected horses was not reported. A review of the literature suggests that castor bean (ricin) toxicosis in human beings and dogs is not nearly as lethal as reported in the literature of the early twentieth century.[5,6] Whether this holds true for horses is unknown.

Pathophysiology

The oil extract of the bean contains ricinoleic acid. Within the small intestine, ricinoleate acts to reduce net absorption of fluid and electrolytes and stimulates peristalsis.[7] The fibrous residue of the seed contains the water-soluble toxalbumin ricin. Ricin is absorbed from the gastrointestinal tract and is a potent inhibitor of protein synthesis. Ricin contains two polypeptide chains. Chain B, a lectin, binds to the cell surface to facilitate toxin entry into the cell. Chain A disrupts protein synthesis by activating the 60S ribosomal subunit. The red blood cell agglutinating properties of ricin are independent of these toxic effects.[8]

Diagnosis

Diagnosis is made by a combination of history of exposure to the plant, clinical signs, and the identification of seeds in feed material, gastric contents, or feces. Analyses for ricin content in urine, as well as other samples, is not readily available and can only be performed at select laboratories.[8]

Treatment

Ricin has no specific antidote. Initial therapy aims to combat shock, alleviating abdominal pain and evacuating the bowel. Maintenance of fluid and electrolyte balance is important. Various sedatives and analgesics may be useful to control abdominal pain, if present. Oral administration of laxatives and protectants such as mineral oil and charcoal may be warranted. Antihistamines also have been recommended.[2]

Pokeweed *(Phytolacca americana)*

Clinical Signs

Pokeweed intoxication in horses is an uncommon occurrence. However, one text reports that horses show signs of gastrointestinal irritation and abdominal discomfort as the primary clinical signs, as is reported in other animal species. The plant also produces a burning sensation of the oral mucous membranes and may cause a hemolytic crisis. Fatalities caused by pokeweed ingestion are said to result from respiratory failure and convulsions.[2]

Pathophysiology

The plant contains phytolaccine, a powerful gastrointestinal irritant that in human beings causes symptoms ranging from a burning sensation of the alimentary tract to severe hemorrhagic gastritis. Five nonspecific mitogens that have hemagglutinating and mitotic activity have been isolated. These substances vary in concentration in the plant throughout the growing season. Noncardiac steroids and triterpenoid glycosides (saponins) also are present in significant quantities, but their role in pokeweed toxicity is unknown.[8] Saponins may potentiate gastrointestinal toxicity and produce vasodilation when given parenterally.

Diagnosis and Treatment

No specific diagnostic test is available. Horses suspected of having toxicosis must be treated symptomatically and supportively. The clinician should attempt to evacuate the gastrointestinal tract using laxatives. Adsorbents such as charcoal and protectants may be useful. If the horse develops a hemolytic crisis, ancillary therapy such as whole-blood transfusions may be lifesaving. Fluid and electrolyte balance must be maintained in these horses in an attempt to prevent or minimize hemoglobin- or hypoxic-induced nephrosis.[2]

Nightshade (*Solanum* spp.)

Clinical Signs

A number of *Solanum* spp. have been incriminated in causing toxicity in horses. However, these plants rarely are a source of natural intoxication to horses. Reported clinical signs are associated with the gastrointestinal and central nervous systems. The primary gastrointestinal signs observed are salivation, abdominal pain, increased borborygmi, and diarrhea. Signs of central nervous system (CNS) dysfunction include mydriasis, dullness, depression, weakness, and progressive paralysis, which can lead to prostration and death.[2,9,10]

Pathophysiology

Solanine is a toxic substance found in *Solanum* species; it is a water-soluble glycoalkaloid capable of producing local

irritation[2,3,8] and is absorbed poorly from the gastrointestinal tract. Intravenous doses have been shown to cause ventricular fibrillation in rabbits, and intraperitoneal doses have caused mild to moderate inhibition of specific and nonspecific cholinesterase activity.[8] It has been shown that exposure to *Solanum* plants can potentiate the effects of ivermectin in horses.[11] It has been hypothesized that the neuronal cytoskeletal alterations with subsequent interference of the axonal development in Purkinje cells may play a relevant role in the pathogenesis of the cerebellar lesions noted in *Solanum*-intoxicated cattle.[12]

Diagnosis and Treatment

No specific diagnostic test is available to confirm a diagnosis of *Solanum* toxicosis. Microscopic evaluation of brain tissue post mortem may reveal cerebellar cortical degeneration with perikaryal vacuolations, axonal swelling, and death primarily of Purkinje cells.[12] Animals suspected of having toxicosis should be treated symptomatically and supportively. Evacuation of the gastrointestinal tract using laxatives and protectants may be indicated. Charcoal also has been recommended for treatment of toxicosis in human beings.[8] The clinician should monitor the fluid, electrolyte, and acid-base status of affected animals and make corrections as needed.

Jimsonweed, Thornapple (*Datura* spp.)

Clinical Signs

Several species of *Datura* grow throughout North America, all of which can produce signs of toxicosis in livestock. However, these plants are rarely a source of natural intoxication in horses, probably because of the unpalatability of the fresh plant.[9,10] One report of equine acute toxicosis resulted when ingested feed was contaminated heavily with jimsonweed seeds. According to this report, one horse was affected acutely and died because of gastric rupture and gas-filled intestinal loops. A second horse was treated for several days before being euthanized. Clinical signs observed in the treated horse included abdominal distention with gas-filled intestinal loops, prolonged ileus, mydriasis, tachycardia, hyperpnea, and dry mucous membranes.[13]

Pathophysiology

The toxic substances found in *Datura* spp. are the tropane alkaloids atropine (a racemic mixture of D- and L-hyoscyamine) and scopolamine (L-hyoscine).[9,10,13] These substances exert an antimuscarinic effect by competitive inhibition with acetylcholine for receptor sites, resulting in attenuation of the physiologic response of neuroeffector junctions to parasympathetic nerve impulses. Blockade of the muscarinic receptors of different tissues accounts for the various clinical signs observed.

Diagnosis and Treatment

Toxicity is suspected when animals exhibit signs compatible with atropine overdose (e.g., dilated pupils, tachycardia, dry tacky mucous membranes, decreased gut sounds). Identification of seeds or plant material in ingesta, gastric lavage contents, or feedstuffs may support a diagnosis. Treatment is largely symptomatic and includes immediate removal of the offending feed or plants, evacuation of the gastrointestinal tract, and supportive care. The use of pilocarpine and physostigmine to counteract the atropine-like effects of these alkaloids has been recommended by some, but this treatment is considered controversial.[9,10]

MISCELLANEOUS AGENTS

Dioctyl Sodium Sulfosuccinate

Dioctyl sodium sulfosuccinate (DSS) is an anionic surface active agent that has been used to treat constipation and intestinal impaction in horses. A recommended dose of DSS is 17 to 66 mg/kg, with a maximum dose of 200 mg/kg.[2,14]

Clinical Signs

Signs of overdose commence within 60 to 120 minutes in affected horses. Initial signs are restlessness and increased intestinal sounds accompanied by steadily increasing heart and respiratory rates. Abdominal pain, watery diarrhea, and dehydration become apparent soon afterward, and horses gradually deteriorate to lateral recumbency and death in 14 to 72 hours.

Pathophysiology

Much information about the pharmacologic action of DSS remains uncertain.[7] The primary organ of involvement is the small intestine, where epithelial denudation, villous atrophy, and submucosal edema and congestion occur. DSS has been suggested possibly to cause epithelial detachment by lowering the surface tension on the basement membranes of intestinal epithelial cells.[15] Once detachment occurs, loss of fluid and electrolytes into the intestinal lumen is possible. The absorptive capacity of the epithelium is lost, and the osmotic effect of intestinal content causes further loss of fluid into the lumen. With extensive mucosal damage, the horse also becomes much more susceptible to endotoxemia. The rapid death in affected animals is caused by hypovolemic shock, endotoxemia, and circulatory collapse resulting from the loss of fluids and electrolytes into the intestinal lumen.

Diagnosis and Treatment

Diagnosis of DSS toxicity depends on observation of the aforementioned clinical signs along with a history of oral DSS administration. No specific antidote for DSS exists, and once clinical signs commence, treatment is aimed at supporting circulation and combating the effects of systemic endotoxin. Specific treatment for circulatory collapse and endotoxemia is covered elsewhere, but medications that might be useful include electrolyte solutions, electrolyte supplementation, corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs), bicarbonate, and orally administered gastrointestinal protectants.

Inorganic Arsenic

Arsenic is found in a number of products, including insecticides, herbicides, defoliants, older rodenticides, livestock dips, medications, wood preservatives, paint pigments, detergents, and certain insulation materials.[14,16] Horses are most likely to be exposed to arsenic by eating contaminated or treated forage or being exposed accidentally to stored or improperly discarded pesticides.[16] Horses cannot receive a significant exposure through cribbing on arsenic-treated wood. However, if arsenic-treated lumber is burned, ingestion of the arsenic-laced ash could be problematic.

Clinical Signs

The clinical signs associated with arsenic toxicity are essentially those of a severe gastrointestinal irritant. Most toxicities result from inorganic forms of arsenic, and signs are similar in all animal species.

In peracute cases the animals may be found dead with no premonitory signs. Acute signs of toxicity include severe colic, staggering, weakness, salivation, diarrhea that may contain blood or shreds of mucosa, and signs of shock that indicate cardiovascular collapse. Death usually occurs in 1 to 3 days.[14,16,17] In subacute poisoning animals may live for several days, exhibiting signs of depression, anorexia, colic, diarrhea that may contain blood and mucus, polyuria followed by anuria, and subsequent shock before death. Horses that are poisoned by topical application of arsenic can show signs of blistering and edema of the skin.[14] Chronic arsenic poisoning rarely occurs in domestic animals.

Pathophysiology

Many factors play a role in the development of arsenic toxicosis in horses. In general, horses that are debilitated, weak, or dehydrated are more susceptible to toxicosis than normal animals. The formulation of the compound (trivalent arsenicals are more toxic than pentavalent forms), the solubility of the compound, the route of exposure, the rate of absorption from the gastrointestinal tract, and the rate of metabolism and excretion by the individual animal are factors that can influence the toxicity of the various arsenic formulations.[14] The most hazardous preparations are products in which the arsenical is in a highly soluble trivalent form, usually trioxide or arsenite. Sodium arsenite is 3 to 10 times more toxic than arsenic trioxide. The average total oral lethal doses of these compounds for the horse are 10 to 45 g of arsenic trioxide and 1 to 3 g of sodium arsenite.[14,16]

Soluble forms of arsenic are absorbed from all body surfaces. Less soluble arsenicals are absorbed poorly from the gastrointestinal tract and essentially are excreted unchanged in the feces. After absorption trivalent arsenic is excreted readily by way of the bile into the intestine, and pentavalent arsenic is excreted by the kidney. Regardless of whether an introduced arsenical is in the trivalent or pentavalent form, all the major actions can be attributed to the trivalent form.[14]

All arsenicals are thought to exert their effects by reacting with sulfhydryl groups in cells, very similar to lead. Trivalent arsenic acts primarily by combining with the two sulfhydryl groups of lipoic acid, thereby inactivating this essential cofactor necessary for the enzymatic decarboxylation of the keto acids pyruvate, ketoglutarate, and ketobutyrate. By inactivating lipoic acid, arsenic inhibits the formation of acetyl, succinyl, and propionyl coenzyme A. The net effect is the blocking of fat and carbohydrate metabolism and cellular respiration.[14,16] Trivalent arsenic may inactivate sulfhydryl groups of oxidative enzymes and the sulfhydryl group of glutathione and other essential monothiols and dithiols. Arsenic also causes a local corrosive action on the intestine.[16]

Arsenic seems to prefer tissues rich in oxidative enzymes such as the liver, kidney, and intestine. The capillary endothelial cells in these organs appear sensitive to arsenic because it relaxes capillaries and increases capillary permeability. Blood vessels with smooth muscle in their walls also dilate. In the intestinal tract the mucosa easily sloughs away because of the accumulation of fluid in the submucosa. In the kidney renal tubular degeneration occurs.[16]

Diagnosis

The clinical signs described previously should cause the practitioner to consider inorganic arsenic poisoning. Antemortem laboratory findings are consistent with gastrointestinal, hepatic, and renal damage. Feces may contain blood, mucus, and increased numbers of white blood cells. The liver enzymes sorbitol dehydrogenase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) may be increased in serum, and urine might contain protein, red blood cells, and casts. Urine arsenic concentration in affected animals often exceeds 2 parts per million (ppm), but this is highly variable.[14]

Postmortem findings are characteristic of severe gastroenteritis and may include hepatic lipidosis and necrosis. In horses in which arsenic poisoning is suspected, the liver, kidneys, stomach, intestinal contents, milk, blood, and urine should be evaluated for arsenic concentrations.

Treatment

Specific therapy for arsenic toxicity is dimercaprol (or British Anti-Lewisite [BAL]). This chelating agent forms a nontoxic and easily excretable complex with arsenic. However, BAL may mobilize stored arsenic in tissues and cause an initial exacerbation of clinical signs by allowing more arsenic to circulate to the intestine and liver. BAL also can be toxic in sufficient doses. Signs of overdose include tremors, convulsions, coma, and death. In horses one recommended dose is 3 mg/kg intramuscularly as a 5% solution in a 10% solution of benzyl benzoate in peanut oil. This dose is administered every 4 hours for the first 2 days, every 6 hours on the third day, and twice daily for the next 10 days until recovery.[16]

Sodium thiosulfate also has been advocated for treatment of arsenic toxicosis, but its efficacy is questionable. The recommended dose for horses is 20 to 30 g orally in 300 mL of water, plus 8 to 10 g intravenously in a 10% to 20% solution.[16]

Symptomatic care of affected animals includes evacuation of the gastrointestinal tract with laxatives and oral demulcents to coat the intestinal tract. The clinician should evaluate fluid, acid-base, and electrolyte indices and provide support if necessary. Aggressive intravenous fluid diuresis is advocated by many to maintain adequate hydration and enhance urinary arsenic excretion. Because endotoxemia may develop as a result of the intestinal and liver lesions, prophylactic administration of flunixin meglumine at a dosage of 0.25 mg/kg every 8 hours may be beneficial. Other therapy to prevent shock and cardiovascular collapse also may be indicated.

Aluminum

One report describes an unexpectedly high incidence of horses on the same farm showing clinical signs compatible with granulomatous enteritis and the presence of abnormally high levels of aluminum in various body organs and tissues of the affected horses.[17] Clinical signs included weight loss with or without diarrhea, hyperkeratosis, ulcerative coronitis, and neurologic deficits compatible with cervical stenotic myelopathy. Laboratory abnormalities included hypoalbuminemia and elevated serum concentration of alkaline phosphatase in some horses. All horses had histologic evidence of granulomatous inflammation of the gastrointestinal tract in varying degrees of severity and distribution. Granulomas occurred in the mucosa, submucosa, and serosa of the small and large intestines and in the abdominal lymph nodes, portal areas of the liver, and pancreas. Aluminum was found within granulomas, and elevated aluminum levels were present in kidney and liver tissue.

Chronic environmental exposure to aluminum was postulated as a cause for the condition. Environmental factors

(e.g., soil pH, moisture content, plants) may have an effect on the bioavailability of aluminum, and it has been suggested that repeated exposure may induce hypersensitivity to aluminum in these horses. In human beings aluminum is known to induce nonimmunologic (foreign body) and immunologic granulomas after administration of aluminum-containing vaccines and hyposensitization products.[18,19] The association between high environmental concentrations of aluminum and increased incidence of generalized granulomatous inflammation in horses warrants further investigation. This is a very rare clinical condition.

Petroleum Distillates

Horses can be exposed to excessive amounts of crude oil or petroleum distillates through contamination of rangeland with byproducts of the oil industry or by iatrogenic application, because petroleum distillates are used as carrier agents for many insecticides.

Clinical Signs

The most frequently noted signs associated with ingestion of petroleum products are essentially those of gastrointestinal and respiratory dysfunction. Petroleum products are irritating to mucous membranes, and hence prominent signs of gastrointestinal dysfunction might include salivation and fluid feces. The feces actually may contain oil or oily substances. Chronically affected animals also may exhibit anorexia and weight loss over several days to weeks.

Signs of respiratory dysfunction are a common manifestation of excessive petroleum exposure. Aspiration of the oil or fumes is irritating to pulmonary tissue, and aspiration pneumonia is probably the most serious consequence of petroleum toxicity.[14] Signs of toxicity include increased respirations, anorexia, depression, weight loss, a variable degree of fever, and possibly increased nasal discharge.

Products that are applied inadvertently to the skin might cause some degree of respiratory impairment, but they are more likely to cause signs related to the absorption of excessive hydrocarbons.[16] Topically applied agents also may cause signs associated with a contact irritant.

Pathophysiology

The toxicity of crude oil is correlated with the gasoline, naphtha, and kerosene content in the oil. Crude oil rich in these low-temperature distillates is more toxic than petroleum containing a great deal of sulfur but less of the low-temperature distillates.[16] Petroleum products are irritating to mucous membranes, and their oiliness makes them difficult to remove from skin and mucous membranes and virtually impossible to remove from the respiratory epithelium. Once aspirated, they serve as a focus for foreign body pneumonia that may progress to abscessing pneumonia, pleuritis and pleural effusion, and death.

Diagnosis

History of possible exposure, clinical signs, and pathophysiologic signs are important in establishing a diagnosis. Suspected contents (i.e., gastrointestinal content or feces) may be mixed with water, and any oil that is present will float to the surface and be readily visible. Analytic chemistry methods sometimes can be used to establish the identity of an oil, but this is challenging and few laboratories offer these analyses on a routine basis.[14]

Treatment

Treatment is supportive. The clinician should evacuate and protect the gastrointestinal tract by using laxatives and demulcents. Products applied to the skin can be removed with warm soapy water followed by thorough rinsing. Products such as mechanics' hand degreasers can be tried first to remove fat-soluble compounds. Aspiration pneumonia may be frustrating to treat, and supportive care such as fluids and electrolytes, NSAIDs, and broad-spectrum antimicrobial therapy is potentially helpful.

Slaframine

Slaframine is an indole alkaloid produced by *Rhizoctonia leguminicola,* a mold that infects red clover, alfalfa, and other legumes.[15,16,20] *R. leguminicola* is a ubiquitous soil fungus that infects certain legumes during conditions of high rainfall or humidity.[20] The toxin can survive and persist in dried and baled hay.[14]

Clinical Signs

The most consistently reported clinical sign is excessive salivation characterized by profuse, viscous, clear saliva.[20] Salivation may begin 30 to 60 minutes after eating the affected feed, and response from one feeding may persist for up to 24 hours or more. Other, less commonly reported clinical signs include anorexia, polyuria, and sometimes watery diarrhea.[14] One case of abortion in an affected mare has been reported.[20] Clinical signs generally abate 48 to 96 hours after the infected feed is removed from the diet.[14]

Pathophysiology

Slaframine apparently is activated by liver microsomes after absorption. The active compound seems to have direct histaminergic effects or possibly a histamine-releasing effect, which is borne out in laboratory animal studies in which clinical signs responded better to antihistamines than to atropine.[16]

Diagnosis

The combination of acute clinical signs of excessive salivation coupled with digestive disturbances and recognition of clover/alfalfa in the diet (and a positive response to removing the offending feed) is adequate to establish a diagnosis. It is possible to analyze slaframine in feeds, but usually such tests are unnecessary.[14]

Treatment

No specific treatment usually is necessary. Animals generally recover uneventfully 48 to 96 hours after withdrawal of the contaminated forage.[14] Atropine and antihistaminic therapies have been suggested to help control clinical signs,[14,16] but their efficacy is questionable.

Pentachlorophenol

Documented instances of horses becoming intoxicated with pentachlorophenol are rare. However, because pentachlorophenol was historically used as a wood preservative and because other domestic animals, including cattle and swine, have been poisoned, some aspects of this toxin require description. Exposure potentially occurs as a result of the horse drinking from a container containing pentachlorophenol or lying on recently treated lumber or contaminated soil.

The chlorophenols (which include pentachlorophenol) generally are not water soluble but are soluble in oils and

organic solvents.[16,21] Pentachlorophenol is volatile and can give off toxic vapors.[16] The chlorophenols are absorbed readily from the gastrointestinal tract, by inhalation, and from intact skin and are excreted rapidly by the kidney.[16,21]

Several factors affect the toxicity of chlorophenols. High ambient temperature, physical activity, poor body condition, oily or organic solvent vehicles, prior exposure, and hyperthyroid states enhance the toxicity in human beings and other animal species. Cold temperatures, antithyroid drugs, and increased amounts of body fat help diminish the toxicity.[16]

The mechanism whereby the chlorophenols exert their toxicity involves the energy production sites of mitochondria, where they uncouple oxidative phosphorylation. The chlorophenols act at sites of adenosine triphosphate (ATP) production to decrease or block their production without blocking the electron transport chain. Free energy from the electron transport chain then is converted to body heat. As the body temperature is increased, the heat-dissipating mechanisms are overcome and metabolism is increased. The electron transport chain responds by using more oxygen in an effort to produce ATP, but much of the free energy is liberated as more body heat. Eventually, the oxygen demand overcomes the oxygen supply, and energy reserves become depleted.[16]

Clinical Signs

Clinical signs, if observed, may include fever, tachycardia, dyspnea, sweating, lethargy, incoordination, weakness, cyanosis, collapse, and death. Less severely affected animals may primarily manifest signs of hyperthermia and oxygen deficiency.[16] Pentachlorophenol in high doses to pregnant animals also is reported to cause embryonic and fetal deaths but is not teratogenic.[14]

Diagnosis and Treatment

Pentachlorophenol toxicity has been associated with the combination of clinical signs and blood pentachlorophenol levels greater than 40 ppm. No specific treatment is available, but saline diuresis has been suggested to be helpful in certain instances of human toxicosis.[14]

Chlorates

Chlorate salts (sodium chlorate or potassium chlorate) are used as herbicides and defoliants. Horses may become exposed by grazing in recently sprayed areas or by ingesting sodium chlorate when it is mistakenly substituted for sodium chloride as a feed additive. Chlorate toxicity in any animal species is rare.

Clinical Signs

Initial signs are those of gastrointestinal irritation and include colic and diarrhea. Hematuria and hemoglobinuria also are present early in the disease course. Within hours the horse may show dyspnea, cyanosis, and increased respiratory effort. Death can occur suddenly, without obvious signs.[14]

Pathophysiology

Chlorates are absorbed readily from the intestine, and once absorbed they continue to exert their damaging effects as long as they are present.[14,16] A dose of 250 g is reported to be lethal to horses.[14]

Chlorates cause toxic changes by three different mechanisms of action:

1. Direct irritation of the gastrointestinal tract
2. Oxidation of hemoglobin to methemoglobin
3. Inducement of hemolysis

The net effect of methemoglobinemia and hemolysis is a severe compromise in the oxygen-carrying capacity of blood, and animals may be so severely affected that they die as a result of anoxia.[16]

Diagnosis

The prolonged, extensive methemoglobinemia found in affected animals should alert the veterinarian to the possibility of chlorate poisoning. A history of exposure to chlorates also should accompany these clinical signs before a presumptive diagnosis is made. Analysis of blood, urine, or ocular fluid can help determine chlorate concentration, and because chlorates normally are not found in animals, their presence in a sample would confirm poisoning if clinical signs, history, lesions, and response to therapy also suggest this diagnosis.[16]

Treatment

After making the diagnosis, the clinician should immediately seek out the chlorate source and remove it from the horse's environment. Methemoglobinemia is treated with methylene blue at a dose of 4.4 mg/kg given as a 1% solution by intravenous drip. This dose may be repeated in 15 to 30 minutes if a clinical response is not obtained.[2,16] Other recommended therapeutic measures include gastric lavage with 1% sodium thiosulfate and the oral administration of intestinal protectants and demulcents.[2,14] Blood transfusion and oxygen supplementation may be beneficial in certain instances.[16]

Pyriminil (Vacor)

Pyriminil currently is not commercially available, but in previous years it was marketed as a rodenticide. Reports of toxicosis in horses are rare,[22] and no deaths caused by pyriminil ingestion have been reported.[22,23]

Clinical Signs

Reported signs in affected horses include severe muscular fasciculation, profuse sweating, dehydration, and mydriasis with a weak pupillary response. Hindlimb weakness, ataxia, persistent inappetence, and abdominal pain also have been reported.[22] Hyperglycemia is a fairly consistent laboratory finding.[14,22]

Pathophysiology

Pyriminil is absorbed from the gastrointestinal tract and excreted in the urine. Pyriminil acts as a nicotinamide antagonist, but its exact mechanism of action is unknown.[2,14,23] Pyriminil also has been shown to damage the pancreatic β-cells and to depress glucose uptake by erythrocytes.

Diagnosis

A presumptive diagnosis is based on compatible clinical signs and history of exposure to the rodenticide.

Treatment

Specific therapy for pyriminil toxicity is reported to be nicotinamide. However, the use of this drug in human beings appears to be effective only when given within 1 hour of ingestion of pyriminil. The reported dosage is 50 to 100 mg of nicotinamide intramuscularly every 4 hours for up to eight injections. This dosage is followed by 25 to 50 mg orally 3 to 5 times daily for 7 to 10 days.[14] Other therapies that may be beneficial include gastric lavage and the oral administration

of mineral oil and activated charcoal.[14,23] Apparently, affected horses recover because no deaths caused by pyriminil toxicosis have been recorded.

Tetrachlorodibenzodioxin

The polychlorinated dibenzodioxins include a large number of isomers that differ chemically only in the number and location of chlorine atoms on the dioxin nucleus but that vary greatly in their toxic potential to various animal species. Of the 75 possible isomers of polychlorinated dibenzodioxin, the specific isomer designated 2,3,7,8-tetrachlorodibenzodioxin (TCDD; dioxin) is most toxic and generally is considered the most toxic synthetic molecule known. TCDD was once a contaminant of certain herbicides and is a byproduct of certain chemical manufacturing and combustion processes.[14] It can also be found in soil as a naturally occurring compound or as a result of contamination by industrial waste. Dioxins have been found in ball clay that had been used as a binding agent for livestock feed. Currently, dioxins are more of a tissue residue issue for production animals than a toxicologic hazard for livestock. The chemical is a highly stable contaminant in the environment, with a half-life in soil of approximately 1 year.[24]

Clinical Signs

In one reported outbreak the initial signs began 4 days after exposure and included abdominal pain, polydipsia, anorexia, severe weight loss, alopecia, skin and oral ulcers, conjunctivitis, dependent edema, joint stiffness, and laminitis. A total of 85 horses were exposed, 58 became ill, and 43 subsequently died. The length of illness varied from 4 to 132 weeks in the terminally ill horses, with those having a heavier exposure exhibiting a shorter disease course (average of 32 weeks) than others (average of 74 weeks). In addition, abortions occurred in pregnant mares, and many foals that were exposed only in utero died at birth or shortly thereafter.[24] Other reported signs in animals included gastrointestinal hemorrhage with necrosis and ulceration of the gastrointestinal mucosa, cerebrovascular hemorrhage, hepatotoxicity, and thymic and peripheral lymph node atrophy.[14,24]

Pathophysiology

TCDD is absorbed readily by oral and dermal routes, and following absorption appears to be retained primarily by liver and adipose tissue. The mechanism of action of TCDD in various organs in not well defined. TCDD is known to induce microsomal mixed function oxidases in liver and kidney, and hepatic δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase, but the role of these processes in the induction of toxicity of TCDD remains to be elucidated.[14] TCDD also induces immunosuppression by causing thymic and peripheral lymph node atrophy.

Diagnosis

A combination of the described clinical signs and possible exposure to industrial waste oil products should alert the veterinarian to the possibility of TCDD toxicity. Dioxin content is confirmed in various tissues by means of gas-liquid chromatography and mass spectroscopy, but few laboratories offer this service, and the analysis is generally expensive.[14]

Liver lesions observed at necropsy in a number of horses included were microscopic evidence of bile stasis, hepatocyte necrosis, bile duct proliferation, and extensive fibrosis that was pronounced around the central veins but minimal in the peripheral liver lobules. Other microscopic changes noted were thickened vascular walls and endothelial proliferation in the smaller blood vessels of several different organs.[24]

Treatment

No known antidote exists for TCDD toxicity once clinical signs develop. After their onset, the clinician can offer only symptomatic and supportive care, and one should use every precaution to prevent laminitis. Activated charcoal appears to bind strongly to TCDD and inhibit its absorption, so if known ingestion has occurred, immediate oral administration of activated charcoal may have beneficial effects by reducing the amount absorbed.[14]

Cantharidin Toxicosis (Blister Beetle Toxicosis)

Cantharidin toxicosis results from ingestion of dead blister beetles that become entrapped in hay during harvesting. Essentially all reports are of horses being fed alfalfa hay or alfalfa products, but anecdotal reports exist of horses intoxicated by ingesting grass hay. More than 200 species of blister beetles inhabit the continental United States, but toxicity results primarily from beetles of the genus *Epicauta*.[25] Depending on the species of beetle, as few as a half dozen ingested beetles can be problematic. The liquid form of cantharidin is suspected in the malicious poisoning of horses.

Cantharidin is the sole toxic principle and is contained in the hemolymph, genitalia, and possibly other tissues of the beetle. Cantharidin is a highly irritating substance that causes acantholysis and vesicle formation when in contact with skin or mucous membranes, and the substance is absorbed from the gastrointestinal tract and rapidly excreted by the kidney. Storage of hay does not appear to reduce the toxicity of cantharidin.[25]

Clinical Signs

The signs associated with toxicosis are many, varied, and dose dependent. Horses affected with a minimal dose may show only signs of lethargy, anorexia, and occasionally polyuria, whereas horses ingesting a lethal dose may show signs of profound shock, gastrointestinal and urinary tract irritation, myocardial dysfunction, and hypocalcemia/hypomagnesemia.[25,26] The onset and duration of clinical signs vary from hours to days, but horses that succumb to cantharidin generally die within 48 hours of onset of signs. Horses that live longer than 48 hours have a better prognosis for recovery if no complications arise.

The most commonly observed clinical signs include varying degrees of abdominal pain, anorexia, lethargy, and repeatedly submerging the muzzle in water or frequently drinking small amounts of water. The respiratory and cardiac rates are elevated, and cardiac contractions are occasionally forceful enough to be observed through the thoracic wall. Mucous membranes are congested and cyanotic, and capillary refill time is prolonged. The feces may be watery in consistency but rarely contain blood or mucus. Profuse sweating is typical of more severely affected horses and may be a sign of severe abdominal pain. Affected horses often make frequent attempts to void urine. The urine is grossly normal early in the disease course but later may become tinged with blood or contain clots of blood. Gross hematuria, if it occurs, is usually in the later stages of the disease process. Less commonly observed signs include synchronous diaphragmatic flutter; erosions of

the gingival and oral mucous membranes; and occasionally a stiff, short-strided gait similar to that seen in acute myositis. Sudden death also has been reported.[25]

Pathophysiology

The mechanism of action of cantharidin at the cellular level has not been elucidated fully. Acantholysis and vesicle formation result from disrupted cell membranes. Cantharidin does not have a direct effect on membranes but is thought to interfere with oxidative enzymes bound to mitochondria. These enzyme systems are involved directly in active transport across the plasma membrane, and their failure results in cell death caused by significant permeability changes in the cell membrane.[25]

Hypovolemic shock and pain develop rapidly in more severely affected horses. The normal transfer of fluid, nutrients, and electrolytes across the intestinal mucosa is disrupted because of the morphologic changes induced by cantharidin. Although renal tubular damage is not severe enough to cause death, changes in the renal tubular epithelium also may be related to the development of fluid, acid-base, and electrolyte abnormalities.[25,26]

Hypoproteinemia develops later in the disease course, probably as a result of protein loss across the damaged intestinal mucosa. Protein also is lost into the peritoneal space, and a minor amount may be lost through the urine.[25]

The profound hypocalcemia and hypomagnesemia that may occur in many horses have not been explained fully. Calcium loss, derangement of calcium homeostasis, or a combination of both is the most likely explanation because the acute onset of the disease eliminates reduced intake as a possible cause. Calcium can be lost through urine and sweat and as protein-bound calcium through the damaged intestinal wall. An influx of intracellular calcium also may occur in certain tissues. Whether cantharidin has an effect on calcium-binding sites on proteins or in cells is unknown.[25]

The low urine specific gravity in most horses may be caused by decreased permeability of the collecting ducts to water. Other findings, however, point to a mild pathologic insult as a cause of the low urine specific gravity. These findings include the facts that a low specific gravity occurs suddenly within hours of toxin exposure; specific gravity returns to normal in 2 to 4 days in surviving horses; only mild to moderate changes are noted in other renal function tests; and the histologic renal lesions are mild, and neither acute nor chronic renal failure is associated with cantharidin toxicosis in horses.[25]

Myocardial necrosis can be observed post mortem in affected horses and may be caused by the direct effect of cantharidin on cardiac muscle. Dose-related intracellular changes involving the mitochondria, cristae, nuclear chromatin, sarcoplasmic reticulum, and myofibrils have been observed in the cardiac muscle of rabbits that were given cantharidin. A proposed mechanism for these changes suggests that an excessive transport of calcium into the myocardial cells occurs, leading to an intracellular calcium overload. This overload may result in a high-energy phosphate deficiency within the cell, leading to necrosis and cell death.[25]

Diagnosis

The clinician should consider cantharidin toxicosis when horses exhibit signs of abdominal pain, lethargy, or polyuria and their diet contains alfalfa hay or alfalfa products. The diagnosis can be made when horses have clinical signs and laboratory findings compatible with cantharidin toxicosis and beetles are found in the hay. Because the beetles can be difficult to identify in hay, it should be thoroughly searched. Cantharidin can be assayed using various techniques.[25,27] Samples to be tested are serum, urine, kidney, and stomach content from horses in which cantharidin toxicosis is suspected.

Laboratory findings are nonpathognomonic, but several abnormalities typically are noted. Packed cell volume (PCV) and serum protein concentrations are elevated early, but hypoproteinemia frequently develops after about 24 hours. Mild hypokalemia can occur but is not a striking feature of this disease. Blood urea nitrogen (BUN) concentration may be elevated moderately, and hyperglycemia is almost always present initially.[25]

Serum calcium and magnesium concentrations can be significantly decreased in some horses and remain low for longer than 48 hours if untreated. The urine generally contains red blood cells and has a low specific gravity, even in the face of clinical dehydration. Abnormal peritoneal fluid findings include increased protein concentration but relatively normal fibrinogen and white blood cell values. Feces are often positive for occult blood. Serum creatine kinase (CK) activity may be elevated in more severely affected horses and augurs a poor prognosis.[25] Although not diagnostic, laboratory findings of prolonged hypocalcemia and hypomagnesemia and elevated CK concentration may help differentiate cantharidin toxicosis from other causes of acute abdominal crisis.

Treatment

No specific antidote is available for cantharidin. Once the diagnosis is suspected, all suspect feed should be removed from the horse's environment and all hay carefully examined for the presence of beetles.

Horses suspected of having cantharidin toxicosis should be given mineral oil as soon as possible. The oil helps evacuate the bowel and also may help reduce the amount of cantharidin available for absorption because cantharidin is lipid soluble. Activated charcoal given through a nasogastric tube also may have beneficial effects.[25]

Fluids should be administered intravenously throughout the disease course to correct dehydration and promote diuresis. Diuretics also may be given once the horse is volume loaded. Analgesics usually are required because of the severity of the abdominal pain, and glucocorticoids may be necessary to aid in treating shock. Calcium gluconate should be administered to elevate the serum calcium concentration, and calculated deficits of magnesium should be replaced by slow intravenous infusion.[25]

Phosphorus

Elemental phosphorus can be available in red and white forms. Red phosphorus is used in manufacturing fertilizers and safety matches and is considered inert and relatively nontoxic. White phosphorus was used as a rodenticide and can be found in pastes containing from 1.5% to 5% phosphorus. The reported toxic dose for horses is 0.5 to 2.0 g.[14] This is a rare toxicosis in livestock.

Clinical Signs

The toxic manifestations of phosphorus poisoning are generally threefold: toxic signs commence within hours of ingestion; are followed by a latent period of 48 hours to several days, when the animal may appear to have recovered; and finally recur with greater severity.

The initial signs are characterized by severe abdominal pain and gastrointestinal irritation, with occasional episodes of diarrhea. Blood may be present in feces. Cardiac arrhythmias may occur during this phase, and if the dose is sufficiently large, cyanosis, shock, incoordination, and coma can develop, and the animal may die before the second and third stages develop.[14]

The latent period may occur from 48 to 96 hours after the onset of clinical signs, and during this time the animal may appear normal. The third stage is characterized as a recurrence of severe abdominal pain, and signs of liver dysfunction may become evident. Icterus and a tendency to bleed from the gingiva, stomach, intestine, or kidney may be evident.[14]

Pathophysiology

Phosphorus is absorbed from the gastrointestinal and respiratory tracts. Although dermal exposure may cause skin irritation or burning, absorption does not occur by this route. The mechanism of action of phosphorus is unknown but is noted for causing irritation and necrosis of affected tissue. Phosphorus is also known to cause peripheral vasodilation.[14]

Diagnosis and Treatment

Clinicopathologic abnormalities reflect hepatic and renal damage. Hypoglycemia may be pronounced, and liver enzymes such as AST, LDH, and sorbitol dehydrogenase are elevated. Renal damage is reflected by increased BUN and creatinine concentrations. Albumin, blood, and increased concentrations of amino acids may be present in the urine. The phosphorus concentration in blood is usually normal. Although elemental phosphorus in tissues can be assayed, in time a large portion may be oxidized to phosphates, thereby making confirmation of poisoning difficult by chemical means.[14]

No specific antidote is available for phosphorus intoxication. Therapy is essentially symptomatic and supportive.

Thallium

Thallium is toxic to all animals, including human beings, but clinical reports of toxicity are extremely rare. However, an oral dose of thallous acetate 27 mg/kg has been suggested as potentially lethal to horses.[16] Thallium has been used historically as a rodenticide, but its use now is limited and restricted only to government agencies.

Clinical Signs

Thallium ingestion can result in acute, subacute, or chronic syndromes. In the acute form clinical signs in animals usually begin within 1 to 4 days of ingestion. Initial signs are those of severe gastrointestinal insult and include vomiting or regurgitation, severe hemorrhagic diarrhea, abdominal pain, and anorexia. Labored breathing is apparent early in the disease course, and motor paralysis and trembling may occur. Signs suggesting renal dysfunction also may occur.[14]

The subacute form generally manifests signs in 3 to 7 days after ingestion. Signs of gastric distress and motor disturbances are less marked than in the acute form, but they persist for a longer time. In this form reddening of the skin and pustule formation occur. A pronounced reddening of the oral mucous membranes also seems to be unique to this particular toxicosis. Other clinical signs observed include conjunctivitis, hair loss, and crusty skin lesions. Secondary bacterial infections also may develop in affected animals.[14]

The chronic stage requires 7 to 10 days to appear. Signs of gastrointestinal and nervous system dysfunction are mild, but hair loss and dry, scaly skin become pronounced.[14]

Pathophysiology

Thallium is absorbed readily from the intestinal tract or through the skin; is distributed to all body tissues, although higher levels accumulate in the kidneys and liver; is excreted principally in feces and to a lesser extent in urine; and undergoes an enterohepatic cycle for resorption and excretion.[16]

Thallium is thought to combine with mitochondrial sulfhydryl enzymes at a specific yet unknown place in the scheme of sulfur metabolism. Thallium therefore interferes with oxidative phosphorylation within cells. Some evidence suggests that thallium exchanges for potassium in muscle and nerve cells primarily and also has a necrotizing effect on the intestinal tract, kidney, and occasionally the brain.[14,16]

Diagnosis

Thallium toxicosis is suspected on the basis of clinical signs, and urine can be assayed for thallium content. The finding of thallium in tissue in any amount is diagnostic, but liver and kidney levels tend to be higher than in other tissues.

Treatment

Chelation therapy using diphenylthiocarbazone (dithizon) at a dosage of 70 mg/kg administered orally thrice daily has been recommended for use in dogs.[14] However, cats react adversely to this agent. Its effect in horses is unknown.

Potassium chloride may aid in the elimination of thallium and can be given intravenously or orally, but the oral route is contraindicated in animals that also are being treated with an ion exchange agent.

One can attempt to trap thallium in the intestine with the ion exchange agent potassium ferric cyanoferrate–II (potassium–Prussian blue). Experimentally, potassium–Prussian blue is not absorbed from the intestinal tract and acts to immobilize the thallus ion by exchanging with the potassium of potassium–Prussian blue. Once trapped, thallium is not released readily from potassium–Prussian blue, and fecal excretion of thallium increases.[16]

Other forms of symptomatic therapy include intestinal protectants and demulcents, fluid and electrolyte support, analgesics, oral activated charcoal, and general nursing care.[16]

Toxicoses Causing Signs of Central Nervous System Stimulation

Dealing with horses showing signs of CNS stimulation can be one of the most difficult and challenging situations faced by the equine veterinarian. Signs can be hard to control, and clients' emotions often run high at the sight of a horse showing severe or uncontrollable signs such as hyperexcitability, exaggerated gait abnormalities, and seizures. The immediate clinical emphasis is usually on minimizing the risk or severity of self-induced trauma. Etiologic diagnosis may be difficult and protracted.

PLANTS

Locoweed

A nervous syndrome in horses, cattle, and sheep has long been associated with eating plants of the genera *Astragalus, Swainsona,* and *Oxytropis.* This group of plants is large. The *Astragalus* species that grow in North America number more than 300. Not all species of *Astragalus* and *Oxytropis* are toxic, however, and some species make nutritious forage for livestock.[28]

The toxic species of locoweed produce three different syndromes in livestock. Some species contain nitroglycosides, which cause methemoglobinemia and competitive inhibition of certain cellular enzymes; others accumulate toxic levels of selenium; and a third group contains alkaloids that cause locoism.[28,29] The first group of plants is of minor importance to the horse, and the selenium concentrators are discussed in the section on selenium toxicosis. A discussion of the clinical syndrome locoism follows.

The species of locoweed that have been associated with locoism include *Astragalus lentiginosus, Astragalus mollissimus, Astragalus wootonii, Astragalus thurberi, Astragalus nothoxys, Oxytropis sericea, Oxytropis lambertii,* and *Oxytropis saximontana.*[28] Additional *Astragalus* species incriminated in causing disease include *Astragalus argillophilus, Astragalus bisulcatus,* and *Astragalus earlei.*[28] Geographically, locoweeds are found from western Canada southward to include the western United States and northern Mexico.[28]

Clinical Signs

Locoweeds cause a number of problems in livestock, including neurologic and reproductive dysfunction, emaciation, and habituation.[28] Typical signs exhibited by affected horses include a slow, staggering gait; lethargy; an unthrifty appearance; emaciation; muscular incoordination; and nervousness, especially when the animal is under stress. The affected horse may become solitary and hard to manage and may have difficulty eating and drinking. In some animals sexual activity may become suppressed. Visual impairment also occurs in some horses, and mares ingesting locoweed during pregnancy have been known to abort or produce foals with various limb deformities.[29,30] Horses that are affected chronically are generally useless for riding or draft purposes because their behavior is so unpredictable.[28]

The onset of clinical signs varies from as short as 2 to 4 weeks to as long as 2 months after the horse starts to graze the plant. Some of these plants are considered unpalatable to horses, but once they start to ingest the plant, they seem to become addicted to it and will search it out.[28] The addiction can extend to subsequent growing seasons, so clinical signs can worsen progressively in successive years if animals continue to graze the plant.[29] Affected horses can recover if feeding or grazing is discontinued before they become too emaciated and if nutritious forage is provided.[28] However, the syndrome eventually may lead to death in chronically affected horses.[29]

Pathophysiology

The indolizidine alkaloids swainsonine and swainsonine *N*-oxide have been suggested as the toxic principles in locoweeds.[29] These alkaloids were first recovered from *Swainsona* species in Australia.[28,29] Evidence suggests that these alkaloids are actually mycotoxins, or toxins produced by endophytes located within the plant. Swainsonine inhibits α-mannosidase, a lysosomal enzyme essential in the cellular metabolism of oligosaccharides. As a result, mannose-rich oligosaccharides accumulate in lysosomes and disrupt cellular function. These accumulations are visible microscopically as intracytoplasmic vacuoles. Vacuolization of the renal cortical tubular cells can occur as early as 4 days after ingestion of locoweed begins, and neurons of the CNS, including Purkinje cells, may show vacuolization by 8 days. The vacuoles disappear shortly after consumption ceases in the early stages of disease, but if grazing is prolonged, permanent cellular damage occurs. Continuous feeding of the plant for 30 days or longer results in vacuolization of almost all tissues of the body except skeletal and cardiac muscle.[29]

Neurologic signs result from vacuolization of the axons, glial cells, and Purkinje cells of the cerebellum and cerebral cortex. The weight loss and emaciation result from impairment of the liver, pancreas, thyroid, and parathyroid glands. Vacuolization of cells of the retina and decreased lacrimation are responsible for impaired vision in some animals. Vacuoles also occur in lymph nodes, placenta, testicles, and lymphocytes.[28,29] The pathogenesis and lesions of locoism are similar to mannosidosis, a heritable lysosomal storage disease of Angus and Murray Grey cattle.[29]

Diagnosis and Treatment

A few laboratories test for swainsonine and mannosidase activity in a variety of biologic specimens. The diagnosis is suspected when horses show clinical signs compatible with locoism and have a history of exposure to the plant. Laboratory testing is nonspecific, and test results are expected to be consistent with multiple organ dysfunction. Microscopic lesions of intracytoplasmic vacuolization in various organs, including the CNS, are compatible with a diagnosis of locoism.[28,29] Peripheral lymphocytes that contain intracytoplasmic vacuoles also are considered indicative of locoism, if clinical signs are present.[29]

No effective cure exists for horses with chronic locoism that have had clinical signs for some time. Mild cases usually resolve in 1 to 2 weeks once ingestion ceases, so successful recovery from locoism depends on early recognition of the disease syndrome and prevention of further consumption. Reserpine has been suggested to be helpful in relieving some of the clinical signs of locoism in horses.[29]

Nervous Ergotism

Two forms of ergotism are observed in domestic animals, a nervous or convulsive form (rare) and a gangrenous form. Horses appear rarely to be affected with ergotism (in the United States, cattle and swine appear to be most commonly affected), but the nervous form is reported to occur much more frequently in horses than the gangrenous form.[31,32] Ergotism is caused by a number of alkaloids contained in *Claviceps purpurea,* a fungus infecting grains such as wheat, barley, rye, and oats and wild grasses such as quackgrass, smooth bromegrass, wheatgrass, bluegrasses, and wild rye. The fungal mass, or sclerotium, replaces the seed or kernel of the plant and may have the same general configuration as the seed but is usually larger, dark colored, and hard. Ergotism is rarely of concern in dry seasons, but abundant fungal growth can occur during wet periods.[4] Although well-documented cases of ergotism caused by *C. purpurea* in horses are absent in the literature, a nervous syndrome typical of *Claviceps paspali* poisoning has

been observed in horses in Australia. However, the cause of the clinical signs in these horses was suggested to have been the tremorogenic mycotoxins in *C. paspali* rather than the alkaloids found in ergot.[33]

Clinical Signs

The first sign of nervous ergotism in animals is reported to be dizziness or an unsteady gait. This phase may be interrupted by convulsions and temporary posterior paralysis and drowsiness.[31] Other behavioral effects that have been described include incoordination, lameness, difficulty in breathing, excessive salivation, and diarrhea.[32]

Pathophysiology

Approximately 40 different alkaloids have been isolated from *C. purpurea*. All of these alkaloids are derivatives of the tetracyclic compound 6-methylergoline, a lysergic acid base structurally similar to a number of biogenic amines such as dopamine, serotonin, and norepinephrine.[31,34] The most pharmacologically potent alkaloids in ergot are ergonovine, ergotamine, ergotsine, ergocristine, ergocryptine, and ergocornine.[31] Additionally, tyrosine, tryptophan, tyramine, histamine, histidine, choline, and acetylcholine have been isolated from ergot sclerotia, but their clinical significance is uncertain.[34]

The mechanism whereby ergot alkaloids induce CNS signs in horses has not been elucidated. Of the ergot alkaloids known to affect the CNS in human beings, bromocriptine is the prototype. Bromocriptine is a long-acting dopamine agonist that has central stimulating effects and may cause hypotension. Another ergot alkaloid, isoergine (lysergic acid amide), has one tenth the mind-altering potency of the structurally related compound lysergic acid diethylamide (LSD). LSD is thought to produce hallucinations by a series of complex agonist and antagonist actions on several central monoamine neurotransmitters, particularly serotonin.[34]

Diagnosis and Treatment

The diagnosis of nervous ergotism in horses is based primarily on clinical signs and eliminating other causes of CNS stimulation. One can assay the ergot alkaloid content of the suspect feed.[31]

No specific antidote exists for ergot alkaloid toxicity. Treatment of affected horses is largely symptomatic. Recommendations for control include using ergot-free feed, rotating crops, plowing deeply (because shallow cultivation and seeding leave the sclerotia near the soil surface, where they can germinate more readily), and mowing surrounding grasses to limit the spread of fungus into the cultivated crop.[32] Pasture grasses that may be infested with ergot should be mowed before the development of seed heads because *Claviceps* replaces the seed or kernel of the plant.

Blue-Green Algae (Cyanobacteria)

The occurrence of cyanobacterial poisoning in livestock is not uncommon in many areas. Most cases of toxicity involve other grazing animals, principally cattle and sheep, but horses are reported to be susceptible.[35] Toxicity may occur during times that favor algal growth in surface water. The most important factors that favor rapid algal growth are a nutrient source (e.g., nitrogen, phosphate, sulfate); quiet, calm weather conditions; and warmish water temperature. Therefore toxicosis is most likely to occur during periods of warm weather (i.e., late spring through fall), when surface water may be contaminated with fertilizer runoff or organic waste high in nitrogen, such as that from feedlots.[35,36] However, it is important to note that livestock toxicities caused by cyanobacteria have been reported in the winter months in the Northern Hemisphere. In more temperate areas cyanobacterial blooms can be a year-round occurrence.

Many genera of cyanobacteria are known to be toxic: *Anabaena, Aphanizomenon, Microcystis (Anacystis), Coelosphaerium, Cylindrospermopsis, Gloeotrichia, Lyngbya, Nodularia, Planktothrix (Oscillatoria), Nostoc, Raphidiopsis,* and *Umezakia;* however, the first three are of most concern in veterinary medicine. Toxins produced by cyanobacteria are either neurotoxic or hepatotoxic; therefore signs of poisoning are typically neurologic or hepatic, but rarely both are observed in an animal (even though some blooms are mixed). Most blooms are composed of one predominant cyanobacterial organism. *Microcystin (Anacystin)* organisms produce the hepatotoxin microcystin, and *Anabaena* and *Aphanizomenon* produce the neurotoxins anatoxin-a and anatoxin-a(s).

Clinical Signs

Common signs of cyanobacterial poisoning associated with microcystins may include rapid appearance of abdominal pain, diarrhea, muscle tremors, dyspnea and cyanosis, prostration, and death. Animals surviving several days may exhibit hemorrhagic diarrhea, muscle tremors, signs of liver damage, and secondary photosensitization.[35,36] Animals exposed to neurotoxic blooms frequently develop signs within a few minutes (less than 1 hour) of ingestion and may show rapid onset of CNS dysfunction with seizures, prostration, and death.

Pathophysiology

Anabaena and *Aphanizomenon* species may contain the low-molecular-weight alkaloid anatoxin-a. This toxin mimics the action of acetylcholine. Death results from respiratory arrest. Blooms of some *Aphanizomenon* species may produce small amounts of the neurotoxin (saxitoxin) and at least three related toxic compounds of unknown structure.[34,35] Saxitoxin blocks sodium conductance through excitable membranes, which subsequently stops nerve action potential. Death resulting from saxitoxin is caused by respiratory neuromuscular paralysis.[35] Anatoxin-a(s) can also be produced by these organisms, whose action is to inhibit acetylcholinesterase activity. *Microcystis* species of cyanobacteria can produce the cyclic hepatotoxin microcystin.[34,35] The immediate cause of death is hemorrhagic shock resulting from massive hepatocellular necrosis and collapse of the hepatic parenchyma.[37]

Diagnosis and Treatment

Analytic methods are now available that can isolate and identify the cyanobacteria organism and the toxins from affected animals (gastric contents, liver) or suspect water. Because the concentrations of cyanobacteria and toxins can vary tremendously over a short time, recovery of the organism or toxin from water may not be possible when clinical signs are noted in exposed animals. A diagnosis can be based on history of exposure to a water source when conditions favoring cyanobacterial overgrowth are present, coupled with onset of compatible clinical signs and analysis of tissues and water for the toxin. One can analyze water samples to identify specific blue-green algae, the presence of which may help support a diagnosis of algal toxicity if toxin testing is not feasible.[35]

No specific antidote is available, and animals often are found dead before treatment can be initiated.[35] Algal growth in surface water can be controlled by a variety of herbicides and copper sulfate. When water is treated with these compounds, it is important to ensure that the compounds are being used in a safe manner for all susceptible livestock.

PESTICIDES

Cholinesterase-Inhibiting Insecticides

The cholinesterase-inhibiting carbamate pesticides are composed of cyclic or aliphatic derivatives of carbamic acid, and numerous ones are commercially available.[35,38] A partial list of these compounds includes carbaryl, aldicarb, carbofuran, methiocarb, methomyl, and oxamyl. Carbamates are absorbed readily through the lungs, gastrointestinal tract, and skin. Carbamates do not accumulate in any particular tissue but do cross the rat placenta and depress fetal acetylcholinesterase; they are not metabolized readily in the fetus.[38] In human beings the carbamates generally penetrate the blood-brain barrier poorly and therefore may produce few CNS signs.[34] Carbamates do not require activation by liver enzymes to exert their effect. Toxicity data are not complete for several domestic animals, but lethal doses of the different compounds vary from less than 1 mg up to several hundred mg/kg of body weight. Carbamates are not stable in the environment and are fairly insoluble in water, but organic solvents and oils can carry the compounds across cell barriers.[38] It is important to note that not all so-called carbamate pesticides inhibit cholinesterase activity, so the clinician must determine exactly which carbamate compound the animal is exposed to and determine its mechanism of action.

Clinical Signs

Clinical signs can commence a few minutes to several hours after exposure, depending on the exposure scenario. The clinical episode frequently is less than 36 to 48 hours in duration, with the animal succumbing or recovering during this time.[35,38]

Signs of toxicity in horses reflect muscarinic and nicotinic cholinergic overstimulation. The signs suggestive of muscarinic cholinergic overstimulation include profuse salivation, severe gastrointestinal disturbances characterized by hypermotility, severe pain, abdominal cramps and diarrhea, excessive lacrimation, miosis, sweating, dyspnea, cyanosis, and urinary and fecal incontinence. Affected animals also may cough frequently as a sign of excessive accumulation of respiratory tract secretions. The signs reflected by nicotinic overstimulation include excessive stimulation of the skeletal muscles. The muscles of the face, eyelids, and tongue, in addition to the general musculature, may twitch. Some animals exhibit signs of generalized tetany that causes them to walk in a stiff-legged fashion. This hyperactivity may be followed by weakness and paralysis of the skeletal muscles.[35]

Signs of CNS involvement in domestic food-producing animals may include hyperactivity reflecting excessive stimulation of the CNS, but domestic animals rarely exhibit convulsive seizures (however, this can occur at high dose exposures). CNS depression is reported to be more common than CNS stimulation.[35]

Pathophysiology

Carbamates induce excessive stimulation of the parasympathetic nervous system by inhibiting acetylcholinesterase and pseudocholinesterase. The carbamate pesticides occupy the anionic and esteratic sites of acetylcholinesterase, with the esteratic site being carbamylated. Acetylcholinesterase can hydrolyze carbamate pesticides but at a slower rate than that for acetylcholine. Therefore carbamates are reversible inhibitors of acetylcholinesterase, but toxicosis occurs when the amount of pesticide is large enough that the rate of carbamylation of acetylcholinesterase exceeds the rate of hydrolysis of pesticide by the enzyme.[38] As a result, acetylcholine accumulates in neuroeffector and synaptic regions, resulting in the observed clinical signs of parasympathetic overstimulation.

The continuous stimulation of secretory glands leads to excessive salivation and accumulation of fluid within the respiratory tract and within the lumen of the bowel. Extensive pulmonary edema may occur and, along with bronchoconstriction, can lead to death in affected animals.[35]

Carbamates are removed from the circulation largely by spontaneous hydrolysis of the carbamate-cholinesterase complex. In addition, blood esterases can inactivate a portion of the circulating carbamate, and certain liver microsomal enzymes break down the compounds within hours of exposure.[34,38] The clinical signs associated with carbamate toxicity are generally rather short-lived, with recovery occurring in less than 36 to 48 hours in most animals.

Diagnosis and Treatment

The clinician is most likely to make a diagnosis on the basis of a history of possible exposure, clinical signs, and response to atropine treatment. Chemical analyses of biologic samples for carbamate residues can be rewarding; finding the pesticide in stomach contents, the liver, or feed samples may confirm the diagnosis.[35]

Cholinesterase activity in blood and brain tissue can also be used as a screening tool to determine exposure to this group of compounds (false inhibition can be observed if the patient is anemic). However, discretion must be used when interpreting these results because recommended therapeutic levels of carbamates applied to animals can result in some depression of blood cholinesterase activity. Because of the reversibility of the binding, one cannot rule out a carbamate exposure if the cholinesterase activity is determined to be normal. In one author's opinion, clinical signs of acute carbamate toxicity are associated with blood cholinesterase activity of less than 50% to 75% of normal values. Because the inactivation of cholinesterase by carbamates involves a much weaker and less stable binding than that by organophosphates, one should not dilute blood samples from suspect animals and should refrigerate them and analyze them as soon as possible.[35]

Affected animals should be treated as quickly as possible. Initial therapy should consist of atropine sulfate at 0.2 mg/kg of body weight. This initial dose should be divided, with approximately one fourth of the dose administered intravenously and the remainder given subcutaneously or intramuscularly. Repeated doses of atropine may be required but should be used only to counteract the parasympathetic signs. The skeletal muscle tremors may not respond to atropine therapy.[35]

Orally administered adsorbents such as activated charcoal may be useful in binding ingested pesticide, and aqueous cathartics may further aid in evacuation of the intestinal tract. Mineral oil probably should not be given orally in suspect

cases because organic solvents and oils may carry the compound across cell barriers. Dermally exposed animals should be washed with soap and water and rinsed thoroughly to prevent further exposure. Oximes such as pralidoxime are of questionable benefit in treating carbamate toxicosis, and their use may worsen the condition of the animals.[35,38] The cost of these drugs may be a prohibiting factor in their use in horses.

Organophosphates

The organophosphate pesticides are being used increasingly in a variety of ways. Some of the typical uses for these compounds include animal insecticides and parasiticides; plant insecticides, soil nematocides, fungicides, herbicides, and defoliants; insect repellents; and chemosterilants.[38] Horses can become intoxicated in several ways because these products are used so commonly in their environment.

A wide variety of organophosphorus compounds have been developed, and their toxicity varies greatly among compounds and among animal species. Osweiler et al.[35] have tabulated a good list of various organophosphorus compounds and their relative toxicities. In addition to variations in compound toxicity, a number of physicochemical factors also can affect the toxicity of organophosphorus compounds. The toxicity of these compounds decreases as they are degraded by sun, water, microbes, alkali, or metal ions such as iron or copper. An increase in toxicity may occur by storage activation, a process in which highly toxic isomers of certain pesticides are formed spontaneously in polar solvents or water. This reaction is speeded by heat. Parathion, malathion, fenthion, chlorpyrifos, diazinon, and coumaphos are some of the compounds that can undergo this type of storage activation.[38] The storage activation phenomena provide good reasons to use only freshly prepared preparations of organophosphate compounds on horses.

Other factors that can influence the toxicity of a particular organophosphorus compound are ambient temperature (higher temperatures may increase the toxicity of certain compounds); the vehicle in which the pesticide is dispersed; the age and sex of the animal; and the presence of other chemicals that may alter organophosphate toxicity. The combined effects of two organophosphates may be synergistic or antagonistic, and drugs that compete with organophosphates for target esterases, such as succinylcholine, phenothiazine, and procaine, may enhance organophosphate toxicity. In addition, drugs that have neuromuscular-blocking properties (e.g., inhalant anesthetics, magnesium ions, certain aminoglycoside antibiotics, and the depolarizing and nondepolarizing neuromuscular-blocking agents) also may enhance organophosphate toxicity. The organophosphates are poorly soluble in water but are soluble in organic solvents, fats, and oils. Oily vehicles or organic solvents also can facilitate passage of the organophosphates through the skin.[38]

Clinical Signs

Clinical signs of organophosphate toxicity are similar to those of carbamate toxicity and essentially are those of overstimulation of the parasympathetic nervous system, skeletal muscles, and CNS.

Overstimulation of muscarinic cholinergic sites results in profuse salivation and lacrimation; serous or seromucous nasal discharge; increased respiratory sounds and coughing resulting from bronchoconstriction and excessive bronchial secretions; profound gastrointestinal disturbances of increased motility, abdominal pain, and diarrhea; bradycardia; miosis; sweating; and frequent urination. Signs of nicotinic cholinergic overstimulation include muscle fasciculations, tremors, twitching, spasms, and a stiff or rigid gait. CNS signs frequently include anxiety, restlessness, and hyperactivity.[35,38] If the exposure is not severe enough to result in the death of the horse, the horse may require several days to recover completely; compounds that are more chlorinated can be stored in fat reserves and therefore have a longer half-life.[38]

Pathophysiology

Organophosphates can be absorbed from the gastrointestinal tract and lungs and through the skin. After absorption, they are distributed throughout the body, but most do not accumulate in any particular tissue. Many of the organophosphates must be activated by hepatic microsomal oxidative enzymes before they become potent esterase inhibitors. Phosphorothiolate and the phosphate class of organophosphates do not require activation and can inhibit esterases immediately after entering the bloodstream.[38]

Organophosphates act as irreversible inhibitors of true cholinesterase and pseudocholinesterase in mammals. They irreversibly phosphorylate the esteratic site of cholinesterases throughout the body. As a result, endogenous acetylcholine is not inactivated. Therefore acetylcholine accumulates in neuromuscular junctions; in parasympathetic postganglionic sites in smooth muscle, cardiac muscle, and glands; in all autonomic ganglia; and in cholinergic synapses within the CNS. The result is overstimulation of these sites, leading to clinical signs of toxicosis.[35,38]

Lethal amounts of organophosphates cause death by the combined effects of nicotinic, muscarinic, and central cholinergic overstimulation or receptor paralysis. These effects include hypotension, bradycardia, bronchoconstriction and excessive bronchial secretion, inability of the respiratory muscles to work properly, cyanosis, and central respiratory depression. The animal actually dies of asphyxia.[38]

Detoxification of organophosphates is accomplished mostly by serum and liver esterases. However, other enzymes in the liver and other tissues may attack the pesticides at rates dependent on the class of pesticide, the species, and the age of the animal. Water-soluble metabolites may be formed rapidly and the pesticide excreted quickly in the urine.[35,38]

Diagnosis and Treatment

The clinician should suspect organophosphate toxicosis when the horse has a history of possible exposure within the past 48 hours, along with characteristic signs of parasympathetic overstimulation. One can analytically test for organophosphate residues in body tissues or specimens and in other suspect materials.

A test for cholinesterase activity in blood or brain tissue is a rapid screening tool that can assist in determining if an animal has been exposed to excessive amounts of a cholinesterase inhibitor. Blood or brain cholinesterase activity values of less than 50% to 75% of normal can be compatible with exposure of the animal to excess organophosphates or other cholinesterase inhibitors (false inhibition can be observed if the patient is anemic). It should be noted that some depression of blood cholinesterase activity occurs when therapeutic levels of organophosphates or carbamates are used, so these results should be considered with discretion.[35,38]

Treatment to help control the muscarinic overstimulation should involve immediate use of atropine sulfate at 0.2 mg/kg of body weight. Approximately one fourth of this dose should be given intravenously, and the remainder should be given subcutaneously or intramuscularly. It may be necessary to repeat this dose at 3- to 6-hour intervals. Because atropine does not block the nicotinic cholinergic effects, the horse may continue to show signs of muscle fasciculation or tremors.[35,38] Atropine should be used with great discretion in the horse so as not to cause further complications, such as gut stasis with atropine overdosage.

The oximes, such as pralidoxime and pralidoxime chloride, act specifically on the organophosphorus-enzyme complex to free the enzyme and also react directly with the organophosphate to form a nontoxic complex that is excreted in urine. However, the use of these products in horses may not be economically feasible. The recommended dose varies between 20 mg/kg and 25 to 50 mg/kg given as a 20% solution intravenously, slowly, over several minutes. Oximes are reported to work best in the presence of atropine, so they should be given to the animal after atropine administration. Treatment with the oximes can be repeated if signs reappear.[38]

Other treatment measures include removal of the source, if possible; use of orally administered activated charcoal; laxatives to aid in evacuation of the bowel; washing with soap and water and thorough rinsing if dermal exposure has occurred; and supportive therapy such as fluids and electrolyte administration, if necessary. Drugs that should not be used when treating organophosphate toxicosis include phenothiazine tranquilizers, succinylcholine, and morphine.[35,38]

Chlorinated Hydrocarbons

The current use of chlorinated hydrocarbon pesticides is limited and severely restricted because of their persistence in the environment and their incorporation into the food chain. However, certain agents still are being used, primarily as contact insecticides and as ectoparasiticides.[35,38,39] A partial list of compounds includes lindane, aldrin, dieldrin, endosulfan, endrin, and methoxychlor.

The chlorinated hydrocarbon insecticides are poorly soluble in water but are soluble in organic solvents and oils. Oily vehicles or organic solvents can facilitate penetration of the insecticide through intact skin. Because this group of compounds also is characterized by volatility, exposure to the pesticide can occur through inhalation of the vaporized compound.[38] Because these compounds accumulate in body tissues, primarily adipose tissue, signs of toxicosis can result from repeated exposure to lesser amounts or after a single excessive dose.[39] Toxicity varies greatly among the different compounds, and Osweiler et al.[35] have tabulated the toxicity of a number of these compounds for various animal species.

Clinical Signs

The chlorinated hydrocarbons generally act as diffuse stimulants (less commonly as depressants) of the CNS, with the onset of signs ranging from several minutes to several days after exposure. The signs displayed may be progressively severe or explosive and fulminating.[35] Initially, the animal may be hypersensitive, apprehensive, or belligerent. These behavioral aberrations may progress to abnormal posturing or frenzied or maniacal behavior. Nervous signs can begin with hypersensitivity and muscle fasciculations beginning around the head and facial area and proceeding caudally eventually to include the hindquarters. These muscle spasms can occur intermittently or continuously. Clonic-tonic seizures often follow and may result in death or be followed by intermittent periods of CNS depression. Autonomic manifestations of profuse salivation, mydriasis, diarrhea, urination, and bradycardia or tachycardia with arrhythmias may occur. Some animals may lose coordination and stumble while walking, walk aimlessly, or move in circles. Other notable signs may include increased rate and depth of respiration and fluid sounds in the lungs. Death may occur within minutes, hours, or days or not at all.[35,38,39]

Pathophysiology

Chlorinated hydrocarbon pesticides gain entry into the body by way of the gastrointestinal and less commonly through the respiratory tracts and absorption through the skin. Once they enter the bloodstream, they are thought to bind to serum lipoproteins and are distributed throughout the body. Eventually, an equilibrium is reached in which the pesticide concentration varies among different body compartments. Most of the absorbed pesticide is stored in fat, but brain and fetus also can accumulate significant amounts.[35,38]

Adipose tissue is the main storage tissue for chlorinated hydrocarbons and as such can retain some of these compounds for an extended period. The pesticide is mobilized slowly from fat, which can account for its presence in blood and milk for weeks to months after a single exposure.[38]

The chlorinated hydrocarbons are broken down by liver microsomal enzymes, and the pesticide and its metabolites are excreted in urine, bile, milk, and feces. This first stage of elimination is fairly rapid and may account for 40% to 50% of the compound being eliminated during the first 3 to 4 days after exposure.[38]

The exact mechanism of action of all of the chlorinated hydrocarbons is unknown, but they act as nonspecific stimulants of the CNS. A suggested mechanism is that some of these compounds easily enter neural membranes and prolong the time during which some of the sodium channels in the membrane are open during depolarization. In addition, potassium efflux from the cell is hindered. The net effect of these ion imbalances is a decreased transmembrane resting potential that causes a decreased firing threshold and an increased neuronal excitability.[38]

An increase in whole-brain free ammonia concentration and brain glutamine also occurs, but whether these changes are a cause or an effect of the sodium-potassium flux defect is unknown. However, the onset and disappearance of convulsions in animals is correlated with an increase and decrease of brain ammonia concentration.[38]

The depression produced by some chlorinated hydrocarbons may result from rapid depolarizing blockade of neurons of the reticular activating system. Excessive depolarization of the medullary neurons may be responsible for respiratory failure, which is the usual cause of death in chlorinated hydrocarbon pesticide toxicosis. The muscle tremors in chlorinated hydrocarbon toxicosis are thought to be partly central in origin and partly caused by direct depolarizing effects on peripheral motor nerves.[38]

Diagnosis and Treatment

The clinician can make a tentative diagnosis when animals are known to have been exposed to an insecticide and are exhibiting compatible signs of toxicosis. Tissue samples

may be assayed for residues of the specific compound, but it is important to exercise caution when interpreting results because some of the compounds may be found in the fat of normal animals as a result of exposure to small concentrations in the environment. However, ppm concentrations may have diagnostic significance if history and clinical signs are consistent with chlorinated hydrocarbon poisoning. Brain and liver concentrations of this category of pesticides are reported to be better correlated with toxicosis than are concentrations in body fat.[38] Other suitable tissue specimens include blood, milk, kidney, and gastrointestinal contents.[35]

Because no specific antidote is available for the chlorinated hydrocarbons, treatment is symptomatic. For animals exhibiting convulsive seizures or neuromuscular hyperactivity, intravenous chloral hydrate or pentobarbital can be administered carefully to effect. Sedative doses of these two agents should control most of the behavioral, nervous, and locomotor signs. Sedation usually can be discontinued after 24 to 48 hours.

Oral exposure should be treated with saline cathartics and an adsorbent such as activated charcoal. If exposure was by the dermal route, the animal requires a thorough bathing with soap and water, followed by a thorough rinsing. As in all cases of pesticide toxicity, the source of contamination should be eliminated if possible.[35,38]

Strychnine

The present-day use of strychnine is primarily as a rodenticide. Although strychnine is available to the public through many retail outlets, instances of horses becoming intoxicated by strychnine are not common.[35,38,40] The approximate oral lethal dose for horses is 0.5 mg/kg.[35]

Clinical Signs

The clinical manifestation of strychnine toxicosis can appear as rapidly as 10 minutes to 2 hours after ingestion. Initial signs include apprehension, nervousness, and muscle stiffness. These signs are followed by stiffness and tetanic spasms that may appear spontaneously or may be initiated by stimuli such as sound, touch, or light. These tetanic spasms may vary from a few seconds to a minute or more and are characterized by extreme extensor muscle rigidity. Apnea frequently occurs during the spasms. Sometimes, intermittent periods of relaxation occur between seizures but become less frequent as the clinical episode progresses. In lethal cases the convulsive spasms become more frequent until death eventually occurs during a seizure or as a result of exhaustion and anoxia. The entire clinical episode typically lasts less than 2 hours.[35,38] Additional signs that are reported in horses include sweating, incoordination, prostration, convulsions, and death within approximately 2 hours of initial exposure.[38]

Pathophysiology

Strychnine is absorbed rapidly from the intestinal tract but not from the stomach. The alkaloid nature of the compound promotes its ionization within an acid medium; hence minimal absorption occurs from the stomach.[35] Once absorbed, strychnine is distributed readily throughout the body. Strychnine does not accumulate in any given tissue, but detectable concentrations can be found in the blood, urine, liver, and kidney.[35,38] Strychnine is metabolized in the liver by hepatic microsomal enzymes, and it and its metabolites are excreted in urine.[35] Excretion of strychnine is rapid, with most of a lethal dose being eliminated within 24 hours.[38]

Glycine is an inhibitory neurotransmitter in the spinal cord and medulla that serves to dampen or modulate efferent motor neuron activity. The purpose of this modulating effect is to provide smooth, coordinated muscle contraction and activity that are appropriate and consistent with the requirements for locomotion and respiration. Strychnine acts competitively to antagonize glycine by blocking its uptake at postsynaptic sites on receptors in the spinal cord and brainstem. The result of this blockade is hyperexcitation of muscle groups caused by lack of normal inhibition. Muscle reflex activity is allowed to proceed in basically an uncontrolled manner. All striated muscles are affected, but the more powerful extensor muscles tend to predominate and produce generalized rigidity and tonic seizures.[35,38]

Diagnosis and Treatment

A tentative diagnosis of strychnine poisoning can be made on the basis of a history of possible exposure, characteristic limb rigidity, and a rapid recovery (i.e., less than 24 hours in many cases) in animals aggressively treated in time. To confirm the diagnosis, the clinician may submit tissue samples for evaluation. Stomach content is the most suitable specimen for analysis.[35,38] Many commercial strychnine baits contain grain (e.g., wheat, oat, barley, sorghum) dyed red or green; seeing this in the stomach contents allows the clinician to pursue strychnine poisoning as a differential diagnosis.

Treatment of strychnine poisoning is symptomatic because no specific antidote is available. The horse should be kept in a quiet environment with minimal stimulation. Of primary importance is maintenance of relaxation and prevention of asphyxia. Pentobarbital or chloral hydrate solutions should be given intravenously to produce effective sedation. Complete anesthetization of the horse usually is not necessary or desired. Other medications have been recommended for use in dogs and should be effective in horses as well. These medications include the centrally acting muscle relaxants methocarbamol (150 mg/kg intravenously) and guaifenesin (110 mg/kg intravenously), repeated as needed; diazepam to control seizures; and inhalation anesthetics if necessary.[35] Oxygen therapy and assisted ventilation may be necessary in some animals.

Additional agents that are beneficial are activated charcoal given orally and followed by a laxative to evacuate the bowel. Acidification of urine with oral ammonium chloride at 132 mg/kg also may enhance excretion of strychnine, but the efficacy of this treatment is not well documented. Although toxic doses of strychnine may be depleted from the body in a short time, it may be necessary to maintain relaxation and sedation in the horse for 24 to 48 hours.[35]

Metaldehyde

Metaldehyde is used primarily as a molluscicide in snail and slug baits in coastal and low-lying areas. The baits are generally in the form of meal, pellets, and liquids and are placed around crops, gardens, or ornamental plants.[35,38] Horses can become poisoned through inadvertent exposure to bags or containers of bait. No specific toxicity studies could be found regarding horses, but reports indicate that horses may be more susceptible to toxicosis than dogs, in which the acute oral LD_{50} may be as little as 60 to 100 mg/kg.[38] In two separate reports, horses died after ingestion of as little as 60 mg/kg and 120 mg/kg.[41,42] Experimentally, a parasitized yearling colt died after exposure to 0.1 mg/kg.[42]

Clinical Signs

The clinical signs reported in horses include acute onset of signs within 1 hour after exposure; excessive sweating, profuse salivation, restlessness, hyperesthesia, incoordination, and tachycardia can be observed. One horse exhibited violent muscle spasms just before death.[41] Other signs include muscle fasciculations, clonic spasms, and rapid and deep respiratory movements.[42] Death occurs rapidly (3 to 5 hours) in horses exposed to a lethal amount of metaldehyde[41,42] and is thought to result from acute respiratory failure.[38] Dogs also are reported to exhibit signs of convulsions and elevated body temperature (often referred to as "shake and bake").[35,38]

Pathophysiology

Metaldehyde is absorbed readily from the gastrointestinal tract. Gastric hydrochloric acid enhances its decomposition to acetaldehyde, and metaldehyde and acetaldehyde are absorbed and readily cross the blood-brain barrier. The exact mechanism of action of metaldehyde is yet to be elucidated.[38]

Diagnosis and Treatment

A history of possible exposure to a molluscicide coupled with the appropriate clinical signs can lead to a tentative diagnosis of metaldehyde toxicity. Stomach contents can be analyzed to detect metaldehyde or acetaldehyde, and a formaldehyde-like odor may be present in the stomach contents.[35,38]

No antidote is available for metaldehyde. Treatment is symptomatic and aimed at sedation; removing the compound from the stomach; and supportive therapy such as maintaining proper fluid, electrolyte, and acid-base indexes. Sedatives such as xylazine, acepromazine, and diazepam may be useful to control convulsive behavior. Methocarbamol may help control muscle spasms and fasciculations, and mineral oil can be given orally to aid evacuation of the gastrointestinal tract.[35,38]

Methiocarb

This molluscicide has been reported to cause toxicity in two horses: one died, and the other fully recovered.[43,44] In each instance the amount ingested was estimated to be from 100 to 125 g of a 4% weight per volume preparation.

Clinical Signs

The onset of signs was rapid, beginning within a few minutes of ingestion. Muscle tremors, which became severe, and profuse sweating and salivation were noted. Both horses had increased heart and respiratory rates, and the surviving horse also exhibited signs of abdominal discomfort. Clinical signs gradually lessened until they became absent approximately 12 hours after onset in the surviving horse.[44] The horse that was fatally intoxicated died approximately 12 hours after initially showing signs. Postmortem findings included severe generalized pulmonary congestion with froth accumulation in the airways and a number of large hemorrhagic areas scattered throughout the intestinal tract.[43] Methiocarb is a cholinesterase-inhibiting carbamate compound; readers may wish to consult the section on carbamate and organophosphate pesticides for more information.

Treatment

The specific antidote for methiocarb is atropine sulfate.[43,44] Repeated dosing may be necessary. Other suggested remedies include supportive therapy consisting of sedatives, calcium solutions, and mineral oil.[44]

4-Aminopyridine

4-Aminopyridine is used commercially as a bird repellent and often is mixed with grain before its distribution. In the one reported instance of 4-aminopyridine toxicity, affected horses were exposed to corn that contained the substance.[45]

Clinical Signs

The two affected horses began showing signs of profuse sweating, severe convulsions, behavioral abnormalities, and rapid fluttering of the third eyelid. Both horses died within 2 hours of the onset of clinical signs and approximately 8 hours after ingesting the contaminated corn. No specific lesions were noted at necropsy. The estimated lethal dose of 4-aminopyridine for these two horses was 2 to 3 mg/kg of body weight.[45]

Pathophysiology

The mechanism whereby 4-aminopyridine causes death has not been elucidated. However, this substance readily crosses the blood-brain barrier and may enhance the release of acetylcholine and other neurotransmitters from prejunctional nerve endings.[38,46] The result is a stimulatory effect on the CNS.

Diagnosis and Treatment

A diagnosis of suspected toxicity is confirmed by testing the suspect material and stomach contents of affected horses.[45] No specific antidote for 4-aminopyridine toxicity is suggested, but the clinician can give affected horses supportive therapy and mineral oil orally to enhance evacuation of the intestinal tract. Given the known mechanism of action of this compound, CNS depressants such as phenobarbital would seem to have some value in treating the CNS signs of affected horses. However, the efficacy of this treatment in unknown.

Levamisole

Levamisole has not gained widespread use as an equine anthelmintic, principally because of its limited efficacy in destroying strongyles and because its toxic dose is close to its therapeutic dose. Levamisole is effective, however, in eliminating lungworms, ascarids, and adult pinworms from horses. The drug also has been used in human beings and other animal species in an attempt to enhance immune system function.

Clinical Signs

Clinical signs associated with levamisole toxicity occur within 1 hour of administration and include hyperexcitability, muscle tremors, hyperactivity, excessive sweating, and lacrimation. Recumbency may follow these signs, but animals that recover generally appear normal within 12 hours after exposure.[47-49] Adverse effects are more likely after subcutaneous injection than oral drenching.[49] The toxic dose of 20 mg/kg is close to the therapeutic dose of 15 mg/kg, and 20 mg/kg may cause death in some horses.[38]

Diagnosis and Treatment

The diagnosis is suspected when horses exhibit clinical signs suggesting levamisole toxicosis, and exposure is known to have occurred. No specific antidote is available, so therapy includes supportive care. Most animals recover uneventfully if a sublethal dose has been given.

Carbon Disulfide

Carbon disulfide is seldom used as an anthelmintic in present times but has enjoyed widespread use in the past for treatment

of infestation caused by *Parascaris equorum* and *Gastrophilus* species.[50] Carbon disulfide is a manufactured product used as a solvent for resins, pesticides, and waxes and as an agent to remove greases. The chemical is used widely as a fumigant to control insects in stored grain.[35] Carbon disulfide is an exceptional fat solvent and in the pure state is a clear, colorless volatile liquid with a sweet, aromatic odor resembling that of decaying cabbage. The chemical is well absorbed through the skin and lungs and after ingestion.[34,35]

Clinical Signs

Reported signs of acute toxicity in animals include dyspnea and cyanosis, spasmodic tremors, vascular collapse, prostration, convulsions, coma, and death.[35] Signs referable to local irritation following inhalation might include salivation and coughing. A combination product of piperazine–carbon disulfide and phenothiazine caused transitory signs of overtranquilization and unsteady gait when dosed at 8 oz per 45 kg of body weight.[50] Whether any of these effects were related directly to carbon disulfide was not determined. Chronic exposure causes neuropsychiatric changes, peripheral neuropathies, and cranial nerve dysfunction in human beings,[34] but chronic exposure in horses is unlikely.

Pathophysiology

Because carbon disulfide is a potent fat solvent, local skin contact results in erythema and pain, and prolonged contact produces chemical burns and vesiculation.[34] The chemical is irritating to mucous membranes when inhaled or ingested.[34]

At the cellular level carbon disulfide acts to block enzymatic processes by reacting with nucleophilic compounds, including pyridoxamine, monoamine oxidase in the cerebrum, and dopamine decarboxylase. Carbon disulfide binds to microsomal enzymes, thereby reducing their activity, and produces a centrilobular hepatic necrosis. In addition, carbon disulfide chelates copper and zinc and therefore can produce disturbances in trace mineral balance.[34]

Diagnosis and Treatment

No specific diagnostic test is available for use in animals suspected of suffering from carbon disulfide toxicosis. In one report affected animals had increased BUN and bilirubin concentrations, increased serum activities of AST and alanine aminotransferase, and increased serum cholesterol concentration. Affected horses also had depressed serum concentrations of protein-bound iodine and magnesium.[50]

In human beings the iodine-azide test is useful to identify carbon disulfide metabolites in urine,[34] but the efficacy of this procedure for diagnostic use in horses is unknown. Treatment of toxicosis primarily involves removal of the source and symptomatic care (Box 21.1).

BOX 21.1 General Rules Regarding Treatment of Suspected Toxicoses

1. Removal of the toxicant or animal from the environment
2. Removal of the toxin from the body of the animal, if possible
3. Cleaning of the skin or contact surface with suitable agents if the route of exposure is dermal
4. Evacuation of the gastrointestinal tract by appropriate means if contamination resulted from ingestion of the toxin
5. Maintenance of normal body functions and physiologic processes by means of basic supportive care measures, such as fluid administration and blood pH and electrolyte modification
6. Elimination of the toxin from the system of the animal as expeditiously as possible
7. Measures to prevent reexposure or recontamination of the animal by the toxic substance

MISCELLANEOUS AGENTS

Nicotine

Nicotine is an alkaloid contained in tobacco leaves. Although toxicosis might result from ingestion of excessive amounts of tobacco leaves or cured tobacco in cigarettes or cigars, this route of toxicosis probably is rare in horses. Horses are more likely to be intoxicated by ingestion or exposure to the salt nicotine sulfate.[35,38] A concentrated solution of nicotine sulfate (Blackleaf 40) has been used to control leaf-eating insects and occasionally as a premise spray to control certain ectoparasites.[35,39] Horses may ingest this substance from spills of the solution, from open containers, or from foliage that has been sprayed with the product.[38] Topical exposure may result when horses are housed in stables where this product has been used for mite control.[51] The lethal dose of nicotine in the horse is 100 to 300 mg.[35]

Clinical Signs

The signs of nicotine toxicity are rapid in onset, often occurring within a few minutes after exposure. Initially, the signs noted are those of cholinergic overstimulation (i.e., excitement, increased respiration, and salivation). Increased peristalsis and diarrhea also can occur.[35,39] These signs are transitory and are followed rapidly by lethargy, muscle weakness and ataxia, slow and shallow respiration, and an increased heart rate. Convulsions also can occur. In fatal cases these signs progress to collapse, coma, and death within minutes to hours after the onset of clinical signs.[35,38,39]

Pathophysiology

Nicotine is absorbed readily from the oral mucosa, respiratory tract, and gastrointestinal tract (excluding the stomach) and through intact skin.[34,35] If ingested, nicotine also can exert a direct, rapid caustic action on the mucosa of the mouth and throat, esophagus, and stomach.[35] The substance is metabolized primarily in the liver, and nicotine and its metabolites are excreted in urine. In human beings urinary acidification greatly enhances clearance of nicotine and its metabolites.[34]

Initially, and only for a short time, nicotine stimulates the autonomic nervous system ganglia, neuromuscular junctions, and some synapses in the CNS by depolarization of the postsynaptic membrane.[34,35] With toxicosis, however, the stimulation is followed rapidly by a depolarizing-type blockade of all these nicotinic cholinergic receptors.[34,35,38] Large doses result in a descending paralysis of the CNS, and death results from respiratory failure caused by paralysis of the diaphragm and chest muscles.[35,38] The effects of a sublethal dose should diminish in a few hours.[38]

Diagnosis and Treatment

No diagnostic lesions are present at necropsy. However, the distinct odor of nicotine may be present in stomach contents.[35,38] One can evaluate urine, blood, liver, kidney, and other tissues for nicotine content in suspect cases.[35]

Treatment often is ineffective because of the rapid course of the toxicosis. However, because no specific antidote exists for nicotine toxicity, affected horses should be treated symptomatically. If exposure has been through the skin, washing with soap and water, followed by thorough rinsing, is indicated.[35] After oral exposure, affected horses can be treated with oral laxatives in an attempt to reduce absorption of the toxin. Activated charcoal also may be beneficial in adsorbing residual nicotine in the intestinal tract.[35,39] Atropine sulfate is reported to be without value in affected horses because it does not protect vital nicotinic receptors in the respiratory muscles and the CNS from the effects of nicotine.[38] Artificial respiration becomes the only means of maintaining life once the respiratory depression reaches a critical level. This last-ditch effort usually fails.[38]

Ammonia

Ammonia toxicosis in the horse can occur in two ways: by primary exposure to ammonia gas or by secondary metabolism of urea to ammonia within the body (see the section on Urea and Nonprotein Nitrogen Substances).[34,35] Primary exposure to toxic concentrations of ammonia gas probably is rare in horses, even though ammonia is the air pollutant most frequently found in high concentrations in animal facilities. The concentrations of ammonia found in stables may be irritating to horses but probably will never reach lethal concentrations. Another source of ammonia toxicosis to horses might be compressed anhydrous ammonia, which is used as an agricultural fertilizer. An accidental spill or spray of anhydrous ammonia near a horse or livestock facility could have disastrous and possibly lethal results.[35] Human beings can detect the odor of ammonia at a concentration of 30 ppm, eye and nasal irritation occurs near 50 ppm, and severe pulmonary dysfunction results from concentrations greater than 1000 ppm. Immediate death occurs at concentrations nearing 1500 ppm.[34]

Clinical Signs

Signs associated with low concentrations of aerial ammonia are those of irritation to the eyes and respiratory tract. Excessive tearing, shallow breathing, coughing, and nasal discharge are common findings. Higher concentrations can induce laryngospasm and pulmonary edema.[34] Exposure to anhydrous ammonia can result in impairment of eyesight, permanent loss of eyesight, respiratory disease, and skin burns.[35]

Pathophysiology

Ammonia is a highly water-soluble, irritating alkaline gas that causes liquefactive necrosis at high concentrations.[34] Because ammonia is so highly water soluble, it readily reacts with the mucous membranes of the eye and the respiratory tract.[35]

Diagnosis and Treatment

A diagnosis of aerial ammonia toxicosis is based primarily on history and physical examination findings. Laboratory evaluation is of little value in establishing a diagnosis of inhalation exposure,[35] but laboratory evaluation can be useful to assess the degree of damage to the respiratory tract.

Treatment involves removing the horse from the source of exposure. If exposure has been severe enough to result in ophthalmic or respiratory tract disease, these conditions should be treated accordingly.

Urea and Nonprotein Nitrogen Substances

Urea and other nonprotein nitrogen substances, including various ammonium salts, are added to ruminant rations as a source of nonprotein nitrogen because ruminants can use these compounds to provide a large percentage of their protein nitrogen requirements. Urea has additional uses as a fertilizer and as a substitute for salt in melting snow and ice.[35,38] Horses are only mildly susceptible to urea toxicosis, and it is highly unlikely that horses would ingest urea or urea-containing feedstuffs in amounts sufficient to cause clinical signs.[35,39] However, horses are more susceptible to toxicosis caused by ingestion of ammonium salts,[35] which may occur after accidental exposure to these substances. In horses urea is lethal when ingested at a rate of 4 g/kg of body weight, and ammonium salts are lethal at a dose of 1.5 g/kg of body weight.[38] Urea and other nonprotein nitrogen formulations are toxic to animals simply because they are hydrolyzed to ammonia, which is responsible for causing the derangements associated with toxicosis.

Clinical Signs

The spectrum and intensity of signs in ruminants with urea toxicosis vary, and the same is probably true for horses. The clinical course is usually acute and rapid, often occurring from a few minutes to a few hours after consumption.[35] Occasionally, animals are found dead, or they may die quickly after exhibiting signs of weakness, dyspnea, colic, bloat, and terminal tonic convulsions. Other varied signs can be present. Behavioral abnormalities such as restlessness and dullness may be present. Excitement and even belligerency may follow these signs. Nervous signs such as hyperesthesia, tremors, and muscle twitching and spasms can occur. Autonomic nervous system derangements may include salivation, bradycardia, hypertension, and severe colic. More terminal signs may include increased and labored respirations, cardiac arrhythmias, frothing at the mouth, and cyanosis. Intermittent tonic-opisthotonic seizures also can be elicited near death. The onset of signs may range from 10 minutes to 4 hours, and death typically occurs within a few hours after exposure.[38]

Pathophysiology

Urea is hydrolyzed to ammonia by the action of the enzyme urease. This reaction is hastened by an alkaline pH, and in the horse these requirements are found in the cecum. In horses urea is absorbed from the small intestine and excreted in the urine. The only urea that might contribute to toxicity in the horse would be that excessive amount that reaches the cecum and is available for hydrolysis.[39]

In normal animals ammonia liberated from nonprotein nitrogen sources can be in the form of the ammonium ion. This ion is soluble, but its charge prevents it from being efficiently absorbed across membranes. Ammonia is also soluble; however, because it lacks an ionic charge, it can be absorbed readily across membranes to enter the bloodstream.[38] Ammonia is a normal byproduct of tissue metabolism, and in hepatocytes it is converted to urea by the urea cycle or is incorporated into glutamic acid in the synthesis of glutamine. Toxicosis occurs when the amount of ammonia absorbed into the bloodstream exceeds the ability of the horse to detoxify it.[38]

The primary mechanism of ammonia toxicosis is thought to be inhibition of the citric acid cycle, but the exact mechanism by which this occurs is not known.[38] Ammonia saturation of the glutamine-synthesizing system has been suggested to have an inhibitory effect on the citrate cycle, creating a decrease in its intermediates and a subsequent decrease in cellular energy production and respiration. As the citrate cycle fails, cells begin to malfunction. Cellular energy and respiration deficits may cause ultrastructural damage leading to degenerative changes and eventual cell death. The role of ammonia in causing signs of encephalopathy is controversial and not well understood.[51]

Laboratory abnormalities associated with ammonia toxicosis include elevated serum ammonia, potassium, phosphorus, lactic acid, glucose, and serum activity of the liver enzyme AST. Urine output decreases, and PCV increases as impending cardiac failure and shock ensue.[38]

The ultimate cause of death is inconsistent in urea toxicosis and poisoning by ammonium compounds. Cardiac failure may be induced by hyperkalemia, or ventricular fibrillation may result from the myocardial effects of ammonia itself. Convulsions may be prolonged and responsible for fatal anoxia. Pulmonary edema may be a complicating factor in some cases. Death also has been postulated to result from asphyxiation.[38]

Diagnosis and Treatment

Animals that die of ammonia toxicosis exhibit no characteristic lesions. Generalized venous stasis and congestion of organs may be present, along with pulmonary edema and scattered petechiation and ecchymoses. A strong odor of ammonia may be present, but this is probably much more characteristic of ruminants than of monogastric animals.[38]

Clinical signs and history can be helpful in establishing a diagnosis. Blood and ocular fluid ammonia can be evaluated in the laboratory, but the results must be interpreted with caution.[38] Storage of the sample, length of time between death and time of sampling, and length of time between sampling and analysis can influence the blood ammonia concentration. Tissue specimens must be frozen immediately if they are to be analyzed.[35] Suspect feeds can also be analyzed for urea or total nitrogen content.

Treatment is often unrewarding because of the rapidity of onset. No specific antidote is available for ammonia, so therapy is mostly symptomatic. Orally administered laxatives such as mineral oil may be beneficial. The clinician should correct any deficits in fluid volume or abnormalities in acid-base or electrolyte concentrations. Horses that are convulsing can be controlled with pentobarbital, and a patent airway should always be maintained. Assisted ventilation, if necessary, is usually futile because of the poor survival rate of severely affected animals.[38]

Toxicoses Causing Signs Relating to Central Nervous System Depression

As with toxic problems associated with other primary clinical signs, it can be difficult to determine the source of CNS depression. The principal task is to rule out the existence of primary cranial disease. Even with the availability of advanced imaging equipment, this can be difficult. Unless the clinical signs or history is definitive, the diagnosis of a toxic problem is often presumptive.

PLANTS

Black Locust *(Robinia pseudoacacia)*

The black locust tree has been described as toxic to horses.[52,53] The toxic principle is classified as a lectin, which is present in seeds, sap, roots, wood, leaves, and bark of the plant. Lectin has been used in cytologic research because of its ability to stimulate glycoprotein biosynthesis and cell proliferation in lymphocytes of various animal species.[54] Horses may become intoxicated by ingesting the bark. Only small amounts of lectin are reported to precipitate clinical disease.[53]

The clinical signs reported are mental depression, weakness, posterior paralysis, irregular heart rate, pale mucous membranes, and anorexia. Abdominal discomfort and diarrhea of varying degrees also may be evident.[52,53]

No definitive diagnostic test is available. Treatment of suspected toxicosis is mostly symptomatic and should include removal of the source; evacuation of the intestinal tract; and maintenance of normal fluid, electrolyte, and acid-base indexes. Honey locust *(Gleditsia triacanthos),* black locust's lookalike, is not thought to be toxic. However, the author is aware of a horse ingesting a large amount of honey locust seeds and suffering from acute colic and impaction.

Bracken Fern *(Pteridium aquilinum)*

Bracken fern is found most commonly in forested areas, burns, or abandoned fields in the northern and western United States.[53] Toxicity can occur at any time of year, but horses are more likely to consume the plant in late summer and fall, when other forage is scarce. However, horses also might acquire a taste for the plant in pastures or when it is incorporated in bedding. Horses also can become intoxicated from hay contaminated with large amounts of bracken fern. The entire plant is considered to be toxic.[52]

Clinical Signs

Signs of toxicosis occur after the horse has been consuming the plant for 30 to 60 days or longer. Horses also can exhibit signs even if they have not ingested bracken fern for 2 to 3 weeks.[52] The signs most frequently reported are incoordination, which may progress to severe ataxia; postural abnormalities, including arching of the back, crouching, and a base-wide stance; muscle fasciculations, which can progress to severe tremors; and bradycardia with cardiac arrhythmias early in the disease course, although tachycardia is most prevalent terminally. Terminal stages of the disease are characterized by signs of opisthotonus and clonic convulsions.[52,53] One report also describes an affected horse showing signs of colic and acute hemolytic anemia.[55]

Pathophysiology

The agent in bracken fern that is toxic to horses is thiaminase.[52,53] Bracken fern also is reported to contain ptaquiloside,[56] which is capable of inducing bone marrow suppression, hematuria, carcinomas, and a β-glucopyranoside that may enhance the release of endogenous histamine.[53] The significance of these last two compounds in the development of toxicity in horses is unknown. Thiamine plays an integral role in carbohydrate, fat, and protein metabolism and acts as a cofactor in enzymatic pathways responsible for energy production. Thiamine is an important cofactor in the decarboxylation of pyruvate to acetyl coenzyme A, which subsequently enters the tricarboxylic acid cycle.

Thiamine deficiency acts to interrupt these cellular energy processes and also limits certain metabolic pathways available for pyruvate metabolism, resulting in the systemic accumulation of a variety of metabolites, including pyruvate and lactate.

Diagnosis and Treatment

History and clinical signs are helpful in arriving at a diagnosis. No pathognomonic lesions or laboratory abnormalities occur, but some laboratory findings may include elevated blood pyruvate concentration and decreased plasma thiamine and red blood cell transketolase concentrations.[53]

Thiamine should be administered daily to affected horses at a dose of 0.25 to 0.5 mg/kg of body weight intravenously, subcutaneously, or intramuscularly for several days. Initially, thiamine can be given at a dose of 5 to 10 mg/kg intravenously, but this dose should be diluted in fluids and administered slowly because of the frequency of adverse reactions when thiamine is given intravenously. Thiamine deficiency usually can be prevented by dietary supplementation of yeast or cereal grains.[53]

Equisetum *(Equisetum arvense)*

Equisetum, commonly called *horsetail, marestail,* or *scouring rush,* has a geographic distribution similar to that of bracken fern. Like bracken fern, *Equisetum* is unpalatable to horses, and toxicosis usually results from hay contaminated with the plant.[52,53] Thiaminase is the toxic principle found in *Equisetum,* and the clinical signs, pathogenesis, and treatment are virtually identical to those of bracken fern.[52,53] Large amounts in the diet are necessary to induce clinical problems.

Milkweed (*Asclepias* spp.)

Several species of *Asclepias* have been reported to be toxic to large animals.[53,57] Clinical signs in affected animals are commonly associated with the nervous, respiratory, gastrointestinal, and cardiac systems. The fresh plants are reported to be distasteful to animals and not commonly grazed, but the plant may become incorporated into hay. Affected animals are reported to have a weak, rapid pulse; dyspnea; loss of muscular control; and muscular spasms. Salivation, bloating, and convulsions also may occur.[53,57] Most animals that reach the convulsive stage die.[57] Numerous compounds have been isolated from *Asclepias* species, including resinoids and cardioactive glycosides. One resinoid produces smooth muscle spasms of the gastrointestinal tract.[53] The mechanism of action of the other toxins has not been elucidated. No specific antidote is available,[53] but supportive care, including evacuation of the gastrointestinal tract, is indicated.

Yellow Starthistle *(Centaurea solstitialis)* and Russian Knapweed *(Acroptilon repens)*

Both yellow starthistle and Russian knapweed cause nigropallidal encephalomalacia in horses. *Centaurea melitensis* is suspected of causing this disease as well. These plants are found scattered over much of the western United States and are most abundant in nonirrigated pastures during the dry seasons of summer and fall. Both plants have a minimal moisture requirement and so may be the only green plants remaining in a dry season. Consequently, most poisonings occur during the summer or fall months.[53,58,59]

Horses apparently reject the plants when more suitable vegetation is available; the disease does not commonly occur in horses grazing improved pastures or grassland range.[59] However, some horses are reported to develop a craving for the plant and selectively seek it out.[53] Horses may eat the weeds, occasionally or frequently, without becoming ill, and continuous and protracted exposure to the plants is necessary for toxicosis to develop under experimental conditions. Feeding trials have shown that horses must consume an amount of weed equivalent to 59% to 200% of their body weight of yellow starthistle and 59% to 63% of their body weight of Russian knapweed for 3 to 11 weeks of continuous feeding before clinical signs develop. The plants retain their toxicity when dried and incorporated into hay. All ages may be affected, but younger horses generally seem more prone to the disease. One study reported a median age of about 2 years in affected horses.[59] Equids appear to be the only animals that develop nigropallidal encephalomalacia when exposed to these plants.

Clinical Signs

The onset of signs is always sudden, beginning with variable degrees of impairment of eating and drinking. Coordinated movements of prehension, mastication, and deglutition are often lacking. Affected horses are unable to chew adequately and propel the food to the back of the mouth. Some horses may show only faulty prehension, whereas others are unable to eat at all. Most horses, however, appear to be able to swallow if feed or water gains access to the posterior pharynx. More severely affected horses may attempt to drink by immersing their muzzles deeply into the water in an attempt to force water into the posterior pharynx.[53,59-61]

Hypertonicity of the facial muscles is a characteristic sign, particularly when feed is offered.[53] The horse often holds the mouth partially open with the lips retracted, resulting in a fixed facial expression. The tongue may protrude from the mouth, and many horses display constant chewing movements—hence the name "chewing disease."[53,61]

Other characteristic signs include weight loss, mild to moderate depression, and yawning. Most horses can be roused readily from somnolence by mild stimulation. Few animals may show aimless, slow walking or circling early in the disease course. The gait is usually normal, but occasional deficits are apparent, including stiffness, slowness, ataxia, tetraparesis, and conscious proprioceptive deficits.[53,61] In cases of prolonged exposure, a wobbly, shuffling gait may occur because of weakness. Sensation and reflexes appear normal, and the animals are afebrile.[61]

Horses less severely affected may adopt unusual means of eating by scooping feed into the mouth. These animals may survive for months, but complete recovery has not been observed in confirmed cases of the disease. In some instances, however, residual signs may become almost undetectable.[59] Death of affected horses is from starvation and dehydration.

Pathophysiology

The pathogenesis of the necrotic brain lesions is unknown, but these plants have been postulated possibly to contain a specifically toxic substance or to lack some nutritional component necessary for the health and well-being of the horse.[60,61] Several sesquiterpene lactones and polyacetylenes have been isolated from these plants, but their significance remains undetermined.[62] Dipyrone has been postulated to be the inciting agent.

Diagnosis and Treatment

The antemortem diagnosis of nigropallidal encephalomalacia is based largely on observation of clinical signs and prolonged exposure of the horse to the plants by grazing or by severely contaminated hay. If available, magnetic resonance imaging can identify characteristic lesions on T1-weighted, T2-weighted, and proton density images. These lesions do not contrast enhance after gadolinium-diethylenetriaminepentaacetic acid administration.[63] Characteristic necropsy findings include unilaterally or, more commonly, bilaterally symmetric softening and necrosis in areas of the globus pallidus and substantia nigra. These areas usually are sharply defined and may be cavitary.[53,61]

No known treatment exists for affected horses. Prevention requires keeping horses from the plant and providing adequate, suitable forage.

Milkvetch and Timber Milkvetch (*Astragalus* spp.)

Most of the variants of *Astragalus miser* are referred to as *timber milkvetch* or *milkvetch.* These plants are found primarily in the western United States from northern Mexico to Canada and are essentially a cause of toxicosis to ruminants. Horses are reported to be poisoned by this group of plants, but there are no reports of the lesions.[64]

The disease in ruminants is characterized primarily by general depression, mental dullness, incoordination, and eventual hindlimb paralysis. Respiratory distress, cyanosis, and acute collapse also may occur. Acute and chronic forms of the syndrome are reported.[64]

The toxic principle, referred to as *miserotoxin,* is a β-D-glucoside of 3-nitro-1-propanol, which is metabolized in the intestinal tract to the highly toxic compound 3-nitro-1-propanol. Miserotoxin is broken down into inorganic nitrite and a three-carbon side chain. The nitrite is responsible for producing methemoglobinemia in animals but is not the primary cause of death.[64]

No specific antidote is recommended. Poisoning is prevented by controlling the plants with herbicides and preventing livestock from grazing the plant.

MISCELLANEOUS AGENTS

Propylene Glycol

Propylene glycol is used commercially as a diluent for injectable drugs and as a glucose precursor in the treatment of hypoglycemia in ruminants. Horses may become intoxicated by the inadvertent use of propylene glycol if it is mistaken for a similar-appearing liquid paraffin preparation. Reports of toxicosis in horses are rare.[65,66]

Propylene glycol has a low oral toxicity in human beings. Approximately 45% of the absorbed dose is excreted unchanged by the kidney, and the remainder is metabolized by hepatic alcohol dehydrogenase to acetate, pyruvate, and lactate.[67]

Clinical Signs

Adverse signs occur within 10 to 30 minutes after a toxic dose of propylene glycol. Initial findings include salivation and profuse sweating, ataxia, depression, and tachypnea. Additional signs can include cyanosis, seizures, and coma.[65] Diarrhea also has been reported in one horse experimentally given a large dose of propylene glycol.[66] Death has occurred 1 to 3 days after ingestion of excessive amounts of the product.[65,66]

Pathophysiology

Propylene glycol is metabolized by hepatic alcohol dehydrogenase to form acetate, lactate, and pyruvate. After a large exposure, excessive amounts of these products accumulate, resulting in severe systemic lactic acidosis.[67] The clinical signs and toxicologic findings result from the effects of this severe acidemia on various body organs and tissues.

The acute oral LD_{50} for horses has not been established, but the doses for rats, rabbits, and dogs are 32, 18, and 9 mL/kg, respectively. One 450-kg horse died after intubation with 3.8 L (7.6 mL/kg) of propylene glycol,[65] but other horses have survived this dose. Toxic signs were reported in horses receiving 1.9 to 7.6 L of propylene glycol, but the only death occurred in the horse getting 7.6 L.[66]

Diagnosis and Treatment

Exposure to propylene glycol can be confirmed by chemical analysis of serum and urine (not commonly run by diagnostic laboratories). Necropsy findings associated with propylene glycol toxicosis may be minimal.[65] The horse given 7.6 L exhibited sloughing of the gastric mucosa, diffuse enterocolitis, renal congestion, and brain edema.[66] Histopathologic findings typically include hepatic necrosis, renal tubular necrosis and infarcts, myocardial perivascular edema, and pulmonary edema.[65,66]

Because no specific antidote is available, treatment of propylene glycol toxicosis aims at alleviating the severe acidemia that develops and providing supportive care for the other organs and tissues that may become compromised. Sodium bicarbonate solutions should be given to treat the acidosis. When possible, the clinician should monitor blood pH and adjust bicarbonate administration according to need. Intravenous fluids should be given to aid diuresis and maintain normal fluid volume. Pulmonary and renal function should be thoroughly evaluated in affected horses, and precautions should be taken to prevent the development of further pulmonary edema. Such precautions may require the use of diuretics and careful monitoring of the fluid administration rate.

Serum electrolyte concentrations should be maintained in normal ranges, and oxygen therapy may be beneficial in horses exhibiting tachypnea and cyanosis. Activated charcoal has been recommended in treating human toxicosis.[67]

Triclopyr

Triclopyr is an herbicide used to control hardwood species on road rights-of-way, industrial sites, and forest planting sites. Horses may become exposed to the herbicide by grazing areas that were inappropriately treated with the product in the past. This toxicosis is rare.

An experimental study was conducted to determine the toxic level of triclopyr to ponies. Ponies given 60 mg/kg per day for 4 days did not show any clinical sign of illness. Ponies given 300 mg/kg per day for 4 days did develop clinical signs. This study indicated that the toxic dosage in ponies was 5 times the estimated maximal intake for the highest recommended usage rate as an herbicide.[68] Therefore poisoning from the proper use of this herbicide is unlikely.

Clinical Signs

Initial signs of depression and decreased gastrointestinal motility were first noticed on the fourth day of the trial. Additional signs that developed were ataxia, weakness, muscle tremors, increased respiratory rate, cyanotic mucous membranes, and normal to slightly elevated body temperature. Some ponies became recumbent as clinical signs progressed. Two ponies died on the fifth and sixth days of the trial, and two other ponies were euthanized on the fifth day. The remaining two ponies were only mildly affected and recovered.

No significant changes were apparent in clinical chemistry values. Gross necropsy lesions consisted of pale livers and pale swollen kidneys, and a few horses had excessive intestinal fluid contents. Microscopic changes were mild and were those of nonspecific hepatosis and nephrosis.[68]

Pathophysiology

The mechanism whereby triclopyr produces clinical disease in horses has not been elucidated.

Diagnosis and Treatment

Toxicosis caused by triclopyr appears highly unlikely under natural circumstances.[68,69] Diagnosis of suspect cases is based on clinical signs and a history of exposure to the herbicide. No specific antidote is available. Affected individuals require supportive and symptomatic care.

Leukoencephalomalacia (Moldy Corn Disease)

Equine leukoencephalomalacia, a sporadically occurring disease of horses, ponies, donkeys, and mules, has a worldwide distribution. The disease usually is seasonal, with most cases occurring from late fall through early spring, and most outbreaks have been associated with a dry growing period followed by a wet period.[70,71]

This malady is caused by the mycotoxin fumonisin B_1, a metabolite of *Fusarium moniliforme*. Equidae become affected usually by ingesting *F. moniliforme*–infected corn or corn screenings, but the problem also has been associated with the consumption of commercially prepared diets.[70-73] Infected kernels often have a pink to reddish-brown discoloration, and damaged kernels and cob parts may have a much greater concentration of fumonisin than do undamaged kernels.[71,73] Feeds containing less than 10 ppm fumonisin B_1 have not been associated with disease, but concentrations of greater than 10 ppm can be lethal to horses.[74]

Clinical Signs

Two clinical syndromes are associated with fumonisin intoxication. The more common is the classic neurotoxic syndrome, but hepatotoxicosis also occurs in some horses. Older animals may be more susceptible than younger animals, and clinical signs become evident approximately 3 to 4 weeks after daily ingestion of contaminated feed. Onset of signs is typically abrupt, and death usually occurs in 2 to 3 days.[70,71] Occasionally, horses that showed few to no premonitory signs are found dead.[70]

The neurologic syndrome is characterized initially by incoordination, aimless walking, intermittent anorexia, lethargy, depression, blindness, and head pressing. These signs may be followed by hyperexcitability, belligerence, extreme agitation, profuse sweating, and delirium.[70,71] Recumbency and clonic-tetanic convulsions may occur before death. Recovery from acute episodes has been reported, but some horses retain neurologic deficits.[71]

Clinical signs associated with the hepatotoxic syndrome are swelling of the lips and nose, somnolence, severe icterus and petechiae of mucous membranes, abdominal breathing, and cyanosis. Affected horses also have acute onset of clinical signs with death occurring within a few hours to days.[70,71]

Pathophysiology

The gross lesions typical of equine leukoencephalomalacia include liquefactive necrosis and degeneration of the cerebral hemispheres, but degenerative changes also can occur in the brainstem, cerebellum, and spinal cord.[70-73] Necrotic areas can vary in size, and regions adjacent to the necrosis often are edematous and rarefied.[71,72] Lesions can be either unilateral or bilateral in appearance.

Gross hepatic lesions in affected horses generally are not pronounced. The liver may be slightly swollen, have a yellowish-brown discoloration, and contain irregular foci or nodules scattered throughout the parenchyma. Histologic abnormalities noted in the liver may include centrilobular necrosis and fibrosis, fatty infiltration of hepatocytes, portal fibrosis, biliary stasis, and bile duct proliferation.[70-72]

The mechanism whereby these changes occur has not been well elucidated but may be related to sphinganine and sphingasine metabolism. The brain lesions may be induced by ingesting smaller quantities of infected corn over a long period of time, whereby ingestion of higher quantities may produce fatal hepatotoxicosis over a shorter time interval.[71,75]

Several metabolites of *F. moniliforme* have been identified in feeds associated with outbreaks of equine leukoencephalomalacia. These include fusarin C, moniliformin, fusaric acid, 2-methoxy-4-ethylphenol, fumonisin B_1, and fumonisin B_2.[72,73] Toxicity information about fumonisin B_2 is unknown, but moniliformin, fusaric acid, and 2-methoxy-4-ethylphenol do not produce leukoencephalomalacia when injected intravenously into donkeys. Their role in the pathogenesis of the liver lesions in equine leukoencephalomalacia is likewise unknown. However, the neurologic and hepatotoxic syndromes can be produced by oral and intravenous administration of fumonisin B_1.[72]

Diagnosis and Treatment

The diagnosis of equine leukoencephalomalacia has been based mostly on observation of clinical signs coupled with a history of exposure to moldy corn. Typical postmortem lesions, when available, help confirm the diagnosis.

Clinicopathologic abnormalities are nonspecific and usually indicate some degree of liver dysfunction. Increased serum concentrations of bilirubin, AST, GGT, and LDH have been reported.[70,72,75] Cerebrospinal fluid abnormalities may include increased protein and total nucleated cell counts and an increased concentration of myelin basic protein.[71,75]

Analytic methods have been developed to assay fumonisin in feed material. Feed containing greater than 10 ppm fumonisin B_1 is not safe to feed to horses.[74] Because the disease requires a fairly prolonged exposure to infected corn, representative feed samples should be submitted for analysis. Feed currently being ingested may not be contaminated with the mold.

Treatment of equine leukoencephalomalacia is largely supportive. Horses that are hyperexcitable should be sedated to minimize injury to themselves and their handlers. Supportive therapy for hepatic dysfunction should be initiated if liver damage is evident, and some horses may require forced feeding and watering

if they become unable to eat and drink. Mannitol or DMSO may be administered to aid resolution of cerebral edema, and laxatives and activated charcoal may be given to eliminate toxins already in the digestive tract, although their usefulness is probably minimal because this disease is not an acute intoxication. Contaminated feed should be removed immediately from all exposed horses, and pastured horses should be moved to pastures without access to corn. Preventive measures include providing suitable feed material to horses and storing grains, particularly corn, under conditions that discourage mold growth.

Trichothecenes

The trichothecenes are a group of compounds elaborated primarily by *Fusarium tricinctum* and other *Fusarium* species. Only four of approximately 40 trichothecene derivatives have been found to occur naturally in feedstuffs. These four include T-2 toxin, deoxynivalenol, diacetoxyscirpenol, and nivalenol.[76,77] Of these, only one reported episode of T-2 intoxication in horses was found in the literature.[77] This group of mycotoxins more commonly causes gastrointestinal problems in other animals, mostly cattle.

In the reported outbreak horses showed clinical signs and laboratory abnormalities similar to those of equine leukoencephalomalacia caused by fumonisin B_1. Gross lesions in necropsied horses were also similar to those of equine leukoencephalomalacia. *F. tricinctum* was isolated from all suspect feed samples, and T-2 toxin was detected in varying concentrations in all examined feed samples. Other *Fusarium* metabolites detected in this outbreak included HT-2, verrucarin A and J, and roridin A. The toxicity of these metabolites in farm animals is not yet determined.[77]

Lead

Lead is reported to be one of the more common toxicants found in veterinary practice (mostly involving cattle), but it rarely is a cause of poisoning for horses. Materials that can serve as a source of lead toxicity to animals include lead-based paints, putty and caulking materials, greases, linoleum, leaded gasoline, solid lead solder, roofing materials, asphalt, discarded automobile batteries, and industrial effluents contaminating streams or forage. Water from lead plumbing also might serve as a source of toxicity.[53,76]

Acute and chronic forms of toxicosis can occur depending on the amount of lead ingested and the time frame in which ingestion occurs. Lead toxicosis in horses has historically been chronic and is associated with some type of forage contamination.[78,79] Foliage near lead smelters often contains excessive lead, and grasses located near busy highways have been reported to contain high lead concentrations.[53,79] Horses appear to be much more sensitive than cattle to prolonged, low-dose exposure to lead, yet they are much less sensitive than cattle to short-term exposure of large doses.[76,78] The acute oral lethal dose of lead acetate in horses is 500 to 750 g total dose, but chronic toxicosis can arise when 1 to 7 mg/kg per day is ingested over a period of days, weeks, or months.[76]

Numerous factors can influence the toxicity of lead in horses. Young animals and malnourished animals are reported to be more susceptible than older animals; solid lead is not as toxic as the more soluble salts, which are absorbed more readily; and concurrent exposure to lead and cadmium results in increased severity of clinical signs of lead poisoning. In addition, lead may interact with other minerals to affect toxicity. High levels of dietary calcium cause decreased gastrointestinal absorption of lead.[53,76,79]

Clinical Signs

The clinical signs of lead toxicity in horses are caused primarily by peripheral nerve dysfunction. The motor nerves are at greater risk, with minimal sensory perception loss in affected horses. Initially, affected horses may appear weak or have slight incoordination. Depression and weight loss become apparent and worsen over the disease course. Laryngeal and pharyngeal paralysis, dysphagia, dysphonia, and proprioceptive deficits occur. As the disease progresses, horses may exhibit flaccidity of the rectal sphincter; paresis of the lower lip; and difficulty in prehension, mastication, and deglutition. Aspiration pneumonia resulting from dysphagia and regurgitation of food is common, and fine muscle tremors may occur intermittently. Terminally, horses may show severe incoordination, anorexia, emaciation, and almost complete pharyngeal and esophageal paralysis with inability to swallow food or water. Seizures also may occur terminally. Colic and diarrhea may be apparent, but they are not common signs.[53,78,79] Progression of these disease signs may require weeks to develop.

Dietary lead crosses the placental barrier, and mares chronically exposed to lead in late pregnancy may deliver premature or small, weak foals. These foals are at greater risk of developing secondary disease complications.[78]

Pathophysiology

Lead enters the body primarily through ingestion. Inorganic lead cannot readily penetrate the skin, but organic forms such as tetraethyl lead and tetramethyl lead are absorbed through the skin. However, exposure of horses to organic lead compounds would seem to be a rare occurrence. Metallic lead shot or bullets lodged in tissues do not dissolve because tissue pH is too high.[76]

Metallic lead and lead sulfide are less absorbed than the acetate, carbonate, hydroxide, oxide, and phosphate salts. Less than 10% of ingested lead is absorbed across the gastrointestinal tract in adults, but if sufficient quantities of soluble salts are ingested, a significantly greater amount of lead can cross into blood. Even though intestinal absorption is inefficient, increases in blood lead concentrations can occur within 3 hours of dosing.[53,76]

Once absorbed, a large portion of lead is carried on erythrocyte membranes, where it is bound irreversibly to erythrocyte proteins. Much of the remaining lead becomes bound to albumin, and only a small proportion of the absorbed lead actually is free in serum. Unbound lead is in equilibrium with lead bound to erythrocytes and albumin, and distribution to various tissues takes place from the unbound fraction.

Much of the blood lead is removed in the liver. Within the liver, cellular trapping of lead is thought to occur by lead binding to cytoplasmic proteins called *metallothioneins*. Lead also accumulates within the renal cortex, where it becomes trapped as intranuclear lead proteinate inclusions in tubular epithelial cells. Unbound lead is excreted into milk, and it readily passes membrane barriers such as the placenta and the blood-brain barrier to become distributed in many body tissues.[76]

Unbound lead becomes immobilized and bound to bone substance, particularly in the physeal region, by an unknown mechanism. However, bone is considered to be the "sink" for lead and eventually may contain greater than 90% of the total body burden of lead. Deposition of lead into bone is a slow, gradual process, entailing redistribution of lead from other soft tissues. In this manner, bone serves as a detoxification mechanism under conditions of chronic exposure to

small concentrations. Bone cannot hold an infinite amount of lead, however, and when saturation occurs, signs of toxicosis may appear suddenly because of rising blood and soft tissue concentrations after continued exposure.[76]

Bile and feces are thought to be the primary pathways of lead excretion, and feces may contain unabsorbed lead and lead that has undergone enterohepatic circulation. Gastrointestinal secretions, including pancreatic secretions, also might be involved in elimination of lead from the body.[53,76]

The mechanism of action of lead at the cellular level is still under scrutiny. The known toxic effects of lead include inhibition of sulfhydryl groups of enzymes essential to cellular metabolism and inhibition of heme synthesis. Lead also is known to cause a decrease in local concentrations of the essential trace metals copper, iron, and zinc. These metals have important functions in mitochondrial enzymes, and interference by lead with these may adversely affect cellular respiration, oxidative phosphorylation, and the ATP synthetase complex.[76]

The peripheral neuropathy associated with lead toxicosis in horses is thought to be caused by peripheral nerve segmental demyelination, which impedes nerve impulse conduction and contributes to the clinical signs observed. The metabolic inhibitory effects of lead are speculated to cause the demyelination.[76,78]

Lead is known to damage the blood-brain barrier and capillary endothelial cells, resulting in cerebral edema and hemorrhage. Additionally, the damaged blood-brain barrier may allow cytotoxic solutes normally excluded from the brain to enter the brain substance.[76] Whether these mechanisms have any appreciable effect on the development of clinical signs observed in the horse is unknown.

Inhibition of heme synthesis is an important aspect of lead toxicosis in several animal species. This pathologic mechanism also occurs in the horse, but its significance to the overall disease course is limited. The result of heme metabolism interference and altered function of other erythrocyte proteins is a shortened erythrocyte half-life that can produce a normochromic, normocytic anemia, which is generally marginal in affected horses (PCV of 25%–30%).[78,79] The anemia also may be accompanied by nucleated red blood cells and Howell-Jolly bodies in peripheral blood.[79]

Two enzymes in the heme synthesis pathway that are particularly susceptible to lead are δ-aminolevulinic acid dehydratase (ALA dehydratase) and ferrochelatase. Inhibition of ALA dehydratase results in reduced levels of porphobilinogen in erythrocytes and an accumulation of ALA dehydratase, which is excreted in urine. Interference with ALA dehydratase also may be partly responsible for brain damage associated with lead toxicity in some species. Inhibition of ferrochelatase limits the formation of heme from protoporphyrin, resulting in an accumulation of unmetabolized porphyrins. These include protoporphyrin I, which is retained in the erythrocyte; uroporphyrins, which are excreted in urine; and coproporphyrins, which are excreted in feces. Lead also interferes with pyrimidine-specific 5′-nucleotidase activity, resulting in basophilic stippling of affected erythrocytes.[53]

Another important implication of lead toxicity is the suggestion that lead may be immunosuppressive by way of its interference with humoral and cell-mediated immune responses.[53,76]

Diagnosis and Treatment

Confirmation of suspected lead poisoning often is based on determination of blood or tissue lead concentrations. Blood levels of 0.30 ppm or greater are diagnostic if horses are showing clinical signs, but blood lead concentrations do not reflect the severity of poisoning.[78,79] Lead concentrations greater than 5 ppm in liver and kidney and 30 ppm in bone are considered diagnostic of lead intoxication in horses.[76]

In instances of chronic toxicity, however, blood lead values may be within the normal reference range. In such instances diagnosis of toxicity may be aided by administration of calcium disodium EDTA, which chelates lead in bone stores and increases the lead concentration in blood. The soluble lead complexes then are excreted in urine, with a resultant manyfold increase in urinary lead concentration within a few hours of EDTA administration.[53,76,77] The recommended dose of calcium disodium EDTA is 75 mg/kg intravenously.[53]

Clinicopathologic aberrations include increased concentrations of erythrocyte ALA and erythrocyte porphyrins and decreased activity of erythrocyte ALA dehydratase. Increased amounts of coproporphyrins, uroporphyrins, and aminolevulinic acid are found in urine, but measurement of erythrocytic ALA dehydratase is considered more diagnostic than measurement of urinary ALA dehydratase content.[53,76,78] Increased blood concentration of zinc protoporphyrin also has been documented in an affected horse.[80] These analyses generally are not routinely run by veterinary diagnostic laboratories.

Hematologic abnormalities in affected horses include a marginal anemia frequently accompanied by metarubricytes and Howell-Jolly bodies in peripheral blood.[53,79] Anisocytosis, poikilocytosis, hypochromasia, polychromasia, and basophilic stippling also can occur. These changes are suggestive but not pathognomonic of lead toxicity in horses.[53] One also can measure the concentration of lead in soil and in forages. Poisoning in horses has been reported when grazed forage contains lead levels in excess of 300 ppm.[53,78]

Treatment of affected horses should include immediate elimination of the lead source, if possible, and prompt initiation of chelation therapy. Calcium disodium EDTA is the chelator of choice; it chelates osseous lead but not tissue-bound lead. The chelated lead then becomes soluble and is excreted by the kidney. The unsaturated bone stores then reequilibrate with the lead in soft tissues. Calcium disodium EDTA can be administered by slow intravenous infusion at a dosage of 75 mg/kg of body weight daily for 3 to 5 days. A 2-day nontreatment period may follow to allow reequilibration of soft tissue and bone and then an additional 5-day treatment regimen if needed. An alternative dosage schedule is 110 mg/kg intravenously twice daily for 2 days. After a 2-day nontreatment period, this same regimen may be repeated. The clinician should base the decision to continue therapy with EDTA on posttreatment blood lead concentrations and renal function tests.[53]

Additional therapy should include fluid and nutritional support. Although thiamine administration has been advocated along with chelation therapy in small animal and ruminant lead toxicosis,[53] its efficacy in treating horses with lead poisoning has not been investigated. Dietary calcium supplementation or sodium-magnesium sulfate may have some beneficial effects by helping to reduce further gastrointestinal absorption of lead.

Methods aimed at reducing exposure to lead and preventing intoxication include appropriate cutting and disposal of contaminated forage, tilling or burning of the stubble, and addition of lime to the soil.[78] Use of alfalfa as a roughage also might be beneficial, possibly because of the high calcium

content in alfalfa, because intoxication is more difficult to produce experimentally in horses when alfalfa is being fed and because increased dietary calcium decreases the gastrointestinal absorption of lead.[53,78]

Toxicoses Causing Signs Relating to the Cardiovascular and Hemolymphatic Systems

Certain plants and ionophores are the most frequently encountered causes of cardiac problems in horses. Manifestations associated with hemolysis and anemia also are possible.

PLANTS

Oleander (*Nerium* spp.)

Clinical Signs

According to most research, sudden death is the most common sign attributed to oleander poisoning. Some reports suggest that affected horses exhibit lethargy, inappetence, and occasional signs of abdominal pain.[81,82] Profuse, watery, catarrhal, or bloody diarrhea also may occur within a few hours of ingestion.[83] Cardiac irregularities, including alternating bradycardia and tachycardia, may be accompanied by a variety of arrhythmias.[83,84] The extremities of the horse may feel cold to the touch, and mucous membranes may appear blanched. Profuse sweating and muscle twitching are followed by weakness and death. Death may occur less than 12 hours after ingestion. One could expect similar problems to occur when horses are exposed to foxglove (*Digitalis* spp.), another cardenolide glycoside–containing plant.

The green plant apparently is unpalatable to horses. Most toxicities occur when leaves have been incorporated into lawn clippings and offered to horses. Drying does not affect the toxicity of the leaves; therefore leaves incorporated into hay also may be toxic. According to reports, 0.005% of an animal's body weight of green oleander is lethal.[81]

Pathophysiology

Common oleander contains several cardiac glycosides that are found in all parts of the plant.[81,83,85] These glycosides (i.e., oleandrin, digitoxigenin, neriin, folinerin, rosagenin) inhibit the Na^+,K^+-ATPase (adenosine triphosphatase) system, resulting in hyperkalemia, conduction abnormalities, and ventricular arrhythmias. Which glycosides or metabolites cause specific abnormalities is unclear because of the undefined pharmacokinetics of the individual glycosides.[85]

Diagnosis and Treatment

Exposure to the plant along with the aforementioned clinical signs should alert the clinician to the possibility of oleander toxicity. The rapidity of onset of clinical signs or the finding of dead animals may preclude any effective treatment. Symptomatic therapy should be initiated in those animals in which toxicosis is suspected. Decontamination measures, such as evacuation of the intestinal tract by laxatives and enemas, may be useful. Atropine and propranolol have been advocated, but they must be used with extreme caution.[83,84] Fluids containing calcium should not be used because they may augment the effects of the glycoside on the myocardium.[81] Postmortem changes may include gastroenteritis, focal myocardial necrosis, and renal necrosis.

White Snakeroot (*Eupatorium rugosum*) and Rayless Goldenrod (*Isocoma wrightii*)

Cases of white snakeroot intoxication have been reported primarily in the eastern half of the United States, from Michigan south to Alabama and eastward.[86] The toxic principle is tremetol, which has been described as a fat-soluble, high-molecular-weight alcohol.[86,87] Tremetol poisoning is reported to be most prevalent in dry years or in circumstances in which animals are subjected to inadequate pasturage. The toxin is excreted slowly and therefore tends to accumulate in animals grazing the plant. Because of this cumulative effect, repeated small doses can result in toxicosis as well as a single, larger exposure to the plant. A total amount of green plant varying between 1% and 10% of body weight may be lethal to horses.[86] The toxic principle remains in the dried plant after freezing.[88] In the southwestern United States, rayless goldenrod is the source of tremetol, and ingestion of this plant produces the same clinical syndrome as that of white snakeroot intoxication.

Clinical Signs

Depression; a stiff gait with frequent crossing of the hindlimbs; and patchy, profuse sweating seem to be the most profound signs of tremetol toxicity. Other, less frequently noted findings include muscle tremors, particularly of the shoulders and limbs; labored or shallow respirations; normal to subnormal body temperature; pupillary dilation; cardiac arrhythmias; and darkly discolored urine.[86,88]

The time of onset of signs varies considerably, from less than 2 days to as long as 3 weeks after the last exposure to the plant. Most horses showing clinical signs die. Recovery is reported to be rare and usually is prolonged and often incomplete. However, a report describes two horses that apparently fully recovered from suspected tremetol toxicity.[86] Death often follows within 1 to 3 days of the appearance of clinical signs.[86]

Noted laboratory abnormalities include hematuria, hemoglobinuria and proteinuria, mild elevations in serum alkaline phosphatase activity, elevated AST concentration, and significant elevation of serum CK activity. Acidosis, hyperglycemia, and glucosuria also have been documented.[86,88]

Postmortem findings primarily consist of mild renal tubular degeneration and necrosis; nonsuppurative colitis; pulmonary congestion; increased amounts of pleural and peritoneal fluid; and moderate to severe centrilobular vacuolar changes in the liver. Other significant findings include pericarditis and extensive, patchy myocardial degeneration and necrosis. Extensive, minute epicardial hemorrhage also has been reported.[86,88] One reported case also described moderate, multifocal degeneration of skeletal muscle.[86]

Pathophysiology

The mechanism whereby tremetol causes the aforementioned lesions and signs remains unknown.

Diagnosis and Treatment

Diagnosis of tremetol toxicity is based on observation of the described clinical signs and concurrent clinicopathologic abnormalities. Additionally, evidence that the affected horses have been exposed to either plant should be present, and, when

available, necropsy findings should be compatible with those described for tremetol toxicity. Analysis for tremetol from suspect samples is not readily available but can be done.[86]

Treatment is symptomatic. The primary goal of therapy is promptly removing the horse from exposure to the plants and providing supportive care. The clinician should attempt to evacuate the horse's gastrointestinal tract with laxatives such as mineral oil. Activated charcoal also has been suggested as being beneficial in removing the toxin.[88] Based on observed histopathologic abnormalities, volume diuresis of affected animals seems appropriate, and the clinician should attempt to maintain normal acid-base and serum electrolyte concentrations. All affected horses should receive adequate and suitable forage and ample fresh water.

Maple (*Acer* spp.)

Several species of maple have been documented as causing hemolysis in horses; however, because of its widespread distribution, red maple is the most commonly identified species. All maples should be considered a potential risk to equids. Intoxication of the horse by leaves of the red maple tree is a seasonal disorder that occurs during the summer and fall months, primarily in the eastern United States.[89,90] Fresh leaves appear to present no problem to the horse, but wilted or dried leaves are toxic, and overnight freezing and storage of dried leaves for 30 days does not alter their toxicity.[91] Experimentally, dried leaves are toxic when administered at a dose of 1.5 mg/kg of body weight.[90,91] The toxins present in red maple leaves are thought to be gallotannins and gallic acid, which are converted in the horse's gut to pyrogallol. The clinical syndrome produced is one of acute hemolytic anemia with methemoglobinemia and Heinz body production.[89-92] Red maple toxicity has been recognized in horses[92] and Grevy's zebras,[93] and horses electively ingest the leaves when other suitable forage is available. Donkeys and mules should be considered potentially susceptible to maple's toxic effects.

Clinical Signs

Signs of toxicity generally commence within 48 hours of ingestion. Affected horses show acute onset of lethargy, anorexia, weakness, and depression. Increased heart and respiratory rates are typical, and the animals are afebrile. Two outstanding characteristics of most affected horses are the obvious presence of icteric, pale, or brown mucous membranes and a brownish discoloration of blood and urine. Many horses appear cyanotic, and petechiae on mucous membranes have been reported. Signs of secondary acute renal failure also have been documented.[92] Death, when it occurs, generally happens 3 to 7 days after ingestion.[89-92] The mortality rate in naturally occurring and experimental cases is approximately 60%.[94,94a]

The aforementioned signs are representative of naturally occurring instances of toxicity. In an experimental study, however, two patterns of toxicity were recognized. One group of ponies given dried leaves accumulated before September 15 exhibited signs of the typical hemolytic syndrome and died 3 to 5 days later. Ponies given leaves collected after September 15 died within 18 hours of dosing and exhibited clinical signs only of cyanosis and depression.[91] The reason for this disparity of signs was not offered.

Pathophysiology

The clinical syndrome is that of acute damage to the red blood cell that produces an acute hemolytic anemia with methemoglobinemia and Heinz body production in affected horses. The mechanism of erythrocyte damage has not been determined, but these hematologic abnormalities are characteristic of an oxidant.[90-92] Gallic acid and tannins have been implicated as possible toxic compounds in red maple leaves.[94b]

Heinz bodies are intracellular precipitates of oxidized hemoglobin that result from oxidant injury to erythrocytes. They damage the erythrocyte membrane and produce intravascular and extravascular hemolysis. Intravascular hemolysis results when erythrocyte membrane functions involving active and passive ion transport become impaired. The hyperpermeability changes that then occur alter the osmotic gradient of the erythrocyte and cause rupture of the affected red blood cell. Extravascular hemolysis occurs when the red blood cell remains intact, but the damaged erythrocyte is removed from circulation by cells of the reticuloendothelial system.[89,90,92]

Methemoglobin is formed when hemoglobin is oxidized from the ferrous to the ferric form of iron. A certain amount of direct oxidation of hemoglobin to methemoglobin occurs naturally, but erythrocytes are able to reduce methemoglobin back to hemoglobin. Excessive production of methemoglobin occurs under conditions of excessive oxidative stress or when methemoglobin reduction is impaired.[89,91] Although methemoglobin itself does not produce hemolysis, it is incapable of transporting oxygen and therefore contributes to hypoxia. When present in sufficient quantities, methemoglobin imparts a brown discoloration to peripheral blood and possibly to mucous membranes.

Diagnosis and Treatment

Maple leaf toxicosis occurs under rather specific conditions but is characterized by an acute onset of hemolytic anemia with methemoglobinemia and Heinz body production. The typical clinical signs and conditions supporting maple leaf intoxication should be in evidence before making a diagnosis.

Hematologic abnormalities noted in affected horses include moderate to severe anemia (PCV often less than 10% in severely affected horses), hemoglobinemia, methemoglobinemia, Heinz bodies, anisocytosis, hyperbilirubinemia, and increased erythrocyte fragility. Blood chemistry analysis may reveal depletion of erythrocyte-reduced glutathione and increased serum concentrations of LDH, creatine phosphokinase, AST, and sorbitol dehydrogenase.[89-92] Transient hypercalcemia has been recorded in some horses,[89] and increases in BUN and creatinine concentrations are expected in horses undergoing significant renal insult secondary to hemolysis.[92] Urinalysis may indicate varying degrees of hemoglobinuria, hematuria, bilirubinuria, and proteinuria.[89-91]

Treatment of affected horses is primarily symptomatic. Exposure to red maple leaves should be eliminated immediately, and affected horses should be kept in a quiet, calm environment. Oxygen therapy may be beneficial in selected cases, and blood transfusions can be administered to severely affected individuals.

Balanced fluid administration is important to maintain renal function and to aid in diuresis because affected horses may be at great risk of developing hemoglobin nephrosis and acute renal failure. Blood electrolyte and acid-base parameters should be monitored and abnormalities corrected as needed. Acute renal failure should be treated appropriately if it develops.

Other symptomatic therapies that have been suggested include nasogastric intubation with activated charcoal to aid in potentially binding to the toxin(s); dexamethasone to aid in stabilizing red blood cell membranes and decrease

phagocytosis of damaged red blood cells; and ascorbic acid (30 mg/kg) given in intravenous fluids twice daily, which may reduce oxidative damage to red blood cells. Whether dexamethasone increases the risk of laminitis in affected horses is unknown, and the efficacy of ascorbic acid is questionable.[94] Treatment with dexamethasone was associated with increased risk of death in one study.[94a]

Prevention of the disease is accomplished by preventing exposure of horses to dried maple leaves.

Onion (*Allium* spp.)

Onion toxicosis in horses is a rare event and occurs when horses are fed large amounts of culled onions (from commercial onion farms) or are forced to eat wild onions because of inadequate available forage. Horses appear to avoid these plants when suitable forage is available.[95,96] The toxic principles in onions, *n*-propyl disulfide and sulfoxide, affect only circulating erythrocytes and cause oxidant injury to the red blood cell, with resultant Heinz body formation and subsequent hemolytic anemia. Severely affected horses succumb to onion toxicosis because of severe anemia or secondary renal failure caused by hypoxic nephropathy.

The clinical signs, laboratory values and pathogenesis of the anemia are all similar to those of maple leaf toxicosis, with the exception that methemoglobin formation does not seem to be nearly as pronounced in onion toxicity as in the former. The carcasses of horses that die as a result of onion toxicosis may have an onion odor at necropsy.[95]

Affected horses may recover from onion toxicity if they are removed from the onion source soon enough and if anemia is not severe. Treatment is largely symptomatic and should include removal of the onion source and provision of adequate, suitable forage. Hematinics are of little value, but oxygen therapy and blood transfusions may be indicated in more severely affected animals.

Maintenance of renal function is of primary concern because affected horses are at risk of developing secondary hypoxic nephropathy. The clinician should give balanced fluids to promote diuresis and should correct electrolyte and acid-base abnormalities. Diuretics should be used with caution, but they may be of value in the volume-loaded horse.

Yew (*Taxus* spp.)

There are both native and ornamental yews distributed throughout the Northern Hemisphere; but by far the vast majority of poisonings in horses is due to accidental consumption of the ornamental shrubs. Yews are evergreen shrubs commonly used in landscaping around houses and property. Horses can be exposed by ingesting the plant directly through accidental access, ingesting plant trimmings thrown in brush piles, or intentionally being fed the plant by well-intentioned neighbors. These plants are popular as landscape shrubs because they stay green all year round and require little maintenance other than occasional trimming.

Pathophysiology

All parts of the plant are toxic (except for the red fleshy aril surrounding the seed), and toxicity is not lost in drying. The cardiotoxic taxines are thought to interfere with atrioventricular cardiac conduction leading to heart block, potentially due to inhibition of sodium and calcium currents in cardiac ells and/or possible potassium channel effects.[97] This inhibition leads to a decrease in the ventricular contraction rate, a variety of cardiac arrhythmias, and death due to cardiac arrest in diastole.[97]

Clinical Signs

Horses can abruptly collapse and die, while exhibiting few to no premonitory signs. Sometimes horses have died while plant material is still present in the oral cavity. Collapse and death can occur within a few minutes of ingesting a lethal dose (approximately 0.1%–0.5% of body weight). Sometimes weakness, tremors, ataxia, and difficulty breathing can be seen just before collapse and death. In many cases, horses are found dead.

Diagnosis and Treatment

Diagnosis relies heavily on identifying the plant material in the mouth or stomach contents, and several diagnostic laboratories can test for the taxine alkaloids. Microscopically one may see areas of multifocal necrosis of the heart tissue, particularly within the ventricles and the papillary muscles. Similar lesions have been reported in cattle exposed to Japanese yew.[98]

Treatment is rarely possible given the acute onset of clinical signs, collapse, and death. Evacuation and decontamination of the gastrointestinal contents with mineral oil and activated charcoal is appropriate, along with basic symptomatic and supportive care, if the patient survives the initial insult. It has been reported that calves who survived an initial nonlethal exposure to yew material died several days later exhibiting histologic changes of myocardial fibrosis.[99] This could conceivably happen in horses as well.

MISCELLANEOUS AGENTS

Coumarin Derivatives

Coumarin normally is present in some species of sweet clover and has no anticoagulant action. However, certain molds present within the plant can convert coumarin to dicoumarol; hence the term *moldy sweet clover poisoning*. Dicoumarol also has been detected in sweet vernal grass *(Anthoxanthum odoratum)*. Coumarin derivatives are used widely as anticoagulants, with bishydroxycoumarin (dicoumarol) and 3-(α-acetonylbenzyl)-4-hydroxycoumarin (warfarin sodium) being the first oral anticoagulants developed from coumarin.[87] These first-generation compounds are used therapeutically as anticoagulants and as rodenticides. There are several anticoagulants on the market, representing both first- and second-generation compounds. In horses warfarin has been used therapeutically to treat thrombotic disorders such as thrombophlebitis and to treat navicular disease.[100,101] Horses may exhibit signs of toxicity while being medicated with warfarin if a therapeutic concentration is exceeded. Rarely are horses exposed to anticoagulants used as rodenticides around buildings or feed storage areas, but it does occur. Second-generation anticoagulant rodenticides (e.g., brodifacoum and bromadiolone) have been developed because of acquired rodent resistance to the first-generation compounds. Because no indications exist for human or veterinary medicinal use of brodifacoum, toxicity associated with this compound is caused by accidental ingestion.[102] Exact toxic doses for horses have not been determined.

Clinical Signs

The clinical signs of toxicity noted with anticoagulants are largely those of a hemorrhagic diathesis. Onset of signs usually is acute and may include hematoma formation, epistaxis, anemia, weakness, pale mucous membranes, or ecchymoses

of mucous membranes. Hematuria and melena can occur, and hemorrhage into various body compartments may result in secondary signs caused by malfunction of the involved organ or tissue. Occasionally, affected animals are found dead.[103] Multiple fractional doses of first-generation compounds given over several days may be more toxic to horses than a single larger dose.[100] Brodifacoum, however, differs from warfarin in that a single oral dose has the potential of causing illness and may even be lethal (estimated LD_{50} of 1–2 kg per adult horse).[102,104]

The onset of clinical signs is delayed from the time of ingestion. In an experimental model the hypothrombogenic effect of warfarin anticoagulation was noticed 60 hours after an acute dose.[105] This effect persisted for approximately 30 hours. The effect of brodifacoum, however, is much longer and may persist for weeks, despite its half-life of 1.22 days.[102] In an experimental study horses given a single dose of brodifacoum (0.125 mg/kg body mass) showed increased partial thromboplastin time (PTT) at 24 hours and one-stage prothrombin time (PT) at 48 hours after exposure. These values returned to pretreatment levels by day 12. However, two horses required 23 days for clotting times to return to normal. In these horses peak plasma concentration of brodifacoum occurred 2 to 3 hours after oral administration. Additionally, four of the six experimental horses showed clinical signs of depression, anorexia, and weight loss.[104]

Pathophysiology

Warfarin is absorbed readily from the gastrointestinal tract but also may be administered intravenously for therapeutic purposes.[87,100] Once absorbed, warfarin is highly bound (greater than 90%) to plasma proteins, and some is stored in the liver.[87,100,105] The degree of anticoagulant protein binding in horses is not known but may be similar to other species.[102] Warfarin is hydroxylated by hepatic enzymes to inactive compounds that are eliminated by the kidney. The metabolites have no anticoagulant effect,[87] and the biologic half-life of warfarin in the horse is approximately 13 hours.[102] Coumarins also cross the placenta and are secreted in milk.[87]

Protein binding of warfarin is reversible, and the protein-bound, pharmacologically inactive warfarin serves as a reservoir. The unbound portion remains fairly constant in plasma.[87,100]

A number of factors can influence the amount of unbound free warfarin in serum. Protein-bound drugs such as phenylbutazone, chloral hydrate, and sulfonamides may enhance the toxicity of warfarin by displacing warfarin from protein-binding sites. This allows a greater proportion of the drug to be in the free form and thus able to exert its pharmacologic effect.[87,103] Corticosteroids and thyroxine may lower the therapeutic dose of warfarin by increasing clotting factor catabolism and receptor site affinity.[100]

Certain physiologic factors also may enhance the toxicity of warfarin. Hypoalbuminemia may result in fewer binding sites available, thereby increasing the amount of free drug. Hepatic dysfunction may impede metabolism of the anticoagulants, and reduced amounts of vitamin K in the diet may predispose to toxicity.[87]

Some drugs reduce the therapeutic response to a given dose of warfarin. Barbiturates, rifampin, and chloramphenicol induce hepatic microsomal enzyme activity, thereby accelerating the metabolism of warfarin. If these drugs are withdrawn during warfarin therapy, toxicosis may result.[87,100] Likewise, excessive dietary intake of vitamin K or vitamin K administration can reduce or inhibit the anticoagulant effects of warfarin.[100]

Warfarin acts as an anticoagulant by inhibiting production of the vitamin K–dependent clotting factors II, VII, IX, and X. Vitamin K is a cofactor in the synthesis of clotting factors II, VII, IX, and X and also acts on all clotting factor precursors to convert glutamyl residues to γ-carboxyglutamyl residues. During this carboxylation process vitamin K_1 is converted to vitamin K_1 2,3-epoxide, an inactive metabolite. This epoxide returns to active vitamin K_1 by the action of the microsomal enzyme vitamin K_1 epoxide reductase. The coumarin anticoagulants inhibit vitamin K_1 epoxide reductase, thereby creating a vitamin K_1 deficiency. The result is decreased production and subsequent deficiency of the vitamin K–dependent clotting factors.[85] The delayed onset of action of the coumarin derivative anticoagulants results from this impediment to clotting factor synthesis rather than from a direct effect on the clotting mechanism per se.

The various blood clotting factors have different plasma half-lives. Factor VII has a shorter half-life than the other clotting factors, so the earliest laboratory indication of anticoagulant toxicity is a prolonged PT. As the other clotting factors become depleted, activated PTT also increases as toxicity continues.[100] The clinician should monitor horses given warfarin therapeutically for clotting abnormalities. Prolongation of PT by 1.5 to 2 times the baseline value has been suggested as the effective range of anticoagulation. However, some horses maintained in this range may show signs of hemorrhage.[101] In fact, reports in horses suggest that factor IX may be depleted from circulation more rapidly than factor VII, indicating that the PTT would be the more sensitive clotting assay to assess in horses after exposure to anticoagulants.[102]

Diagnosis and Treatment

The diagnosis of warfarin or other coumarin-derivative anticoagulant intoxication is based on evidence of exposure to the compound, presence of a bleeding diathesis, and prolongation of PTT and PT. The platelet count, fibrinogen concentration, fibrin degradation product (FDP) concentration, and antithrombin III activity remain within their respective normal ranges of activity.[100,101] However, in dogs exposed to toxic doses of anticoagulant rodenticides, fibrinogen and FDPs have been found to be elevated 50% of the time.

Blood, liver, stomach and intestinal contents, and feces can be submitted for analytic testing for the anticoagulant residues. Specimens should be frozen for transportation to the laboratory.[103,105]

In horses intoxicated by warfarin, discontinuance of warfarin therapy or removal of the product from the environment is of primary importance. Mineral oil may be used to enhance fecal excretion of any orally ingested warfarin products. Vitamin K_1 should be given at a dosage of 300 to 500 mg subcutaneously every 4 to 6 hours until PTT and PT return to their baseline values (5–7 days is generally sufficient). PTT and PT should be monitored daily for 3 to 4 days thereafter to ensure stability. The subcutaneous route of vitamin K_1 administration is preferable because of the possibility of adverse reactions if the product is given intravenously or intramuscularly. Intravenous injection of vitamin K_1 may result in transient restlessness, tachypnea, tachycardia, sweating, and anaphylactic reactions.[106,107] Intramuscular administration of vitamin K_1 results in erratic response times and therefore may be

inappropriate for a hemorrhaging patient.[106] Alternative doses for vitamin K_1 administration are 0.3 to 0.5 mg/kg intravenously, and a dosage of greater than 0.5 mg/kg intravenously has been suggested to inhibit coumarin activity for several days.[100] If the intravenous route is chosen, vitamin K_1 should be diluted in 5% dextrose or saline and administered slowly.[103]

In horses intoxicated with long-acting anticoagulant rodenticides, treatment with vitamin K_1 may be required for 2 to 4 weeks or until coagulation status is normal 2 to 3 days after the last dose of vitamin K_1. Affected horses were given vitamin K_1 (2.5 mg/kg) subcutaneously every 12 hours for 36 hours and then orally twice daily for the duration of treatment.[102]

If bleeding is serious, fresh blood or fresh plasma given intravenously may be necessary to control hemorrhage and help correct hypovolemia. Other supportive measures such as bandaging and keeping the animal in a quiet environment may be helpful. Alfalfa hay added to the diet may provide a natural source of vitamin K_1.[102] Organ dysfunction induced by hemorrhage and hypoxia should be treated appropriately. The prognosis for horses intoxicated with anticoagulant compounds is good if the disorder is recognized early and appropriate therapy is instituted. For symptomatic patients the prognosis is highly dependent on the severity, site, and extent of blood loss.

To prevent warfarin toxicosis, the clinician should strive to minimize exposure to the product and carefully monitor the therapeutic use of the drug in the horse. Contraindications to its use include any clinical or laboratory suggestion of hepatic disease, hypoproteinemia, or other condition that might increase the risk of toxicity. The practitioner should evaluate the concurrent use of other medications, because they may affect the potential for toxicosis to develop, and reevaluate the dosage of warfarin administered if other concurrent medications are changed. Additionally, the potential for traumatic injury in horses undergoing warfarin therapy should be minimized.

Dimethyl Sulfoxide

Dimethyl sulfoxide (DMSO), a byproduct of the paper-making industry, is a colorless liquid originally used as an industrial solvent. The chemical is a polar compound that readily mixes with ethyl alcohol and many organic solvents; it is extremely hygroscopic and can absorb more than 70% of its weight of water from air. DMSO possesses some antimicrobial and antifungal activity, but its primary medical use has been as an antiinflammatory agent and a transdermal transport agent.[108] More recently, DMSO has been used as a diuretic and has shown promising results when used to treat acute cranial and spinal cord trauma.[108,109]

The systemic toxicity of DMSO is considered to be low, and its greatest toxic potential appears to result from its combination with other agents.[110] However, in an experimental study rapid infusion of DMSO in 20% and 40% concentrations caused hemolysis, hemoglobinuria, diarrhea, muscle tremors, and signs of colic in some horses.[111] The LD_{50} of DMSO has not been established for horses, but ranges between 2.5 and 9.0 g/kg as a single intravenous dose have been reported in a number of animal species.[110] A dose of 1 g/kg intravenously has been suggested for use in horses. This dose should be diluted to a 10% to 20% solution and administered slowly intravenously.[109,110]

DMSO produces hemolysis when given intravenously in concentrations of 20% to 50% or greater.[108-110] If hemolysis is severe, affected horses may be at increased risk of developing hypoxic nephrosis. Concentrations of 10% or less are considered suitable for intravenous injection in horses.[108,109,111] Additionally, increased white blood cell adherence and fibrinogen precipitation have been reported when concentrations greater than 50% were administered.[108]

DMSO is a mild cholinesterase inhibitor, and its concurrent use with organophosphates or other cholinesterase inhibitors is not recommended.[108,110] DMSO also is known to induce histamine release from mast cells, but the significance of this phenomenon is unclear.[110]

Skin reactions to topically applied DMSO sometimes occur. Varying degrees of erythema, pruritus, drying, hardening, and desquamation of normal skin may be evident. These reactions are usually self-limiting and typically diminish with repeated applications.[110]

The greatest risk of toxicity resulting from DMSO is probably a consequence of its concomitant use with other toxic or potentially toxic agents. DMSO may aid transport of a variety of toxic compounds across skin, thereby inducing toxicosis from the transported agent. For example, mercury toxicity has been reported in a horse that had a blister after DMSO and mercury were applied topically to a leg.[112] In such instances, the clinician should treat the specific toxic reaction (or reactions) appropriately. No specific antidote is recognized for DMSO toxicosis, and it should always be used judiciously and conscientiously. The practitioner should heed the aforementioned precautions when administering the drug.

Bicarbonate

Sodium bicarbonate is one of the most common alkalizing agents used in animals, including horses. Sodium bicarbonate is indicated specifically to treat acute, severe metabolic acidosis because of its rapid effect on blood pH when given intravenously.[113] Sodium bicarbonate also has been used in performance horses to treat exertional myopathies and in attempts to prevent rhabdomyolysis. More recently, sodium bicarbonate administration in performance horses has been investigated because of its suggested role in limiting or preventing systemic lactic acidosis, thereby enhancing performance level.[114,115] The adverse effects of bicarbonate administration occur when it is given too rapidly or in excessive quantities or when it is given in the presence of certain systemic abnormalities.

Clinical Signs

Animals, including horses, that suffer from an acute overdose of bicarbonate may exhibit signs of delirium, depression, and coma. Rapidly induced alkalosis also has been associated with cardiac dysrhythmias.[113]

Horses such as endurance horses or racehorses that are volume depleted and have sustained excessive electrolyte loss through sweat often have clinicopathologic changes of hypokalemia, hypochloremia, hypocalcemia, and metabolic alkalosis. When such horses are given bicarbonate, dramatic deleterious effects occur. These animals may exhibit signs of muscle fasciculation, synchronous diaphragmatic flutter, bruxism, and decreased respiratory rate. One also may note clinical evidence of further dehydration, such as increased capillary refill time, delayed jugular distensibility, diminished skin turgor, and decreased arterial pulse.[115]

Pathophysiology

The rapid administration or overdose of sodium bicarbonate has been associated with extracellular hyperosmolality, intracranial

hemorrhage, and cerebrospinal fluid (CSF) acidosis. Hyperosmolality results from hypernatremia because sodium bicarbonate dissociates into sodium ions and bicarbonate ions. An abrupt increase in serum osmolality can lead to intracranial hemorrhage as intracellular water moves into the extracellular space. This extracellular fluid accumulation may result in engorgement of perivascular spaces, with subsequent tearing of bridge veins and resultant hemorrhage. The CSF acidosis results from the rapid diffusion of generated carbon dioxide into the CSF. Carbon dioxide enters the CSF almost instantaneously, establishing new steady-state levels within minutes. Bicarbonate ion, however, is slow to enter the CSF and requires hours to days to achieve new steady-state levels. With sodium bicarbonate administration the increasing amount of carbon dioxide generated readily enters the CSF disproportionately more than does the bicarbonate ion. As a consequence, the CSF becomes acidic.[116] These mechanisms, individually or collectively, are thought to be responsible for development of the clinical signs observed with rapid or excessive administration of sodium bicarbonate solution.

Another effect of alkalosis is a shift to the left of the oxyhemoglobin dissociation curve. This left shift indicates an increased affinity of hemoglobin for oxygen, with a resultant decrease in the amount of oxygen available for cellular use. This change in hemoglobin affinity for oxygen has been associated with cardiac dysrhythmias following rapidly induced alkalosis.[113]

Horses undergoing extensive exercise or horses that are treated with furosemide (primarily as prerace medication for exercise-induced pulmonary hemorrhage) are at risk of developing hypochloremic, hypokalemic metabolic alkalosis. When sodium bicarbonate is given to these volume-depleted horses with electrolyte loss and concurrent metabolic alkalosis, another deleterious series of events can result. Further reduction of the circulating fluid volume may occur, along with development of serum hyperosmolality; electrolyte abnormalities of hypernatremia, hypokalemia, hypochloremia, and hypocalcemia; and further development of metabolic alkalosis.[115]

Excessive sodium bicarbonate produces hyperosmolality caused by hypernatremia because sodium bicarbonate dissociates into sodium and bicarbonate ions. The hypokalemia is explained partially by the intracellular shift of potassium in response to metabolic alkalosis, and the bicarbonate ion adds to the alkalosis already present. Furosemide administration causes urinary loss of potassium, chloride, and calcium, and sweating also can result in significant loss of chloride and calcium. Additionally, rapid intravenous infusion of 5% sodium bicarbonate in normal horses results in hypochloremia.[117]

The muscle fasciculations and diaphragmatic flutter noted in some horses likely result from hypocalcemia.[115] The total serum calcium concentration may be within a normal range, but alkalosis causes a reduction in the amount of circulating ionized calcium. Because ionized calcium is responsible for neuromuscular function, reduction in this fraction can cause clinical signs of hypocalcemia.

Diagnosis and Treatment. Horses being treated with sodium bicarbonate solutions should be monitored closely for clinical signs of alkalosis. Blood pH obviously is elevated, but rapid infusion of sodium bicarbonate or excessive use in horses already sustaining fluid and electrolyte loss can result in a number of clinicopathologic alterations. Affected horses typically exhibit increases in PCV, total serum protein, and serum bicarbonate and sodium concentrations. Additional findings of hypokalemia, hypochloremia, and hypocalcemia usually are present.

Treatment of affected horses involves cessation of bicarbonate administration and correction of the alkalosis and electrolyte abnormalities. Potassium chloride administration is indicated in horses with hypokalemic, hypochloremic metabolic alkalosis and is much more effective in correcting these electrolyte abnormalities than is sodium chloride. Potassium chloride also has been shown to cause a prompt, significant decline in venous blood pH in these horses.[115]

Apparently, hypertonic sodium bicarbonate solutions should not be used in dehydrated horses. The concurrent use of furosemide and sodium bicarbonate also appears to be contraindicated; if this is necessary, they must be used with extreme caution and close observation with laboratory assessment. Sodium bicarbonate should not be mixed with fluids containing calcium because insoluble complexes may form. The use of sodium bicarbonate orally in horses subjected to short-term intense exercise remains controversial.

Ionophore (Monensin)

Monensin is one of several biologically active compounds categorized as ionophore antibiotics (others include lasalocid, salinomycin, narasin, laidlomycin, maduramycin, and virginiamycin) because they can form lipid-soluble complexes with specific metal cations and transport them across biologic membranes. Monensin is produced by the fungus *Streptomyces cinnamonensis* and is selective in transporting sodium and potassium ions between intracellular and extracellular spaces.[103,118,119] Monensin is used routinely as a poultry coccidiostat and as a feed additive to improve feed efficiency in pasture and feedlot cattle. Horses are the domestic animals most sensitive to monensin toxicosis. The LD_{50} and possibly a single acute toxic dose of monensin for the horse is 1 to 2 mg/kg.[103]

Clinical Signs

Several syndromes of toxicity occur and seem to be dose related. Peracute toxicity may manifest as progressive, severe hemoconcentration; hypovolemic shock; and death within a few hours of ingestion. The acute form of the disorder is characterized by partial to complete feed aversion, abdominal pain, occasional watery diarrhea, intermittent profuse sweating, stiffness, progressive muscle weakness (especially in the hindquarters), progressive ataxia, tachycardia, hypotension, dyspnea, and polyuria. Affected horses may show clinical signs for 1 to 4 days before death.[119-121] Horses surviving sublethal doses of monensin exhibit signs of reduced athletic performance, unthriftiness, and cardiac failure. Cardiac arrhythmias, including atrial fibrillation and tachycardia, a prominent jugular pulse, and pleural and pericardial effusion, are apparent.[103] Intravascular hemolysis also may occur to a limited degree, but this is an uncommon finding.[118]

Pathophysiology

The primary action of monensin is the selective transport of sodium and potassium ions between the intracellular and extracellular spaces. Two mechanisms have been suggested to explain the toxic action.

According to one theory, monensin interacts with the mechanism regulating potassium entry into cell organelles, especially the mitochondria.[103] Low concentrations of monensin lead to a net accumulation of potassium within the cell, whereas higher

doses cause a net loss of potassium from the cell.[118] Because potassium is required for ATP hydrolysis by the mitochondria, the effect of monensin might be to inhibit ATP hydrolysis in mitochondria. As a result, cell energy production is decreased and can result in loss of cell function and death.[103,118]

The second hypothesis suggests that increased intracellular calcium concentration is the mechanism responsible for cell death. When the intracellular calcium concentration is increased, the mitochondria are forced to maintain calcium homeostasis by sequestering the excess calcium. This requires energy, which could take priority over ATP production. When the mitochondria become overloaded with calcium, oxidative phosphorylation is inhibited and less energy is produced to pump calcium out of the cell. When intracellular calcium levels reach a critical level, degradative enzymes are released, swelling of the mitochondria and sarcoplasmic reticulum occur, and cell necrosis and death follow.[103,118]

The heart is the primary target organ of monensin toxicosis, and electron microscopic studies of acute monensin toxicosis in ponies have shown structural changes in myocardial cells consistent with severe mitochondrial damage.[122] In horses ingesting a sublethal dose of monensin, the myocardial sarcolemma is damaged and is replaced by fibrous tissue in the healing process. Myocardial lesions are characterized microscopically by pale myofibers, loss of fiber striation, multifocal vacuolar degeneration, and scattered areas of necrosis. The result is a structurally weakened heart that can succumb to stress and cause acute collapse of the horse. Other lesions that may be present in affected horses include pericardial, pleural, and peritoneal effusions; hemopericardium; and epicardial hemorrhage. Chronically affected horses also may have hepatic congestion with centrilobular necrosis and hydropic degeneration of the renal tubules.[103,118]

Diagnosis

Monensin toxicosis is suspected when horses show clinical signs of feed refusal, abdominal discomfort, muscle weakness, and heart failure and when a possible exposure to contaminated feed has occurred. The practitioner can evaluate feeds, serum, liver, gastrointestinal content, and feces for the presence of monensin.

Clinicopathologic abnormalities are nonpathognomonic but include early signs of severe hemoconcentration and dehydration in horses affected peracutely. Serum potassium and calcium may be decreased moderately in the first 12 to 16 hours but then tend to come back to normal levels. BUN and creatinine concentrations are elevated in horses acutely affected but return to normal in surviving animals. Other enzymes that show elevated serum activity include CK, AST, and LDH isoenzyme fractions 1 (cardiac muscle) and 2 (red blood cell origin). Total serum bilirubin also may be elevated.[103,118] Troponin levels are often elevated, along with abnormal echocardiographic changes.

Abnormal findings in urine can include a progressive decrease in urine osmolality during the initial few hours of the disease course.[103] One also might expect elevations in urinary activity of renal tubular enzymes such as γ-glutamyl transferase and *N*-acetylglucosaminidase. Urinalysis abnormalities tend to correlate well with the degree of renal insult and are nonspecific indicators of renal damage.

Treatment

No specific antidote exists for monensin. Horses that have ingested a large amount should be treated early and aggressively with fluids to combat hemoconcentration and hypovolemic shock. Electrolyte and acid-base analysis should be performed, if possible, and any deficiencies corrected. The clinician should attempt to evacuate the bowel of the horse using orally administered laxatives such as mineral oil; activated charcoal may help decrease the absorption of monensin. Affected horses should be kept as quiet and nonstressed as possible for weeks after exposure to allow the damaged myocardium to heal.[103,118] Administration of selenium and vitamin E should be considered; these compounds have been shown to be somewhat protective against the toxic effects of monensin in swine.

Digitalis glycosides should never be used in acutely affected horses because they and monensin have been shown to be synergistic and immediately fatal to cardiac muscle cells. Digitalis glycosides should be used only with great caution in the weeks after recovery from the toxic episode. Likewise, calcium should not be given to acutely affected horses for two reasons. First, serum hypocalcemia is transitory, and serum calcium usually recovers to normal by 24 hours. Second, calcium can be dangerously irritating to an already injured myocardium.[118]

It is important to note that affected horses are susceptible to cardiac damage, which is often permanent. A critical evaluation of cardiac function and the integrity of any previously intoxicated horse that is destined to return to some form of athletic endeavor is judicious.

Ionophore (Lasalocid)

Lasalocid, another carboxylic ionophore antibiotic, is a fermentation product of the mold *Streptomyces lasaliensis* that is used commercially as a poultry coccidiostat and as a feed additive to improve feed efficiency and rate of weight gain in ruminants.

Clinical Signs

The signs observed in horses given toxic amounts of lasalocid are similar to those of monensin toxicosis. Affected horses exhibit lethargy, ataxia, paresis, and paralysis with partial to complete feed aversion. Once recumbent, some horses rise when given assistance. Most horses that survive appear normal 2 to 3 days after exposure. The lowest dose that has been reported to cause fatality was 15 mg/kg of body weight, but the LD_{50} for a single oral dose of lasalocid was estimated to be 21.5 mg/kg. Death occurred between 31 and 96 hours after oral dosing in the nonsurvivors.[123]

Results of a toxic feeding study indicated that poultry rations containing approved concentrations of lasalocid (75 to 125 g per metric ton) are not toxic or lethal to horses. This study revealed that horses voluntarily reduced their feed intake with increasing amounts of lasalocid in the ration and refused to eat the commercial premix when it was offered in place of their normal ration.[123]

Pathophysiology

The mechanism of action of lasalocid is thought to be similar to that of monensin. Lasalocid is the least toxic of the ionophores and differs from monensin in that it accepts divalent as well as monovalent cations.[87]

Diagnosis

No signs or laboratory findings are pathognomonic for lasalocid toxicity. In horses in which it is suspected, feedstuffs, stomach contents, serum, liver, and feces can be analyzed for lasalocid content.

Abnormal laboratory findings in affected horses may include hypocalcemia, hypophosphatemia, and hypokalemia early in the disease course (within 24 hours of exposure), but these values return to normal ranges by 120 hours after ingestion. Serum activity of AST frequently is increased, as are total serum bilirubin, glucose, and troponin concentrations. Occasionally, the BUN concentration is increased.[123] Echocardiographic and electrocardiographic changes can be observed.

Treatment

Initial treatment should include removal of all suspected feed sources and the oral administration of laxatives, along with activated charcoal, to enhance evacuation of the gastrointestinal tract and decrease absorption of the lasalocid. Other nonspecific supportive care may be helpful, but horses receiving a sublethal dose probably will recover with minimal assistance. If a lethal dose is forced into an animal inadvertently, oral laxatives and adsorbents such as activated charcoal may help bind lasalocid and reduce the amount absorbed. Administration of selenium and vitamin E before the onset of signs may help alleviate the severity of the clinical signs once they occur.

Ionophore (Salinomycin)

Salinomycin is an ionophore, also marketed as a coccidiostat, which is related more closely to monensin than is lasalocid. The ionic affinity of salinomycin is predominantly to sodium and potassium ions, and its mode of action and cellular effects are similar to those of monensin.[87]

In one report of affected horses, the clinical signs were similar to those of the other ionophore toxicoses. The range of clinical signs included partial to complete feed aversion, depression, occasional sweating, colic, dyspnea, weakness, ataxia, and recumbency. Occasionally, horses showed reduced performance for several weeks after exposure, but many horses that became recumbent were destroyed humanely. The clinicopathologic abnormalities exhibited by affected horses included elevated serum activities of CK, AST, and alkaline phosphatase.[124]

The diagnosis of salinomycin toxicity is hampered by the fact that none of the clinical signs are pathognomonic. However, one may assay suspect feed, gastrointestinal content, serum, liver, and feces for the presence and quantity of salinomycin.

Treatment of affected horses is mostly symptomatic because no specific antidote is available. Evacuation of the bowel by laxatives along with the use of activated charcoal may be helpful in reducing the amount of toxic material available for absorption. Fluid balance and electrolyte and acid-base indices should be maintained within normal ranges. Administration of selenium and vitamin E should be considered. Affected horses generally require an extended convalescence, and the clinician should consider the possibility of a persistent cardiomyopathy.[124] Affected horses should undergo a rigorous cardiac examination before returning to performance events.

Nitrates and Nitrites

Nitrates are an important component in the naturally occurring nitrogen cycle and as such are present in soils, groundwater, forages, row crops, weeds, animal tissues, and excreta. Nitrates also are used widely in fertilizers. Toxicity problems can arise when animals consume plants, feed, or water containing excessive amounts of nitrites. Nitrates naturally undergo microbial decomposition to nitrites, so nitrite toxicity can occur when animals ingest feed or water in which the nitrates have decomposed to yield large amounts of nitrites. This decomposition can occur in moist haystacks, water troughs, farm ponds, silages, and pig swills. Nitrite also is administered intravenously to treat cyanide poisoning, and overzealous use of this therapy can result in toxicity.[87] It is important to note that nitrate poisoning is common in ruminants but rare in horses and other monogastrics. As described, the risk is when horses are exposed to preformed nitrite.

The primary exposure to nitrite for most animals is the plants they consume. Many plants and forages are known to be nitrate accumulators, and a number of factors influence the uptake of nitrates in plants. Nitrate concentration by plants is enhanced by low soil pH; low soil molybdenum, sulfur, or phosphorus content; low soil temperature; drought; soil aeration; decreased light; and use of phenoxyacetic acid herbicides such as 2,4-dichlorophenoxyacetic acid. Nitrate and nitrite accumulation in ponds and groundwater is caused by water runoff from nitrate-rich soils or by direct contamination with nitrates and nitrites.[87,103] Nitrates and nitrites are water soluble and are carried easily from feedlots, pigpens, and fertilized areas into the soil and subsequently into plants, wells, and ponds.

The most important aspect of nitrates is their ease of microbial conversion to nitrites. Nitrite is responsible for the primary signs associated with toxicity in nitrate poisoning.

Clinical Signs

Although apparently rare, horses are reported to be susceptible to nitrate intoxication. Experimentally, an oral dose of 1 g/kg potassium nitrate caused illness but not death in horses. However, nitrites have been associated with death of horses under field conditions.

In monogastric animals, nitrate ingestion is reported to produce gastrointestinal irritation with resulting emesis/regurgitation or enteritis. Salivation, diarrhea, colic, and frequent urination may be evident if the nitrate is sufficiently concentrated.

Signs of acute poisoning with nitrites usually begin 30 minutes to 4 hours after ingestion of high-nitrite feed or water. The most characteristic signs are those referable to respiratory insufficiency and include dyspnea, cyanosis, rapid and weak pulse, and anxiety. Exertion may exacerbate these signs and induce muscle tremors and collapse. The horse may have terminal clonic convulsions. The blood of affected animals is usually brown or chocolate colored because of the formation of methemoglobin, and this imparts a cyanotic or pale appearance to mucous membranes. Death may occur in several hours or be delayed until 12 to 24 hours after ingestion.[87,103]

Pathophysiology

Nitrites are absorbed rapidly from the gastrointestinal tract into the bloodstream. The nitrite ion acts directly on vascular smooth muscle to cause relaxation and easily enters erythrocytes in exchange for chloride ion. Nitrite also can pass the placenta to enter fetal erythrocytes, which are especially sensitive to nitrite. The biologic half-life of blood nitrate in horses is 4.8 hours. Only small amounts of nitrate or nitrite are bound to plasma proteins.[87,125]

Nitrite causes acute poisoning by two mechanisms. The primary action of nitrite is to interact with hemoglobin to form methemoglobin. One molecule of nitrite interacts with two molecules of hemoglobin, causing the oxidation of normal

ferrous hemoglobin to ferric hemoglobin, which is called *methemoglobin*. Methemoglobin is incapable of transporting oxygen to tissues, and if sufficient quantities are formed, severe oxygen deficiency can occur. Clinical signs become evident when methemoglobin levels approach 30% to 40%, and death occurs when 80% to 90% of hemoglobin is oxidized to methemoglobin.[87,103] However, death can occur in active animals with only 50% to 60% methemoglobin.[87]

Normally, methemoglobin is converted back to ferrous hemoglobin by two reducing enzyme systems. This conversion occurs slowly, and in instances of nitrate toxicity, methemoglobin formation far exceeds the ability of these enzyme systems to regenerate hemoglobin.[87]

The second action of nitrite is to cause direct relaxation of smooth muscle, particularly vascular smooth muscle. The mechanism by which this occurs is unknown, but the physiologic changes brought about by the vasodilating action of nitrite include pulmonary arterial, central venous, and systemic arterial hypotension and decreased cardiac output. These changes may contribute to tissue anoxia and act to enhance tissue oxygen starvation already initiated by methemoglobin.[87]

Diagnosis and Treatment

The diagnosis of nitrate or nitrite toxicity is based on compatible clinical signs; presence of methemoglobinemia; history of exposure to potential nitrate- or nitrite-containing plants, water, or fertilizers; and nitrate quantitation in blood, plasma, or serum. One should perform blood methemoglobin determinations soon after collection because methemoglobin is not stable in refrigerated, heparinized blood for more than a few hours. However, blood mixed with a phosphate buffer preserves the methemoglobin and allows shipment to a diagnostic laboratory. Forage, hay, and water samples can be analyzed for nitrate or nitrite content, as well as other body fluids such as ocular fluid collected postmortem.[103]

Treatment aims to reduce methemoglobin back to hemoglobin. Mildly affected animals may recover spontaneously if the toxic source is removed and if they are given sufficient time for normal methemoglobin reduction processes to occur. Intravenous methylene blue may be used to treat more severely affected horses at a suggested dosage of 4.4 mg/kg, given slowly as a 1% solution in isotonic saline. The dose may be repeated in 30 minutes if clinical response is unsatisfactory. Caution is necessary, however, because excessive amounts of methylene blue may directly oxidize hemoglobin to methemoglobin. Methylene blue is converted to leukomethylene blue by an $NADPH_2$-dependent system. Leukomethylene then reduces methemoglobin to hemoglobin. In this reaction leukomethylene blue is oxidized back to methylene blue but can be reconverted to leukomethylene blue as long as sufficient $NADPH_2$ is available. If this $NADPH_2$ system becomes saturated with methylene blue, excessive methylene blue may oxidize hemoglobin directly to more methemoglobin.[87] Because methylene blue is difficult to obtain, ascorbic acid has been suggested as a less effective alternative.

Other nonspecific therapies include blood transfusion, oxygen therapy, and laxatives such as mineral oil to aid evacuation of the gastrointestinal tract.[87]

Cyanide

Hydrogen cyanide (HCN), cyanide, hydrocyanic acid, and *prussic acid* are terms that refer to the same toxic substance. Horses become exposed primarily through ingestion of certain plants that contain cyanogenic glycosides, but compounds containing cyanide also have been used as fumigants, rodenticides, and fertilizers.[87,103]

Numerous plants or plant parts may accumulate large quantities of cyanide or cyanogenic glycosides, and a more complete description of them can be found elsewhere.[126] When these cyanogenic glycosides undergo hydrolysis, free HCN is formed. Plant cells contain degradative enzymes that can hydrolyze these glycosides, but under natural conditions the enzymes are kept separated spatially from the glycosides in intact cells. Damage to the plant cells by wilting, freezing, or stunting allows enzymatic degradation of the glycoside.[103] Rapid hydrolysis and release of HCN occur only when the plant cell structure is disrupted. When the glycoside is exposed to an acid medium, or when maceration of the plant occurs within the intestinal tract, hydrolysis and subsequent formation of HCN also occur.

A number of factors can influence the toxic potential of cyanogenic plants. Because plant cyanogenic glycosides and degradative enzymes are controlled genetically by a dominant gene, selectively breeding plants with low cyanogenic potential is possible. Therefore different species and varieties of forage contain varying cyanogenic content. The ability to genetically remove plants with high cyanogenic potential is one reason that plant-related cyanide toxicity is an uncommon event. Sudan grass and Johnson grass historically have been problematic forages for livestock. High-nitrogen fertilization, nitrogen and phosphorus imbalance in soil, and drought conditions also can influence cyanide potential in plants.[103]

Most of the cyanogenic activity in the plant is located in the leaves and seeds, and immature and rapidly growing plants have the greatest potential for high glycoside levels. Conditions that damage the plant, such as drought, wilting, or freezing, may allow for more rapid combination of glycoside and enzyme, thereby enhancing plant toxicity. Other factors that may affect toxicity are the size of the animal, speed of ingestion, the type of food ingested along with the cyanogen, and the presence of active degradative enzymes in the plant and in the digestive tract of the horse.[103]

Clinical Signs

Because cyanide is such a potent, rapidly acting poison, affected animals commonly are found dead. On observation clinical signs may range from mild tachypnea and anxiousness to severe panting, gasping, and behavioral excitement. Salivation, lacrimation, muscle tremors, defecation, urination, and mydriasis may be evident. These signs are followed by prostration, clonic convulsions, and death. The mucous membranes may have a bright red appearance, and blood color may be bright cherry red, although this is not commonly reported in cases. Clinical signs may last from only a few minutes to a few hours, but horses that survive longer than 90 to 120 minutes after exposure usually survive.[84,87,103,126]

Pathophysiology

HCN is absorbed rapidly from the gastrointestinal tract or from the lungs. After absorption, endogenous thiosulfate combines with the cyanide ion to form thiocyanate, which is relatively harmless. This reaction occurs in the liver and other tissues, and the generated thiocyanate is excreted in urine. Another inherent detoxification mechanism involves inactivation of HCN in the bloodstream by combining with the ferric iron of methemoglobin. However, because a small amount of

methemoglobin normally is present in blood and endogenous stores of thiosulfate can be depleted rapidly, these two endogenous mechanisms of detoxification are overcome rapidly in cases of clinical toxicity.[103]

Excessive cyanide ion reacts readily with the trivalent (ferric) iron of cytochrome oxidase to form a stable cyanide-cytochrome oxidase complex. When iron is maintained in the ferric form, electron transport can no longer occur, and the chain of cellular respiration is brought to a halt. As a consequence, hemoglobin is unable to release its oxygen to the electron transport system, and cellular hypoxia results. This action occurs despite a large concentration of oxygen in the bloodstream. Cytochrome oxidase is most concentrated in tissues that have a high oxidative metabolic rate, such as the CNS and cardiac muscle. All tissues can be affected from this lack of usable oxygen, but death primarily is caused by anoxia in the brain.[87,103] The acute oral LD_{50} of HCN is 2 to 2.3 mg/kg, and rapid intake of plant material equivalent to about 4 mg/kg is thought to be a lethal amount.[87]

Diagnosis and Treatment

Cyanide poisoning should be considered when animals consuming cyanogenic plants are affected with acute signs of oxygen starvation and bright red blood (rarely present). The presence of cyanide is confirmed chemically, and samples of forage, blood, liver, muscle, brain, and heart may be submitted. All samples should be frozen as soon as possible and shipped.[87,103] Plant materials containing greater than 200 ppm HCN and concentrations in brain and ventricular myocardium greater than 100 mg/100 g wet weight are considered significant.[87]

Treatment aims at splitting the cyanide-cytochrome oxidase complex, with subsequent removal of the cyanide complex, and augmenting available thiosulfate in the bloodstream. Sodium nitrite displaces the cyanide molecule from the cytochrome enzyme and changes some of the hemoglobin to methemoglobin, which then competes with cytochrome oxidase for the cyanide ion. In this process methemoglobin and the cyanide ion form cyanomethemoglobin, and cytochrome oxidase subsequently is regenerated. Sodium nitrite should be used cautiously because of the possible danger of producing nitrite toxicosis, but it can be administered intravenously at dosages ranging from 6 mg/kg, given as a 20% solution,[87] to 15 to 25 mg/kg.[87]

Sodium thiosulfate reacts with the cyanide ion in the blood or liberated from cyanomethemoglobin and forms thiocyanate, which is essentially harmless and is excreted in urine. Sodium thiosulfate also can be given intravenously at dosages ranging from 60 to 660 mg/kg, as a 20% solution,[84] to 1.25 g/kg. Sodium nitrite and thiosulfate are not easy to obtain; thus these compounds rarely are used to treat clinically affected animals. In small animals a commercially available kit containing a vitamin B_{12} precursor, hydroxocobalamin, is currently recommended. An additional recommended therapy includes large doses of mineral oil. The cobalt in the vitamin B_{12} preparation may bind additional cyanide in the circulation, and mineral oil aids evacuation of the gastrointestinal tract. Animals that survive 24 hours usually do not require further treatment.[87]

Sodium Fluoroacetate (Compound 1080)

Sodium fluoroacetate and fluoroacetamide are highly toxic to many animal species. They have been used as rodenticides and in predator control, and because of their toxicity, their use in the United States is highly restricted. The compounds are odorless, tasteless, and water soluble and typically are incorporated into baits composed of carrot chunks, bread, bran, or meats. In the United States these compounds often are mixed with a dye before being placed in baits. Horses may become intoxicated by inadvertent exposure to these baits.[87,103]

Clinical Signs

Sodium fluoroacetate causes signs primarily related to cardiac dysfunction in herbivores. The onset of signs usually occurs from 30 minutes to 2 hours after ingestion. When signs begin, they usually have an acute onset and follow a rapid, usually violent course. Significant cardiac arrhythmias with rapid, weak pulse and eventual ventricular fibrillation are typical findings. Horses may exhibit staggering, trembling, restlessness, urination, and defecation. Moaning and bruxism may occur along with profuse sweating and signs of colic. Terminal convulsions also may occur. Ventricular fibrillation is the cause of death, and some horses may be found dead with no outward signs of struggle.[87,103]

Pathophysiology

Fluoroacetate is absorbed readily from the gastrointestinal tract, lungs, or open wounds but not through intact skin. After absorption, fluoroacetate is distributed throughout the body and does not accumulate in any specific tissue. The acute oral LD_{50} of fluoroacetate in horses is 0.35 to 0.55 mg/kg.[87]

After entry into cells, fluoroacetate can replace acetyl coenzyme A, combining with oxaloacetate to form fluorocitrate. Fluorocitrate then competes with citrate for the active site of aconitase, a Krebs cycle enzyme, and also inhibits succinate dehydrogenase, which catalyzes succinate metabolism. The inhibition of these two enzymes and the subsequent accumulation of citrate block the Krebs cycle, leading to decreased glucose metabolism, energy stores, and cellular respiration. These actions occur in all cells, but organs with high metabolic rates (e.g., brain, gastrointestinal tract, and heart) are affected most severely.[85,87,103]

The short latent period between ingestion and onset of signs occurs because fluoroacetate must be converted to the more toxic fluorocitrate. The accumulation of toxic levels of fluorocitrate therefore requires some time.[103]

Diagnosis and Treatment

Diagnosis of fluoroacetate toxicity must rely heavily on history of potential exposure, compatible clinical signs, and absence of other pathologic findings. Laboratory detection of these compounds can be difficult, but suspect baits, stomach contents, kidney, and urine are the best samples to submit for evaluation.[87,103] Significantly increased kidney citrate levels suggest compound 1080 toxicity.[87]

Laboratory abnormalities in affected animals might include hyperglycemia and lactic acidemia.[103] However, these findings are inconclusive and accompany many disease states in the horse.

Therapy is largely supportive and apparently unrewarding in horses already showing signs of toxicity. The practitioner may attempt intestinal decontamination using orally administered mineral oil and activated charcoal. If hypocalcemia develops, calcium gluconate or calcium chloride may be administered. Glycerol monoacetate at 0.1 to 0.5 mg/kg, administered intramuscularly with treatments repeated hourly, has been suggested but may not be effective after onset of clinical signs.[84,103]

Toxicoses Causing Signs Relating to the Epithelium, Skeletal System, and General Body Condition

Toxicoses causing these clinical signs can be among the most difficult to diagnose. Signs often are mild or moderate and quite nonspecific. In some cases the history is nonspecific, and the possibility of exposure to a toxin is difficult to evaluate.

PLANTS

Black Walnut *(Juglans nigra)*

Ingestion of shavings and aqueous extracts from black walnut trees is responsible for a toxic syndrome in horses characterized by acute onset of laminitis and variable degrees of limb edema.[127-130]

Clinical Signs

Horses begin showing signs of toxicity within 10 to 12 hours of being bedded with black walnut shavings. The primary signs are those of laminitis and include reluctance to move, shifting weight from limb to limb, increased digital pulse and temperature of the hoof, and positive response to hoof testers. The laminitis may vary from mild to severe.[127-129] Another characteristic finding is that of limb edema, which can become pronounced. Additional signs noted may include increased respiratory rate with flared nostrils, anorexia and lethargy, and abdominal pain.[128] Horses removed from the bedding after clinical signs have developed have a good prognosis for full recovery.[127,128] Ingestion of the plant hoary alyssum *(Berteroa incana)* by horses can elicit the exact same clinical syndrome.

Pathophysiology

The toxic principle involved in this toxicosis is unidentified. Juglone, a naphthoquinone found in roots, bark, and nuts of black walnut trees, was suggested in the past to be the causative agent.[127-129] Further work has shown, however, that an aqueous soluble toxin other than juglone that is found in the heartwood is more likely to be responsible for generation of clinical signs.[129] The mechanism of action of this soluble toxin has not been elucidated fully, but it has been shown to reversibly enhance vasoconstriction of isolated digital vessels in vitro induced by administration of epinephrine potentiated with hydrocortisone.[130]

Diagnosis and Treatment

Diagnosis of black walnut–induced laminitis is based primarily on known ingestion of shavings containing the plant and subsequent development of clinical signs. The treatment of laminitis is covered elsewhere in this text, but horses have a favorable prognosis for recovery if they are removed quickly from the offending bedding. Black walnut shavings should not be used as bedding for horses.

Wild Jasmine *(Cestrum diurnum)*

Cestrum diurnum (wild jasmine, day cestrum, day-blooming jessamine, king-of-the-day, Chinese inkberry) is a tropical to subtropical plant native to the West Indies that has been introduced and cultivated widely as an ornamental in warmer parts of the United States, including Florida, Texas, and southern California. The plant grows rapidly from seeds, and birds may contribute to its spread because of their appetite for ripe berries that contain seeds. The plant is naturalized in Hawaii and India. It also tends to multiply along fence rows, on roadsides, and in neglected pastures and fields. Horses show signs of disease after ingestion of the plant for several weeks to months.[131]

Clinical Signs

Affected horses show signs primarily of weight loss and lameness. Weight loss occurs over several weeks to months in spite of normal appetite. Lameness usually is of increasing severity and may begin with signs of generalized stiffness. Eventually, the fetlock joints may become overextended, and horses may exhibit kyphosis. The flexor tendons and particularly the suspensory ligament become sensitive to palpation. The lameness may grow so severe that euthanasia becomes necessary.[131] Signs of renal failure also may become evident in isolated cases.

Pathophysiology

C. diurnum and certain other members of the Solanaceae family contain a potent steroid glycoside with vitamin D–like activity. The toxic agent is a 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$) glycoside found in the leaves of the plant.[132,133] Normally, vitamin D_3 is acquired from the diet or is produced in the skin by a reaction dependent on ultraviolet light. Vitamin D_3 is hydroxylated in the liver to yield 25-hydroxycholecalciferol, which subsequently is hydroxylated in the kidney to $1,25(OH)_2D_3$. This compound is the most active form of the vitamin and acts to increase calcium absorption and stimulate production of calcium-binding protein in the intestine.[132]

The normal rate of production of $1,25(OH)_2D_3$ is regulated by a negative feedback mechanism. Calcium or phosphorus deprivation stimulates the production of $1,25(OH)_2D_3$, and a decreased rate of production occurs when calcium or phosphorus is present in adequate amounts.[132]

This natural feedback mechanism is bypassed when $1,25(OH)_2D_3$ is supplied exogenously, as in the case of ingestion of *C. diurnum.* As a consequence, excessive calcium-binding protein is synthesized in the intestine, and excessive amounts of calcium and phosphorus are absorbed. If the calcium load exceeds the capacity of the kidney to excrete it, soft tissue mineralization (dystrophic calcification) and osteopetrosis occur.[132] In horses that have ingested toxic amounts of *C. diurnum,* calcification of the flexor tendons, suspensory ligament, and other elastic tissues appears to predominate over deposition of calcium into other soft tissues. Osteopetrosis is thought to result from sustained hypercalcemia and secondary elevation of calcitonin.[131]

Diagnosis and Treatment

A diagnosis of toxicosis is suspected when horses exhibit signs of weight loss and lameness and they are known to have had prolonged access to *C. diurnum.* This is a very uncommon problem. Affected horses typically are hypercalcemic, but serum phosphorus concentration usually is within a normal range. Renal dysfunction, if present, may be characterized by elevations in BUN and creatinine concentrations. Urine analysis may indicate an increased fractional excretion of sodium, potassium, and phosphorus, along with other laboratory findings associated with renal failure. Horses should be denied access to *C. diurnum* plants.

Hairy Vetch *(Vicia villosa)*

One report of toxicosis suspected to result from ingestion of hairy vetch is described.[134] The affected horse was a 1-year-old crossbred female that required euthanasia because of severe weight loss and bilateral corneal ulceration with perforation.

The clinical signs reported were weight loss despite good appetite, fluctuating body temperature, subcutaneous swelling that started around the lips and spread to involve the rest of the body, and bilateral corneal ulceration with eventual perforation.

The only recorded abnormal laboratory findings consisted of elevated serum concentrations of LDH and AST measured 2 weeks after onset of clinical signs. Histologic lesions consisted of multifocal to diffuse granulomatous inflammation of the heart, lungs, kidneys, skin, lymph nodes, ileum, colon, skeletal muscle, and choroid.

The toxic substance has not been identified, and no specific therapy has been recommended. A similar toxic condition occasionally occurs in other livestock grazing hairy vetch, with most cases of toxicosis occurring between April and July.

Photosensitizing Plants

Many plants can cause photosensitive reactions in horses. Some plants contain photodynamic substances that are absorbed from the gastrointestinal tract intact or in a form metabolically altered into an active compound (primary photosensitizing plants). Other plants may cause photodermatitis after liver dysfunction, in which the photodynamic toxin is a metabolite normally excreted in bile (secondary photosensitivity). Because of hepatic damage induced by the plant, these metabolites enter the circulation, and subsequent interaction with light results in clinical manifestation of disease. Box 21.2 lists plants[135-137] known to induce photosensitization in herbivores.

Clinical Signs

Signs of photosensitization are similar, regardless of the cause, and vary in degree of severity. Factors that influence the severity of signs include the amount of reactive pigment present in the skin at a given time; the degree of exposure to light of appropriate wavelength; and the severity of hepatic damage, if hepatogenous photosensitization is the cause.[137]

Restlessness and discomfort usually are the first evident signs. Erythema may be apparent, followed by edema of affected areas. Blister formation and subsequent serum exudation and scab formation occur as the condition progresses. Affected sites usually are painful when touched, and animals often attempt to protect themselves from direct sunlight.[135,137]

The light-colored or nonpigmented areas of skin are affected most severely, particularly the face, nose, back, escutcheon, and coronary band. Severely affected animals also may have involvement of pigmented areas of skin, and secondary self-trauma and bacterial infection may arise from attempts to rub the affected areas. Horses may lose patches of skin that slough in large, leathery plaques.[135,137]

Pathophysiology

Plants causing primary photosensitization contain a photodynamic agent that is absorbed from the gastrointestinal tract intact or that is later metabolically altered into an active compound. Secondary photosensitizing plants induce hepatic damage of sufficient magnitude to inhibit adequate clearance of photodynamic agents. Normally, these photodynamic toxins

BOX 21.2 Plants Inducing Photosensitization in Herbivores

PRIMARY PHOTOSENSITIZERS

Ammi majus (bishop's weed): contains furocoumarins
Astragalus cicer (chickpea milk vetch)
Avena fatua (oatgrass)
Brassica spp. (mustards)
Cooperia pedunculata
Cymopterus watsonii (spring parsley): contains furocoumarins
Erodium spp. (filaree)
Fagopyrum sagittatum (buckwheat): contains the naphthodianthrone derivative fagopyrin
Heracleum spp. (cow parsnip)
Hypericum perforatum (St. John's wort, Klamath weed): contains the naphthodianthrone derivative hypericin
Lotus spp. (trefoil)
Medicago (alfalfa)
Perennial ryegrass
Poaceae family (oats, sandbur, bermudagrass, barnyard grass, cupgrass, barley, rye, foxtail, sorghum, wheat, ryegrass)
Polygonum spp. (smartweed, lady's thumb, knotweed)
Ricinus communis (castor bean)
Rutaceae
Spenosciadium spp. (whiteheads)
Thamnosma spp. (blisterweed)
Trifolium spp. (clover)
Umbelliferae
Zephyranthes spp. (evening rain lily)

SECONDARY OR HEPATOGENOUS PHOTOSENSITIZERS

Agave lecheguilla (lecheguilla)
Blue-green algae
Brachiaria brizantha
Brassia hyssopifolia
Brassica napus (cultivated rape)
Holocalyx glaziovii
Lantana spp.
Lippia rehmanni
Myoporum laetum (ngaio)
Narthecium ossifragum (bog asphodel)
Nolina texana (sacahuiste)
Panicum spp. (panic grass, kleingrass)
Pithomyces chartarum and *Pithomyces minutissima*
Senecio spp. (ragwort, groundsel)
Tetradymia canescens (gray horsebrush)
Tetradymia glabrata (spineless horsebrush)
Tribulus terrestris (puncturevine)

OTHER PHOTOSENSITIZERS

Avena (oats)
Euphorbia maculata (milk purslane)
Kochia scoparia (summer cyprus, fireweed)
Medicago (alfalfa)
Polygonum spp. (smartweed)
Sorghum vulgare (Sudan grass)
Trifolium (clover)
Vicia spp. (vetches)

are metabolites excreted in bile. When the liver is damaged sufficiently or bile flow is hindered, these metabolites enter the circulation. Phylloerythrin, a normal chlorophyll breakdown product, is considered the only important photodynamic substance in instances of secondary photosensitization.[138] These photodynamic agents then are circulated throughout the body, eventually reaching the dermal capillaries.

Interaction of long-wave ultraviolet light with these photodynamic agents circulating in the skin capillaries results in chemical excitement of these substances. As a consequence, free radicals form that are highly inflammatory and cause degradation of cell phospholipid membranes, polypeptide proteins, and nucleic acids.[135,139] These processes disrupt the cells and ultimately result in the dermal lesions noted with this toxicity.

Diagnosis and Treatment

Clinical signs of photosensitivity are fairly typical and seldom are confused with other afflictions of skin. When clinical signs become evident, the horse should be evaluated thoroughly for evidence of hepatopathy because primary and secondary photosensitivity produce the same clinical signs. If hepatic disease is present, the clinician should ascertain the cause and institute appropriate therapy.

Primary photodynamic agents can be identified with several biologic assay systems, but this is rarely done. A mouse assay system and a microbial assay using *Candida albicans* are available for this purpose but, again, are rarely done.[135]

Specific therapy for photodermatitis is not available. Horses should be removed from direct sunlight until skin lesions are healed and the offending plant is removed from the environment. Various topical agents may be used to facilitate skin healing. Superficial bacterial dermatitis should be treated with appropriate antibiotics, and NSAID therapy may be beneficial during the initial stages of the disease process.

Tall Fescue *(Festuca arundinacea)*

The causative agent of the disorder associated with grazing tall fescue is *Neotyphodium coenophialum*[140] (formerly identified as *Acremonium coenophialum*), a fungal endophyte that you cannot visually see that grows within the stem, leaf sheaths, and seeds of tall fescue.[141,142] This endophyte is highly indigenous to many areas of the United States and may contaminate as much as 90% of fescue pastures in certain geographic areas.[141]

Clinical Signs

Horses grazing or being fed tall fescue hay preparations may develop a condition termed *summer slump* or *summer syndrome.*[135,143] The disorder is characterized by anorexia, weight loss, poor hair quality, pyrexia, and hypersalivation. Additionally, mares may sustain a variety of pregnancy- and reproduction-related disorders. Typical findings include the presence of a thick, tough placenta; prolonged gestation; abortion; birth of dead or weak dysmature foals; and high perinatal foal mortality.[144] Mares frequently are affected with agalactia, retained placenta, and rebreeding difficulties as well.[135,139,141]

Pathophysiology

The fungus produces multiple toxins, including peramines, lolines, and ergopeptine alkaloids. Peramines have no apparent effect on animal health, but the lolines (*N*-acetyl loline and *N*-formyl loline) present in *Neotyphodium* are pyrrolizidine alkaloids. However, the hepatotoxicity characteristic of pyrrolizidine alkaloids has not been observed with tall fescue toxicosis in any species.[142]

The ergopeptine alkaloids appear to be responsible for most of the abnormalities associated with fescue toxicosis. Ergotamine, ergosine, ergovaline, ergoine, ergocristine, ergocryptine, and ergocornine have been isolated, but ergovaline and ergosine are most prominent. Ergovaline is thought to account for 84% to 97% of the ergopeptine concentration of infected tall fescue. The concentration of ergot alkaloids in tall fescue tends to increase with nitrogen fertilization and drought stress, and toxicity varies from season to season and year to year depending on the percentage of endophyte present, drought stress, nitrogen fertilization, and probably other factors.[142]

Agalactia occurs for a variety of reasons. First, ergopeptines are dopamine D_2-receptor agonists, and dopamine is thought to be the major inhibitor of prolactin secretion in the body. Second, ergot alkaloids inhibit adrenocorticotropic hormone secretion, resulting in reduced fetal cortisol concentration that subsequently causes reduced progesterone secretion by the placenta. Third, these alkaloids decrease tissue binding of estradiol, which can lead to an elevated serum concentration of estradiol-17β[142] (normally, serum estradiol concentrations decline near parturition). The interaction of appropriate levels of prolactin, progesterone, and estradiol-17β plays a major role in preparing the mammary gland for lactation. The combination of reduced concentrations of prolactin and progesterone and elevated concentration of estradiol-17β likely is the major cause of the agalactia and impaired udder development in affected mares.

The gestation period may be prolonged because ergopeptines have been hypothesized to block corticotropin-releasing hormone activity in the foal, which results in a lack of fetal production of adrenocorticotropic hormone and cortisol. Because increased fetal cortisol concentration acts to signal the mare for parturition, lack of fetal production of corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol may contribute to extended gestation periods in affected mares.[142]

The placental abnormalities frequently noted have been suspected to be associated with vasoconstriction. Edema, fibrosis, and mucoid degeneration of arteries in placentae from affected mares have been observed.[143] These changes were thought to be consistent with anoxia, which was hypothesized to be associated with vasoconstriction. Ergovaline and *N*-acetylloline, which are present in infected fescue, have vasoconstrictive properties.[142]

Ergot alkaloids also may have a negative effect on fertilized ovum implantation in the endometrium, resulting in reduced reproductive efficiency of affected mares. In the mare the effects of ergopeptines on implantation are inconclusive, but ergocryptine, ergocornine, ergosine, and ergovaline are capable of interrupting early pregnancy in the rat.[142]

Foals born alive to affected mares may be hypothyroid (and dysmature), although the mechanism responsible for this is unknown.[144] High perinatal foal mortality probably also is influenced by agalactia and poor colostral antibody production by the mare, resulting in failure of passive transfer with associated septicemia and failure of the foal to thrive.[142]

Diagnosis and Treatment

Diagnosis often is empiric, based on clinical signs and access to fescue pasture or hay during late gestation. Affected mares

typically have reduced serum concentrations of prolactin and progesterone and elevated levels of estradiol-17β. Reduced serum concentrations of triiodothyronine, adrenocorticotropic hormone, cortisol, and total progestogens may be present in affected foals. Hay and pasture samples also may be evaluated for the presence of endophytes and ergovaline.[142]

Treatment of gravid mares past their foaling date should include removal from fescue as quickly as possible. Domperidone given orally once daily at 1.1 mg/kg during the last 15 days of gestation may be efficacious in helping establish udder development and lactation. Postfoaling mares may benefit from domperidone (1.1 mg/kg orally) administered once daily for several days in an attempt to stimulate milk production.

Prevention requires removing mares from fescue pasture or hay during late gestation. In one study fescue removed from the diet at 300 days of gestation was not associated with any problems in mares.[143] Thus the most critical time for exposure to infected fescue in pregnant mares seems to be during the last 30 days of pregnancy. Local or regional authorities can provide important information regarding current methods of managing infected pastures.

Hoary Alyssum *(Berteroa incana)*

Toxicosis in horses related to ingestion of hoary alyssum was first reported in 1992.[145,146] This plant is a member of the Cruciferae (mustard) family and can behave as an annual, biannual, or perennial. Hoary alyssum grows primarily in the northeastern and north-central United States and Canada but also is reported in the states of Oklahoma, Washington, Oregon, Idaho, and California and in Europe. The plant tends to flourish under conditions of drought, frost, and overgrazing and in areas of poor soil.[146,147] Toxicity has been reported in horses grazing contaminated pasture, eating plant-contaminated hay, and being fed oats containing hoary alyssum seed.[145-148] Reported cases are more prevalent during summer months after ingestion of recently cut hay, but toxicosis has resulted from feeding hay stored for 9 months.[146]

Clinical Signs

Not all horses exposed to hoary alyssum develop clinical signs of toxicosis. An estimated 45% of horses exposed to the plant under field conditions did not develop any signs of illness. However, the most common clinical signs observed under field and experimental conditions were varying degrees of fever, edema of one to four limbs, and laminitis. Horses affected with laminitis rarely are reported to develop rotation of the distal phalanges. These signs typically commence within 24 hours of ingestion (although this varies depending on the amount of plant material ingested) and in most cases subside within 2 to 4 days after removal of the source.[146] Other signs noted have been lethargy, short-term diarrhea, and abdominal discomfort.[148] Early parturition and abortion are rare occurrences in pregnant mares.[145,147] Rarely, individual horses are affected more severely and exhibit clinical signs of endotoxemia, hypovolemic shock, acute renal failure, and ventral rotation of the distal phalanges. Death can occur in these horses.[146,148]

Pregnant mares may be more susceptible to toxicity and develop more severe clinical signs, however. In one report 23 of 29 broodmares developed signs of fever, tachycardia, tachypnea, distal limb edema, and mild to severe laminitis.[147] Of these mares 15 subsequently developed moderate to profuse bloody diarrhea, dehydration, abdominal pain, hematuria, and oliguria. Four of these mares were euthanized, and two had necropsy findings of hemoperitoneum, hemothorax, and ventral rotation of distal phalanges. Three of the mares aborted spontaneously, but no abnormalities were noted in the placentae or fetuses.

Pathophysiology

The toxic agent in hoary alyssum remains unknown,[146] as does the mechanism of action of the toxic substance (or substances). However, red blood cell destruction appears to occur by way of hemolysis or some other mechanism.[146,147] No consistent laboratory abnormalities have been noted in an experimental feeding trial and in most field cases.[146] However, laboratory findings in more severely affected horses include significant red blood cell hemolysis; elevations in serum concentrations of creatinine, urea nitrogen, phosphorus, alkaline phosphatase, AST, CK, sorbitol dehydrogenase, and total bilirubin; neutropenia; proteinuria; hematuria; hemoglobinuria; and occult blood in feces and gastric fluid.[146-148] Which of these abnormalities are the direct result of the toxin and which result from physiologic deterioration of various organs is unknown.

Diagnosis and Treatment

A diagnosis is suspected on the basis of history of exposure to the plant and clinical signs. No specific therapy is indicated, but horses immediately should be denied access to the plant, and all contaminated hay and grain should be removed. One should initiate symptomatic treatment with fluids, NSAIDs, and gastrointestinal tract evacuation. Activated charcoal (1 to 3 g/kg administered through a nasogastric tube) has been suggested to help prevent absorption of the unknown toxin.[148] Intravenous DMSO should be used with caution because it has the potential to cause intravascular hemolysis, thereby compounding the red blood cell destruction caused by the toxin.[146,148] Most horses recover uneventfully in 2 to 4 days after removal of the plant and provision of supportive care.

MISCELLANEOUS AGENTS

Iodine

Iodine toxicosis or iodism is a rarely reported cause of toxicosis in horses. When iodism occurs, it most likely is caused by iatrogenic administration of iodine-containing substances. Many rations contain iodine in the form of various iodized salts, and sodium iodide and potassium iodide, along with the organic iodide compound ethylenediamine dihydroiodide, are used to treat various medical conditions.[135,149]

Clinical Signs

Nonpruritic generalized alopecia and diffuse scaliness of the skin were reported in a horse given 45 g of ethylenediamine dihydroiodide twice daily for 14 days.[150] Other reported clinical signs include goiter after excessive iodine intake, increased secretions of the respiratory tract, nasal discharge, intermittent nonproductive cough, and excessive lacrimation.[135,138,150] Pregnant mares receiving excessive amounts of iodine may produce weak foals with enlarged thyroid glands. Such foals have a high mortality rate.[135]

Pathophysiology

Organic and inorganic forms of iodine are absorbed rapidly and almost completely from the gastrointestinal tract in the ionic form and are distributed throughout the body. Iodine is

excreted primarily in the urine, but smaller amounts are present in feces, sweat, and milk.[138,149] The only known metabolic role of iodine is involvement in synthesis of the thyroid hormones thyroxine and triiodothyronine.[138]

Oral iodine salts, whether organic or inorganic, taken in higher doses stimulate nerve receptors in the stomach wall. Subsequently, the vagus nerve becomes stimulated and causes reflex secretion by cells in the upper respiratory tract.[138,151] Excessive iodine intake causes thyroidal organic iodine formation to increase to a maximal amount, and then a sharp decline in organic iodine formation occurs. Excessive iodine also inhibits release of organic iodine from the thyroid gland if the gland is stimulated by thyroid-stimulating hormone. The net results are clinical signs of increased amount and viscosity of respiratory tract secretions and occasional goiter development.[151] The mechanism underlying the development of the dermal lesions associated with iodism is unknown.[150]

Diagnosis and Treatment

Diagnosis of iodism is based on a history of exposure to high levels of iodine for a prolonged time coupled with compatible clinical signs of nasal discharge and excessive lacrimation, intermittent nonproductive cough, and nonpruritic generalized alopecia and scaling. Serum iodine concentrations, which are elevated in cases of iodism, can be measured, but blood levels decrease rapidly to near background levels within a few days if iodine exposure ceases.[138] Serum concentrations of thyroxine and triiodothyronine may be below normal reference values in affected horses.[150]

Treatment consists of removing the source of the iodine. Because iodine is mobilized rapidly and excreted from tissues, clinical signs usually subside rapidly when excessive iodine is removed.[138,149,150]

Snake Venom

The venomous snakes in North America belong to the families Crotalidae (pit vipers), Elapidae (cobra), and Viperidae (true vipers). Most poisonous snakebites reported in human beings are inflicted by members of the Crotalidae family, and the same is probably true for horses as well. Of this family *Crotalus* (rattlesnakes), *Agkistrodon* (copperhead, cottonmouth), and *Sistrurus* (pigmy rattlesnake, massasauga) are the three genera most commonly involved in snakebites of livestock. The eastern coral snake *(Micrurus fulvius)* and the Arizona coral snake *(Micruroides euryxanthus)* are two members of the Elapidae family indigenous to the United States, but they account for only about 3% of poisonous bites reported in human beings.[135,152]

Venom injected into prey is an aid to digestion and greatly reduces the time for complete digestion to occur in the snake. The amount of venom injected at a given time is under voluntary control, and larger amounts are injected into larger prey or when the snake strikes for defensive purposes. Not all bites result in envenomation, and rattlesnakes are estimated to fail to inject venom in up to 20% of bites (referred to as dry bites).[135,152]

Snake venom is a highly complex mixture of enzymes, lipids, biogenic amines, free amino acids, metal ions, proteins, and polypeptides. Most snake venoms contain up to 25 different fractions, yet many of them are not yet identified. Venom composition and toxic properties vary among *Crotalus* species and among individuals of the same species. Factors influencing venom composition include age, time from last feeding, and seasonal influences related to changes in feeding patterns or physiologic responses such as hibernation. The LD_{50} in mice exposed to venom of *Crotalus* species ranges from 0.23 mg/kg for the Mojave rattlesnake *(Crotalus scutulatus)* to 3.77 mg/kg for the red diamond rattlesnake *(Crotalus ruber ruber)*. The LD_{50} for mice for the copperhead *(Agkistrodon contortrix)* and cottonmouth *(Agkistrodon piscivorus)* is 10.92 mg/kg and 4.17 mg/kg, respectively.[152] This large variation in dose and chemical composition of venom accounts for the extreme amount of variation in the physiologic responses of animals to these substances.

Clinical Signs

Snakebites in horses are most common on or near the muzzle, but bites also may occur on the limbs or other parts of the body. The classic signs noted in most horses are acute onset of swelling and edema at the bite site. The muzzle and nasal passages may become swollen to the extent that respiration can become extremely labored, necessitating a tracheotomy. Epistaxis also may be apparent. Initially, fang marks may be evident, but they soon disappear with the onset of extensive swelling. Skin discoloration at the injection site is a common occurrence with many rattlesnakes but rarely occurs with copperhead and Mojave rattlesnake bites.[135] Varying degrees of necrosis of local tissue may occur, as well as secondary bacterial infections at the wound site. In the author's experience, bites involving the distal extremities of horses frequently have a prolonged convalescent period that may be accompanied by residual lameness.

Systemic manifestations of snake envenomation may be apparent when venom is injected intravascularly or perivascularly. In a retrospective study of rattlesnake venom poisoning in 32 horses, manifestations included fever, tachycardia, tachypnea, cardiac arrhythmia, hemolytic anemia, thrombocytopenia, hemorrhage, thrombosis of venipuncture sites, colic, diarrhea, and prehensile and masticatory dysfunction. Chronic problems included cardiac disease, pneumonia, laminitis, pharyngeal paralysis, and wound complications. The most common chronic problem observed in these horses was cardiac disease.[153] Labored respiration may result from pulmonary edema caused by passive congestion after vascular hypotension or precipitation of pulmonary emboli. Muscle fasciculation may be evident.[135] The venom of the Mojave rattlesnake produces respiratory paralysis that may result in death in human beings.[152] The overall mortality rate in 32 horses acutely affected with bites of prairie rattlesnake was 25%.[153]

The venom of coral snakes produces neurologic signs in affected human beings, with death occurring within 24 hours as a result of respiratory depression, hypotension, and cardiovascular collapse.[152] However, the coral snake requires prolonged contact (30 seconds or longer) to work the venom into the skin of its prey. Therefore it seems improbable that horses would become exposed to coral snake venom except under extremely unusual circumstances.

Pathophysiology

The venoms of Crotalidae are rich in enzymes. Proteases cause severe tissue damage by digesting tissue proteins and peptides, and hyaluronidase allows rapid spread of venom through tissue by hydrolyzing connective tissue hyaluronic acid. L-Amino acid oxidase, L-arginine ester hydrolases, and 5′-nucleotidase also may contribute to tissue destruction. Phospholipases A,

B, C, and D hydrolyze lipids and cause hemolysis by destroying lecithin in the red blood cell membranes. They also disrupt neurotransmission at the presynaptic and postsynaptic junctions. Other enzymes present in crotalid venom include ribonuclease, deoxyribonuclease, transaminase, phosphomonoesterase, phosphodiesterase, ATPase, DNAse, alkaline phosphatase, acid phosphatase, and endonuclease.[152]

Crotalid venom contains a number of polypeptides in addition to the enzymes. These polypeptides are low-molecular-weight proteins that are 5 to 20 times more lethal than crude venom in animal models, and they do not have enzymatic activity. They are present in higher concentrations in cobra venom than in rattlesnake venom and are mainly responsible for blood dyscrasias and coagulopathies. Small peptides are partly responsible for the generation of disseminated intravascular coagulation, and a venom fraction of the timber rattlesnake *(Crotalus horridus horridus)* causes platelet aggregation with resultant thrombocytopenia. Additionally, the venoms of the Mojave and Southern Pacific rattlesnakes contain a direct cardiotoxin.[152]

Rattlesnake venom contains substances with anticoagulant, procoagulant, and plasminogen-induced fibrinogenolytic properties. The coagulopathy that occurs in a given instance varies and depends on the content of the various venom components and on the dose of venom injected. The anticoagulant activity of crotalid venom appears to result from reversible binding of venom to prothrombin. Thrombin-like enzymes produce hypofibrinogenemia and increased concentration of FDPs. They also are capable of directly converting fibrinogen to fibrin, which may result in excessive fibrin formation and rapid disseminated intravascular coagulation. The fibrinolytic characteristics of crotalid venom occur directly or indirectly through the activation of endogenous plasminogen.[135,152]

Diagnosis and Treatment

Diagnosis of snakebite usually depends on clinical signs and accessibility to poisonous snakes. Laboratory abnormalities that may be present in affected horses include thrombocytopenia, hypofibrinogenemia, anemia, prolonged PT and PTT, hematuria, proteinuria, and myoglobinuria.[135,152]

Affected horses should be kept calm and quiet. Incision over the fang marks and suction are rarely indicated and probably of minimal value except in the immediate time frame of the bite, because the venom is absorbed almost immediately into the surrounding tissues. Tracheotomy is indicated in those horses that develop excessive edema and swelling of the head and external nares to the point that respiration becomes impaired. Topical cold therapy may have some beneficial effect if applied early and for short periods. Prolonged or excessive cold application, however, may enhance further tissue necrosis. Antivenom therapy commonly is used in instances of human snakebite but often is considered unnecessary in horses because of the low mortality rate, financial considerations, and availability of other therapies. An exception may be the horse or foal that is extremely valuable.

The clinician should initiate tetanus prophylaxis and systemic antibiotic therapy. Broad-spectrum antimicrobial drugs should be used because gram-positive and gram-negative organisms are found in the mouth of North American pit vipers. The most common organisms isolated include *Proteus vulgaris, Escherichia coli, Corynebacterium* spp., *Streptococcus* spp., and Enterobacteriaceae.[152]

NSAIDs should not be used during the early stages of the bite wound because they may enhance the defects in primary hemostasis frequently induced by snake venom. NSAIDs may be used in the later stages of the disease to help reduce pain, swelling, and inflammation. Corticosteroids should be used with caution because they may diminish the clearance of FDPs from the peripheral vasculature by the reticuloendothelial system, and they may increase susceptibility to wound infection at the site. Corticosteroids may be beneficial, however, in treating severe hypotensive shock in young animals or in patients with intravenous envenomation. Heparin therapy also has been suggested to be helpful in instances of thrombus formation.[135]

Animals affected with systemic hypotension and cardiac dysrhythmias should be treated appropriately. Treatment may include the administration of intravenous fluids and plasma and the use of specific antiarrhythmic medications.

Fluoride

Fluoride toxicosis in horses appears to be a rare event.[154] Although acute and chronic fluoride toxicosis have been described in various animals, chronic fluorosis appears more common. Common sources of fluoride in chronic fluorosis include forages subjected to airborne contamination from nearby industrial plants such as aluminum smelters, steel mills, or fertilizer plants that heat fluorine-containing materials and discharge fluorides; drinking water containing excessive fluoride; feed supplements and vitamin and mineral additives with high fluoride concentration; and vegetation grown on soils containing high fluoride levels.[138]

Animals normally ingest small amounts of fluoride throughout their lives, and it accumulates in the body as long as constant or increasing amounts are ingested. Chronic toxicosis can result from prolonged ingestion of sufficiently high levels. The long-term dietary tolerance for fluoride in the horse is reported to be 40 to 60 ppm dietary fluoride.

Fluoride is absorbed almost totally from the gastrointestinal tract. Once absorbed, approximately half is excreted rapidly in urine, with the remaining half being stored in bone and teeth. Fluoride accumulates in calcified tissues, but once exposure ceases, bone fluoride is depleted slowly over months to years.

Clinical Signs

Chronic fluorosis in the horse is a rare event. In one suspected case the affected horse exhibited chronic weight loss of months' duration; poor growth; difficulty in mastication; and deformed, discolored, and absent deciduous incisors. The horse also was missing some deciduous premolars and molars.[154] Classic dental abnormalities reported in other species include mottled, hypoplastic enamel and brown discoloration with uneven wear of teeth.[138] Additional signs associated with chronic fluorosis in other animals include hyperostosis, enlargement and roughening of involved bones, intermittent lameness and generalized stiffness, dry and roughened hair coat, and decreased weight and milk production.[154] Because of the insidious nature of chronic fluorosis, it is important to remember that a time lag may occur between ingestion of excessive fluoride and the appearance of clinical signs.

Pathophysiology

Excessive fluoride produces dental abnormalities by affecting the teeth during development. The primary effect of fluorine is thought to be a delaying and alteration of normal

mineralization of the preenamel, predentine, and precementum. High fluoride levels appear to cause specific ameloblastic and odontoblastic damage. The matrix laid down by these damaged cells fails to accept minerals normally, resulting in faulty mineralization of the tooth bud. Once the tooth is formed fully, the ameloblasts have lost their constructive ability and the enamel lesions cannot be repaired. However, odontoblasts can produce secondary dentine to compensate for deficiencies brought about by excessive fluoride.[138] The brown to black discoloration of affected teeth results from oxidation of organic material in the teeth.

The pathogenesis of bone lesions associated with fluoride toxicosis is still debated. One theory is that high fluoride levels lead to inadequate matrix and defective, irregular mineralization of bone. Another theory is that hydroxyl radicals in the hydroxyapatite crystal structure are replaced by fluoride ions, resulting in a decrease in crystal lattice dimensions. The pathologic results of skeletal fluorosis include dissociation of normal sequences of osteogenesis, production of abnormal bone, accelerated remodeling of bone, and occasional accelerated bone resorption.[138]

Diagnosis and Treatment

Diagnosis of chronic fluorosis is based primarily on clinical findings and history of possible exposure to fluorides. Fluorosis usually is confirmed by analysis of skeletal or dental tissues for fluoride content and evaluation of fluoride concentration in urine. Water and feed consumed by the animals also should be analyzed for fluoride content.

Treatment of chronic fluorosis aims mainly at dietary restriction of fluoride-containing substances. Aluminum sulfate, aluminum chloride, calcium aluminate, calcium carbonate, and defluorinated phosphate have been used to reduce the toxic effects of fluoride, but no substance completely prevents the toxic effects of ingesting excessive amounts of fluorides.

Zinc

Excessive exposure to dietary zinc can be a problem for young, growing horses. Sources of excessive zinc usually are soil and forage contamination from nearby smelters and pastures that were top dressed with zinc oxide.[155,156] Classic signs of copper deficiency associated with excessive zinc ingestion have been produced by experimental feeding of excessive zinc to young foals.[157,158] Skeletally mature horses do not appear to be susceptible to the effects of zinc-contaminated pastures.[158]

Clinical Signs

Signs associated with zinc-induced copper deficiency include swelling at the physeal region of long bones, gradual onset of lameness and stiffness that may become so severe that affected animals are reluctant to rise from lateral recumbency, swollen joints resulting from synovial effusion, unthriftiness, and weight loss despite normal appetite.[155-157] Anemia also may develop in more chronically affected individuals.[157] The joint swellings in affected horses are typical of those of osteochondrosis dissecans, and severe generalized osteochondrosis also develops in copper-deficient horses.[155,156,158]

Pathophysiology

In horses this disease actually appears to be a manifestation of copper deficiency and the subsequent development of hypocupremia-induced articular cartilage disease.[155,158] Copper is an essential cofactor for lysyl oxidase, an enzyme involved in the formation of collagen cross-links. Copper deficiency interferes with collagen metabolism and results in production of weak connective tissue. This allows articular cartilage fractures and growth physeal fractures to occur in the zone of hypertrophic cells, producing the clinical syndrome of osteochondritis dissecans.

The mechanism of zinc-induced copper deficiency is not totally understood but is most likely due to altered absorption from the gastrointestinal tract. Experimentally, affected foals at necropsy had high hepatic copper content despite low serum copper concentration, suggesting that the hepatic copper was not readily available for production of ceruloplasmin or could not be mobilized rapidly enough for use by other tissues.[158] Excessive zinc stimulates production of metallothionein, an intestinal cell protein that binds to excessive zinc, copper, and other divalent metal ions and facilitates their excretion in bile, feces, and saliva. Copper has a higher affinity for metallothionein than does zinc, and increased production of metallothionein, with subsequent binding to copper, may lead to copper deficiency through increased copper excretion.[159]

Diagnosis and Treatment

Once signs of osteochondritis develop, the condition should be appropriately treated. Diagnosis of excessive zinc intake may be difficult in that zinc is excreted rapidly after absorption, and blood and tissue zinc concentrations tend to decline quickly to normal levels with cessation of intake. Liver, kidney, and serum zinc concentrations can be measured, but fecal samples collected from affected horses may be more suitable. Feed and water supplies also can be evaluated for zinc content.[160]

Treatment of affected horses aims to restore proper copper concentration in the diet and remove the source of excessive zinc. Diets containing 7.7 mg of copper per kilogram and 250 mg of zinc per kilogram of dry weight were sufficient to maintain normal serum copper and zinc concentrations and did not induce disease in treated foals. Diets containing 1000 mg/kg or greater of zinc caused hypocupremia and subsequent osteochondrosis dissecans when fed to foals over a period of several weeks.[158] Osteochondrosis dissecans can be treated surgically.

Selenium

Selenium toxicity in horses usually results from prolonged ingestion of plants containing excessive amounts of selenium. Intoxication can result from horses foraging on soil with high levels of selenium and ingesting selenium-accumulating plants growing on soils with minimal amounts of selenium.[160-163] Acute toxicosis also can occur through inadvertent overdosing of selenium supplements added to rations or given by parenteral injection.[164] The acute single oral toxic dosage of selenium given as sodium selenite is between 3.3 and 6 mg/kg for the horse.[160,165]

Clinical Signs

Three different syndromes are attributed to selenium intoxication: acute toxicity and two chronic forms described as "blind staggers" and "alkali disease." Signs of acute toxicity develop within 6 hours of ingestion and include sweating, diarrhea, tachycardia, tachypnea, mild pyrexia, lethargy, and mild to severe colic. Clinical signs are attributable to selenium's effect on both the heart (myocardial necrosis) and lung (pulmonary necrosis and edema). Death may occur within 24 hours, and some horses exhibit a dumb attitude before death. Head pressing before death is suggested as a classic sign of acute selenosis.[160,165]

Chronic selenium intoxication described as blind staggers results from frequent ingestion of plants over weeks to months. Signs associated with this syndrome include aimless wandering or circling, muscle weakness, incoordination, respiratory difficulty, and decreased vision. In classic cases blindness eventually develops and is followed by paralysis and death.[166] Some have hypothesized that blind staggers is not due to selenium at all but is actually polioencephalomalacia associated with excess sulfur intake.

Alkali disease, the more common presentation of chronic selenosis in horses, results from ingestion of seleniferous plants, and signs may develop weeks to months after exposure. Initially, lameness and swelling of the coronary band regions are apparent, along with anorexia and mild depression. These signs progress to transverse cracking of the hoof wall distal to the coronary band with associated lameness. The hooves eventually may be sloughed. Loss of hair from the mane and tail occurs because the hairs become brittle and are broken easily, hence the term *bob-tailed disease.* Compromised reproductive and immune function also may develop in affected horses.[161-164]

Pathophysiology

Selenium is absorbed readily from the gastrointestinal tract, but organic forms of selenium generally are retained in greater amounts than are inorganic forms. Elimination occurs rapidly through urine, sweat, feces, and exhaled air. The clinician also must recognize hoof and hair as routes of excretion because excess selenium is deposited in these structures.[164] This latter fact has important diagnostic implications.

Selenium functions in a number of enzymatic and physiologic processes. The toxic effects of selenium have been associated with its affinity for reacting with sulfur-containing amino acid residues such as cysteine that are incorporated synthetically into biologically active glycoproteins and polypeptides. As a result, various selenosulfides are formed as a substitute for disulfide bonds.[167]

Diagnosis and Treatment

A presumptive diagnosis of selenium intoxication can be made on the basis of typical clinical signs and history of possible exposure to selenium iatrogenically in the form of selenium supplementation or through ingestion of appropriate plants. The clinician can attempt to make a definitive diagnosis by assaying blood, serum, hair, and hoof material for selenium content. In acute fatal selenosis blood selenium concentration may exceed 1 ppm.[160,165] A selenium concentration greater than 5 ppm in hair and hoof wall is considered diagnostic of selenosis.[160,161,163] It also is possible to measure selenium concentration in liver and kidney samples.

Treatment of affected horses involves removal of the selenium source, symptomatic treatment of lesions, and provision of good nursing care. Oral dosing of naphthalene at 4.5 g/day for 5 days, waiting 5 days, and then repeating the dose for an additional 5 days has been suggested for treatment of adult horses; however, this is not commonly done, and its efficacy is questionable.[160,161] Prevention of selenosis has been attempted by adding copper,[164] methionine,[168] or sodium arsenite[160,161] to the diet of at-risk animals.

Gangrenous Ergotism *(Claviceps purpurea)*

Claviceps purpurea is a fungal parasite that invades the developing ovary of the grass flower and cereal grains. This fungus commonly parasitizes rye, oats, wheat, and Kentucky bluegrass, which are associated most often with outbreaks of gangrenous ergotism. The fungus replaces the seed ovary with a dark brown to purple oblong body called a *sclerotium.* Sclerotia are slightly larger than the original whole grain seed, and their growth is promoted by warm, moist conditions. Horses rarely are affected by this condition, in part because of the distasteful nature of affected feedstuffs and because most fungal elements are removed during commercial grain processing procedures.

Clinical Signs

Signs of intoxication may become apparent if infected feeds are ingested over several days to weeks. An early sign observed in livestock following exposure to high levels of ergot include feed refusal. Dry gangrene of the extremities is the classic sign associated with *C. purpurea* toxicosis and can affect the limbs, nose, ears, and tail. Early signs of toxicosis are lameness and cool extremities. The hindlimbs often are affected first, with swelling and tenderness in the fetlock area. The involved tissues become dark and discolored, and a transverse line of demarcation may occur between normal skin and the distal parts of the limb. Eventually, the hoof and associated bones and tissue may slough. This same sequence may involve the nose, ears, and tail. Gangrenous signs may be preceded by colic and constipation or diarrhea. Possible subacute effects include depression, partial anorexia, general unthriftiness, and increased pulse and respiratory rates.[169] Abortions can also occur.

Pathophysiology

The major toxic alkaloids in ergot are a group of structurally similar ergopeptides. They are absorbed slowly and incompletely from the gastrointestinal tract, reaching peak plasma concentrations in approximately 2 hours. The liver is the primary site of metabolism, and approximately 90% of the metabolites are excreted in bile. Small amounts of unmetabolized alkaloids are excreted in urine. The total concentration and proportions of alkaloids present in ergot sclerotia may vary with species and environmental conditions.

Ergotamine, the most abundant of the alkaloids, is a polypeptide derivative of lysergic acid. The varied physiologic effects of ergot are caused primarily by mixtures of levoisomers of ergotamine, coupled with smaller amounts of acetylcholine, histamine, and tyramine.[169]

Ergotamine is a vasoactive substance that causes arterial and venous constriction. Ergotamine also may damage capillary endothelium. The combined effects of vasoconstriction and endothelial damage produce increased blood pressure, decreased blood flow through the extremities, vascular stasis, thrombosis, and eventually gangrene.[169]

The ergotoxine group of alkaloids produce α-adrenergic blockade and antagonize 5-hydroxytryptamine. This produces an increase in blood pressure as a result of peripheral vasoconstriction, particularly in postcapillary vessels.[169]

Diagnosis and Treatment

A tentative diagnosis of ergotism is based on clinical signs and exclusion of other disease processes. An experienced person can readily identify ergot sclerotia in grains. However, once grinding or pelleting of feed has occurred, ergot can be recognized only by microscopic examination or chemical analysis for ergot alkaloids. Ergot alkaloids can be identified and quantitated by chromatographic methods, and a sample of the

individual grain components should be obtained for analysis whenever possible.

No specific treatment exists for gangrenous ergotism. The offending grain should be removed, and affected animals should be kept warm to avoid cold-induced vasoconstriction in the extremities. Supportive therapy in the form of antibiotics and analgesics may be indicated. The use of anti–α-adrenergic pharmaceuticals to effect vasodilation also may be helpful. Such agents might include acepromazine, isoxsuprine, phenoxybenzamine, and similar products. [139]

Toxicoses Causing Signs Relating to Liver Disease or Dysfunction

PLANTS

Pyrrolizidine Alkaloids

Pyrrolizidine alkaloid toxicity results from consumption of plants that contain various pyrrolizidine alkaloids. Horses can become intoxicated after ingesting fresh plants and dried plants incorporated into hay. As with many toxic plants, however, these plants are frequently unpalatable to most horses, although certain conditions may render them more appetizing. This toxicity is characterized by a chronic, progressive disorder manifested by signs of liver failure. The more common plants that cause toxicosis in horses include *Senecio jacobaea* (tansy ragwort; also known as *Jacobaea vulgaris*), *Senecio vulgaris* (common groundsel), *Senecio longilobus* (groundsel), and *Cynoglossum officinale* (houndstongue).[169,170] Other plants that contain pyrrolizidine alkaloids but are not commonly associated with clinical disease in horses include *Amsinckia intermedia* (fiddleneck tarweed), *Crotalaria* spp. (rattlebox), *Echium plantagineum* (viper's bugloss), and *Heliotropium europaeum* (common heliotrope).

Clinical Signs

Signs of pyrrolizidine alkaloid intoxication in horses are essentially those of liver failure. The most frequently noted signs include weight loss of weeks to months in duration, icterus, and behavioral abnormalities. The behavioral changes indicate hepatoencephalopathy and may include aimless pacing or wandering, ataxia, licking inanimate objects, blindness, head pressing, and uncharacteristic aggression. Convulsions and coma may precede death. Clinical signs of abnormal behavior usually are a terminal event in the disease process and typically have an acute onset.[170,171] Other signs less frequently reported include diarrhea, photosensitization of nonpigmented areas of skin, hemoglobinuria, and inspiratory dyspnea.[170,172] Abortion and poor exercise tolerance (reduced athletic performance) also have been observed in horses after ingestion of sublethal amounts.[173] Because toxicity of these plants is related to liver dysfunction, clinical signs of disease may not become apparent for weeks to months after ingestion. A terminal hemolytic event is common in horses during end-stage liver failure.

Pathophysiology

A number of pyrrolizidine alkaloids are present in various plant species, and some plants may contain multiple alkaloids. These substances are absorbed from the gastrointestinal tract and carried to the liver, where they are metabolized by hepatic microsomal enzymes to pyrroles. These pyrroles then can cross-link double-stranded deoxyribonucleic acid (DNA) and bind to proteins and nucleic acid within hepatocytes.[169,170]

The cross-linking of DNA has an antimitotic effect on hepatocytes. The hepatocytes are unable to divide and subsequently become megalocytes. As these cells die, they are replaced by fibrous tissue rather than normal hepatocytes. Binding of protein and nucleic acid results in inhibition of cytoplasmic protein synthesis. These changes may lead to more rapid death of hepatocytes and cause centrilobular necrosis. Eventually, liver function begins to fail because of progressive hepatocellular death and subsequent fibrosis.[169,170] As the disease progresses, generalized fibrosis develops. Once connective tissue bridges form between portal areas, the disease is fatal.[169]

Variation in the dosage and frequency of administration of alkaloids results in a wide spectrum of hepatic lesions. Acute toxicosis resulting from massive doses is more likely to produce centrilobular necrosis with hemorrhage; however, this type of exposure is extremely rare. Chronic doses tend to produce hepatocellular death in the portal areas, along with megalocytosis, fibrosis, biliary hyperplasia, and occlusion of hepatic veins.[169,170] Liver failure is thought to be ultimately responsible for the clinical signs observed in pyrrolizidine alkaloid toxicosis.

The toxic dose of dried *Senecio* is estimated to be 5% of the body weight of the horse. This amount does not need to be ingested all at once, however, because the effects are cumulative. The total dose of alkaloids consumed determines the toxic effect, regardless of the amount of time in which the alkaloids were consumed.[169,170]

Diagnosis and Treatment

Most cases of pyrrolizidine alkaloid toxicosis are diagnosed on the basis of history, compatible clinical signs, serum liver enzyme activities, and liver biopsy findings. When active hepatocellular damage is occurring early in the disease process, sorbitol dehydrogenase and LDH usually are elevated, but they often decline to normal values by the time the horse is showing clinical signs. Serum GGT, alkaline phosphatase, and AST activities tend to be elevated throughout the disease course. Serum concentration of bile acids also is reported to be elevated in affected horses. Serum bilirubin concentration tends to be elevated in later stages of the disease, and hypoglycemia and hypoalbuminemia rarely are seen except in cases of severe hepatic disease.[169,170,172,173]

Other diagnostic aids that may be useful from a diagnostic and prognostic standpoint include measurement of the ratio of branched-chain amino acids to aromatic amino acids in serum. The branched-chain amino acids isoleucine, leucine, and valine are catabolized primarily in muscle, and the aromatic amino acids phenylalanine and tyrosine are catabolized mainly in the liver. The ratio of the sums of these amino acids (branched-chain to aromatic) decreases progressively from normal in horses affected with pyrrolizidine alkaloid toxicosis. If the ratio is below normal range and the horses are exposed continually to alkaloid-containing plants, they have a poor chance of survival. Some affected horses also showed a dramatic decrease in this ratio just before death.[174] Liver biopsy findings of a triad of fibrosis, bile duct proliferation, and megalocytosis are highly suggestive of pyrrolizidine alkaloid toxicosis. Liver biopsy samples also can help in establishing prognosis because the presence of advanced or generalized hepatic fibrosis warrants a grave prognosis.[169,170]

Feed samples may be analyzed for pyrrolizidine alkaloid content, but the process is often time consuming and unnecessary.[169] Pyrrole testing of liver tissue can be helpful in confirming exposure to the plant in the early stages of the disease; however, this analysis is offered by only a few laboratories.

No specific treatment for pyrrolizidine alkaloid toxicity exists. Affected horses may survive if they are denied further exposure to alkaloid-containing plants and are fed a suitable diet. However, some horses still may show signs of liver disease even though they have not had exposure to the plant for some time. Specific treatment of acute and chronic liver failure is discussed elsewhere.

Prevention entails keeping horses away from pasture or feed contaminated with pyrrolizidine alkaloid–containing plants.

Alsike and Red Clover *(Trifolium hybridum, Trifolium pratense)*

Both alsike and red clover are members of the Leguminosae family. Both are perennials that often are found in varying percentages in pastures and in seed pasture mixes, along with grasses. Both plants are very cold hardy, and tolerant of poorly drained wet, acidic, or clay soils, and often outcompete other grasses over time.

Liver disease associated with these two clovers has only been reported in the horse. Feed material that caused liver disease in horses has been fed safely to adult cattle. Toxicities can be the result of grazing pastures or ingesting contaminated hay. The toxic principle is unknown, but it has been hypothesized that it perhaps is a mycotoxin associated with the "black blotch" or "sooty blotch" fungus, *Cymodothea trifolii.* As little as 20% alsike clover in the diet has been associated with signs of poisoning, and depending on the percentage of clover in the diet, clinical signs of illness can be seen as early as 2 to 4 weeks after initial exposure.

Clinical Signs

Initial signs often recognized are lesions associated with a secondary photosensitivity. Sunburned lesions of the nonpigmented areas of the body, along with mucous membranes and cornea, are the sites most commonly affected. These lesions include vesicular swelling, erythema, edema, ulceration, and ultimately scab formation. This condition has been described in the literature as trifoliosis or dew poisoning and was thought to be the result of contact dermatitis. It is now recognized as a form of hepatogenous photosensitization.

As the disease progresses, nonspecific signs of hepatic disease start to develop. These may include decreased appetite, loss of body condition, weakness, sluggishness, weight loss, and poor hair coat. Acute onset of neurologic signs can occur in the more severe cases, which can include depression, aimless wandering and circling, head pressing, ataxia, yawning, teeth grinding, and colic. A rapid progression to recumbency, coma, and death has been reported.

Diagnosis and Treatment

Most cases of alsike and red clover poisoning are based on confirmation of exposure to the plant, clinical pathologic changes, and histologic lesions of either biopsy or necropsy tissue. Significant elevations in GGT, aspartate transaminase, sorbitol dehydrogenase, and alkaline phosphatase typically are seen. Serum bile acid and bilirubin concentrations will also be increased, although prominent icterus is not a consistent feature with this disease. Blood ammonia concentrations are also usually elevated, whereas hemograms are typically normal. Urinalyses may reveal bilirubinuria.

The liver often appears enlarged on ultrasound, along with irregular borders and texture. This feature had led to this disease once being referred to as "big liver disease." However, this change is not consistently observed, and small, shrunken, fibrotic livers have also been described.

Microscopic lesions reported include bile duct proliferation and perilobular, centrilobular, and periportal fibrosis. The severity of these findings is time dependent and can change depending on whether the exposure has been acute or chronic. There typically are mild inflammatory changes, mild hepatocellular swelling, and vacuolization, along with subtle and minimal necrosis. Mild megalocytosis has been reported in a few cases.

Specialists trained in plant identification can readily determine the presence of plants in a pasture or hay sample.

Mushroom (*Amanita* spp.)

One report describes fatal intoxication of a horse by ingestion of mushrooms (*Amanita* spp.).[175] Mushrooms apparently are unpalatable to livestock species, but the described horse also suffered from meningioangiomatosis, a rare benign tumor of the meninges and brain. The authors speculated that the presence of the tumor may have resulted in abnormal mentation and altered the normal eating behavior of the horse, resulting in mushroom consumption.

Clinical Signs

The described horse had acute onset of depression, head pressing, ataxia, and repeated recumbency with difficulty in rising. Before the onset of liver disease, many poisoned animals display signs of gastrointestinal distress. Clinical signs of shock, including hypothermia, poor capillary perfusion, and weak pulse, also were apparent. The horse was euthanized because of continued deterioration and lack of response to supportive therapy.

Pathophysiology

Some species of *Amanita* mushrooms contain a number of biologically active cyclic peptides such as amatoxins, phallotoxins, phallolysin, and antaminide. Some of these peptides affect actin polymerization and cause hepatocytes to lose their cytoskeletal organization and cellular attachments, resulting in hepatocellular dissociation and necrosis. Amanitins are peptides that inhibit ribonucleic acid (RNA) polymerase II within the nucleus, thereby preventing DNA transcription and subsequent protein synthesis. The result is acute submassive hepatic necrosis, which was present in the described horse.

Diagnosis and Treatment

Diagnosis in the described horse was based on histologic findings of submassive hepatic necrosis, acute onset of clinical signs, and presence of partially digested mushrooms in stomach contents. No specific therapy is recommended, although prompt evacuation of the gastrointestinal tract coupled with supportive care would seem prudent. The quantity of mushrooms ingested appears to be the primary prognostic indicator. If sufficient amounts of toxin are absorbed, the prognosis is grave. Hepatic coma often ensues in other species, and renal necrosis has been reported as well. Amatoxins can be tested for in various biologic samples (urine, kidney).

MISCELLANEOUS AGENTS

Iron

Iron toxicosis in horses is rare and can result from iatrogenic overdose of injected or oral products given to foals or from accidental consumption of iron-containing supplements. Supplemental iron is available in injectable and oral formulations, and more than 100 different iron preparations are on the market.[176] Iron dextran, iron polysaccharide, iron sorbitol, and ferric ammonium citrate are available injectable preparations. Oral formulations include iron salts such as ferrous sulfate, citrate, and ammonium citrate; ferrous sulfate, chloride, glutamate, lactate, fumarate, and carbonate; and ferric phosphate with sodium citrate. Chelated iron compounds are about one fourth as toxic as other compounds. Some forms of iron are more bioavailable than others.

The toxicity of iron is least by the oral route, with intramuscular and intravenous routes being increasingly more toxic. Because most animals do not have a mechanism for iron excretion, the toxicity of iron depends on the amount of iron already present in the body.

Clinical Signs

Two syndromes of iron toxicosis in animals are reported. A peracute syndrome is represented by sudden death a few minutes to hours after injection. This syndrome may resemble an anaphylactic reaction, but the triggering mechanism is unknown. A subacute reaction characterized by progressive depression, icterus, and disorientation leading to coma and death seems to be the more typical syndrome seen in horses.[176-178]

Pathophysiology

Experimentally, 5% to 10% of some forms of oral iron is absorbed in the small intestine, primarily from the duodenum and jejunum, by a rate-limited mucosal transfer system. The ferrous forms are absorbed to a greater extent than the ferric forms, but both can be absorbed if they are in the ionized state. Phosphates reduce the absorption of iron, and a high-sugar diet increases absorption. Once absorbed, iron is bound in serum by transferrin.[176]

Toxic doses of orally administered iron overwhelm the mechanism controlling absorption of iron from the intestine, resulting in massive iron absorption. Toxicity occurs when serum iron levels exceed the iron-binding capacity of transferrin. Free circulating iron then damages blood vessels, may cause erosion and ulceration of the stomach and intestine, causes hepatocellular necrosis and fatty degeneration of the myocardium, and can produce cerebral edema.[176,179] In horses hepatic failure appears to be the cause of death.[178]

At the cellular level, excessive iron causes extensive peroxidation of lipids in biologic membranes. A resulting decline in the ratio of unsaturated to saturated fatty acids leads to increased membrane rigidity, reduction of membrane potential, and increased permeability to various ions, leading to rupture of the membrane. The intracellular organelles adversely affected include the mitochondria and the lysosomal and sarcoplasmic membranes.[180] Elevated serum iron also inhibits the thrombin-induced conversion of fibrinogen to fibrin, thereby adversely affecting coagulation and enhancing any hemorrhagic process.[178] Histologically, affected livers are characterized by small size and have prominent bile duct proliferation, periportal fibrosis, and hepatocellular necrosis.[179]

Diagnosis

Measurement of serum iron concentration is the best method of confirming a diagnosis of iron toxicity.[176] In addition, a history of iron administration coupled with clinical signs and laboratory evidence of hepatocellular damage and cardiovascular collapse is highly suggestive of toxicity.

Abnormal laboratory findings include prolonged PTT and PT, high concentrations of aromatic amino acids (tyrosine, phenylalanine, tryptophan, methionine), elevated plasma ammonia concentration, and increased activities of the liver-derived enzymes alkaline phosphatase and GGT. In addition, a high ratio of aromatic to branched-chain amino acids and an elevated total serum bilirubin content can be found.[177]

Treatment

Treatment of peracute iron toxicosis usually is unrewarding. Once clinical signs become evident, major organ damage usually is present. No specific treatment for horses affected with iron toxicity is known. Treatment of affected dogs has included supportive therapy with glucose and norepinephrine and magnesium oxide given orally to help complex the ingested iron. Experimentally, a specific chelator of ferric iron, deferoxamine, has been used at 0.75 mg/kg/min intravenously to chelate the circulating iron in dogs. This drug should be administered slowly by intravenous drip because it can cause a sharp decline in blood pressure. Plasma extenders and intravenous fluids also have been used to counteract cardiovascular shock present in some dogs.[176]

In cases of human toxicosis, gastric lavage, chelating agents, and cathartics (sodium sulfate and magnesium sulfate) have been used. The use of oral bicarbonate solutions to decrease iron absorption is controversial, and activated charcoal is given orally to absorb the iron-deferoxamine complex, even though charcoal does not effectively bind free iron.[179]

The chelator of choice in human toxicosis is deferoxamine. Deferoxamine is administered intravenously for maximum effect; the drug has a plasma half-life of about 1 hour. Deferoxamine is detoxified by the liver, but the iron-deferoxamine complex is excreted by the kidneys.[179] There are other iron chelators used in humans (deferiprone and deferasirox), but their use has not been evaluated in horses.

Aflatoxin

Aflatoxicosis is a rarely documented disorder of horses, as demonstrated by the paucity of clinical cases reported in the literature.[181-183] In reported instances toxicosis developed in horses being fed contaminated feedstuffs, but horses also have been affected experimentally by forced feeding of aflatoxin-contaminated material.[184,185]

Aflatoxins are toxic metabolites primarily produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These molds are ubiquitous and normally can be found in stored feeds. The molds are not inherently toxigenic, but they can grow rapidly and produce large amounts of aflatoxin under environmental conditions of adequate temperature and humidity. Many feedstuffs can support the growth of these molds, but cereal grains, cottonseed meal and cake, and peanuts seem to be affected most commonly.[186]

These molds produce five major aflatoxins: B_1 and B_2, which fluoresce blue under long-wave ultraviolet light; G_1 and G_2, which fluoresce green; and M_1 aflatoxin, which is present in milk. B_1 is the most important of these because of its toxicity and it abundance under natural conditions.[186]

Aflatoxins are a group of polycyclic, unsaturated compounds that have a coumarin nucleus coupled to a reactive bifuran system and a pentenone or lactone. These toxins are insoluble in water and are heat resistant; they are absorbed rapidly from the gastrointestinal tract and bound to serum albumin. Most of the toxins are removed from the bloodstream in the liver, where aflatoxins bind to macromolecules such as DNA, endoplasmic steroid-binding sites, and certain enzymes within the hepatocytes. In the liver a variety of metabolites are produced at rates that vary among species. The metabolites may be lipid-soluble or water-soluble conjugates and are excreted in bile. At least some of the metabolites undergo an enterohepatic cycle of absorption and excretion. The aflatoxins and their metabolites are excreted in urine and feces, and complete elimination may take several days. The aflatoxins are not known to be stored in any particular tissue, other than perhaps liver and kidney.[186]

Acute and chronic aflatoxicoses are reported in a variety of animal species, including human beings. However, clinical reports of equine disease are concerned primarily with acute intoxication. The acute oral LD_{50} of aflatoxin B_1 in horses is reported to be 2 mg/kg or greater. One author reported signs of toxic hepatopathy and gastrointestinal upset in horses that consumed feed containing 2 to 50 ppb of aflatoxin B_1. Toxicity of aflatoxins also is reported to be enhanced by riboflavin; exposure to light; and a diet low in protein, choline, and vitamin B_{12}.[186]

Clinical Signs

Clinical signs associated with acute aflatoxicosis include anorexia, elevated temperature, increased heart and respiratory rates, ataxia, depression, lethargy, convulsions, icterus, colic and abdominal straining, bloody feces, and death. These signs were exhibited by horses experimentally intoxicated with aflatoxin at dosages of 2 to 5 mg/kg. Onset of signs began as early as 4 hours after dosing, and deaths occurred from 68 hours to 32 days after intoxication.[182,184,185] An additional sign observed in a naturally occurring instance of toxicosis was subcutaneous hemorrhages.[182]

The feed concentration of aflatoxin B_1 necessary to cause signs of chronic aflatoxicosis in horses has not been established, and reports of chronic aflatoxicosis were not found in the literature. Signs of chronic aflatoxicosis in other species have included reduced feed efficiency, rough hair coats, anemia, anorexia, depression, mild jaundice, and occasionally abortion.[186] It should be noted that experimental trials have indicated that some horses refuse mold-contaminated grain and well-fleshed animals may be at lesser risk of developing, aflatoxicosis than unthrifty animals.[182,184]

Pathophysiology

The hepatic cytotoxicity of aflatoxins is thought to be related to their binding to intracellular macromolecules. Aflatoxin B_1 binds to nuclear DNA to inhibit RNA synthesis, which subsequently inhibits synthesis of intracellular enzymes and other proteins. Aflatoxin also binds to endoplasmic steroidal ribosome-binding sites, resulting in ribosomal disaggregation. Metabolites of aflatoxin B_1 also can bind to cellular macromolecules, and most of the cytotoxicity of aflatoxin B_1 appears to result from binding of certain of these metabolites rather than from aflatoxin B_1 itself. This impairment of protein synthesis and the related ability to mobilize fats is thought to cause the early lesions of hepatic necrosis and fatty degeneration of the liver in aflatoxicosis.[176]

The possibility of other mechanisms being responsible for the other signs and lesions noted with aflatoxicosis has been suggested. Aflatoxins are known to be carcinogenic, they can be immunosuppressive, and they also can inhibit synthesis of clotting proteins. The mechanisms whereby these changes take place have not been well described.[186]

Diagnosis and Treatment

Definitive diagnosis of aflatoxicosis can be difficult because of the nonspecificity of many clinical signs and because the disease can mimic many other conditions. Experimentally intoxicated horses have elevations in serum concentrations of AST, alanine aminotransferase, GGT, iditol dehydrogenase, and arginase.[182,184,185] Other laboratory abnormalities include hypoglycemia, hyperlipidemia, lymphopenia, and elevated PT.[181,182,185]

Gross necropsy lesions typically consist of variable degrees of hepatic degeneration and necrosis, visceral petechial hemorrhages, and hemorrhagic enteritis.[181,182,185] Other lesions noted at necropsy include encephalomalacia of the cerebral hemispheres, myocardial degeneration, fatty infiltration of the kidney, and subcutaneous and intramuscular hemorrhage.[181,182] Histopathologic abnormalities may include fatty degeneration and necrosis of hepatocytes, bile duct hyperplasia, periportal fibrosis, and inflammatory cell infiltration into the liver. Renal lesions have included lipid accumulation in the tubular epithelial cells of the proximal tubules and protein precipitation in the tubular lumen.[181,185]

Although metabolites of *Aspergillus* species fluoresce under ultraviolet light, the presence of fluorescence in suspect feed samples is not pathognomonic for aflatoxin. Aflatoxin can be assayed definitively in feed samples by a variety of analytic methods.[182] It also is possible to quantitate aflatoxin in animal tissues, although this test is not routinely available through diagnostic laboratories.[181,185] Tissue residue testing can come up negative because of the long delay between time of exposure and demise of the patient. Mold culture is nondiagnostic.[186]

Treatment is largely symptomatic and must include removal of the offending feed material, if it still is being fed. An easily digested, low-fat diet containing appropriate protein has been recommended. Multiple vitamin supplementation might be beneficial, and treatment of specific organ dysfunction should be initiated. In acutely intoxicated horses orally administered charcoal has been recommended.[186] Prevention of the condition requires storing feeds in a suitable environment that discourages mold growth and the feeding of noncontaminated feedstuffs.

Toxicoses Causing Signs Relating to the Urinary System

Detection of renal disease can be difficult, regardless of cause. Whereas nephrotoxicity associated with equine administration of medications is well established and usually recognized, that linked to ingestion of plants is less commonly detected, which makes it more difficult to make a definitive diagnosis. Although heavy metals such as cadmium and mercury have long been known for their nephrotoxic effects, clinical signs are nonspecific, and the diagnosis will be made only if the clinician performs diagnostic tests to specifically rule these problems in or out.

PLANTS

Oxalate Toxicosis

The most common source of oxalates for livestock is plants, particularly those of the Chenopodiaceae family. These plants contain varied amounts of soluble oxalates, usually in the form of sodium or potassium salts. However, because plants containing oxalates (Box 21.3) are generally unpalatable to horses, plant-associated oxalate intoxication is rare.

Of the plants listed in Box 21.3, *Halogeton* and *Sarcobatus* seem to be the primary offenders in range animals in the western United States.[187]

Because oxalates accumulate in the plants throughout the growing season, the incidence of toxicosis may be highest in the fall and winter months.[188] The oxalate content is highest in the leaves, with a lesser amount in seeds and a minimal amount present in the stems. The nonfatal toxic dosage of sodium oxalate for adult horses is approximately 200 g/day for 8 days.[187]

Clinical Signs

Affected horses may begin to show signs of depression, mild to moderate colic, muscular weakness, and irregular gait 2 to 6 hours after ingestion. Weakness may proceed to lateral recumbency, unconsciousness, and death in 10 to 12 hours. Some animals may exhibit convulsions before succumbing to death.[187] The observed clinical signs of acute toxicity are typical of those of hypocalcemia.

Pathophysiology

Oxalates combine with serum calcium ions to form insoluble calcium oxalate. The result is a functional hypocalcemia in acute cases, with associated signs of altered behavior and neuromuscular abnormalities.

Acute and chronic ingestion of oxalates can result in renal failure. Insoluble calcium oxalate crystals can lodge in the renal tubules, producing tubular blockage and necrosis. Oxalates also may crystallize in the vasculature and infiltrate blood vessel walls, producing necrosis and hemorrhage.[187]

Diagnosis and Treatment

In instances of acute toxicosis, consistent clinicopathologic abnormalities include moderate to significant hypocalcemia and varied electrolyte alterations. Urinalysis may reveal the presence of characteristic calcium oxalate crystals on microscopic examination. Impending renal failure also is characterized by increases in BUN and creatinine concentrations.[187]

Treatment usually is of little value once clinical signs have appeared. Calcium gluconate can be administered intravenously, but usually it provides only temporary relief of signs. Balanced electrolyte solutions are indicated to aid diuresis, and diuretics also may have benefit in the volume-loaded patient. Prevention primarily entails keeping horses from the plants by providing adequate suitable sources of feed.[187]

Sorghum

Ingestion of *Sorghum* species and certain hybrid Sudan grasses has been associated with the development of an ataxia-cystitis syndrome.[189,190] This is, however, a very rare event. The toxicity occurs when horses graze the plants. More cases occur when the plant is young and rapidly growing, but mature and second-growth plants also have been incriminated. Horses being fed well-cured *Sorghum* species hay have not developed signs of toxicity. Occurrence of toxicity may increase during seasons of medium to high rainfall, but no cases have been recognized following the date of the first frost. Signs of toxicity may develop following a grazing period of 1 week to several months.[189]

BOX 21.3 Plants that Contain Large Amounts of Soluble Oxalates

Amaranthus spp.	Pigweed
Beta vulgaris	Beet, mangold
Chenopodium album	Lambsquarters
Halogeton glomeratus	Halogeton
Oxalis spp.	Wood sorrel, soursob
Portulaca oleracea	Purslane
Rheum rhaponticum	Rhubarb
Rumex spp.	Sorrel, dock
Salsola kali	Russian thistle
Sarcobatus vermiculatus	Black greasewood

Clinical Signs

The primary clinical signs are those of posterior ataxia and urinary incontinence or cystitis. The neurologic signs usually develop first and begin as posterior ataxia and incoordination. Affected horses may sway from side to side if forced to move, and signs tend to worsen on backing the animal. Occasionally, the rear quarters may drop almost to the ground, and flaccid paralysis of the tail and the rear legs may develop within 24 hours of the onset of neurologic signs. Affected horses remain alert and afebrile and have a normal appetite and pulse and respiratory rates. Mares frequently exhibit continual opening and closing of the vulva and relaxation of the perineal muscles. Males typically have a relaxed and extended penis.[189,190]

Urinary incontinence exhibited by continual urine dribbling is prominent in both sexes, and urine scalding on dependent skin becomes pronounced. The urinary bladder typically is distended and atonic, resulting in moderate to severe cystitis. Urethritis and ureteritis also may develop, and in horses that die because of the disease, ascending pyelonephritis usually is the cause of death. Other clinical signs include abortion and birth of foals with arthrogryposis.[189,190]

Pathophysiology

The clinical signs result from axonal degeneration and demyelination of nerve fibers in the spinal cord, particularly in the lumbar and sacral segments. The toxic substance in *Sorghum* species responsible for causing this change is not definitive. Most *Sorghum* species are cyanogenic plants and contain various amounts of HCN. Exposure to multiple sublethal doses of HCN has been suggested to induce axonal degeneration and demyelination.[189] Another hypothesis is that sorghum plants contain lathrogenic precursors and that this toxicosis may be caused by the ingestion of lathrogenic nitriles present in rapidly growing plants.[187,190]

Diagnosis and Treatment

Diagnosis of sorghum ataxia-cystitis is based primarily on appropriate clinical signs, history of grazing the plants, and exclusion of other known causes of posterior ataxia or paresis.

No specific diagnostic tests are available. Cystitis and pyelonephritis are diagnosed by standard laboratory methods.

No specific treatment is available. The offending feed should be removed immediately. Once the feed is removed, affected horses usually show gradual improvement over several weeks to months, but complete recovery may not occur. Supportive and symptomatic therapy should include appropriate antibiotic treatment of bacterial urinary tract infections and topical treatment of urine scald dermatitis. Periodic manual decompression of the urinary bladder may be helpful. Catheterization and frequent aspiration of bladder contents may be necessary to aid resolution of cystitis.

MISCELLANEOUS AGENTS

Vitamins D_2 and D_3

Horses are capable of meeting their requirement for vitamin D if they are exposed to sunlight or have access to sun-cured forages. Although dietary requirements for the horse have not been established, a maximum safe level of 44 IU/kg of body mass daily has been proposed for long-term feeding (more than 60 days).[191] Most cases of vitamin D intoxication are iatrogenic, resulting from overzealous use of vitamin supplements or improperly formulated vitamin D–supplemented feeds. Ingestion of *C. diurnum* (wild jasmine) also can result in vitamin D toxicosis because this plant contains a metabolically active glycoside of 1,25-dihydroxycholecalciferol (see the section on Wild Jasmine [*C. diurnum*] earlier in this chapter).

Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are potentially toxic, but vitamin D_3 is much more active and results in more severe lesions with wider tissue distribution than does an equivalent dose of vitamin D_2.[192,193] Other variables that may affect toxicosis include duration of treatment and route of administration. High concentrations of dietary calcium also might enhance the effects of excessive amounts of vitamin D. The effect of vitamin D supplementation is cumulative, and signs of toxicity may occur weeks after supplementation has begun.

Clinical Signs

The clinical signs associated with vitamin D toxicosis are associated with impairment of the renal, cardiovascular, and musculoskeletal systems. Signs may include depression, anorexia, weakness, polyuria and polydipsia, cardiac murmurs and tachycardia, limb stiffness with impaired mobility, and recumbency. Calcification of tendons, ligaments, and other soft tissue structures may be palpable on physical examination.[192] Ultrasonographic examination of these structures also may demonstrate abnormal mineralization within the tissues.

The toxicity of excessive amounts of vitamin D_3 results from extensive dystrophic mineralization rather than from any inherent toxicity of vitamin D itself. Soft tissue sites most frequently affected include the kidneys, the endocardium and walls of large blood vessels, and tendons and ligaments.[192,193]

Laboratory findings associated with toxicosis vary with the organ system affected but generally include persistent hypercalcemia and hyperphosphatemia, although the latter may not be especially pronounced. Serum calcium concentration may remain within a normal range in some horses. Other laboratory evidence of chronic renal failure may become evident with progression of toxicosis. A definitive diagnosis is made by measuring serum concentrations of vitamins D_2 and D_3 and 1,25-dihydroxycholecalciferol.[193]

Treatment

Treatment of vitamin D intoxication should include removal of all exogenous sources of vitamin D. A cation chelator such as sodium phytate may be helpful in reducing intestinal absorption of calcium, but the efficacy of this product has not been determined. If necessary, symptomatic therapy for renal insufficiency and failure should be instituted. Recovery may take months in less severely affected horses,[193] but treatment usually is unrewarding if excessive mineralization has occurred.

Menadione Sodium Bisulfite (Vitamin K_3)

Vitamin K_3 is a reported cause of acute renal failure in horses, but the product has been withdrawn from the U.S. market. When the product was given at the manufacturer's recommended dosage of 2.2 to 11 mg/kg intravenously or intramuscularly, signs of toxicity became evident 6 to 48 hours after injection in affected horses.[194] Clinical signs included depression, anorexia, colic, hematuria, and stranguria. Azotemia, electrolyte abnormalities, proteinuria, and isosthenuria also were apparent. Pathologic lesions noted at necropsy were those of acute tubular necrosis. Interstitial fibrosis and chronic renal failure also were present in one horse.[194] Treatment of affected horses is symptomatic for acute or chronic renal failure.

Cadmium

Intoxication with cadmium rarely is reported in horses but has been seen in animals raised near smelting operations.[195] Environmental contamination of soil and forage by cadmium and zinc was the cause of excessive intake.

Affected horses exhibited signs of unthriftiness, lameness, and swollen joints. Some of these signs were attributed to excess zinc in the diet, but the horses also had pronounced osteoporosis and nephrocalcinosis, which along with proteinuria are typical findings of cadmium toxicosis in human beings.[195]

Serum concentrations of zinc and potassium were elevated in these horses, and the serum magnesium concentration was low in one foal. Sodium, calcium, chloride, and bicarbonate concentrations also were decreased. Extensive nephrocalcinosis was characterized by multifocal loss of cortical tubules, which were replaced by dense deposits of calcium phosphate crystals.

Cadmium induces changes in proximal renal tubular cells by an unknown mechanism. However, increased numbers of lysosomes and mitochondrial swelling in the proximal tubular cells are early changes. Proteinuria usually is the first abnormality noted in human beings and laboratory animals. With continued chronic exposure, fibrosis and atrophy resulting from interstitial nephritis may ensue, leading to chronic renal failure.[196] Cadmium exposure can be confirmed by analyzing blood and various biologic tissues, including liver and kidney.

In human beings treatment essentially is supportive, with elimination of exposure to cadmium being imperative. Research data suggest a possible beneficial role of zinc, vitamin B complex, and nickel preparations, but their clinical efficacy is unproven.[196]

Mercury

Mercury exists in a variety of organic and inorganic forms. Both forms can be toxic to horses, but more recently reported cases involve acute toxicity resulting from inorganic mercury-containing blistering agents topically applied to skin.[197,198] Ingestion of feed or seed grain contaminated with organic mercurial seed preservatives has been a source of contamination in previous years.

Acute and chronic forms of toxicosis can occur in horses. The acute toxic dose of inorganic mercury in adults is 5 to 10 g.[187] Experimentally, chronic toxicity from inorganic mercury has been produced by ingestion of mercuric chloride, 0.8 mg/kg per day, over a 14-week period.[199] Chronic organic mercury toxicosis also has been produced experimentally by feeding methylmercury at 0.4 mg/kg per day for 10 weeks.[200]

Clinical Signs

Signs associated with toxicity of the various mercurial compounds differ, but they all include some degree of renal dysfunction. Acute toxicity resulting from inorganic mercury can cause signs of acute renal failure, including oliguria and depression, and signs of gastrointestinal irritation. Ulcerative stomatitis, excessive salivation, colic, and diarrhea are common findings associated with gastrointestinal tract disturbances.[197,198] Chronic intoxication with inorganic mercury can result in signs of oral ulceration, reduced appetite and weight loss, alopecia, progressive respiratory difficulty, gradually increasing urine production, and terminal azotemia.[199] Signs reported with chronic organic mercury toxicity include development of neurologic dysfunction characterized by proprioceptive deficits, exudative dermatitis, reluctance to move, reduced appetite and weight loss, dullness, and renal changes exhibited by a steadily increasing BUN concentration and glucosuria.[200]

Pathophysiology

Inorganic mercury compounds are absorbed from the lungs and gastrointestinal tract and absorbed poorly through the skin. After ingestion and absorption, accumulation in the liver and particularly the kidney occurs. Some forms of organic mercury are degraded in the body to inorganic forms, which then also accumulate in the kidney before excretion.[187]

Inorganic mercury is concentrated to high levels within the proximal renal tubular cells. Metallothionein, a low-molecular-weight metal-binding protein, is synthesized within 48 hours after exposure to heavy metals. This protein binds mercuric ions within the endoplasmic reticulum of the tubular epithelial cells and then slowly releases mercury. This slow release of sequestered mercury can cause continuing damage to tubular cells after the source of mercury is removed.[201] Hence the development of mercury nephropathy appears to be a function of the amount of protein-bound mercury concentrated in the renal tubules. Bound mercury can persist in the kidneys for several weeks after exposure.[188] Acute toxicity results in massive tubular necrosis and acute renal failure, and chronic exposure may cause renal interstitial fibrosis leading to chronic renal failure.

Methylmercury can be biotransformed in the body to inorganic mercury, but methylmercury also accumulates in the brain to a much greater extent than do other forms of mercury.[187] The exact mechanism whereby methylmercury and other alkylmercurials damage the nervous system is not well understood.[200]

At the cellular level mercury combines with sulfhydryl groups within cells. As a result, sulfhydryl enzyme systems essential to cellular metabolism and respiration are inhibited, resulting in cell death.

Diagnosis and Treatment

Mercury intoxication is suspected when horses show compatible clinical signs and have a history of exposure. Laboratory abnormalities are similar to those of other causes of acute or chronic renal failure and irritative gastrointestinal diseases. Definitive diagnosis usually is based on measurement of mercury concentrations in blood, kidney, and liver.[187] Stomach and intestine samples also may be submitted for analysis in acute cases.

Treatment of mercury intoxication initially involves removal of the source. In acute toxicity evacuation of the bowel with a mild laxative may be helpful. The oral administration of 500 g of activated charcoal might help block absorption of mercury, but its efficacy has not been demonstrated. Dimercaprol (used to inactivate circulating mercury) can be given at a dosage of 3 mg/kg intramuscularly every 4 hours for the first 2 days, 4 times on the third day, and twice daily for the next 10 days until recovery is complete.[197] The clinician also should follow other principles of therapy for acute or chronic renal failure. Treatment of chronic mercury intoxication usually is unrewarding.

REFERENCES

1. Anderson GA, Mount ME, Vrins AA, et al. Fatal acorn poisoning in a horse: pathologic findings and diagnostic considerations. *J Am Vet Med Assoc*. 1983;182:1105–1110.
2. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
3. Robinson NE, ed. *Current therapy in equine medicine*. 6th ed. St. Louis: Saunders; 2009.
4. McCunn J. Castor bean poisoning in horses. *Vet J*. 1945;101:136.
5. Rauber A, Heard J. Castor bean toxicity re-examined: a new perspective. *Vet Hum Toxicol*. 1985;27:498–502.
6. Albretsen JC, Gwaltney-Brant SM, Khan SA. Evaluation of castor bean toxicosis in dogs: 98 cases. *J Am Anim Hosp Assoc*. 2000;36:229–233.
7. Gilman AG, Rall TW, Nies AS, et al. *Goodman and Gilman's the pharmacological basis of therapeutics*. 8th ed. Elmsford, NY: Pergamon Press; 1990.
8. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
9. Hart CR, Garland T, Barr AC, et al. *Toxic plants of Texas*. College Station: Texas Agricultural Extension Service; Texas A&M University System; 2001.
10. Knight AP, Walter RG. *A guide to plant poisoning of animals in North America*. Jackson, WY: Teton NewMedia; 2001.
11. Garland T, Barr AC, eds. *Toxic plants and other natural toxicants*. New York: CAB International; 1998.
12. Verdes JM, Marquez M, Calliari A, et al. A novel pathogenic mechanism for cerebellar lesions produced by *Solanum bonariense* in cattle. *JVDI*. 2015;27(3):278–286.
13. Schulman ML, Bolton LA. Datura seed intoxication in two horses. *J S Afr Vet Assoc*. 1998;69:27–29.
14. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
15. Moffatt RE, Kramer LL, Lerner D, et al. Studies on dioctyl sodium sulfosuccinate toxicity: clinical, gross and microscopic pathology in the horse and guinea pig. *Can J Comp Med*. 1975;39:434–441.
16. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
17. Pace LW, Turnquist SE, Casteel SW, et al. Acute arsenic toxicosis in five horses. *Vet Pathol*. 1997;34:160–164.
18. Fogarty U, Perl D, Good BS, et al. A cluster of equine granulomatous enteritis cases: the link with aluminum. *Vet Hum Toxicol*. 1998;40(5):297–305.
19. Garcia-Patos V, Pujol RM, Alomar A, et al. Persistent subcutaneous nodules in patients hypersensitized with aluminum-containing allergen extracts. *Arch Dermatol*. 1995;131:1421–1424.

20. Sockett DC, Baker JC, Stowe CM. Slaframine *(Rhizoctonia leguminicola)* intoxication in horses. *J Am Vet Med Assoc*. 1982;181:606.
21. Exon JH. A review of chlorinated phenols. *Vet Hum Toxicol*. 1984;26:508–520.
22. Russel SH, Monin T, Edwards WC. Rodenticide toxicosis in a horse. *J Am Vet Med Assoc*. 1978;172:270–271.
23. Peoples SA, Maddy KT. Poisoning of man and animals due to ingestion of the rodent poison, vacor. *Vet Hum Toxicol*. 1979;21:266–268.
24. Kimbrough RD, Carter CD, Liddle JA, et al. Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. *Arch Environ Health*. 1977;32:77–86.
25. Schmitz DG. Cantharidin toxicosis in horses. *J Vet Intern Med*. 1989;3:208–215.
26. Schoeb TR, Panciera RJ. Blister beetle poisoning in horses. *J Am Vet Med Assoc*. 1978;173:75–77.
27. Ray AC, Kyle ALG, Murphy MJ, et al. Etiologic agents, incidence, and improved diagnostic methods of cantharidin toxicosis in horses. *Am J Vet Res*. 1989;50:187–191.
28. James LF, Hartley WJ, Van Kampen KR. Syndromes of *Astragalus* poisoning in livestock. *J Am Vet Med Assoc*. 1981;178: 146–150.
29. Knight AP. Locoweed poisoning. *Compend Cont Educ Pract Vet*. 1987;9:F418.
30. McIlwraith CW, James LF. Limb deformities in foals associated with ingestion of locoweed by mares. *J Am Vet Med Assoc*. 1982;181:255–258.
31. Burfening PJ. Ergotism. *J Am Vet Med Assoc*. 1973;163: 1288–1290.
32. Hintz HF. Ergotism. *Equine Pract*. 1988;10:6.
33. Wyllie TD, ed. *Mycotoxic fungi, mycotoxins, mycotoxicoses*. New York: LG Moorehouse; 1978.
34. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
35. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
36. Zin LL, Edwards WC. Toxicity of blue-green algae in livestock. *Bovine Pract*. 1979;14:151.
37. Steyn PS, Vleggaar R, eds. *Mycotoxins and phycotoxins: a collection of invited papers presented at the sixth International IUPAC Symposium on Mycotoxins and Phycotoxins*. Amsterdam: Elsevier Science; 1986.
38. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
39. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
40. Lilley CW. Strychnine poisoning in a horse. *Equine Pract*. 1985;7:7.
41. Sutherland C. Metaldehyde poisoning in horses. *Vet Rec*. 1983;112:64–65.
42. Harris WF. Metaldehyde poisoning in three horses. *Mod Vet Pract*. 1975;56:336–337.
43. Edwards HG. Methiocarb poisoning in a horse. *Vet Rec*. 1986;119:556.
44. Alexander KA. Methiocarb poisoning in a horse. *Vet Rec*. 1987;120:47.
45. Ray AC, Dwyer JN, Fambro GW, et al. Clinical signs and chemical confirmation of 4-aminopyridine poisoning in horses. *Am J Vet Res*. 1978;39:329–331.
46. Kitzman JV, Wilson RC, Hatch RC, et al. Antagonism of xylazine and ketamine anesthesia by 4-aminopyridine and yohimbine in geldings. *Am J Vet Res*. 1984;45:875–879.
47. Drudge JH, Lyons ET, Swerczek TW. Critical tests and safety studies on a levamisole-piperazine mixture as an anthelmintic in the horse. *Am J Vet Res*. 1974;35:67–72.
48. DiPietro JA, Todd KS. Anthelmintics used in treatment of parasitic infections of horses. *Vet Clin North Am Equine Pract*. 1987;3:1–14.
49. Marriner S. Anthelmintic drugs. *Vet Rec*. 1986;118:181–184.
50. Glenn MW, Burr WM. Toxicity of a piperazine-carbon disulfide-phenothiazine preparation in the horse. *J Am Vet Med Assoc*. 1972;160:988–992.
51. Morris DD, Henry MM. Hepatic encephalopathy. *Compend Cont Educ Pract Vet*. 1991;13:1153.
52. Robinson NE, ed. *Current therapy in equine medicine*. 6th ed. St. Louis: Saunders; 2009.
53. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Saunders; 2009.
54. Fleischmann G, Rudiger H. Isolation, resolution and partial characterization of two *Robinia pseudoacacia* seed lectins. *Biol Chem Hoppe Seyler*. 1986;367:27–32.
55. Kelleway RA, Geovjian L. Acute bracken fern poisoning in a 14-month-old horse. *Vet Med Small Anim Clin*. 1978;73: 295–296.
56. Hintz HF. Bracken fern. *Equine Pract*. 1990;12:6.
57. Sperry OE, Dollahite JW, Hoffman GO, et al: Texas plants poisonous to livestock, Texas Agricultural Experiment Station Pub No. B-1028, College Station, TX, Agricultural Extension Service.
58. Farrell RK, Sande RD, Lincoln SD. Nigropallidal encephalomalacia in a horse. *J Am Vet Med Assoc*. 1971;158:1201–1204.
59. Young S, Brown WW, Klinger B. Nigropallidal encephalomalacia in horses caused by ingestion of weeds of the genus *Centaurea*. *J Am Vet Med Assoc*. 1970;157:1602–1605.
60. Mettler FA, Stern GM. Observations on the toxic effects of yellow star thistle. *J Neuropathol Exp Neurol*. 1963;22:164–169.
61. Cordy DR. Nigropallidal encephalomalacia in horses associated with ingestion of yellow star thistle. *J Neuropathol Exp Neurol*. 1954;13:330–342.
62. Stevens KL, Wong RY. Structure of chlororepdiolide, a new sesquiterpene lactone from *Centaurea repens. J Nat Prod*. 1986;49:833.
63. Sanders SG, Tucker RL, Bagley RS, et al. Magnetic resonance imaging features of equine nigropallidal encephalomalacia. *Vet Radiol Ultrasound*. 2001;42:291–296.
64. James LF, Hartley WJ, Van Kampen KR. Syndromes of *Astragalus* poisoning in livestock. *J Am Vet Med Assoc*. 1981;178:146–150.
65. Dorman DC, Haschek WM. Fatal propylene glycol toxicosis in a horse. *J Am Vet Med Assoc*. 1991;198:1643–1644.
66. Myers VS, Usenik EA. Propylene glycol intoxication of horses. *J Am Vet Med Assoc*. 1841;155:1969.
67. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
68. Osweiler GD. Toxicology of triclopyr herbicide in the equine. *Proc Am Assoc Vet Lab Diagn*. 1983;26:193.
69. Whisenant SG, McArthur ED. Triclopyr persistence in northern Idaho forest vegetation. *Bull Environ Contam Toxicol*. 1989;42:660.
70. Buck WB, Haliburton JC, Thilsted JP, et al. Equine encephalomalacia: comparative pathology of naturally occurring and experimental cases. *Proc Am Assoc Vet Lab Diagn*. 1979;22:239.
71. McCue PM. Equine leukoencephalomalacia. *Compend Cont Educ Pract Vet*. 1989;11:646.
72. Marasas WFO, Kellerman TS, Gelderblom WCA, et al. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme. Onderstepoort J Vet Res*. 1988;55:197–203.
73. Wilson TM, Ross PF, Rice LG, et al. Fumonisin B1 levels associated with an epizootic of equine leukoencephalomalacia. *J Vet Diagn Invest*. 1990;2:213–216.
74. Ross PF, Rice LG, Reagor JC, et al. Fumonisin B1 concentrations in feeds from 45 confirmed equine leukoencephalomalacia cases. *J Vet Diagn Invest*. 1991;3:238–241.
75. Brownie CF, Cullen J. Characterization of experimentally induced equine leukoencephalomalacia (ELEM) in ponies *(Equus caballus)*: preliminary report. *Vet Hum Toxicol*. 1987;29:34–38.

76. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
77. Gabal MA, Awad YL, Morcos MB, et al. Fusariotoxicoses of farm animals and mycotoxic leucoencephalomalacia of the equine associated with the finding of trichothecenes in feedstuffs. *Vet Hum Toxicol*. 1986;28:207–212.
78. Burrows GE. Lead poisoning in the horse. *Equine Pract*. 1982;4:30.
79. Burrows GE, Borchard RE. Experimental lead toxicosis in ponies: comparison of the effects of smelter effluent–contaminated hay and lead acetate. *Am J Vet Res*. 1982;43:2129–2133.
80. Kowalczyk DF, Naylor JM, Gunson D. The value of zinc protoporphyrin in equine lead poisoning: a case report. *Vet Hum Toxicol*. 1981;23:12–15.
81. Knight AP. Oleander poisoning. *Compend Cont Educ Pract Vet*. 1988;10:262.
82. Oleander poisoning in equines. *J R Army Vet Corps*. 1971;42:8.
83. Robinson NE, ed. *Current therapy in equine medicine*. 6th ed. St. Louis: Saunders; 2009.
84. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
85. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
86. Olson CT, Keller WC, Gerken DF, et al. Suspected tremetol poisoning in horses. *J Am Vet Med Assoc*. 1984;185:1001.
87. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
88. Smetzer DL, Coppock RW, Ely RW, et al. Cardiac effects of white snakeroot intoxication in horses. *Equine Pract*. 1983;5:26.
89. Tennant B, Dill SG, Glickman LT, et al. Acute hemolytic anemia, methemoglobinemia, and Heinz body formation associated with ingestion of red maple leaves by horses. *J Am Vet Med Assoc*. 1981;179:143–150.
90. Divers TJ, George LW, George JW. Hemolytic anemia in horses after the ingestion of red maple leaves. *J Am Vet Med Assoc*. 1982;180:300–302.
91. George LW, Divers TJ, Mahaffey EA, et al. Heinz body anemia and methemoglobinemia in ponies given red maple *(Acer rubrum L.)* leaves. *Vet Pathol*. 1982;19:521–523.
92. Plumlee KH. Red maple toxicity in a horse. *Vet Hum Toxicol*. 1991;33:66–67.
93. Weber M, Miller RE. Presumptive red maple *(Acer rubrum)* toxicosis in Grevy's zebra *(Equus greyvi)*. *J Zoo Wildl Med*. 1997;28:105–108.
94. Corriher CA, Parviainen AKJ, Gibbons DS, et al. Equine red maple leaf toxicosis. *Compend Cont Educ Pract Vet*. 1999;21:74–80.

94a. Alward A, Corriher CA, Barton MH, et al. Red maple (*Acer rubrum*) leaf toxicosis in horses: a retrospective study of 32 cases. *J Vet Intern Med*. 2006;20:11974–1201.

94b. Agrawal K, Ebeler JG, Altier C, et al. Identification of protoxins and a microbial basis for red maple (*Acer rubrum*) toxicosis in equines. *J Vet Diagn Invest*. 2013;25:112–119.

95. Pierce KR, Joyce JR, England RB, et al. Acute hemolytic anemia caused by wild onion poisoning in horse. *J Am Vet Med Assoc*. 1972;160:323–327.
96. Hutchison TWS. Onion toxicosis. *J Am Vet Med Assoc*. 1978;172:1440.
97. Burrows GE, Tyrl RJ. *Toxic plants of North America*. 2nd ed. Ames, IA: John Wiley & Sons; 2013.
98. Sula MJM, Morgan S, Bailey KL, et al. Characterization of cardiac lesions in calves after ingestion of Japanese yew *(Taxus cuspidate)*. *JVDI*. 2013;25(4):522–526.
99. Burcham GN, Becker KJ, Tahara JM, et al. Myocardial fibrosis associated with previous ingestion of yew (*Taxus* sp.) in a Holstein heifer: evidence for chronic yew toxicity in cattle. *JVDI*. 2013;25(1):147–152.
100. Vrins A, Carlson G, Feldman B. Warfarin: a review with emphasis on its use in the horse. *Can Vet J*. 1983;24:211–213.
101. Scott EA, Byars TD, Lamar AM. Warfarin anticoagulation in the horse. *J Am Vet Med Assoc*. 1980;177:1146–1151.
102. McConnico RS, Copedge K, Bischoff KL. Brodifacoum toxicosis in two horses. *J Am Vet Med Assoc*. 1997;211:882–886.
103. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
104. Boermans HJ, Johnstone I, Black WD, et al. Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. *Can J Vet Res*. 1991:21–27.
105. Thijssen HHW, van den Bogaard AEJM, Wetzel JM, et al. Warfarin pharmacokinetics in the horse. *Am J Vet Res*. 1983;44:1192–1196.
106. Byars TD, Greene CE, Kemp DT. Antidotal effect of vitamin K1 against warfarin-induced anticoagulation in horses. *Am J Vet Res*. 1986;47:2309–2312.
107. Mount ME, Feldman BF, Buffington T. Vitamin K and its therapeutic importance. *J Am Vet Med Assoc*. 1982;180:1354–1356.
108. Alsup EM, DeBowes RM. Dimethyl sulfoxide. *J Am Vet Med Assoc*. 1984;185:1011–1014.
109. Blythe LL, Craig AM, Appell LH, et al. Intravenous use of dimethyl sulfoxide (DMSO) in horses: clinical and physiologic effects. *Proc Am Assoc Equine Pract*. 1986;32:441–446.
110. Brayton CF. Dimethyl sulfoxide (DMSO): a review. *Cornell Vet*. 1986;76:61–90.
111. Blythe LL, Craig AM, Christensen JM, et al. Pharmacokinetic disposition of dimethyl sulfoxide administered intravenously to horses. *Am J Vet Res*. 1986;47:1739–1743.
112. Schuh JCL, Ross C, Meschter C. Concurrent mercuric blister and dimethyl sulfoxide (DMSO) application as a cause of mercury toxicity in two horses. *Equine Vet J*. 1988;20:68–71.
113. Hartsfield SM, Thurmon JC, Benson GJ. Sodium bicarbonate and bicarbonate precursors for treatment of metabolic acidosis. *J Am Vet Med Assoc*. 1981;179:914–916.
114. Lawrence L, Kline K, Miller-Graber P, et al. Effect of sodium bicarbonate on racing Standardbreds. *J Anim Sci*. 1990;68:673.
115. Freestone JF, Carlson GP, Harrold DR, et al. Furosemide and sodium bicarbonate–induced alkalosis in the horse and response to oral KCl or NaCl therapy. *Am J Vet Res*. 1989;50:1334–1339.
116. Posner JB, Swanson AG, Plum F. Acid-base balance in cerebrospinal fluid. *Arch Neurol*. 1965;12:479–496.
117. Rumbaugh GE, Carlson GP, Harrold D. Clinicopathologic effects of rapid infusion of 5% sodium bicarbonate in 5% dextrose in the horse. *J Am Vet Med Assoc*. 1981;178:267–271.
118. Amend JF, Mallon FM, Wren WB, et al. Equine monensin toxicosis: some experimental clinicopathologic observations. *Compend Cont Educ Pract Vet*. 1980;11:S173.
119. Matsuoka T. Evaluation of monensin toxicity in the horse. *J Am Vet Med Assoc*. 1976;169:1098–1100.
120. Blomme EAG, La Perle KMD, Wilkins PA, et al. Ionophore toxicity in horses. *Equine Vet Educ*. 1999;11:153–158.
121. Bila CG, Perreira CL, Gruys E. Accidental monensin toxicosis in horses in Mozambique. *J S Afr Vet Assoc*. 2001;72:163–164.
122. Mollenhauer HH, Rowe LD, Cysewski SJ, et al. Ultrastructural observations in ponies after treatment with monensin. *Am J Vet Res*. 1981;42:35–40.
123. Hanson LJ, Eisenbeis HG, Givens SV. Toxic effects of lasalocid in horses. *Am J Vet Res*. 1981;42:456–461.
124. Rollinson J, Taylor FGR, Chesney J. Salinomycin poisoning in horses. *Vet Rec*. 1987;121:126–128.
125. Schneider NR, Yeary RA. Nitrite and nitrate pharmacokinetics in the dog, sheep, and pony. *Am J Vet Res*. 1975;36:941–947.
126. Kingsbury JM. *Poisonous plants of the United States and Canada*. Englewood Cliffs, NJ: Prentice-Hall; 1964.
127. Ralston SL, Rich VA. Black walnut toxicosis in horses. *J Am Vet Med Assoc*. 1983;183:1095.
128. Uhlinger C. Black walnut toxicosis in ten horses. *J Am Vet Med Assoc*. 1989;195:343–344.
129. Minnick PD, Brown CM, Braselton WE, et al. The induction of equine laminitis with an aqueous extract of the heartwood of black walnut *(Juglans nigra)*. *Vet Hum Toxicol*. 1987;29:230–233.

130. Galey FD, Beasley VR, Schaeffer D, et al. Effect of an aqueous extract of black walnut *(Juglans nigra)* on isolated equine digital vessels. *Am J Vet Res*. 1990;51:83–88.
131. Krook L, Wasserman RH, Shively JN, et al. Hypercalcemia and calcinosis in Florida horses: implication of the shrub, *Cestrum diurnum*, as the causative agent. *Cornell Vet*. 1975;65:26–56.
132. Keeler RF, Van Kampen KR, James LF, eds. *Effects of poisonous plants on livestock*. New York: Academic Press; 1978.
133. Hughes MR, McCain TA, Chang SY, et al. Presence of 1,25-dihydroxy-vitamin D3-glycoside in the calcinogenic plant *Cestrum diurnum*. *Nature*. 1977;268:347–349.
134. Anderson CA, Divers TJ. Systemic granulomatous inflammation in a horse grazing hairy vetch. *J Am Vet Med Assoc*. 1983;183:569–570.
135. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
136. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
137. Kingsbury JM. *Poisonous plants of the United States and Canada*. Englewood Cliffs, NJ: Prentice-Hall; 1964.
138. Johnson AE. Toxicologic aspects of photosensitization in livestock. *J Natl Cancer Inst*. 1982;69:253–258.
139. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
140. Glenn AE, Bacon CW, Price R, et al. Molecular phylogeny of *Acromonium* and its taxonomic implications. *Mycologia*. 1996;88:369–383.
141. Putnam MR, Bransby DI, Schumacher J, et al. Effects of the fungal endophyte *Acremonium coenophialum* in fescue on pregnant mares and foal viability. *Am J Vet Res*. 1991;52:2071–2074.
142. Blodgett DJ. Fescue toxicosis. *Vet Clin North Am Equine Pract*. 2001;17(3):567–577.
143. Poppenga RH, Mostrom MS, Hascheck WM, et al. Mare agalactia, placental thickening, and high foal mortality associated with the grazing of tall fescue: a case report. In Proceedings of the twenty-seventh annual meeting of the American Association of Veterinary Laboratory Diagnosticians. *Fort Worth, TX*; 1984: Texas, pp 325–336.
144. Boosinger TM, Brendemuehl JP, Bransby DL, et al. Prolonged gestation, decreased triiodothyronine concentration, and thyroid gland histomorphologic features in newborn foals of mares grazing *Acremonium coenophialum*–infected fescue. *Am J Vet Res*. 1995;56:66–69.
145. Ellison SP. Possible toxicity caused by hoary alyssum *(Berteroa incana)*. *Vet Med*. 1992;87(5):472–475.
146. Goer RJ, Becker RL, Kanara EW, et al. Toxicosis in horses after ingestion of hoary alyssum. *J Am Vet Med Assoc*. 1992;201(1): 63–67.
147. Hovda LR, Rose ML. Hoary alyssum *(Berteroa incana)* toxicity in a herd of broodmare horses. *Vet Hum Toxicol*. 1993;35(1): 39–40.
148. Kanara EW, Murphy MJ. Ingestion of hoary alyssum as a cause of laminitis in horses, In Proceedings of the thirteenth annual meeting of the American College of Veterinary Internal Medicine. Lake Buena Vista, FL; 1995:571–573.
149. Stowe CM. Iodine, iodides, and iodism. *J Am Vet Med Assoc*. 1981;179:334–336.
150. Fadok VA, Wild S. Suspected cutaneous iodism in a horse. *J Am Vet Med Assoc*. 1983;183:1104–1106.
151. Schwink AL. Toxicology of ethylenediamine dihydriodide. *J Am Vet Med Assoc*. 1981;178:996–997.
152. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
153. Dickinson CE, Traub-Gargatz JL, Dargatz DA, et al. Rattlesnake venom poisoning in horses: 32 cases (1973–1993). *J Am Vet Med Assoc*. 1996;208:1866–1871.
154. Stewart KA, Genetzky RM. Odontodysplasia in a horse. *Mod Vet Pract*. 1984;65:87.
155. Gunson DE, Kowalczyk DF, Shoop CR, et al. Environmental zinc and cadmium pollution associated with generalized osteochondrosis, osteoporosis, and nephrocalcinosis in horses. *J Am Vet Med Assoc*. 1982;180:295–299.
156. Messer NT. Tibiotarsal effusioin associated with chronic zinc intoxication in three horses. *J Am Vet Med Assoc*. 1981;178: 294–297.
157. Willoughby RA, MacDonald E, McSherry BJ, et al. Lead and zinc poisoning and the interaction between Pb and Zn poisoning in the foal. *Can J Comp Med*. 1972;36:348–359.
158. Bridges CH, Moffitt PG. Influence of variable content of dietary zinc on copper metabolism of weanling foals. *Am J Vet Res*. 1990;51:275–280.
159. Ringenberg QS, Doll DC, Patterson WP, et al. Hematologic effects of heavy metal poisoning. *South Med J*. 1988;81: 1132–1139.
160. Ruckebusch Y, Toutain PL, Koritz GD, eds. *Veterinary pharmacology and toxicology*. Lancaster, England: MTP Press; 1983.
161. Hultine JD, Mount ME, Easley KJ, et al. Selenium toxicosis in the horse. *Equine Pract*. 1979;1:57.
162. Crinion RAP, O'Connor JP. Selenium intoxication in horses. *Ir Vet J*. 1978;32:81.
163. Traub-Dargatz JL, Knight AP, Hamar DW. Selenium toxicity in horses. *Compend Cont Educ Pract Vet*. 1986;8:771.
164. Dewes HF, Lowe MD. Suspected selenium poisoning in a horse. *N Z Vet J*. 1987;35:53–54.
165. Stowe HD. Effects of copper pretreatment upon the toxicity of selenium in ponies. *Am J Vet Res*. 1980;41:1925–1928.
166. James LF, Van Kampen KV, Hartley WJ. *Astragalus fisulcatus:* a cause of selenium or locoweed poisoning. *Vet Hum Toxicol*. 1983;25:86–89.
167. Painter EP. The chemistry and toxicity of selenium compounds, with special reference to the selenium problem. *Chem Rev*. 1941;28:179.
168. Sellers EA, Vou RW, Lucas CC. Lipotropic agents in liver damage produced by selenium or carbon tetrachloride. *Proc Soc Exp Biol Med*. 1950;75:118–121.
169. Smith BP, ed. *Large animal internal medicine*. 4th ed. St. Louis: Mosby; 2009.
170. Knight AP, Kimberling CV, Stermitz FR, et al. *Cynoglossum officinale* (hound's-tongue): a cause of pyrrolizidine alkaloid poisoning in horses. *J Am Vet Med Assoc*. 1984;185:647–650.
171. Giles CJ. Outbreak of ragwort *(Senecio jacobea)* poisoning in horses. *Equine Vet J*. 1983;15:248–250.
172. Pearson EG. Liver failure attributable to pyrrolizidine alkaloid toxicosis and associated with inspiratory dyspnea in ponies: three cases (1982–1988). *J Am Vet Med Assoc*. 1991;198: 1651–1654.
173. Lessard P, Wilson WD, Olander HJ, et al. Clinicopathologic study of horses surviving pyrrolizidine alkaloid *(Senecio vulgaris)* toxicosis. *Am J Vet Res*. 1986;47:1776–1780.
174. Gulick BA, Liu IKM, Qualls CW, et al. Effect of pyrrolizidine alkaloid-induced hepatic disease on plasma amino acid patterns in the horse. *Am J Vet Res*. 1980;41:1894–1898.
175. Frazier K, Liggett A, Hines M, et al. Mushroom toxicity in a horse with meningioangiomatosis. *Vet Hum Toxicol*. 2000;42(3): 166–167.
176. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
177. Divers TJ, Warner A, Vaala WE, et al. Toxic hepatic failure in newborn foals. *J Am Vet Med Assoc*. 1983;183:1407.
178. Arnbjerg J. Poisoning in animals due to oral application of iron with description of a case in a horse. *Nord Vet Med*. 1981;33:71.
179. Ellenhorn MJ, Schonwald S, Ordog G, et al. *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1997.

180. Hershko C. Mechanism of iron toxicity and its possible role in red cell membrane damage. *Semin Hematol*. 1989;26:277–285.
181. Angsubhakorn S, Poomvises P, Romruen K, et al. Aflatoxicosis in horses. *J Am Vet Med Assoc*. 1981;178:274–278.
182. Asquith RL, Edds GT. Investigations in equine aflatoxicosis. *Proc Am Assoc Equine Pract*. 1980;26:193–200.
183. Greene HJ, Oehme FW. A possible case of equine aflatoxicosis. *Vet Toxicol*. 1975;17:76.
184. Aller WW, Edds GT, Asquith RL. Effects of aflatoxins in young ponies. *Am J Vet Res*. 1981;42:2162–2164.
185. Bortell R, Asquith RL, Edds GT, et al. Acute experimentally induced aflatoxicosis in the weanling pony. *Am J Vet Res*. 1983;44:2110–2114.
186. Riviere JE, Papich MG, eds. *Veterinary pharmacology and therapeutics*. 9th ed. Philadelphia: Wiley-Blackwell; 2010.
187. Osweiler GD, Carson TL, Buck WB, et al. *Clinical and diagnostic veterinary toxicology*. 3rd ed. Dubuque, IA: Kendall/Hunt; 1985.
188. Hulbert LC, Oehme FW. *Plants poisonous to livestock*. 3rd ed. Manhattan, KS: Kansas State University; 1968.
189. Adams LG, Dollahite JW, Romane WM, et al. Cystitis and ataxia associated with sorghum ingestion by horses. *J Am Vet Med Assoc*. 1969;155:518–524.
190. Van Kampen KR. Sudan grass and sorghum poisoning of horses: a possible lathyrogenic disease. *J Am Vet Med Assoc*. 1970;156:629–630.
191. *Nutrient requirements of horses*. Washington, DC: National Academy of Sciences; 1989.
192. Harrington DD. Acute vitamin D2 (ergocalciferol) toxicosis in horses: case report and experimental studies. *J Am Vet Med Assoc*. 1982;180:867–873.
193. Harrington DD, Page EH. Acute vitamin D3 toxicosis in horses: case reports and experimental studies of the comparative toxicity of vitamins D2 and D3. *J Am Vet Med Assoc*. 1983;182:1358–1369.
194. Rebhun WC, Tennant BC, Dill SG, et al. Vitamin K3–induced renal toxicosis in the horse. *J Am Vet Med Assoc*. 1984;184:1237–1239.
195. Gunson DE, Kowalczyk DF, Shoop CR, et al. Environmental zinc and cadmium pollution associated with generalized osteochondrosis, osteoporosis, and nephrocalcinosis in horses. *J Am Vet Med Assoc*. 1982;180:295–299.
196. Roxe DM, Krumlovsky FA. Toxic interstitial nephropathy from metals, metabolites, and radiation. *Semin Nephrol*. 1988;8:72–81.
197. Schuh JCL, Ross C, Meschter C. Concurrent mercuric blister and dimethyl sulfoxide (DMSO) application as a cause of mercury toxicity in two horses. *Equine Vet J*. 1988;20:68–71.
198. Markel MD, Dyer RM, Hattel AL. Acute renal failure associated with application of a mercuric blister in a horse. *J Am Vet Med Assoc*. 1984;185:92–94.
199. Roberts MC, Seawright AA, Ng JC, et al. Some effects of chronic mercuric chloride intoxication on renal function in a horse. *Vet Hum Toxicol*. 1982;24:415–420.
200. Seawright AA, Roberts MC, Costigan P. Chronic methylmercurialism in a horse. *Vet Hum Toxicol*. 1978;20:6.
201. Massry SG, Glassock RJ, eds. *Massry and Glassock's textbook of nephrology*. 4th ed. Baltimore: Lippincott Williams & Wilkins; 2001.

APPENDIX I

Aspects of Clinical Relevance in Donkeys

Francisco J. Mendoza, Ramiro E. Toribio, and Alejandro Perez-Ecija

The donkey belongs to the order Perissodactyla, suborder Hippomorpha, family Equidae, and genus *Equus*. In this genus there is the horse and Przewalski's horse (*E. ferus caballus* and *E. ferus przewalskii*); the donkey or ass *(E. africanus asinus);* the onager or Asiatic wild ass *(E. hemionus onager);* the plains, common, or Burchell's zebra (*E. quagga,* formerly *E. burchellii*); the mountain and Hartmann's zebras (*E. zebra zebra* and *E. zebra hartmannae*); and the imperial or Grévy's zebra *(E. grevyi)*.[1] Donkeys and asses have 31 chromosomes (2n = 62), horses have 32 chromosomes (2n = 64), zebras have a variable number of chromosomes depending on the species (2n = 32 to 2n = 46), and mules have 63 chromosomes. Approximately 200 breeds of donkeys and asses are listed in the Food and Agricultural Organization Domestic Animal Diversity Information System (http://dad.fao.org/).

Donkeys and alike, including wild assess were domesticated before the horse and have contributed to social, economic, and cultural advances; religious customs; and human geographic expansion. Donkeys often live longer than horses, and their size, calm and stoic behavior, thriftiness, ability to perform hard work, and resistance to diseases made the donkey the preferred species for pack and draft work.[2,3]

In addition to physical work, donkeys are used for leisure and touristic activities; are a source of food (meat and milk) in some countries; their byproducts are used in the cosmetic industry; and more recently, donkey-assisted therapy is being used for people with mental and physical disabilities.[4] Because of its claimed hypoallergenic properties, excellent palatability, and nutritional value, donkey milk has been proposed to be a good breast milk substitute for infants with allergic disorders.[5]

Asinotherapy (onotherapy) is gaining popularity in treating and supporting people with mental and emotional disorders (Alzheimer's disease, autism, attention deficit hyperactivity disorder, and Down syndrome) and physical disabilities as well as in geriatric medicine as a therapeutic tool to improve physical and mental health and quality of life.[6] Behavioral tests to select donkeys for this purpose have been developed.[7]

Because interest in donkeys by the general public is increasing, it is important to generate donkey-specific information to provide better veterinary care. When facing the diagnosis and treatment of pathologic conditions of donkeys and asses, it should be considered that there are anatomic, physiologic, and metabolic differences from horses. From this perspective, extrapolating data for diagnostic and therapeutic purposes could be fine in most instances, but in others it may lead to misdiagnosis and unnecessary or inadequate treatment.

DISORDERS

Cardiovascular System

When their electrocardiogram is compared to horses', donkeys display shorter P waves (lead II), pronounced QRS complex–positive waves, and different QRS vectors, which could be related to cardiac size or cardiac location relative to the thorax.[8-10] Tricuspid and mitral peak flow velocities are lower in donkeys than horses.[11,12] These values must be taken with caution because they are likely influenced by breed, age, size, and fitness. Donkey gender and body weight affect cardiac dimensions on two-dimensional and M-mode echocardiography.[13] Standardized imaging protocols for M-mode and Doppler measurements described for horses also apply to donkeys.[11-13]

A low prevalence of cardiac diseases in donkeys was reported.[14] Third-degree atrioventricular block seems to be a common cardiac conduction disorder in donkeys, particularly in small breeds.[15] It has been reported that 2% of donkeys have an audible murmur,[14] which is lower than horses (50%–80%).[16]

Respiratory Tract

Respiratory disorders documented in horses also affect donkeys, although the presentation and severity of signs varies. Because of their stoic nature, signs of respiratory disease in donkeys tend to be less evident, and when present, the disease process is often advanced. Lower respiratory tract cytologic distribution is similar between donkeys and horses.[17,18]

Pulmonary fibrosis and tracheal collapse are common in geriatric donkeys.[19,20] An association between tracheal collapse and pulmonary fibrosis has been proposed.[21] Asinine herpesvirus 4 (AHV-4) and AHV-5 have been isolated and proposed as potential causative agents.[22] One can speculate that this condition is equivalent to equine multinodular pulmonary fibrosis, which has been linked to equine herpesvirus 5 (EHV-5) in horses.

Donkeys and mules can be reservoirs for infectious and parasitic diseases that affect horses (e.g., herpesviruses, lungworms). Therefore, their cohabitation can be problematic, particularly in farms with poor sanitary management or frequent animal movements.[23] Influenza is the most common respiratory infectious disease in donkeys[24] and they are apparently more susceptible than horses to the influenzavirus.[25]

Donkeys can be infected by EHV-1 and EHV-4,[26] as well as specific asinine herpesviruses (AHV-1, AHV-2, AHV-3, AHV-4, AHV-5, and AHV-6). Not every asinine variant causes clinical respiratory disease in donkeys. Mules can be infected but rarely show signs.[27] There are no vaccines for AHVs, and vaccination protocols are based on the use of vaccines for horses.

Based on serologic surveys, donkeys and mules develop antibodies against equine arteritis virus (EAV).[28] However, naturally occurring EAV has not been reported in donkeys or mules. There are donkey-specific EAV strains (asinine-94) with limited transmission to horses,[29] but reports of clinical disease in donkeys are lacking. Fever, mild depression, nasal and ocular discharge, conjunctivitis, and dependent edema were reported in donkeys experimentally infected with the KY-84 strain.[29]

Donkeys and mules appear to be more prone to glanders *(Burkholderia mallei)* than horses.[30,31] African horse sickness is less severe and rarely fatal in donkeys,[32] which may have epidemiologic implications, because donkeys and mules can act as carriers.[33]

Donkeys can host high numbers of adult lungworms *(Dictyocaulus arnfieldi)* with minimal to no clinical signs compared with horses, thus, acting as carriers. Prevalences of 68% to 87.5% for donkeys and 29% for mules were reported.[34,35] Therefore having horses and donkeys grazing together should be avoided, particularly when a parasite control program is not established. Diagnosis is achieved via fecal examination (Baermann method), and bronchoalveolar cytology may show eosinophilic inflammation and larval stages. Macrocyclic lactones and benzimidazoles are effective against *D. arnfieldi.*[36]

Alimentary Tract

A recent report based on postmortem examination of aged donkeys at a sanctuary described a 40% prevalence of gastric ulcers.[37] A gastric ulcer scoring system for donkeys has not been developed, but the one for horses seems valid. *Draschia megastoma* is considered the most pathogenic gastric parasite in donkeys.[37]

For equivalent intestinal lesions, donkeys exhibit less pain than horses, which is a factor that should be taken into consideration when deciding management, including exploratory laparotomy. In a large population of donkeys, among all types of colic, the prevalence of large colon impaction was 55% and the mortality risk was 51%.[38] The prevalence of enteroliths is geographic and is more frequent in areas with hard water and access to alfalfa (e.g., Mediterranean basin, California).[39]

Donkey-specific information on drugs that influence gastrointestinal function, including gastric secretion, is scarce. Management of colitis relies on the same principles as for other equids.

Endocrine and Metabolic Disorders

Nutritional Requirements

Donkeys can handle water deprivation longer than horses,[40] and their tolerance to dehydration has been equated to that of camels and Zebu cattle.[41] Dehydrated donkeys when allowed free access to water are very effective at restoring their hydration status, with minimal risk for overhydration.

Donkeys are thrifty animals, with low energy requirements and a natural ability to digest poor quality forages.[42-45] Concentrates and caloric-rich diets are unnecessary and should be avoided unless the animal is performing heavy work.

Metabolic Disorders

Hyperlipemia. The prevalence of dyslipidemias is higher in donkeys (10%–20%) than horses and ponies, with higher mortality rates (up to 80%).[42,46] Liver disease (19.4%) followed by colic (13.8%) was the most common condition in 449 dyslipidemic donkeys.[47] Transport can be stressful for some donkeys, which may explain their susceptibility to hyperlipemia after transportation.[48] For details on the pathogenesis of hyperlipemia, see Chapter 13.

Baseline triglyceride concentrations (range: 53–248 mg/dL; 0.6–2.8 mmol/L) in healthy donkeys are higher than values reported for healthy horses and ponies.[49] Serum leptin concentrations in healthy donkeys (2.7 ± 0.3 ng/mL) are similar to values reported for horses.[50] Therapeutic principles of hyperlipemia in small equids apply to donkeys.

Endocrine Disorders

Pathophysiologic principles of obesity, insulin resistance, metabolic syndrome, endocrinopathic laminitis, and pituitary pars intermedia dysfunction (PPID) apply to donkeys[50,51]; however, donkey-specific information for these conditions is minimal.

Donkey or Asinine Metabolic Syndrome. Obesity is common in donkeys in developed countries. When high-quality or calorie-rich feed is provided, donkeys easily accumulate adipose tissue. From the equine perspective, they can be considered "easy keepers."

A donkey-specific Body Condition Scoring (BCS) system ranging from 1 to 9 points (<3 = underweight; 4–6 = optimal condition; >7 = overweight) has been developed.[51] The Donkey Sanctuary, a charitable organization in England, developed a formula to estimate body weight in donkeys. For adult donkeys, weight (kg) = 0.000252 × height 0.24 × heart girth 2.575.[52] In donkeys younger than 2 years of age, the formula is weight (kg) = 0.000283 × heart girth 2.778.[52] A neck scoring system for donkeys has also been developed.[51] This score ranges from 0 (thin neck without palpable crest) to 4 (thick neck, rounded, cresty).

A link between obesity, insulin resistance, and laminitis has been documented in donkeys.[53] However, detailed information on asinine metabolic syndrome (AMS) and insulin resistance is limited. Other signs of equine metabolic syndrome reported in horses, such as hypertension or reproductive disturbances, have not been documented in donkeys. Insulin concentrations are often high in obese donkeys, suggesting insulin resistance. Unlike horses, hyperinsulinemia as a direct cause of laminitis has not been demonstrated in donkeys.[54] Similar to horses, geriatric donkeys have lower insulin concentrations that could result in reduced β-cell mass or less β-cell sensitivity to glucose.[51]

The diagnosis of insulin resistance relies on the same principles for horses. Few studies have evaluated glucose and insulin dynamics in donkeys.[53,55-57] In one study, insulin concentrations >20 μIU/mL were associated with laminitis.[58] The clinical relevance of this or higher insulin cutoff values to diagnose insulin resistance and evaluate their risk for endocrinopathic laminitis remains unclear. Reference values for glucose ratios and surrogates have been described in healthy donkeys.[57]

When dynamic diagnostic testing is performed in donkeys, validated protocols should be used.[56,57,59,60] Donkeys have an inherently delayed insulin-mediated glucose uptake compared with horses and ponies.[57] Their glucose curve is right-shifted

in the combined glucose insulin test (CGIT), intravenous glucose tolerance test (IVGTT), and oral glucose tolerance test compared with horses.[59,61-63] These differences support delaying glucose sampling for 60 or 75 minutes in donkeys versus 45 minutes for horses in the CGIT and for 150 minutes in the IVGTT.[64]

Serum leptin concentrations are higher in donkeys with high BCS and neck scores,[51] as well as in old donkeys compared with young donkeys.[51] The association between blood adiponectin, glucagon, ghrelin, and insulin growth factor–1 concentrations with age, gender, and obesity was recently reported in healthy donkeys.[51]

Specific treatments for AMS are not described, but principles for horses seem to apply. Grazing should be reduced when pastures may be rich in carbohydrates. A grazing muzzle could be useful in some animals. A weight-loss program should be oriented toward reducing body weight by 2% monthly until an optimal weight and BCS are achieved. Pharmacologic treatments used in horses to promote weight loss and increase insulin sensitivity have not been critically evaluated in donkeys.

Pituitary Pars Intermedia Dysfunction. Epidemiologic information for PPID in donkeys is lacking. PPID is more prevalent in donkeys older than 15 years of age.[65] No gender or breed predisposition has been demonstrated.

Clinical signs are similar to horses, with hypertrichosis (hirsutism) being the most frequent sign, considering that the hair-coat is longer and the hair is thicker in donkeys than horses. Laminitis, insulin resistance, abnormal fat distribution, and muscle wasting (sarcopenia, potbelly) are also signs of PPID in donkeys. Polyuria is rarely seen. Other signs described for horses (reproductive disturbances, blindness, seizures, secondary infections, and lethargy) have not been reported in donkeys.[65]

The diagnosis of PPID in donkeys is based on protocols described for horses.[65] Basal plasma cortisol concentrations in healthy donkeys and horses are similar[66]; however, adrenocorticotropic hormone (ACTH) concentrations are higher in donkeys than in horses.[66] This indicates that using equine-specific ACTH cutoff values for a healthy donkey could potentially cause misdiagnosis of PPID. Seasonal ACTH reference values for donkeys have not been generated. Dynamic diagnostic testing protocols for PPID in horses (the thyrotropin releasing hormone stimulation test) also appear to be valid for PPID donkeys.[67]

Based on clinical results, pergolide dosing for horses also works in donkeys with PPID.

Thyroid Gland Diseases. Information on thyroid gland disorders in donkeys is minimal. Thyroxine (T4) and triiodothyronine (T3) concentrations are occasionally measured, but result interpretation can be misleading. Healthy donkeys have higher plasma free T3, total T3, free T4, total T4, and reverse T3 concentrations than horses.[68] This is a good example in which values from horses should not be extrapolated to donkeys because it could lead to misdiagnoses. Sexual dimorphism for thyroid hormones has not been demonstrated in donkeys or horses.[69,70] Similar to horses,[71] age affects thyroid hormones, with younger animals having higher thyroid hormone concentrations.[68]

Drugs known to reduce thyroid hormone concentrations, such as phenylbutazone and glucocorticoids, should be taken into consideration for data interpretation,[72-74] particularly if these drugs have been administered within a month of testing.

Neurology

The neurologic examination of donkeys with mild spinal ataxia can be challenging for multiple reasons, including differences in gait, type of lesion, and spinal cord response to disease. Cervical stenotic myelopathy is uncommon in donkeys and mules but may develop from developmental, traumatic, or inflammatory processes. Information on viral (Borna; EHV-1; rabies; coronavirus; West Nile virus; Japanese viral encephalitis; and Eastern, Western, and Venezuelan equine encephalitis),[75-81] bacterial (e.g., neuroborreliosis or Lyme disease),[82] and parasitic (e.g., *Neospora* spp., *Strongylus vulgaris*)[83,84] disorders of donkeys is lacking. Clinical signs of Venezuelan equine encephalitis are usually mild in donkeys and mules,[85] but they could act as amplifying vectors, propagating the virus to horses and people. There are no published cases of equine protozoal myeloencephalitis *(Sarcocystis neurona)* in donkeys, and they are considered to be relatively resistant to the disease.[86]

Nutritional and metabolic imbalances leading to neurologic disorders have been reported in donkeys and mules. Signs similar to equine degenerative myelopathy from vitamin E deficiency were reported in donkeys.[87,88]

Urinary System

Urinary tract diseases are uncommon in donkeys, with urolithiasis being the most common.[89-92] In one postmortem survey of renal lesions in 100 donkeys, tubulointerstitial nephritis (50%), cystitis (19%), and renal fibrosis and cysts (13%) were the most common lesions found in the urinary tract.[89] Dysuria, stranguria, pollakiuria, hyperkalemia and hyponatremia, and urinary tract infections were common signs of urinary tract disease in donkeys.[89,90]

Most drugs used in horses and ponies to treat renal disease have not been investigated in donkeys, and dosing protocols are derived from horses.

Ophthalmology

Corneal ulceration is the most frequent ocular pathology in donkeys.[93,94] Equine recurrent uveitis prevalence seems to be lower in donkeys than horses, which could be explained by a lower predisposition to this condition or reflect infrequent ocular examinations in donkeys.[95] Old donkeys are prone to lens pathologies such as cataracts and luxation[96] or even colobomas.[97] Nonulcerative eosinophilic keratitis was reported in mules and donkeys.[98]

Ocular parasites described in donkeys include *Onchocerca* spp.[99] and *Setaria equina*.[100] Habronemiasis mainly affects the conjunctiva and occurs sporadically.[93]

Blindness can go unnoticed in donkeys, which likely reflects their ability to memorize their surroundings better than horses.

Dermatology

Congenital dermatoses such as junctional epidermolysis bullosa,[101] epitheliogenesis imperfecta, or an Ehlers-Danlos–like syndrome[102] have been documented in donkeys. Immune-mediated dermatopathies are rare in donkeys. Pemphigus foliaceus was reported in a pregnant jennie.[101] Atopic dermatitis, characterized by pruritus and urticaria on the face, trunk, and legs, was

reported in a Miniature donkey.[103] Traumatic skin lesions are a frequent problem in working donkeys.

Sarcoids are frequent cutaneous tumors in donkeys. Invasive and fibroblastic types are the most reported in donkeys, whereas the occult and verrucose forms are rare.[102] Other skin tumors of donkeys include squamous cell carcinomas, fibromas, fibrosarcomas, melanomas, and epitheliotropic lymphomas.[104] Melanomas are usually located in periocular areas and rarely related to haircoat color. Squamous cell carcinomas tend to be ocular and are less frequently located in the oral and nasal cavities, prepuce, vulva, or proximal gastrointestinal tract.

Musculoskeletal

There are foot anatomic and radiologic differences between donkeys and horses.[105] For a more detailed description of the donkey hoof, see Thiemann and Rickards.[106]

Foot lesions are the main cause of lameness (65% of the cases), and endocrinopathic laminitis is the main one.[107] Pedal abscesses are the second cause of lameness in donkeys. Long hooves are a frequent cause of coffin bone remodeling. White line disease, interphalangeal joint contracture, luxations, and keratomas are infrequent problems.[106,108,109] Hip fractures and coxofemoral luxations in small- to medium-sized donkeys (<250 kg) may be seen sporadically.[110,111]

Deep and superficial digital flexor tendinitis (23%–47%), suspensory desmitis (16%–31%), and digital flexor tendon sheath swelling (62%–77%) are the main soft tissue problems associated with lameness. Exertional rhabdomyolysis, polysaccharide storage myopathy, grass sickness, and atypical myopathy have been described in donkeys.[112-116]

CLINICAL PATHOLOGY

Interpretation of hematologic and biochemical information using equine reference values can be misleading because of the lack of donkey-specific information in most veterinary laboratories.

Hematology

Hematologic variables from donkeys are listed in Table A.1. The donkey red blood cell (RBC) count tends to be lower, but erythrocytes are larger than in horses. RBC count, hematocrit (HCT), and hemoglobin (Hb) values are closer to ponies than horses.[129] Reticulocytes are not found in peripheral circulation. Large- and medium-sized lymphocytes are more frequent in blood smears from donkeys than horses. Eosinophils and basophils are slightly larger in donkeys.

No effect of gender on hematologic variables has been documented. RBC, HCT, and Hb concentrations are higher in newborn donkey foals and then decrease in the first week postpartum.[130] Erythrocyte volume, corpuscular Hb concentration, and red cell distribution width remain unchanged in the first week of life. White blood cell count is lower in the immediate postpartum period, ranging from 4 to 12,000/μL (birth to day 3) to 6 to 15,000/μL (days 3–7).[130] The neutrophil:lymphocyte ratio can be as high as 2.8 at birth in donkey foals, but it declines to 1 by 12 months of age. Eosinophils and basophils change minimally in the first weeks of life, although their numbers tend to be higher than those for equine foals. Platelet parameters do not change in the first week of life, and the platelet number is higher than in adults.

Neonatal isoerythrolysis is a frequent problem in mule foals.[131] The presence of a donkey-specific erythrocyte antigen (donkey factor) is suspected to be responsible for this disorder. Considering that horses do not have alloantibodies against the donkey factor, mule foals with isoerythrolysis could receive blood transfusion from a horse with no history of breeding to donkeys. Washed erythrocytes from the dam can also be used. Neonatal alloimmune thrombocytopenia is an occasional finding in sick newborn mules. This condition usually develops within 24 to 48 hours after colostrum ingestion.[132] Immune-mediated thrombocytopenia can be a serious complication in donkey foals receiving plasma transfusion of equine origin.

Biochemistry

Biochemical variables from a number of studies are listed in Table A.2. Hepatic enzyme activities and bilirubin concentrations are lower in donkeys compared with horses, whereas creatine kinase, γ-glutamyl transpeptidase, and plasma triglyceride concentrations are higher in donkeys.[130] In one study, jennies had higher aspartate aminotransferase activity, total protein, total calcium, and indirect bilirubin concentrations, whereas male donkeys had greater alkaline phosphatase activity, albumin, phosphorus, glucose, creatinine, cholesterol, magnesium, and ionized calcium concentrations.[135] Pregnancy, foaling, and lactation can influence biochemical variables.[136] There are age-related differences for a number of biochemical variables.[130] Coagulation profiles vary between donkeys and horses.[137] Fibrinolytic subproducts (fibrin/fibrinogen degradation products [D-dimers]) were higher in healthy donkeys compared with horses.[137]

PHARMACOLOGY

Drugs commonly used in horses do not necessarily have similar pharmacokinetic and pharmacodynamic properties in donkeys and mules, and donkey-specific information is lacking (Tables A.3 and A.4). Depending on the drug, caution must be used when extrapolating doses and frequency from horses to avoid inadequate dosing and toxicity.

Antiinflammatory Drugs

There are differences in nonsteroidal antiinflammatory drug metabolism between donkeys and horses (Table A.3).[163] Phenylbutazone has a shorter half-life in Miniature donkeys, requiring shorter treatment intervals compared with regular-sized donkeys and horses.[164] Flunixin meglumine showed an earlier entrance of the drug into the exudate in donkeys compared with horses, although its maximal concentration and durability was shorter.[165,166] Information on steroid pharmacology in donkeys is lacking.

Antibiotics

There are pharmacologic data for a number of antimicrobial drugs in donkeys (Table A.4). Donkeys show a higher metabolism for most antibiotics, with faster clearance rates and shorter half-lives for many of them compared with horses (Table A.4).

Antiparasitic Drugs

Pharmacologic data for antiparasitic drugs used in donkeys are compiled in Table A.5. It is important to note that most of these drugs are not approved by regulatory agencies for the specific use in donkeys. Close monitoring is indicated for drugs lacking species-specific literature. Donkeys are more

TABLE **A.1** **Hematologic Values in Adult Donkeys**

Reference	Mean ± SD[117]	Mean ± SD[118]	Mean ± SD[119]	Mean ± SD[120]	CI[121]	CI[49]	Mean[122]	Mean[123]	Mean[124]	Mean[125]	Mean[126]	CI[127]	Mean ± SD[128]
RBC ($\times 10^6/\mu L$)	5.41 ± 0.9	6.02 ± 0.9	6.77 ± 1.2	5.25 ± 0.7	5.9–7.1	4.4–7.1	6.05	6.82	6.65	5.5	7.62	4.51–7.67	5.9 ± 0.9
Hb (g/dL)	11.1 ± 1.6	11.4 ± 1.5	12.4 ± 2.5	11.4 ± 1	11.2–12.5	8.9–14.7	10.65	12.87	13.1	11.6	15	10.1–14.6	11.8 ± 1.4
HCT (%)	32.1 ± 4.5	37 ± 4.2	36 ± 5	28.5 ± 3	34–38	27–42	37.6	37.6	38	33	45	28–42	33.1 ± 5.4
MCV (fL)	59.6 ± 3.0	—	54.1 ± 7.6	54.6 ± 2.6	53–57.5	53–67	52.47	55.45	57.9	64	59	47.5–63	56.9 ± 7.3
MCH (pg)	—	—	19.1 ± 2.1	22.1 ± 0.9	17.1–18.8	17.6–23.1	18.01	18.85	19.9	21.9	—	17.2–20	20.3 ± 2.5
MCHC (g/dL)	34.46 ± 0.73	—	34.6 ± 1.3	40.6 ± 0.8	31.9–33.2	31–37	34.36	34.2	34.3	34.8	33.3	33.8–37.5	34 ± 2.4
WBC ($\times 10^3/\mu L$)	10.9 ± 3.2	10.39 ± 2.08	9.6 ± 1.8	9.83 ± 2.29	11.9–17	—	12.14	8.22	10.3	10.2	10.2	6.5–16.67	10.1 ± 3.3
Neutrophils ($\times 10^3/\mu L$)	4.9 ± 1.6	5.07 ± 1.4	4.3 ± 1.2	4.58 ± 1.6	—	2.4–6.3	5.61	3.39	4.7	5.0	6.12	1.57–8.48	4.2 ± 1.9
Eosinophils ($\times 10^3/\mu L$)	1.38 ± 0.7	0.47 ± 0.3	0.63 ± 0.5	0.74 ± 0.4	—	0.1–0.9	0.96	0.38	0.58	0.38	0.35	0–1.86	0.5 ± 0.3
Basophils ($\times 10^3/\mu L$)	0.06 ± 0.0	0.00 ± 0.0	0.02 ± 0.06	—	—	0–0.06	0.07	0.03	0.04	0	0.05	0–0.23	0.05 ± 0.02
Monocytes ($\times 10^3/\mu L$)	0.38 ± 0.1	0.26 ± 0.1	0.21 ± 0.1	0.32 ± 0.2	—	0–0.75	0.52	0.13	0.51	0.13	0.51	0–0.76	0.8 ± 0.5
Lymphocytes ($\times 10^3/\mu L$)	4.17 ± 0.1	4.43 ± 1.5	4.2 ± 1.2	4.01 ± 1.1	4.6–7.5	2.2–9.6	4.94	4.15	4.4	4.2	3.79	3.03–10.22	4.4 ± 1.9
Platelets ($\times 10^3/\mu L$)	248 ± 75	324 ± 114	236 ± 82.1	220 ± 72.1	171–283	95–384	280	—	—	—	140	75–368	220 ± 89
N	68	69	71	30	52	137	83	38	165	>200	62	189	84

Results are expressed either as median ± standard deviation (SD), 95% confidence interval (CI), or mean value, depending on the referenced study.
N, Sample size; *RBC,* red blood cell count; *Hb,* hemoglobin; *HCT,* hematocrit; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration; *WBC,* white blood cell count.

TABLE **A.2** **Biochemical Values in Adult Donkeys**

Reference	Mean ± SD[117]	CI[121]	CI[49]	Mean[122]	Mean ± SD[133]	Mean ± SD[124]	CI[125]	Mean ± SD[126]	Mean ± SD[134]	CI[126]
Albumin (g/dL)	2.91 ± 0.29	2.3–2.95	2.15–3.16	2.88	3.13 ± 0.2	3.3 ± 0.3	2.0–3.4	4.0 ± 0.5	2.75 ± 0.3	2.2–3.2
ALP (IU/L)	409 ± 112	398–528	98–252	345	345 ± 66	236 ± 75	150–563	—	—	126–342
Amylase(IU/L)	—	—	1–10.6	—	—	—	—	—	—	—
AST (IU/L)	225 ± 64	193–299	238–536	317	295 ± 62	487 ± 119	59–199	433 ± 80	251 ± 47	281–442
Bile acids (μmol/L)	4.7 ± 1.9	—	2.6–18.6	4.1	—	—	—	—	—	—
Bilirubin (mg/dL)	1.5 ± 0.5	3.2–5.1	0.1–3.7	0.16	—	1 ± 0.5	1.4–7.7	—	0.05 ± 0.03	0.1–2.1
Calcium (mmol/L)	—	2.7–3.1	2.2–3.4	3.04	2.13 ± 0.04	2.94 ± 0.2	—	2.9 ± 0.4	—	—
Chloride (mmol/L)	—	—	96–106	—	106 ± 3.2	102 ± 3	—	—	—	—
Cholesterol (mmol/L)	—	0.7–1.3	1.4–2.9	—	2.28 ± 0.2	2.79 ± 0.7	—	—	1.74 ± 0.33	1.4–2.3
CK (IU/L)	153 ± 59	143–272	128–525	158	158 ± 77	64 ± 43	15–149	225 ± 50	191 ± 109	86–251
Creatinine (mg/dL)	1.02 ± 0.15	0.66–0.95	0.59–1.33	1.12	1.8 ± 0.1	1.1 ± 0.3	0.59–1.59	1.0 ± 0.5	1.1 ± 0.2	1.0–1.8
Fibrinogen (mg/dL)	280 ± 110	—	—	284	—	300	—	—	—	120–478
GGT (IU/L)	28 ± 9	18–31	14–69	45	45 ± 13	69 ± 29	8–49	—	—	13–28
GLDH (IU/L)	—	—	1.2–8.2	—	—	—	0.4–8	—	—	—
Globulins (g/dL)	4.36 ± 0.8	—	3.2–4.8	3.56	—	3.9	2.9–5.3	—	—	—
Glucose (mg/dL)	—	—	—	—	58.3 ± 10	84 ± 22	—	—	—	74–87
LDH (IU/L)	—	323–534	—	—	576 ± 156	427 ± 161	—	450 ± 80	318 ± 151	211–387
Lipase (IU/L)	—	—	7.8–27.3	—	—	—	—	—	—	—
Phosphorus (mmol/L)	—	0.8–1.2	—	—	0.89 ± 0.12	1.38 ± 0.4	—	1.6 ± 0.5	1.12 ± 0.22	—
Potassium (mmol/L)	—	—	3.2–5.1	—	3.7 ± 0.4	4.2 ± 0.6	—	—	—	—
SDH (IU/L)	—	—	—	—	—	—	0–1.5	—	—	0.1–2.2
Sodium (mmol/L)	—	—	128–138	—	121.5 ± 4.1	141 ± 4	—	—	—	—
Total protein (g/dL)	7.25 ± 0.64	5.9–7	5.8–7.6	6.82	6.8 ± 0.4	7.2 ± 0.7	5.8–8.2	7.5 ± 1.0	—	6.1–7.6
Triglyceride (mmol/L)	—	0.4–1.5	0.6–2.8	—	—	—	0.2–4.3	—	0.93 ± 0.37	0.2–1.3
Urea (mg/dL)	38 ± 9	24.6–36.1	10–31.2	34	24 ± 5.3	38 ± 10	5.3–22	15.1 ± 8.1	37.2 ± 7.2	16–37
N	68	52	136	83	40	108	>200	62	71	189

Results are expressed either as median ± standard deviation, 95% confidence interval (CI), or mean values, depending on the study.
N, Sample size; *ALP,* alkaline phosphatase; *AST,* aspartate aminotransferase; *CK,* creatine kinase; *GGT,* γ-glutamyl transpeptidase; *GLDH,* glutamate dehydrogenase; *LDH,* lactate dehydrogenase; *SDH,* sorbitol dehydrogenase.

TABLE **A.3** **Pharmacokinetic Analysis of Nonsteroidal Antiinflammatory Drugs Studied in Donkeys**

Drug	Animal	AUC (μg/mL/h)	Clearance (mL/kg/h)	MRT (h)	Vd (mL/kg)	*N*	Dose (mg/kg)	Reference
Carprofen	D	148.7 ± 30.8	4.9 ± 0.9	—	214.1 ± 18.9	5	0.7, IV	138, SD
	H	22.5 ± 10.5	36.6 ± 18.4	—	517 ± 208.9	3		
Cimicoxib	D	0.13 ± 0.1	13,861 ± 7147	3.1 ± 0.8	46,639 ± 8379	3	2, PO	139, SD
	D	0.14 ± 0.1	32,402 ± 22,358	3.9 ± 0.9	175,005 ± 18,814	6	5, PO	
Flunixin meglumine	D	23.6 ± 5.0	50.3 ± 9.4	3.4 ± 0.5	171.1 ± 12.6	3	1.1, IV	140, SE
	D	38.8 ± 8.9	108 ± 30	0.9 ± 0.1	85 ± 27	5	1.1, IV	141, SD
	M	51.6 ± 20.6	84 ± 30	1.5 ± 0.5	127 ± 79	5		
	H	58.6 ± 10.1	66 ± 12	1.8 ± 0.4	117 ± 16.4	3		
Ketoprofen	D	17.1 ± 7.4	200 ± 70	2.4 ± 0.5	610 ± 160	8	3, IV	142, SE
	D	—	414.1 ± 98.7	0.7 ± 0.2	263.1 ± 55.4	7	2.2, IV	143, SD
	H	9.6 ± 2.7	300 ± 95	2.7 ± 1.1	1250 ± 825	8	3, IV	144, SD
Meloxicam	D	6.01 ± 0.1	90 ± 0.01	1.4 ± 0.05	130 ± 0.01	8	0.6, IV	145, SE
	D	4.5 ± 2.5	187.9 ± 147.3	0.6 ± 0.3	93.2 ± 33.7	5	0.6, IV	146, SD
	H	18.8 ± 7.3	34.7 ± 9.2	9.6 ± 9.2	270 ± 160	5		
Phenylbutazone	D	19.2 ± 2.1	214.2 ± 18.1	0.7 ± 0.1	146.7 ± 17.6	3	4.4, IV	147, SE
	D	12.6 ± 2.6	360 ± 66	1.4 ± 0.9	545 ± 358	6	4.4, IV	148, SD
	D	28.3 ± 9.5	170.3 ± 54.4	1.7 ± 0.7	242 ± 97.6	4	4.4, IV	149, SD
	H	118.32 ± 12.4	29 ± 4.6	3.6 ± 0.2	174 ± 12.4	6		

D, Donkey; *M,* mule; *H,* horse; *AUC,* area under the curve; *MRT,* mean residence time; *Vd,* volume of distribution; *N,* number of animals sampled; *IV,* intravenously; *PO,* orally; *SD,* data expressed as mean ± standard deviation; *SE,* data expressed as mean ± standard error.

TABLE **A.4** **Pharmacokinetic Parameters of Antibiotics Studied in Donkeys**

Drug	Animal	AUC (μg/mL/h)	Clearance (mL/kg/h)	MRT (h)	Vd (mL/kg)	*N*	Dose (mg/kg)	Reference
Amikacin	D	—	58	2.8	150.2	3	6, IV	150
	H	—	45.2	4	206.6	3		
Ampicillin	D	24.5 ± 4	418.8 ± 69.1	0.7	422.5 ± 214.1	3	10, IV	151, SE
	H	42 ± 3.9	240.5 ± 21.8	0.7	210.8 ± 16	3		
Amoxicillin	D	20.6 ± 12.6	288 ± 78	0.8 ± 0.1	113 ± 26	4	10, IV	152, SD
	D	—	333	—	—	3	15, IV	153
	H	63.7	239	—	—	5		
Enrofloxacin	D	22.7 ± 9.9	249.6 ± 90	7.5 ± 2.3	1700 ± 200	5	5, IV	154, SD
	H	13.2 ± 3.1	510 ± 110	5.1 ± 0.7	2300 ± 500	6		155, SE
Gentamicin	D	30.3 ± 4.2	73.2 ± 10.8	2.6 ± 0.2	127 ± 25.5	3	2.2, IV	156, SD
	D	28.6 ± 1.43	77.4 ± 4.2	2.6 ± 0.1	88 ± 28.1	4		
	D	22 ± 5.8	100.2 ± 28.8	2.1 ± 0.3	203 ± 58	3		157, SD
	D	28.4 ± 1.8	76.2 ± 4.8	2.9 ± 0.3	220 ± 9.8	3		
	H	30.8	71.5	3.2	240	7		158
Penicillin	D	21.9 ± 0.5	462.9 ± 40.04	0.4 ± 0.03	204.5 ± 3	3	10, IV	159, SE
	D	—	393	—	84	5	20,000 IU, IV	153
	H	22.4 ± 5.3	514.5 ± 145.6	0.6 ± 0.08	362.3 ± 106.5	3	10, IV	159, SE
Oxytetracycline	D	96.9	91.4	—	649.3	3	10, IV	160
	H	—	39.5	—	348.8	3		
Sulfadimidine	D	—	67.8 ± 10.8	9.5 ± 1.15	680 ± 80	5	20, IV	161, SD
Sulfadiazine	D	—	102 ± 8.4	5.8 ± 1.05	630 ± 70	5		
Sulfadimethoxine	D	—	45 ± 2.4	9.9 ± 1.4	460 ± 50	5		
Sulfamethoxazole	D	96 ± 9	132 ± 12	2.7 ± 0.4	335 ± 46	5	12.5, IV	162, SD
	M	216 ± 54	60 ± 18	5.9 ± 1.3	337 ± 94	5		
	H	138 ± 6	90 ± 6	3.9 ± 0.1	301 ± 20	3		
Trimethoprim	D	1.8 ± 0.6	1680 ± 540	1 ± 0.5	1426 ± 209	5	2.5, IV	
	M	2.4 ± 0.18	942 ± 66	1.4 ± 0.4	1351 ± 358	5		
	H	2.4 ± 0.3	1224 ± 180	1.5 ± 0.1	1819 ± 189	3		

D, Donkey; *M,* mule; *H,* horse; *AUC,* area under the curve; *MRT,* mean residence time; *Vd,* volume of distribution; *N,* number of animals sampled; *IV,* intravenously; *SD,* data expressed as mean ± standard deviation; *SE,* data expressed as mean ± standard error.

TABLE **A.5 Antiparasitic Drugs Evaluated in Donkeys**

Drug	Indication	Investigated Dose	Reference
Doramectin	Roundworms *Dictyocaulus arnfieldi*	0.2 mg/kg, PO	167
Eprinomectin	*D. arnfieldi* Lice	0.5 mg/kg, pour on	168,169
Fenbendazole	Roundworms	30–60 mg/kg, PO	170
Imidocarb	*Babesia caballi, Theileria equi*	<2.2 mg/kg, IM q 24 h (*B. caballi*) and 48 h (*T. equi*)	171
Ivermectin	Roundworms *D. arnfieldi* Arthropods	0.2 mg/kg, PO	167
Mebendazole	Roundworms	10 mg/kg, PO	172
Moxidectin	Roundworms	0.2 mg/kg, IM	173
Oxfendazole	Roundworms *D. arnfieldi*	10 mg/kg, PO	170
Praziquantel	Cestodes	1 mg/kg, PO	174
Pyrantel pamoate	Roundworms Cestodes	19 mg/kg, PO	175
Secnidazole	*Balantidium coli*	10 mg/kg, PO	176
Triclabendazole	*Fasciola hepatica*	12 mg/kg, PO	177
		38 mg/kg, PO	170

IM, Intramuscularly; *PO,* orally.

sensitive to imidocarb toxicity than horses, so lower doses are recommended. Caution must be taken with macrocyclic lactones, particularly in animals in poor body condition, because of the risk of neurologic toxicity.

REFERENCES

1. Kruger K, Gaillard C, Stranzinger G, Rieder S. Phylogenetic analysis and species allocation of individual equids using microsatellite data. *J Anim Breed Genet*. 2005;122(suppl 1):78–86.
2. Svendsen ED. Work to improve the condition of donkeys and mules worldwide. In: Fielding D, Pearson RA, eds. *Donkeys, mules and horses in tropical agricultural development*. University of Edinburgh, U.K; 1991.
3. Taylor TS, Matthews NS. Mammoth asses–selected behavioural considerations for the veterinarian. *Appl Anim Behav Sci*. 1998;60:283–289.
4. Bough J. The Mirror Has Two Faces: contradictory Reflections of Donkeys in Western Literature from Lucius to Balthazar. *Animals*. 2011;1:56–68.
5. Vincenzetti S, Foghini L, Pucciarelli S, et al. Hypoallergenic properties of donkey's milk: a preliminary study. *Vet Ital*. 2014;50:1596–1600.
6. Borioni N, Marinaro P, Celestini S, et al. Effect of equestrian therapy and onotherapy in physical and psycho-social performances of adults with intellectual disability: a preliminary study of evaluation tools based on the ICF classification. *Disabil Rehabil*. 2012;34:279–287.
7. Gonzalez-De Cara CA, Perez-Ecija A, Aguilera-Aguilera R, et al. Temperament test for donkeys to be used in assisted therapy. *Appl Anim Behav Sci*. 2017;186:64–71.
8. Rezakhani A, Yazdanmehr MM. Normal electrocardiographic parameters of domestic donkeys (Equus asinus). *Anim Physiol Pathol Clin Vet Med*. 1977;24:672–679.
9. Kojouri GA, Rezakhani A, Torki E. The effects of verapamil hydrochloride on electrocardiographic (ECG) parameters of domestic donkey (Equus asinus). *J Equine Vet Sci*. 2007;27:499–503.
10. Escudero A, Gonzalez JR, Benedito JL, et al. Electrocardiographic parameters in the clinically healthy Zamorano-leones donkey. *Res Vet Sci*. 2009;87:458–461.
11. Amory H, Bertrand P, Delvaux V, Sandersen C. Doppler echocardiographic reference values in healthy donkeys. In: Matthews NS, Taylor T, eds. *Veterinary care of donkeys International Veterinary Information Service*. Ithaca, NY; 2004.
12. Hassan EA, Torad FA. Two-Dimensional and M-Mode Echocardiographic Measurements in the Healthy Donkey (Equus asinus). *J Equine Vet Sci*. 2015;35:283–289.
13. Roberts SL, Dukes-McEwan J. Echocardiographic reference ranges for sedentary donkeys in the UK. *Vet Rec*. 2016;179:332.
14. Roberts SL, Dukes-McEwan J. Assessment of cardiovascular disease in the donkey: clinical, echocardiographic and pathological observations. *Vet Rec*. 2016;179:384.
15. Decloedt A. Prevalence of cardiovascular disease in donkeys. *Vet Rec*. 2016;179:382–383.
16. Young LE, Rogers K, Wood JL. Heart murmurs and valvular regurgitation in thoroughbred racehorses: epidemiology and associations with athletic performance. *J Vet Intern Med*. 2008;22:418–426.
17. Delvaux V, Kirschvink N, Amory H, et al. Spécificités de la fonction cardiorespiratoire de l'âne (Equus asinus). *Pratique Vét Equine*. 2001;33:21–28.
18. Campi C, El Mohaine B, Lamrini H, Crane M. Working mules and donkeys: preliminary observations on videoendoscopic anatomy of the upper airway tract and BAL cytology. In: *Proceedings of the 10th International Congress of the World Equine Veterinary Association*. 2008:548–549.
19. Powell RJ, du Toit N, Burden FA, Dixon PM. Morphological study of tracheal shape in donkeys with and without tracheal obstruction. *Equine Vet J*. 2010;42:136–141.
20. Morrow LD, Smith KC, Piercy RJ, et al. Retrospective analysis of post-mortem findings in 1,444 aged donkeys. *J Comp Pathol*. 2011;144:145–156.
21. Thiemann AK. Respiratory disease in the donkey. *Equine Vet Educ*. 2012;24:469–478.

22. Kleiboeker SB, Schommer SK, Johnson PJ, et al. Association of two newly recognized herpesviruses with interstitial pneumonia in donkeys (Equus asinus). *J Vet Diagn Invest*. 2002;14:273–280.
23. Finney S, Collins JA, Duggan V. An investigation of the equine infectious disease threat represented by the presence of donkeys at mixed equestrian events in Ireland. *Ir Vet J*. 2015;68:11.
24. Rose MA, Round MC, Beveridge WI. Influenza in horses and donkeys in Britain, 1969. *Vet Rec*. 1970;86:768–769.
25. Newton JRMJ. Equine influenza. In: Coetzer JAW, RC T, eds. *Infectious diseases of livestock*. Oxford, UK: Oxford University Press; 2005:766–774.
26. Yildirim Y, Yilmaz V, Kirmizigul AH. Equine herpes virus type 1 (EHV-1) and 4 (EHV-4) infections in horses and donkeys in northeastern Turkey. *Iran J Vet Res*. 2015;16:341–344.
27. Bell SA, Pusterla N, Balasuriya UBR, et al. Isolation of a gammaherpesvirus similar to asinine herpesvirus-2 (AHV-2) from a mule and a survey of mules and donkeys for AHV-2 infection. *Vet Microbiol*. 2008;130:176–183.
28. Paweska JT, Binns MM, Woods PSA, Chirnside ED. A survey for antibodies to equine arteritis virus in donkeys, mules and zebra using virus neutralisation (VN) and enzyme linked immunosorbent assay (ELISA). *Equine Vet J*. 1997;29:40–43.
29. McCollum WH, Timoney PJ, Tengelsen LA. Clinical, virological and serological responses of donkeys to intranasal inoculation with the KY-84 strain of equine arteritis virus. *J Comp Pathol*. 1995;112:207–211.
30. Naureen A, Saqib M, Muhammad G, Hussain MH, Asi MN. Comparative evaluation of rose bengal plate agglutination test, mallein test, and some conventional serological tests for diagnosis of equine glanders. *J Vet Diagn Invest*. 2007;19:362–367.
31. Khan I, Ali S, Gwida M, et al. Prevalence of Burkholderia mallei in equids of remount depot, Sargodha, Pakistan. *Pak J Zool*. 2013;45:1751–1756.
32. Williams R, Duplessis DH, Vanwyngaardt W. Group-reactive ELISAS for detecting antibodies to African horsesickness and equine encephalosis viruses in horse, donkey, and zebra sera. *J Vet Diagn Invest*. 1993;5:3–7.
33. Hamblin C, Salt JS, Mellor PS, et al. Donkeys as reservoirs of African horse sickness virus. *Arch virol Suppl*. 1998;14:37–47.
34. Andersen S, Fogh J. Prevalence of lungworm D. arnfieldi (Cobbold 1884) in donkeys in Denmark and in horses in herds together with donkeys. *Nord Vet Med*. 1981;33:484–491.
35. Lyons ET, Tolliver SC, Drudge JH, et al. Lungworms (Dictyocaulus arnfieldi)–prevalence in live equids in Kentucky. *Am J Vet Res*. 1985;46:921–923.
36. Veneziano V, Di Loria A, Masucci R, et al. Efficacy of eprinomectin pour-on against Dictyocaulus arnfieldi infection in donkeys (Equus asinus). *Vet J*. 2011;190:414–415.
37. Al-Mokaddem AK, Ahmed KA, Doghaim RE. Pathology of gastric lesions in donkeys: a preliminary study. *Equine Vet J*. 2015;47:684–688.
38. Cox R, Proudman CJ, Trawford AF, et al. Epidemiology of impaction colic in donkeys in the UK. *BMC Vet Res*. 2007;3:1.
39. Hassel DM, Langer DL, Snyder JR, et al. Evaluation of enterolithiasis in equids: 900 cases (1973-1996). *J Am Vet Med Assoc*. 1999;214:233–237.
40. Maloiy GMO, Boarer CDH. Response of Somali donkey to dehydration-hematological changes. *Am J Physiol*. 1971;221:37–41.
41. Maloiy GMO. Water economy of Somali donkey. *Am J Physiol*. 1970;219:1522–1527.
42. Pearson RA, Merritt JB. Intake, digestion and gastrointestinal transit time in resting donkeys and ponies and exercised donkeys given ad libitum hay and straw diets. *Equine Vet J*. 1991;23:339–343.
43. Tisserand JL, Faurie F, Toure M. A comparative-study of donkey and pony digestive physiology. In: Fielding D, Pearson RA, eds. *Donkeys, mules and horses in tropical agricultural development*. University of Edinburgh, U.K; 1991:67–72.
44. Pearson RA, Archibald RF, Muirhead RH. The effect of forage quality and level of feeding on digestibility and gastrointestinal transit time of oat straw and alfalfa given to ponies and donkeys. *Br J Nutr*. 2001;85:599–606.
45. Smith DG, Pearson RA. A review of the factors affecting the survival of donkeys in semi-arid regions of sub-Saharan Africa. *Trop Anim Health Prod*. 2005;37:1–19.
46. McKenzie HC. Equine hyperlipidemias. *Vet Clin North Am Equine Pract*. 2011;27:59–72.
47. Burden FA, Du Toit N, Hazell-Smith E, Trawford AF. Hyperlipemia in a population of aged donkeys: description, prevalence, and potential risk factors. *J Vet Intern Med*. 2011;25:1420–1425.
48. Fazio E, Medica P, Cravana C, et al. Comparative endocrinological responses to short transportation of Equidae (Equus asinus and Equus caballus). *Anim Sci J*. 2013;84:258–263.
49. Burden FA, Hazell-Smith E, Mulugeta G, et al. Reference intervals for biochemical and haematological parameters in mature domestic donkeys (Equus asinus) in the UK. *Equine Vet Educ*. 2016;28:134–139.
50. Frank N. Insulin resistance and equine metabolic syndrome. In: *Current Therapy in Equine Medicine*. 6th ed. St. Louis, MO: Saunders Elsevier; 2009:159–160.
51. Mendoza FJ, Estepa JC, Gonzalez-De Cara CA, et al. Energy-related parameters and their association with age, gender, and morphometric measurements in healthy donkeys. *Vet J*. 2015;204:201–207.
52. Pearson RA, Ouassat M. Estimation of live weight. In: *A Guide to Live Weight Estimation and Body Condition Scoring of Donkeys*. 1st ed. Glasgow, U.K.: Thomson Colour Printers; 2000:17–20.
53. Du Toit N, Trawford AF. Determination of serum insulin and insulin resistance in clinically normal donkeys and donkeys with a history of laminitis (obese and non-obese). In: *American College of Veterinary Internal Medicine Forum*. Anaheim: California; 2010:779.
54. Asplin KE, Sillence MN, Pollitt CC, McGowan CM. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet J*. 2007;174:530–535.
55. Pleasant RS, Suagee JK, Thatcher CD, et al. Adiposity, plasma insulin, leptin, lipids, and oxidative stress in mature light breed horses. *J Vet Intern Med*. 2013;27:576–582.
56. McLean AKNB, Yokoyama M, O'Connor-Robison CI, et al. Insulin resistance in standard donkeys (Equus asinus) of three body conditions-thin, moderate, and obese. *J Equine Vet Sci*. 2009;29:406–407.
57. Mendoza FJ, Aguilera-Aguilera R, Gonzalez-De Cara CA, et al. Characterization of the intravenous glucose tolerance test and the combined glucose-insulin test in donkeys. *Vet J*. 2015;206:371–376.
58. Carter RA, Geor RJ, Staniar WB, et al. Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *Vet J*. 2009;179:204–210.
59. June V, Soderholm V, Hintz HF, Butler WR. Glucose-tolerance in the horse, pony and donkey. *J Equine Vet Sci*. 1992;12:103–105.
60. Forhead AJ, Dobson H. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. *Res Vet Sci*. 1997;62:265–269.
61. Eiler H, Frank N, Andrews FM, et al. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. *Am J Vet Res*. 2005;66:1598–1604.
62. Funk RA, Wooldridge AA, Stewart AJ, et al. Seasonal changes in the combined glucose-insulin tolerance test in normal aged horses. *J Vet Intern Med*. 2012;26:1035–1041.
63. Bertin FR, Sojka-Kritchevsky JE. Comparison of a 2-step insulin-response test to conventional insulin-sensitivity testing in horses. *Domest Anim Endocrinol*. 2013;44:19–25.
64. Frank N. Equine metabolic syndrome. *Vet Clin North Am Equine Pract*. 2011;27:73–92.

65. McFarlane D. Equine pituitary pars intermedia dysfunction. *Vet Clin North Am Equine Pract*. 2011;27:93–113.
66. Dugat SL, Taylor TS, Matthews NS, Gold JR. Values for triglycerides, insulin, cortisol, and ACTH in a herd of normal donkeys. *J Equine Vet Sci*. 2010;30:141–144.
67. Mejia-Moreira LS, Mendoza FJ, Buchanan B, et al. Evaluation of Diagnostic Tests for Pituitary Pars Intermedia Dysfunction in Donkeys. In: *Havemeyer International Equine Endocrinology Summit*. Coral Gables, FL; Jan 3-7, 2017.
68. Mendoza FJ, Perez-Ecija RA, Toribio RE, Estepa JC. Thyroid hormone concentrations differ between donkeys and horses. *Equine Vet J*. 2013;45:214–218.
69. Chen CL, Riley AM. Serum thyroxine and triiodothyronine concentrations in neonatal foals and mature horses. *Am J Vet Res*. 1981;42:1415–1417.
70. McCall CA, Potter GD, Kreider JL, Jenkins WL. Physiological-responses in foals weaned by abrupt or gradual methods. *J Equine Vet Sci*. 1987;7:368–374.
71. Malinowski K, Christensen RA, Hafs HD, Scanes CG. Age and breed differences in thyroid hormones, insulin-like growth factor (IGF)-I and IGF binding proteins in female horses. *J Anim Sci*. 1996;74:1936–1942.
72. Ramirez S, Wolfsheimer KJ, Moore RM, et al. Duration of effects of phenylbutazone on serum total thyroxine and free thyroxine concentrations in horses. *J Vet Intern Med*. 1997;11:371–374.
73. Rothschild CM, Hines MT, Breuhaus B, Gay J, Sellon DC. Effects of trimethoprim-sulfadiazine on thyroid function of horses. *J Vet Intern Med*. 2004;18:370–373.
74. Abraham G, Allersmeier M, Schusser GF, Ungemach FR. Serum thyroid hormone, insulin, glucose, triglycerides and protein concentrations in normal horses: association with topical dexamethasone usage. *Vet J*. 2011;188:307–312.
75. Zhang L, Wang X, Zhan QL, et al. Evidence for natural Borna disease virus infection in healthy domestic animals in three areas of western China. *Arch Virol*. 2014;159:1941–1949.
76. Faizee N, Hailat NQ, Ababneh MMK, et al. Pathological, immunological and molecular diagnosis of rabies in clinically suspected animals of different species using four detection techniques in Jordan. *Transboundary Emerg Dis*. 2012;59:154–164.
77. Fielding CL, Higgins JK, Higgins JC, et al. Disease associated with equine coronavirus infection and high case fatality rate. *J Vet Intern Med*. 2015;29:307–310.
78. Garcia-Bocanegra I, Jaen-Tellez JA, Napp S, et al. West Nile fever outbreak in horses and humans, Spain, 2010. *Emerg Infect Dis*. 2011;17:2397–2399.
79. Erlanger TE, Weiss S, Keiser J, et al. Past, present, and future of Japanese encephalitis. *Emerg Infect Dis*. 2009;15:1–7.
80. Gonzalez-Salazar D, Estrada-Franco JG, Carrara AS, et al. Equine amplification and virulence of subtype IE Venezuelan equine encephalitis viruses isolated during the 1993 and 1996 Mexican epizootics. *Emerg Infect Dis*. 2003;9:161–168.
81. Gibbs EPJ, Long MT. Equine alphaviruses. In: Sellon DC, Long MT, eds. *Equine infectious diseases*. St. Louis, MO: Saunders/Elsevier; 2007:191–197.
82. Veronesi F, Morganti G, Ravagnan S, et al. Molecular and serological detection of tick-borne pathogens in donkeys (Equus asinus) in Italy. *Vet Microbiol*. 2014;173:348–354.
83. Mayhew IG, Brewer BD, Reinhard MK, Greiner EC. Verminous (Strongylus vulgaris) myelitis in a donkey. *Cornell Vet*. 1984;74:30–37.
84. Machacova T, Bartova E, Di Loria A, et al. Seroprevalence and risk factors of Neospora spp. in donkeys from Southern Italy. *Vet Parasitol*. 2013;198:201–204.
85. Mackenzie RM, Desiger J, Parrav D. Venezuelan equine encephalitis virus comparison of infectivity and virulence of strains V-38 and P676 in donkeys. *Am J Trop Med Hyg*. 1976;25:494–499.
86. Dubey JP, Lindsay DS, Saville WJA, et al. A review of Sarcocystis neurona and equine protozoal myeloencephalitis (EPM). *Vet Parasitol*. 2001;95:89–131.
87. Miller MM, Collatos C. Equine degenerative myeloencephalopathy. *Vet Clin North Am Equine Pract*. 1997;13:43–52.
88. Verdonck F, Merlevede I, Goddeeris BM, et al. Vitamin E deficiency and decreased serum immunoglobulin concentrations in a population of donkeys. *Vet Rec*. 2007;160:232–233.
89. Bakeer AM, Shaheed IB, RMS K. Survey on Lesions of Urinary System in Donkeys. *Egypt J Comp Pathol Clin Pathol*. 2008;21:147–166.
90. Mekibib B, Abrham E, Kassaye A, et al. Clinicopathologic findings associated with urinary tract problems in working donkeys, Southern Ethiopia: a case study. *Comp Clin Pathol*. 2015;24:417–422.
91. Van den Brand JM, Hendriks-Onstein WK, Kik MJ, et al. Renal cysts, nephrolithiasis, and ovarian cysts in a donkey suspected of having been sexually abused. *Tijdschr Diergeneeskd*. 2006;131:730–735.
92. Frei S, Kummer M, Furst A, Eser MW. Cystoliths in the horse–A retrospective study. *Pferdeheilkunde*. 2016;32:124–131.
93. Scantlebury CE, Aklilu N, Reed K, et al. Ocular disease in working horses in Ethiopia: a cross-sectional study. *Vet Rec*. 2013;172:99–104.
94. Mancuso LA, Lassaline M, Scherrer NM. Porcine urinary bladder extracellular matrix grafts (ACell Vet Corneal Discs) for keratomalacia in 17 equids (2012–2013). *Vet Ophthalmol*. 2016;19:3–10.
95. Grobe VS. Conditions of the eye. In: Svendsen ED, Duncan J, Hadrill D, eds. *The professional handbook of the donkey*. 4th ed. Wiltshire, U.K: Whittet books limited; 2008.
96. Misk NA, Makady FM, Sayed AM. Cataract extraction in the donkey–evaluation of 19 cases. *Assiut Vet Med J*. 1992;27:209–221.
97. Martin-Suarez E, Galan A, Gallardo Galero JM, Molleda J. Bilateral typical complete colobomas in a donkey: Retinographic and fluorangiographic description. *Vet Ophthalmol*. 2009;12:338–342.
98. Edwards S, Clode AB, Gilger BC. Equine eosinophilic keratitis in horses: 28 cases (2003–2013). *Clin Case Rep*. 2015;3:1000–1006.
99. Hussein HS, Elsammani SE. Onchocerca raillieti prevalence and pathology in Sudanese donkeys. *J Comp Pathol*. 1985;95:345–351.
100. Marzok MA, Desouky A-RY. Ocular infection of donkeys (Equus asinus) with Setaria equina. *Trop Anim Health Prod*. 2009;41:859–863.
101. White SD. Donkey dermatology. *Vet Clin North Am Equine Pract*. 2013;29:703–708.
102. Knottenbelt DC. Skin disorders of donkeys. In: Matthews NS, Taylor TS, eds. *Veterinary care of donkeys*. Ithaca, NY: International Veterinary Information Service; 2005.
103. Falk EF, Restifo M, Lam ATH, Ferrer L. Use of cyclosporin modified to treat atopic dermatitis in a miniature donkey. In: *Abstracts of the North American Veterinary Dermatology Forum 15-18 April 2015 Nashville, TN*. 2015:133–159.
104. Kaleibar MT, Eshghi D, Helan JA. A survey on the status of equine skin tumors and associated epidemiological factors in Iran. *Comp Clin Pathol*. 2015;24:1407–1415.
105. Collins SN, Dyson SJ, Murray RC, et al. Radiological anatomy of the donkey's foot: Objective characterisation of the normal and laminitic donkey foot. *Equine Vet J*. 2011;43:478–486.
106. Thiemann A, Rickards K. Donkey hoof disorders and their treatment. *Practice*. 2013;35:134–140.
107. Collins SN, Dyson SJ, Murray RC, et al. Development of a quantitative multivariable radiographic method to evaluate anatomic changes associated with laminitis in the forefeet of donkeys. *Am J Vet Res*. 2012;73:1207–1218.
108. Walmsley JP. Flexural deformities of distal interphalangeal joints in a group of young donkeys. *Equine Vet Educ*. 1995;7:4–6.
109. Brogniez L, Launois MT, Perrin RAR, et al. Treatment of a severe distal forelimb wound presenting with extensive laceration and distal interphalangeal joint luxation in a donkey. *Pferdeheilkunde*. 2012;28:160–166.

110. Garcia-Lopez JM. Coxofemoral luxations in the horse: surgical options and challenges. *Equine Vet Educ*. 2010;22:554–556.
111. Toth F, Adair HS, Holder TEC, Schumacher J. Femoral head ostectomy to treat a donkey for coxofemoral luxation. *Equine Vet Educ*. 2007;19:478–481.
112. Valentine BA, Habecker PL, Patterson JS, et al. Incidence of polysaccharide storage myopathy in draft horse-related breeds: a necropsy study of 37 horses and a mule. *J Vet Diagn Invest*. 2001;13:63–68.
113. Kay G, Knottenbelt DC. Tetanus in equids: a report of 56 cases. *Equine Vet Educ*. 2007;19:107–112.
114. Valentine BA. Pathologic findings in equine muscle (excluding polysaccharide storage): a necropsy study. *J Vet Diagn Invest*. 2008;20:572–579.
115. Van Galen G, Pitel CM, Saegerman C, et al. European outbreaks of atypical myopathy in grazing equids (2006–2009): Spatiotemporal distribution, history and clinical features. *Equine Vet J*. 2012;44:614–620.
116. Mellor NE, Bladon B, Foote AK, O'Meara B. Successful treatment of chronic grass sickness in a donkey. *Equine Vet Educ*. 2013;25:628–632.
117. Courouce-Malblanc A, Moulin M, Valette JP, et al. Reference values on hematologic and biochemical parameters in French donkeys. In: *10th International Congress of World Equine Veterinary Association*. 2008.
118. Girardi AM, Marques LC, Pereira CZ, Campos E. Hematological variables of the Pêga donkey (Equus asinus) breed: influence of age and sex. *Comp Clin Pathol*. 2015;24:335–342.
119. Folch P, Jordana J, Cuenca R. Reference ranges and the influence of age and sex on haematological values of the endangered Catalonian donkey. *Vet J*. 1997;154:163–168.
120. Caldin M, Furlanello T, Solano-Gallego L, et al. Reference ranges for haematology, biochemical profile and electrophoresis in a single herd of Ragusana donkeys from Sicily (Italy). *Comp Clin Pathol*. 2005;14:5–12.
121. Stanišić L, Dimitrijević V, Simeunović P, et al. Morphological, biochemical and hematological characterization of endangered Balkan donkey breed. *Acta Vet*. 2015;65:125–136.
122. Pitel P, Moulin M, Valette J, et al. Approche des valeurs hématologiques et biochimiques chez deux races asines. *Pratique Vét Équine*. 2006;38:19.
123. Mori E, Mirandola RMS, Ferreira RR, et al. Reference values on hematologic parameters of the Brazilian donkey (Equus asinus) breed. *J Equine Vet Sci*. 2004;24:271–276.
124. Zinkl JG, Mae D, Guzman Merida P, et al. Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (Equus asinus). *Am J Vet Res*. 1990;51:408–413.
125. French JM, Patrick VH. Reference values for physiological, haematological and biochemical parameters in domestic donkeys (Equus asinus). *Equine Vet Educ*. 1995;7:33–35.
126. Turnbull A, Wernery U, Wernery R, et al. Survey of six infectious diseases of feral donkeys in the United Arab Emirates. *Equine Vet Educ*. 2002;14:33–38.
127. Laus F, Faillace V, Paggi E, et al. Reference values for hematological and biochemical parameters of mixed breed donkeys (Equus asinus). *Wulfenia J*. 2015;22:294–304.
128. Perez-Ecija A, Gonzalez-De Cara CA, Aguilera-Aguilera R, et al. Comparison of donkey hemogram using the LaserCyte hematology analyzer, an impedance system, and a manual method. *Vet Clin Pathol*. 2014;43:525–537.
129. Burden F, Thiemann A. Donkeys are different. *J Equine Vet Sci*. 2015;35:376–382.
130. Veronesi MC, Gloria A, Panzani S, et al. Blood analysis in newborn donkeys: hematology, biochemistry, and blood gases analysis. *Theriogenology*. 2014;82:294–303.
131. Traub-Dargatz JL, McClure JJ, Koch C, Schlipf Jr JW. Neonatal isoerythrolysis in mule foals. *J Am Vet Med Assoc*. 1995;206:67–70.
132. Ramirez S, Gaunt SD, McClure JJ, Oliver J. Detection and effects on platelet function of anti-platelet antibody in mule foals with experimentally induced neonatal alloimmune thrombocytopenia. *J Vet Intern Med*. 1999;13:534–539.
133. Mori E, Fernandes WR, Mirandola RMS, et al. Reference values on serum biochemical parameters of Brazilian donkey (Equus asinus) breed. *J Equine Vet Sci*. 2003;23:358–364.
134. Jordana J, Folch P, Cuenca R. Clinical biochemical parameters of the endangered Catalonian donkey breed: normal values and the influence of sex, age, and management practices effect. *Res Vet Sci*. 1998;64:7–10.
135. Girardi AM, Marques LC, Toledo CZP, et al. Biochemical profile of the Pêga donkey (Equus asinus) breed: Influence of age and sex. *Comp Clin Pathol*. 2014;23:941–947.
136. Bonelli F, Rota A, Corazza M, et al. Hematological and biochemical findings in pregnant, postfoaling, and lactating jennies. *Theriogenology*. 2015;85:1233–1238.
137. Mendoza FJ, Perez-Ecija RA, Monreal L, Estepa JC. Coagulation profiles of healthy Andalusian donkeys are different than those of healthy horses. *J Vet Intern Med*. 2011;25:967–970.
138. Mealey KL, Matthews NS, Peck KE, et al. Pharmacokinetics of R(−) and S(+) carprofen after administration of racemic carprofen in donkeys and horses. *Am J Vet Res*. 2004;65:1479–1482.
139. Kim T-W, Della Rocca G, Di Salvo A, et al. Pharmacokinetics of the novel cyclooxygenase 2 inhibitor cimicoxib in donkeys. *J Equine Vet Sci*. 2014;34:923–925.
140. Cheng Z, McKellar Q, Nolan A, Lees P. Preliminary pharmacokinetic and pharmacodynamic studies on flunixin meglumine in donkeys. *Vet Res Commun*. 1996;20:469–472.
141. Coakley M, Peck KE, Taylor TS, et al. Pharmacokinetics of flunixin meglumine in donkeys, mules, and horses. *Am J Vet Res*. 1999;60:1441–1444.
142. Anwer MN, Rasheed MA, Ashraf M. Pharmacokinetics of ketoprofen in healthy donkeys in Pakistan. *J Anim Plant Sci*. 2012;22:966–969.
143. Oukessou M, Bouljihad M, Van Gool F, Alvinerie M. Pharmacokinetics of ketoprofen in the donkey (Equus asinus). *Zentralblatt fur Veterinarmedizin Reihe A*. 1996;43:423–426.
144. Rehman ZU, Ashraf M, Khan MA, et al. Pharmacokinetcis of ketoprofen in healthy horses in Pakistan. *J Anim Plant Sci*. 2012;22:584–587.
145. Mahmood KT, Ashraf M. Pharmacokinetics of meloxicam in healthy donkeys. *Pak J Zool*. 2011;43:897–901.
146. Sinclair MD, Mealey KL, Matthews NS, et al. Comparative pharmacokinetics of meloxicam in clinically normal horses and donkeys. *Am J Vet Res*. 2006;67:1082–1085.
147. Cheng Z, McKellar QA, Nolan A, Lees P. Pharmacokinetics and pharmacodynamics of phenylbutazone and oxyphenbutazone in the donkey. *J Vet Pharmacol Ther*. 1996;19:149–151.
148. Matthews NS, Peck KE, Taylor TS, Mealey KL. Pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in miniature donkeys. *Am J Vet Res*. 2001;62:673–675.
149. Mealey KL, Matthews NS, Peck KE, et al. Comparative pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in clinically normal horses and donkeys. *Am J Vet Res*. 1997;58:53–55.
150. Horspool LJI, Taylor DJ, McKellar QA. Plasma disposition of amikacin and interaction with gastrointestinal microflora in Equidae following intravenous and oral-administration. *J Vet Pharmacol Ther*. 1994;17:291–298.
151. Horspool LJ, Sarasola P, McKellar QA. Disposition of ampicillin sodium in horses, ponies and donkeys after intravenous administration. *Equine Vet J Suppl*. 1992:59–61.
152. Lavy E, Ziv G, Aroch I, Glickman A. Pharmacokinetics, intramuscular bio-availability, and bioequivalence of amoxicillin in donkeys. *J Vet Med B*. 1995;42:284–292.
153. Oukessou M, Aissa M, Hmidouch A. Pharmacokinetics study of benzylpenicillin and amoxicillin in the donkey (Equus asinus). *Indian Vet J*. 1994;71:1077–1081.

154. Sekkin S, Gokbulut C, Kum C, Karademir U. Plasma disposition of enrofloxacin following intravenous and intramuscular administration in donkeys. *Vet Rec*. 2012;171.
155. Kaartinen L, Panu S, Pyorala S. Pharmacokinetics of enrofloxacin in horses after single intravenous and intramuscular administration. *Equine Vet J*. 1997;29:378–381.
156. Miller SM, Matthews NS, Mealey KL, et al. Pharmacokinetics of gentamicin in Mammoth asses. *J Vet Pharmacol Ther*. 1994;17:403–406.
157. Welfare RE, Mealey KL, Matthews NS, Taylor TS. Pharmacokinetics of gentamicin in donkeys. *J Vet Pharmacol Ther*. 1996;19:167–169.
158. Whittem T, Firth EC, Hodge H, Turner K. Pharmacokinetic interactions between repeated dose phenylbutazone and gentamicin in the horse. *J Vet Pharmacol Ther*. 1996;19:454–459.
159. Horspool LJI, McKellar QA. Disposition of penicillin-G sodium following intravenous and oral-administration to Equidae. *Br Vet J*. 1995;151:401–412.
160. Lizarraga I, Sumano H, Brumbaugh GW. Pharmacological and pharmacokinetic differences between donkeys and horses. *Equine Vet Educ*. 2004;16:102–112.
161. Oukessou M, Alsouss L. Pharmacokinetics of sulfonamides and trimethoprim in the donkey (Equus asinus). *Physiol Pathol Clin Med*. 1998;45:191–198.
162. Peck KE, Matthews NS, Taylor TS, Mealey KL. Pharmacokinetics of sulfamethoxazole and trimethoprim in donkeys, mules, and horses. *Am J Vet Res*. 2002;63:349–353.
163. Grosenbaugh DA, Reinemeyer CR, Figueiredo MD. Pharmacology and therapeutics in donkeys. *Equine Vet Educ*. 2011;23:523–530.
164. Lizarraga I, Sumano H, Brumbaugh GW. Pharmacological and pharmacokinetic differences between donkeys and horses. *Equine Vete Educ*. 2004;16:102–112.
165. Landoni MF, Lees P. Comparison of the antiinflammatory actions of flunixin and ketoprofen in horses applying PK/PD modeling. *Equine Vet J*. 1995;27:247–256.
166. Cheng Z, McKellar Q, Nolan A, Lees P. Preliminary pharmacokinetic and pharmacodynamic studies on flunixin meglumine in donkeys. *Vet Res Commun*. 1996;20:469–472.
167. Gokbulut C, Boyacioglu M, Karademir U. Plasma pharmacokinetics and faecal excretion of ivermectin (Eqvalan paste) and doramectin (Dectomax, 1%) following oral administration in donkeys. *Res Vet Sci*. 2005;79:233–238.
168. Veneziano V, Galietti A, Mariani U, et al. Field efficacy of eprinomectin against the sucking louse Haematopinus asini on naturally infested donkeys. *Vet J*. 2013;197:512–514.
169. Veneziano V, Di Loria A, Masucci R, et al. Efficacy of eprinomectin pour-on against Dictyocaulus arnfieldi infection in donkeys (Equus asinus). *Vet J*. 2011;190:414–415.
170. Grosenbaugh DA, Reinemeyer CR, Figueiredo MD. Pharmacology and therapeutics in donkeys. *Equine Vet Educ*. 2011;23:523–530.
171. Kumar S, Kumar R, Sugimoto C. A perspective on Theileria equi infections in donkeys. *Japanese J Vet Res*. 2009;56: 171–180.
172. Gokbulut C, Aksit D, Santoro M, et al. Plasma disposition, milk excretion and parasitological efficacy of mebendazole in donkeys naturally infected by Cyathostominae. *Vet Parasitol*. 2016;217:95–100.
173. Trawford AF, Burden F, Hodgkinson JE. Suspected moxidectin resistance in cyathostomin in two donkey herds at the Donkey Sanctuary, UK. In: *20th International conference of the World Association for the advancement of veterinary parasitology*. 2005.
174. Getachew AM, Innocent G, Proudman CJ, et al. Field efficacy of praziquantel oral paste against naturally acquired equine cestodes in Ethiopia. *Parasitol Res*. 2013;112:141–146.
175. Gokbulut C, Aksit D, Smaldone G, et al. Plasma pharmacokinetics, faecal excretion and efficacy of pyrantel pamoate paste and granule formulations following per os administration in donkeys naturally infected with intestinal strongylidae. *Vet Parasitol*. 2014;205:186–192.
176. Khan A, Khan MS, Avais M, et al. Prevalence, hematology, and treatment of balantidiasis among donkeys in and around Lahore, Pakistan. *Vet Parasitol*. 2013;196:203–205.
177. Kinabo LD, Bogan JA. Disposition of triclabendazole in horses, ponies and donkeys. *Equine Vet J*. 1989;21:305–307.

Index

A
AAA. *see* Aromatic amino acids
Abdomen
  auscultation of, 709
  distention of, 709
  percussion of, 709
  radiography of, 712–713
  ultrasonography of, 713
Abdominal distention, in bloat, 241
Abdominal pain, hepatic insufficiency and, 855
Abdominocentesis
  in critical care, 171–172, 171b
  for uroperitoneum, 1407
Abducens nerve, 622, 624
Abiotrophy, cerebellar, 585, 630–631, 631f
Abortion, 1278–1285
  early fetal loss and, 1279–1280
  embryonic loss in, 1278–1279
  leptospirosis and, 1282
  mare reproductive loss syndrome and, 1280–1281
  noninfectious causes of, 1284–1285
  placentitis-induced, 1281–1282
  protozoal, 1282
  twins and, 1284
  umbilical cord compromise and, 1284–1285
  viral, 1282–1283
Abscessation, hepatic, 879
Abscesses
  after trocarization, 172
  bacterial capsules and formation of, 7
  cerebellar, 632
  corynebacterial, 1165t–1167t, 1174
  ovarian, 1245
  pulmonary, 1395–1396
  retropharyngeal, 336
  in *S. equi* infection, 335–336
  stromal, 1148–1149, 1149f
Absorbent powders, 761
Absorption
  of cerebrospinal fluid, 590
  of drugs, 80
    aminoglycosides, 106
    aminopenicillins, 101
    amphenicols, 109
    antipseudomonal penicillins, 102
    cephalosporins, 104
    fluoroquinolones, 117
    macrolides and azalides, 114–115
    metronidazole, 119
Absorption *(Continued)*
    penicillin G, 100
    rifampin, 119
    sulfonamides, 110
    tetracyclines, 112–113
  test of
    in disorders of the gastrointestinal system, 714
    in gastrointestinal disorders, 283
Acanthocyte, 993
Accelerated idioventricular (idionodal) rhythms, 510f–511f, 511–512
Accessory sex glands, male, 1315–1316
  abnormalities of, 1338–1339
Accidental hypothermia, 238
Acepromazine
  for acute rhabdomyolysis, 549
  for esophageal obstruction, 799
  for pain management, 148
  for recurrent exertional rhabdomyolysis, 553
  for West Nile virus, 661–662
Acer toxicity, 1485–1486
Acetazolamide, for hyperkalemic periodic paralysis, 573
N-acetyl-β-D-glucosaminidase (NAG), 913–914
N-acetylcysteine (NAC), for endometritis, 1255, 1255t
Acetylcysteine retention enema, for meconium impaction, 1401
Acetylpromazine, for seizures, 607
Acetylsalicylic acid (Aspirin), 1005
Acid detergent fiber (ADF), 194b
Acid-base status, assessment of, 180–181
Acidosis
  bicarbonate, supplemental, 164–165
  metabolic, 180–181, 711
    in chronic kidney disease, 940–941
  renal tubular, 966–969, 967t
Acid-stable picornavirus, 344–345
Acid-suppressive drugs, for gastric ulceration, 1405–1406, 1405t
Acquired conditions
  of accessory glands, 1338–1339
  of epididymides, 1337
  of penis, 1333, 1333f
  of scrotum, 1334–1335
  of semen and spermatozoa, 1339–1340
  of spermatic cord, 1338
Acquired equine polyneuropathy, in Scandinavia, 653
Acquired hemophilia A, in plasma-based coagulation system, 1014
Acquired immunodeficiency, 59
Acroptilon repens, 1479–1480
Actinobacillus spp., 484–485
  systemic manifestations of, 1165t–1167t
Action potentials
  cardiac, 413, 413f
  motor unit, 592, 593f
    polyphasic, 593–594, 594f
Activated charcoal, for tetrachlorodibenzodioxin toxicity, 1466
Activated partial thromboplastin time
  in hepatic disease, 859
  hepatic insufficiency and, 857t
Activity, thyroid gland function and testing and, 1062
Acupressure, 1112–1113
Acupuncture, 1112–1113
  for endometritis, 1256
  for pain management, 150
Acute blood loss
  approach to, 1002, 1002b
  disorders associated with, 1006–1007
Acute diarrhea, 747
  differentials and diagnosis of, 748t
  infectious diseases and, 747–755
    clostridiosis, 752–753
    cyathostomiasis, 754
    Potomac horse fever, 750–752
    salmonellosis, 747–750, 748t
    strongylosis, 753–754
  intestinal anaphylaxis and, 758–759
  pain control in, 761
  probiotics, 761
  sand enteropathy and, 748t, 759
  therapy for, 759–762
    absorbent powders and mineral oil, 761
    antibiotics, 761
    anticoagulation, 761
    fluid replacement and circulatory support, 760
    mucosal repair and protection, 760
    nutrition, 761
  toxicologic diseases and, 755–758
    antibiotic-associated, 755–756
    arsenic in, 748t, 758
Acute diarrhea *(Continued)*
    cantharidin in, 748t, 757–758
    nonsteroidal antiinflammatory drugs in, 756–757
Acute generalized hepatic injury, 850
Acute glomerulonephritis, 926–927
Acute inflammatory response, 20–23
  acute phase proteins, proinflammatory cytokines, and complement in, 20–23, 20t, 21f
  chemotaxis and leukocyte trafficking in, 22–23
  lipid mediators in, 21–22
Acute interstitial nephritis, 924, 926
Acute kidney injury, in foals, 1409–1410
Acute lung injury, 1387–1391, 1389b
  equine neonatal, 1389–1390
  veterinary, 1390–1391
Acute phase proteins
  acute inflammatory response, 20–23, 20t
  drugs bound to, 85
  in endotoxemia, 731
Acute phase response, 235
  in endotoxemia, 731
Acute renal failure (ARF), 923, 1044
  causes of, 923–924
  clinical signs of, 927
  diagnosis of, 927–929
  in foals, 1409–1410
  pathophysiology of, 924–927
    acute glomerulonephritis in, 926–927
    acute interstitial nephritis in, 926
    acute tubular necrosis in, 926
  prognosis of, 930
  treatment of, 929–930
Acute respiratory distress syndrome, 1387–1391, 1389b
  neonatal equine, 1388, 1388b–1389b
  veterinary, 1390–1391
Acute tubular necrosis (ATN), 924, 926
Acyclovir, 650
  for foals, 1422t
Adaptive immunity, 23–41, 23f
  in foals, 39–40
  immunoglobulins in, 24–27, 24f, 25t, 26f
  major histocompatibility complex in, 28–30, 29f, 31f–32f

Note: Page numbers followed by *f* indicate figures, *t* indicate tables, and *b* indicate boxes.

Adaptive immunity *(Continued)*
signaling through antigen-specific receptor, 30–31, 32f
T-cell receptor and CD_3 complex in, 27
T-lymphocyte subsets in, 27, 28f
Adaptive responses, 20
Adenocarcinoma, thyroid tumors and, 1068
Adenoma
pituitary, magnetic resonance imaging of, 601, 601f
thyroid tumors and, 1068
Adenosine monophosphate-activated protein kinase (AMPK), 1080
ADF. *see* Acid detergent fiber
Adhesins
afimbrial, 6
biofilm, 6
Adhesion
in bacteria, 6
fungal, 8
intestinal, 785–786
uterine, 1231–1232, 1232f, 1243
Adipose tissue, 1080
Adjunctive therapies, for endometritis, 1256–1257
Adjuvants, for immunomodulation, 62
Adrenal Cushing syndrome, 1109
Adrenal gland, 1070–1071
disorders of, 1078–1079
Adrenal gland cortex, 1071
Adrenal insufficiency, 1072–1076
in foals, 1074
in horses, 1073–1074
pathophysiology of, 1074–1075
Adrenal steroids, 1071
Adrenergic receptors, 174
β_2, renal, 889–890
in urinary system, 889–890
α-adrenergic receptors
in control of micturition, 973
in urinary system, 889–890
vascular, 392
β-adrenergic receptors, 253–254
vascular, 391–392
β-adrenoceptor blocker, for heart disease, 457t–461t
Adrenocortical neoplasia, 1078
Adrenocortical steroidogenesis, 1071f
Adrenocorticotropic hormone (ACTH), pituitary pars intermedia dysfunction and, 1100
α_2-adrenoreceptor agonists, for pain management, 144t–145t, 146–147
α_2-adrenoreceptor antagonists, for gastrointestinal ileus, 778
Adventitious pulmonary sounds, 316
AEF. *see* Assisted enteral feeding
Aerosolized antimicrobials, for respiratory disorders, in foals, 1397, 1397t, 1424
Afferent pathways regulating cough, 245–246
Afimbrial adhesins, 6
Aflatoxin, 1504–1505
African horse sickness (AHS), 345
Afterload, ventricular, 396
Agalactia
during pregnancy, 1293–1294
tall fescue toxicity and, 1496
Agammaglobulinemia, 55
Aganglionosis, congenital, 1401
Age
drug dosage regimens and, 89–91, 90t
immunodeficiency and, 57–58
parasitic infection resistance related to, 17
thyroid gland function and testing and, 1060–1064, 1060t
vulvar pathology and, 1239
Agenesis, renal, 891–893, 893f
Agricola, 220
AHS. *see* African horse sickness
Air bronchograms, 320–322, 321f
Airway cytology
for cough, 250
for respiratory distress, 258
Airway diameter, control of, 253–254
Airway hyperresponsiveness, measurements of, for pulmonary function testing, 322
Airway obstruction
in cough, 248
recurrent, hypersensitivity disease and, 41–42, 45
Alanine aminotransferase (ALT)
hepatic insufficiency and, 857t
in liver disease, 860
Albumin
in cerebrospinal fluid, 590, 591t
drugs bound to, 85
hepatic insufficiency and, 857t
Albuterol
as bronchodilator, 1396–1397, 1397t
for interstitial pulmonary disease, 352–353
Aldosterone, 1071
Alfalfa
for horse, 205t
nutrients from, 199t
Alimentary lymphosarcoma, 742, 743t–744t
Alkaline phosphatase (ALP), 914
hepatic insufficiency and, 857t
in liver disease, 860–861
Allergen, in type I hypersensitivity, 41
Allergen-specific immunotherapy, 1193
Allergy, 41
skin
contact, 1192
food, 1191
insect bite hypersensitivity, 45, 1190
treatment for, 1193–1194
Allium toxicity, 1486
Allodynia, 138b
Allogenic whole blood transfusion, for hematopoietic disorders, 1003, 1003b
Allograft rejection, major histocompatibility complex in, 28
Alloimmune neonatal neutropenia, 1008
Alloimmune thrombocytopenia, 45–46
Allopregnanolone, 1076
Allopurinol, for neonatal encephalopathy, 1386
Allotetrahydrodeoxycorticosterone, 1076
Aloe vera, for burns, 1198
Alopecia areata, 1169, 1191f
ALP. *see* Alkaline phosphatase
Alphaherpesviruses, 339–340
Alphavirus, 655, 656t
Alphaxalone, 1077
ALS. *see* Amyotrophic lateral sclerosis
Alsike toxicity, 1503
ALT. *see* Alanine aminotransferase
Altered platelet function, with systemic disease, 1013
Alternate pathway, of complement system, 21
Alternative oxygen carriers, for hematopoietic disorders, 1003–1004
Aluminum toxicity, 1463–1464
Alveolar dead space, 254–255
Alveolar radiographic pattern, 320–322, 321f
Amanita toxicity, 1503
Amblyomma species, 1184
Ambulatory (Holter) ECG, 415, 415f
Amikacin
in acute renal failure, 930
for foals, 1422t
pharmacology of, 105–106, 107t
for pleuropneumonia, 350t
Amikacin sulfate
for endometritis, 1254t
for infective endocarditis, 484
Amino acids, hepatic, 845
γ-Aminobutyric acid (GABA)
in hepatic encephalopathy, 852
seizures and, 604
Aminoglycoside antibiotics, renal critical care, 183
Aminoglycosides, 105–109, 107t, 108f
adverse effects and drug interactions of, 107–109, 108f
clinical use of, 109
for foals, 1423
formulations of, 109
mechanism of action of, 106
mechanism of resistance of, 106–107
spectrum of activity, 106
Aminopenicillins, 101–102
4-aminopyridine toxicity, 1475
Amiodarone
for atrial fibrillation, 506–507
for heart disease, 457t–461t
for ventricular tachycardia, 513–514
Ammonia
hepatic elimination of, 845–846
in hepatic encephalopathy, 852
testing of, in hepatic disease, 857t, 859
toxic effects of, 1477
Ammonium chloride
for cystitis, 947
in renal tubular acidosis, 967t
Amoxicillin, 95, 96f
for foals, 1421, 1422t
Amphotericin, for guttural pouch mycosis, 326
Amphotericin B
for endometritis, 1258, 1259t
for fungal pneumonia, 355, 356t
Ampicillin
for endometritis, 1253, 1254t
for foals, 1421, 1422t
Ampicillin sodium, for pleuropneumonia, 350t
AMPK. *see* Adenosine monophosphate-activated protein kinase
Ampullae, 622, 1315–1316
evaluation of, 1322
Amsinckia intermedia, 874t
Amyloidosis
in chronic kidney disease, 937
hepatic, 880
Amyotrophic lateral sclerosis (ALS), 665
Anaerobic bacteria, capsules of, 7
Anagen defluxion, 1170, 1170f
Anagen phase, of hair cycle, 1160, 1161f
Anal sphincter paralysis, 652
Analgesia, nonsteroidal antiinflammatory drugs for, 121
Analgesics
critically ill horse and, 185
for duodenitis-proximal jejunitis, 740
Anal/tail clamp reflex, of neonatal foals, 1382t
Anaphylatoxins, 719
Anaphylaxis, systemic, 43–45
Anaplasma phagocytophila, 996, 1011–1012
Anatomic dead space, 177
Ancillary diagnostic aids
for body temperature, 236–237
for collapse, 268
for dysphagia, 272
for polyuria and polydipsia, 288–289
Ancillary diagnostic tests
for colic, 276–277
for cough, 249–250
for edema, 263
for hypothermia, 238
for respiratory distress, 258
for weight gain, 241
for weight loss, 244
Androstane, 1076
Androstanediol, 1076
Anemia
aplastic, 1017
equine infectious, 1008
in hepatitis, 869
in Fell pony syndrome, 53
hemolytic, 1010–1011
immune-mediated hemolytic, 1007–1008

Anemia *(Continued)*
of inflammation/chronic disease, 1017
iron deficiency, 1016
neonatal isoerythrolysis and, 1415
nonregenerative/hypoproliferative, 1016–1018
paraneoplastic disease and, 372
secondary to other deficiencies, 1016–1017
secondary to recombinant human erythropoietin administration, 1017
Anesthesia
halothane, interaction with aminoglycosides, 108–109
local, for pain management, 144t–145t
for traumatic brain injury, 617
Anestrus
lactational, 1225
winter, 1221
Angiocardiography, 398b, 418, 419f
Angiostrongylus cantonensis, verminous encephalomyelitis and, 682
Angiotensin II, in acute renal failure, 925
Angiotensin-converting enzyme, in chronic kidney disease, 943–946
Angiotensin-converting enzyme (ACE) inhibitors, 457t–461t
Anhidrosis, 233, 1110, 1211
clinical signs of, 1112
diagnosis of, 1112
epidemiology of, 1110
pathophysiology of, 1111–1112
physiology of, 1110–1111
prevention of, 1112–1113
prognosis of, 1112–1113
systemic manifestations of, 1165t–1167t
treatment of, 1112–1113
Anisocytosis, 993
Anoplocephala perfoliata infections, 14
Anorexia, weight loss associated with, 241
Anovulatory season, 1221
Antacids, for gastroduodenal ulceration, 807–808
Antiarrhythmics, 457t–461t, 513–514
Antibacterial shampoo, for dermatophilosis, 1174
Antibiotics
beta-lactam, 99–100
for corneal disorders, 1146
critically ill horse and, 186
for dermatophilosis, 1174
for diarrhea, 755–756
for endometritis, 1252–1253
for equine herpesvirus-1 myeloencephalopathy, 650
for infective endocarditis, 484
for pleuropneumonia, 350, 350t
for spinal cord injury, 621
for tetanus, 671
for toxic injury, 490
Antibodies
in adaptive immunity, 23–24
in immune response, in foals, 40
Antibody-mediated hypersensitivity diseases, 44–46
Antibody-mediated immunity
in foals, 40–41
tests for, 48–49, 49f–50f
Anticholinergic (vagolytic) agents, 457t–461t
Anticoagulant rodenticide toxicity, 1014
Anticoagulants, for hemostatic dysfunction, 1005
Anticoagulation, hemostasis and, 1001
Anticollagenases, for corneal disorders, 1146–1147
Anticomplement factor, bacterial spread and, 7
damage and diseased tissues and, 6t
Anticonvulsants, 606
for pain management, 148
Antidiuretic hormone challenge test, 919–921, 921f
Antiendotoxin therapy, for duodenitis proximal jejunitis, 740
Antifibrinolysis, hemostasis and, 1001
Antifungals, for corneal disorders, 1146
Antigenic determinants, in adaptive immunity, 23
Antigenic shifts, 14
Antigens
in adaptive immunity, 23
major histocompatibility, 28, 29f
Antigen-specific immunomodulation, 62–63
Antigen-specific receptors
of B lymphocytes, 24–27
signaling through, 30–31, 32f
of T cells, 27
Antihistamines, for allergic skin disease, 1193
Antiinflammatory drugs
for cervical vertebral compressive myelopathy, 636
for duodenitis-proximal jejunitis, 740
for equine herpesvirus-1 myeloencephalopathy, 650
for equine protozoal myeloencephalitis, 644
for gastrointestinal ileus, 776
for hepatic insufficiency, 864
for pain management, 143–145
for placentitis, 1366–1367
for temporohyoid osteoarthropathy, 626
for traumatic brain injury, 617
for verminous encephalomyelitis, 684–685
Antimicrobial therapy, 94
aminoglycosides in, 105–109, 107t, 108f
aminopenicillins in, 101–102
amphenicols in, 109–110
Antimicrobial therapy *(Continued)*
antipseudomonal penicillins in, 102
benzylpenicillin in, 100–101
beta-lactam antibiotics in, 99–100
beta-lactamase inhibitors in, 99–100
carbapenems in, 105
cephalosporins in, 99–100, 102–105
chloramphenicol (CPHC) in, 109
concurrent use of additional antimicrobials in, 99
designing of dosage regimens of, 95, 97–98
for duodenitis-proximal jejunitis, 740
for endotoxemia, 733
florfenicol (FLF) in, 109
fluoroquinolones in, 116–118
for foals, 1420–1424, 1422t
aerosol administration in, 1424
aminoglycosides as, 1423
cephalosporins as, 1421–1423
chloramphenicol as, 1424
fluoroquinolones as, 1424
β-lactams as, 1421
macrolides as, 1423–1424
metronidazole as, 1424
potentiated sulfonamides as, 1423
rifamycins as, 1424
tetracyclines as, 1423
therapeutic targets of, 1420–1421
for gastrointestinal ileus, 777–778
for infective endocarditis, 484
macrolides and azalides in, 114–116
metronidazole in, 119–120
prophylactic use of, 99
rational use of, 94–97
bactericidal *versus* bacteriostatic antimicrobials, 96–97, 97b
infection documentation and, 95
minimum inhibitory concentration interpretation in, 95–96, 96f
pharmacokinetic-pharmacodynamic relationships in, 97, 98f, 98b
postantibiotic effect of, 97, 97t
rifampin in, 118–119
sulfonamides in, 110–112, 111t
for tetanus, 671
tetracyclines in, 112–114
Antioxidants, for endotoxemia, 736
Antiprotozoal medication, 644
Antipseudomonal penicillins, 102
Antithrombin III, hepatic insufficiency and, 856
Anus, neurologic examination of, 584, 588
Aorta, 441b–443b, 464
Aortic valve
auscultation of, 402f, 403
echocardiographic and Doppler studies of, 441b–443b
infective endocarditis, 481–484, 482f–483f
regurgitation of, 476–479, 476f, 478f–479f
causes of, 401t–402t
Aortocardiac fistula, 493f, 496–497
Aortoiliac thrombosis, as cardiovascular disorder, 493f–494f, 495–496
Aphthovirus, 344–345
Aplastic anemia, 1017
Apoptosis
in bacteria, 7
in fungi, 8
in spinal cord injury, 619
Apparent volume, of distribution, 82
Appetite, regulation of, 181
Aquaporins, 260
Arachidonic acid, in pathophysiology of colitis, 722
Arbor vitae, 628
ARF. *see* Acute renal failure
Argasid ticks, 1184
Arginase (ARG), hepatic insufficiency and, 857t, 860
Arginine vasopressin
measurement of, for polyuria and polydipsia, 289
role of, 284–285, 285f
Aromatic amino acids (AAAs), 853, 853f, 857t
Arrhythmias, 387, 389b, 498b
atrial, 498–509, 500f–502f
auscultation of, 401t
exercise-induced, 412
fibrillation, 503–509, 505b, 507f–508f
flutter, 500f, 502–503
premature complexes, 500–502, 500f
tachycardia, 500f–502f, 502
conduction disturbances, 514–517, 515f–516f
junctional, 511
poor performance and, 291, 399b
sinus rhythms, 497–498, 499f
sudden cardiovascular death due to, 399b
ventricular, 510f–511f, 512
premature complexes, 512–513
tachycardia, 512f, 513–514
Arsenic
gastrointestinal toxic effects of, 1462–1463
toxicity, 1187
systemic manifestations of, 1165t–1167t
Arsenic toxicosis, 748t, 758
Arterial blood gases
analysis, in critical care, 178
during exercise testing, 293–294
normal values in foals, 1387, 1388t
in traumatic brain injury, 616
Arterial blood pressure (ABP)
examination of, 409–410
monitoring of, in critical care, 168–169, 168f–169f, 168t, 176
values of, 410
Arterial pulse, 404, 409–410, 477
Arterial rupture, 497
Arteriovenous fistulas, 496
Arteritis, equine viral, 869, 1340

Arthritis, in severe combined immunodeficiency, 52
Arthropathies, 118
Article-level metrics, 221–222
Artificial insemination
  with cooled shipped semen, 1266
  with fresh semen, 1266
  of frozen semen, 1266–1267
Artificial vagina (AV)
  care of, 1324
  selection of, 1323–1324
Arytenoid chondritis, 317f, 330
Ascarid impactions, 784
Ascites
  abdominal distention and, 240
  in hepatic insufficiency, 855
Asclepias toxicity, 1479
Aspartate aminotransferase (AST)
  in cerebrospinal fluid, 591t
  hepatic insufficiency and, 857t
  in liver disease, 860
Aspartate transaminase
  for diagnosis, of musculoskeletal system disorders, 545–546
  in type 1 polysaccharide storage myopathy, 554
Aspergillus (Emericella) fumigatus, guttural pouch mycosis from, 325–327
Aspergillus species, toxic effects of, 1504
Asphyxia, 1048
Aspiration
  meconium, 1391–1392
  milk, 1392
  pneumonia due to, 799
Aspiration pneumonia, 352
  in foals, 1391
Aspirin
  for infective endocarditis, 484
  pharmacology of, 84, 123
Assisted enteral feeding (AEF), 204–205, 204t
Assisted reproductive techniques
  for mares, 1310
  for stallions, 1312–1313
Astragalus toxicity, 1480
Ataxia
  abnormal gaits associated with, 586
  cerebellar, 585, 632
  in cervical vertebral compressive myelopathy, 633
  dysphagia and, 272
  in equine herpesvirus-1 myeloencephalopathy, 648
  in vestibular disease, 624
Atelectotrauma, in foals, 1399–1400
Atheroma, 1211
ATN. *see* Acute tubular necrosis
Atopic dermatitis, 43, 1191
Atopy, 42, 1191
  systemic manifestations of, 1165t–1167t
Atresia
  biliary, 881
  pulmonary, with ventricular septal defect, 470–471, 470f
  tricuspid, 471
Atresia ani, 1401
Atresia coli, 789
Atrial arrhythmias, 498–509, 500f–502f
  auscultation of, 401t
  exercise-induced, 412
  fibrillation, 503–509, 505b, 507f–508f
  flutter, 500f, 502–503
  premature complexes, 500–502, 500f
  tachycardia, 500f–502f, 502
Atrial repolarization wave (Ta wave), 414, 414f
Atrial septal defects, 463f, 468
Atrial septum, 441b–443b, 463
Atrial sound, 392, 403
Atrioventricular block, 514, 515f, 517f
  auscultation of, 401t
  electrocardiogram in, 395f
Atrophy
  gonadal, 1336
  muscular, 596
  pathophysiology of, 545, 545f
Atropine
  for cardiopulmonary resuscitation, of foals, 1373
  for heart disease, 457t–461t
  narcolepsy and, 610
Atropine sulfate, for methiocarb toxicity, 1475
Atypical equine thrombasthenia, 1013
Auscultation
  abdominal, 709
  of cardiovascular disorders, 399–409
    algorithm for, 406t–407t
    of cardiac murmurs, 403f–404f, 405–408, 408b
    clinical method for, 399–403, 400t–402t, 402f–403f
    of heart rate and rhythm, 395f, 401t, 404–405
    of heart sounds, 403–404, 404f
    pulmonary, 408–409, 409f
    tricuspid regurgitation, 479–480
    in valvular heart disease, 471–472
  for respiratory sounds, 258
Autoimmune diseases, 41
  pemphigus foliaceus as, 1194–1195, 1195f
  virally induced, 12
Autologous blood transfusion, for hematopoietic disorders, 1003
Autologous conditioned serum, for pain management, 149
Autonomic bladder, 973
Autonomic nervous system
  equine grass sickness and, 676
  heart and, innervation of, 391–392
Autumnal transition, of reproductive cycle, 1224
Axillary nodular necrosis, equine, 1212
Azalides, 114–116
  adverse effects and drug interactions of, 115–116
  clinical use of, 116
  formulations of, 116
  mechanism of action of, 114
  mechanism of resistance of, 114
  pharmacokinetics of, 114–115
Azathioprine, as immunosuppressor, 60
Azithromycin, 114
  for foals, 1422t, 1423–1424
Azotemia, 910–911
  in acute renal failure, 923–924
  in chronic kidney disease, 942
  hyperlipemia and, 872
  neonatal sepsis and, 1377
  prerenal, 911, 911t
  uroperitoneum and, 1407

B

B cell receptors (BCR), signaling through, 30–31
B cells, in adaptive immunity, 23–24
B lymphocytes, antigen-specific receptors of, 24–27, 24f, 25t, 26f
Babesiosis, in hemolysis, 1008–1009
Bacteria, as normal flora, in upper respiratory tract, 314, 352
Bacterial cystitis, 946
Bacterial derivatives, for immunosuppression, 60
Bacterial granuloma, 1175, 1175f
Bacterial hepatitis, 868–869
  in foals, 1417–1418
Bacterial infections
  cutaneous
    botryomycosis, 1175, 1175f
    corynebacterial infections, 1174–1175
    dermatophilosis, 1173–1174
    pastern dermatitis, 1181, 1181f
    staphylococcal pyoderma, 1174
  entry of, 5–6
  infective endocarditis, 481–484, 482f–483f
  pathogenesis of, 5–7, 6t
  spread of bacteria, 7
    survival in host environment, 7
Bacterial meningitis, 1383–1384
Bacterial pneumonia, 346–352
  clinical manifestation of, 347
  diagnosis of, 347–349, 347f
  etiologic agents of, 347
  prognosis and outcome in, 352
  treatment of, 349–352, 350t
Bacterial products, for immunostimulation, 61–62
Bacterial rhinitis, 323
Bacterial urethritis, 946
Bactericidal permeability-increasing protein (BPI), 734–735
Bacteriostatic antimicrobials, 96–97, 97b
Bacteroides fragilis, in peritonitis, 763
Baermann fecal flotation technique, for suspected lungworm infection, 250
BAL. *see* Bronchoalveolar lavage
Balance, caloric, 558
Balanced electrolyte solution, 164
Ballistic shockwave lithotripsy, for cystic calculi, 954–955
Ballooning degeneration, 850
Barbiturates
  for seizures, 606
  for traumatic brain injury, 617
Barium sulfate, for radiography, 712–713
Barrier function
  gastric, 766–767
  intestinal, 767
  mucosal, 766–767
Bartonella henselae, bacterial hepatitis due to, 1417–1418
Basal layer, epidermal, 1159
Baseline cortisol concentrations, in pituitary pars intermedia dysfunction, 1105–1106
Basement membrane, 1159
Basioccipital and basisphenoid bones, fracture of, 627
Basophils, 997
BCS. *see* Body condition scoring
Beclomethasone, for interstitial pulmonary disease, 352–353
Bedding, for recumbent horses, 186
Bees, envenomation by, in hemolysis, 1010
Behavior
  in mare breeding soundness examination, 1226
  of neonatal foals, 1380, 1382t
  in neurologic examination, 584
  of stallions
    abnormal, 1326t, 1331–1332, 1331t
    sexual, 1318
Benign essential hematuria, 960
Benign primary hematuria, 960
Benzamides, for gastrointestinal ileus, 777
Benzimidazole
  for fungal pneumonia, 355
  for parasitic pneumonitis, 357
Benzodiazepines, in tetanus, 671
Benzylpenicillin, 100–101. *see also* Penicillin G.
Bermuda hay, ileal impaction and, 784
Bernoulli equation, 451
Berteroa incana, 1497
Beta-lactam antibiotics, 99–100
Beta-lactamase inhibitors, 99–100
Bethanechol, for gastrointestinal ileus, 777
Bicarbonate
  supplemental, in fluid therapy, 164–165
  toxic effects of, 1488–1489
Bilateral bog spavin, 580
Bilateral osteochondritis dissecans, 580
Bile, excretion of, 848–849, 848f
Bile acids, 848
  concentration of, hepatic insufficiency and, 857t, 858
Biliary atresia, 881

Biliary obstruction
acute, 871
neonatal acquired, 879
Bilirubin, 848–849
hepatic insufficiency and, 857–858, 857t
in neonatal foals, 1385
Bioavailability, of drugs, 84
Biofilm, 6, 6t
Biofilm infections, endometritis and, 1248
Biological abstracts, 220
Biopsy
endometrial, 1234–1236, 1235f, 1242
in fungal pneumonia, 355
for immune-mediated myositis in Quarter horse-related breeds, 567
liver, 862–863, 877–878
lung, 322–323
for malignant hyperthermia, 554
muscle, for musculoskeletal system disorders, 547, 547f
for myofibrillar myopathy, 559–560
rectal, 741–742
renal, 922–923
skin
in dermatophilosis, 1173–1174
in dermatophytosis, 1177–1178
in drug reactions, 1188
in equine axillary nodular necrosis, 1212
in insect hypersensitivity, 1191
in lice infestations, 1181
in seborrhea, 1171
of thyroid gland, 1065
Bioresorbable cisplatin beads, 1205
Biotransformation
of drugs, 88, 88f
hepatic, 849
Biphasic fever, 236
Bisphosphonates
for acute tubular necrosis, 926
for pain management, 149
Bites, venomous, 1189
Biting midges, hypersensitivity to, 1190
Black flies, 1165t–1167t
hypersensitivity to, 1190
Black locust toxicity, 1478
Black or white follicle dystrophy, 1169
Black walnut toxicity, 1494
Bladder
defects, 896–897
displacement of, 957
distention, 897
in equine herpesvirus-1 myeloencephalopathy, 648
dysfunction, 973
clinical signs of, 974–975
diagnosis of, 975
treatment of, 975–976
postpartum
eversion of, 1309
prolapse of, 1309
rupture of, 1309
ultrasonography in, 917, 917f
Blastomyces dermatitidis
adhesins in, 8
in fungal pneumonia, 354
Blind-ending ductules, 1337
Blindfolding
during gait evaluation, 585
in vestibular disease, 584, 624
Blindness, equine recurrent uveitis, 46–47
Blister beetle toxicosis, 1466–1467
rhabdomyolysis from, 568
Blood flow
intestinal, 768
renal, 903–905, 903f, 904t
in traumatic brain injury, 611–613
Blood gas
analysis
in critical care, 180
during exercise, 295
in critical care, 159
Blood loss
acute
approach to, 1002b
disorders associated with, 1006–1007
chronic, disorders associated with, 1006–1007
differential diagnoses for, in horse, 994b
intestinal, 1006
periparturient, in mares, 1007
Blood transfusion
for traumatic brain injury, 617
whole blood, 167
Blood urea nitrogen (BUN), 846, 857t
measurement of, 183
in urinary system, examination of, 910
Blood work
for respiratory distress, 258
Blue-green algae toxicity, 1470–1471
Body condition scoring (BCS), 191, 195t
Body fluids, 900, 900t
Body temperature
changes in, 232–238
causes of, 233b
control of, 232
diagnostic approach to elevations in, 236–237
fever and, 234–236, 234f
increased, 232–237
laboratory testing for, 236–237
pathogenesis of fever, 234, 234f
Body weight
alterations in, 216
changes in, 238–244, 239t
decreased, mechanisms and selected differential diagnoses for, 243b
measurement of, in nutritional status, 191
mechanisms controlling, 238–239
vulvar pathology and, 1238–1239
Bone, parathyroid hormone effects on, 1036–1037, 1038f, 1040t
Bone marrow, hematopoiesis in, 998
Bornavirus, 662
Borrelia burgdorferi, 678
Botryomycosis, 1175, 1175f
Bottle feeding, of orphan foals, 208
Botulism, 672–676
in foals, 1384
toxins in, 672–674
BPI. *see* Bactericidal permeability-increasing protein
Brachial plexus injury, 596, 597f
Bracken fern toxicity, 1478–1479
Bradycardia
auscultation of, 401t
sinus, 498, 499f
Bradykinin
in gastrointestinal inflammation, 719
in pathophysiology of colitis, 722t
Brain-heart syndrome, 613
Brainstem, in central control of ventilation, 253
Brainstem auditory evoked response (BAER) testing, 597–598, 598f–599f
in vestibular disease, 628
Brainstem lesions, localization of, 586
Breath frequency, measurement of, 322
Breath sounds, 316
in cardiovascular disorders, 408–409, 409f
Breed, thyroid gland function and testing and, 1060–1062
Breeding
artificial insemination in
with cooled shipped semen, 1266
with fresh semen, 1266
of frozen semen, 1266–1267
equine embryo transfer in, 1267–1268
mare soundness examination for, 1225
behavior in, 1226
external genitalia examination in, 1226–1227, 1227f
general physical examination in, 1226
hormonal assays in, 1236–1238, 1238f
identification in, 1225
ovarian pathology in, 1228–1230, 1232f
restraint in, 1226
signalment and history in, 1225–1226, 1225t
transrectal palpation and ultrasound examination in, 1227–1228, 1228t, 1229f–1230f
uterine pathology in, 1230–1236
mount, selection of, 1323
optimizing pregnancy rates in, 1264–1265
pasture, 1265
stallions, 1327–1330
exercise for, 1327
herd health management for, 1327–1328
Breeding *(Continued)*
measuring reproductive performance of, 1330
methods for, 1328–1330
novice or maiden, 1330
nutrition of, 1327
records of, 1318–1319
vaginal trauma during, 1240
Bretylium tosylate, for heart disease, 457t–461t
Bridging fibrosis, 850
Bridging necrosis, hepatic, 850, 876
Broad-spectrum antimicrobials, for umbilical remnant infections, 1408–1409
Bromide, for seizures, 607
Bromocriptine
for hepatic insufficiency, 864
for pituitary pars intermedia dysfunction, 1108
Bromsulphthalein (BSP), 857t, 861
Bronchial sounds, 316
Bronchiolar pattern, radiographic, 320–322, 321f
Bronchoalveolar lavage (BAL)
in recurrent airway obstruction, 358
sampling for, 318–319, 319t
Bronchoconstriction, in cough reflex, 246
Bronchodilators
for interstitial pulmonary disease, 352–353
for respiratory disorders, in foals, 1396–1397, 1397t
Bronchopleural fistulae, 351
Bronchopneumonia, alveolar radiographic pattern in, 320–322, 321f
Bronchus-associated lymphoid tissue, 38
Bruising, perineal, 1310
BSP. *see* Bromsulphthalein
Bulbourethral glands, 1315
evaluation of, 1322
Bullous pemphigoid, 1165t–1167t, 1195–1196
BUN. *see* Blood urea nitrogen
Bupivacaine, for pain management, 144t–145t
Buprenorphine, for pain management, 144t–145t
Burkholderia mallei, systemic manifestations of, 1165t–1167t
Burns, 1165t–1167t, 1197–1198
Butorphanol
for acute rhabdomyolysis, 549
for meconium impaction, 1401
for pain management, 145
N-butylscopolamine, for pain management, 144t–145t, 148

C

CAB abstracts, 220
Cachexia, 242
Cadmium toxicity, 1507
Calbindins, 1039
Calcitonin, 1040
Calcitonin-gene-related peptide (CGRP), 767

Calcium
absorption of, 1031–1032, 1031t–1032t, 1032f
in cardiac cell electrical activity, 413
deficiency, 1033, 1040–1049
disorders in, 1040–1049
distribution of, 1029–1033, 1030f
elimination of, 1032–1033, 1033t
homeostasis, 1035–1037, 1035f
hypercalcemia, 1048
hypocalcemia, 1040–1042, 1040t–1041t
physical properties of, 1029–1033, 1030f
regulation of, intracellular, 551
requirements in, 1030–1031, 1030t–1031t
supplementation of, 164
Calcium borogluconate, for hypocalcemic tetany, 574
Calcium channel blocker, 457t–461t
Calcium cycling, aberrant, rhabdomyolysis and, 544–545
Calcium disodium EDTA, for lead toxicity, 1483
Calcium metabolism, in acute renal failure, 930
California encephalitis, 662
Caloric balance, 558
Caloric intake, 182
of neonatal foals, 203
restriction of, 212
Caloric testing, in vestibular disease, 628
Calvarium, bony fractures of, 615–616
Campylorhinus lateralis, 794
Candida albicans
adhesins in, 8
in fungal pneumonia, 354
macrophages and, 8
Candida spp. infections, respiratory, in foals, 1394
Cannon keratosis, 1171
Cantharidiasis, 1043–1044
Cantharidin toxicity, 748t, 757–758
Cantharidin toxicosis, 1466–1467
Capsid, 8
viral infection and, 9f
Capsules
bacterial, 7
fungal, 8
Carbamate pesticides, 1471
Carbamazine, 681, 681t
Carbapenems, pharmacology of, 105
Carbohydrates
acute diarrhea associated with, 748t, 759
classification and nomenclature of, 197–198
digestion and, 199–201
minimizing, recommendations for, 201–202
in equine nutrition, 197–202
evaluation of, in feeds, 198
metabolism of
liver's role in, 846, 846f
testing of, in hepatic insufficiency, 859
Carbohydrates *(Continued)*
nonstructural, 194b, 198, 199t
nutrition, colic and, 199
Carbon disulfide toxicity, 1475–1476
Carcinoma
hepatocellular, 879
medullary, 1068–1069
renal cell, 969, 970t–971t
squamous cell
of conjunctiva and third eyelid, 1143, 1143f
of cornea, 1150–1151, 1150f
cutaneous, 1206–1208
of eyelid, 1141–1142, 1141f
Cardiac catheterization, 398b, 451–453, 452f
Cardiac cycle, 392–395, 393f–395f
Cardiac impulse, 392–395
Cardiac index, 396
Cardiac output
cardiovascular circulation and, 173
in congestive heart failure, 457
measurement of, 177
in ventricular function, 396
Cardiac tamponade
examination in, 397
pericardial disease and, 485
sudden cardiovascular death and, 399b
Cardiac troponin-I, 398b
Cardiogenic shock, 173
Cardiometabolic syndrome, 1085–1086
Cardiomyopathy, dilated, 447f, 491
Cardiopulmonary arrest (CPA), in foals, 1370–1371
Cardiopulmonary resuscitation, for neonatal foals, 1370–1373, 1372f, 1372b
Cardiovascular diseases
anatomic correlates of, 387–392, 388b–389b, 390f–391f
arrhythmias, 497–517. *see also* Arrhythmias.
auscultation in, 399–409
algorithm for, 406t–407t
of cardiac murmurs, 403f–404f, 405–408, 408b
clinical method for, 399–403, 400t–402t, 402f–403f
of heart rate and rhythm, 395f, 401t, 404–405
of heart sounds, 403–404, 404f
pulmonary, 408–409, 409f
of conduction systems, 391–392, 391f
congenital heart disease, 462–471
atrial septal defects, 463f, 468, 469f
clinical pathophysiology of shunts in, 464–465
patent ductus arteriosus, 468–469, 469f
pathogenesis of, 463–464, 463f
pulmonary atresia with ventricular septal defect, 470–471, 470f
tetralogy of Fallot, 469–470, 470f
Cardiovascular diseases *(Continued)*
tricuspid atresia, 471
truncus arteriosus communis, 471
ventricular septal defects, 463f, 465–468, 465f–467f
congestive heart failure, 455–462
causes of, 455, 455f
clinical recognition of, 456–457
examination of, 411f
therapy for, 457–462, 457t–461t
diagnostic and laboratory studies in, 388b, 398b, 411–454
echocardiography, 419–451. *see also* Echocardiography.
electrocardiography, 413–418. *see also* Electrocardiography.
exercise testing, 411–413
intravascular pressures and cardiac catheterization, 451–453, 452f
laboratory studies, 453–454
thoracic radiography, 418–419, 419f
dilated cardiomyopathy, 447f, 491
endocardial, 390–391, 391f
examination in, 397–454
general approach to, 397, 398b
history in, 397–398, 399b
myocardial disease, 388–390, 389b, 390f, 486–491, 487f–489f
myocarditis, 490
pericardial disease, 388, 484–486, 484f–485f
peripheral vasculature, examination of, 409–411
poor performance and, 291
toxic injury of the myocardium, 399b, 490–491
toxicoses and, 1484
valvular heart disease, 390–391, 391f, 471–484, 472f
aortic regurgitation, 476–479, 476f, 478f–479f
infective endocarditis, 481–484, 482f–483f
mitral regurgitation, 472–476, 473f
pulmonary regurgitation, 481, 481f
tricuspid regurgitation, 479–480, 480f
vascular diseases, 389b, 392, 491–497
aortocardiac and aortopulmonary fistula, 493f, 496–497
aortoiliac thrombosis, 493f–494f, 495–496
arteriovenous fistulas, 496
parasitic vascular lesions, 494f, 496
pulmonary artery rupture, 497
thrombophlebitis, 482, 492f, 493–495
Cardiovascular support, endotoxemia and, 737–738
Cardiovascular system
critical care in
cardiovascular insufficiency and, 173
cardiovascular physiology and, 173
monitoring, 175–176
therapies for, 174–175, 176t
vasoactive drugs and, 174
disorders of, 387–541. *see also* Cardiovascular diseases.
physiology of, 392–397
cardiac cycle in, 392–395, 393f–395f
ventricular function in, 395–397, 395b
tetracyclines and, 114
Carnitine, for polysaccharide storage myopathy, 559
Carpal flexion, causing dystocia, 1302
Carprofen, pharmacology of, 123
Carrier, in adaptive immunity, 23
Case reports, 225–227
Case-control study, 227
Caslick vulvoplasty, 1239
Cassia obtusifolia, rhabdomyolysis from, 568
Castor bean toxicity, 1461
Catagen phase, of hair cycle, 1160, 1161f
Cataplexy
in foals, 1385
narcolepsy and, 609
Cataract, 1153–1154, 1153f–1154f
Catecholamines, 1080
pain and, 138
Catheterization
cardiac, 398b, 451–453, 452f
in equine herpesvirus-1 myeloencephalopathy, 650
for fluid administration, 161, 162t
ureteral, 919, 921f
urinary, 173
Cattle management, for insect control, 1163b–1164b
Caudal medulla oblongata, traumatic injury to, 615t
Caudal (posterior) presentation, 1297, 1302–1303
CBC. *see* Complete blood count
CD_3 complex, 27
$CD4^+$ T lymphocyte, 27, 28f
$CD8^+$ T lymphocyte, 27, 28f
in foals, 39
CD28 antigen, 31–32
CD40 antigen, 31–32
CDAD. *see* Clostridium difficile-associated diarrhea
CDE. *see* Chronic degenerative endometritis
Cecal impaction, 786
Cefadroxil, 103t, 105
Cefazolin, 103t
for foals, 1422t
Cefepime, for foals, 1421–1423, 1422t
Cefotaxime, for foals, 1422t
Cefoxitin, 102–104, 103t

Cefpodoxime, for foals, 1421–1423, 1422t
Cefquinome, for foals, 1421–1423
Ceftiofur
  as aerosolized antimicrobials, 1397t
  for equine herpesvirus-1 myeloencephalopathy, 650
  for foals, 1422t
  for Lyme disease, 679
  pharmacology of, 102–104, 103t
Ceftiofur crystalline-free acid
  for endometritis, 1253, 1254t
  for pleuropneumonia, 350t
Ceftiofur sodium
  for endometritis, 1253, 1254t
  for foals, 1421–1423, 1422t
  for pleuropneumonia, 350t
Ceftriaxone, 102–104, 103t
Celiotomy, for hemoperitoneum, 1006
Cell death
  in bacteria, 7
  in fungi, 8, 12
  in virus, 9
Cell-based coagulation model, 1001f
Cell-mediated immunity, 32–33
  in foals, 39–40
  tests of, 49–52
Cell-mediated type IV hypersensitivities, 44
CEM. *see* Contagious equine metritis
Centaurea solstitialis toxicity, 1479–1480
Central compartment
  in pharmacokinetics, 79–80
  volume of, 82
Central control, of ventilation, 253
Central nervous system (CNS)
  depression of, toxicoses and, 1478
  examination of, 580–588
    form for, 581f–582f
    gait evaluation in, 585–586
    localization of lesion in, 586, 587t
    normal and abnormal gaits, 586–588
  increased body temperature and disorders of, 233
  in *S. equi* infection, 336
  stimulation of, toxicoses and, 1468
  trauma, 610–622
    spinal cord and vertebral injury, 618–622, 618t
    traumatic brain injury. *see* Traumatic brain injury
Central regulation, of cough, 246
Central sensitization, 138
Central venous pressure
  monitoring, 169, 176–177
  in traumatic brain injury, 617–618
Central vestibular disease, 624f, 627–628
Centrilobular zonal injury, hepatic, 850
Cephalexin, for foals, 1422t
Cephaloridine, in acute tubular necrosis, 926
Cephalosporins, 99–100, 102–105
  in acute tubular necrosis, 926
  clinical use of, 105
  critically ill horse and, 186
  for foals, 1421–1423
  formulations of, 105
  pharmacokinetics of, 104–105
  spectrum of activity, 102–104, 103t
Cephalothin, 103t
Cephapirin, 103t
Cephradine, 103t
Ceragyn, for endometritis, 1255t, 1256
Cerebellar abiotrophy, 630–631, 631f
  in foals, 1385
  neurologic examination of, 585
Cerebellar cortex, 628
Cerebellar medulla, 628–629
Cerebellar peduncles, 628, 629f
  damage to, 624
Cerebellum, 622
  diseases of, 628–632
    abiotrophy and degeneration, 630–631, 631f
    clinical signs of, 630
    developmental abnormalities, 631–632
    Gomen disease, 631
    infectious conditions, 632
  neurologic examination of, 583
  structure and function of, 628–630, 629f
  traumatic injury to, 611, 615t
Cerebral cortex, 622
  seizures and, 603
Cerebrospinal fluid
  in equine herpesvirus-1 myeloencephalopathy, 649
  in equine motor neuron disease, 667
  in equine protozoal myeloencephalitis, 643
  evaluation of, 588–591, 589f, 591t
  in polyneuritis equi, 652
  in spinal cord and vertebral injury, 620
  in temporohyoid osteoarthropathy, 626
  in traumatic brain injury, 613, 615f
  in verminous encephalomyelitis, 683, 684t
  in West Nile virus, 661
Cerebrum, traumatic injury to, 615t
Cervical spinal cord, localization of lesion in, 586
Cervical vertebral compressive myelopathy, 633–636, 633f
  clinical signs of, 634
  diagnosis of, 634–636, 635f, 636t
  pathogenesis of, 634
  treatment of, 636
Cervicitis, 1241
Cervicofacial reflex
  of neonatal foals, 1381, 1382t
  neurologic examination of, 584
Cervix, 1218, 1219f, 1240–1241
  dilation of, hormones in, 1262–1263
  examination of, 1232–1233
Cestrum diurnum, 940, 1494
C-fibers, 245–246
CGRP. *see* Calcitonin-gene-related peptide
Champignon, 1338
Charcoal, activated, for tetrachlorodibenzodioxin toxicity, 1466
Cheek, teeth, 791
Chemicals, rhabdomyolysis from, 568–569
Chemokines
  in gastrointestinal inflammation, 717f
  in immune response, 22
  in type I hypersensitivity, 43
Chemoreceptors, in ventilation control, 251–253
Chemotaxis, in acute inflammatory response, 22–23
Chemotherapy
  for sarcoids, 1142
  for squamous cell carcinoma, 1142
Chenodeoxycholate, 848
Chest compression, in neonatal resuscitation, 1371–1373
Chestnut, skin structures and, 1160
Chewing disease, 1479
Chirality, of nonsteroidal antiinflammatory drugs, 121–122
Chiropractic manipulations, for pain management, 151
Chloral hydrate, for traumatic brain injury, 617
Chloramphenicol (CPHC)
  for foals, 1422t, 1424
  pharmacology of, 109
  for pleuropneumonia, 350t
Chlorate toxicity, 1465
Chlorhexidine, for dermatophilosis, 1173
Chlorhexidine diacetate, for patent urachus, 897
Chloride, in pathophysiology of colitis, 721, 722t
Chlorinated hydrocarbons toxicity, 1473–1474
Chlorpheniramine, for allergic skin disease, 1193
Chlortetracycline, pharmacology of, 112
Choanal atresia, in foals, 1391
Choking down, in dorsal displacement of soft palate, 328
Cholangiocarcinoma, 879
Cholangiohepatitis, 869, 879
Cholangitis, 850
Cholate, 848
Cholelithiasis, 876–878, 877f–878f
Cholesteatoma, 628
Cholinesterase-inhibiting insecticides, 1471–1472
Cholinomimetics, for gastrointestinal ileus, 777
Chondroids, in guttural pouch empyema, 327
Chorioptes equi infestation, 1182–1183, 1182f–1183f
Chorioretinitis, 1154
Chromium, for polysaccharide storage myopathy, 559
Chronic active hepatitis, 850, 875–876
Chronic blood loss, disorders associated with, 1006–1007
Chronic degenerative endometritis (CDE), 1247, 1247f–1248f
Chronic generalized hepatic injury, 850
Chronic infiltrative endometritis. *see* Endometriosis
Chronic interstitial nephritis (CIN), 936–937, 937f
Chronic kidney disease (CKD), 930
  clinical signs of, 938–939, 939f
  diagnosis and laboratory evaluation in, 939–943, 940t, 941f–944f
  of other cause, 937
  predisposing conditions in, 930–937
    chronic interstitial nephritis, 936–937, 937f
    end-stage kidney disease, 937
    glomerulonephritis, 931–936, 931f–935f, 936t
  progression of, 943–944
  treatment of, 944–946
  uremic syndrome in, 937–938
  uremic toxins in, 937–938
Chronic megalocytic hepatopathy, 874–875, 874f, 874t
Chronic progressive lymphedema, 1211
Chrysops, hypersensitivity to, 1190
Cimetidine
  for gastroduodenal ulceration, 806
  for melanoma, 1209
CIN. *see* Chronic interstitial nephritis
Ciprofloxacin, 116
CIRCI. *see* Critical illness-related corticosteroid insufficiency
Circulation, 392
  fetal, 464
Cirrhosis, 850
Cisapride, for gastrointestinal ileus, 777
Cisplatin
  for sarcoids, 1205
  for squamous cell carcinoma, of eyelid, 1142
CKD. *see* Chronic kidney disease
Clarithromycin, 114
  for foals, 1422t, 1423–1424
Classical pathway, of complement system, 21
Claviceps purpurea, 1501–1502
Clavulanic acid (CA), 102
  for foals, 1421
Clearance, of drugs, 86, 87t
  hepatic, 87–88, 861–862
  renal, 87, 87f, 87t
Cleft palate, 794
  deformities, in foals, 1391
Clenbuterol, for uterine relaxation, during late gestation, 1367
Climbing fibers, 629

Clinical assessment, for malabsorption and maldigestion, 741–747
Clitoris, 1219–1220, 1222f, 1239
Clonal thrombocytosis, 1018
Clopidogrel, 1005
Closed model, 79–80
Clostridial infections, systemic manifestations of, 1165t–1167t
Clostridial myonecrosis, 564
  clinical signs of, 564, 565f
  diagnosis of, 564
  pathophysiology of, 564
  treatment of, 564
Clostridium botulinum, 672–673
  botulism due to, 1384
Clostridium difficile, acute diarrhea associated with, 748t, 752
Clostridium difficile-associated diarrhea (CDAD), 1403
Clostridium organisms, acute diarrhea associated with, 748t, 752
Clostridium perfringens
  acute diarrhea associated with, 748t, 752
    fecal evaluation in, 282
  necrotizing enterocolitis due to, 1403
Clostridium piliforme, 868
  Tyzzer's disease due to, 1417
Clostridium tetani, 668
  tetanus due to, 1384
Clot formation, 999
Clotrimazole
  for endometritis, 1258, 1259t
  for guttural pouch mycosis, 326
Clover poisoning, 875
Cluster differentiation antigen 14 (CD14), 726
Coagulation
  cell-based, model of, 1001f
  disseminated intravascular, in endotoxemia, 730–731, 738
  factors in, 999t
  hemostasis and, 999–1000
Coccidioides immitis, toxin of, 8
Coccidioidomycosis, for interstitial pulmonary disease, 354
Cochlea, 624, 628
Codocyte, 993
Cohort study, 227
Coil sets, for fluid administration, 162
Coital exanthema, equine, 1177, 1239
  transmission of, through semen, 1340
Colchicine, for hepatic insufficiency, 864
Colic, 272–278
  ancillary diagnostic tests for, 276–277
  approach to, 274–278
  carbohydrate nutrition and, 199
  clinical pathology of, 276–277
  conditions associated with, 273–274
  cyathostomins, 15
  definition of, 272
Colic *(Continued)*
  diagnostic imaging for, 277
  endoscopy for, 277
  in foals, 1400
  gastrointestinal causes of, 274t
  in hepatic insufficiency, 855
  mechanisms of, 273
  Parascaris spp. and, 14
  physical examination of, 275–276
  in polyneuritis equi, 652
  referral indications of, 277–278
  signalment and history of, 274–275
  Strongylus vulgaris and, 14
Colitis, 721. *see also* Diarrhea
  pathophysiology of, 721
Collagen disorders, 1171–1173
Collapsibility
  conditions causing, 266–267, 266b
  diagnostic approach to, 267–268
  diagnostic imaging of, 268
Colloid osmotic pressures, 259–260, 261f, 261b
  monitoring of, in critical care, 177
Colloids, for fluid therapy, 166–167, 167t
  in shock, 175
Colon
  physical examination of, 710
  trocarization of, 172, 172b
Colonoscopy, for gastrointestinal disorders, 1401
Colony-stimulating factors (CSFs), 22
Colorado State University model artificial vagina, 1323
Color-coded Doppler imaging, 440–443, 449f
  in mitral regurgitation, 471–472
  in tricuspid regurgitation, 479
  in ventricular septal defects, 467
Colostrum, in passive transfer of maternal antibodies, 40, 55–56
Commensal bacteria, 3
Common variable immunodeficiency, 54
Compartment models, 79–80
Compensated insulin resistance, 1088
Complement
  activation, in endotoxemia, 731
  acute inflammatory response, 20–23, 20t
  in foals, 38–39
Complement cascade, in gastrointestinal inflammation, 719
Complete blood count (CBC), 992f, 999–1001
Complete malabsorption, 714
Complex repetitive discharges, 595
Compound 1080 toxicity, 1493
Computed tomography
  gastrointestinal, 713
  in respiratory disorders, 317–318, 318f
Concentration-dependent antimicrobials, 1420
Concentric fibrosis, hepatic, 878f
Conduction disturbances, 514–517, 515f–516f
Conduction systems, 391–392, 391f
  disorders of, anatomic correlates of, 391–392, 391f
Congenital abnormalities
  esophageal, 802
  hepatic, 880–881
  oral, 794–796
Congenital aganglionosis, 1401
Congenital conditions
  of accessory glands, 1338
  of epididymides, 1337
  of spermatic cord, 1338
Congenital enzyme defect, in hemolysis, 1010
Congenital heart disease, 389b, 462–471
  atrial septal defects, 463f, 468, 469f
  clinical pathophysiology of shunts in, 464–465
  patent ductus arteriosus, 468–469, 469f
  pathogenesis of, 463–464, 463f
  pulmonary atresia with ventricular septal defect, 470–471, 470f
  tetralogy of Fallot, 469–470, 470f
  tricuspid atresia, 471
  truncus arteriosus communis, 471
  ventricular septal defects, 463f, 465–468, 465f–467f
Congenital neurologic disorders, 1385
  cataplexy as, 1385
  cerebellar abiotrophy as, 1385
  epilepsy as, 1385
  hydrocephalus as, 1385
  narcolepsy as, 1385
  occipitoatlantoaxial malformation as, 1385
Congenital patent urachus, 897
Congenital upper respiratory tract disorders, 1391
Congenital urinary tract disorders, 1406
Congestive heart failure, 455–462
  causes of, 455, 455f
  clinical recognition of, 456–457
  examination of, 411f
  therapy for, 457–462, 457t–461t
Conjugated bilirubin, 848–849
  in hepatic insufficiency, 857
Conjunctival disorders, 1142–1143
Connecting peptide, 1080
Consciousness, traumatic brain injury and, 615t
CONSULTANT, 220
Contact dermatitis
  allergic, 1192–1193
  irritant, 1188
Contact hypersensitivity, 1192–1193
Contact photosensitization, 1198
Contact system of plasma, 719
Contagious equine metritis (CEM)
  causing endometritis, 1248–1249
  transmission of, through semen, 1340
Contemporary veterinary clinical practice, 218
Continuous positive airway pressure (CPAP), for acute respiratory distress syndrome, 1389
Continuous rate infusion (CRI), 91, 210
  for acute renal failure, 1409–1410
Continuous-wave (CW) Doppler, 443–444, 449f, 467–468
  in tricuspid regurgitation, 470f
Contractility, myocardial, 396, 413
Contraction, intestinal, 774
Contrast echocardiography, 440
Cooled shipped semen, 1266
Coordination
  loss of, cerebellar disease and, 630
  neurologic examination of, 583–585
Cor pulmonale, 389b, 454–455
Corn oil, 201
Cornea, anatomy of, 1143–1144
Corneal disorders, 1143–1151
  eosinophilic keratitis in, 1149–1150
  Friesian stromal loss in, 1148, 1148f
  grafting biomaterials for, 1149
  idiopathic nonulcerative keratitis in, 1150
  ulcer in, 1143–1144, 1144f
Corneal grafting biomaterials, for cornea, 1149
Corneal ulceration, in vestibular disease, 624–625
Cornified layer, epidermal, 1160
Coronary ligament, of liver, 843
Corpora nigra cyst, 1151, 1151f
Cortex
  cerebellar, 628
  cerebral, 622
    seizures and, 603
Corticoid:creatinine ratio, in pituitary pars intermedia dysfunction, 1106
Corticosteroids
  for acute rhabdomyolysis, 549–550
  for chronic kidney disease, 945
  for critically ill horse, 185
  for cutaneous habronemiasis, 1186
  for diarrhea, 283
  for endotoxemia, 736
  for equine protozoal myeloencephalitis, 644
  for immune-mediated myositis in Quarter horse-related breeds, 567
  for immunosuppression, 58
  as immunosuppressor, 59–60, 60t
  for interstitial pulmonary disease, 352–353
  for pain management, 143
  for spinal cord injury, 621
  for traumatic brain injury, 617
  for verminous encephalomyelitis, 684–685
Cortisol, 1071
  pain and, 138
Corynebacterial infections, 1174–1175

Corynebacterium pseudotuberculosis
purpura hemorrhagica and, 46
systemic manifestations of, 1165t–1167t
in ulcerative lymphangitis, 1174
Corynebacterium species, afimbrial adhesins in, 6
Cosyntropin, 1075
Cough, 244–250
in bacterial pneumonia, 346
causes of, associated with fever, 247–248, 247b
conditions associated with, 247–249
cycle of, 245, 246f
diagnostic approach to, 249–250
diagnostic imaging of, 250
efficiency/adverse effects, 247
mechanisms of, 244–247
neurophysiology of, 245–247, 246f
Coumarin toxicity, 1486–1488
CpG sequences, DNA motifs, 62
Crackles, 258, 316
Cranial (anterior) presentation, 1297, 1301–1302
Cranial nerves
involvement of, in polyneuritis equi, 652
of neonatal foals, 1381
neurologic examination of, 583
in traumatic brain injury, 614–615
Craniocervical dislocation, 1277–1278
Creatine kinase, in cerebrospinal fluid, 591t
Creatinine
in acute kidney injury, 1409
measurement of, 183
metabolism, 899
Cresty neck score, 239t
CRI. *see* Continuous rate infusion
Crista, 622
Critical appraisal, 222–223
Critical care, 158–190
basic procedures in, 161
abdominocentesis, 171–172, 171b
arterial blood pressure, monitoring, 168–169, 168f–169f, 168t
fluid therapy, 163–165, 166t
planning, 163–165
vascular access and administration of, 161–163, 162t
cardiovascular, 173
cardiovascular insufficiency and, 173–174
cardiovascular physiology and, 173
monitoring, 175–176
therapies for, 174–175
vasoactive drugs and, 174
central venous pressure, 169
equations in, 164t
gastrointestinal, 181
intensive care units, 158–161
emergency drugs in, 159, 160t
equipment in, 159, 159b
Critical care *(Continued)*
nasogastric intubation, 170–171
neurologic, 184
renal, 182
respiratory, 177–178
acid-base status, assessment of, 180–181
arterial blood gas analysis and, 178
blood gas analysis, 180
hypercapnia, 178
hypoxemia, 178
hypoxia and, 178
inhalation therapy, 179
oxygen delivery to tissues and, 177
oxygen therapy, 178–179, 179f
pharmacologic ventilation, 179–180
pulse oximetry, 181
respiratory distress and, 178–181, 179b
thoracocentesis, 170
tracheostomy, 169–170, 170b
trocarization, 172, 172b, 173f
urinary catheterization, 173
Critical illness-related corticosteroid insufficiency (CIRCI), 1072, 1411
Cross-sectional studies, 227
Crotalaria species, 874f, 874t
Crude protein (CP), 194b
Crusting dermatosis
cannon keratosis, 1171
generalized granulomatous dermatitis, 1213, 1213f
linear keratosis, 1171
seborrhea, 1170–1171
Cryopreservation, of oocytes and embryos, 1312
Cryotherapy
for endotoxemia, 738
for sarcoids, 1204–1205
Cryptococcus neoformans, capsule of, 8
Cryptorchidism, 1317, 1335–1336
Cryptosporidium parvum, in severe combined immunodeficiency, 52
Cryptosporidium spp., in foal diarrhea, 1404
Crystalloid fluids, for traumatic brain injury, 617
Crystalloid solutions, for fluid therapy, 165t, 166, 1425
in shock, 175
CSF. *see* Colony-stimulating factors
Culicoides, hypersensitivity to, 45, 1190, 1190f
Culicoides imicola, in African horse sickness, 345
Curli fimbriae, 6t
Curly coat, 1169
Curschmann's spirals, 358
Cutaneous drug reactions, 1188
Cutaneous lymphoma, 1210
Cutaneous trunci reflex, of neonatal foal, 1382t
Cyanide, 1492–1493
Cyanobacterial poisoning. *see* Blue-green algae toxicity
Cyathostomiasis
acute diarrhea and, 754
therapies for, 762
Cyathostomins, 15
resistance and, 17–18
Cyclic adenosine monophosphate (cAMP), fever and, 235
Cyclooxygenase, in inflammatory response, 21–22
Cyclooxygenase (COX)
fever and, 235
in inflammatory response, 143–145
inhibition of, 120
Cyclooxygenase 1 (COX-1), 143
renal function, 183
Cyclophosphamide, as immunosuppressor, 60
Cyclosporine, for immunosuppression, 60
Cyproheptadine, for trigeminal-mediated headshaking, 681
Cystic calculi, 954–956, 954f–956f
Cystitis, 946–947
in equine herpesvirus-1 myeloencephalopathy, 648
Cystometrography, 923
Cysts
cervical, 1241
corpora nigra, 1151, 1151f
dermoid, 1211
endometrial, 1231, 1232f, 1242–1243
esophageal duplication, 802
oral, 794
renal, 893
serous hepatic, 881
Cytogenetic testing, for stallions, 1326
Cytokines, 23f, 32–33, 33t
acute inflammatory response, 20–23, 20t
fever and, 234
in gastrointestinal inflammation, 717f
for immunostimulation, 60
as inflammatory mediators, 726–727
in paraneoplastic disease, 372
in viral infection, 12–13
Cytotoxic drugs, as immunosuppressor, 59–60

D

Daily rhythm, thyroid gland function and testing and, 1062
Damage-associated molecular patterns, 20–21
Damalinia equi, 1181
Dandy-Walker syndrome, 631
Danofloxacin, 116
Dantrium sodium
for acute rhabdomyolysis, 549–550
for focal myopathy, 569–570
for recurrent exertional rhabdomyolysis, 553
Datura toxicity, 1462
Dazzle reflexes, in neonatal foals, 1381
De novo antibody production, 40–41
Death, sudden cardiovascular, 399b
Decay, dental, 794
Deciduous teeth, 790
Decompression, gastric, 740, 740f
Decubital ulcers, 186, 1197
Deep compartments, 79–80
Deep intrauterine insemination, 1312–1313
Deer flies, hypersensitivity to, 1190
Deferoxamine, for iron toxicity, 1504
Defibrillation, in neonatal resuscitation, 1373
Degeneration
cerebellar, 630–631, 631f
hepatic, 850
Degenerative left-shift, 996
Deglutition, in normal swallowing, 268
Dehydration
in acute renal failure, 924
evaluation of, in fluid therapy, 163, 163t
Dehydroepiandrosterone sulfate, 1076
Delayed hypersensitivity, 46
Demodex species, 1184
Demodicosis, 1184
Dendritic cells, 30, 31f
Dental decay, 794
Dental disease, sinusitis and, 323, 324f
Dentition, 790–791
Deoxycholate, 848
Deoxyribonucleic acid (DNA), viral infection and, 8, 9f
Deoxyribonucleic acid-protein kinase (DNA-PK), 52
Depolarization
of cell, 413, 413f
P wave and, 416–418, 417f
Depression, central nervous system, toxicoses and, 1478
Dermacentor species, 1184
Dermanyssus gallinae infestation, 1184
Dermatitis
atopic, 43, 1191
contact
allergic, 1192–1193
irritant, 1188
equine exfoliative eosinophilic, 1165t–1167t
generalized granulomatous, 1213, 1213f
pastern, 1181, 1181f
Dermatophilosis, 1173–1174, 1173f
systemic manifestations of, 1165t–1167t
Dermatophytosis, 1177–1178, 1178f, 1180t
Dermis, 1159
Dermoid, conjunctival, 1142
Dermoid cysts, 1211
Descemetocele, 1144, 1144f
Descriptive observational studies, 225–227
O-desmethyl-tramadol, for pain management, 148
Desmopressin, for diabetes insipidus, 965
Desmopressin acetate, 921, 921f

Detachment, retinal, 1154
Detomidine
  for acute rhabdomyolysis, 549
  for esophageal obstruction, 799
  gastrointestinal ileus and, 775
  for hepatic insufficiency, 863
  for pain management, 144t–145t
Detoxification, hepatic, 849
Detrusor muscle, 973
Developmental abnormalities, cerebellar, 631–632
Developmental conditions
  of penis, 1332–1333
  of scrotum, 1334
  of semen and spermatozoa, 1339
Developmental malformations, of urinary system
  bladder defects in, 896–897
  ectopic ureter in, 894–895
  patent urachus in, 897–898
  pendulant kidney in, 894
  polycystic kidney disease, glomerulocystic disease, and other hereditary nephropathies in, 893–894, 894f
  rectourethral and rectovaginal fistulae in, 896, 896f
  renal agenesis, hypoplasia, and dysplasia in, 891–893, 893f
  renal cysts in, 893
  ureteral defects or tears in, 895–896
  vascular anomalies in, 894, 895f
Dexamethasone
  for cutaneous habronemiasis, 1186
  for immune-mediated myositis in Quarter horse-related breeds, 567
  for interstitial pulmonary disease, 352–353
  for lymphoid pharyngeal hyperplasia, 327
  for spinal cord injury, 621
  for verminous encephalomyelitis, 684–685
Dexamethasone suppression test, 1106
Dexmedetomidine, for pain management, 144t–145t
Dextrose
  in hyperkalemia, 517
  in parenteral mixtures, 205t
D-Glucose absorption test, 714
Diabetes insipidus, 287, 1050
  dipsogenic, 963
  nephrogenic, 964–966
  polyuria and polydipsia in, 964–966
Diabetes mellitus, 287, 1084
  polyuria and polydipsia in, 966
Diaphragmatic hernias, 781
Diarrhea, 278–283
  acute, differential diagnoses for, 279t
  chronic, differential diagnoses for, 280t
  conditions associated with, 279–281
Diarrhea *(Continued)*
  definition of, 278
  diagnostic approach to, 281–283
    clinical pathology of, 281–282
    diagnostic imaging of, 283
    feces evaluation in, 282–283
    history and physical examination in, 281b
  in foals, 1401–1404
    differential diagnoses for, 280t
  in hepatic insufficiency, 855
  mechanisms of, 278–279
  pathophysiology of, 721, 722t
  seasonal, 15
  therapy for, evaluation of response to, 283
  weight loss associated with, 242–243
Diastole
  function of, 395b, 397
  murmur and, 400t, 402f
Diathesis, hemorrhagic, 855–856
Diazepam
  for seizures, 606
  for traumatic brain injury, 617
DIC. *see* Disseminated intravascular coagulation
Diclazuril, 644
Diclofenac, 123
  for pain management, 144t–145t
Dictyocalus arnfieldi infections, in respiratory system, 16
Diencephalon, traumatic injury to, 615t
Diestrus, 1224, 1229f
Diet
  for cervical vertebral compressive myelopathy, 636
  evaluation of, 196–197
  hypoallergenic, 1192
  for polysaccharide storage myopathy, 558–559
  recurrent exertional rhabdomyolysis and, 552
Dietary carbohydrates, for body weight, 238–239
Dietary imbalances, sporadic exertional rhabdomyolysis and, 549
Dietary management, for cystic calculi, 956
Dietary protein, in chronic kidney disease, 944
Dietary supplementation, 1193
Diethylcarbamazine, 684
Diffusion impairment, 178, 255
Difloxacin, 116
Digallic acid, 1460
Digestible energy (DE), 194b, 211t
  requirements
    for mature horses, 203
    for thin and starved horses, 213
Digestion, 181
  carbohydrates and, 199–201
  foregut, 199–200
  hindgut, 200–201
  test of, in disorders of the gastrointestinal system, 714
Digestive disturbances, minimizing, 201–202
Digitalis glycosides, 457t–461t
Digoxin
  for atrial fibrillation, 501f–502f, 504
  for congestive heart failure, 457t–461t, 461–462
Dihydrostreptomycin, 627
Dilated cardiomyopathy, 447f, 491
Dilation
  cervical, hormones in, 1262–1263
  gastric, 808
Diltiazem, 457t–461t
Dimercaprol
  for arsenic toxicity, 1463
  for mercury toxicity, 1508
Dimethyl sulfoxide (DMSO)
  for acute rhabdomyolysis, 549–550
  for allergic skin disease, 1194
  for cervical vertebral compressive myelopathy, 636
  for critically ill horse, 185–186
  for cutaneous habronemiasis, 1186
  for endometritis, 1255, 1255t
  for endotoxemia, 736
  for equine herpesvirus-1 myeloencephalopathy, 650
  for equine protozoal myeloencephalitis, 644
  for focal myopathy, 569–570
  for hepatic insufficiency, 864
  for lymphoid pharyngeal hyperplasia, 327
  for neonatal encephalopathy, 1386
  for spinal cord injury, 621
  toxic effects of, 1488
  for verminous encephalomyelitis, 684–685
Dioctophyma renale, in urinary tract infections, 949
Dioctyl sodium sulfosuccinate, toxic effects of, 1462
Diphenhydramine, for allergic skin disease, 1193
Diphtheritic membrane, 320–322, 325
Dipsogenic diabetes insipidus, 963
Direct Coombs test, 44f
Discoid lupus erythematosus, 1196
Disorders of sexual development (DSD), 1332
Displacement
  dorsal, of soft palate, 328–329, 328t
  of large intestine, 710
  rostral, of palatopharyngeal arch, 330
Disposable artificial vagina liners, 1324
Disseminated hemangiosarcoma, 371
Disseminated intravascular coagulation (DIC)
  in endotoxemia, 730–731, 738
  in plasma-based coagulation system, 1015
Distention
  abdominal, 240, 709
  gastrointestinal ileus and, 775
Distichiasis, 1141
Distillates, petroleum, 1464
Distribution, of drugs in the body, 82–85, 82f–83f
  aminoglycosides, 106–107
  aminopenicillins, 101
  amphenicols, 109
  antipseudomonal penicillins, 102
  cephalosporins, 104
  drug dosage regimens and, designing, 89–90
  fluoroquinolones, 117
  macrolides and azalides, 115
  metronidazole, 119
  minimum inhibitory concentrations and, 95–96, 96f
  penicillin G, 100
  rifampin, 119
  sulfonamides, 110–111
  tetracyclines, 113
Diuretics
  for acute renal failure, 929
  for congestive heart failure, 457–461
  for critically ill horse, 185
  for heart disease, 457t–461t
Dive-bomber potentials, 595, 595f
Diverticula, esophageal, 802
DNA-PK. *see* Deoxyribonucleic acid-protein kinase
Dobutamine
  in critical care, 174
  endotoxin and, 737–738
  for foals, 1429
  for heart disease, 457t–461t
Documentation of infection, 95
Dog-sitting posture, 1301
Domperidone
  for gastrointestinal ileus, 777
  for tall fescue toxicity, 1497
Domperidone response test, 1108
Donor mare, in equine embryo transfer, 1268–1269
Dopamine
  in critical care, 174
  endotoxin and, 737–738
  for foals, 1429–1430
  for heart disease, 457t–461t
Dopamine antagonists, for gastrointestinal ileus, 777
Doppler studies
  in aortic regurgitation, 477
  in arteriovenous fistulas, 496
  in congestive heart failure, 456–457
  echocardiographic, 394f, 419, 440, 441b–443b, 449f
  in infective endocarditis, 483–484
  in mitral regurgitation, 474f, 475
  in patent ductus arteriosus, 469
  in thrombophlebitis, 492f, 494–495
  in tricuspid regurgitation, 480
  in valvular heart disease, 471–472
  in ventricular septal defects, 466f, 467–468
Doppler ultrasound
  in critical care, 169
  for monitoring pregnant mares, 1288

Dosage
adjustment of, 91–92
in renal failure, 92–94
intervals greater than half-life, 89, 90f
intervals less than half-life, 89, 89f
opioids, 145–146
pharmacokinetics for determination of, 79–80
regimen design of, 89–91, 90t
for antimicrobials, 95, 97–98
Dose-reduction method, 93
Dourine
causing endometritis, 1249
transmission of, through semen, 1340
Down horses, in critical care, 186–187
Doxapram, for cardiopulmonary resuscitation, of foals, 1373
Doxepin, for allergic skin disease, 1193
Doxycycline
for foals, 1422t, 1423
for Lyme disease, 679
pharmacology of, 112
for pleuropneumonia, 350t
rifampin and, 114
Draft crosses, polysaccharide storage myopathy and, 555
Draft horse, polysaccharide storage myopathy and, 555
Drainage, in pleural effusion, 170
Draschia megastoma, in verminous encephalomyelitis, 682–683
Drug toxicities, 627
Drug-induced immunodeficiency, 58
Drugs, 79
accumulation of, 89, 89f
bioavailability of, 84
cutaneous reactions to, 1188
designing of dosage regimens of, 89–91, 90t
distribution of, 82–85, 82f–83f
elimination of, 79–80, 85
rate constant and half-life, 85–89, 85f–86f, 86t
emergency, 159, 160t
for heart disease, 457t–461t
increased body temperature and, 233–234
lipid solubility and ionization of, 84, 84b
in pain management, 140–149, 144t–145t
α_2-adrenoreceptor agonists, 144t–145t, 146–147
antiinflammatory drugs, 143–145
opioids, 144t–145t, 145–146
pharmacokinetics of, 79
definitions in, 79–80
protein binding, 85
rates and orders of reactions of, 80–81, 80f
clinical application of compartmental modeling and, 81–82, 81f–82f
renal failure and, 92–94
Drugs *(Continued)*
therapeutic drug monitoring and, 91–92, 92t
toxicity of, hepatic, 870
variation between drug dose and plasma drug concentration, 79
vasoactive, 174
Dry gangrene, 1197
Dry matter (DM), in feed, 194b
DSD. *see* Disorders of sexual development
Duodenitis-proximal jejunitis, 738
clinical and clinicopathologic signs of, 739–740
pathophysiology of, 738–739
prognosis for, 741
treatment of, 740–741, 740f
Dutasteride, 1077
D-Xylose absorption test, 714
Dynamic compliance (C_{dyn}), measurement of, 322
Dysautonomia, equine. *see* Equine grass sickness
Dysmaturity syndrome, with hypothyroidism, 1066–1067, 1067f
Dysmetria, 585, 630
Dysmorphism, 958
Dysphagia, 268–272, 584, 791–793, 792t–793t
conditions associated with, 269–271
diagnostic approach to, 271–272
diagnostic imaging of, 272
in equine grass sickness, 676
in foals, 1391
functional causes of, 270–271, 270b
guttural pouch mycosis and, 326–327
mechanisms of, 268–269
morphologic causes in, 269–270, 269b
neurologic examination of, 272
nutritional myodegeneration and, 1419–1420
weight loss associated with, 241–242
Dysplasia
cerebellar, 632
renal, 891–893, 893f
in foals, 1406
Dyspnea, 250–251
Dystocia
etiology of, 1299
management of, 1299–1303
caudal (posterior) presentation, 1302–1303
cranial (anterior) presentation, 1301–1302
fetal anomalies, 1303
transverse presentation, 1303
Dystrophy
black or white follicle, 1169
equine neuroaxonal, 636–640
mane and tail, 1169

E

E protein, 660
Eastern equine encephalomyelitis (EEE), 655, 656t
Easy keepers, 203, 1088
Eating
normal, 268
problems with. *see* Dysphagia
EBM. *see* Evidence-based medicine
Ecbolics, for endometritis, 1253–1255
Echinocyte, 993
Echium plantagineum, 874t
Echocardiography, 419–451, 421t–431t, 440b–443b, 444f–449f
in aortic regurgitation, 477, 478f–479f
in atrial fibrillation, 504
in atrial septal defects, 468, 469f
in congestive heart failure, 456
in diagnostic studies, 398b
in infective endocarditis, 483–484
in mitral regurgitation, 474f, 475
in pericardial disease, 484f, 485
pharmacologic stress and, 413
in poor performance evaluation, 291
in pulmonary regurgitation, 481, 481f
in tricuspid regurgitation, 480, 480f
ventricular function and, 394f
in ventricular septal defects, 466f, 467
Eclampsia, postpartum, 1310
Ectopic cilia, 1141
Ectopic rhythms, auscultation of, 401t
Ectopic testis, 1335
Ectopic ureter, 894–895
in foals, 1406
Eczema, 43
Edema, 258–263
compensatory mechanisms limiting, 262
conditions associated with, 262–263, 263t
definition of, 258–259
diagnostic approach to patient with, 263
in hepatic insufficiency, 856
mechanisms of formation of, 261–262
physiology of fluid movement, 259–261
ventral, in infective endocarditis, 483f
EEE. *see* Eastern equine encephalomyelitis
Efferent pathways, activation of, 246–247
Effusion, pleural, 170
EHM. *see* Equine herpesvirus myeloencephalopathy
EHV-1, as latent viral infection, 10
EHV-3, 645
EHV-4, 645
as latent viral infection, 10
EHV-5, 645
EIPH. *see* Exercise-induced pulmonary hemorrhage
Ejaculation
failure of, 1331–1332
pharmacologically induced, for semen collection, 1324
Ejection fraction, 392–395
Ejection murmur, systolic, 392–395
Ejection period, of cardiac cycle, 392–395
Electrocardiography, 413–418
in atrial fibrillation, 506, 507f
in atrial premature complexes, 500f
cardiac cycle and, 392, 395f
clinical, 415–418, 415f, 415b–416b, 416t, 417f–418f
for collapse, 268
in diagnostic studies, 398b
genesis of, 414, 414f
monitoring of, in critical care, 177
normal cardiac cell electrical activity and, 413–414, 413f
P wave in, 392
for uroperitoneum, 1407
Electrocution, 399b
Electroencephalography (EEG), 598–599, 599f
for collapse, 268
for hypoxic ischemic encephalopathy, 1382–1383
Electrohydraulic lithotripsy, 953–954
Electrolytes
abnormalities of, 687
balance of, 901t, 902–903
in endotoxemia, 732
fractional clearance of, 915–917, 916t
imbalances, sporadic exertional rhabdomyolysis and, 549
in penicillin G therapy, 101
for recumbent horses, 186–187
therapy, for gastrointestinal ileus, 776
Electromyography, 591–595, 592t
abnormal potentials in, 593–595
in focal and multifocal myelopathies, 595–596
in laryngeal hemiplegia, 596–597
musculoskeletal system disorders and, 547
normal potentials in, 592–593
in radial and suprascapular nerve injury, 596
Electrophoresis, serum protein, 49
Elimination, of drugs, 79–80
aminoglycosides, 107
aminopenicillins, 101
amphenicols, 109
antipseudomonal penicillins, 102
cephalosporins, 104
erythromycin, 115
fluoroquinolones, 117–118
macrolides and azalides, 115
metronidazole, 119
penicillin G, 100
rate constant and half-life, 85–89, 85f–86f, 86t
rifampin, 119
sulfonamides, 111
tetracyclines, 113

ELISA. *see* Enzyme-linked immunosorbent assay
Elliptocyte, 993
Eltenac, for endotoxemia, 735
Embase, 220
Embolism, sudden cardiovascular death and, 399b
Embryo
  cryopreservation of, 1312
  loss of, 1278–1279
  transfer of, 1268–1269
EMND. *see* Equine motor neuron disease
EMS. *see* Equine metabolic syndrome
Enalapril, 457t–461t, 462
Encephalitis, viral, 654–659, 655t
Encephalomyelitis, verminous, 682–685, 684t
Encephalopathy
  hepatic, 851–854, 851t, 853f, 864t
  hypoxic ischemic, 1381–1383
  metabolic, 1384–1385
  neonatal, 1381–1383
    treatment of, 1385–1386, 1386t–1387t
  sepsis-associated, 1383
End-diastolic dimensions, 396
Endocardial diseases, anatomic correlates of, 390–391, 391f
Endocarditis
  chronic, 475
  infective, 481–484, 482f–483f
Endocrine system disorders, 1029–1138
  anhidrosis in, 1110
    clinical signs of, 1112
    diagnosis of, 1112
    epidemiology of, 1110
    pathophysiology of, 1111–1112
    physiology of, 1110–1111
    prevention of, 1112–1113
    prognosis of, 1112–1113
    treatment of, 1112–1113
  calcium in, 1040–1049
    hypercalcemia, 1048
    hypocalcemia, 1040–1042, 1040t–1041t
  equine metabolic syndrome in, 1085
    clinical pathology of, 1090
    clinical signs of, 1089–1090, 1089f–1090f
    definition of, 1088
    diagnostic testing in, 1090–1100
    differential diagnoses of, 1089–1090, 1089f–1090f
    etiology of, 1088–1089
    genetics in, 1089
    management of, 1098–1099
    treatment of, 1100
  insulin dysregulation in, 1085
    clinical pathology of, 1090
    clinical signs of, 1089–1090, 1089f–1090f
    definition of, 1088
    diagnostic testing in, 1090–1100
Endocrine system disorders *(Continued)*
    differential diagnoses of, 1089–1090, 1089f–1090f
    etiology of, 1088–1089
    genetics in, 1089
    management of, 1098–1099
    treatment of, 1100
  magnesium in, 1052
    hypermagnesemia, 1058
    hypomagnesemia, 1056–1057
  phosphorus in, 1029, 1046f, 1049–1052, 1051t
    parathyroid hormone in, 1035
  pituitary pars intermedia dysfunction in, 1085, 1100
    clinical signs of, 1100–1102, 1101f
    diagnostic testing of, 1105–1108
    epidemiology of, 1102
    laboratory testing of, 1104
    pathogenesis of, 1102–1103, 1104f
    pathologic findings of, 1103–1104, 1105f
    treatment of, 1101f, 1108–1109
  pituitary-independent hyperadrenocorticism (adrenal Cushing syndrome), 1109
  thyroid gland in, 1058–1069
    hyperthyroidism, 1068
    hypothyroidism, 1061t, 1065
    thyroid tumors in, 1068–1069
Endocrinologic testing
  for stallions, 1326
Endocrinology
  of parturition, 1272–1273
  of pregnancy, 1271–1272
  of stallions, 1317
Endocrinopathic laminitis, 1088
Endogenous creatinine clearance, 1409
Endogenous pyrogens, in fever, 234
Endometriosis, 1241–1242
Endometritis, 1230–1231, 1231t, 1242
  diagnostics of, 1249–1252
    adjunctive techniques in, 1252
    clinicopathologic techniques in, 1233t, 1250
    history in, 1249–1250
  fungal, 1257–1258, 1257f–1258f, 1259t
  pathophysiology of, 1246–1249
  treatment strategies for, 1252–1257
    adjunctive therapies for, 1256–1257
    antibiotics for, 1252–1253
    ecbolics for, 1253–1255
    lavage and nonantibiotic therapies for, 1255–1256, 1255t
  uterine therapy and, 1246
Endometrium
  assessment of, 1250–1252
  biopsy of, 1242
    in endometritis, 1251–1252
Endometrium *(Continued)*
    in mare breeding soundness examination, 1234–1236, 1235f
  culture and cytology of
    in endometritis, 1233t, 1250–1251
    in mare breeding soundness examination, 1233–1234, 1233t
  cysts in, 1242–1243
    mare breeding soundness examination and, 1231, 1232f
Endoscopy
  for body temperature, 237
  for colic, 277
  for cough, 250
  for disorders of the gastrointestinal system, 713–714
  for duodenitis-proximal jejunitis, 740f
  for dysphagia, 272
  for gastrointestinal disorders, diarrhea, 283
  for pharyngeal and laryngeal region, 584
  for respiratory disorders, 316–317, 317f
    in guttural pouch tympany, 325
    for *S. equi* infections, 335
    in sinusitis, 323
  for respiratory distress evaluation, 258
  for urinary system, 919
  for weight loss, 244
Endothelial cells, 22
  in cellular effectors of inflammation, 716–719
  dysfunction and damage, in endotoxemia, 729
Endotoxemia, 181, 723–725
  causes, 725–731
  cellular activation by lipopolysaccharide in, 726
  clinical pathologic testing in, 732–733
  clinical signs and diagnosis of, 731–732
  cryotherapy for, 738
  endotoxins and, 723–724
  in hepatic insufficiency, 856
  inflammatory mediators in, 726–727, 727t–728t, 729f
  management of, 733–738
    antibodies directed against tumor necrosis, 737
    inhibition of endotoxin release, 733
    inhibition of mediator synthesis, 735–736
    platelet-activating factor receptor antagonists, 737
    scavenging of lipopolysaccharide, 733
    supportive care, 737–738
  pathogenesis of, 729
  polyuria and polydipsia in, 966
Endotoxin
  in diarrhea, 722t, 723
  in endotoxemia, 723–724
  in gastrointestinal ileus, 775
  translocation of, 725
End-stage kidney disease (ESKD), 931, 937
End-stage liver disease, 850
Energy
  alternative sources of, 201
  increased, causes of demand, 241–242
  requirements of
    for foals, 207, 209t
    for mature horses, 211t
Enilconazole, for fungal pneumonia, 356t
Enoxaparin, 484
Enrofloxacin
  for endometritis, 1253, 1254t
  for foals, 1424
  pharmacology of, 116
  for pleuropneumonia, 350t
Enteral feeding
  assisted, 204–205, 204t
    transition to voluntary feeding, 207
  in critically ill horse, 182
  withdrawal, 204
Enteral nutrition (EN), 204
  for sick neonatal foals, 203
Enteric nervous system, 715
Enteritis
  granulomatous, 742–745, 743t–744t
  in severe combined immunodeficiency, 52
Enterocentesis, 172
Enterocolitis
  acute, 749–750
  eosinophilic, 743t–744t, 745
  lymphocytic-plasmacytic, 743t–744t, 745
Enteroinsular axis, 1081
Enteroliths, 787
Enteropathy
  proliferative, 743t–744t, 745–746
  protein-losing, weight loss associated with, 242–243
  sand, 748t, 759
Entrapment
  epiglottic, 330
  epiploic foramen, 779
Entropion, 1141
Enucleation, orbital, 1156
Enveloped viruses, 8, 11f
Envenomation, 1189
  by bees or snakes, in hemolysis, 1010
Environment, recurrent exertional rhabdomyolysis and, 552
Enzyme electrophoresis, for *Streptococcus equi* subsp. *equi* infection, 331
Enzyme-linked immunosorbent assay (ELISA)
  for antibody-mediated immunity, 48
  in botulism, 675

Enzyme-linked immunosorbent assay (ELISA) *(Continued)*
in equine herpesvirus-1 myeloencephalopathy, 649
in equine protozoal myeloencephalitis, 643
in passive immunity, transfer of, 56–57
in West Nile virus, 661
Enzymes
in cerebrospinal fluid, 590–591
drugs that induce or inhibit, 88, 88b
liver, 860–861
Enzymuria, 913–915, 1409
Eosinophilic dermatitis and stomatitis, equine exfoliative, systemic manifestations of, 1165t–1167t
Eosinophilic enterocolitis, 743t–744t, 745
Eosinophilic epitheliotropic disease, multisystemic, 743t–744t, 745, 1213
Eosinophilic granuloma, 1212–1213, 1212f
Eosinophilic keratitis, corneal, 1149–1150, 1149f
Eosinophils, 996–997
in bronchoalveolar lavage fluid, 319t
in type I hypersensitivity, 43
EPE. *see* Equine proliferative enteropathy
Epidemic, togaviral encephalitis as, 656
Epidemiology, evidence-based medicine and, 218–231
evidence, acquire, 219
evidence to practice, apply, 219
experimental studies, 227
information, sourcing, 221
intervention, assessing, 219
evidence, and quality of evidence, 219
identifying information, 219–220
literature, searching, 221–228
veterinary literature database, 220–221
veterinary scientific literature, 219
limitations of, 222t–223t
methodology for, 225t
observational studies, 225–227
pertinent question, ask, 218–219
quality of evidence
appraise, 219
evaluating, 221–225
levels of, 225t
recommendation, 218–219
synthesis of evidence, reviews, 228
recommendation, practice guidelines, 228–229, 229t
Epidermis, 1159
Epididymides, 1315
abnormalities of, 1337–1338
Epididymis, 1314
Epidural catheters, for pain management, 149, 149f
Epiglottic entrapment, 330
Epilepsy, 603
in foals, 1385
Epileptic seizures, 603
Epinephrine
for cardiopulmonary resuscitation, 1373
in critical care, 174
for foals, 1430
for heart disease, 457t–461t
for systemic anaphylaxis, 45
Epiploic foramen entrapment, 779, 779f
Epistaxis, in horse, 994b
Epithelial cells, in bronchoalveolar lavage fluid, 319t
Epithelial restitution, 770–771
Epithelium
inflammation of, 715
toxicoses and, 1494
Epitopes, in adaptive immunity, 23
Epoxide hydrolase inhibitors, for pain management, 148
Equids, demonstrating facial expressions of pain, 143f
Equine adenovirus infections, in foals, 1393
Equine arteritis virus infection, 344
Equine axillary nodular necrosis, 1212
Equine bone fragility syndrome, 1048–1049
Equine coital exanthema, 1177
Equine coronavirus, 741
diarrhea due to, 1402
Equine Cushing's disease, 963
Equine cutaneous amyloidosis, systemic manifestations of, 1165t–1167t
Equine degenerative myeloencephalopathy, 636–640
clinical signs of, 636–637
diagnosis of, 639
pathology of, 637
pathophysiology of, 637–639, 638f–639f
prognosis of, 640
treatment and prevention of, 639–640
Equine DNA virus, 867
Equine dysautonomia. *see* Equine grass sickness
Equine encephalosis virus, 663
Equine endocrine pancreas, 1079, 1081–1083
anatomy of, 1079
disease in, 1084
insulin secretion from, 1083–1084
physiology of, 1079–1081
and pituitary pars intermedia dysfunction, 1085
role of, in equine metabolic syndrome, 1084–1085
Equine exfoliative eosinophilic dermatitis and stomatitis, systemic manifestations of, 1165t–1167t
"Equine gastric ulcer syndrome," 803
in adults, 805–806
diagnosis of, 806, 806t
neonatal, 805
pathophysiology of, 803–804, 804f
prevalence of, 803
risk factors of, 804–805
in sucklings and early weanling foals, 805, 805f–806f
treatment of, 806–808
Equine grass sickness, 676–678
Equine herpesvirus infection, 339–344
clinical signs of, 340
diagnosis and treatment of, 340–341
in hepatitis, 869
pathogenesis of, 340
Equine herpesvirus myeloencephalopathy (EHM), 340–341
Equine herpesvirus type 3 (EHV-3)
causing endometritis, 1249
transmission of, through semen, 1340
Equine herpesvirus-1, 1418
associated with myelitis, 340
infection from, clinical signs of, 340
in myeloencephalopathy, 645–652
clinical signs of, 648
control and prevention of, 651–652
diagnosis of, 649
epidemiology and immunity in, 646–647
laboratory findings in, 648–649
pathogenicity and pathogenesis of, 647–648
pathologic findings in, 650–651
polyneuritis equi *versus*, 653
treatment and prognosis for, 649–650
virologic findings in, 645–646
upper respiratory disease from, 339–340
viral strain of, 340
Equine herpesvirus-2, 341
Equine herpesvirus-4, upper respiratory disease from, 339–340
Equine herpesvirus-5
equine multinodular pulmonary fibrosis and, 341–342, 343f
interstitial pulmonary disease and, 352–353
Equine immunology, 19–41
innate immunity and the acute inflammatory response in, 20–23
acute phase proteins, proinflammatory cytokines, and complement in, 20–23, 20t, 21f
chemotaxis and leukocyte trafficking in, 22–23
lipid mediators in, 21–22
Equine infectious anemia (EIA)
in hemolysis, 1008
in hepatitis, 869
Equine influenza virus (EIV), 338–339
pneumonia due to, 1393
Equine insulin resistance, 1090–1098, 1091t
baseline blood tests in, 1090–1092
clinical assessment of, 1090
diagnostic testing for, 1092–1093, 1093f
Equine intestinal clostridiosis, 752–753
Equine metabolic syndrome (EMS), 212, 1085
clinical pathology of, 1090
clinical signs of, 1089–1090, 1089f–1090f
definition of, 1088
diagnostic testing in, 1090–1100
differential diagnoses of, 1089–1090, 1089f–1090f
etiology of, 1088–1089
genetics in, 1089
management of, 1098–1099
treatment of, 1100
Equine motor neuron disease (EMND), 562–563
as neurologic disorder, 665–668, 665f
Equine multinodular pulmonary fibrosis, 321f, 341–342, 343f
Equine neuroaxonal dystrophy, 636–640
Equine pegivirus, 867
Equine picornavirus infection, 344–345
Equine polyneuropathy, acquired, in Scandinavia, 653
Equine proliferative enteropathy (EPE), 1404
Equine protozoal myeloencephalitis, 640–645
clinical signs of, 642
diagnosis of, 643–644
differential diagnosis for, 644
epidemiology of, 640–641
magnetic resonance imaging in, 601–602, 602f
pathogenesis of, 641–642
pathology of, 642
prevention of, 645
Sarcocystis spp. in, life cycle of, 641
treatment of, 112, 644–645
Equine recurrent uveitis, 46–47, 1152–1153
Equine rhinitis B virus, 344–345
Equine rhinovirus 1, 344–345
Equine rhinovirus 2, 344–345
Equine rhinovirus 3, 344–345
Equine sarcoidosis, 1213–1214, 1213f
Equine viral arteritis (EVA), 1012. *see also* Equine arteritis virus infection
causing endometritis, 1249
transmission of, through semen, 1340

Equipment
in intensive care units, 159, 159b
in magnetic resonance imaging, 600
Equisetum toxicity, 1479
Erbovirus, 344–345
Erection failure, 1331
Ergopeptines, 1496
Ergot, 1160
Ergotamine, 1501
Ergotism
gangrenous, 1501–1502
nervous, 1469–1470
systemic manifestations of, 1165t–1167t
Eruption, of teeth, 790
disorders of, 793–794
Erythema multiforme, systemic manifestations of, 1165t–1167t
Erythematosus
discoid lupus, 1196
systemic lupus, 1165t–1167t, 1196
Erythrocytes, 991–994
equine, oxidant-induced damage in, 1009
evaluation of, 991–993, 992b, 993t
interpretation of, 993–994, 994b–995b
physiology of, 991
Erythrocytosis, 1018
Erythromycin
for foals, 1422t, 1423–1424
for gastrointestinal ileus, 777
pharmacology of, 114
Erythromycin estolate, for infective endocarditis, 484
Erythropoiesis, 991
ESC. *see* Ethanol soluble carbohydrates
Escape complexes, 511
Escape rhythms, 511, 515f
Escherichia coli, inhibition of, by amoxicillin, 95, 96f
ESCs. *see* Ethanol soluble carbohydrates
ESKD. *see* End-stage kidney disease
Esophageal disorders, weight loss associated with, 242
Esophageal duplication cysts, 802
Esophagitis, 800–801, 800f
Esophagus, 796–803
disorders of, 789–791, 797t
congenital, 802
esophagitis, 800–801, 800f
motility disorders in, 801
diverticula, 802
obstruction, 796, 798f–799f, 798t
role of, in normal eating, 268
stricture, 801–802
Estrous cycle, synchronizing, hormones for, 1259–1260
Estrus, 1219f, 1222–1224, 1227f
suppression of, hormones for, 1260–1261
Etamsylate, for hemostatic dysfunction, 1004–1005
Ethacrynic acid, in acute renal failure, 929
Ethanol soluble carbohydrates (ESCs), 194b, 198
Ethylenediamine dihydroiodide, organic, for fungal pneumonia, 356t
Ethylenediaminetetraacetic acid (EDTA), platelet counts on, 997–998
Etiocholanolone, 1076
Eupatorium rugosum, 1484–1485
rhabdomyolysis from, 568
Euthyroid syndrome, 1063
EVA. *see* Equine viral arteritis
Evans syndrome, 1008
Evaporation, in heat loss, 232
Evidence-based medicine (EBM), 218–231
evidence, acquire, 219
evidence to practice, apply, 219
experimental studies, 227
information, sourcing, 221
intervention, assessing, 219
evidence, and quality of evidence, 219
identifying information, 219–220
literature, searching, 221–228
veterinary literature database, 220–221
veterinary scientific literature, 219
limitations of, 222t–223t
methodology for, 225t
observational studies, 225–227
pertinent question, ask, 218–219
quality of evidence
appraise, 219
evaluating, 221–225
levels of, 225t
recommendation, 218–219
synthesis of evidence, reviews, 228
recommendation, practice guidelines, 228–229, 229t
Evidentiary value, in evidence-based medicine, 224–225, 224f
Examination
in cardiovascular disorders, 397–454
auscultation in, 399–409
algorithm for, 406t–407t
of cardiac murmurs, 403f–404f, 405–408, 408b
clinical method for, 399–403, 400t–402t, 402f–403f
of heart rate and rhythm, 395f, 401t, 404–405
of heart sounds, 403–404, 404f
pulmonary, 408–409, 409f
in congestive heart failure, 411f
general approach to, 397, 398b
history in, 397–398, 399b
in mitral regurgitation, 474–475
peripheral vasculature, 409–411
clinical, in nutritional status, 191–196
in disorders of the gastrointestinal system, 709–711
endoscopic, uterine, 1236
mare breeding soundness, 1225
neurologic, 580–588
Examination *(Continued)*
form for, 581f–582f
gait evaluation in, 585–586
localization of lesion in, 586, 587t
normal and abnormal gaits, 586–588
in traumatic brain injury, 613
of newborn foal, 1367–1369
ophthalmic, 1139–1140
of urinary system, 909
Exanthema, equine coital, 1177, 1239
transmission of, through semen, 1340
Excessive bladder distention, 897
Excitotoxicity, 619
Excretion
of bile, 848–849, 848f
renal, of drugs, 87, 87f, 87t
of solute and water, 908–909
Exercise
aortoiliac thrombosis and, 495–496
for breeding stallions, 1327
for endometritis, 1257
exertional myopathies from, 548
hypocalcemia due to, 1043
immunodeficiency and, 57
for polysaccharide storage myopathy, 558
regimens of, 558
recurrent exertional rhabdomyolysis and, 550–552
thyroid gland function and testing and, 1062
Exercise testing
in cardiovascular disorders, 398b, 411–413
in electrocardiography, 415–416
for poor performance, 293–295
Exercise-associated hematuria, 961
Exercise-induced pulmonary hemorrhage (EIPH), 248, 365–368
clinical signs of, 366–367
definition of, 365
diagnosis of, 367, 367t
epidemiology of, 365–366
pathology of, 366
treatment of, 367–368
Exercise-related hyperthermia, 236
Exertional rhabdomyolysis, 1044
Exfoliative dermatoses
cannon keratosis, 1171
contact dermatitis
allergic, 1192–1193
irritant, 1188
dermatophilosis, 1173–1174, 1173f
dermatophytosis, 1177–1178, 1178f
linear keratosis, 1171
pemphigus foliaceus, 1194–1195, 1194f–1195f
seborrhea, 1170–1171
Exhaustion, sporadic exertional rhabdomyolysis and, 549
Exogenous compounds, thyroid gland function and testing and, 1063–1064
Experimental studies, in evidence-based medicine, 227
External device, for dorsal displacement of soft palate, 329
External genitalia
examination of, in mare breeding soundness examination, 1226–1227, 1227f
male, 1316
evaluation of, 1319–1322, 1322t
External hemorrhage, in horse, 994b
Extracellular fluid, 900
Extracellular matrix proteins, 718
Extracorporeal shock wave therapy, for pain management, 150–151
Extrasystoles, 415f
Exudate, tracheal, 317f
Eye disorders, 1139–1158
of conjunctiva and third eyelid, 1142–1143, 1143f
corneal. *see* Corneal disorders
of eyelids, 1139–1158, 1141f
of lens, 1153–1154
of nasolacrimal system, 1143
of optic nerve, 1154–1155, 1155f
orbital, 1155–1156
retinal, 1154
uveal, 1151–1153
Eyelids, disorders of, 1141–1142, 1141f
third, 1142–1143
Eyes
corneas, anatomy of, 1143–1144
disorders. *see* Eye disorders
examination of, 1139–1140
imaging for, 1140–1141
neurologic examination of, 583
topical anesthetics for, 1140

F

Face, neurologic examination of, 583
Facial nerve, 623
neurologic examination of, 584
paralysis of, 586, 624–625
in peripheral vestibular disease, 625, 626f
in traumatic brain injury, 613
Failure of transfer, of passive immunity, 55–57
Falciform ligament, of liver, 843
False-negative results, 643
Familial myeloid, 1017–1018
Farmer's lung, 43–44
Fasciculation potentials, 594–597
Fasciculations, pathophysiology of, 545
Fat necrosis, 1211–1212
Fats
controlling body weight and, 238–239
selection of source of, polysaccharide storage myopathy and, 559
Fatty acids
for allergic skin disease, 1193
for chronic kidney disease, 945

Fatty acids *(Continued)*
short-chain, 846, 847f
ω-3, 736
for endotoxemia, 736
FBP. *see* Fibronectin binding protein
Fecal examination, in disorders of the gastrointestinal system, 712
Feces
evaluation of, in diarrhea, 282–283
normal, characteristics of, 279
Feed additives, 202
Feed products
hyperkalemic periodic paralysis and, 573
polysaccharide storage myopathy and, 559
Feeding
frequency, in digestive disturbances, 201
obese horses, 212–213
thin and starved horses, 213–214
thyroid gland function and testing and, 1062–1063
Feeding programs
for breeding stallions, 1327
evaluation of, 212
diet and ration, 196–197
nutritional status and, 191–197, 192f–194f, 195t
terminology relevant to feed composition data, 194b
worksheet for, 192f–194f
minimizing digestive disturbances, 201–202
Feeds
botulism and, 673
diet and ration evaluation of, 196–197
evaluation of, carbohydrate content in, 198, 199t
requirements of estimating, 203–204
for mature horses, 203
terminology relevant to, 194b
withdrawal without ill effect, 202
Fell pony syndrome, 53
Fenbendazole, for parasitic pneumonitis, 357
Fenestrations, for clostridial myonecrosis, 564
Fentanyl
for gastrointestinal ileus, 775
for pain management, 144t–145t, 145–146
Fenthion, for cutaneous habronemiasis, 1186
Fertility
equine embryo transfer and, 1268
markers of, 1326
Fertilization, in vitro, 1312
Festuca arundinacea, 1496–1497
Fetal adrenal gland, 1071
Fetal circulation, 464
Fetal fluids, hydrops of, 1289–1290
Fetal heart rates, of neonatal foals, 1366
Fetal membranes, retained, 1306–1308
Fetal sexing, 1285–1286
Fetus
anomalies of, parturition and, 1303
early loss of, 1279–1280
kinetics of, during parturition, 1297–1298
Fever
causes of cough associated with, 247–248
causes of cough without, 248–249
conditions associated with, 236
documentation of, 236
in hepatic insufficiency, 856
increased body temperature, 234–236, 234f
in infective endocarditis, 483
paraneoplastic disease and, 371
pathogenesis of, 234–236, 234f
Fever of unknown origin, 236
Fiber, adequate, 201
Fibrillar adhesins (FAs), 5–6
Fibrillation, atrial, 503–509, 505b, 507f–508f
auscultation of, 401t
Fibrillation potentials, 594
Fibrils, 6t
Fibrinogen, hepatic disease and, 859
Fibrinolysis, hemostasis and, 1001
Fibrinolytic drugs, for hemostatic dysfunction, 1005–1006
Fibroblast growth factor 23 (FGF23), 1040
Fibroblastic sarcoids, 1203, 1203f
Fibroids, uterine, 1243
Fibronectin binding protein (FBP), 6
Fibrosis
congenital, 1418–1419
hepatic, 850
pulmonary, equine multinodular, 321f, 341–342, 343f
Fibrotic myopathy, 560
abnormal gaits associated with, 586–587
Filiform papillae, 790
Filtration *(Kf)* and reflection (σ) coefficients, 259
Fimbriae, 5–6
type I, 6t
Finasteride, 1077
Fire ant bites, 1189
Firocoxib, 123–124
for pain management, 144t–145t
for pleuropneumonia, 350t
First heart sound, 392–395, 403
auscultation of, 403, 404f
First order reactions, 88
First pass effect, 87
First-degree AV block, 514
First-degree burns, 1198
Fistulas
aortocardiac, 493f, 496–497
arteriovenous, 496
rectovaginal, 1240
postpartum, 1309
Flat sarcoids, 1204
Flavivirus encephalitis, 659–663
Flecainide
for atrial fibrillation, 506–507
for heart disease, 457t–461t
Fleroxacin, 116
Flexible rods, 6t
Flexor reflex, of neonatal foals, 1381, 1382t
Flick reflexes, of neonatal foals, 1382t
Flip-flop kinetics, 86, 87f
Flocculonodular lobe, 622
Flora, gastrointestinal, sulfonamides and, 112
Florfenicol (FLF), 109
for foals, 1424
Flow cytometry, in cellular immunity assessment, 51f
Fluconazole
for endometritis, 1258, 1259t
for foals, 1422t
for fungal pneumonia, 356t
for guttural pouch mycosis, 326
Fluid therapy, 163–165, 166t
for acute renal failure, 1409–1410
for endotoxemia, 737–738
for foals, 1424–1429
dosage of, 1427–1429
patient requirement for, 1425
routes of administration for, 1426–1427
types of fluids for, 1425–1426, 1426t
for gastrointestinal ileus, 776
for neonatal sepsis, 1379
orally administered, 165–167, 166t
planning, 163–165
for shock, 175
for traumatic brain injury, 617
vascular access and administration of, 161–163, 162t
Flumazenil, for hepatic insufficiency, 864
Flunixin meglumine
for acute rhabdomyolysis, 549
for equine herpesvirus-1 myeloencephalopathy, 650
for hepatic insufficiency, 864
for infective endocarditis, 484
for pain management, 144t–145t
for periparturient hemorrhage, 1305
for peritonitis, 765
pharmacology of, 124
for placentitis, 1366–1367
for pleuropneumonia, 350t
in retained fetal membranes, 1307
for spinal cord injury, 621
for thrombophlebitis, 495
for verminous encephalomyelitis, 684–685
Fluorescent antibody testing, in rabies, 664
Fluoride toxicity, 1499–1500
Fluoroquinolones, 116–118
adverse effects and drug interactions of, 118
clinical use of, 118
for foals, 1424
formulations of, 118
mechanism of action of, 116–117
mechanism of resistance of, 117
pharmacokinetics of, 117–118
Fluphenazine
for recurrent exertional rhabdomyolysis, 553
toxicity, 686
Flutter, atrial, 500f, 502–503
Foal heat diarrhea, 1401–1402
Foals
aminoglycosides in, 107
antiinflammatory and analgesic therapy for, 1431–1432, 1432t
antimicrobial therapy for, 1420–1424, 1422t
botulism in, 673–674
cerebellar abiotrophy in, 630
disorders of, 1365–1459
dysmaturity of, 1369–1370
endocrine disorders of, 1410–1414
dysfunction of somatotropic axis and energy regulation as, 1412–1414
hypothalamic-pituitary-adrenal axis function and dysfunction as, 1410–1412
hypothalamic-pituitary-thyroid axis function and dysfunction as, 1412
endotoxemia in, 737
examination of, 1367–1369
failure of transfer of passive immunity in, 55–57
Fell pony syndrome in, 53
fetal assessment, monitoring, and management of, 1365–1367
fluid therapy in, 1424–1429
dosage of, 1427–1429
patient requirement for, 1425
routes of administration for, 1426–1427
types of fluids for, 1425–1426, 1426t
gastric ulceration in, 805
gastrointestinal disorders of, 1400–1406
diarrhea as, 1401–1404
gastric ulceration as, 1404–1406, 1405t
hepatic disorders of, 1416–1419, 1417t
bacterial hepatitis as, 1417–1418
congenital fibrosis as, 1418–1419
due to equine herpesvirus 1, 1418
glycogen storage disease type IV as, 1418
hepatic lipidosis as, 1418
iron toxicity as, 1418
neonatal isoerythrolysis as, 1418
persistent hyperammonemia as, 1418
portosystemic shunts as, 1419
Tyzzer's disease as, 1417
hypothyroidism in, 1065–1066, 1066f
dysmaturity syndrome in, 1066–1067, 1067f
immune responses in

Foals *(Continued)*
adaptive cell-mediated, 39–40
antibody-mediated, 40–41
innate, 38–39
immunocompetence in, 38
immunologic disorders of, 1414–1416
failure of transfer of passive immunity as, 1414
neonatal alloimmune thrombocytopenia and neutropenia as, 1416
neonatal isoerythrolysis as, 1414–1416
inotrope and vasopressor therapy for, 1429–1430, 1429t
intrauterine growth retardation of, 1369–1370
muscular disorders of, 1419–1420
neonatal resuscitation of, 1370–1373, 1372f, 1372b
neonatal sepsis in, 1373–1380
clinical presentation of, 1377–1379
identification of, 1377–1379
management of, 1379
pathophysiology of, 1374–1377, 1374t, 1375f, 1375b
prognosis of, 1379–1380
risk factors for, 1377
neonatology of, 1365
neurologic disorders of, 1380–1386
bacterial meningitis as, 1383–1384
botulism as, 1384
congenital, 1385
hypoxic ischemic encephalopathy as, 1381–1383
metabolic encephalopathies as, 1384–1385
neonatal encephalopathy as, 1381–1383
seizures as, 1383
sepsis-associated encephalopathy as, 1383
tetanus as, 1384
trauma as, 1384
neurologic examination of, 1380, 1382t
nutritional management of, metabolism and nutrient requirements, 207
nutritional support of, 1432–1437, 1435t–1436t
orphan, 207–208
sick neonatal, 208–210, 209t
prematurity of, 1369–1370
respiratory disorders of, 1386–1400
acute lung injury as, 1387–1391, 1389b
acute respiratory distress syndrome as, 1387–1391, 1389b
bacterial, 1394–1396
congenital upper respiratory tract disorders as, 1391
Foals *(Continued)*
fungal pneumonia as, 1394
hemothorax as, 1392–1393
meconium aspiration as, 1391–1392
milk aspiration as, 1392
parasitic pneumonia as, 1394
persistent pulmonary hypertension as, 1391
pneumothorax as, 1392–1393
respiratory distress as, 1387
rib fractures as, 1392–1393
therapeutics for, 1396–1400
transient tachypnea as, 1392
viral pneumonia as, 1393
seizures in, 604, 604t
selective IgM deficiency in, 54–55
urogenital disorders of, 1406–1410
acute kidney injury as, 1409–1410
acute renal failure as, 1409–1410
congenital, 1406
umbilical, 1407–1409
vaccination in, tetanus, 672
vertebral trauma in, 618
Foamy degeneration, 850
Focal hepatic injury, acute, 850
Focal myelopathies, 595–596, 596f
Focal myopathy, 569–570
clinical signs of, 569
diagnosis of, 569
pathogenesis of, 569
prevention of, 570
treatment of, 569–570
Folia, 628
Foliate papillae, 790
Folic acid supplementation, 644
Follicular lymphoid hyperplasia, associated with cough, 249
Food allergies, 1191
Food-borne botulism, 673
Foot processes, 931–932
Foot-nape posture, 1301–1302
Forages, 196, 201
selection of
for polysaccharide storage myopathy, 558–559
recurrent exertional rhabdomyolysis and, 552
Forced oscillation method, for pulmonary function testing, 322
Foregut digestion, 199–200
Foreign body, conjunctival, 1142
Formaldehyde, for hemostatic dysfunction, 1004–1005
Formalin injection, 324
Fostering, orphan foals and, 207
Founder lines, 1088
Fourth heart sound, auscultation of, 403f
Fraction of inspired oxygen, 180
Fractional Cr clearance, 915
Fractional electrolyte clearance, 915–917, 916t
Fractional excretion, 915
Fractures
bony, of calvarium, 615–616
orbital, 1155
temporohyoid osteoarthropathy and, 625
Frank blood, in fecal examination, 282
Free radicals
in equine degenerative myeloencephalopathy, 639f
in spinal cord injury, 619
Fresh semen, 1266
Friesian stromal loss, 1148, 1148f
Frontal sinuses, sinonasal disease in, 323
Frostbite, 1198
Frozen equine plasma, 1004
Frozen semen, 1266–1267, 1325
Fructan, 198
Full-thickness burns, 1198
Fumonisin B1, 686
Functional diastolic murmurs, 408
Functional protodiastolic heart murmur, 395
Functional systolic ejection murmur, 392–395, 393f, 408
Fungal derivatives, for immunosuppression, 60
Fungal endometritis, 1257–1258, 1257f–1258f, 1259t
Fungal infections, 1177–1181, 1180t
damage to host tissues, 8
dermatophytosis as, 1177–1178, 1178f, 1180t
mycetomata as, 1179, 1180t
pathogenesis of, 7–8
phaeohyphomycosis as, 1179, 1180t
piedra as, 1178, 1180t
pythiosis as, 1180–1181, 1180f
sporotrichosis as, 1178, 1179f, 1180t
stachybotryotoxicosis as, 1181
Fungal keratitis, 1145f–1146f
Fungal pneumonia, 354–356, 1394
clinical signs of, 354
diagnosis of, 354–355
in immunosuppressed patients, 354
pathogenesis of, 354
treatment and prognosis in, 355–356, 356t
Fungiform papillae, 790
Furosemide
for acute renal failure, 1409–1410
in acute renal failure, 929
in congestive heart failure, 457–461
for heart disease, 457t–461t
for traumatic brain injury, 617–618
Fusobacterium spp., 7

G

Gabapentin, for pain management, 144t–145t, 148
Gait
in cerebellar disease, 630
evaluation, in neurologic examination, 585–586
Gait *(Continued)*
of neonatal foals, 1380, 1382t
normal and abnormal, 586–588
Gallic acid, 1460
Gallop, normal, 586
Gamete intrafallopian transfer (GIFT), 1313
Gamithromycin, 114
Gammaherpesviruses, 341
Ganglionopathy, peripheral, in Fell pony syndrome, 53
Gangrene, 1197
systemic manifestations of, 1165t–1167t
Gangrenous ergotism, 1501–1502
Garlic, consumption of, in hemolysis, 1010
Gastric decompression, for duodenitis-proximal jejunitis, 740
Gastric emptying
delayed, 808
evaluation of, 714
Gastric impaction, 808
Gastric ulceration, in foals, 1404–1406, 1405t
Gastrin, 804
Gastroduodenal ulcer syndrome (GDUS), 1405
Gastroduodenal ulceration, 803–806
in adults, 805–806
diagnosis of, 806, 806t
neonatal, 805
pathophysiology of, 803–804, 804f
prevalence of, 803
risk factors of, 804–805
in sucklings and early weanling foals, 805, 805f–806f
treatment of, 806–808
Gastrointestinal complications, postpartum, 1308–1309
Gastrointestinal hemorrhage, in horse, 994b
Gastrointestinal system
critical care in, 181
parasitic infections in, 14–16
tetracyclines and, 113
toxicoses related to, 1460
Gastrointestinal system disorders, 709–842, 1400–1406
in critically ill horse, 181
diarrhea as, 1401–1404
duodenitis-proximal jejunitis, 738
clinical and clinicopathologic signs of, 739–740
pathophysiology of, 738–739
prognosis for, 741
treatment of, 740–741, 740f
endotoxemia as, 723–725
causes, 725–731
cellular activation by lipopolysaccharide in, 726
clinical pathologic testing in, 732–733
clinical signs and diagnosis of, 731–732
endotoxins and, 723–724
inflammatory mediators in, 726–727, 727t–728t, 729f

Gastrointestinal system disorders *(Continued)*
management of, 733–738
pathogenesis of, 729
esophagus and, 796–803, 797t
examination in
absorption and digestion, 714
clinical pathology, 711–712
cross-sectional imaging in, 713
endoscopy for, 713–714
gastric emptying, 714
histopathologic, 715
laparoscopy for, 715
nuclear scintigraphy for, 713
physical, 709–711
radiography for, 712–713
ultrasonography for, 713
gastric ulceration as, 1404–1406, 1405t
ileus, 773–774
diagnosis of, 776
manifestation of, 774
pathophysiology of, 774
physiology of, 774–776
treatment of, 776–778
inflammation, 715
cellular effectors of, 716–719, 717f
humoral mediators of, 719
initiation of, 715–716
tissue injury during, 719–720
vascular response during, 716
ischemic, 778
of large intestine, 710
nonstrangulating, 783–784
rectal prolapse, 783f, 783t
strangulating obstruction, 778, 779f
malabsorption and maldigestion in, 741–747, 743t–744t
mucosal injury and repair, 766–767
growth factors and, 773
intestinal barrier function, 767
intestinal ischemia/ reperfusion injury, 768–770, 769f–770f, 769t
mechanisms of, 770–772, 771f
mediators of repair, 772–773, 772f
mucosal barrier function, 766–767
neoplasia, 809
oral, 789–790
congenital and developmental, 794–796
dental, 794
dysphagia, 791–793, 792t–793t
periodontal, 794
salivary glands and, 795–796
stomatitis and glossitis, 795
stomach and, 803–808
dilation and rupture, 808
gastroduodenal ulceration, 803–806
in adults, 805–806
diagnosis of, 806, 806t
neonatal, 805
Gastrointestinal system disorders *(Continued)*
pathophysiology of, 803–804, 804f
prevalence of, 803
risk factors of, 804–805
in sucklings and early weanling foals, 805, 805f–806f
treatment of, 806–808
impaction, 808
pyloric obstruction and delayed gastric emptying, 808
Gaze, neurologic examination of, 583
GDUS. *see* Gastroduodenal ulcer syndrome
Gender
determination of, 1285–1286
thyroid gland function and testing and, 1060–1062
Gene expression studies, molecular and, of endometritis, 1247–1248
General anesthesia, for traumatic brain injury, 617
Generalized granulomatous dermatitis, 1213, 1213f
Generalized postanesthetic myopathy, 570
clinical signs of, 570
diagnosis of, 570
pathogenesis of, 570
prevention of, 570
treatment of, 570
Generalized seizures, 603
Genetic factors
in obesity, 211–212
in viral infection, susceptibility, 14
Genetic screening, for malignant hyperthermia, 570
Genetic testing
for malignant hyperthermia, 554
for musculoskeletal system disorders, 546–547
for polysaccharide storage myopathy, 554–555
Genitalia
female, examination of, in mare breeding soundness examination, 1226–1227, 1227f
male
external, 1316
internal, 1314–1316
Genome, viral, 9
Gentamicin
in acute renal failure, 930
as aerosolized antimicrobials, 1397t
auditory system and, 627
for endometritis, 1253, 1254t
for foals, 1422t, 1423
pharmacology of, 105–106, 107t
for pleuropneumonia, 350, 350t
Gentamicin sulfate, for infective endocarditis, 484
Geriatric, definition of, 90
Germ cells, 1314–1315
Gestation
late, complications in, 1288–1294
hydrops of fetal fluids, 1289–1290
uterine torsion, 1291–1292
vaginal hemorrhage, 1292–1293
ventral body wall hernias and prepubic tendon rupture, 1290–1291
length of, 1294
physiology of, 1269–1274
GFR. *see* Glomerular filtration rate
Giant cell hepatopathy, 869
GIFT. *see* Gamete intrafallopian transfer
Gilbert's syndrome, 854–855
Glanders, systemic manifestations of, 1165t–1167t
Glanzmann's thrombasthenia, 1013
Glaucoma, 1154–1155, 1155f
Globulins, hepatic insufficiency and, 857t
Glomerular filtration, 905–907, 906t–907t
Glomerular filtration rate (GFR)
in acute renal failure, 925
in chronic kidney disease, 943, 944f
renal drug clearance and, 87
values of, in horses and ponies, 906t–907t
Glomerulocystic disease, 893–894, 894f
Glomerulonephritis, 924, 931–936, 931f–935f, 936t
acute, 926–927
Glomerulosclerosis, 933–934, 935f
Glossitis, 795
Glucagon, 1080
Glucocorticoids, 45
for cervical vertebral compressive myelopathy, 636
Gluconate, 457t–461t
Gluconeogenesis, 845, 1079–1080
Glucose
in cerebrospinal fluid, 591t
in equine motor neuron disease, 667
in hepatic insufficiency, 857t
in hyperkalemia, 517
for recumbent horses, 186–187
in traumatic brain injury, 617
Glucose-6-phosphate, 846
Glucose-to-insulin ratio, 1091–1092
Glutamate, hepatic formation of, 845–846
Glutamate dehydrogenase (GLDH), hepatic insufficiency and, 857t, 860
γ-glutamyltransferase (GGT), 276, 914
hepatic insufficiency and, 857t
in liver disease, 861
Glutathione peroxidase (GSH-Px), 561
Glycemic index, 1083
Glycine, in strychnine, 1474
Glycogen branching enzyme deficiency, 560–561, 881, 1419
clinical signs of, 560, 561f
diagnosis of, 560
pathogenesis of, 560
prevalence of, 560
treatment of, 561
Glycogen storage disease type IV, in foals, 1418
Glycogen synthase 1 gene *(GYS1)*, 554
Glycogenesis, 1079–1080
Glycogenolysis, 1079–1080
Glycolysis, 1079–1080
Glycopyrrolate, 457t–461t
for cardiopulmonary resuscitation, of foals, 1373
Gomen disease, 631
Gonadal atrophy, 1336
Gonadal hypoplasia, 1336
Google Scholar, 220
GRADE. *see* Grading of Recommendations Assessment, Development and Evaluation
Grading of Recommendations Assessment, Development and Evaluation (GRADE), 218
Grading quality, of evidence, in evidence-based medicine, 221
Gradual dietary changes, 202
Grafting biomaterials, corneal, 1149
Graft-*versus*-host disease, 1165t–1167t
Grain-concentrate meals, size of, 201
Gram-negative organisms, neonatal sepsis and, 1378, 1378t
Gram-positive organisms, neonatal sepsis and, 1378, 1378t
Granular cell tumors, 368
Granular layer, epidermal, 1159–1160
Granulocyte-colony stimulating factor, for immunostimulation, 60
Granuloma
bacterial, 1175, 1175f
cholesterol, 685
eosinophilic, 1212–1213, 1212f
Granulomatous dermatitis, generalized, 1213, 1213f
Granulomatous enteritis, 742–745, 743t–744t
Grass sickness, equine, 271, 676–678
Greasy heel. *see* Pastern dermatitis
Griseofulvin, for dermatophytosis, 1178
Growth factors, in mucosal repair, gastrointestinal, 773
Growth hormones, 1080–1081
Grüenhagen's space, 769, 769f
Guaifenesin, 617
Gut-derived incretin hormones, 1083
Guttural pouch
anatomy of, 324–325
dysphagia associated with, 269
empyema, 327
mycosis, 325–327
tympany, 325

Guttural pouch disease
neurologic examination in, 584
in temporohyoid osteoarthropathy, 625, 626f

H

H2RA, for gastric ulceration in foals, 1405–1406, 1405t
Habronemiasis
conjunctival, 1142
cutaneous, 1185–1186, 1185f
Haematobia, hypersensitivity to, 1190
Haematopinus asini, 1181
Hair
disorders of, 1169–1170
follicle abnormalities, 1169
pigmentation, 1161–1169
structure of, 1160, 1161f
Hairy vetch toxicity, 1495
Hairy vetch toxicosis, 1165t–1167t
Half-life elimination, of drugs, 85–89, 85f–86f, 86t
dosage intervals greater than, 89, 90f
dosage intervals less than, 89, 89f
Halicephalobus gingivalis
cerebellar, 632
in urinary tract infections, 948–949
in verminous encephalomyelitis, 682
Halothane anesthesia, interaction with aminoglycosides, 108–109
Hamartoma, mixed, 880
Hand rearing, orphan foals, 208
Hand walking, acute rhabdomyolysis and, 549
Hanover model artificial vagina, 1323
Haptens, in adaptive immunity, 23
Hard palate, 790
Hay dust, from recurrent airway obstruction and, 45
Hay nets, 213
Hays, with good nutritional value, 196
Head
neurologic examination of, 580–582
tilt of, in vestibular dysfunction, 623, 623f–624f
trauma to, 627
temporohyoid osteoarthropathy, 625–627
traumatic brain injury. *see* Traumatic brain injury
Head shakers, photic, 680–681
Headshaking
syndrome, 323
trigeminal-mediated, 680–682, 680t–681t
Hearing loss
brainstem auditory evoked response for, 597–598, 598f–599f
in peripheral vestibular disease, 628
Heart
physiology of, 392–397
cardiac cycle in, 392–395, 393f–395f
ventricular function in, 395–397, 395b
sagittal view of, 390f
Heart disease
anatomic correlates of, 387–392, 388b–389b, 390f–391f
arrhythmias, 497–517. *see also* Arrhythmias.
congenital heart disease, 462–471
atrial septal defects, 463f, 468, 469f
clinical pathophysiology of shunts in, 464–465
patent ductus arteriosus, 468–469, 469f
pathogenesis of, 463–464, 463f
pulmonary atresia with ventricular septal defect, 470–471, 470f
tetralogy of Fallot, 469–470, 470f
tricuspid atresia, 471
truncus arteriosus communis, 471
ventricular septal defects, 463f, 465–468, 465f–467f
congestive heart failure, 455–462
causes of, 455, 455f
clinical recognition of, 456–457
examination of, 411f
therapy for, 457–462, 457t–461t
diagnostic and laboratory studies in, 388b, 398b, 411–454
echocardiography, 419–451. *see also* Echocardiography
electrocardiography, 413–418. *see also* Electrocardiography
exercise testing, 411–413
intravascular pressures and cardiac catheterization, 451–453, 452f
laboratory studies, 453–454
thoracic radiography, 418–419, 419f
dilated cardiomyopathy, 447f, 491
examination in, 397–454
auscultation in, 399–409
algorithm for, 406t–407t
of cardiac murmurs, 403f–404f, 405–408, 408b
clinical method for, 399–403, 400t–402t, 402f–403f
of heart rate and rhythm, 395f, 401t, 404–405
of heart sounds, 403–404, 404f
pulmonary, 408–409, 409f
general approach to, 397, 398b
history in, 397–398, 399b
peripheral vasculature, 409–411
myocardial disease, 388–390, 389b, 390f, 486–491, 487f–489f
myocarditis, 490
Heart disease *(Continued)*
pericardial disease, 388, 484–486, 484f–485f
toxic injury of the myocardium, 399b, 490–491
valvular heart disease, 390–391, 391f, 471–484, 472f
aortic regurgitation, 476–479, 476f, 478f–479f
infective endocarditis, 481–484, 482f–483f
mitral regurgitation, 472–476, 473f
pulmonary regurgitation, 481, 481f
tricuspid regurgitation, 479–480, 480f
Heart rate
auscultation of, 401t, 404–405
electrocardiography and, 416b
during exercise
poor performance and, 294
testing, 293–294
in exercise testing, 411
for monitoring pregnant mares, 1287–1288
normal, 416t
Heart sound
atrial, 392, 403
auscultation of, 403, 404f
first, 392–395, 403
origins of, 393f
second, 392–395, 403–404
third, 395
Heat stroke, 233
Heimlich valve, in thoracocentesis, 319, 319f
Heinz bodies, 994, 1485
Heliotropium europaeum, 874t
Helminths, infection, 1184–1187
Hemangioma, 1019, 1165t–1167t, 1210–1211, 1210f
Hemangiosarcoma, 371, 1019
systemic manifestations of, 1165t–1167t
Hematocrit analysis, during exercise, 295
Hematologic testing, for stallions, 1326
Hematology, in urinary system, 909–912, 910f, 911t
Hematomas
ovarian, 1245–1246
vulvar, postpartum, 1310
Hematopoiesis, disorders of, 1016–1019
Hematopoietic system, disorders of, 991–1028
basophils in, 997
bone marrow, 998
complete blood count, 992f, 999–1001
eosinophils in, 996–997
erythrocytes in, 991–994, 992b, 993t, 994b–995b
investigation of, 991–1002
iron metabolism in, 994–995, 996t
leukocytes in, 995–998
lymphocytes in, 997
Hematopoietic system, disorders of *(Continued)*
mast cells in, 997
neutrophils in, 995–996
platelets in, 997–998, 998b, 1012–1013
treatment of, 1002–1006
to acute blood loss, 1002, 1002b
allogenic whole blood transfusion for, 1003, 1003b
alternative oxygen carriers for, 1003–1004
autologous blood transfusion for, 1003
plasma transfusion for, 1004
platelet transfusion for, 1004
transfusion medicine for, 1002–1004
Hematuria, 957–958
exercise-associated, 961
in horse, 994b
idiopathic renal, 960–961, 961f
pigmenturia associated with systemic disease, 961
toxicities, 958
urethral defects in, 958–960, 959f–960f
Hemiplegia, laryngeal, 596–597
Hemodialysis
for acute renal failure, 1409–1410
in acute renal failure, 930
Hemodynamic changes, in endotoxemia, 731
Hemoglobin
pulse oximetry and, 181
renal critical care, 183
Hemoglobin-based oxygen carriers, for hematopoietic disorders, 1003–1004
Hemoglobinuria, 924
Hemogram, 711
Hemolysis
alloimmune neonatal neutropenia and, 1008
babesiosis and, 1008–1009
congenital enzyme defect and, 1010
differential diagnoses for, 995b
disorders associated with, 1007–1011
envenomation in, 1010
Evans syndrome and, 1008
garlic and onion consumption in, 1010
hemolytic-uremic syndrome and, 1010
hemotropic *Mycoplasma* infection and, 1009
in hepatic insufficiency, 856
immune-mediated, 1007–1008
infectious causes of, 1008–1009
leptospirosis and, 1009
neonatal isoerythrolysis and, 1008
piroplasmosis and, 1008–1009
Pistacia leaf toxicity and, 1010
red maple leaf toxicity and, 1009–1010
secondary to oxidative injury, 1009–1010

Hemolytic anemia, 1010–1011
Hemolytic icterus, 854
Hemolytic-uremic syndrome, 961, 1010
Hemoperitoneum, 994b, 1006
Hemophilia A, in plasma-based coagulation system, 1014
Hemorrhage
  periparturient, postpartum, 1304–1305
  pulmonary, 317f, 1006–1007
  renal and urethral, 1007
  in spinal cord injury, 619
  sudden cardiovascular death and, 399b
  in temporohyoid osteoarthropathy, 625
  in traumatic brain injury, 613
  vaginal, 1292–1293
Hemorrhagic diathesis, in hepatic insufficiency, 855–856
Hemorrhagica, purpura, 1011, 1196
Hemospermia, 1339–1340
Hemostasis, 999–1002, 999t
  anticoagulation and, 1001
  antifibrinolysis and, 1001
  coagulation and, 999–1000, 1000t, 1001f
  disorders of, 1011–1016
    Anaplasma phagocytophila in, 1011–1012
    equine viral arteritis in, 1012
    purpura hemorrhagica in, 1011
    vasculitis in, 1011
  dysfunctions of, pharmacologic treatment of, 1004–1006
    anticoagulants for, 1005
    fibrinolytic drugs for, 1005–1006
    procoagulants for, 1004–1005
  fibrinolysis and, 1001
  parameters, interpretation of, 1000t
  values of, reference ranges for, 1000t
Hemothorax, 1006, 1392–1393
Hemotropic *Mycoplasma* infection, in hemolysis, 1009
Henderson-Hasselbalch equations, 84
Hendra virus infection, 345–346
Henneke BCS system, 191–194
  in obesity, 210–211
Henneke system, horse body condition scores on, 239t
Heparin
  endotoxemia and, 738
  for hyperlipemia, 873
  for infective endocarditis, 484
  for peritonitis, 765
Hepatic clearance, of drugs, 87–88
Hepatic diseases, 865b
  acute, 865–874
    bacterial hepatitis, 868–869
    biliary obstruction, 871
    hyperlipemia and hepatic lipidosis, 871–874, 873f
    infectious necrotic hepatitis, 868–869
Hepatic diseases *(Continued)*
    parasitic hepatitis, 869
    Theiler's disease, 865–866, 866f
    toxic hepatopathy, 869–871
    viral hepatitis, 866–867, 869
  chronic, 874–880
    abscessation, 879
    amyloidosis, 880
    cholelithiasis, 876–878, 877f–878f
    chronic active hepatitis, 875–876
    clover poisoning, 875
    hypoxemia, 880
    megalocytic hepatopathy, 874–875, 874f, 874t
    neonatal acquired biliary obstruction, 879
    neoplasia, 879–880
  clinical signs of, 851b
  congenital, 880–881
  definition of, 849–850
  diagnosis and laboratory findings of, 856–863, 857t
  neonatal, 1416–1419, 1417t
    bacterial hepatitis as, 1417–1418
    congenital fibrosis as, 1418–1419
    due to equine herpes virus 1, 1418
    glycogen storage disease type IV as, 1418
    hepatic lipidosis as, 1418
    iron toxicity as, 1418
    neonatal isoerythrolysis as, 1418
    persistent hyperammonemia as, 1418
    portosystemic shunts as, 1419
Hepatic duct, 845
Hepatic encephalopathy, 851–854, 851t, 853f, 864t
Hepatic icterus, 854–855
Hepatic injury, patterns and pathology of, 850–851
Hepatic insufficiency
  clinical signs of, 851–856, 851b
  definition of, 849–850
  diagnosis and laboratory findings of, 856–863, 857t
  prognosis for, 864–865
  treatment of, 863–864
Hepatic lobules, 843–845, 844f
Hepatitis, 850
  bacterial, 868–869
  chronic active, 875–876
  infectious necrotic, 868–869
  parasitic, 869
  serum-associated, 865
  viral, 866–867, 869
Hepatoblastoma, 880
Hepatocellular carcinoma, 879
Hepatocyte growth factor, in mucosal repair, gastrointestinal, 773
Hepatocytes, histology of, 843–845
Hepatoencephalopathy, 686–687
Hepatogenous photosensitization, 1165t–1167t, 1198
  causes of, 1200t
Hepatopathy
  megalocytic, chronic, 874–875, 874f, 874t
  toxic, 869–871
Hepatorenal ligament, 843
Hepatorenal syndrome, in hepatic insufficiency, 856
Hepcidin, 372, 845
HERDA. *see* Hereditary equine dermal asthenia
Hereditary disorders, in plasma-based coagulation system, 1013
Hereditary equine dermal asthenia (HERDA), 1171–1172
Hereditary nephropathies, in urinary system, 893–894, 894f
Hermaphroditism, 1332
Hernias
  diaphragmatic, 781
  inguinal, 780, 1338
  pericardial, 484–485
  scrotal, 1338
  strangulating umbilical, 780
  ventral body wall, 1290–1291
Herpesvirus-1, in myeloencephalopathy, 645–652
  clinical signs of, 648
  control and prevention of, 651–652
  diagnosis of, 649
  epidemiology and immunity in, 646–647
  laboratory findings in, 648–649
  pathogenicity and pathogenesis of, 647–648
  pathologic findings in, 650–651
  polyneuritis equi *versus*, 653
  treatment and prognosis for, 649–650
  virologic findings in, 645–646
Herpesviruses, as latent viral infection, 10
Hetastarch, 167, 176t, 1426
  endotoxemia and, 737
Hickey-Hare test, in diabetes insipidus, 965
High clearance drugs, 87
High endothelial venule, 35
High-density lipoproteins, 847, 847f
Higher brain centers, input of, in central control of ventilation, 253
High-speed treadmill exercise, 411
Hindgut digestion, 200–201
Hirsutism, 1169–1170, 1169f
  in pituitary pars intermedia dysfunction, 1100
Histamine, in pathophysiology of colitis, 722t
Histamine bronchoprovocation testing, 322
Histoplasma capsulatum
  in fungal pneumonia, 354
  fungal pneumonia due to, 1394
Histoplasma farciminosa, systemic manifestations of, 1165t–1167t
History
  of body temperature, 236
  of cardiovascular disorders, 397–398, 399b
  of colic, 274–275
  of collapse, 267
  of cough, 249
  of diarrhea, 281
  of dysphagia, 271
  of edema, 263
  of endometritis, 1249–1250
  of hypothermia, 238
  in mare breeding soundness examination, 1225–1226, 1225t
  of polyuria and polydipsia, 288
  of respiratory distress, 257
  of skin disorders, 1160–1161, 1162f
  of urinary disorders, 909
  of weight gain, 240
  of weight loss, 243–244
Hives, 43, 1190, 1190f
Hoary alyssum toxicity, 1497
Holliday-Segar formula, 1427
Holter ECG, 415, 415f
Hoofs, structure of, 1160
Hormones
  appetite, regulation of, 181
  in mare, manipulation of, 1258
    for cervical dilation, 1262–1263
    for estrous cycle synchronization, 1259–1260
    for estrus suppression, 1260–1261
    for lactation induction, in barren mare, 1264
    for oviductal contraction, 1263
    for ovulation induction, 1261–1262
    for superovulation, 1262, 1262b
    for support of pregnancy, 1263–1264
    for uterine contractility, 1263
    for vernal transition, 1258–1259
  therapy, for recurrent exertional rhabdomyolysis, 553
  thyroid gland function and testing and, 1062
Horn flies, hypersensitivity to, 1190
Horner syndrome, 583, 623
Horse flies, hypersensitivity to, 1190
Horsepox, 1177
Host immune responses, viral replication and, 9
Houseflies, hypersensitivity to, 1190
Howell-Jolly bodies, 993
Human metabolic syndrome, 1085–1086
Hurdling posture, 1301
Hyaluronic acid capsules, of *Streptococcus equi*, 333
Hydatidosis, 372
Hydralazine
  for congestive heart failure, 462
  for heart disease, 457t–461t

Hydranencephaly, increased CSF volume in, 590
Hydration
for recumbent horses, 186–187
status
clinical assessment of, 1428t
of foals, 1425
Hydrocarbons, chlorinated, 1473–1474
Hydrocephalus
fetal, 1303
in foals, 1385
increased CSF volume in, 590
magnetic resonance imaging in, 602, 602f
Hydrochlorothiazide, for hyperkalemic periodic paralysis, 573
Hydrocortisone, for critical illness-related corticosteroid insufficiency, 1411–1412
Hydrogen peroxide, for endometritis, 1255, 1255t
Hydrops, of fetal fluids, 1289–1290
Hydrostatic osmotic pressures, 259–260, 261f, 261b
Hydroxyzine, for insect hypersensitivity, 1193
Hyperadrenocorticism, 1078
Hyperalgesia, 138b
Hyperalimentation, 203
Hyperammonemia, in neonatal foals, 1385
Hyperbilirubinemia, 854
in foals, 1415
Hypercalcemia, in paraneoplastic disease, 372
Hypercalcemic disorders, 1044–1049
asphyxia and, 1048
calcinosis in, 1048
equine bone fragility syndrome in, 1048–1049
hypercalcemia of malignancy in, 1048
hyperparathyroidism in
primary, 1044
secondary, 1044
nutritional, 1045–1047, 1045f, 1047f
renal, 1044–1045
hypervitaminosis D in, 1047–1048
idiopathic systemic granulomatous disease in, 1048
neonatal hypercalcemia and, 1048
Hypercapnia, 178
in respiratory distress, 254–255
Hyperelastosis cutis, 1171–1172, 1172f
Hyperesthesia, 138b
Hyperesthetic leukotrichia, 1169
Hyperglycemia
in neonatal foals, 1413
PN, associated with, 182
Hyperinsulinemia, 1093–1098
Hyperkalemia
conduction disturbances and, 516–517, 516f
in foals, 1407
Hyperkalemic periodic paralysis, 572–573
clinical signs of, 572–573, 572f
clinicopathology of, 573
control of, 573
diagnosis of, 573
electrolyte imbalances in, 573–574
pathogenesis of, 572
prevalence of, 572
prognosis of, 573
risk factors of, 572–573
treatment of, 573
Hyperlipemia, 871–874, 873f
Hyperlipidemia, 203
Hypermagnesemia, 1058
Hypermetria, 630
Hypernatremia, CNS dysfunction and, 687
Hyperosmolar therapy, 617–618
Hyperpathia, 138b
Hyperpigmentation, 1168
Hyperplasia, 1336–1337
Hypersensitivity, 41–47
insect bite, 45
reactions, 1189–1194
contact, 1192–1193
food, 1191–1192
insect bite, 1190–1191, 1190f–1191f
recurrent airway obstruction, 45
systemic anaphylaxis, 43–45
type I, 41–43, 41t
type II, 41, 41t, 43, 44f
type III, 41, 41t, 43–44, 44f
type IV, 41, 41t, 44
Hypertension, pulmonary, 389b, 454–455
in equine multinodular pulmonary fibrosis, 344
Hyperthermia, 233–234, 236
exercise-related, 233
malignant, 233, 553–554, 570
Hyperthyroidism, 1068
Hypertonic crystalloid fluids, for fluid therapy, 1425
Hypertonic saline
solution, 166
critically ill horse and, 185
in shock, 175
for traumatic brain injury, 617–618
for verminous encephalomyelitis, 684–685
Hypertonic sodium bicarbonate, for fluid therapy, 1425
Hypertrophic osteopathy, 372
Hypertrophy, 1336–1337
ileal, 784–785
ventricular, 469–470
Hyperventilation, 180
in traumatic brain injury, 617
Hyperviscosity, in disorders of the gastrointestinal system, 711
Hypervitaminosis D, 1047–1048
Hypoadrenalism, 1072
Hypoalbuminemia, in hepatic insufficiency, 858
Hypocalcemia
cantharidin toxicosis and, 1467
in neonatal foals, 1385
Hypocalcemic disorders, 1042–1044
acute renal failure in, 1044
cantharidiasis in, 1043–1044
exertional rhabdomyolysis in, 1044
in foals, 1043
hypoparathyroidism in, 1042
primary, 1042–1043
secondary, 1043
oxalate toxicity in, 1044
pancreatitis in, 1044
sepsis and, 1043
systemic inflammation and, 1043
Hypocalcemic seizures, 1041
Hypocalcemic tetany, 573–574
clinical signs of, 573–574
diagnosis of, 574
pathogenesis of, 573
treatment of, 574
Hypoderma species, in verminous encephalomyelitis, 683
Hypodermiasis, 1187
Hypofibrinogenemia, 859
Hypogammaglobulinemia, transient, 55
Hypoglycemia
CNS dysfunction and, 687
in neonatal foals, 1373, 1384, 1413
Hypoglycin A myopathy, 568–569
clinical signs of, 569
diagnosis of, 569
pathogenesis of, 568, 569f
prevalence of, 568–569
treatment and prognosis of, 569
Hypomagnesemia, 1056–1057
cantharidin toxicosis and, 1467
Hypometria, 637
Hyponatremia
CNS dysfunction and, 687
in neonatal foals, 1384–1385
Hypoparathyroidism, 1042
Hypophysis, 1070
Hypopigmentation, 1168
Hypoplasia
cerebellar, 632
iris stromal, 1151
renal, 891–893, 893f
Hypoplasia, gonadal, 1336
Hypoproliferative anemia, 1016–1018
Hypoproteinemia
cantharidin toxicosis and, 1467
edema and, 262
Hyposensitization, 62–63
for allergic skin disease, 1193
Hyposthenuria, 1409
Hypothalamic-pituitary-adrenal (HPA) axis
activation of, 1367, 1410
function and dysfunction of, 1410–1412
maturation of, 1410–1411
physiology, 1069–1078
sepsis and, 1072
Hypothalamic-pituitary-thyroid axis, function and dysfunction of, 1412
Hypothalamus, 1069, 1220
in thermoregulation, 232
Hypothermia, 237–238
approach to, 238
in traumatic brain injury, 617
Hypothyroidism, 1061t, 1065
in foals, 1412
increased body weight and, 238
systemic manifestations of, 1165t–1167t
Hypotonic crystalloid solutions, for fluid therapy, 1425–1426
Hypotonic maintenance solutions, 1427
Hypoventilation, 178
in hypoxemia, 254
Hypovolemia, in acute renal failure, 924
Hypovolemic shock, 173
Hypoxemia, 178
caused by, 178, 180
CNS dysfunction and, 687
hepatic, 880
role of, 254–255
Hypoxia, 178
Hypoxic ischemic encephalopathy, 1381–1383
Hysteroscopy
in endometritis, 1252
for insemination, 1313
in mare breeding soundness examination, 1236

I

Iatrogenic adrenal insufficiency, 1073
Iatrogenic polyuria, 966
ICAM. *see* Intracellular adhesion molecules
Icterus, 854–855
IDE. *see* Insulin degrading enzyme
Idiopathic immune-mediated/inflammatory myopathies, 568
Idiopathic renal hematuria, 960–961, 961f
Idiopathic systemic granulomatous disease, 1048
Idiopathic vasculitis, 1196
IFAT. *see* Indirect fluorescent antibody test
Ileocecal intussusception, 781
Ileocolonic aganglionosis, 789
Ileus, 773–774
diagnosis of, 776
hypertrophy in, 784–785
hypocalcemia and, 1041
impaction in, 784
manifestation of, 774
pathophysiology of, 774–776
physiology of, 774
treatment of, 776–778
IMHA. *see* Immune-mediated hemolytic anemia
Imipenem, for foals, 1421, 1422t
Imipramine, for narcolepsy, 610
Imiquimod
for equine sarcoid, 1206
for immunostimulation, 61–62
Immune complex
disease, 46
in type III hypersensitivity, 43, 44f
Immune expulsion, of parasitic infections, 17–18

Immune serum, for *Strongylus vulgaris* infection, 14–15
Immune system, 19
  immunodeficiency and, 47–59
    agammaglobulinemia as, 55
    age, 57–58
    common variable, 54
    drug-induced, 58
    exercise, 57
    failure of transfer of passive immunity as, 55–57
    foal, 53–54
    immunomodulators, 59–60
    infectious disease and, 58–59
    leukoproliferative disease-associated, 58
    primary, 52–55
    secondary immunodeficiency, 55–59
    selective IgM deficiency, 54–55
    severe combined, 52–53
    transient hypogammaglobulinemia as, 55
    undifferentiated, 59
  immunology, equine, 19–41
    acute inflammatory response, 20–23
    adaptive immunity, 23–41
    cytokines, 23f, 32–33, 33t
    innate immunity, 20–23
    lymphocyte trafficking pathways in, 35–36
    T-helper paradigms, 34–35
Immune thrombocytopenic purpura, 1012
Immune-mediated glomerulonephritis, 932, 933f
Immune-mediated hemolysis, 1007–1008
Immune-mediated hemolytic anemia (IMHA), 1007–1008
Immune-mediated myositis, in Quarter horse-related breeds, 566–567
  diagnosis of, 567
  history and clinical signs of, 566–567, 567f
  pathogenesis of, 566
  treatment and prevention of, 567
Immune-mediated reactions, in benzylpenicillin therapy, 100
Immune-mediated thrombocytopenia (IMT), 1012
Immune-mediated vasculitis, 1011
Immunity, 19
  antibody-mediated, in foals, 40–41
  cellular, tests of, 49–52
  immunology, equine, 19–41
    acute inflammatory response, 20–23
    adaptive immunity, 23–41
    cytokines, 23f, 32–33, 33t
    innate immunity, 20–23
    lymphocyte trafficking pathways in, 35–36
    T-helper paradigms, 34–35
Immunity *(Continued)*
  innate, acute inflammatory response and
    in foals, 40–41
  mechanisms of disease and, 1–78
  mucosal, 36–38, 37f
Immunoblot analysis, 643
Immunocompetence, in foals, 38
Immunodeficiency, 47–59
  agammaglobulinemia as, 55
  age, 57–58
  common variable, 54
  diarrhea and, 723
  drug-induced, 58
  exercise, 57
  failure of transfer of passive immunity as, 55–57
  foal, 53–54
  immunomodulators, 59–60
  infectious disease and, 58–59
  leukoproliferative disease-associated, 58
  primary, 52–55
  secondary immunodeficiency, 55–59
  selective IgM deficiency, 54–55
  severe combined, 52–53
  tests for, 48–52, 48t
  transient hypogammaglobulinemia as, 55
  undifferentiated, 59
Immunodominant, 30
Immunoglobulin A, 36
  as antibody isotype, 24–25, 25t
  secretory, in respiratory tract, 314
Immunoglobulin D, as antibody isotype, 24–25, 25t
Immunoglobulin E
  as antibody isotype, 24–25, 25t
  type I hypersensitivity mediated by, 41, 41t
Immunoglobulin G
  as antibody isotype, 24–25, 25t
  in antibody-mediated immunity of foals, 40
  in cerebrospinal fluid, 591t
  in failure of passive transfer, 55–56
  in hypersensitivity
    type II, 41t, 43
    type III, 41t, 43
Immunoglobulin M
  as antibody isotype, 24–25, 25t
  deficiency, 54–55
Immunoglobulins, in adaptive immunity, 24–27, 24f, 25t, 26f
Immunology
  adaptive immunity, 23–41, 23f
    immunoglobulins in, 24–27, 24f, 25t, 26f
    major histocompatibility complex in, 28–30, 29f, 31f–32f
    signaling through antigen-specific receptor, 30–31, 32f
    T-cell receptor and CD_3 complex in, 27
    T-lymphocyte subsets in, 27, 28f
Immunology *(Continued)*
  cytokines, 23f, 32–33, 33t
  equine, 19–41
    innate immunity and the acute inflammatory response in, 20–23
  lymphocyte trafficking pathways in, 35–36
  T-helper paradigms, 34–35
Immunomodulators, 59–60
  antigen-specific, 62–63
  for endometritis, 1256–1257
  for equine protozoal myeloencephalitis, 644
  immunostimulants as, 60–63
  immunosuppressors as, 59–60, 60t
Immunoreceptor tyrosine based activation motif (ITAM), 30–31
Immunostimulants, 60–63
Immunosuppressors, 59–60, 60t
Immunotherapy
  for endotoxemia, 733–735
  for pythiosis, 1181
Impaction
  ascarid, 784
  cecal, 786
  ileal, 784
  large colon, 786–787
  meconium, in foals, 1400–1401
  for recumbent horses, 187
  small colon, 789
Impaired lymph drainage, 262
Impulse-oscillation method, for pulmonary function testing, 322
IMT. *see* Immune-mediated thrombocytopenia
In vitro fertilization, 1312
Incontinence, urinary, 973
Indirect fluorescent antibody test (IFAT), 643
Indocyanine green, 862
Indomethacin, 1077
Inducers, in biotransformation, 849
Infarctive purpura hemorrhagica, 566
  clinical signs of, 566, 566f
  diagnosis of, 566, 566f
  pathophysiology of, 566
  treatment of, 566
Infections. *see also specific infections*
  assessment, for cough, 250
  documentation and, 95
  nosocomial, 5
Infectious conditions
  of accessory glands, 1339
  of epididymides, 1337–1338
  of penis, 1334
  of scrotum, 1335
  of semen and spermatozoa, 1339–1340
  of spermatic cord, 1338
Infectious diseases, 747–755
  acute diarrhea associated with, 748t
  bacterial
    entry of bacteria, 5–6
    pathogenesis of, 5–7, 6t
    spread of bacteria, 7
Infectious diseases *(Continued)*
  cerebellar, 632
  cyathostomiasis, 754
  equine intestinal clostridiosis, 752–753
  fungal
    damage to host tissues, 8
    pathogenesis of, 7–8
  immunodeficiency and, 58–59
  nosocomial, 5
  parasitic
    gastrointestinal, 14–16
    pathogenesis of, 14
    protective resistance, 17–18
    regulatory responses and, 18–19
    respiratory, 16
    skin, 16–17
  potomac horse fever, 750–752
  salmonellosis, 747–750, 748t
  strongylosis, 753–754
  viral
    immunologic avoidance, 14
    pathogenesis of, 8–14, 10f–12f
    pathology of, 12
    spread of, 10–11
    virulence and, 10–11
    virus life cycle and, 9, 12f
Infectious endometritis (IE), 1246
Infective endocarditis, 481–484, 482f–483f
Inflammation
  central vestibular disease and, 627
  in disorders of the gastrointestinal system, 715–716
  gastrointestinal, 715
    cellular effectors of, 716–719, 717f
    diarrhea in, 278
    humoral mediators of, 719
    initiation of, 715–716
    tissue injury during, 719–720
    vascular response during, 716
  in gastrointestinal ileus, 774–775
  myocardial, 490
  nonsteroidal antiinflammatory drugs on, effect of, 121
Inflammatory airway disease, 358, 361–365
  clinical presentation of, 361–362
  diagnosis of, 362, 362t
  treatment of, 363–365, 363t
Inflammatory bowel disease, 743t–744t
Influenza
  exercise and, 57
  viral respiratory, 338–339
Information, sourcing, in evidence-based medicine, 221
Infraspinatus muscle, electromyography of, 593f
Inguinal hernia, 780, 1338
Inhalation therapy, 179
In-hand mating. *see* Live cover breedings
Injuries
  radial and suprascapular nerve, 596, 597f
  spinal cord and vertebral, 618–622, 618t

Injuries *(Continued)*
thermal, 1197–1198
traumatic brain. *see* Traumatic brain injury
Innate immunity, acute inflammatory response and, 20–23
acute phase proteins, proinflammatory cytokines, and complement in, 20–23, 20t, 21f
chemotaxis and leukocyte trafficking in, 22–23
in foals, 38–39
lipid mediators in, 21–22
tests of, 52
Innate responses, 20
Inotropes
in critical care, 174
for heart disease, 457t–461t
Insect allergies, 1195
Insect bite hypersensitivity, 45, 1190–1191, 1190f–1191f
Insecticides, 1188
cholinesterase-inhibiting, 1471–1472
Insemination
artificial
with cooled shipped semen, 1266
with fresh semen, 1266
of frozen semen, 1266–1267
deep intrauterine, 1312–1313
hysteroscopic, 1313
in oocyte transfer, 1311–1312
Insertional activity, 592–593, 593f
decreased, 593
prolonged, 593
Insulin, 1080
administration of, in critically ill foals, 210
for foals, 1437, 1438f, 1438b
in hyperkalemia, 517
Insulin degrading enzyme (IDE), 1094–1095
Insulin dysregulation, 1085
clinical pathology of, 1090
clinical signs of, 1089–1090, 1089f–1090f
definition of, 1088
diagnostic testing in, 1090–1100
differential diagnoses of, 1089–1090, 1089f–1090f
etiology of, 1088–1089
genetics in, 1089
management of, 1098–1099
treatment of, 1100
Insulin resistance syndrome, 1085–1086
Integrins, in neutrophil adherences, 22
Intensive care units, 158–161
emergency drugs in, 159, 160t
equipment in, 159, 159b
Interferon-α, for immunostimulation, 60
Interferon-γ, in pathophysiology of colitis, 722t
Interleukin-1
in endotoxemia, 727t–728t
in pathophysiology of colitis, 722t
Interleukin-1 receptor antagonist protein, for pain management, 149
Interleukin-6, as inflammatory mediators, 727t–728t
Interleukin-8 (IL-8), 717–718
as inflammatory mediators, 727t–728t
Intermediate lipoproteins, 847
Internal abscessation, in *S. equi* infection, 335–336
Internal genitalia, of stallions, 1314–1316
evaluation of, 1322–1323
Internal medicine, clinical nutrition and, 191–217
Internist's tumor, 969
Interstitial fluid, 258–259
ionic composition of, 900t
Interstitial pattern, radiographic, 320–322, 321f
Interstitial pulmonary disease, 352–354
clinical findings of, 353
diagnosis of, 353, 353f
etiology of, 352–353
prognosis of, 354
treatment of, 353
Interval-extension method, 93–94, 93f–94f
Intestinal anaphylaxis, 758–759
Intestinal blood loss, 1006
Intestinal microbiota, 723
Intestinal tract, ischemic disorders of, 778
strangulating obstruction, 778, 779f
Intestine
barrier function of, 767
ischemia/ reperfusion injury, 768–770, 769f–770f, 769t
parathyroid hormone effects on, 1037
strangulation, 172
Intracellular adhesion molecules (ICAMs), 717–718
Intracellular calcium, 1029
Intracellular fluid, 900, 900t
Intracytoplasmic sperm injection, 1310
Intrarenal vasoconstriction, in acute renal failure, 925
Intrauterine antibiotics, for endometritis, 1253, 1254t
Intrauterine growth retardation, of foals, 1369–1370
Intravenous catheters, central venous pressure measurement and, 169
Intraventricular conduction blocks, 516f
Intubation, nasogastric, in critical care, 170–171
Intussusceptions, 781, 781f–782f
postpartum, 1306
Iodine, toxic effects of, 1497–1498
Iodine toxicity, 1187
Iodine-azide test, 1476
Iodism, 1165t–1167t
Ionization, drug, 84, 84b
Ionophore toxicity, 1489–1491
rhabdomyolysis and, 568
in toxic injury, 490–491
Ipratropium, for interstitial pulmonary disease, 352–353
Ipratropium bromide, as bronchodilator, 1396–1397, 1397t
Iris prolapse, 1148, 1149f
Iris stromal hypoplasia, 1151
Iron
metabolism, 994–995, 996t
toxic effects of, 1504
in foals, 1418
hepatic, 870–871
Iron deficiency anemia, 1016
Irreversible adrenal insufficiency, 1072
Ischemia
CNS dysfunction and, 687
intestinal, 778
epithelial injury, 768–769
nonstrangulating, 783–784
rectal prolapse, 783f, 783t
reperfusion injury in, 768–770, 769f–770f, 769t
strangulating obstruction, 769–770, 778, 779f
Ischemic coagulative necrosis, 850
Isocoma wrightii, 1484–1485
rhabdomyolysis from, 568
Isoerythrolysis, neonatal, 45–46, 46f
as hepatic disorder, 1418
as immunologic disorder, 1414–1416
Isosthenuria, 1409
Isotonic crystalloids, 166
Isotonic electrolyte-containing fluids, for fluid therapy, 1425
Isotonic fluids, for traumatic brain injury, 617–618
Isotonic replacement fluid, 1428
Isotonic saline, 164
Isotypes, of antibody, 24–25
Isovolumic period, 392–395
Isovolumic relaxation, 392–395
ITAM. *see* Immunoreceptor tyrosine based activation motif
Itraconazole
for dermatophytosis, 1178
for endometritis, 1258, 1259t
for fungal pneumonia, 356t
Ivermectin
for cutaneous habronemiasis, 1186
for demodicosis, 1184
for lice, 1181–1182
for onchocerciasis, 1185
for parasitic pneumonitis, 357
for scabies, 1182
for verminous encephalomyelitis, 684
Ixodid ticks, 1184

J

Japanese encephalitis, 659
Jasmine, wild, 1494
Jaundice, hepatic, 854
Jaundiced foal agglutination (JFA) test, for neonatal isoerythrolysis, 1415
Jejunitis, duodenitis-proximal, 738
clinical and clinicopathologic signs of, 739–740
pathophysiology of, 738–739
prognosis for, 741
treatment of, 740–741, 740f
Jejunojejunal intussusception, 781, 781f
Jimsonweed toxicity, 1462
Journal reputation, in evidence-based medicine, 221–222
Juglans nigra, 1494
Jugular pulsations, 410–411
Jugular pulse, 392
Jugular veins
compression of, CSF pressure and, 589
examination of, 176
pulse of, 392
abnormalities of, 410–411
thrombophlebitis of, 491, 492f, 493–494
Junctional arrhythmias, 511
Junctional epidermolysis bullosa type 1 (JEB-1), 1173
Junctional epidermolysis bullosa type 2 (JEB-2), 1173
Juvenile idiopathic epilepsy, in neonatal foals, 1385

K

Kallikrein-bradykinin system, 719
Kanamycin, auditory system and, 627
Keratinization disorders, 1170–1171, 1171f
Keratitis, 624–625
eosinophilic, 1149–1150, 1149f
fungal, 1145f–1146f
idiopathic nonulcerative, 1150
Keratomalacia, 1145f
Keratosis
cannon, 1171
linear, 1171
Kernicterus, 1385
Kerosene, for endometritis, 1255, 1255t
Ketamine
for pain management, 144t–145t, 147–148
for seizures, 607
for traumatic brain injury, 617
Ketoconazole
for endometritis, 1258, 1259t
for guttural pouch mycosis, 326
Ketoprofen
for acute rhabdomyolysis, 549
for pain management, 144t–145t
pharmacology of, 124–125
Kidneys
acute renal failure. *see* Acute renal failure
anatomy of, 888
chronic kidney disease. *see* Chronic kidney disease

Kidneys *(Continued)*
developmental malformations of, 891–898
agenesis, hypoplasia, and dysplasia in, 891–893, 893f
cysts in, 893
polycystic kidney disease, glomerulocystic disease, and other hereditary nephropathies in, 893–894, 894f
vascular anomalies in, 894, 895f
pendulant, 894
physiology of, 898
nitrogenous and organic wastes, production and elimination of, 898–900, 898b
polycystic kidney disease in, 893–894, 894f
ultrasonography in, 917–918, 918f
Kinetics
of drug metabolism, 88–89
fetal, during parturition, 1297–1298
flip-flop, 86, 87f
Klebsiella spp., 1234
Klossiella equi, in urinary tract infections, 949
Klotho, 1040
Kupffer cells, 843–845, 849, 856

L

Laboratory studies
in cardiovascular disorders, 398b, 411–454
in atrial fibrillation, 504
in infective endocarditis, 482–483
in myocardial disease, 489
in pericardial disease, 485
failure of passive transfer and, 56
Fell pony syndrome, 54
severe combined immunodeficiency and, 52–53
Lacerations
eyelid, 1141
perineal, postpartum, 1309
uterine, 1243–1244
vaginal, 1309
β-Lactams, for foals, 1421
Lactate dehydrogenase (LDH), 545
in cerebrospinal fluid, 591t
in liver disease, 861
Lactate dehydrogenase-5, hepatic insufficiency and, 857t
Lactated Ringer's solution, 166–167
for endometritis, 1255t
for hyperkalemia, 517
Lactation
induction of, in barren mare, 1264
tetany, 1310
Lactic acid
in cerebrospinal fluid, 591, 591t
recurrent exertional rhabdomyolysis and, 551
Lactose tolerance testing, 714
Lactulose, for hepatic insufficiency, 863–864
Lameness, in poor performance, 289
Laminitis, 1088
Laparocystotomy, for cystic calculi, 954–955
Laparoscopy, gastrointestinal, 715
Large colon impaction, 786–787
Large colon volvulus, 781–783
Large intestine, displacement of, 710
Large-vessel thrombosis, in plasma-based coagulation system, 1015–1016
Larval cyathostomiasis, 15
Laryngeal hemiplegia, in *S. equi* infection, 336
Laryngeal tie-forward procedure, for dorsal displacement of soft palate, 328–329
Larynx
hemiplegia, 596–597
innervation to, neurologic examination of, 584
recurrent neuropathy of, 329–330, 329t
Lasalocid
in toxic injury, 490
toxicity, 1490–1491
Latency, defined, 10
Lateral thoracic vein catheterization, 161–162
Lavage
pericardial, 485–486
uterine, for endometritis, 1255–1256, 1255t
Lawsonia intracellularis
equine proliferative enteropathy due to, 1404
weight loss and, 242–243
Lead toxicity, 1482–1484
Leads, electrocardiographic, 415, 415b
Lectin pathway, 21
Lectins, 6–7
Lens, disorders of, 1153–1154, 1153f–1154f
Leptocyte, 993
Leptospirosis
in abortion, 1282
in hemolysis, 1009
in neonatal foals, 1417–1418
Leucinosis, systemic manifestations of, 1165t–1167t
Leukemia, 1019
Leukocyte trafficking, in acute inflammatory response, 22–23
Leukocytes, 995–998
fecal, in diarrhea assessment, 282
Leukocytoclastic vasculitis, 1201–1202, 1202f
Leukoderma, 1168
Leukoencephalomalacia, 686, 1481–1482
Leukopenia
in endotoxemia, 732
in neonatal foals, 1370
Leukoproliferative disease-associated immunodeficiency, 58
Leukotrichia, 1168–1169, 1168f–1169f
Leukotrienes, in inflammatory response, 21–22
Levamisole
for immunostimulation, 62
toxic effects of, 1475
Levator veli palatini, 328t
Level of consciousness, in traumatic brain injury, 613–614
Levofloxacin, 116
Levothyroxine sodium, 1099
Leydig cells, 1314
Libido, in breeding stallions
frequency of service, 1331
low, 1331
Lice infestations, 1165t–1167t, 1181–1182
Lidocaine
for endotoxemia, 736
for gastrointestinal ileus, 778
for heart disease, 457t–461t
for pain management, 144t–145t, 147
for ventricular tachycardia, 513–514
Light breeds, polysaccharide storage myopathy in, 555
Limb strength, of neonatal foals, 1382t
Lime sulfur dip
for dermatophytosis, 1178
for lice, 1182
Linear keratosis, 1171
Lip, 790
Lipid A-proximal, of lipopolysaccharides, 724, 724f
Lipid mediators, inflammatory response and, 21–22
Lipid metabolism, 846–848, 846f–847f
in hepatic insufficiency, 859–860
Lipid solubility, of drugs, 84, 84b
Lipidosis, hepatic, 871–874, 873f
in foals, 1418
Lipoma, strangulation by, 780
Lipophilic antimicrobials, for equine proliferative enteropathy, 1404
Lipopolysaccharide (LPS)
capsule and, 7
in endotoxemia, 724, 724f
Escherichia coli, 724–725, 725f
in gastrointestinal inflammation, 715
inhibition of, 735
scavenging of, 733
Lipoteichoic acid, 6t
Lipoxygenase products, in pathophysiology of colitis, 722t
Lithocholate, 848
Live cover breedings, 1328, 1328f–1329f
Liver
anatomy of, 843
histology of, 843–845, 844f
physiology of, 845–849
protein metabolism in, 845–846, 846f–847f
Liver disorders, 843–887
in plasma-based coagulation system, 1014–1015
toxicoses and, 1502–1503
Liver enzymes, in hepatic insufficiency, 860–861
Liver lobe torsion, 881
L-lactate, in critical patients, monitoring of, 176
Llewellyn procedure, for dorsal displacement of soft palate, 328
Loading dose, 91
Local anesthetics
for gastrointestinal ileus, 778
for pain management, 144t–145t, 147
Locomotor disorders, in poor performance, 290–291
Locoweed toxicity, 1469
Loop diuretics, in acute renal failure, 929
Low-density lipoproteins, 847
Lower airway disease, 178, 179b
Lower respiratory tract
bronchoalveolar lavage in, 318–319, 319t
diagnostic sampling of, 318–323
examination of, 316–318
endoscopy for, 316–317, 317f
radiography for, 317–318, 318f
lung biopsy in, 322–323
pulmonary function testing in, 322
thoracic radiography for, 320–322, 321f
thoracocentesis in, 319–320, 319f
thoracoscopy in, 323
tracheobronchial aspiration in, 318
Low-level laser therapy, for pain management, 151
Low-molecular-weight heparin (LMWH), for hemostatic dysfunction, 1005
Lufenuron, for endometritis, 1258, 1259t
Lung biopsy, 322–323
Lung fields
auscultation of, 316
ultrasonographic examination of, 320
Lung movement, measurements of, 322
Lupus erythematosus, systemic, 1165t–1167t, 1196
Luxation, lens, 1153, 1153f
Lyme disease, in horses, 678–680
Lymphadenopathy, from *S. equi* infections, 334
Lymphangioma, 1019–1020
Lymphangiosarcoma, 1019–1020
Lymphangitis, ulcerative, 1174
Lymphatics, 261
Lymphedema, chronic progressive, 1211
Lymphocytes, 997
in adaptive immunity, 23–24
in bronchoalveolar lavage fluid, 319t
function testing, 50–51, 52f
of respiratory tract, 314
trafficking pathways, 35–36
Lymphocytic-plasmacytic enterocolitis, 743t–744t, 745

Lymphoid hyperplasia
B-cell lymphoma and, 327
pharyngeal, 327, 328t
Lymphoma, 1018–1019
B-cell, 317f
cutaneous, 1210
equine, 370
classification of, 371
in IgM deficiency, 58
immunodeficiency and, 58
Lymphomatoid granulomatosis, 370
Lymphoproliferative disorders, 1018–1019
Lymphosarcoma, 1018–1019
alimentary, 742, 743t–744t
systemic manifestations of, 1165t–1167t

M
M cells, 36
in salmonellosis, 748–749
M protein, 660
Macrolides, 114–116
adverse effects and drug interactions of, 115–116
clinical use of, 116
for foals, 1423–1424
formulations of, 116
mechanism of action of, 114
mechanism of resistance of, 114
pharmacokinetics of, 114–115
for tetanus, 671
Macrophages
alveolar, 346
in bronchoalveolar lavage fluid, 319t
in gastrointestinal inflammation, 715–716
Macula, 622
Magnesium, 1052
absorption of, 1036f, 1053–1054, 1054f
central nervous system and, 1055–1056
deficiency of, 1057
disorders of, 1056
distribution of, 1053, 1053f
electrolytes and, 1057
excretion of, 1036f, 1053f–1054f, 1054–1055
homeostasis, 1055
hypermagnesemia, 1058
hypomagnesemia, 1056–1057
diagnosis of, 1057
treatment of, 1057–1058
for pain management, 148
requirements in, 1052–1053
Magnesium sulfate
for heart disease, 457t–461t
for ventricular tachycardia, 513–514
Magnetic resonance imaging (MRI)
in hypoxic ischemic encephalopathy, 1382–1383
in neurologic examination, 599–603
in respiratory disorders, 318
Main drain virus, 662–663
Maintenance dose regimen, 91
Major histocompatibility complex (MHC), 24
in type I hypersensitivity disease, 42
Malabsorption
diarrhea and, 278
diseases associated with, 741–747, 743t–744t
in failure of passive transfer, 56
weight loss associated with, 241
Malassimilation, weight loss associated with, 241
Maldigestion, diseases associated with, 741–747, 743t–744t
Maldisposition, fetal, 1297
Maldistributive shock, 173–174
Malevolent sarcoid, 1204f
Malignant hyperthermia, 233, 553–554, 570
clinical signs of, 553, 570
diagnosis of, 554
pathogenesis of, 553, 570
prevalence and risk factors of, 553
prevention of, 570
treatment of, 554, 570
Mammary gland, 1220
Mane and tail dystrophy, 1169
Mange. *see* Mite infestations
Mannitol
for acute renal failure, 1409–1410
for neonatal encephalopathy, 1386
for traumatic brain injury, 617–618
for verminous encephalomyelitis, 684–685
for West Nile virus, 661–662
Manual reduction, of twins, 1276
Maple toxicity, 1485–1486
Marbofloxacin, 116
for foals, 1424
Mare reproductive loss syndrome, 1280–1281
Mares
assisted reproductive techniques for, 1310
breeding soundness examination of, 1225
behavior in, 1226
external genitalia examination in, 1226–1227, 1227f
general physical examination in, 1226
hormonal assays in, 1236–1238, 1238f
identification in, 1225
ovarian pathology in, 1228–1230, 1232f
restraint in, 1226
signalment and history in, 1225–1226, 1225t
transrectal palpation and ultrasound examination in, 1227–1228, 1228t, 1229f–1230f
uterine pathology in, 1230–1236
embryonic losses in, 1279t
in equine embryo transfer, 1267–1268
Mares *(Continued)*
donor, 1268–1269
recipient, 1269
hormonal manipulation of, 1258
for cervical dilation, 1262–1263
for estrous cycle synchronization, 1259–1260
for estrus suppression, 1260–1261
for lactation induction, in barren mare, 1264
for oviductal contraction, 1263
for ovulation induction, 1261–1262
for superovulation, 1262, 1262b
for support of pregnancy, 1263–1264
for uterine contractility, 1263
for vernal transition, 1258–1259
periparturient blood loss, 1007
pregnant, 1269. *see also* Pregnancy
gestation in, physiology of, 1269–1274
management of, 1296–1303
monitoring, 1286–1288
parturition in, endocrinology of, 1272–1273
reproductive tract of
anatomy and physiology of, 1217, 1220–1225
hormones of, 1220, 1223t, 1224f
pathology of, 1238
phases of, 1220–1225
Marie's disease, 372
Masseter muscle myodegeneration, 562
clinical signs of, 562
diagnosis of, 562
pathogenesis of, 562, 562f
treatment of, 562
Masseter myopathy, weight loss associated with, 242
Mast cell tumor, 1209–1210, 1210f
Mast cells, 997
in bronchoalveolar lavage fluid, 319t
degranulation of, in allergic reaction, 42
gastrointestinal inflammation and, 718–719
Maternal antibodies, passive transfer of, 40
Mathematical models, 79–80
MBC. *see* Minimum bactericidal concentration
Mean arterial blood pressure, 168
examination of, 409
traumatic brain injury and, 617
Mean residence time (MRT), 85
Mechanical ventilation, for respiratory disorders, of foals, 1397–1400
Meckel's diverticulum, 785
Meconium aspiration, 1391–1392
Meconium impaction, in foals, 1400–1401
Medetomidine, for pain management, 144t–145t
Medicated shampoos, 1193–1194
MEDLINE, 220
Medulla oblongata, traumatic injury to, 615t
Medullary carcinoma, thyroid tumors and, 1068–1069
Medullary rim sign, 918
MEED. *see* Multisystemic eosinophilic epitheliotropic disease
Megaesophagus, 801
Megakaryocytic hypoplasia, 1017–1018
Megalocytic hepatopathy, chronic, 874–875, 874f, 874t
Megavesica, 897
Melanin pigments, 1161–1167
Melanocytes, 1161–1167
Melanoma, 1208–1209, 1208f–1209f
in eyelid, 1141
Melanosomes, 1161–1167
Meloxicam, 125
for pain management, 144t–145t
for pleuropneumonia, 350t
Menace response, in neonatal foals, 1381
Menadione sodium bisulfite, 1507
Meningitis, bacterial, 1383–1384
Meperidine, for pain management, 144t–145t
Mepivacaine, for pain management, 144t–145t
Mercury toxicity, 1188, 1507–1508
systemic manifestations of, 1165t–1167t
Mesenteric portography, operative, 862
Mesothelioma, 484–485
Messenger ribonucleic acid (mRNA), 9
Metabolic acidosis, 180–181, 711
bicarbonate, supplemental, 164–165
endotoxemia and, 737
Metabolic alkalosis, 180–181
Metabolic disorders, CNS dysfunction and, 686–687
hepatoencephalopathy, 686–687
hypoglycemia, hypoxemia, and ischemia, 687
Metabolic encephalopathies, in foals, 1384–1385
Metabolic syndrome
equine, 1086–1088
human, 1085–1086
Metabolism
of carbohydrate
liver's role in, 846, 846f
testing of, in hepatic insufficiency, 859
of creatinine, 899
foal, nutrient requirements and, 207
hepatic, of drugs, 88, 88f
sulfonamides, 111
induction and inhibition of, 88, 88b
of lipid
in hepatic insufficiency, 859–860

Metabolism *(Continued)*
liver's role in, 846–848, 846f–847f
liver's role in, 845, 846f–847f
of nitrogenous and organic compounds, 900
of protein, 845–846, 846f–847f
of urea, 898–899
Metaldehyde, 1474–1475
Metformin, 1099–1100
Methadone, for pain management, 144t–145t
Methemoglobin
maple toxicity and, 1485
nitrite toxicity and, 1491–1492
Methiocarb, 1475
Methocarbamol
for acute rhabdomyolysis, 549–550
for pain management, 144t–145t, 148
Methylmercurial poisoning, 632
Methylprednisolone sodium succinate (MPSS)
critically ill horse and, 185
for spinal cord injury, 621
Methylprednisolone succinate, for acute rhabdomyolysis, 549–550
Metoclopramide
for gastrointestinal ileus, 777
for reflux esophagitis, 801
Metritis
contagious equine, 1340
toxic, postpartum, 1306–1308
Metronidazole
for *C. perfringens* enterocolitis, 1403
for foals, 1422t, 1424
pharmacology of, 119–120
for pleuropneumonia, 350, 350t
for tetanus, 671
for thrombophlebitis, 495
Metyrapone, 1076
MHC. *see* Major histocompatibility complex
MIC. *see* Minimum inhibitory concentration
Michaelis-Menten kinetics, 88
Miconazole
for endometritis, 1258, 1259t
for fungal pneumonia, 356t
Microbial surface components recognizing adhesive matrix molecules, 6
Microbiologic testing, for stallions, 1326
Microbiome, 3–5
fungal, 5
intestinal, 4
nosocomial infections, 5
oral, 3–4
pharyngeal and respiratory, 4
skin, 3
urogenital, 4–5
Microfold, 36
Microfold (M) cells, of respiratory tract, 314
Microscopic hematuria, 957–958
Microsomes, in biotransformation, 849
Micturition, control of, 973
Midbrain, traumatic injury to, 615t
Middle gluteal muscle, electromyography of, 593f
Middle molecules, 938
Migrating motility complexes, 774
Mild ventral edema, with chronic kidney disease, 939
Milk aspiration, 1392
Milkvetch toxicity, 1480
Milkweed toxicity, 1479
Milrinone, 457t–461t
Mineral oil
for cantharidin toxicosis, 1467
for hepatic insufficiency, 863
Minimum bactericidal concentration (MBC)
of antimicrobials, 95
bactericidal *versus* bacteriostatic, 96–97, 97b
Minimum inhibitory concentration (MIC)
of antimicrobials, 95
bactericidal *versus* bacteriostatic, 96–97, 97b
interpretation of, in antimicrobial therapy, 95–96, 96f
Minocycline
for foals, 1423
pharmacology of, 112
for pleuropneumonia, 350t
Minute volume, measurement of, 322
Miserotoxin, 1480
Misoprostol, for gastric ulceration in foals, 1405–1406, 1405t
Missouri University model artificial vagina, 1323
Mite infestations, 1182–1184, 1182f
Mitochondrial myopathy, 560
Mitomycin-C, for sarcoid, 1205
Mitral valve, 390, 391f
echocardiographic and Doppler studies of, 441b–443b
infective endocarditis, 481–484, 482f–483f
regurgitation of, 472–476, 473f
causes of, 401t–402t
Mixed hamartoma, 880
M-mode echocardiogram, 394f, 420, 444f, 447f, 449f
in aortic regurgitation, 477
in mitral regurgitation, 474f
Mobile crash carts, 159
Models, in pharmacokinetics, 79–80
Modified water deprivation test, 919–921
Moisture
dermatophilosis and, 1173
dermatophytosis and, 1177
Moldy corn disease, 1481–1482
Monensin toxicity, 1489–1490
Monensin toxicosis, myocardial, 490
Monitoring
arterial blood pressure, 168–169, 168f–169f, 168t
cardiovascular, 175–176
central venous pressure, 169
in intensive care units, 159
therapeutic drug, 91, 92b
Monitoring, pregnant mares, 1286–1288
Mononuclear cells, in cerebrospinal fluid, 590
Mononuclear phagocyte system, hepatic, 849
Morbillivirus, 345
Morphine
gastrointestinal ileus and, 775
for pain management, 144t–145t, 145
Mosquitoes, hypersensitivity to, 1191
Mossy fibers, 629
Motility
abnormal, diarrhea and, 278–279
esophageal, disorders of, 801
gastrointestinal, in diarrhea, 723
Motor neuron disease, equine, as neurologic disorder, 665–668, 665f
Motor unit, disease affecting, 595–597
Motor unit action potentials, 592, 593f
polyphasic, 593–594, 594f
Mouldy sweet clover toxicity, 1014
Mouth. *see* Oral cavity
Moxidectin, for parasitic pneumonitis, 357
Moxifloxacin, 116
MPSS. *see* Methylprednisolone sodium succinate
MRT. *see* Mean residence time
Mucociliary escalator, 346
Mucocutaneous vesicles, irritant and toxic causes of, 1188–1189
Mucosal immune system, 36
Mucosal immunity, 36–38, 37f
Mucosal-associated lymphoid tissue, 36
Mucous gel, 313
Mucous membranes, in defense against infection, 20
Mud fever. *see* Pastern dermatitis
Multifocal hepatic injury, acute, 850
Multifocal myelopathies, 595–596, 596f
Multimodal therapy, 138b
Multiple dose regimen, 91
Multiple endocrine neoplasia, thyroid tumors and, 1069
Multiple myeloma, 1019
Multiple organ dysfunction syndrome (MODS), 724
in foals, 1374
Multisystem neuropathy, 676
Multisystemic eosinophilic epitheliotropic disease (MEED), 743t–744t, 745, 1213
Muramyl dipeptide, for immunostimulation, 61
Murmur, cardiac
of aortic regurgitation, 476
auscultation of, 399–400, 400t, 402f, 404f
causes of, 401t–402t
functional systolic ejection, 392–395, 393f, 408
of infective endocarditis, 482
Murmur, cardiac *(Continued)*
of mitral regurgitation, 472f, 474
phonocardiograms of, 472f
poor performance and, 399b
of tricuspid regurgitation, 479
of ventricular septal defects, 466–467
Murray Valley virus, 659
Musca, hypersensitivity to, 1190
Muscle
atrophy, 596
cramping, 573–574
form and function of, 542–548, 543f
skeletal, 542
Muscular dystrophy, nutritional, 561
Musculoskeletal system
associated with poor performance, 290
disorders of, 542–579
atrophy and, pathophysiology of, 545, 545f
bacterial-associated myositis as, 564
classification of, 544–545, 544b
diagnostic approach to, 542–543
diagnostic tools for, 545–548
electrolytes as, 546
electromyography as, 547
genetic testing as, 546–547
muscle biopsy as, 547, 547f
renal parameters as, 546
selenium and vitamin E as, 546
serum CK exercise response test as, 546
serum creatine kinase and aspartate transaminase activities as, 545–546
urinalysis as, 546
exertional myopathies as, 543
chronic, 550–553
idiopathic chronic, 560
fasciculations and, pathophysiology of, 545
glycogen branching enzyme deficiency as, 560–561
history of, 542
hyperkalemic periodic paralysis as, 572–573
hypocalcemic tetany as, 573–574
idiopathic immune-mediated/inflammatory myopathies, 568
imaging for, 548
nuclear scintigraphy as, 548
ultrasonography as, 548, 548f
immune-mediated myopathies as, 566–568
immune-mediated myositis in Quarter horse-related breeds as, 566–567
infarctive purpura hemorrhagica as, 566
inflammatory myopathies as, 563–568
inspection of, 542–543, 544f

Musculoskeletal system *(Continued)*
locomotor and neurologic examination of, 543
malignant hyperthermia as, 553–554, 570
masseter muscle myodegeneration, 562
muscle fasciculations in, 571b
muscle form and function in, 542–548, 543f
myopathy as
focal, 569–570
generalized postanesthetic, 570
hypoglycin A, 568–569
mitochondrial, 560
myofibrillar, 559–560
polysaccharide storage, 554–559
postanesthetic, 569–570
Streptococcus Equi subsp.*Equi*-associated myopathy as, 564–565
vitamin E-deficient, 562–563
myotonia as, 570–571
ear tick-associated, 571–572, 571f
myotonic, 570–572
nonexertional myopathies as, 560–563, 560b
nutritional myodegeneration as, 561–562
physical examination of, 543
rhabdomyolysis and
acute, treatment of, 549–550
anaplasma-associated, 564
pathophysiology of, 544–545, 545f
recurrent exertional, 550–553
severe, 550
sporadic exertional, 548–550
toxic causes of, 568–569
sarcocystosis as, 563–564
synchronous diaphragmatic flutter as, 574
systemic calcinosis as, 567–568
tetracyclines and, 114
Mushroom toxicity, 1503
Mutant prevention concentration (MPC), of antimicrobials, 95
Mutation, obstetric, 1301
Mycetomata, 1179, 1180t
Mycobacterial products, for immunostimulation, 61
Mycotoxins, 871
Myeloencephalitis, equine protozoal, 640–645
clinical signs of, 642
diagnosis of, 643–644
differential diagnosis for, 644
epidemiology of, 640–641
magnetic resonance imaging in, 601–602, 602f
pathogenesis of, 641–642
pathology of, 642
polyneuritis equi *versus*, 653
prevention of, 645
Sarcocystis spp. in, life cycle of, 641
treatment of, 644–645
treatment of, 112
Myeloencephalopathy, equine herpesvirus-1, 645–652
clinical signs of, 648
control and prevention of, 651–652
diagnosis of, 649
epidemiology and immunity in, 646–647
laboratory findings in, 648–649
pathogenicity and pathogenesis of, 647–648
pathologic findings in, 650–651
polyneuritis equi *versus*, 653
treatment and prognosis for, 649–650
virologic findings in, 645–646
Myelography
in cervical vertebral compressive myelopathy, 635
in spinal cord and vertebral injury, 620
Myelopathies, focal and multifocal, 595–596, 596f
Myelophthisis, 998
Myocardial necrosis, cantharidin toxicosis and, 1467
Myocarditis, 490
Myocardium
contractility of
cardiac cycle and, 393f
measurement of, 396
disease of, 388–390, 389b, 390f, 486–491, 487f–489f
anatomic correlates of, 387–392, 388b–389b, 390f–391f
oxygen demand of, 397
toxic injury of, 399b, 490–491
Myofibrillar myopathy, 559–560
Myogenic atrophy, 545
Myoglobin, renal critical care, 183
Myoglobinuria, 924
Myoid cells, 1314
Myometrial activity, during pregnancy, 1273–1274
Myopathy
fibrotic, abnormal gaits associated with, 586–587
in poor performance, 293
Streptococcus-associated, 337
Myositis
EHV-1 - associated, 340
immune-mediated, 337
in *S. equi* infection, 337
Myotonia, 570–571
clinical signs of, 571
diagnosis of, 571
ear tick-associated, 571–572, 571f
myotonia congenita, 571
myotonia dystrophica, 571, 571f
pathogenesis of, 570
treatment and prognosis of, 571
Myotonic potentials, 595, 595f

N

Naive T lymphocytes, 35
Nalbuphine, for pain management, 144t–145t
Naloxone, for gastrointestinal ileus, 778
Naphthalene, for selenium toxicity, 1501
Narcolepsy, 265–266, 609–610
in foals, 1385
Narrative review, in evidence-based medicine, 228
Nasal discharge, in sinusitis, 323
Nasogastric intubation, 272, 275–276
in assisted enteral feeding, 205
in critical care, 170–171
Nasogastric reflux, 171
Nasogastric tube
for esophageal obstruction, 798–799
for fluid therapy, 1427
Nasolacrimal system disorders, 1143
Nasopharyngeal cicatrix, 330–331
Natural endotoxin-binding substances, 734–735
Natural killer cells, in inflammatory response, 21
Natural reduction, of twins, 1276–1277
Natural service. *see* Live cover breedings
NDF. *see* Neutral detergent fiber
Neck posture, neurologic examination of, 583
Necrosis
equine axillary nodular, 1212
fat, 1211–1212
hepatic, 850
myocardial, 490
Necrotic hepatitis, infectious, 868–869
Necrotizing enterocolitis (NEC), in foals, 1402
Necrotizing skin disorders, 1197–1202
burns, 1197–1198
decubital ulcers, 1197
envenomation, 1189
frostbite, 1198
gangrene, 1197
panniculitis and fat necrosis, 1211–1212
stachybotryotoxicosis, 1181
Neomycin
auditory system and, 627
for endometritis, 1254t
for hepatic insufficiency, 863–864
Neonatal acquired biliary obstruction, 879
Neonatal alloimmune thrombocytopenia, 1012–1013
Neonatal foals. *see also* Foals
bacterial infections in, 1394
examination of, 1367–1369
failure of transfer of passive immunity in, 55–57
gastric ulceration in, 805
gastrointestinal disorders of, 1400–1406
hepatic disorders of, 1416–1419
immunologic disorders of, 1414–1416
muscular disorders of, 1419–1420
neurologic disorders of, 1380–1386
Neonatal foals *(Continued)*
respiratory disorders of, 1386–1400
resuscitation of, 1370–1373, 1372f, 1372b
sepsis in, 1373–1380
sick, nutritional support for, 208–210, 209t
urogenital disorders of, 1406–1410
Neonatal isoerythrolysis, 45–46, 1008
Neonate, definition of, 90
Neoplasm
adrenocortical, 1078
of eyelid, 1141
hepatic, 879–880
neurologic, 685–686
orbital, 1155–1156
ovarian, 1244–1245
of urinary tract, 969–972, 970t–971t, 972f
uterine, 1243
vaginal, 1240
viral infections and, 9
vulvar, 1239
Neoplastic conditions
of accessory glands, 1339
of penis, 1333–1334
of scrotum, 1335
of spermatic cord, 1338
Neoplastic skin disorders, 1202–1211
atheroma, 1211
dermoid cysts, 1211
lymphoma, 1210
mast cell tumor, 1209–1210
melanoma, 1208–1209
pseudolymphoma, 1210
sarcoids, 1202–1206
squamous cell carcinoma, 1206–1208
Neostigmine, for gastrointestinal ileus, 777
Neotyphodium coenophialum, 1064
Nephrogenic diabetes insipidus, 964–966
Nephrolithiasis, 951–954, 953f
Nephrons, 889, 889f, 903f
Nephropathies, hereditary, in urinary system, 893–894, 894f
Nephrotic-range proteinuria, 942
Nephrotoxicity, 107–108, 108f
Nerium spp., 1484
Nerve conduction studies, 595, 595f–596f
Nervous ergotism, 1469–1470
Neuroactive steroids, 1076–1077
equine disease and, 1077–1078
Neuroaxonal dystrophy, equine, 636–640
Neuroelectrodiagnostics, 591–599
Neuroendocrine, pain and, 138
Neurogenic atrophy, 545
Neurologic critical care, 184
Neurologic disorders, 580–708
botulism, 672–676
cerebellum, diseases of, 628–632
cerebrospinal fluid, evaluation of, 588–591, 589f, 591t

Neurologic disorders *(Continued)*
cervical vertebral compressive myelopathy, 633–636
in critically ill horse, 184
electrolyte abnormalities, 687
equine grass sickness, 676–678
equine herpesvirus-1 myeloencephalopathy, 645–652
clinical signs of, 648
control and prevention of, 651–652
diagnosis of, 649
epidemiology and immunity in, 646–647
laboratory findings in, 648–649
pathogenicity and pathogenesis of, 647–648
pathologic findings in, 650–651
polyneuritis equi *versus*, 653
treatment and prognosis for, 649–650
virologic findings in, 645–646
equine motor neuron disease, 665–668, 665f
equine protozoal myeloencephalitis, 640–645
clinical signs of, 642
diagnosis of, 643–644
differential diagnosis for, 644
epidemiology of, 640–641
pathogenesis of, 641–642
pathology of, 642
prevention of, 645
Sarcocystis spp. in, life cycle of, 641
treatment of, 644–645
flavivirus encephalitis, 659–663
fluphenazine toxicity, 686
of foals, 1380–1386
bacterial meningitis as, 1383–1384
botulism as, 1384
congenital, 1385
hypoxic ischemic encephalopathy as, 1381–1383
metabolic encephalopathies as, 1384–1385
neonatal encephalopathy as, 1381–1383
seizures as, 1383
sepsis-associated encephalopathy as, 1383
tetanus as, 1384
trauma as, 1384
focal and multifocal myelopathies, 595–596, 596f
hepatoencephalopathy, 686–687
hypoglycemia, hypoxemia, and ischemia, 687
laryngeal hemiplegia, 596–597
leukoencephalomalacia, 686
Lyme disease, in horses, 678–680
magnetic resonance imaging in, 599–603
narcolepsy, 609–610
neuroelectrodiagnostics in, 591–599
Neurologic disorders *(Continued)*
brainstem auditory evoked response in, 597–598, 598f–599f
electroencephalography in, 598–599, 599f
electromyography in. *see* Electromyography
neurologic neoplasia, 685–686
nigropallidal encephalomalacia, 686
polyneuritis equi, 652–653
rabies, 663–665
radial and suprascapular nerve injury, 596, 597f
seizures, 603–607
clinical signs and diagnosis of, 605–606
etiology/pathogenesis of, 604–605, 604t–605t
treatment of, 606–607
spinal cord and vertebral injury, 618–622, 618t
tetanus, 668–672
traumatic brain injury, 611–618
neurologic evaluation of, 613–616, 614f–615f, 615t
pathophysiology of, 611–613, 612f
treatment and prognosis in, 616–618, 616f
trigeminal-mediated headshaking, 680–682, 680t–681t
verminous encephalomyelitis, 682–685, 684t
vestibular, 622–628
brainstem auditory evoked response in, 628
caloric testing in, 628
central vestibular disease, 627–628
clinical signs of, 623–625, 623f–624f
peripheral vestibular disease, 625–627
viral encephalitis, 654–659, 655t
Neurologic examination, 580–588
form for, 581f–582f
gait evaluation in, 585–586
localization of lesion in, 586, 587t
normal and abnormal gaits, 586–588
in traumatic brain injury, 613
Neurologic neoplasia, 685–686
Neurologic system
central. *see* Central nervous system
disorders of. *see* Neurologic disorders
of foals, 1367
Neuromuscular blockade, aminoglycosides and, 108
Neuromuscular disorders, 653–654, 654t
Neuropathy
in guttural pouch mycosis, 326
recurrent laryngeal, 329–330, 329t
Neurosteroids, 1076
Neurotoxins, in hepatic encephalopathy, 852
Neurotransmitters, false, 853, 853f
Neutral detergent fiber (NDF), 194b
Neutropenia, neonatal alloimmune, 1416
Neutrophils, 995–996
in bronchoalveolar lavage fluid, 319t
in endotoxemia, 730
in foals, 38–39
in gastrointestinal ileus, 775
in gastrointestinal inflammation, 716–718, 717f, 720
in inflammatory response, 22
Newborn foals. *see* Neonatal foals
NFCs. *see* Nonfiber carbohydrates
Nicotinamide, for pyriminil toxicity, 1465–1466
Nicotine toxicity, 1476–1477
Nightshade toxicity, 1461–1462
Nigropallidal encephalomalacia, 686
Nipah virus, 663
Nitrate toxicity, 1491–1492
Nitric oxide, 729
inhaled, for persistent pulmonary hypertension, 1391
Nitrite toxicity, 1491–1492
Nitrofurazone, in lymphoid pharyngeal hyperplasia, 327
Nitrogen substances, nonprotein, toxic effects of, 1477–1478
Nitrogenous compounds, metabolism of, 900
Nitrogenous wastes, production and elimination of, 898–900, 898b
Nitroglycerine, 457t–461t
Nociception, 138, 138b, 139f
Nociceptive pain, 138
management of, 140
Nodular interstitial disease, radiographic patterns in, 320–322, 321f
Nodular regeneration, hepatic, 850
Nodular skin disorders
bacterial granuloma, 1175, 1175f
corynebacterial infections, 1174–1175
equine axillary nodular necrosis, 1212
mycetomata, 1179, 1180t
phaeohyphomycosis, 1179, 1180t
pythiosis, 1180–1181, 1180f
sporotrichosis, 1178, 1179f, 1180t
urticaria, 1190, 1190f
Nonadrenergic-noncholinergic (NANC) innervation, 254
Nonantibiotic therapies, for endometritis, 1255–1256, 1255t
Nonenveloped viruses, 8
Nonfiber carbohydrates (NFCs), 194b, 199t
Nonfibrillar adhesins (NFAs), 5–6
Nonpregnant mares, reproductive tract of
anatomy and physiology of, 1217, 1220–1225
hormones of, 1220, 1223t, 1224f
pathology of, 1238
phases of, 1220–1225
lactational anestrus in, 1225
Nonpregnant mares, reproductive tract of *(Continued)*
ovulatory receptivity/breeding season in, 1222–1224
postpartum period of, 1225
vernal transition/anovulatory receptivity in, 1222
winter anestrus/anovulatory season in, 1221
Nonprimate hepacivirus, 867
Nonprotein nitrogen substances, toxic effects of, 1477–1478
Nonpulmonary respiratory distress, 258
Nonregenerative anemia, 995b, 1016–1018
in chronic kidney disease, 940
Nonselective *versus* COX-2-selective inhibitors, 120–121
Nonsteroidal antiinflammatory drugs (NSAIDs), 120, 120f, 350, 350t
acute diarrhea associated with, 748t
adverse effects of, 122–123
aspirin, 123
carprofen, 123
chirality, 121–122
for chronic kidney disease, 945
diclofenac, 123
drug interactions and, 122
for duodenitis-proximal jejunitis, 740
for endotoxemia, 735
for equine protozoal myeloencephalitis, 644
firocoxib, 123–124
flunixin meglumine, 124
for infective endocarditis, 484
ketoprofen, 124–125
mechanism of action of, 120–121
meloxicam, 125
for pain management, 140, 144t–145t
phenylbutazone, 125–126
physical properties of, 122
renal critical care, 183
systemic, for corneal disorders, 1147
vedaprofen, 126
for verminous encephalomyelitis, 684–685
Nonstructural carbohydrate (NSC), 194b, 198, 199t
exertional rhabdomyolysis and, 548–550
polysaccharide storage myopathy and, 555–556
Nonthyroidal illness syndrome (NTIS), 1063, 1412
Norepinephrine
in critical care, 174
for foals, 1430
for heart disease, 457t–461t
Normosol-R, 166
Nosocomial infections, 5
Noxious stimulus, 138b
NTIS. *see* Nonthyroidal illness syndrome

Nuclear medicine imaging, in cardiovascular disorders, 419
Nuclear scintigraphy
  for musculoskeletal system disorders, 548
  in urinary system, 919, 920f
Nurse mares, 207
Nutrition
  carbohydrates
    colic and, 199
    in equine, 197–202
  clinical, 191–217
  diarrhea and, 723
  evaluation of, feeding programs and, 191–197
    clinical examination in, 191–196, 195t
    diet and ration, 196–197
    terminology relevant to feed composition data, 194b
    worksheet for, 192f–194f
  for foals
    metabolism and nutrient requirements of, 207
    orphan, 207–208
    sick neonatal, 207–210, 209t
  in malabsorption and maldigestion, 746
  for recumbent horses, 186–187
  skin disorders and, 1187–1189
Nutritional muscular dystrophy, 561
Nutritional myodegeneration, 561–562, 1419–1420
  clinical pathology of, 561
  clinical signs of, 561
  diagnosis of, 561
  pathogenesis of, 561
  pathology of, 561
  prevention and control of, 562
  treatment and prognosis for, 561–562
Nutritional support
  assisted enteral feeding and, 204–205, 204t
  candidates for, 203
  for critically ill horse, 181–182
  for duodenitis-proximal jejunitis, 740
  enteral nutrition, 204, 204t
  estimating nutrient and feed requirements, 203–204
  for foals
    assisted enteral feeding, 204t
    enteral nutrition, 208–209
    metabolism and nutrient requirements, 207
    orphaned, 207–210
    parenteral nutrition, 205–207, 206t, 209–210, 210t
    sick neonatal, 208–210
  for pleuropneumonia, 350–351
  for sick horses, 202–207
  for thin and starved horses, 213
  in traumatic brain injury, 618
Nutritional therapy, modes of, 204–207
Nystagmus, 622, 624
Nystatin, for endometritis, 1258, 1259t

O

Oak toxicity, 1460–1461
Obesity, 1088
  alterations in body weight, 239–241
  causes of, 240b
  management of, 210–213, 211t
Observational studies, in evidence-based medicine, 225–227
Obstruction
  airway
    recurrent, 41–42, 45
    in respiratory distress, 255
  biliary
    acute, 871
    neonatal acquired, 879
  esophageal, 796, 798f–799f, 798t
  intestinal, strangulating, 769–770, 778, 779f
  pyloric, 808
Obstructive disease, of urinary system, 949
  bladder displacement in, 957
  cystic calculi in, 954–956, 954f–956f
  epidemiology of, 949
  nephrolithiasis and ureterolithiasis in, 951–954, 953f
  pathophysiology of, 949–951, 951f–952f, 951t
  penile trauma in, 957
  sabulous urolithiasis in, 957
  urethral calculi in, 956–957, 956f
Obstructive shock, 173
Occipitoatlantoaxial malformation, in foals, 1385
Occult sarcoids, 1202, 1203f
Oculocephalic reflex, 622
Oculomotor nerve, 622
Oleander toxicity, 1484
Oligosaccharides, 197
OLWS. *see* Overo lethal white syndrome
Omeprazole, 807
  for gastric ulceration in foals, 1405–1406, 1405t
Omphaloarteritis, 1408
Omphalophlebitis, 1408
  severe combined immunodeficiency and, 52
Onchocerciasis
  conjunctival, 1142–1143
  cutaneous, 1184–1185, 1185f
Onion
  consumption of, in hemolysis, 1010
  toxicity, 1486
Ontogeny, of equine immune system, 38
Oocyte
  culture, 1311
  donors, 1311
  transfer, 1310–1312
    success of, 1312
Open-ended model artificial vagina, 1323–1324
Operative mesenteric portography, 862
Ophthalmology, 1139
Opioids
  critically ill horse and, 185
  for pain management, 144t–145t, 145–146
Optic nerve, disorders of, 1154–1155
Oral cavity, 789–790
  dentition in, 790–791
  diseases of, 791
    congenital and developmental abnormalities in, 794–796
    dental, 794
    dysphagia, 791–793, 792t–793t
    periodontal, 794
    salivary glands and, 795–796
    stomatitis and glossitis, 795
  morphology and function of, 790
  physical examination of, 710
Oral glucose tolerance test, 714
Orbifloxacin, 116
Orbit, disorders of, 1155–1156
Orbivirus, in African horse sickness, 345
Organ failure, in endotoxemia, 731
Organic compounds, metabolism of, 900
Organic ethylenediamine dihydroiodide, for fungal pneumonia, 356t
Organic wastes, production and elimination of, 898–900, 898b
Organophosphate pesticides, 1472–1473
Organum vasculosum laminae terminalis (OVLT), 235
Oropharynx, 789–790
Orphan foals, nutritional support for, 207–208
Oscillometric sphygmomanometry, 169, 169f
Osmotic diuretics
  critically ill horse and, 185
  for traumatic brain injury, 617–618
Osmotic overload, in diarrhea, 279
O-specific chains, of endotoxins, 724, 724f
Osteochondritis dissecans, bilateral, 580
Otobius megnini, 571–572, 1184
Ototoxicity, 108
Ovarian hematomas, associated with hemoperitoneum, 1006
Ovaries, 1217, 1244–1246
  pathology of, in mare breeding soundness examination, 1228–1230, 1232f
Overexertion, sporadic exertional rhabdomyolysis and, 549
Overfeeding, 211
Overo lethal white syndrome (OLWS), 1168
  in foals, 1401
Over-the-wire (OTW) catheters, 161–162, 162t
  for blood pressure measurement, 168
  for fluid therapy, 1427
Oviduct, 1217–1218, 1218f
  contraction, hormones in, 1263
OVLT. *see* Organum vasculosum laminae terminalis
Ovulation, inducing, hormones for, 1261–1262
Ovulatory receptivity/breeding season, 1222–1224
Oxalate toxicity, 1032t, 1044
Oxalate toxicosis, 1506, 1506b
Oxidative injury, hemolysis secondary to, 1009–1010
Oximes, for organophosphate toxicity, 1473
Oxygen
  demand of, myocardial, 397
  inspired, reduction of, 255
  partial pressure, 177, 177t
  pulse, maximum, 294
  uptake, 294
    in poor performance, 289–290
Oxygen saturation (SaO_2), 180
Oxygen therapy
  for congestive heart failure, 461
  in critical care, 178–179, 179f
Oxytetracycline
  for foals, 1423
  for Lyme disease, 679
  pharmacology of, 112
  for pleuropneumonia, 350t
Oxytocin
  for endometritis, 1254–1255
  for esophageal obstruction, 799
Oxyuris equi, 1186

P

P wave, in electrocardiography, 392, 403, 414, 414f, 417f
Pace, 586
Pacemaker
  activity, 414
  for atrioventricular conduction block, 517f
Packed cell volume (PCV), for colic, 276
$PaCO_2$, respiratory and, 177, 177t
Pain
  abdominal, hepatic insufficiency and, 855
  assessment of, 139–140
  behavioral indicators of, 140b
  central sensitization, 138
  complementary therapies, 150–151
  definition of, 138–157, 138b
  equids, demonstrating facial expressions of, 143f
  multidimensional pain scale, 142f
  multifactorial numerical rating composite pain scale, 141f
  nociception and perception, 138, 139f
  pathophysiology of, 138–139
  peripheral sensitization, 138
  physiologic, 138
  specific analgesic techniques for, 149–150
  topical drug administration of, 149–150, 150f
  transdermal drug administration of, 149–150, 150f

Pain *(Continued)*
treatment of drugs used for, 140–149, 144t–145t
α_2-adrenoreceptor agonists, 144t–145t, 146–147
antiinflammatory drugs, 143–145
opioids, 144t–145t, 145–146
treatment plan for, 140
Palate
morphology and function of, 790
soft, dorsal displacement of, 328–329, 328t
Palatinus, 328t
Palatopharyngeal arch, rostral displacement of, 330
Palatopharyngeus, 328t
Palpation
of arterial pulses, 409
during neurologic examination, 587–588
transrectal, 710
in mare breeding soundness examination, 1227–1228, 1228t
in uterine torsion, 1291
Pancreas, 1085
Pancreatic polypeptide, 1080
Pancreatitis, 1044
Pancytopenia, 1017
Panniculitis, 1165t–1167t, 1211–1212
Pantoprazole, 806
for gastric ulceration in foals, 1405–1406, 1405t
Papillae, of tongue, 790
Papillomatosis, viral, 1175–1176
Paradoxic vestibular disease, 624
Paralysis
facial, 586, 624–625
neurologic examination in, 584
in peripheral vestibular disease, 625, 626f
in traumatic brain injury, 613
in polyneuritis equi, 652
Paramembranous ventricular septal defects, 402f
Paranasal sinuses, sinonasal disease in, 323
Paraphimosis, 1333
Parapoxvirus ovis, for immunostimulation, 61
Parascaris equorum
colic due to, 1401
in hepatitis, 869
parasitic pneumonia due to, 1394
Parascaris spp.
colic and, 14
in respiratory infection, 16
Parasite migration, in central nervous system, 628
Parasitic diseases
conjunctival, 1142–1143
skin, 1181–1187
systemic manifestations of, 1165t–1167t
Parasitic hepatitis, 869
Parasitic infections
gastrointestinal, 14–16
pathogenesis of, 14
protective resistance, 17–18
regulatory responses and, 18–19
respiratory, 16
skin, 16–17
of urinary tract, 948–949
Parasitic pneumonia, 1394
Parasitic pneumonitis, 356–357
Parasitic rhinitis, 323
Parasitic vascular lesions, 494f, 496
Parathyroid hormone, 1035
physiologic actions of, 1035–1036, 1035f–1037f
Parathyroid hormone-like hormone, 372
Parathyroid hormone-related protein, 1040
Parelaphostrongylus tenuis, in verminous encephalomyelitis, 682
Parenteral nutritional support, 205–207, 206t
in critically ill horse, 182
for sick neonatal foals, 209–210, 210t
transition to voluntary feeding and, 207
Parity, vulvar pathology and, 1239
Parotid duct, 795
"Paroxysmal depolarization shift," 604
Parrot mouth, 795
Partial malabsorption, 714
Partial parenteral nutrition, 202–203
Partial pressure of oxygen (PaO_2)
in hypoxemia, 178
respiratory and, 177, 177t
Partial seizures, 603
Parturition
dystocia
etiology of, 1299
management of, 1299–1303
endocrinology of, 1272–1273
fetal kinetics and, 1297–1298
induction of, 1295–1296
in hydrops of fetal fluids, 1289
normal, 1297–1299
stages of, 1298–1299
terminology in, 1297
Passive immunity, failure of transfer of, 55–57
in foals, 1414
Pastern dermatitis, 1181, 1181f
Pasture breeding, 1265
Pasture grazing, 201–202
Patella, upward fixation of, 586–587
Patent ductus arteriosus, 468–469, 469f
Patent urachus, 897–898
in foals, 1408
Pathogen-associated molecular patterns (PAMPs), 20–21
in foals, 1374
Pathogenicity islands, 749
Pathogens, opportunistic, 1341
Peak running speed, in poor performance, 295
Pediculosis. *see* Lice
Pelvic urethra, 1323
Pemphigoid, bullous, 1195–1196
Pemphigus foliaceus, 1165t–1167t, 1194–1195, 1194f–1195f
Pemphigus vulgaris, 1195
Pendulant kidney, 894
Penicillin
antipseudomonal, 102
for critically ill horse, 186
for guttural pouch empyema, 327
for infective endocarditis, 484
pharmacology of, 99–100
for pleuropneumonia, 350
for tetanus, 671
for type I hypersensitivity, 43
Penicillin G
for foals, 1421, 1422t
pharmacology of, 100–101
Penicillin-binding proteins (PBPs), 99
Penile trauma, 957
Penile urethra, 1323
Penis, 1316
abnormalities of, 1332–1334
Pentachlorophenol toxicity, 1464–1465
Pentastarch, 1426
Pentobarbital
for seizures, 606
for traumatic brain injury, 617
Pentoxifylline
for allergic skin disease, 1193
for endotoxemia, 736
for hepatic insufficiency, 864
for neonatal encephalopathy, 1386
for oxygen delivery, in utero, 1367
Peptides, trefoil, 766–767, 773
Perception, of pain, 138, 139f
Percussion
of abdomen, 709
thoracic, in respiratory distress evaluation, 258
of thorax, 316
Perforation, esophageal, 803
Periacinar zonal injury, hepatic, 850
Peribronchiolar pattern, radiographic, 320–322
Pericardial disease, 484–486, 484f–485f
anatomic correlates of, 388
Pericardial effusion, 441b–443b
Pericardial hernias, 484–485
Pericardial space, echocardiographic and Doppler studies of, 441b–443b
Pericardiocentesis, 485–486
Pericarditis, 484–485
examination in, 397
Pericardium, echocardiographic and Doppler studies of, 441b–443b
Perinatal asphyxia-associated gastrointestinal dysfunction, diarrhea due to, 1402
Perineum, postpartum bruising and laceration of, 1309–1310
Perineural catheters, for pain management, 149
Periodontal disease, 794
Periparturient blood loss, in mares, 1007
Periparturient hemorrhage, postpartum, 1304–1305
Peripheral compartments, 79–80
Peripheral ganglionopathy, in Fell pony syndrome, 53
Peripheral nerve, in electromyography, 592t
Peripheral sensitization, 138
Peripheral vasculature, examination of, 409–411
Peripheral vestibular disease, 625–627
clinical signs of, 623, 623f
Peritoneal fluid, in disorders of the gastrointestinal system, 711–712
Peritoneal lavage, for peritonitis, 765
Peritoneum, 762
Peritonitis, 172, 762
after trocarization, 172
clinical signs of, 764
clinicopathologic findings in, 764–765
etiopathogenesis of, 763–765
fever of unknown origin and, 236
prognosis of, 765
treatment of, 765
Permanent teeth, 790
Persistent hyperammonemia, 1418
Persistent pulmonary hypertension, 1391
Persistent right aortic arch, 802
Persistent-mating-induced endometritis (PMIE), 1246–1247, 1247f
Pesticides, toxic effects of, 1471–1476
Pethidine, for pain management, 144t–145t
Petroleum distillates toxicity, 1464
Petrous temporal bone, 623
fractures of, 625
PGD_2, in inflammatory response, 22
PGE_2, in inflammatory response, 22
PGF_2, in inflammatory response, 22
PGI_2, in inflammatory response, 22
pH, drugs classified by, 84, 84b
Phaeohyphomycosis, 1179, 1180t
Phagocyte
function testing, 51–52
mononuclear, hepatic, 849
Phagocytosis, 6–7, 6t
Phagolysosomal survival, spread of bacteria and, 6t
Phantom, breeding, for stallion mounting, 1328–1330, 1330f
Pharmacokinetics, 79
definitions in, 79–80
drug dosage regimens based on, 90–91
drug protein binding and, 85
Pharmacologic alteration, in gastrointestinal ileus, 775
Pharmacologic principles, 79–137
of antimicrobial therapy, 94
aminoglycosides in, 105–109, 107t, 108f

Pharmacologic principles *(Continued)*
aminopenicillins in, 101–102
amphenicols in, 109–110
antipseudomonal penicillins in, 102
benzylpenicillin in, 100–101
beta-lactam antibiotics in, 99–100
beta-lactamase inhibitors in, 99–100
carbapenems in, 105
cephalosporins in, 99–100, 102–105
chloramphenicol (CPHC) in, 109
concurrent use of additional antimicrobials in, 99
designing of dosage regimens of, 95, 97–98
florfenicol (FLF) in, 109
fluoroquinolones in, 116–118
macrolides and azalides in, 114–116
metronidazole in, 119–120
prophylactic use of, 99
rational use of, 94–97
bactericidal *versus* bacteriostatic antimicrobials, 96–97, 97b
infection documentation and, 95
minimum inhibitory concentration interpretation in, 95–96, 96f
pharmacokinetic-pharmacodynamic relationships in, 97, 98f, 98b
postantibiotic effect of, 97, 97t
rifampin in, 118–119
sulfonamides in, 110–112, 111t
tetracyclines in, 112–114
designing of dosage regimens in, 89–91, 90t
distribution of drugs in the body and, 82–85, 82f–83f
drug accumulation and, 89, 89f
elimination of drugs from body and, 85
rate constant and half-life, 85–89, 85f–86f, 86t
lipid solubility and ionization of, 84, 84b
of nonsteroidal antiinflammatory drugs, 120, 120f
adverse effects of, 122–123
aspirin, 123
carprofen, 123
chirality, 121–122
diclofenac, 123
drug interactions and, 122
firocoxib, 123–124
flunixin meglumine, 124
ketoprofen, 124–125
mechanism of action of, 120–121
Pharmacologic principles *(Continued)*
meloxicam, 125
phenylbutazone, 125–126
physical properties of, 122
vedaprofen, 126
pharmacokinetics in, 79
definitions in, 79–80
rates and orders of reactions of, 80–81, 80f
clinical application of compartmental modeling and, 81–82, 81f–82f
renal failure and, 92–94
therapeutic drug monitoring and, 91–92, 92t
variation between drug dose and plasma drug concentration and, 79
Pharmacologic stress testing, 413
Pharyngeal foreign bodies, weight loss associated with, 242
Pharyngitis, associated with cough, 249
Pharynx
innervation to, neurologic examination of, 584
lymphoid hyperplasia in, 327, 328t
Phenobarbital
for neonatal encephalopathy, 1385–1386
for seizures, 606
for traumatic brain injury, 617
Phenoxybenzamine, for bladder dysfunction, 975–976
Phenylbutazone
for acute rhabdomyolysis, 549
for pain management, 144t–145t
penicillin G therapy and, 101
pharmacology of, 125–126
for pleuropneumonia, 350t
in retained fetal membranes, 1307
for spinal cord injury, 621
for verminous encephalomyelitis, 684–685
Phenylephrine
in critical care, 174–175
for foals, 1430
for heart disease, 457t–461t
Phenylephrine-associated bleeding, 1007
Phenytoin
for heart disease, 457t–461t
for recurrent exertional rhabdomyolysis, 553
for seizures, 606–607
Pheochromocytoma, 1078–1079
Phimosis, 1333
Phonocardiograms (PCG), 404f, 472f
Phosphatonin, 1040
Phosphorus, 1033–1040, 1034f
absorption of, 1031–1032, 1031t–1032t, 1032f
deficiency in, 1033
homeostasis, 1035–1037, 1035f
requirements in, 1030–1031, 1030t–1031t
toxic effects of, 1467–1468
Photic head shakers, 680–681
Photodynamic therapy (PDT), for squamous cell carcinoma, of eyelid, 1142
Photoplethysmography, 169
Photosensitization, 1198–1201
causes of, 1199t
diagnosis of, 1201, 1201f
hepatogenic, 855
hepatogenous, 1165t–1167t, 1198
pathogenesis of, 1198–1201
treatment for, 1201
Photosensitizing plants, 1495–1496, 1495b
Phthisis bulbi, 1152, 1152f
Phycomycosis. *see* Pythiosis
Phylloerythrin, 855, 1198
Physical examination
for body temperature, 236
in cardiovascular critical care, 176–177
for cardiovascular disorders, 397–454
auscultation in, 399–409
algorithm for, 406t–407t
of cardiac murmurs, 403f–404f, 405–408, 408b
clinical method for, 399–403, 400t–402t, 402f–403f
of heart rate and rhythm, 395f, 401t, 404–405
of heart sounds, 403–404, 404f
pulmonary, 408–409, 409f
in congestive heart failure, 411f
general approach to, 397, 398b
history in, 397–398, 399b
in mitral regurgitation, 474–475
peripheral vasculature, 409–411
for colic, 275–276
for collapse, 267–268
for cough, 249
for diarrhea, 281
for disorders of the gastrointestinal system, 709–711
for dysphagia, 271–272
for edema, 263
for mare breeding soundness, 1226
for polyuria and polydipsia, 288
for respiratory disorders, 315–316
for respiratory distress, 257–258
for skin disorders, 1161
for stallions, 1319–1323
of urinary system, 909
for weight gain, 241
for weight loss, 244
Physiologic dead space, 177
Physiologic pain, 138
Physiologic shunting, 255
Physiologic status, thyroid gland function and testing and, 1062
Physiotherapy, 187
for spinal cord injury, 621–622
Physostigmine salicylate, 610
Phytolacca americana toxicity, 1461
Piedra, 1178, 1180t
Pigmentation disorders, 1161–1169
Pigmenturia, 957–958
associated with systemic disease, 961
Pili, 5–6
type 4, 6t
Pineal gland, 1317
Pinky syndrome. *see* Vitiligo, of Arabian horses
Pinworms, 1161, 1186–1187
Piroplasmosis
causing endometritis, 1249
in hemolysis, 1008–1009
transmission of, through semen, 1340–1341
Piroxicam, for squamous cell carcinoma, 1208
Pistacia leaf toxicity, 1010
Pituitary adenoma, 601, 601f
Pituitary gland, 1070, 1220
Pituitary pars intermedia dysfunction (PPID), 287, 1085, 1100
clinical signs of, 1100–1102, 1101f
diagnostic testing of, 1105–1108
epidemiology of, 1102
laboratory testing of, 1104
pathogenesis of, 1102–1103, 1104f
pathologic findings of, 1103–1104, 1105f
polyuria and polydipsia in, 963
systemic manifestations of, 1165t–1167t
treatment of, 1101f, 1108–1109
Pituitary-independent hyperadrenocorticism (adrenal Cushing syndrome), 1109
PKD. *see* Polycystic kidney disease
Placenta
assessment of, 1365
retained, 1041–1042
Placentation, in pregnancy, 1270–1271
Placentitis, 1281–1282, 1286
treatment for, 1283–1284
Plants
hepatotoxic, 870
photosensitizing, 1495–1496, 1495b
product, for immunostimulation, 61–62
rhabdomyolysis from, 568–569
toxic
cardiovascular effects of, 1484–1486
central nervous system depression and, 1478–1480
central nervous system stimulation and, 1469–1471
epithelium and skeletal system and, 1494–1497
gastrointestinal effects of, 1460–1462
liver disease or dysfunction and, 1502–1503
urinary system and, 1506–1507
Plasma, 167
for endotoxemia, 737
ionic composition of, 900t

Plasma cells, in adaptive immunity, 23–24
Plasma drug concentrations as therapeutic guideline, 79
Plasma inhibitors, 1001
Plasma lactate measurement, 294
Plasma proteins, in interstitial fluid, 259
Plasma transfusion, for hematopoietic disorders, 1004
Plasma-based coagulation system, abnormalities of, 1013–1016
  disseminated intravascular coagulation in, 1015
  hemophilia A in, 1014
    acquired, 1014
  liver disease in, 1014–1015
  mouldy sweet clover toxicity in, 1014
  prekallikrein deficiency in, 1014
  thrombosis in, 1015–1016
    large-vessel, 1015–1016
  vitamin K deficiency bleeding in, 1014
  Von Willebrand's disease in, 1013–1014
  warfarin/anticoagulant rodenticide toxicity in, 1014
Plasma-lyte148, 166
Platelet transfusion, for hematopoietic disorders, 1004
Platelet-activating factor (PAF)
  in endotoxemia, 737
  in pathophysiology of colitis, 721, 722t
  in vascular response during inflammation, 716
Platelets, 997–998
  disorders of, 1012–1013
  evaluation of, 997
  function of, changes in, 1013
  interpretation of, 997–998, 998b
  physiology of, 997
Pleural effusion
  drainage in, 170
  in fungal pneumonia, 354–355
Pleural fluid
  diagnostic sampling of, 319
  in pleuropneumonia, 348–349
Pleuritic friction rubs, 316
Pleuritis, pleuropneumonia and, 346
Pleuropneumonia, 346–352
  antibiotic therapy for, 350, 350t
  clinical manifestation of, 347
  diagnosis of, 347–349, 347f–348f
  etiologic agents of, 347
  extrathoracic complications of, 351–352
  prognosis and outcome in, 352
  treatment of, 349–352, 350t
Pleuroscopy, 323
PMIE. *see* Persistent-mating-induced endometritis
Pneumatosis intestinalis, 277
Pneumocystis carinii, in severe combined immunodeficiency, 52
Pneumocystis jirovecii, in fungal pneumonia, 354
Pneumonia, in foals
  fungal, 1394
  parasitic, 1394
  viral, 1393
Pneumothorax, 1392–1393
  in critical care, 170
  pleuropneumonia and, 347–348, 351
Pneumovagina, 1240
Podocytes, 931–932
Poikilocyte, 993
Pokeweed toxicity, 1461
Polish model artificial vagina, 1323–1324
Pollakiuria, 284
Polyamines, 772–773
  in mucosal repair, gastrointestinal, 772–773
Polychromasia, 993
Polycystic kidney disease (PKD), 893–894, 894f
Polycythemia, 711
Polycythemia vera, 1018
Polydipsia, 283–289, 961–962, 962t
  causes of, 284t
  in chronic kidney disease, 939
  conditions associated with, 287–288
  in diabetes insipidus, 964–966
  in diabetes mellitus, 966
  diagnostic approach to, 288–289
  diagnostic imaging of, 289
  in endotoxemia, 966
  excessive salt consumption in, 964
  general mechanisms of, 286
  in hepatic insufficiency, 856
  mechanisms of, 284–286
  in pituitary pars intermedia dysfunction, 963, 1101
  primary, 963–964
  as primary problem, 286
  in renal failure, 962–963
  in sepsis, 966
Polymerase chain reaction (PCR)
  for equine herpesvirus infection, 340–341
  for *S. equi* infection, 340–341
  testing, for rhodococcal pneumonia, 1396
Polymerases, viral, 9
Polymyxin B
  in acute tubular necrosis, 926
  for endometritis, 1254t
  for endotoxemia, 734
Polyneuritis equi, 652–653
  equine protozoal myeloencephalitis and, 644
Polyneuropathy, acquired equine, in Scandinavia, 653
Polyphasic motor unit action potentials, 593–594, 594f
Polyps, cervical, 1241
Polysaccharide capsules, fungal, 8
Polysaccharide storage myopathy, 554–559
  in foals, 1419
  management of, 557–559
    caloric balance in, 558
    diets in, 558–559
Polysaccharide storage myopathy *(Continued)*
    exercise in, 558
    low-starch, high-fat concentrates and, 559
    rest in, 558
    selection of fat source in, 559
    selection of forage in, 558–559
    supplements in, 559
  terminology in, 554
  type 1, 554–556
    acute clinical signs of, 555, 557f
    diagnosis of, 554–555, 555f–556f
    in draft horses and draft crosses, 555
    genetics of, 554
    in light breeds, 555
    pathophysiology of, 555–556
    prevalence of, 554
    risk factors of, 554
  type 2, 556–557
    clinical signs of, 557
    diagnosis of, 557, 557f
    pathogenesis of, 557
    prevalence of, 557
Polysaccharides, structural, 197–198
Polyuria, 283–289, 961–962, 962t
  causes of, 284t
  in chronic kidney disease, 939
  conditions associated with, 287–288
  in diabetes insipidus, 964–966
  in diabetes mellitus, 966
  diagnostic approach to, 288–289
  diagnostic imaging of, 289
  in endotoxemia, 966
  excessive salt consumption in, 964
  general mechanisms of, 286
  in hepatic insufficiency, 856
  iatrogenic, 966
  mechanisms of, 284–286
  in pituitary pars intermedia dysfunction, 963, 1101
  as primary problem, 286
  in renal failure, 962–963
  in sepsis, 966
Ponazuril, 644
Pons, traumatic injury to, 615t
Poor performance, 289–295
  ancillary diagnostic aids of, 293–295
  cardiovascular association of, 399b
  cardiovascular examination for, 292
  causes of, 290b
  clinical pathology/selected laboratory tests for, 293
  conditions associated with, 290–291
  diagnostic approach to, 292–295
  diagnostic imaging for, 293
  endoscopy of, 293
  exercise testing in, 293–295
  genetic factors of, 289
  locomotion in, 289
  mechanisms affecting, 289–290
  musculoskeletal and neurologic examination, 292–293
Poor performance *(Continued)*
  physical examination of, 292–293
  respiratory disorders in, 291
  respiratory examination for, 292
  signalment and history of, 292
Portal vein thrombosis, 881
Portography, operative mesenteric, 862
Portosystemic shunts, 880–881
  in foals, 1419
Position, fetal, 1297
Positive sharp waves, 594, 594f
Postanesthetic myopathy, 569–570
Postantibiotic effect, 97, 97t
Postmortem examination
  in African horse sickness, 345
  in cerebellar abiotrophy, 631
  in equine degenerative myeloencephalopathy, 637
  in equine herpesvirus, 340
  in equine motor neuron disease, 667
  in equine multinodular pulmonary fibrosis, 342
  in granular cell tumors, 369
  in metastatic pulmonary neoplasia, 370
  in polyneuritis equi, 652
  in *Streptococcus*-associated myopathy, 337
  in thymic tumors, 369
  in verminous encephalomyelitis, 684
  in West Nile virus, 662
Postmortem images
  in aortoiliac thrombosis, 494f
  in mitral regurgitation, 473f
Postpartum mare, 1303–1310
  eclampsia (lactation tetany) in, 1310
  gastrointestinal complications in, 1308–1309
  partial inversion (intussusception) of uterine horn in, 1306
  perineal bruising and vulvar hematomas in, 1310
  periparturient hemorrhage in, 1304–1305
  rectovaginal fistulas and perineal lacerations in, 1309
  retained fetal membranes and toxic metritis in, 1306–1308
  urinary bladder eversion in, 1309
  urinary bladder rupture in, 1309
  uterine prolapse (eversion) in, 1305–1306
  uterine rupture in, 1306
  vaginal lacerations and bladder prolapse in, 1309
Postpartum period, 1225
Postrenal obstructive failure, 924
Posture
  in fetal, 1297
  in gait evaluation, 585
  neurologic examination of, 583
Potassium
  in cardiac cell electrical activity, 413
  in cerebrospinal fluid, 591t
  in hyperkalemia, 516
  supplementation of, 164

Potassium bromide, for neonatal encephalopathy, 1385–1386
Potassium channel blocker, 457t–461t
Potassium chloride
  for bicarbonate toxicity, 1489
  for thallium toxicity, 1468
Potassium iodide, for fungal pneumonia, 356t
Potassium penicillin
  for gastrointestinal ileus, 777–778
  for infective endocarditis, 484
  pharmacology of, 101
Potassium penicillin G
  for endometritis, 1253, 1254t
  for pleuropneumonia, 350t
Potassium supplementation, in acute renal failure, 929–930
Potentials
  electromyographic
    abnormal, 593–595
    normal, 592–593
  fasciculation, 594–597
  fibrillation, 594
  motor unit action, 592, 593f
    polyphasic, 593–594, 594f
  myotonic, 595, 595f
  positive sharp waves, 594, 594f
Potentiated sulfonamides, for foals, 1423
Potomac horse fever
  acute diarrhea associated with, 750–752
  therapy for, 116
Povidone-iodine solution, for endometritis, 1255t
Powassan virus, 659
PPID. *see* Pituitary pars intermedia dysfunction
Prececal starch digestibility, 201
Precordial thrill, 403, 476
Prednisolone
  for allergic skin disease, 1193
  for cutaneous habronemiasis, 1186
  for immune-mediated myositis in Quarter horse-related breeds, 567
  for onchocerciasis, 1185
  for spinal cord injury, 621
  for verminous encephalomyelitis, 684–685
Prednisolone acetate, 650
Prednisolone therapy, 185
Pre-ejection period, of cardiac cycle, 392–395
Preemptive analgesia, 138b
Preexcitation, 515–516, 516f
Pregabalin, for pain management, 148
Pregnancy
  complications in late gestation during, 1288–1294
    hydrops of fetal fluids, 1289–1290
    uterine torsion, 1291–1292
    vaginal hemorrhage, 1292–1293
    ventral body wall hernias and prepubic tendon rupture, 1290–1291
Pregnancy *(Continued)*
  diagnosis, 1274–1275
  endocrinology of, 1271–1272
  fescue toxicity and agalactia in, 1293–1294
  foaling management of, 1294–1295
  gender determination in, 1285–1286
  hormonal support of, 1263–1264
  loss
    early, 1278–1285
    early fetal loss, 1279–1280
    embryonic loss, 1278–1279
    leptospirosis, 1282
    mare reproductive loss syndrome, 1280–1281
    placentitis, 1281–1282
    protozoal abortion, 1282
    twins and, 1284
    umbilical cord compromise and, 1284–1285
    viral abortion, 1282–1283
  management of twins during, 1275–1278
  maternal recognition of, 1269–1270
  myometrial activity during, 1273–1274
  parturition in
    dystocia. *see* Dystocia
    endocrinology of, 1272–1273
    fetal anomalies and, 1303
    fetal kinetics and, 1297–1298
    induction of, 1295–1296
    normal, 1297–1299
    stages of, 1298–1299
    terminology in, 1297
  placentation in, 1270–1271
  postpartum period following, 1303–1310
    eclampsia (lactation tetany) in, 1310
    gastrointestinal complications in, 1308–1309
    partial inversion (intussusception) of uterine horn in, 1306
    perineal bruising and vulvar hematomas in, 1310
    periparturient hemorrhage in, 1304–1305
    rectovaginal fistulas and perineal lacerations in, 1309
    retained fetal membranes and toxic metritis in, 1306–1308
    urinary bladder eversion in, 1309
    urinary bladder rupture in, 1309
    uterine prolapse (eversion) in, 1305–1306
    uterine rupture in, 1306
    vaginal lacerations and bladder prolapse in, 1309
  teratogenesis, 1274
  termination, with prostaglandin, 1277
Pregnane, 1076
Pregnant mare, 1269
  gestation in, physiology of, 1269–1274
  management of, 1296–1303
    twins in, 1275–1278
  monitoring, 1286–1288
  parturition in, endocrinology of, 1272–1273
Pregnenolone, 1071–1072
Pregnenolone sulfate, 1076
Prehension, in normal eating, 268
Prehepatic icterus, 854
Prekallikrein deficiency, 1014
Prelaminitic metabolic syndrome, 1088
Prelaminitic syndrome, 212
Preload, in ventricular function, 396
Premature complexes
  atrial, 500–502, 500f
  junctional, 511
  ventricular, 512–513
Prenatal development, of equine immune system, 38
Prepubic tendon rupture, 1290–1291
Prepuce, 1316
  abnormalities of, 1332–1334
Prerenal azotemia, 911, 911t
Prerenal failure, 911, 923–924
Presentation, fetal
  caudal (posterior), 1297, 1302–1303
  cranial (anterior), 1297, 1301–1302
  definition of, 1297
  transverse, 1303
Pressure sores, 1197
Pressure ulcers, 186
Priapism, 1333
Primary adrenal insufficiency, 1073
Primary cystitis, 946
Primary erythrocytosis, 1018
Primary hemostasis, 999
Primary immunodeficiency, 52–55
Primary photosensitization, 1198
Primary polydipsia, 963–964
Primary pulmonary neoplasms, 369
Primary thrombocytosis, 1018
Primidone, for seizures, 606–607
Principle of superposition, 89
Probiotics, definition of, 202
Procainamide
  for heart disease, 457t–461t
  for ventricular tachycardia, 513–514
Procaine penicillin, for dermatophilosis, 1174
Procaine penicillin G
  for endometritis, 1253, 1254t
  for foals, 1421
  pharmacology of, 100
  for pleuropneumonia, 350t
Procoagulants, for hemostatic dysfunction, 1004–1005
Prodrug, 88
Progesterone
  pregnancy and, 1271
  recurrent exertional rhabdomyolysis and, 553
Progressive ethmoidal hematoma, 324
Proinflammatory cytokines, for gastrointestinal ileus, 775
Prokinetics
  for duodenal disease, 807
  for duodenitis-proximal jejunitis, 740–741
Prolapse
  aortic valve, 476, 478f–479f
  bladder, postpartum, 1309
  iris, 1148, 1149f
  mitral valve, 472f
  uterine, postpartum, 1305–1306
Proliferative enteropathy, 743t–744t, 745–746
Proopiomelanocortin (POMC) peptides, 1108
Propafenone
  for atrial fibrillation, 506–507
  for heart disease, 457t–461t
Prophylactic use, of antimicrobials, 99
Propionibacterium acnes, for immunostimulation, 61
Propranolol, 457t–461t
Propylene glycol toxicity, 1480
Prospective cohort studies, 227
Prostacyclin, in inflammatory response, 21–22
Prostaglandin E2 (PGE2), in pathophysiology of colitis, 721, 722t
Prostaglandins, 772, 772f
  in acute renal failure, 925
  in fever, 235
  in inflammatory response, 21–22, 143
  in pathophysiology of colitis, 721
Prostanoids, in inflammatory response, 21–22
Prostate glands, 1315
  evaluation of, 1322
Protein
  acute phase, in inflammatory response, 20–23, 20t
  for body weight, 238–239
  in cerebrospinal fluid, 590, 591t
  metabolism of, liver's role in, 845–846, 846f–847f
  synthesis of, testing of, in hepatic insufficiency, 858–859
Protein C
  in disseminated intravascular coagulation, 730
  hepatic insufficiency and, 856
Protein malnutrition, 1187, 1187f
Protein secretion systems, 5–6
Protein-calorie malnutrition, in chronic kidney disease, 944
Proteinuria, 1409
Proteus mirabilis, 947
Prothrombin time, 857t, 859
Protodiastolic heart murmur, 395
Proton pump inhibitors, for gastric ulceration in foals, 1404, 1405t
Protozoal abortion, 1282

Protozoal myeloencephalitis
equine, 640–645
clinical signs of, 642
diagnosis of, 643–644
differential diagnosis for, 644
epidemiology of, 640–641
magnetic resonance imaging in, 601–602, 602f
pathogenesis of, 641–642
pathology of, 642
prevention of, 645
Sarcocystis spp. in, life cycle of, 641
treatment of, 644–645
treatment of, 112
Provocative testing
for contact allergy, 1192–1193
for contact hypersensitivity, 1192–1193
Pruritic dermatoses, causes of
cannon keratosis, 1171
contact dermatitis, irritant, 1188
dermatophilosis, 1173–1174, 1173f
dermatophytosis, 1177–1178, 1178f
generalized granulomatous dermatitis, 1213, 1213f
linear keratosis, 1171
parasitic infections, 1181–1187
pemphigus foliaceus, 1194–1195, 1194f–1195f
seborrhea, 1170–1171
Pruritic skin disease, differential diagnosis of, 1163f
Pruritus
in hepatic insufficiency, 856
paraneoplastic disease and, 371–372
regional, 1163f
Pseudohyperparathyroidism, 1048
Pseudolymphoma, 1210
Pseudotruncus arteriosus, 470–471
Psoroptes equi infestation, 1183
Pteridium aquilinum toxicity, 1478–1479
Puberty, in stallions, 1317
PubMed, 220
Pulmonary abscess, 1395–1396
formation, 352
Pulmonary artery, 464
echocardiographic and Doppler studies of, 441b–443b
obstructed flow in, 448f
pressure of, cardiac catheterization and, 451
rupture of, 475–476
Pulmonary atresia, with ventricular septal defect, 470–471, 470f
Pulmonary auscultation, 408–409, 409f
Pulmonary consolidation, examination of, 316
Pulmonary fibrosis, equine multinodular, 321f, 341–342, 343f
Pulmonary function
evaluation, during exercise, 295
testing, 322
Pulmonary hemorrhage, 1006–1007
Pulmonary hypertension, 389b, 454–455
congestive heart failure and, 456
in equine multinodular pulmonary fibrosis, 344
Pulmonary regurgitation, 481, 481f
causes of, 401t–402t
Pulmonary resistance, measurement of, 322
Pulmonic valve
auscultation of, 403
regurgitation of, 481, 481f
Pulpitis, 794
Pulse oximetry, 181
Pulse pressure
examination of, 409
monitoring of, in critical care, 168
Pulsed-wave (PW) Doppler, 440–443
in infective endocarditis, 483–484
Pupillary light reflexes, of neonatal foals, 1381, 1382t
Pupils
abnormalities of, 1151
neurologic examination of, 583
in traumatic brain injury, 613, 615t
Purkinje neurons, 629, 629f
in cerebellar abiotrophy, 631
Purpura hemorrhagica, 46, 1011, 1196
Pyelonephritis, 947–948, 948f
Pyemotes tritici infestation, 1184
Pyloric stenosis, 808
Pyometra, 1231, 1243
Pyospermia, 1339
Pyrantel pamoate, for parasitic pneumonitis, 357
Pyrethrin dips, 1181–1182
Pyrethroids, 1181–1182
Pyrilamine maleate, for allergic skin disease, 1193
Pyrimethamine, for equine protozoal myeloencephalitis, 644
Pyriminil toxicity, 1465–1466
Pyrogallol, 1460
Pyrogens, in fever, 234
Pyrrolizidine alkaloid toxicity, 1502–1503
Pyrrolizidine alkaloid-containing plants, toxicity of, 874–875, 874f, 874t
Pyrrolizidine alkaloids, 352, 1199
Pythiosis, 1180–1181, 1180f
Pythium insidiosum, 1180

Q

QRS complex
in cardiac cycle, 392–395
in electrocardiography, 414f, 418
in pericardial disease, 485
QT interval, in electrocardiography, 414
Quasi-species, 9
Queckenstedt's phenomenon, 589
Quercus toxicity, 1460–1461
Quinapril, 457t–461t, 462
Quinidine
for atrial fibrillation, 501f–502f, 504–505
Quinidine *(Continued)*
for congestive heart failure, 457t–461t, 462
for ventricular tachycardia, 513–514

R

R wave, in electrocardiography, 418, 421t–431t
Rabies, 663–665
central vestibular disease and, 627–628
Radial immunodiffusion (RID), 48
Radial nerve, injury to, electromyography in, 596, 597f
Radiographic methods, in pituitary pars intermedia dysfunction, 1108
Radiographic patterns, 320–322, 321f
Radiography
for dysphagia, 272
for gastrointestinal system, 712–713
for guttural pouch tympany, 325
for respiratory disorders, 317–318, 318f
for sinusitis, 323, 324f
thoracic, 418–419, 419f
for cardiovascular disorders, 398b
for congestive heart failure, 456–457
for diagnostic sampling, 320–322, 321f
for urinary disorders, 918–919
Radionucleotide imaging, in hepatic disease, 862
RAI. *see* Relative adrenal insufficiency
Ramipril, 457t–461t, 462
Randomized, controlled clinical trials, 223, 226t–227t
Range of motion, regulation of, 583
Rapid expulsion, of parasitic infections, 17–18
Rapid-adapting receptors, 245
Rapidly fermented substrate, to hindgut, 201–202
Rate constants, 80
Rattlesnake bites, 1189, 1189f
Raw semen, evaluation of, 1324–1325
Rayless goldenrod
rhabdomyolysis from, 568
toxicity, 1484–1485
Reaction rates and orders, 80
in pharmacokinetics, 80–81, 80f
clinical application of compartmental modeling and, 81–82, 81f–82f
Reactive oxygen mediators, 722t
Reactive thrombocytosis, 1018
Reagent strip analysis, 912–913
Recipient mare, in equine embryo transfer, 1269
Recombinant human erythropoietin (rhEPO)
anemia secondary to, 1017
in chronic kidney disease, 939
Recombinant human tissue plasminogen activator (rt-PA), 1005–1006
Recommendation, practice guidelines, in evidence-based medicine, 228–229, 229t
Rectal prolapse, 783, 783f, 783t
Rectourethral fistulae, 896, 896f
Rectovaginal fistulas, 896, 896f, 1240
postpartum, 1309
Rectum, physical examination of, 710
Recumbent horses, in critical care, 186
Recurrent airway obstruction, 357–361
alternative bronchodilatory options in, 361
antiinflammatory therapy for, 359–360
bronchodilator therapy for, 360–361
clinical presentation of, 357–358
diagnosis of, 358–359
hypersensitivity disease and, 41–42, 45
Recurrent exertional rhabdomyolysis, 550–553
adjunct therapies for, 553
diagnosis of, 551–552
diet and, 552
environment and, 552
exercise and, 550–552
hormone therapy for, 553
intracellular calcium regulation and, 551
lactic acid and, 551
low-starch, high-fat concentrates and, 552–553
management of, 552–553
medications for, 553
prevalence and risk factors of, 551
selection of forage and, 552
supplements and, 553
Recurrent laryngeal neuropathy, 329–330, 329t
Red blood cells, in cerebrospinal fluid, 590, 591t
Red clover toxicity, 1503
Red maple leaf toxicity, 1009–1010
Reduced fibrinogen binding, 1013
Refeeding syndrome, 214
Reflexes, neural, in gastrointestinal ileus, 775
Reflux, nasogastric, 171
Regeneration, nodular, hepatic, 850
Regional adiposity, 1088
Regurgitation
icterus, 855
valvular, 450–451
aortic, 476–479, 476f, 478f–479f
auscultation of, 402f
causes of, 401t–402t
mitral, 472–476, 473f
pulmonary, 481, 481f
tricuspid, 479–480, 480f
Relative adrenal insufficiency (RAI), 1072
Relative adrenocortical insufficiency, 1411

Relaxin, pregnancy and, 1272
Remifentanil, for pain management, 144t–145t
Renal agenesis, 891–893, 893f
Renal arteriovenous malformations, 894
Renal biopsy, 922–923
Renal blood flow, 903–905, 903f, 904t
Renal cell carcinoma, 969, 970t–971t
Renal clearance, of drugs, 87, 87f, 87t
Renal critical care, 182
Renal cysts, 893
Renal dysplasia, 891–893, 893f
  in foals, 1406
Renal failure
  dosage adjustment in, 92–94
  in polyuria and polydipsia, 287, 962–963
Renal function, quantitative measures of, 921–922
Renal hemorrhage, 1007
Renal hyperosmotic medullary interstitium, role of, 285–286
Renal hypoplasia, 891–893, 893f
Renal parameters, for musculoskeletal system disorders, 546
Renal replacement therapy, 184
Renal system
  anatomy of, 888–890, 889f
  development of, 890–891, 890f–892f
  developmental malformations of, 891–898
    agenesis, hypoplasia, and dysplasia in, 891–893, 893f
    cysts in, 893
    pendulant kidney in, 894
    polycystic kidney disease, glomerulocystic disease, and other hereditary nephropathies in, 893–894, 894f
    vascular anomalies in, 894, 895f
  physiology of, 898
    blood flow, 903–905, 903f, 904t, 906t–907t
    body fluids volume and composition of, 900, 900t
    creatinine metabolism in, 899
    electrolyte balance in, 901t, 902–903
    excretion of solute and water in, 908–909
    glomerular filtration, 905–907
    nitrogenous and organic wastes, production and elimination of, 898–900, 898b
    renal tubular function, 907–908
    urea metabolism in, 898–899
    water balance in, 901–902, 901t
  tetracyclines and, 113
Renal tubular acidosis (RTA), 966–969, 967t
Renal tubular function, 907–908
Renal tubules, necrosis of, 113
Renal water reabsorption, 908–909
Reperfusion injury
  in hypoxia, 1381–1382
  intestinal ischemia and, 768–770, 769f–770f, 769t
Replication, viral, 9, 12f
Repolarization, of cell, 413, 413f
Reproductive tract
  anatomy of, 1217–1220
  assisted techniques for
    for mares, 1310
    for stallions, 1312–1313
  cervix, 1218, 1219f, 1240–1241
  clitoris, 1219–1220, 1222f
  disorders of, 1217–1364
  endometritis and, 1230–1231, 1231t
    diagnostics of, 1249–1252
    fungal, 1257–1258, 1257f–1258f, 1259t
    pathophysiology of, 1246–1249
    treatment strategies for, 1252–1257
    uterine therapy and, 1246
  equine embryo transfer and, 1267–1268
  hormonal manipulation of mare and, 1258
    for cervical dilation, 1262–1263
    for estrous cycle synchronization, 1259–1260
    for estrus suppression, 1260–1261
    for lactation induction, in barren mare, 1264
    for oviductal contraction, 1263
    for ovulation induction, 1261–1262
    for superovulation, 1262, 1262b
    for support of pregnancy, 1263–1264
    for uterine contractility, 1263
    for vernal transition, 1258–1259
  hypothalamus, 1220
  mammary gland, 1220
  mare breeding soundness examination of, 1225
    behavior in, 1226
    external genitalia in, examination of, 1226–1227, 1227f
    general physical examination in, 1226
    hormonal assays in, 1236–1238, 1238f
    identification of, 1225
    ovarian pathology in, diagnosis of, 1228–1230, 1232f
    restraint in, 1226
    signalment and history in, 1225–1226, 1225t
    transrectal palpation and ultrasound examination in, 1227–1228, 1228t, 1229f–1230f
    uterine pathology in, diagnosis of, 1230–1236
  of nonpregnant mares
    anatomy and physiology of, 1217, 1220–1225
Reproductive tract *(Continued)*
    hormones of, 1220, 1223t, 1224f
    pathology of, 1238
    phases of, 1220–1225
  optimize pregnancy rates and, breeding management to, 1264–1265
  ovaries, 1217, 1244–1246
  oviduct, 1217–1218, 1218f
  pituitary gland, 1220
  pregnant mare and, 1269. *see also* Pregnancy
    gestation in, physiology of, 1269–1274
    management of, 1296–1303
    monitoring, 1286–1288
    parturition in, endocrinology of, 1272–1273
  of stallion, 1313
    anatomy of, 1314–1316
    assisted reproductive techniques for, 1312–1313
    breeding, management of, 1327–1330
    clinical evaluation of, 1318–1327, 1319f–1322f
    physiology of, 1316–1318
  uterine tubes, 1244
  uterus, 1218, 1218f, 1241–1244
  vagina, 1218–1219, 1220f, 1239–1240
  vestibule, 1219, 1220f–1221f
  vulva, 1219, 1222f, 1238–1239
Reserpine, for recurrent exertional rhabdomyolysis, 553
Respiratory disorders, 311–386
  classification of, 315, 315f
  diagnostic considerations for, 314–315, 315f
  of foals, 1386–1400
    acute lung injury as, 1387–1391, 1389b
    acute respiratory distress syndrome as, 1387–1391, 1389b
    bacterial, 1394–1396
    congenital upper respiratory tract disorders as, 1391
    fungal pneumonia as, 1394
    hemothorax as, 1392–1393
    meconium aspiration as, 1391–1392
    milk aspiration as, 1392
    parasitic pneumonia as, 1394
    persistent pulmonary hypertension as, 1391
    pneumothorax as, 1392–1393
    respiratory distress as, 1387
    respiratory therapeutics for, 1396–1400
    rib fractures as, 1392–1393
    transient tachypnea as, 1392
    viral pneumonia as, 1393
  in guttural pouch
    anatomy of, 324–325
    empyema, 327
    mycosis, 325–327
    tympany, 325
  lower
Respiratory disorders *(Continued)*
    diagnostic sampling for, 318–323
    examination of, 316–318
  lymphoid pharyngeal hyperplasia in, 327, 328t
  patient assessment in, 314–316
  physical examination for, 315–316
  progressive ethmoidal hematoma in, 324
  recurrent laryngeal neuropathy as, 329–330, 329t
  rostral displacement of palatopharyngeal arch as, 330
  signalment, 314, 314f
  sinusitis in, 323–324, 324f
  of upper respiratory tract, 323–324
Respiratory distress, 169–170, 178–181, 179b, 250–258
  approach to, 257–258
  causes of, 257b
  conditions associated with, 256–257
  control of ventilation and, 251–253, 252f
  definition of, 250–251
  diagnostic imaging of, 258
  in foals, 1387
  hypercapnia in, 254–255
  mechanisms of, 251–256
  nonpulmonary, 256
  obstructive disease in, 255–256
  restrictive disease in, 256
  in traumatic brain injury, 613
Respiratory system
  anatomic protection for, 313
  critical care in, 177–178
    acid-base status, assessment of, 180–181
    arterial blood gas analysis, 178
    blood gas analysis, 180
    hypercapnia, 178
    hypoxemia, 178
    hypoxia and, 178
    inhalation therapy, 179
    oxygen delivery to tissues and, 177
    oxygen therapy, 178–179, 179f
    pharmacologic ventilation, 179–180
    pulse oximetry, 181
    respiratory distress and, 178–181, 179b
  disorders of. *see* Respiratory disorders
  parasitic infections of, 16
  tumors of, 368–373
    mesothelioma, 369–370
    metastatic pulmonary neoplasia, 370–371
    other considerations in, 372–373
    other types of, 369
    paraneoplastic disease, 371–372
    primary thoracic neoplasia, 368–369
    thymic tumors, 369
Respiratory system reactance (X_{rs}), 322

Respiratory system resistance (R_{rs}), 322
Respiratory tract receptors, 251–253
Rest, for polysaccharide storage myopathy, 558
Resting activity, 592, 593f
Resting insulin concentrations, in pituitary pars intermedia dysfunction, 1107–1108
Resting membrane potential, 413
Resting requirements, 214
Restraint, in mare breeding soundness examination, 1226
Resuscitation
  fluid, 175
  of neonatal foals, 1370–1373, 1372f, 1372b
    defibrillation in, 1373
    monitoring of, 1373
    postresuscitation support in, 1373
  volume, for spinal cord injury, 621
Retained fetal membranes, 1306–1308
Retained placenta, hypocalcemia and, 1041–1042
Retention icterus, 854–855
Retina, disorders of, 1154
Retropharyngeal abscess, in *S. equi* infection, 336
Retrospective cohort studies, 227
Retrovirus, oncogenic, 9
Rhabdomyolysis
  acute, 564–565
    Streptococcus Equi subsp.*Equi*-associated, 564–565
      clinical signs of, 565
      diagnosis of, 565
      pathophysiology of, 564–565
      treatment of, 565
    treatment of, 549–550
  anaplasma-associated, 564
  exertional, 1044
  pathophysiology of, 544–545, 545f
  recurrent exertional, 550–553
    adjunct therapies for, 553
    diagnosis of, 551–552
    diet and, 552
    environment and, 552
    exercise and, 550–552
    hormone therapy for, 553
    intracellular calcium regulation and, 551
    lactic acid and, 551
    low-starch, high-fat concentrates and, 552–553
    management of, 552–553
    medications for, 553
    prevalence and risk factors of, 551
    selection of forage and, 552
    supplements and, 553
  severe, 550
  sporadic exertional, 548–550
    dietary imbalances and, 549
    electrolyte imbalances and, 549
    exhaustion and, 549
    focal or generalized trauma and, 549
Rhabdomyolysis *(Continued)*
    overexertion in, 549
    signalment, history, and diagnosis in, 548–549
    vitamin E and selenium concentrations and, 549
  toxic causes of, 568–569
Rhinitis, 323
Rhodococcus equi
  bacterial pneumonia due to, 1394–1395
  enteric disease due to, 1404
  in severe combined immunodeficiency, 52
Rhythms, cardiac
  auscultation of, 401t, 406t–407t
  electrocardiography and, 416b
Rib fractures, in foals, 1392–1393
Ribonucleic acid (DNA), viral infection and, 8, 9f
Rice bran, polysaccharide storage myopathy and, 559
Richter's hernia, 780
Ricin, 1461
Ricinus communis toxicity, 1461
Rifampin
  doxycycline and, 114
  for foals, 1422t
  pharmacology of, 118–119
  for pleuropneumonia, 350t
  for rhodococcal pneumonia, 1396
Rifamycins, for foals, 1424
Robinia pseudoacacia toxicity, 1478
Romifidine, for pain management, 144t–145t
Ronnel, for cutaneous habronemiasis, 1186
Ropivacaine, for pain management, 144t–145t
Rostral displacement of palatopharyngeal arch, 330
Rostral hyoid muscles, in DDSP, 328
Rotaviral diarrhea, in foals, 1402
Rotavirus, in diarrhea, fecal examination and, 283
Round ligament, of liver, 843
Ruptures
  of artery, 497
  of mitral valve, 472–474, 473f
  prepubic tendon, 1290–1291
  uterine, postpartum, 1306
  uterine artery, 1244
Russian knapweed, 686
  toxicity, 1479–1480
Ryanodine receptor 1 gene *(RYR1)*, mutation in, 553, 570

S

Sabulous urolithiasis, 947, 957
Sacculation, ventral uterine, 1232, 1241
Saddle galls, 1197
Saddle sores, 1197
Safety, in magnetic resonance imaging, 600–601
Saline
  for endometritis, 1255, 1255t
  for shock, 175
  for traumatic brain injury, 617
Salinomycin
  in toxic injury, myocardial, 490
  toxicity, 1491
Salivary glands, 795–796
Salmonella enterica, acute diarrhea associated with, 747
Salmonella enteritidis, 5
Salmonella spp., membrane ruffling in entry, 6–7
Salmonellosis, 747–750, 748t
Salt consumption, in polyuria and polydipsia, 964
Sand enteropathy, 748t, 759
SAP. *see* Secreted aspartyl proteinase
Sarcocystis fayeri, 563
Sarcocystis neuroma, in equine protozoal myeloencephalitis, 641
Sarcocystis spp., in equine protozoal myeloencephalitis, 641
Sarcocystosis, 563–564
  clinical signs of, 563
  diagnosis of, 564
  pathogenesis of, 563
  treatment and control of, 564
Sarcoidosis, equine, 1213–1214, 1213f
Sarcoids, 1202–1206, 1203f–1204f
  in eyelid, 1142
Sarcoptes scabiei infestations, 1182
"Sawhorse" stance, in tetanus, 669, 669f
Scabies mites, 1182
Scaling dermatosis
  cannon keratosis, 1171
  generalized granulomatous dermatitis, 1213, 1213f
  linear keratosis, 1171
  seborrhea, 1170–1171
SCC. *see* Squamous cell carcinoma
SCID. *see* Severe combined immunodeficiency
Scintigraphy
  in disorders of the gastrointestinal system, 713
  in gastric emptying, 714
  nuclear, in spinal cord and vertebral injury, 620
  in thyroid gland function, 1065
Scirrhous cord, 1338
Scopus, 220
Scratches. *see* Pastern dermatitis
Scrotal hernia, 1338
Scrotum, 1316
  abnormalities of, 1334–1335
Season, thyroid gland function and testing and, 1062
Seborrhea, 1170–1171
  in hepatic insufficiency, 856
Second heart sound, 392–395, 403–404
  auscultation of, 403, 403f
Secondary adrenal insufficiency, 1073
Secondary erythrocytosis, 1018
Secondary hemostasis, 999
Secondary immunodeficiency, 55–59
Secondary thrombocytosis, 1018
Second-degree AV block, 514, 515f
Second-degree burns, 1198
Secreted aspartyl proteinase (SAP), 8
Secretion, mucus, 313
Sedation
  α_2-adrenoreceptor agonists and, 146
  critically ill horse and, 185
  in tetanus, 671
Sediment examination, 913, 914f
Seizures, 264–267, 603–607
  clinical signs and diagnosis of, 605–606
  etiology/pathogenesis of, 604–605, 604t–605t
  in foals, 1383, 1386t
  hypocalcemic, 1041
  in traumatic brain injury, 617
  treatment of, 606–607
Selectins, in neutrophil adherences, 22
Selective IgM deficiency, 54–55
Selenium
  concentrations, sporadic exertional rhabdomyolysis and, 549
  deficiency of
    in dilated cardiomyopathy, 491
    nutritional myodegeneration and, 561
  for masseter muscle myodegeneration, 562
  for musculoskeletal system disorders, 546
  for nutritional myodegeneration, 1420, 1419–1420
  toxic effects of, 1500–1501
  toxicosis, 1187, 1188f
Selenosis, 1010–1011, 1165t–1167t
Self-cure, parasitic, 17
Self-mutilation, in breeding stallions, 1332
SeM protein, of *Streptococcus equi*, 334
Semen
  abnormalities of, 1339–1340
  collection of, 1323–1326
    miscellaneous methods of, 1324
    using live mare, 1328
  cooled shipped, 1266
  evaluation of, 1324–1326
  fresh, 1266
  frozen, 1266–1267, 1325
Seminal vesicles, 1316
  evaluation of, 1322–1323
Senecio species, 874, 874t
Sensitization
  central, 138
  peripheral, 138
Sensory afferents, in neuromuscular physiology of ventilation, 251
Sepsis, 724. *see also* Endotoxemia
  hypocalcemia and, 1043
  neonatal, 1373–1380
    clinical presentation of, 1377–1379
    identification of, 1377–1379
    management of, 1379
    pathophysiology of, 1374–1377, 1374t, 1375f, 1375b

Sepsis *(Continued)*
prognosis of, 1379–1380
risk factors for, 1377
in polyuria and polydipsia, 966
Sepsis-associated encephalopathy, 1383
Septal defects, 388
atrial, 463f, 468
echocardiogram of, 469f
ventricular, 463f, 465–468, 465f–467f
pulmonary atresia with, 470–471, 470f
Septic funiculitis, 1338
Septic shock, 724
Septicemia
in foals, 1373–1374
immunodeficiency and, 59
Serologic testing
for cough, 250
for equine herpesvirus-1 myeloencephalopathy, 649
for fungal pneumonia, 355
for *S. equi* infections, 335
Serotonin, in pathophysiology of colitis, 722t
Serous cysts, hepatic, 881
Sertoli cells, 1314
Serum allergy, of atopic dermatitis, 1191
Serum amyloid A, in endometritis, 1252
Serum biochemistry, in urinary system, 909–912, 910f, 911t
Serum CK exercise response test, for musculoskeletal system disorders, 546
Serum creatine kinase
for diagnosis, of musculoskeletal system disorders, 545–546
in recurrent exertional rhabdomyolysis, 551–552
in type 1 polysaccharide storage myopathy, 554
Serum creatinine, in chronic kidney disease, 942, 942f–943f
Setaria species, in verminous encephalomyelitis, 683
Severe combined immunodeficiency (SCID), 52–53
Sexual behavior, of stallions, 1318
Sexual differentiation, of stallion, 1316–1317
Sexually transmitted diseases (STDs)
endometritis and, 1248–1249
transmission of, through semen, 1340–1341
"Shaker foal syndrome," 673, 1384
Shampoo therapy
for dermatophilosis, 1174
for dermatophytosis, 1178
for seborrhea, 1171
Shedding, abnormal, 1170, 1170f
Shivering, in thermoregulation, 232
Shivers, 632–633
Shock
categories of, 173
in endotoxemia, 731
septic, 724
Shock wave therapy, extracorporeal, for pain management, 150–151
Short-chain fatty acids, hepatic metabolism of, 846, 847f
Shoulder flexion posture, 1302
Shunts
in congenital heart disease, 464–465
in hypoxemia, 255
in ventricular septal defects, 466
Sicklepod, rhabdomyolysis from, 568
Signal "aliasing," 443–444, 449f
Signaling, through antigen-specific receptors, 30–31, 32f
Signalment
in body temperature, 236
in colic, 274–275
in collapse, 267
in cough, 249
in diarrhea, 281
in dysphagia, 271
in edema, 263
in hypothermia, 238
in mare breeding soundness examination, 1225–1226, 1225t
in polyuria and polydipsia, 288
in respiratory disorders, 314, 314f
in respiratory distress, 257
in weight gain, 240
in weight loss, 243–244
Silicosis, 352
Silver nitrate sticks, for patent urachus, 1408
Simple sugars, 197
Single-dose drug regimen, 90–91
Single-nephron GFR, 943
Sinoatrial node, 391, 391f
Sinonasal disease, 323
Sinus rhythms, 497–498, 498b, 499f
auscultation of, 401t
Sinuscopy, for sinusitis, 323
Sinusitis, 323–324, 324f
Sinusoids, hepatic, 843–845, 844f
SIRS. *see* Systemic inflammatory response syndrome
16S rRNA gene sequencing, for pleuropneumonia, 349
Skeletal muscle, 542
Skeletal system, toxicoses and, 1494
Skin
biopsy of
in dermatophilosis, 1173–1174
in dermatophytosis, 1177–1178
in drug reactions, 1188
in insect hypersensitivity, 1191
in lice infestations, 1181
in seborrhea, 1171
insect bite hypersensitivity, 45
parasitic infection on, 16–17
reactions, to dimethyl sulfoxide, 1488
structure of, 1159–1160
testing, intradermal, for atopy, 1191
Skin disorders, 1159–1216
approach to, 1160–1161
collagen disorders, 1171–1173
drug reactions causing, 1188
Skin disorders *(Continued)*
history in, 1160–1161, 1162f
hypersensitivity, 1189–1194
contact, 1192–1193
food, 1191–1192
insect bite, 1190–1191
immune-mediated, 1189–1197
infectious, 1173–1187
bacterial, 1173–1175
fungal, 1177–1181
parasitic, 1181–1187
viral, 1175–1177
miscellaneous and idiopathic, 1211–1214
mucocutaneous vesicles, 1188–1189
necrotizing, 1197–1202
burns, 1197–1198
decubital ulcers, 1197
frostbite, 1198
gangrene, 1197
panniculitis and fat necrosis, 1211–1212
stachybotryotoxicosis, 1181
neoplastic, 1202–1211
nodular
bacterial granuloma, 1175, 1175f
corynebacterial infections, 1174–1175
equine axillary nodular necrosis, 1212
mycetomata, 1179, 1180t
phaeohyphomycosis, 1179, 1180t
pythiosis, 1180–1181, 1180f
sporotrichosis, 1178, 1179f, 1180t
urticaria, 1190, 1190f
nutritional and toxic, 1187–1189
physical examination of, 1161
with systemic manifestations, 1165t–1167t
Skull fracture, 615f
Slaframine toxicity, 1464
Sleep, 607–608
definition of, 265
deprivation, 608, 609t
Sleep disorders, 265–267, 608–610
Sling, for recumbent horses, 187
Slow-adapting receptors, 245
Small intestine
palpation of, 710
simple obstruction, 784–786
strangulation, 778–781
Smooth lipopolysaccharide, 724
Snake bites, 43–44, 1189, 1189f
Snake venom toxicity, 1498–1499
Snakes, envenomation by, in hemolysis, 1010
SNAP test, 56–57
Snowshoe hare virus, 662
Soapy water enemas, for meconium impaction, 1400–1401
Sodium
in cardiac cell electrical activity, 413
in cerebrospinal fluid, 591t
Sodium bicarbonate
for chronic kidney disease, 945
in hyperkalemia, 517
Sodium channel blocker, 457t–461t
Sodium chloride (NaCl), for chronic kidney disease, 945
Sodium fluoroacetate toxicity, 1493
Sodium iodide, for fungal pneumonia, 356t
Sodium iodine, for dermatophytosis, 1178
Sodium penicillin G, for pleuropneumonia, 350t
Sodium phosphate enemas, for meconium impaction, 1400–1401
Sodium thiosulfate, for cyanide toxicity, 1493
Soft palate
dorsal displacement of, 328–329, 328t
muscles controlling, 328t
Soft tissue mass, radiographic imaging of, 318f
Solanine, 1461–1462
Solanum toxicity, 1461–1462
Solute, excretion of, 908–909
Somatostatin (SST), 1081
Somatotropic axis, dysfunction of, energy regulation and, 1412–1414
Sorbitol dehydrogenase (SDH), hepatic insufficiency and, 857t, 860
Sorghum, toxic effects of, 1506–1507
Space of Disse, 843–845
Spasmolytic peptide, 773
Spasms, muscle, in tetanus, 671
Spastic bladder, 973
Spasticity, 585, 637
Specific gravity, urine, 159
Speculum examination, vaginal, 1232–1233
Spermatic cord, 1315
abnormalities of, 1338
Spermatocytogenesis, 1317–1318
Spermatogenesis, 1317–1318
Spermatozoa, abnormalities of, 1339–1340
Spermiation, 1317–1318
Spherocyte, 993
Sphygmomanometry, oscillometric, 169, 169f
Spider bites, 1189
Spinal cord
cervical, localization of lesion in, 586
compression of
in cervical vertebral compressive myelopathy, 635
magnetic resonance imaging in, 602, 603f
injury, 618–622, 618t
Spinal nerve root, in electromyography, 592t
Spinal reflexes, of neonatal foals, 1381
Spinous layer, epidermal, 1159

Sporadic exertional rhabdomyolysis, 548–550
dietary imbalances and, 549
electrolyte imbalances and, 549
exhaustion and, 549
focal or generalized trauma and, 549
overexertion in, 549
signalment, history, and diagnosis in, 548–549
vitamin E and selenium concentrations and, 549
Sporotrichosis, 1178, 1179f, 1180t
SPORTDiscus, 220
Squamous cell carcinoma
of conjunctiva and third eyelid, 1143, 1143f
of cornea, 1150–1151, 1150f
cutaneous, 1206–1208, 1207f
of eyelid, 1141–1142, 1141f
St. Louis encephalitis virus, 659
ST segment, in electrocardiography, 418
Stable flies, hypersensitivity to, 1190
Stabling, for insect control, 1163b–1164b
Stachybotryotoxicosis, 1181
Stallion reproductive evaluations (SREs), 1318
Stallions, 1313
anatomy of, 1314–1316
assisted reproductive techniques for, 1312–1313
breeding, 1327–1330
exercise for, 1327
herd health management for, 1327–1328
measuring reproductive performance of, 1330
methods for, 1328–1330
novice or maiden, 1330
nutrition of, 1327
records of, 1318–1319
clinical evaluation of, 1319f–1322f
physiology of, 1316–1318
Staphylococcal pseudomycetoma. *see* Botryomycosis
Staphylococcal pyoderma, 1174
Staphylococcus aureus, toxins of, 7
Starch, 194b, 198
Starling equation, 259–260
Starling forces, 259–260, 260f–261f, 261b
alterations in, 261–262
Starvation
feeding thin and starved horses, 213
metabolic consequences of, 202
Status epilepticus, 603
Steatorrhea, in hepatic insufficiency, 855
Stenosis, pyloric, 808
Stercobilin, 849
Stereoisomers, nonsteroidal anti-inflammatory drugs as, 121
Steroidogenesis, 1071–1072, 1071f–1072f
Stethoscope examination, 400–403, 401t–402t, 402f–403f
Stimulation, central nervous system, toxicoses and, 1468
Stings, venomous, 1189
Stomach
diseases of, 803–808
dilation and rupture, 808
gastroduodenal ulceration, 803–806
in adults, 805–806
diagnosis of, 806, 806t
neonatal, 805
pathophysiology of, 803–804, 804f
prevalence of, 803
risk factors of, 804–805
in sucklings and early weanling foals, 805, 805f–806f
treatment of, 806–808
impaction, 808
pyloric obstruction and delayed gastric emptying, 808
mucosal barrier of, 766
Stomatitis
equine exfoliative eosinophilic dermatitis and, 1165t–1167t
oral, 795
vesicular, 1176–1177, 1176f
Stomoxys calcitrans, hypersensitivity to, 1190
Strabismus
in traumatic brain injury, 614–615
in vestibular disease, 624
Strangles, 331–338, 1165t–1167t
Strangulating obstruction, 769–770
Strangulating umbilical hernias, 780
Stratified squamous, barrier function of, 766
Streptococcus equi
in central vestibular disease, 627–628
in cerebellar abscess, 632
guttural pouch empyema from, 327
hyaluronic acid capsules of, 333
purpura hemorrhagica and, 46
virulence factors of, 334
Streptococcus equi subsp. *equi* infection, 331–338
associated myopathy in, 337
clinical management of, 335
clinical pathology of, 334
clinical signs of, 334
diagnosis of, 334–335
disease prevention in, 337–338
epidemiology of, 331–332, 332f–333f
general control measures in, 338
internal abscessation in, 335–336
other complications of, 336
outbreak management of, 337
pathogenesis of, 332–334
transmission of, 332
treatment of, 335
Streptococcus equi subsp. *zooepidemicus*, 331
in bacterial pneumonia, 346–347
bacterial pneumonia due to, 1394
Streptococcus-associated myopathy, 337
Streptokinase, 1005–1006
Streptolysin-like toxin, 334
Streptomycin, 627
Stricture, esophageal, 801–802
Stride length
evaluation in poor performance, 293–294
during exercise, 295
Stringhalt, abnormal gaits associated with, 586–587
Stroke, heat, 233
Stroke volume, 173
in cardiac cycle, 392–395, 394f
Stromal abscess, corneal, 1148–1149, 1149f
Stromal cells, in adaptive immunity, 23–24
Strongyloides westeri
acquired resistance to, 17
in foal diarrhea, 1404
Strongylosis
acute diarrhea and, 753–754
acute diarrhea associated with, 748t
therapies for, 762
Strongylus equinus, in verminous encephalomyelitis, 682
Strongylus vulgaris
in aortoiliac thrombosis, 493f, 495
colic and, 14
in parasitic vascular lesions, 494f, 496
resistance to, 17
in verminous encephalomyelitis, 682
Strontium-90, for squamous cell carcinoma, 1208
Strychnine, 1474
ST-T wave, in electrocardiography, 414
Styloglossus muscles, in DDSP, 328
Stylopharyngeus muscle, 328
Subepiglottic cyst, 314f
Subpalpebral lavage catheters, for corneal disorders, 1147–1148
Suck reflex, in sick, recumbent foals, 208–209
Suckle reflex, of neonatal foals, 1380, 1382t
Suckling, gastroduodenal ulcer disease in, 805, 805f–806f
Sucralfate, 807
for gastric ulceration in foals, 1405–1406, 1405t
Sudden cardiovascular death, 399b
Sugars, simple, 197
Sulfadiazine
for endometritis, 1254t
for equine protozoal myeloencephalitis, 644
Sulfadiazine-trimethoprim, for foals, 1422t
Sulfonamides, 110–112
adverse effects and drug interactions of, 111–112
clinical use of, 112
critically ill horse and, 186
for equine herpesvirus-1 myeloencephalopathy, 650
Sulfonamides *(Continued)*
for equine protozoal myeloencephalitis, 644
formulations of, 112
mechanism of action of, 110
mechanism of resistance of, 110
pharmacokinetics of, 110–111, 111t
Summer pasture-associated obstructive pulmonary disease, 358, 361
Superovulation, hormones in, 1262, 1262b
Superposition, principle of, 89
Supplements
for endometritis, 1257
for polysaccharide storage myopathy, 559
recurrent exertional rhabdomyolysis and, 553
Supportive care
for duodenitis-proximal jejunitis, 740
for endotoxemia, 737–738
for tetanus, 671–672
Suprascapular nerve injury, electromyography in, 596, 597f
Surfactant proteins, 313–314
Surgery
for cholelithiasis, 878
for traumatic brain injury, 616
Surgical removal, of progressive ethmoidal hematoma, 324
Surgical therapy
for duodenitis-proximal jejunitis, 741
for guttural pouch mycosis, 326
Survival scoring system, for hospitalized foals, 1379–1380, 1380t
Swainsonine, 1469
Swallow reflex, of neonatal foals, 1382t
Swallowing, in normal eating, 268
Sway reaction, in gait evaluation, 588
Sweating
neurologic examination and, 583
thermoregulation and, 232
Synaptobrevin, 668–669
Synchronous diaphragmatic flutter, 574, 1041
clinical signs of, 574
control of, 574
risk factors for, 574
treatment of, 574
Syncope, 264, 266
Syndrome X, 1085–1086
Synechiae, in uveitis, 1152
Synergy, ventricular, 396
Synthetic colloids, 167
for fluid therapy, 1426
Systematic reviews, in evidence-based medicine, 228
Systemic anaphylaxis, 43–45
Systemic antibiotics, for endometritis, 1253, 1254t
Systemic calcinosis, 567–568
clinical signs of, 567, 568f
diagnosis of, 567–568

Systemic calcinosis *(Continued)*
pathogenesis of, 567
pathophysiology of, 567
treatment and prognosis of, 568
Systemic infections, viral, 12, 13f
Systemic inflammation, hypocalcemia and, 1043
Systemic inflammatory response syndrome (SIRS), 273, 723–724, 1074
in foals, 1374, 1374t–1375t
Systemic lupus erythematosus, 1165t–1167t, 1196
Systemic reactive amyloidosis, 937
Systole
in cardiac cycle, 392–395
function of, 395b
Systolic murmur
auscultation of, 403, 403f–404f
functional ejection, 392–395, 393f, 408
of tricuspid regurgitation, 479–480

T

T helper cells, in type I hypersensitivity, 43
T wave, in electrocardiography, 418
T_3 suppression test, 1065
Tabanus, hypersensitivity to, 1190
Tachycardia
atrial, 500f–502f, 502
auscultation of, 401t
ventricular, 512f, 513–514
Tail
dystrophy of, 1169
neurologic examination of, 584, 588
paralysis of, in polyneuritis equi, 652
Tall fescue toxicity, 1496–1497
Tamponade, cardiac
examination in, 397
pericardial disease and, 485
sudden cardiovascular death and, 399b
Tannic acid, 1460
Tapeworms, in intussusception, 781
Tartrate-resistant acid phosphatase (TRAP), 1036–1037
Taxonomy, viral, 8
Taxus spp., 1486
T-cell receptors (TCRs)
CD_3 and, 27
signaling through, 30–31
T-cell-rich B-cell lymphoma (TCRBCL), 371
T-cells
in adaptive immunity, 23–24
antigen-specific receptors of, 27
flow cytometric analysis of, 50, 52f
in intestinal parasite expulsion, 17
regulatory, 27
Tears, ureteral, 895–896
Teeth
disorders of, 793–794
Teflon catheters, 161, 162t
Telemetry-based ECG, 415–416
Telogen defluxion, 1170, 1170f
Telogen phase, of hair cycle, 1160, 1161f
Temperament, rhabdomyolysis and, 551
Temporohyoid osteoarthropathy, 625–627, 626f
Tenesmus, in hepatic insufficiency, 855
Tensor veli palatini, 328t
Teratogenesis, 1274
Tertiary adrenal insufficiency, 1073
Testes
abnormalities of, 1335–1337
acquired conditions of, 1336–1337
cells, interactions of, 1315
infectious conditions of, 1337
neoplastic conditions of, 1337
Testicles, 1314–1315
descent of, 1317
Testicular degeneration. *see* Gonadal atrophy
Testosterone, recurrent exertional rhabdomyolysis and, 553
Tetanolysin, 669
Tetanospasmin, 668–669
Tetanus, 668–672
clinical signs and course of, 669–670, 669f–670f, 670t
diagnosis of, 670–671
in foals, 1384
prevention of, 672
prognosis of, 672
risk factors for, 669
toxins in, 668–669
treatment of, 671–672
Tetanus antitoxin (TAT), 671
Tetany, hypocalcemic, 1041
Tetrachlorodibenzodioxin toxicity, 1466
Tetrachlorvinphos, rhabdomyolysis from, 568
Tetracyclines, 112–114
adverse effects and drug interactions of, 113–114
clinical of, 114
for foals, 1423
formulations of, 114
mechanism of action of, 112
mechanism of resistance of, 112
pharmacokinetics of, 112–113
for tetanus, 671
Tetralogy of Fallot, 469–470, 470f
T_H1 CD_4+ effector T cells, 33, 34f
T_H2 CD_4+ effector T cells, 33–34, 34f
T_H17 CD_4+ effector T cells, 35
Thalamus, traumatic injury to, 615t
Thallium toxicity, 1468
Theileria equi, 1249
Theiler's disease, 865–866, 866f
Theiler's disease-associated virus, 867
Therapeutic drug monitoring (TDM), 91–92, 92t
of aminoglycosides, 108
Thermal injuries, 1197–1198
Thermoregulation, 233
in neonatal foals, 1369
Thiabendazole, 1186
for parasitic pneumonitis, 357
Thiamine
for bracken fern toxicity, 1479
deficiency, refeeding syndrome and, 214
in traumatic brain injury, 618
Thin and starved horses, feeding for, 213–214
Third eyelid, disorders of, 1142–1143
Third heart sound, 395
auscultation of, 403f
Third-degree burns, 1197
Third-degree or complete AV block, 514, 515f
Thirst, contributes to water balance, 286
Thoracic percussion, 316
Thoracic radiography, 418–419, 419f
in cardiovascular disorders, 398b
in congestive heart failure, 456–457
Thoracic ultrasonographic examination, 320
Thoracocentesis, 170, 319–320, 319f
in pleuropneumonia, 348
Thoracoscopy, 323
Thoracotomy, for pleuropneumonia, 351
Thorax, auscultation of, 402f
Thornapple toxicity, 1462
Thrombin activatable fibrinolysis inhibitor (TAFI), 1001
Thrombocytopenia, 1012
alloimmune, 45–46
differential diagnoses for, 998b
immune-mediated, 1012
neonatal alloimmune, 1012–1013, 1416
secondary to systemic disease, 1012
Thrombocytosis, 997–998, 1018–1019
Thrombophlebitis, 482, 492f, 493–495
Thrombosis
aortoiliac, as cardiovascular disorder, 493f–494f, 495–496
in plasma-based coagulation system, 1015–1016
vascular, hyperlipemia and, 872
Thromboxane
as inflammatory mediators, 727t–728t
in pathophysiology of colitis, 722t
Through-the-needle catheters, 162t
Thymus, development of, 38
Thyroid gland, 1058–1069
diagnostic tests in, 1060t–1061t, 1064–1065
factors that affect, 1060–1064, 1060t
hyperthyroidism in, 1068
hypothyroidism in, 1061t, 1065
imaging of, 1065
tumors in, 1068–1069
Thyroid hormones
function of, 1058–1059
metabolism of, 1059
molecular actions of, 1059
Thyroid suppression test, 1065
Thyroid-stimulating hormone stimulation test, 1064
Thyrotropin-releasing hormone stimulation test, 1064–1065
Thyroxine, recurrent exertional rhabdomyolysis and, 553
Tiamulin, 114
Ticarcillin, 102
for foals, 1421, 1422t
Ticarcillin + clavulanic acid, for endometritis, 1253, 1254t
Tick infestations, 1184
Ticks, in Lyme disease, 678
Tidal volume, measurement of, 322
Tilmicosin, 114
Timber milkvetch toxicity, 1480
Tissue injury, during inflammation, 719–720
Togaviral encephalitis, 655–659, 656t, 658t
Toll-like receptors (TLRs), 20–21, 726
Tongue, 790
Torsion
ovarian, 1245
umbilical cord torsion, 1284
uterine, 1291–1292
Total bilirubin, in hepatic insufficiency, 857, 857t
Total fluid replacement, for rhabdomyolysis, 550
Total nonstarch polysaccharides (NSPs), 197–198
Total protein, in cerebrospinal fluid, 590, 591t
Total red cell volume analysis, during exercise testing, 293–294
Total run time, in poor performance, 295
Toxic disorders, 686
Toxic hepatopathy, 869–871
Toxic injury, to heart, 399b, 490–491
Toxic metritis, postpartum, 1306–1308
Toxicity
fescue, during pregnancy, 1293–1294
oxalate, 1032t
in urinary system, 958
Toxicoinfectious botulism, 673
Toxicologic problems, 1460–1512
Toxicoses
blister beetle, 1466–1467
cantharidin, 1466–1467
cardiovascular and hemolymphatic systems and, 1484
central nervous system depression and, 1478
stimulation and, 1468
epithelium and skeletal system and, 1494
gastrointestinal tract and, 1460
hairy vetch, 1165t–1167t
liver disease or dysfunction and, 1502–1503
oxalate, 1506, 1506b
selenium, 1187, 1188f
suspected, rules regarding treatment of, 1476b
urinary system and, 1505

Toxins
aflatoxin, 1504–1505
alsike, 1503
aluminum, 1463–1464
4-aminopyridine, 1475
ammonia, 1477
arsenic
acute diarrhea associated with, 748t, 758
gastrointestinal effects of, 1462–1463
bicarbonates, 1488–1489
black locust, 1478
black walnut, 1494
blue-green algae, 1470–1471
in botulism, 672–674
bracken fern, 1478–1479
cadmium, 1507
cantharidin, 1466–1467
acute diarrhea associated with, 748t, 757–758
carbamates, 1471
carbon disulfide, 1475–1476
castor beans, 1461
chlorates, 1465
chlorinated hydrocarbons, 1473–1474
coumarin derivatives, 1486–1488
cyanide, 1492–1493
damage to host tissue by
in bacterial infection, 7
in fungal infection, 8
dimethyl sulfoxide, 1488
dioctyl sodium sulfosuccinate, 1462
Equisetum, 1479
ergotism due to
gangrenous, 1501–1502
nervous, 1469–1470
fluoride, 1499–1500
hairy vetch, 1495
hepatotoxins, 870
hoary alyssum, 1497
increased body temperature and disorders of, 233–234
iodine, 1497–1498
iron, 1504
hepatic, 870–871
jimsonweed, 1462
lasalocid, 1490–1491
lead, 1482–1484
leukoencephalomalacia, 1481–1482
levamisole, 1475
locoweed, 1469
maple, 1485–1486
menadione sodium bisulfite, 1507
mercury, 1507–1508
metaldehyde, 1474–1475
methiocarb, 1475
milkvetch, 1480
milkweed, 1479
monensin, 1489–1490
mushroom, 1503
mycotoxins, 871
nicotine, 1476–1477
nightshade, 1461–1462
nitrates and nitrites, 1491–1492
nonprotein nitrogen substances, 1477–1478
oak, 1460–1461
Toxins *(Continued)*
oleander, 1484
onion, 1486
organophosphates, 1472–1473
oxalates, 1506, 1506b
pentachlorophenol, 1464–1465
pesticides, 1471–1476
petroleum distillates, 1464
phosphorus, 1467–1468
photosensitizing plants, 1495–1496, 1495b
pokeweed, 1461
propylene glycol, 1480
pyriminil, 1465–1466
from pyrrolizidine alkaloid-containing plants, 874–875, 874f, 874t
pyrrolizidine alkaloids, 1502–1503
rayless goldenrod, 1484–1485
red clover, 1503
Russian knapweed, 1479–1480
salinomycin, 1491
selenium, 1500–1501
slaframine, 1464
snake venom, 1498–1499
sodium fluoroacetate, 1493
sorghum, 1506–1507
strychnine, 1474
tall fescue, 1496–1497
in tetanus, 668–669
tetrachlorodibenzodioxin, 1466
thallium, 1468
timber milkvetch, 1480
trichothecenes, 1482
triclopyr, 1480–1481
urea, 1477–1478
vitamins D_2 and D_3, 1507
white snakeroot, 1484–1485
wild jasmine, 1494
yellow starthistle, 1479–1480
yew, 1486
zinc, 1500
Tracheobronchial aspiration, 318
Tracheostomy, 169–170, 170b
Tramadol
for hemostatic dysfunction, 1004–1005
for pain management, 148
Tranexamic acid, for hemostatic dysfunction, 1004–1005
Tranquilizers, for recurrent exertional rhabdomyolysis, 553
Transabdominal ballottement, 709
Transcutaneous (abdominal) ultrasound-guided fetal puncture, 1278
Transfer
of equine embryo, 1267–1268
gamete intrafallopian, 1313
oocyte, 1310–1312
success of, 1312
Transforming growth factor-β (TGF-β), 773
Transfusion
medicine, for hematopoietic disorders, 1002–1004
reactions, 1165t–1167t
whole blood, 167
Transient hypogammaglobulinemia, 55
Transient tachypnea, 1392
Transition state, 1089
Translocation, of endotoxins, 725
Transport, in poor performance, 289–290
Transrectal palpation, 710
in mare breeding soundness examination, 1227–1228, 1228t
in uterine torsion, 1291
Transtracheal wash (TTW), 316–318
Transvaginal ultrasound-guided twin reduction, 1277
Transverse presentation, 1303
TRAP. *see* Tartrate-resistant acid phosphatase
Trauma
cardiac, sudden cardiovascular death and, 399b
cervical, 1241
in foals, 1384
focal or generalized, sporadic exertional rhabdomyolysis and, 549
vaginal, during breeding, 1240
Traumatic brain injury, 611–618
neurologic evaluation of, 613–616, 614f–615f, 615t
pathophysiology of, 611–613, 612f
spinal cord and vertebral injury, 618–622, 618t
treatment and prognosis in, 616–618, 616f
verminous encephalomyelitis, 682–685, 684t
vestibular disease and, 622–628
Traumatic optic neuropathy, 1155
Trefoil peptides, 766–767, 773
Tremetone, rhabdomyolysis from, 568
Tremors
in cerebellar disease, 630
neurologic examination of, 583
Triangular ligaments, of liver, 843
Trichlorfon, for cutaneous habronemiasis, 1186
Trichophyton species, 1177
Trichorrhexis nodosa, 1169
Trichothecenes, 1482
Tricide, for endometritis, 1255t
Triclopyr, 1480–1481
Tricuspid atresia, 471
Tricuspid valve, 390
auscultation of, 402f, 403
echocardiographic and Doppler studies of, 441b–443b
infective endocarditis, 481–484, 482f–483f
regurgitation, 479–480, 480f
causes of, 401t–402t
Trifolium hybridum, 1503
Trifolium pratense, 1503
Trigeminal nerve
neurologic examination of, 584
paralysis, 624
Trigeminal-mediated headshaking, 680–682, 680t–681t
Triglyceride, hepatic insufficiency and, 857t
Triheptanoin, polysaccharide storage myopathy and, 559
Trilostane, 1078
Trimethoprim, for endometritis, 1254t
Trimethoprim sulfa, for dermatophilosis, 1174
Trimethoprim (TMP), 110, 111t
Trimethoprim-sulfamethoxazole
critically ill horse and, 186
for equine herpesvirus-1 myeloencephalopathy, 650
Trimethoprim-sulfonamide, for pleuropneumonia, 350t
Trocarization, 172, 172b, 173f
Trochlear nerve, 622
Trombiculiasis, 1183
Trot
gait evaluation during, 585
normal, 586
Truncus arteriosus, 470–471
Trypanosoma equiperdum, 1340
Tubuloglomerular feedback, in acute renal failure, 925
Tumor necrosis factor
antibodies directed against, 737
in endotoxemia, 726–727, 727t–728t
Tumor necrosis factor alpha (TNF-α), in fever, 235
Turbid CSF, 590
Twins
abortion of, 1284
management of, 1275–1278
Two-dimensional (2D) echocardiography, 420–440, 444f, 448f
in aortic regurgitation, 477
in atrial fibrillation, 507, 508f
cardiac output and, 453
in infective endocarditis, 483f
in pericardial disease, 484f
in tricuspid regurgitation, 480f
in ventricular function, 396
in ventricular septal defects, 467
TXA_2, in inflammatory response, 22
Tylosin, 114
Tympany, guttural pouch, 325
Tyrosine, 1161–1167
Tyzzer's disease, 868, 1417

U

Ulceration
corneal, 1144
antibiotics for, 1146
anticollagenases for, 1146–1147
antifungals for, 1146
complicated, 1144–1146
descemetocele and, 1144f
fungal keratitis and, 1145f–1146f
laceration and, 1144f–1145f
melting, 1145f
nonhealing, 1144
subpalpebral lavage catheters for, 1147–1148
systemic NSAIDs for, 1147
uncomplicated, 1144
decubital, 1197

Ulceration *(Continued)*
gastroduodenal, 803–806
diagnosis of, 806, 806t
neonatal, 805
pathophysiology of, 803–804, 804f
prevalence of, 803
risk factors of, 804–805
treatment of, 806–808
Ulcerative lymphangitis, 1174
Ultrapurified polymerized bovine hemoglobin, 1426
Ultrasonography
for aortoiliac thrombosis, 494f, 496
body temperature and, 237
for cardiovascular disorders, 420
for cholelithiasis, 877, 877f
for disorders of the gastrointestinal system, 713
for gastrointestinal disorders, 1401
for hepatic disorders, 862, 1416–1417
for intensive care units, 159–160
for mare breeding soundness examination, 1227–1228, 1228t, 1229f–1230f
for musculoskeletal system disorders, 548, 548f
ocular, 1140–1141
for pleuropneumonia, 347, 347f, 349
for respiratory disorders, 320
for rhodococcal pneumonia, 1395–1396
for rib fractures, 1392–1393
for thrombophlebitis, 492f, 494–495
transcutaneous (abdominal)
for fetal puncture, 1278
for monitoring pregnant mares, 1286–1287
in uterine torsion, 1291
transvaginal, in twin reduction, 1277
for umbilical cord compromise, 1285
for umbilical remnant infections, 1408
for urinary system, 917–918, 917f–918f
for uroperitoneum, 1407
for viral pneumonia, 1395
Umbilical cord compromise, abortion and, 1284–1285
Umbilical remnant infections, 1408
Uncompensated insulin resistance, 1088
Unconjugated bilirubin, 848–849
in hepatic insufficiency, 857
Undifferentiated immunodeficiencies, 59
Unfractionated heparin, for hemostatic dysfunction, 1005
Upper respiratory tract disorders, 323–324
congenital, 1391
Upward fixation of patella, abnormal gaits associated with, 586–587
Urachitis, 1408
Urachus, patent, 897–898
Urate, metabolism of, 900
Urea
hepatic formation of, 846, 847f
metabolism, 898–899
toxic effects of, 1477–1478
Uremic syndrome, 937–938
Uremic toxins, 937–938
Ureteral catheterization, 919, 921f
Ureterolithiasis, 951–954, 953f
Ureterorrhexis, 895–896
Ureters
anatomy of, 888
defects or tears of, 895–896
ectopic, 894–895
Urethra
anatomy of, 888
defects in, 958–960, 959f–960f
Urethral calculi, 956–957, 956f
Urethral hemorrhage, 1007
Urethral rents, 958
Urethritis, 946, 974
Urge incontinence, 974
Uric acid, metabolism of, 900
Urinalysis, 912–917
for acute kidney injury, 1409
enzymuria in, 913–915
fractional clearance of electrolytes in, 915–917, 916t
for musculoskeletal system disorders, 546
reagent strip analysis in, 912–913
sediment examination in, 913, 914f
urine concentration, assessment of, 912
Urinary bladder
distention of, in equine herpesvirus-1 myeloencephalopathy, 648
postpartum
eversion of, 1309
rupture of, 1309
Urinary catheterization, in critical care, 173
Urinary corticoid:creatinine ratio, in pituitary pars intermedia dysfunction, 1106
Urinary incontinence, 973
Urinary system
anatomy of, 888–890, 889f
development of, 890–891, 890f–892f
renal physiology of, 898
blood flow, 903–905, 903f, 904t, 906t–907t
body fluids volume and composition of, 900, 900t
creatinine metabolism in, 899
electrolyte balance in, 901t, 902–903
excretion of solute and water in, 908–909
glomerular filtration, 905–907
nitrogenous and organic wastes, production and elimination of, 898–900, 898b
Urinary system *(Continued)*
renal tubular function, 907–908
urea metabolism in, 898–899
water balance in, 901–902, 901t
toxicoses and, 1505
Urinary system disorders, 888–990
acute renal failure. *see* Acute renal failure
chronic kidney disease in. *see* Chronic kidney disease
congenital, 1406
developmental malformations of, 891–898
bladder defects in, 896–897
ectopic ureter in, 894–895
patent urachus in, 897–898
pendulant kidney in, 894
polycystic kidney disease, glomerulocystic disease, and other hereditary nephropathies in, 893–894, 894f
rectourethral and rectovaginal fistulae in, 896, 896f
renal agenesis, hypoplasia, and dysplasia in, 891–893, 893f
renal cysts in, 893
ureteral defects or tears in, 895–896
vascular anomalies in, 894, 895f
examination of, 909
hematology and serum biochemistry in, 909–912, 910f, 911t
history and physical, 909
imaging techniques in, 917–919
renal biopsy in, 922–923
renal function in, quantitative measures of, 921–922
specialized diagnostic techniques in, 919–923
ureteral catheterization in, 919, 921f
urinalysis in, 912–917
urodynamic procedures in, 923
water deprivation and antidiuretic hormone challenge test in, 919–921, 921f
hematuria in, 957–958
exercise-associated, 961
idiopathic renal hematuria, 960–961, 961f
pigmenturia associated with systemic disease, 961
toxicities, 958
urethral defects, 958–960, 959f–960f
neoplasia of, 969–972, 970t–971t, 972f
obstructive disease of, 949
bladder displacement in, 957
cystic calculi in, 954–956, 954f–956f
epidemiology of, 949
nephrolithiasis and ureterolithiasis in, 951–954, 953f
Urinary system disorders *(Continued)*
pathophysiology of, 949–951, 951f–952f, 951t
penile trauma in, 957
sabulous urolithiasis in, 957
urethral calculi in, 956–957, 956f
polyuria and polydipsia in, 961–962, 962t
diabetes insipidus, 964–966
diabetes mellitus, 966
iatrogenic, 966
pituitary pars intermedia dysfunction, 963
primary polydipsia, 963–964
renal failure, 962–963
sepsis and endotoxemia, 966
renal tubular acidosis in, 966–969, 967t
urinary incontinence and bladder dysfunction in, 973
urinary tract infections in, 946
Urinary tract infections (UTI), 946
cystitis in, 946–947
hematuria and, 958
parasitic infections in, 948–949
pyelonephritis in, 947–948, 948f
urethritis in, 946
in urolithiasis, 951
Urine
concentration, assessment of, 912
pooling. *see* Urovagina
production of, monitoring of, 176
volume, water intake and, in polyuria and polydipsia, 288
Urine pooling. *see* Urovagina
Urobilinogen, 849
Urocanic acid, 1160
Urodynamic procedures, in urinary system, 923
Urokinase, 1005–1006
Urolithiasis
epidemiology of, 949
pathophysiology of, 949–951, 951f–952f, 951t
sabulous, 957
Uroperitoneum, 896–897
in foals, 1406–1407
Urospermia, 1339
Urovagina, 1240
Ursodeoxycholic acid, for hepatic insufficiency, 864
Ursodiol, for hepatic insufficiency, 864
Urticaria, 43, 1190, 1190f
Uterine artery rupture, 1244
Uterine horn, partial inversion (intussusception) of, 1306
Uterine therapy, endometritis and, 1246
Uterine tubes, 1244
Uterus, 1218, 1218f, 1241–1244
contractility of, hormones in, 1263
pathology of, in mare breeding soundness examination, 1230–1236
prolapse of, postpartum, 1305–1306
rupture of, postpartum, 1306
torsion of, obstetric, 1291–1292

UTI. *see* Urinary tract infections
Uvea
  anatomy of, 1151
  disorders of, 1151–1153
    corpora nigra cyst as, 1151, 1151f
    iris stromal hypoplasia as, 1151
Uveitis, 1151–1152
  equine recurrent, 46–47, 1152–1153

V

Vaccination
  for alphavirus encephalitis, 658–659
  for immunomodulation, 62
  in lymphocyte function assessment, 51
  for *S. equi* infection, 337
  for tetanus, 672
  for West Nile virus, 662
Vacor toxicity, 1465–1466
Vagina, 1218–1219, 1220f, 1239–1240
  artificial, for semen collection
    care of, 1324
    selection of, 1323–1324
  examination of, 1232–1233
  hemorrhage in, obstetric, 1292–1293
  lacerations of, postpartum, 1309
Vaginitis, 1240
Vagus nerve, neurologic examination of, 584
Valacyclovir
  for equine herpesvirus myeloencephalopathy, 341
  for equine multinodular pulmonary fibrosis, 342–344
  for foals, 1422t
Vallate papillae, 790
Valvular heart disease, 390–391, 391f, 471–484, 472f
  aortic regurgitation, 476–479, 476f, 478f–479f
  infective endocarditis, 481–484, 482f–483f
  mitral regurgitation, 472–476, 473f
  pulmonary regurgitation, 481, 481f
  tricuspid regurgitation, 479–480, 480f
Valvular regurgitation, 450–451
  aortic, 476–479, 476f, 478f–479f
    auscultation of, 402f
  causes of, 401t–402t
  mitral, 472–476, 473f
  pulmonary, 481, 481f
  tricuspid, 479–480, 480f
VAMP. *see* Vesicle-associated membrane protein
Vancomycin, auditory system and, 627
Varicose veins, vaginal, 1240
Vascular cell adhesion molecules, 717–718
Vascular endothelium, 260
Vascular pattern, radiographic, 320–322
Vascular permeability, changes in, 262
Vascular system
  disorders of
    anatomic correlates of, 387–392, 388b–389b, 390f–391f
    aortocardiac and aortopulmonary fistula, 493f, 496–497
    aortoiliac thrombosis, 493f–494f, 495–496
    arteriovenous fistulas, 496
    parasitic vascular lesions, 494f, 496
    poor performance and, 399b
    pulmonary artery rupture, 497
    thrombophlebitis, 482, 492f, 493–495
  peripheral, examination of, 409–411
Vascular tumors, 1019–1020
Vasculitis, 1011
  cutaneous, systemic manifestations of, 1165t–1167t
  edema and, 262
  equine herpesvirus-1 myeloencephalopathy and, 650
  immune-mediated, 1196–1197, 1196f
  leukocytoclastic, 1201–1202, 1202f
Vasoactive drugs, in critical care, 174
Vasoactive intestinal peptide, colitis and, 722t
Vasodilators
  for congestive heart failure, 462
  for heart disease, 457t–461t
Vasopressin
  for acute renal failure, 925
  in critical care, 175
  for diabetes insipidus, 965
  for foals, 1430
Vasopressors, in critical care, 174–175
V(D)J recombination, 52
Vedaprofen, 126
VEE. *see* Venezuelan equine encephalomyelitis
Vegetable oils, 201, 204
Veins
  cutaneous, examination of, 410
  echocardiographic and Doppler studies of, 441b–443b
  varicose, vaginal, 1240
Venezuelan equine encephalomyelitis (VEE), 655, 656t
Venom, snake, 1498–1499
Venomous bites and stings, 1189
Venous blood gas, monitoring of, in critical care, 177
Ventilation
  control of, 251–253, 252f
  effectors of, 253
Ventilation-perfusion (V-Q) mismatch, 254–255
Ventilator-induced lung injury (VILI), in foals, 1399–1400
Ventipulmin, for interstitial pulmonary disease, 352–353
Ventral body wall hernias, 1290–1291
Ventral edema
  in infective endocarditis, 483f
  in lymphoma, 370
Ventral sacculation, uterine, 1232
Ventricles, 390f
  arrhythmias of, 510f–511f, 512
    premature complexes, 512–513
    tachycardia, 512f, 513–514
  cardiac cycle and, 392–395
  echocardiographic and Doppler studies of, 441b–443b
  function of, 395–397, 395b
Ventricular hypertrophy, 469–470
Ventricular septal defects, 463f, 465–468, 465f–467f
  echocardiography in, 466f, 467
  paramembranous, 402f
  pathophysiology of, 466
  pulmonary atresia with, 470–471, 470f
  thoracic radiography in, 419f
Ventricular septum, 463
  echocardiographic and Doppler studies of, 441b–443b
Ventricular synergy, 396
Verapamil, 457t–461t
Verminous encephalomyelitis, 682–685, 684t
Vermis, 628
Vernal transition, 1222
  hormonal manipulation of, 1258–1259
Verrucous sarcoids, 1202, 1204f
Vertebral injury, 618–622, 618t
Very-low-density lipoproteins, 847
Vesicle-associated membrane protein (VAMP), 668–669
Vesicoureteral reflux, 947–948
Vesicovaginal reflux. *see* Urovagina
Vesicular sounds, in respiratory tract examination, 316
Vesicular stomatitis, 1176–1177, 1176f
Vestibular system, 622–623
  disease of, 622–628
    brainstem auditory evoked response in, 628
    caloric testing in, 628
    central vestibular disease, 627–628
    clinical signs of, 623–625, 623f–624f
    peripheral vestibular disease, 625–627
  neurologic examination of, 580–582
Vestibule, 1219, 1220f–1221f
Vestibulocochlear nerve
  neurologic examination of, 583
  temporohyoid osteoarthropathy and, 625
VetMed Resource, 220
VetSRev, 221
Vicia villosa, 1495
Videoendoscopic examination, of upper respiratory tract, 317
Vincristine, 627
Vinegar, for endometritis, 1255, 1255t
Viral agents, of rhinitis, 323
Viral encephalitis, 654–659, 655t
Viral hepatitis, 869
Viral infections
  abortion and, 1282–1283
  cutaneous, 1175–1177
  immunologic avoidance, 14
  in neonatal foals, 1393
  papillomatosis, 1175–1176
  pathogenesis of, 8–14, 10f–12f
  pathology of, 12
  spread of, 10–11
  virulence of, 10–11
  virus life cycle and, 9, 12f
Viral products, for immunostimulation, 61–62
Viral-associated myopathies, 563
Virologic latency, 10
Virulence
  bacterial infection and, 5–6
  fungal infection and, 7–8
  viral infection and, 10–11
Visceral pain, 138
Vital HN, 204
Vitamin D
  actions of, 1037f, 1039
  in calcium and phosphate homeostasis, 1037–1038
  metabolism of, 1038–1039
Vitamin D_2 toxicity, 1507
Vitamin D_3 toxicity, 1507
Vitamin E
  deficiency of
    in dilated cardiomyopathy, 491
    in equine degenerative myeloencephalopathy, 637–638
    nutritional myodegeneration and, 561
  deficient myopathy, 562–563
    clinical signs of, 563, 563f, 563b
    diagnosis of, 563
    pathogenesis of, 562–563
    treatment of, 563
  in musculoskeletal system disorders, 546
  for nutritional myodegeneration, 1420, 1419–1420
  for pregnant mare, 1367
  sporadic exertional rhabdomyolysis and, 549
  supplementation of
    in equine degenerative myeloencephalopathy, 639–640
    in equine motor neuron disease, 667
Vitamin K, 1000
  deficiency, 1000
    bleeding, in neonatal foals, 1014
Vitamin K_3 toxicity, 1507
Vitiligo, of Arabian horses, 1168, 1168f

Volatile fatty acids (VFAs), 278
Volume of distribution (Vd)
  clinical use of different values, 83, 83b
  conditions that affect, 83–84
  of drug, 82, 82f–83f
Volutrauma, in foals, 1399–1400
Volvulus
  large colon, 781–783
  small intestinal, 780
Von Willebrand's disease, 1013–1014
Voriconazole, for fungal pneumonia, 356t
Vulva, 1219, 1222f
  hematomas of, postpartum, 1310
  pathology of, 1238–1239
Vulvoplasty, Caslick, 1239

W

Walking
  compulsive, 582
  gait evaluation during, 585
  normal, 586
Warbles, 1187
Warfarin
  toxic effects of, 1486–1488
  toxicity, 1014
Warts, 1175, 1176f
Wasp, hypersensitivity to, 1190
Wastes, production and elimination of, 898–900, 898b
Water
  balance, 901–902, 901t
    normal, maintenance of, 284–286
  deprivation, 919–921, 921f
    testing, for polyuria and polydipsia, 288–289
  excretion of, 908–909
  intake of. *see* Polydipsia
  management, for insect control, 1163b–1164b
Water-soluble carbohydrates (WSCs), 194b, 198
Weakness, gait evaluation and, 585
Weanling, gastroduodenal ulcer disease in, 805, 805f–806f
Web of Science, 221
WEE. *see* Western equine encephalomyelitis
Weight, changes in, 238–244, 239t
Weight gain
  alterations in, 239–241
  causes of, 240b
  conditions associated with, 240
  diagnostic approach to, 240–241
  mechanisms of, 239–240
Weight loss, 241–244
  approach to, 243–244
  in chronic kidney disease, 938–939
  clinical pathology of, 244
  conditions associated with, 242–243
  in hepatic insufficiency, 855
Weight loss *(Continued)*
  in infective endocarditis, 483f
  laboratory testing for, 244
  mechanisms of, 241–242
Weight management programs, 212–213
West Nile virus, 659–660
  clinical signs in, 660–661
  diagnosis of, 661
  pathologic findings in, 661
  prevention of, 662
  therapy in, 661–662
Western blot, 643
Western equine encephalomyelitis (WEE), 655, 656t
Wet gangrene, 1197
Wheal-and-flare reaction, 43
Wheezes, 316
White blood cell count
  in cerebrospinal fluid, 590, 591t
  hepatic insufficiency and, 857t
White muscle disease, 561, 1419–1420
White snakeroot
  rhabdomyolysis from, 568
  toxicity, 1484–1485
Whole blood, transfusion, 167
Wiggers' cardiac cycle, 392, 393f
Wikipedia, 221
Wild jasmine toxicity, 1494
Winter anestrus, 1221
Wire and pulley system, critically ill horse and, 185, 186f
WNV encephalitis, equine protozoal myeloencephalitis and, 644
Wolbachia, 16
Wounds
  botulism and, 673
  oral, 795

X

Xanthine oxidase, in reperfusion injury, intestinal, 770, 770f
Xanthochromia, cerebrospinal fluid, 590
Xylazine
  for acute rhabdomyolysis, 549
  for esophageal obstruction, 799
  for gastrointestinal ileus, 775
  for hepatic insufficiency, 863
  for pain management, 144t–145t
  for sedation, in electromyography, 591–592
  for seizures, 607

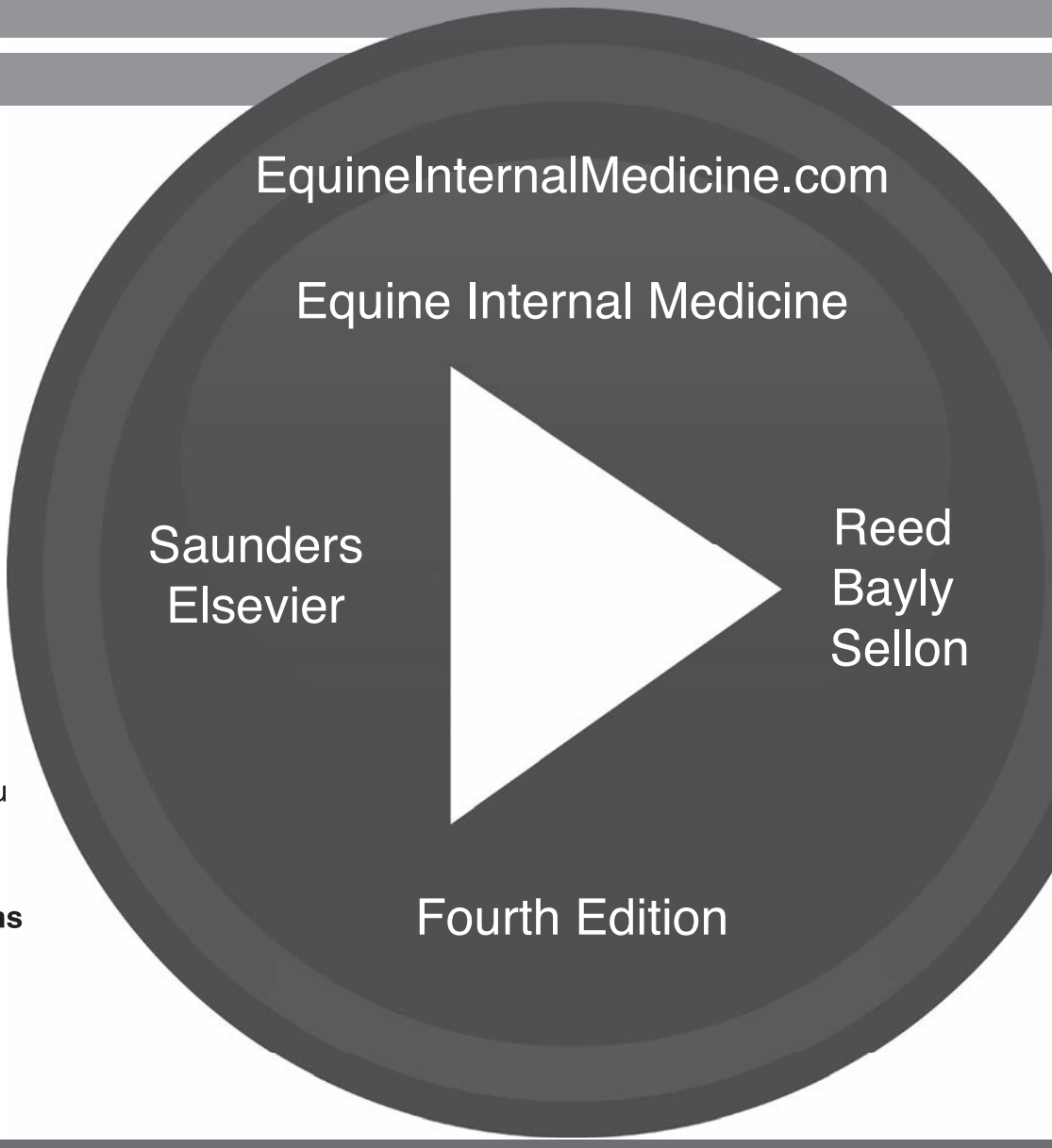
Y

Yellow starthistle, 686
  toxicity, 1479–1480
Yersinia spp., 6–7
Yew toxicity, 1486

Z

Zero order reactions, 80, 80f
Zinc, toxic effects of, 1500
Zona fasciculata, 1071
Zona glomerulosa, 1071

See detailed videos of diseases and procedures you read about!



More than 130 video clips on the **companion website** bring to life the symptoms and treatment of diseases covered in the text. These clips demonstrate problems and procedures related to the cardiovascular and neurologic systems for greater comprehension.

The Companion Website includes:

- **Video clips of different procedures** relating what you read to real-life examples of diseases and their treatment.
- **Audio-visual demonstrations** that familiarize you with all aspects of carrying out procedures.

Start using the website now!